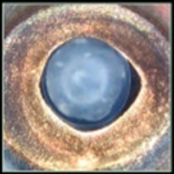


ENCYCLOPEDIA OF

FISH PHYSIOLOGY

FROM GENOME TO ENVIRONMENT

EDITOR-IN-CHIEF

ANTHONY P. FARRELL

ASSOCIATE EDITORS

E. DON STEVENS, JOSEPH J. CECH, JR. & JEFFREY G. RICHARDS

ENCYCLOPEDIA OF
FISH PHYSIOLOGY: FROM GENOME TO ENVIRONMENT

How to go to your page

This eBook set contains 3 volumes.

Each volume has its own page numbering scheme for the front matter.

For example, to go to Volume 1, page ii, type 'Vol1:ii' in the "page #" box at the top of the screen and click "Go".

To go to Volume 2, page v, type 'Vol2:v' in the "page #" box at the top of the screen and click "Go".

The chapter numbers are continuous between the all three volumes.

Please refer to the eTOC for any further clarification.

ENCYCLOPEDIA OF FISH PHYSIOLOGY: FROM GENOME TO ENVIRONMENT

Volume 1

THE SENSES, SUPPORTING TISSUES, REPRODUCTION, AND BEHAVIOR

Editor-in-Chief
ANTHONY P. FARRELL
Department of Zoology,
University of British Columbia,
Vancouver, BC, Canada

ELSEVIER
AMSTERDAM BOSTON HEIDELBERG LONDON NEW YORK OXFORD
PARIS SAN DIEGO SAN FRANCISCO SINGAPORE SYDNEY TOKYO
Academic Press is an imprint of Elsevier
ACADEMIC PRESS

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NWI 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

Copyright © 2011 Elsevier Inc. All rights reserved

The following articles are US government works in the public domain and are not subject to copyright:
Energetic Models: Bioenergetics in Ecosystems
Hormonal Control of Metabolism and Ionic Regulation: The Hormonal Control of Osmoregulation in Teleost Fish
Swimming and Other Activities: Applied Aspects of Fish Swimming Performance
The Skin: Functional Morphology of the Integumentary System in Fishes; The Many Functions of Fish Integument

The following articles are Crown Copyright:
Fish Migrations: Eel Migrations
Social and Reproductive Behaviors: Sexual Behavior in Fish

No part of this publication may be reproduced, stored in a retrieval system
or transmitted in any form or by any means electronic, mechanical, photocopying,
recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at (http://elsevier.com/locate/permissions), and selecting *Obtaining permission to use Elsevier material*

Notice
No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein, Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Catalog Number: 2011923567

ISBN: 978-0-12-374545-3

For information on all Elsevier publications
visit our website at books.elsevier.com

Printed and bound in Italy

11 12 13 14 15 10 9 8 7 6 5 4 3 2 1

For supplementary material please visit the companion site:
http://www.elsevierdirect.com/companions/9780123745453

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER BOOK AID International Sabre Foundation

Editorial: Kate Mittell
Production: Nicky Carter

EDITORS

EDITOR-IN-CHIEF

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ASSOCIATE EDITORS

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island,
Charlottetown, PE, Canada

This page intentionally left blank

EDITORS' BIOGRAPHIES

Editor-in-Chief

Anthony P. Farrell, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Tony Farrell is a graduate of Bath University, where he was fortunate to study with Peter Lutz. His fortunes grew further when he moved in 1974 to Canada and the Zoology Department at the University of British Columbia (UBC) to complete his PhD degree under the excellent tutelage of Dave Randall. In 2004, Tony returned to UBC when he accepted an endowed research chair in sustainable aquaculture. In between these positions at UBC, Tony was employed at the University of Southern California (PDF), the University of New Brunswick (sessional lecturer), Mount Allison University (first real job), and Simon Fraser University (moving through the ranks to a full professor). In addition to highly controlled laboratory experiments on fish cardiorespiratory physiology, Tony is committed to working on animals in their own environment. Therefore, his research on fish physiology has taken him on an *Alpha Helix* expedition to the Amazon, the University of Gothenburg and the Kristineberg Marine Research Station in Sweden, the Portobello Marine Biological Station in New Zealand, the University of Christchurch and Massey University in New Zealand, the Bamfield Marine Science Station and the Huntsman Marine Station in Canada, the University of Aarhus in Denmark, the University of Adelaide Charles and Darwin University in Australia, and to the Danish Arctic Marine Station on Disco Island in Greenland. These travels have allowed him to work with many superb collaborators wordwide, as well as study the physiology of over 70 different species of fish. Tony has received a number of awards for his scientific contributions: an honorary degree from the University of Gothenburg in Sweden; Awards of Excellence from the American Fisheries Society for Fish Physiology, Conservation and Management; the Fry Medal from the Canadian Society of Zoologists; and the Beverton Medal from the Fisheries Society of the British Isles.

Associate Editor

Joseph J. Cech, Jr., Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

Joe Cech grew up in northern Illinois <1 km from the Salt Creek Preserve, where he spent many hours as a boy. Summer trips to northern Wisconsin, northern Minnesota, and southern Ontario increased his interest in aquatic habitats and their fishes. He graduated from the University of Wisconsin (UW, Madison), where he was fortunate to study oceanography under Robert Ragotzkie and fish biology under Art Hasler and Fran Henderson in the UW Laboratory of Limnology. Before completing his BS degree in zoology, he was fortunate to serve as the resident zoologist aboard the R/V *Dante Deo*, for a year, as it crossed the Caribbean Sea and the South Pacific Ocean. Completing his degree upon his return, he was hired as a research assistant at the University of Texas' Marine Science Institute (UTMSI), where he earned his MA and PhD degrees in zoology, under the supervision of Curly Wohschlag. During his graduate education in Austin (Texas), he studied fish biology under Clark Hubbs and physiology under Jack Myers and Jim Larimer. After a 2-year postdoctoral position in Maine, he was hired as a fish physiological ecologist faculty member at the University of California (UC), Davis, in the Department of Wildlife, Fish, and Conservation Biology.

During his 32 years as an active faculty member at UC Davis, his research on the physiological ecology of fishes took him to Canada (University of British Columbia, Bamfield Marine Station), Finland (University of Helsinki), and to other laboratories in the USA (University of Tennessee, Scripps Institution of Oceanography, R/V *David Starr Jordan* in the North Pacific Ocean, UTMSI, Oregon Institute of Marine Biology, and the UC Bodega Marine Laboratory) where he collaborated with many excellent scientists. During this period, he taught courses in physiology of fishes, physiological ecology, and field studies in fish biology; he supervised 25 graduate students (10 MS and

15 PhD) in the disciplinary areas of ecology, physiology, zoology, and pharmacology/toxicology at UC Davis. He is a fellow of the AIFRB and AAAS, and he received Awards of Excellence from the American Fisheries Society (Physiology Section, and California-Nevada Chapter) and the UC Davis Prize for Undergraduate Teaching and Scholarly Achievement. Finally, he co-authored a popular ichthyology textbook, with Peter Moyle, and he published more than 160 peer-reviewed publications on the physiological ecology of fishes. He is currently Professor Emeritus of Fish Biology at the University of California Davis.

Associate Editor

Jeffrey G. Richards, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Jeffrey Richards is an associate professor in the Department of Zoology at the University of British Columbia, Canada. He started his academic career in the Department of Biology at Wilfrid Laurier University obtaining a BSc in 1995. He then went on to do an MSc at the University of Waterloo under the guidance of the late Prof. Richard Playle. With Prof. Playle, he studied environmental physiology and toxicology in fish. Following his MSc, he entered the laboratory of Prof. Chris Wood at McMaster University and focused his research on energy metabolism in swimming fish. He completed his PhD in 2002 and took up a postdoctoral fellowship at the University of British Columbia with Prof. Patricia Schulte. After a 2-year postdoctoral work, he started at the University of British Columbia as an assistant professor in 2004. His lab studies the relationship between plasticity and adaptive variation in the mechanisms that initiate, maintain, and coordinate metabolic energy balance in animals during exposure to environmental stress. He is particularly interested in the adaptations to low oxygen (hypoxia) in fish and other lower vertebrates. He prefers, whenever possible, to study animals in their natural habitat; thus, he travels extensively to unique and challenging environments, including the Amazon and the Tibetan plateau.

Associate Editor

E. Don Stevens, Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

Don Stevens did an MSc with the late Edgar Black on the effects of severe exercise on metabolites in trout in the Physiology Department at UBC after an undergraduate degree in mathematics and zoology at the University of Victoria. He then moved across the street to do a PhD on effects of moderate exercise on respiration and circulation in trout with Dave Randall, who just had been hired in the Zoology Department at UBC (summarized in a JEB classics article, *Journal of Experimental Biology* 211: 1521). His first position was at the Zoology Department at the University of Hawaii where he discovered that there was more to the fish world than trout, and that there was no such thing as the physiology of fish, rather there was a physiology of fishes. In Hawaii, he worked mostly on the biology of tropical tunas with a focus on their warm-bodiedness, in particular how they achieved higher than ambient body temperatures and the adaptive advantage of them. He then joined the Zoology Department at the University of Guelph working in the general area of comparative physiology with a focus on exercise effects until he retired in 2004. He now has an adjunct position at the Atlantic Veterinary College and continues to do research in fish physiology.

SECTION EDITORS

C Brauner
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

AB Butler
Department Molecular Neuroscience, George Mason University, Fairfax, VA, USA

BA Carlson
Department of Biology, Washington University, St. Louis, MO, USA

BM Casper
Department of Biology, University of Maryland, College Park, MD, USA

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

G Goss
Biological Sciences, University of Alberta, Edmonton, AB, Canada

JB Graham
Department of Economics, University of California at San Diego, La Jolla, CA, USA

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

S Holmgren
Zoophysiology, University of Goteberg, Goteberg, Sweden

N Maclean
Biological Sciences, University of Southampton, Hampshire, UK

JA Nelson
Department of Biological Sciences, Towson University, Towson, MD, USA

KR Olson
Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA

C Olsson
Zoophysiology, University of Goteberg, Goteberg, Sweden

AN Popper
Department of Biology, University of Maryland, College Park, MD, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

PM Schulte
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

RE Shadwick
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

K Sloman
University of the West of Scotland, Paisley, Scotland, UK

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

AP Summers
Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, USA

M Vijayan
Department of Biology, University of Waterloo, Waterloo, ON, Canada

H-J Wagner
Universitaet Tuebingen, Tuebingen, Germany

This page intentionally left blank

CONTRIBUTORS

MV Abrahams
Memorial University of Newfoundland, St. John's, NL, Canada

RMcN Alexander
University of Leeds, Leeds, UK

D Alsop
McMaster University, Hamilton, ON, Canada

N Aluru
Woods Hole Oceanographic Institution, Woods Hole, MA, USA

D Álvarez
Universidad de Oviedo, Oviedo, Spain

B Austin
University of Stirling, Stirling, UK

M Axelsson
University of Gothenburg, Gothenburg, Sweden

R Baker
New York University Medical Center, New York, NY, USA

AM Bakke
Norwegian School of Veterinary Science, Oslo, Norway

JS Ballantyne
University of Guelph, Guelph, ON, Canada

S Balshine
McMaster University, Hamilton, ON, Canada

AH Bass
Cornell University, Ithaca, NY, USA

TL Beitinger
University of North Texas, Denton, TX, USA

TJ Benfey
University of New Brunswick, Fredericton, NB, Canada

M Berenbrink
University of Liverpool, Liverpool, UK

D Bernal
University of Massachusetts, Dartmouth, MA, USA

TR Binder
Hammond Bay Biological Station, Millersburg, MI, USA

BA Block
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

A Böhne
Université de Lyon, Lyon, France

NC Bols
University of Waterloo, Waterloo, ON, Canada

JK Bowmaker
University College London, London, UK

I Braasch
University of Oregon, Eugene, OR, USA

VA Braithwaite
Penn State University, State College, PA, USA;
University of Bergen, Norway

MH Braun
University of Calgary, Calgary, AB, Canada

CJ Brauner
University of British Columbia, Vancouver, BC, Canada

DL Breitburg
Smithsonian Environmental Research Center, Edgewater, MD, USA

F Brunet
Université de Lyon, Lyon, France

J Buckley
University of Plymouth, Plymouth, UK

T Burt de Perera
University of Oxford, Oxford, UK

D Burton
Memorial University of Newfoundland, St. John's, NL, Canada

AB Butler
George Mason University, Fairfax, VA, USA

DC de Caprona
University of Iowa, Iowa City, IA, USA

AA Caputi
Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

BA Carlson
Washington University in St. Louis, St. Louis, MO, USA

MA Caruso
North Dakota State University, Fargo, ND, USA

MR de Carvalho
Universidade de São Paulo, São Paulo, Brazil

BM Casper
University of Maryland, College Park, MD, USA

T Castro-Santos
USGS Biological Resources Discipline, Turners Falls, MA, USA

JJ Cech Jr.
University of California, Davis, CA, USA

JN Cech
Howard Hughes Medical Institute, Boston, MA, USA

D Chabot
Institut Maurice-Lamontagne, Mont-Joli, QC, Canada

MJ Chacron
McGill University, Montreal, QC, Canada

K Cheney
The University of Queensland, Brisbane, QLD, Australia

KM Claeson
University of Texas at Austin, Austin, TX, USA

SJ Cooke
Carleton University, Ottawa, ON, Canada

SL Coombs
Bowling Green State University, Bowling Green, OH, USA

AJ Cotel
University of Michigan, Ann Arbor, MI, USA

LI Crawshaw
Portland State University, Portland, OR, USA

GT Crossin
Simon Fraser University, Burnaby, BC, Canada

S Currie
Mount Allison University, Sackville, NB, Canada

CP Cutler
Georgia Southern University, Statesboro, GA, USA

MN Dean
Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

J-P Denizot
CNRS, Gif sur Yvette, France

RH Devlin
Fisheries and Oceans Canada, West Vancouver, BC, Canada

RJ Díaz
Virginia Institute of Marine Science, Gloucester Point, VA, USA

KA Dickson
California State University Fullerton, Fullerton, CA, USA

P Domenici
CNR – IAMC, Torregrande (Or), Italy

SI Doroshov
University of California, Davis, CA, USA

RH Douglas
City University London, London, UK

WW Dowd
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

K Dunlap
Trinity College, Hartford, CT, USA

S Egginton
University of Birmingham, Birmingham, UK

DJ Ellerby
Wellesley College, Wellesley, MA, USA

DG Elliott
Western Fisheries Research Center, US Geological Survey, Seattle, WA, USA

J Engelmann
University of Bielefeld, Bielefeld, Germany

DH Evans
University of Florida, Gainesville, FL, USA

DS Faber
Albert Einstein College of Medicine, Bronx, NY, USA

AP Farrell
University of British Columbia, Vancouver, BC, Canada

RR Fay
Loyola University Chicago, Chicago, IL, USA

LA Ferry
Arizona State University, Phoenix, AZ, USA

PA Fields
Franklin & Marshall College, Lancaster, PA, USA

IP Forster
Fisheries and Oceans Canada, West Vancouver, BC, Canada

ME Forster
University of Canterbury, Christchurch, New Zealand

ES Fortune
Johns Hopkins University, Baltimore, MD, USA

B Fritzsch
University of Iowa, Iowa City, IA, USA

DS Fudge
University of Guelph, Guelph, ON, Canada

D Galiana-Arnoux
Université de Lyon, Lyon, France

GLJ Galli
University of Manchester, Manchester, UK

AK Gamperl
Memorial University of Newfoundland, St. John's, NL, Canada

DP German
University of California, Irvine, CA, USA

TE Gillis
University of Guelph, Guelph, ON, Canada

KM Gilmour
University of Ottawa, Ottawa, ON, Canada

D Giordano
National Research Council (CNR), Naples, Italy

J Godwin
North Carolina State University, Raleigh, NC, USA

A González
University Complutense of Madrid, Madrid, Spain

RJ Gonzalez
University of San Diego, San Diego, CA, USA

A Gräns
University of Gothenburg, Gothenburg, Sweden

AY Gracey
University of Southern California, Los Angeles, CA, USA

JB Graham
University of California at San Diego, La Jolla, CA, USA

S Grillner
Karolinska Institutet, Stockholm, Sweden

H Guderley
Université Laval, Montreal, QC, Canada

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

MC Hastings
Georgia Institute of Technology, Atlanta, GA, USA

AD Hawkins
Loughine Ltd., Aberdeen, Scotland, UK

LP Hernandez
George Washington University, Washington, DC, USA

JE Herr
University of Guelph, Guelph, ON, Canada

MJ Herrero-Turrión
Institute of Neurosciences of Castilla y León, Salamanca, Spain

TE Higham
Clemson University, Clemson, SC, USA

SD Hillyard
UNLV School of Dental Medicine, Las Vegas, NV, USA

EJ Hilton
Virginia Institute of Marine Science, Gloucester Point, VA, USA

SG Hinch
University of British Columbia, Vancouver, BC, Canada

MH Hofmann
University of Bonn, Bonn, Germany

S Holmgren
University of Gothenburg, Gothenburg, Sweden

DR Huber
University of Tampa, Tampa, FL, USA

P-P Hwang
Academia Sinica, Taipei, Taiwan, China

JM Jørgensen
University of Aarhus, Aarhus, Denmark

WR Jeffery
University of Maryland, College Park, MD, USA

E Jönsson
University of Gothenburg, Gothenburg, Sweden

M Jobling
University of Tromsø, Tromsø, Norway

DR Jones
University of British Columbia, Vancouver, BC, Canada

MG Jonz
University of Ottawa, Ottawa, ON, Canada

F Jutfelt
University of Gothenburg, Gothenburg, Sweden

IM Kaatz
Stamford, CT, USA

I Katsiadaki
Cefas Weymouth Laboratory, Weymouth, UK

A Kawano
University of Waterloo, Waterloo, ON, Canada

M Kawasaki
University of Virginia, Charlottesville, VA, USA

CJ Kennedy
Simon Fraser University, Burnaby, BC, Canada

P Kiilerich
Institut National de la Recherche Agronomique (INRA), Rennes, France

SS Killen
University of Glasgow, Glasgow, Scotland, UK

M Kinoshita
Kyoto University, Kyoto, Japan

F Kirschbaum
Humboldt-Universität zu Berlin, Berlin, Germany

CS Krahforst
East Carolina University, Greenville, NC, USA

Å Krogdahl
Norwegian School of Veterinary Science, Oslo, Norway

RHH Kröger
Lund University, Lund, Sweden

D Kültz
University of California, Davis, CA, USA

F Ladich
University of Vienna, Vienna, Austria

LEJ Lee
Wilfrid Laurier University, Waterloo, ON, Canada

B Levavi-Sivan
Hebrew University of Jerusalem, Rehovot, Israel

MW Levine
University of Illinois at Chicago, Chicago, IL, USA

D Liedtke
University of Würzburg, Würzburg, Germany

JH Long Jr.
Vassar College, Poughkeepsie, NY, USA

Z Lu
University of Miami, Coral Gables, FL, USA

JJ Luczkovich
East Carolina University, Greenville, NC, USA

WI Lutterschmidt
Sam Houston State University, Huntsville, TX, USA

P Machnik
University of Bayreuth, Bayreuth, Germany

N Maclean
University of Southampton, Southampton, UK

CP Madenjian
Great Lakes Science Center, Ann Arbor, MI, USA

H Malte
Aarhus University, Aarhus, Denmark

F Marino
University of Messina, Messina, Italy

NJ Marshall
The University of Queensland, Brisbane, QLD, Australia

GB McClelland
McMaster University, Hamilton, ON, Canada

CA McCormick
Oberlin College, Oberlin, OH, USA

SD McCormick
USGS, Conte Anadromous Fish Research Center,
Turners Falls, MA, USA

DJ McKenzie
Université Montpellier 2, Montpellier, France

DB McMillan
University of Western Ontario, London, ON, Canada

M Megías
University of Vigo, Vigo, Spain

AF Mensinger
University of Minnesota Duluth, Duluth, MN, USA

JD Metcalfe
Centre for Environment, Fisheries and Aquaculture
Science, Lowestoft, UK

WK Milsom
University of British Columbia, Vancouver, BC, Canada

CMR Le Moine
University of Ottawa, Ottawa, ON, Canada

JC Montgomery
University of Auckland, Auckland, New Zealand

TW Moon
University of Ottawa, Ottawa, ON, Canada

PC Morris
Heriot-Watt University, Edinburgh, UK

CD Moyes
Queen's University, Kingston, ON, Canada

CA Myrick
Colorado State University, Fort Collins, CO, USA

JA Nelson
Towson University, Towson, MD, USA

M Nikinmaa
University of Turku, Turku, Finland

GE Nilsson
University of Oslo, Oslo, Norway

S Nilsson
University of Gothenburg, Gothenburg, Sweden

RH Nóbrega
Utrecht University, Utrecht, The Netherlands

RG Northcutt
University of California, San Diego, La Jolla, CA, USA

D Northmore
University of Delaware, Newark, DE, USA

KR Olson
Indiana University School of Medicine – South Bend,
South Bend, IN, USA

C Olsson
University of Gothenburg, Gothenburg, Sweden

K Pappas
Trinity College, Hartford, CT, USA

JC Partridge
University of Bristol, Bristol, UK

MA Peck
University of Hamburg, Hamburg, Germany

B Pelster
University of Innsbruck, Innsbruck, Austria

AE Pereda
Albert Einstein College of Medicine, Bronx, NY, USA

SF Perry
University of Ottawa, Ottawa, ON, Canada

S Pieperhoff
University of British Columbia, Vancouver, BC, Canada

F Piferrer
Institut de Ciències del Mar, Consejo Superior de
Investigaciones Científicas (CSIC), Barcelona, Spain

JE Podrabsky
Portland State University, Portland, OR, USA

MA Pombal
University of Vigo, Vigo, Spain

H-O Pörtner
Alfred-Wegener-Institute, Bremerhaven, Germany

AN Popper
University of Maryland, College Park, MD, USA

G di Prisco
National Research Council (CNR), Naples, Italy

P Prunet
Institut National de la Recherche Agronomique (INRA),
Rennes, France

DJ Randall
City University of Hong Kong, Hong Kong, China

BJ Reading
North Carolina State University, Raleigh, NC, USA

SG Reebs
Université de Moncton, Moncton, NB, Canada

SG Reid
University of Toronto Scarborough, Toronto, ON, Canada

JG Richards
University of British Columbia, Vancouver, BC, Canada

DA Righton
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

JW Robinson
University of Guelph, Guelph, ON, Canada

RE Rodríguez
Institute of Neurosciences of Castilla y León, Salamanca, Spain

I Rodríguez-Moldes
Universidad de Santiago de Compostela, Santiago de Compostela, Spain

PJ Rombough
Brandon University, Brandon, MB, Canada

I Rønnestad
University of Bergen, Bergen, Norway

GG Rosenthal
Texas A&M University, College Station, TX, USA

JL Rummer
University of British Columbia, Vancouver, BC, Canada

R Russo
National Research Council (CNR), Naples, Italy

FM Sánchez-Simón
Institute of Neurosciences of Castilla y León, Salamanca, Spain

PM Schulte
University of British Columbia, Vancouver, BC, Canada

RW Schulz
Utrecht University, Utrecht, The Netherlands

W Schulze
University of Bayreuth, Bayreuth, Germany

S Schuster
University of Bayreuth, Bayreuth, Germany

CJW Scott
Heriot-Watt University, Edinburgh, UK

M Sebire
Cefas Weymouth Laboratory, Weymouth, UK

SM Secor
University of Alabama, Tuscaloosa, AL, USA

O Seppälä
EAWAG, Swiss Federal Institute of Aquatic Science and Technology, and ETH-Zürich, Institute of Integrative Biology (IBZ), Dübendorf, Switzerland

H Seth
University of Gothenburg, Gothenburg, Sweden

RS Seymour
University of Adelaide, Adelaide, SA, Australia

MA Sheridan
North Dakota State University, Fargo, ND, USA

HA Shiels
University of Manchester, Manchester, UK

KR Siegfried
Massachusetts General Hospital, Boston, MA, USA

JA Sisneros
University of Washington, Seattle, WA, USA

KA Sloman
University of the West of Scotland, Paisley, Scotland, UK

LU Sneddon
University of Chester, Chester, UK

MC Soares
Universidade de São Paulo, São Paulo, Brazil

GN Somero
Stanford University, Pacific Grove, CA, USA

PW Sorensen
University of Minnesota, St. Paul, MN, USA

MW Sprague
East Carolina University, Greenville, NC, USA

N Stacey
University of Alberta, Edmonton, AB, Canada

EM Standen
McGill University, Montreal, QC, Canada

ED Stevens
University of Prince Edward Island, Charlottetown, PE, Canada

H Straka
Ludwig-Maximilians-Universität, Martinsried-Planegg, Germany

CV Sullivan
North Carolina State University, Raleigh, NC, USA

A Sundby
Norwegian School of Veterinary Science, Oslo, Norway

KS Sundell
University of Gothenburg, Gothenburg, Sweden

F Syeda
University of Birmingham, Birmingham, UK

DA Syme
University of Calgary, Calgary, AB, Canada

EW Taylor
University of Birmingham, Birmingham, UK

H Thorarensen
Holar University College, Saudarkrokur, Iceland

KB Tierney
University of Alberta, Edmonton, AB, Canada

L Tort
Universitat Autonoma de Barcelona, Cerdanyola, Spain

ED Tytell
Johns Hopkins University, Baltimore, MD, USA

C Verde
National Research Council (CNR), Naples, Italy

J-N Volff
Université de Lyon, Lyon, France

H Volkoff
Memorial University of Newfoundland, St. John’s, NL,
Canada

G von der Emde
University of Bonn, Bonn, Germany

M Vornanen
University of Eastern Finland, Joensuu, Finland

H-J Wagner
Universität Tübingen, Tübingen, Germany

MM Walker
University of Auckland, Auckland, New Zealand

KN Wallace
Clarkson University, Potsdam, NY, USA

T Wang
Aarhus University, Aarhus, Denmark

Y Wang
Queen’s University, Kingston, ON, Canada

W Waser
University of Turku, Turku, Finland

JF Webb
University of Rhode Island, Kingston, RI, USA

PW Webb
University of Michigan, Ann Arbor, MI, USA

NC Wegner
Scripps Institution of Oceanography, La Jolla, CA, USA

SE Wendelaar Bonga
Radboud University Heiweg 8, EB Malden,
The Netherlands

CR White
The University of Queensland, St. Lucia, QLD, Australia

MP Wilkie
Wilfrid Laurier University, Waterloo, ON, Canada

TM Winegard
University of Guelph, Guelph, ON, Canada

JM Wilson
Centre for Interdisciplinary Marine and Environmental
Research (CIIMAR), Porto, Portugal

RW Wilson
University of Exeter, Exeter, UK

RJ Wootton
Aberystwyth University, Aberystwyth, Wales, UK

PA Wright
University of Guelph, Guelph, ON, Canada

Y Yamamoto
University College London, London, UK

Z Yaron
Tel-Aviv University, Tel Aviv, Israel

D Zaccone
University of Messina, Messina, Italy

G Zaccone
University of Messina, Messina, Italy

DG Zeddies
Marine Acoustics, Inc., Arlington, VA, USA

IV Zhdanova
Boston University School of Medicine,
Boston, MA, USA

LI Zon
Howard Hughes Medical Institute, Boston, MA, USA

This page intentionally left blank

HOW TO USE THE ENCYCLOPEDIA

STRUCTURE OF THE ENCYCLOPEDIA

The material in the Encyclopedia is arranged as a series of articles within a certain topic.

There are three features to help you easily find the topic you're interested in: a contents list, cross-references to other relevant articles in the Encyclopedia, and a full subject index.

1. CONTENTS LIST

The contents list, which appears at the front of all volume, lists the entries in the order that they appear in the Encyclopedia. It includes both the volume number and the page number of each entry.

2. CROSS-REFERENCES

All of the entries in the Encyclopedia have been extensively cross-referenced. The cross-references which appear at the end of an entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere
ii. To draw the readers attention to parallel discussions in other entries
iii. To indicate material that broadens the discussion

Example

The following list of cross-references may appear in text and at the end of the entry
The Muscles|Cartilaginous Fishes Cranial Muscles

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **The Muscles**: Bony Fish Cranial Muscles. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

3. INDEX

The index includes page numbers for quick reference to the information you're looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4. CONTRIBUTORS

At the start of each volume there is list of the authors who contributed to the Encyclopedia.

This page intentionally left blank

CONTENTS

VOLUME 1 – THE SENSES, SUPPORTING TISSUES, REPRODUCTION, AND BEHAVIOR

SENSING THE ENVIRONMENT

BRAIN AND NERVOUS SYSTEM

Functional Morphology of the Nervous System: An Introduction *AB Butler* 1

Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern *B Fritzsch and DC de Caprona* 6

Functional Morphology of the Brains of Agnathans *MA Pombal and M Megías* 16

Functional Morphology of the Brains of Cartilaginous Fishes *I Rodríguez-Moldes* 26

Functional Morphology of the Brains of Ray-Finned Fishes *AB Butler* 37

Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and *Latimeria* *A González and RG Northcutt* 46

Motor Control Systems of Fish *S Grillner* 56

Physiology of the Mauthner Cell: Discovery and Properties *AE Pereda and DS Faber* 66

Physiology of the Mauthner Cell: Function *DS Faber and AE Pereda* 73

Autonomic Nervous System of Fishes *S Nilsson* 80

Opioids and Opioid Receptors in Fishes *MJ Herrero-Turrión, FM Sánchez-Simón, and RE Rodríguez* 89

VISION

Vision in Fishes: An Introduction *H-J Wagner* 98

Physiological Optics in Fishes *RHH Kröger* 102

Photoreceptors and Visual Pigments *JK Bowmaker* 110

Adaptations of Photoreceptors and Visual Pigments *JK Bowmaker* 116

Inner Retina and Ganglion Cells *MW Levine* 123

Optic Tectum *D Northmore* 131

Behavioral Assessment of the Visual Capabilities of Fish *S Schuster, P Machnik, and W Schulze* 143

Color Vision and Color Communication in Reef Fish *J Marshall and K Cheney* 150

Extraretinal Photoreception *H-J Wagner* 159

Visual Adaptations to the Deep Sea *RH Douglas and JC Partridge* 166

see Communication Behavior: Visual Signals, Volume 1, p. 692
see Coloration and Chromatophores in Fishes, Volume 1, p. 489
see Bioluminescence in Fishes, Volume 1, p. 497

SMELL, TASTE, AND CHEMICAL SENSING

Chemoreception (Smell and Taste): An Introduction *TJ Hara* 183
Morphology of the Gustatory (Taste) System in Fishes *TJ Hara* 187
Morphology of the Olfactory (Smell) System in Fishes *TJ Hara* 194
Neurophysiology of Olfaction *TJ Hara* 208
Neurophysiology of Gustation *TJ Hara* 218
Chemosensory Behavior *TJ Hara* 227
see The Effects of Toxicants on Olfaction in Fishes, Volume 3, p. 2078
see Oxygen Sensing in Fish, Volume 2, p. 871

HEARING AND LATERAL LINE

Fish Bioacoustics: An Introduction *AN Popper and BM Casper* 236
Vestibular System Anatomy and Physiology *H Straka and R Baker* 244
Auditory System Morphology *AN Popper* 252
The Ear and Hearing in Sharks, Skates, and Rays *BM Casper* 262
Biomechanics of the Inner Ear in Fishes *MC Hastings* 270
Psychoacoustics: What Fish Hear *RR Fay* 276
Auditory/Lateral Line CNS: Anatomy *CA McCormick* 283
Physiology of the Ear and Brain: How Fish Hear *Z Lu* 292
Sound Source Localization and Directional Hearing in Fishes *DG Zeddies, RR Fay, and JA Sisneros* 298
Effects of Human-Generated Sound on Fish *AD Hawkins* 304
Acoustic Behavior *J Luczkovich, MW Sprague, and CS Krahforst* 311
Vocal Behavior of Fishes: Anatomy and Physiology *F Ladich and AH Bass* 321
Lateral Line Neuroethology *JC Montgomery and S Coombs* 329
Lateral Line Structure *J Webb* 336
see How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling, Volume 1, p. 684

DETECTION AND GENERATION OF ELECTRIC SIGNALS

Detection and Generation of Electric Signals in Fishes: An Introduction *BA Carlson* 347
Morphology of Electroreceptive Sensory Organs *JM Jørgensen* 350
Physiology of Ampullary Electrosensory Systems *MH Hofmann* 359
Physiology of Tuberous Electrosensory Systems *ES Fortune and MJ Chacron* 366
Active Electrolocation *G von der Emde and J Engelmann* 375
Electric Organs *AA Caputi* 387
Generation of Electric Signals *M Kawasaki* 398

Development of Electroreceptors and Electric Organs *F Kirschbaum and J-P Denizot* 409
see Shocking Comments: Electrocommunication in Teleost Fish, Volume 1, p. 699

MUSCLES, SKELETON, SKIN, AND MOVEMENT

Functional Anatomy of Bones, Muscle, and Skin: An Introduction *ED Stevens* 416

THE SKELETON

Cartilaginous Fish Skeletal Anatomy *KM Claeson and MN Dean* 419
Cartilaginous Fish Skeletal Tissues *MN Dean* 428
Bony Fish Skeleton *EJ Hilton* 434

THE MUSCLES

Cartilaginous Fishes Cranial Muscles *DR Huber, MC Soares, and MR de Carvalho* 449
Bony Fish Cranial Muscles *LA Ferry and L Hernandez* 463

THE SKIN

The Many Functions of Fish Integument *DG Elliott* 471
Functional Morphology of the Integumentary System in Fishes *DG Elliott* 476
Coloration and Chromatophores in Fishes *D Burton* 489
Bioluminescence in Fishes *AF Mensinger* 497
Hagfish Slime *DS Fudge, JE Herr, and TM Winegard* 504

BUOYANCY, LOCOMOTION, AND MOVEMENT IN FISHES

Buoyancy, Locomotion, and Movement in Fishes: An Introduction *ED Stevens* 516
Buoyancy in Fishes *RMcN Alexander* 520
Swimbladder Function and Buoyancy Control in Fishes *B Pelster* 526
Experimental Hydrodynamics *ED Tytell* 535
Undulatory Swimming *DJ Ellerby* 547
Functional Properties of Skeletal Muscle: Work Loops *DA Syme* 555
Paired Fin Swimming *EM Standen* 564
Maneuverability *PW Webb* 575
Stability and Turbulence *PW Webb and AJ Cotel* 581
Fast Start *P Domenici* 587
Feeding Mechanics *TE Higham* 597
Biomimetics: Robotics Based on Fish Swimming *JH Long Jr.* 603
see Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems, Volume 2, p. 935
see Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder, Volume 2, p. 929
see The Retia, Volume 2, p. 1119
see Deep-Sea Fishes, Volume 3, p. 1953

see Applied Aspects of Fish Swimming Performance, Volume 3, p. 1652
see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215
see The Effect of Exercise on Respiration, Volume 2, p. 812

REPRODUCTION

The Diversity of Fish Reproduction: An Introduction *KA Sloman* 613

THE REPRODUCTIVE ORGANS AND PROCESSES

Anatomy and Histology of Fish Testis *RW Schulz and RH Nóbrega* 616
Regulation of Spermatogenesis *RW Schulz and RH Nóbrega* 627
Vitellogenesis in Fishes *BJ Reading and CV Sullivan* 635
see Parental Care in Fishes, Volume 1, p. 670
see Endocrine Regulation of Fish Reproduction, Volume 2, p. 1500
see Socially Controlled Sex Change in Fishes, Volume 1, p. 662
see Nutritional Provision During Parental Care, Volume 1, p. 678
see Sexual Behavior in Fish, Volume 1, p. 656

BEHAVIOR AND PHYSIOLOGY

Linking Fish Behavior and Physiology: An Introduction *KA Sloman* 647

SOCIAL AND REPRODUCTIVE BEHAVIORS

Dominance Behaviors *KA Sloman* 649
Sexual Behavior in Fish *I Katsiadaki and M Sebire* 656
Socially Controlled Sex Change in Fishes *J Godwin* 662
Parental Care in Fishes *S Balshine and KA Sloman* 670
Nutritional Provision During Parental Care *KA Sloman and J Buckley* 678
see Hormonal Pheromones, Volume 2, p. 1553
see The Diversity of Fish Reproduction: An Introduction, Volume 1, p. 613

SENSORY SYSTEMS, PERCEPTION, AND LEARNING

How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling *IM Kaatz* 684
Communication Behavior: Visual Signals *GG Rosenthal and NJ Marshall* 692
Shocking Comments: Electrocommunication in Teleost Fish *K Pappas and K Dunlap* 699
Fish Learning and Memory *VA Braithwaite* 707
Nociception or Pain in Fish *LU Sneddon* 713
Spatial Orientation: How do Fish Find their Way Around? *T Burt de Perera* 720
Magnetic Sense in Fishes *MM Walker* 726
Circadian Rhythms in Fish *SG Reebs* 736
see Bioluminescence in Fishes, Volume 1, p. 497

see Acoustic Behavior, Volume 1, p. 311
see Behavioral Assessment of the Visual Capabilities of Fish, Volume 1, p. 143
see Chemosensory Behavior, Volume 1, p. 227

BEHAVIORAL RESPONSES TO THE ENVIRONMENT

A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food *MV Abrahams* 744
Effects of Compensatory Growth on Fish Behavior *D Álvarez* 752
Temperature Preference: Behavioral Responses to Temperature in Fishes *LI Crawshaw and JE Podrabsky* 758
Behavioral Responses to Hypoxia *KA Sloman* 765
Sleep in Fish *IV Zhdanova* 772
Manipulation of Fish Phenotype by Parasites *O Seppälä* 777
Anthropogenic Influences on Fish Behavior *KA Sloman* 783
see The Biology of Fish Migration, Volume 3, p. 1921

VOLUME 2 – GAS EXCHANGE, INTERNAL HOMEOSTATIS, AND FOOD UPTAKE

GAS EXCHANGE

Respiration: An Introduction *JJ Cech Jr. and CJ Brauner* 791

VENTILATION AND ANIMAL RESPIRATION

Plasticity in Gill Morphology *GE Nilsson* 796
Gill Respiratory Morphometrics *NC Wegner* 803
The Effect of Exercise on Respiration *H Thorarensen* 812
Efficiency of Gas Exchange Organs *H Malte* 820
Respiratory Gas Exchange During Development: Models and Mechanisms *PJ Rombough* 829
Respiratory Gas Exchange During Development: Respiratory Transitions *PJ Rombough* 838
Techniques in Whole Animal Respiratory Physiology *JJ Cech Jr. and CJ Brauner* 846
see Branchial Anatomy, Volume 2, p. 1095

CONTROL OF RESPIRATION

Generation of the Respiratory Rhythm in Fish *EW Taylor* 854
The Ventilatory Response to CO_2/H^+ *WK Milsom* 865
Oxygen Sensing in Fish *MG Jonz* 871

TRANSPORT AND EXCHANGE OF RESPIRATORY GASES IN THE BLOOD

Red Blood Cell Function *M Nikinmaa* 879
Hemoglobin *M Nikinmaa* 887
O_2 Uptake and Transport: The Optimal P_{50} *T Wang and H Malte* 893
Carbonic Anhydrase in Gas Transport and Exchange *KM Gilmour* 899

Carbon Dioxide Transport and Excretion *D Randall* 909
Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange *CJ Brauner and JL Rummer* 916
Evolution of the Bohr Effect *M Berenbrink* 921
Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder *W Waser* 929
Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems *M Berenbrink* 935
Hemoglobin Differentiation in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 944
see Cellular Composition of the Blood, Volume 2, p. 984

TISSUE RESPIRATION

Cellular Respiration *GB McClelland* 951
Mitochondrial Respiration *CD Moyes and CMR Le Moine* 959
Specializations in Mitochondrial Respiration of Fish *CD Moyes and CMR Le Moine* 966

CIRCULATION

The Circulatory System: An Introduction *AP Farrell* 973
Circulatory System Design: Roles and Principles *KR Olson* 977

BLOOD

Cellular Composition of the Blood *AP Farrell* 984
Erythropoiesis in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 992
see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Red Blood Cell Function, Volume 2, p. 879
see Hemoglobin, Volume 2, p. 887

DESIGN AND PHYSIOLOGY OF THE HEART

Cardiac Anatomy in Fishes *AP Farrell and S Pieperhoff* 998
Cellular Ultrastructure of Cardiac Cells in Fishes *GLJ Galli* 1006
The Outflow Tract from the Heart *DR Jones and MH Braun* 1015
Physiology of Cardiac Pumping *AP Farrell* 1030
Action Potential of the Fish Heart *M Vornanen* 1038
Cardiac Excitation–Contraction Coupling: Routes of Cellular Calcium Flux *HA Shiels* 1045
Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element *TE Gillis* 1054
Cardiac Cellular Length–Tension Relationship *HA Shiels* 1060
Intracardiac Neurons and Neurotransmitters in Fish *G Zaccone, A Mauceri, and D Zaccone* 1067
Accessory Hearts in Fishes *AP Farrell* 1073
The Coronary Circulation *AP Farrell* 1077

DESIGN AND PHYSIOLOGY OF ARTERIES AND VEINS

Anatomical Pathways and Patterns *KR Olson* 1085
Branchial Anatomy *KR Olson* 1095
Physiology of Resistance Vessels *KR Olson* 1104

Physiology of Capacitance Vessels *KR Olson* 1111

The Retia *ED Stevens* 1119

The Gastrointestinal Circulation *M Axelsson and H Seth* 1132

see Secondary Circulation and Lymphatic Anatomy, Volume 2, p. 1161
see The Coronary Circulation, Volume 2, p. 1077

DESIGN AND PHYSIOLOGY OF CAPILLARIES AND SECONDARY CIRCULATION

Capillaries, Capillarity, and Angiogenesis *S Egginton and F Syeda* 1142

Circulatory Fluid Balance and Transcapillary Exchange *KR Olson* 1154

Secondary Circulation and Lymphatic Anatomy *KR Olson and AP Farrell* 1161

INTEGRATED CONTROL AND RESPONSE OF THE CIRCULATORY SYSTEM

Integrated Control of the Circulatory System *KR Olson* 1169

Central Control of Cardiorespiratory Interactions in Fish *EW Taylor* 1178

Hormone Metabolism in the Circulation *KR Olson* 1190

Integrated Responses of the Circulatory System to Temperature *AK Gamperl* 1197

Integrated Responses of the Circulatory System to Digestion *H Seth and M Axelsson* 1206

Integrated Cardiovascular Responses of Fish to Swimming *AP Farrell* 1215

Integrated Responses of the Circulatory System to Hypoxia *AK Gamperl* 1221

see Hormone Metabolism in the Circulation, Volume 2, p. 1190
see Accessory Hearts in Fishes, Volume 2, p. 1073
see Respiratory Adaptations for Air-Breathing Fishes, Volume 3, p. 1861
see The Osmorespiratory Compromise, Volume 2, p. 1389

THE GUT

The Gastrointestinal Tract: An Introduction *C Olsson and S Holmgren* 1229
Appendix A 1232

GUT ANATOMY AND MORPHOLOGY

Gut Anatomy *C Olsson* 1268

Pancreas *MA Caruso and MA Sheridan* 1276

Development of Fish Gut *KN Wallace* 1284

see The Gastrointestinal Circulation, Volume 2, p. 1132

INTEGRATED FUNCTION AND CONTROL OF THE GUT

Gut Motility *A Gräns and C Olsson* 1292

Gut Secretion and Digestion *Å Krogdahl, A Sundby, and AM Bakke* 1301

Intestinal Absorption *KS Sundell* *I Rønnestad* 1311

Barrier Function of the Gut *F Jutfelt* 1322

Nervous System of the Gut *S Holmgren and C Olsson* 1332
Endocrine Systems of the Gut *E Jönsson and S Holmgren* 1341
see Integrated Responses of the Circulatory System to Digestion, Volume 2, p. 1206

OSMOTIC, IONIC AND NITROGENOUS-WASTE BALANCE

Osmoregulation in Fishes: An Introduction *DH Evans* 1348
Mechanisms of Gill Salt Secretion in Marine Teleosts *DH Evans* 1354
Mechanisms of Ion Transport in Freshwater Fishes *P-P Hwang* 1359
Water Balance and Aquaporin *CP Cutler* 1366
Osmosensing *D Kültz* 1373

ROLE OF THE GILLS

Morphology of Branchial Ionocytes *JM Wilson* 1381
The Osmorespiratory Compromise *RJ Gonzalez* 1389

ROLE OF THE KIDNEYS

Histology of the Kidney *DB McMillan* 1395
The Kidney *SF Perry* 1411

ROLE OF THE GUT

Gut Ion, Osmotic and Acid-Base Regulation *RW Wilson* 1419
Dogfish Rectal Gland *WW Dowd* 1429
see The Hormonal Control of Osmoregulation in Teleost Fish, Volume 2, p. 1466
see Barrier Function of the Gut, Volume 2, p. 1322
see Endocrine Systems of the Gut, Volume 2, p. 1341
see Intestinal Absorption, Volume 2, p. 1311

NITROGENOUS-WASTE BALANCE

Excretion of Ammonia *D Randall* 1437
Ureotelism *PA Wright* 1444

HORMONAL CONTROLS

THE PITUITARY

Development of the Hypothalamus-Pituitary-Interrenal Axis *D Alsop and N Aluru* 1450
Pituitary Gland or Hypophysis *DB McMillan* 1457

HORMONAL CONTROL OF METABOLISM AND IONIC REGULATION

The Hormonal Control of Osmoregulation in Teleost Fish *SD McCormick* 1466
Corticosteroids *P Kiilerich and P Prunet* 1474

HORMONAL CONTROL OF REPRODUCTION AND GROWTH

Endocrinology of Fish Growth *MA Sheridan* 1483

Endocrine Control of Sex Differentiation in Fish *F Piferrer* 1490

Endocrine Regulation of Fish Reproduction *Z Yaron and B Levavi-Sivan* 1500

Control of Appetite in Fish *H Volkoff* 1509

HORMONAL RESPONSES TO STRESS

Hormone Response to Stress *SE Wendelaar Bonga* 1515

Catecholamines *SG Reid* 1524

Stress Effect on Growth and Metabolism *TW Moon* 1534

Impact of Stress in Health and Reproduction *L Tort* 1541

HORMONES IN COMMUNICATION

Hormonal Pheromones *N Stacey and P Sorensen* 1553

see Hormone Metabolism in the Circulation, Volume 2, p. 1190

see Integrated Control of the Circulatory System, Volume 2, p. 1169

VOLUME 3 – ENERGETICS, INTERACTIONS WITH THE ENVIRONMENT, LIFESTYLES, AND APPLICATIONS

ENERGETICS

Energetics: An Introduction *JA Nelson* 1563

General Energy Metabolism *JA Nelson and D Chabot* 1566

Physiological Functions that Scale to Body Mass in Fish *CR White and RS Seymour* 1573

FOOD ACQUISITION AND DIGESTION

Energetics of Prey Capture: From Foraging Theory to Functional Morphology *LA Ferry* 1583

Energetics of Foraging Decisions and Prey Handling *S Killen* 1588

Digestive Efficiency *DP German* 1596

Cost of Digestion and Assimilation *SM Secor* 1608

Dietary Requirements of Fish Under Culture Conditions *IP Forster* 1617

see A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food, Volume 1, p. 744

ENERGY UTILIZATION IN GROWTH

Energetics of Growth *RJ Wootton* 1623

Growth: Environmental Effects *RJ Wootton* 1629

see Effects of Compensatory Growth on Fish Behavior, Volume 1, p. 752

SWIMMING AND OTHER ACTIVITIES

Energetics of Fish Swimming *DJ McKenzie* 1636

Cellular Energy Utilization: Environmental Influences on Metabolism *H-O Pörtner* 1645

Applied Aspects of Fish Swimming Performance *T Castro-Santos* 1652

see Dominance Behaviors, Volume 1, p. 649
see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215
see The Effect of Exercise on Respiration, Volume 2, p. 812
see The Osmorespiratory Compromise, Volume 2, p. 1389
see Functional Properties of Skeletal Muscle: Work Loops, Volume 1, p. 555

ENERGETIC MODELS

Bioenergetics in Aquaculture Settings *M Jobling* 1664

Bioenergetics in Ecosystems *CP Madenjian* 1675

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

General Principles of Biochemical Adaptations *GN Somero* 1681

TEMPERATURE

Effects of Temperature: An Introduction *PM Schulte* 1688

Measures of Thermal Tolerance *TL Beitinger and WI Lutterschmidt* 1695

Proteins and Temperature *PA Fields* 1703

Mitochondria and Temperature *H Guderley* 1709

Temperature and Excitable Membranes *M Vornanen* 1717

Membranes and Temperature: Homeoviscous Adaptation *CD Moyes and JS Ballantyne* 1725

Heat Shock Proteins and Temperature *S Currie* 1732

Effects of Climate Change *H-O Pörtner and MA Peck* 1738

see Temperature Preference: Behavioral Responses to Temperature in Fishes, Volume 1, p. 758
see Integrated Responses of the Circulatory System to Temperature, Volume 2, p. 1197

HYPOXIA

The Expanding Hypoxic Environment *RJ Díaz and DL Breitburg* 1746

Respiratory Responses to Hypoxia in Fishes *SF Perry* 1751

Anaerobic Metabolism in Fish *Y Wang and JG Richards* 1757

Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia *JG Richards* 1764

see General Principles of Biochemical Adaptations, Volume 3, p. 1681
see Behavioral Responses to Hypoxia, Volume 1, p. 765
see Integrated Responses of the Circulatory System to Hypoxia, Volume 2, p. 1221

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

HAGFISHES AND LAMPREY

Hagfishes *ME Forster* 1771
Lampreys: Energetics and Development *MP Wilkie* 1779
Lampreys: Environmental Physiology *MP Wilkie* 1788
see Hagfish Slime, Volume 1, p. 504
see Functional Morphology of the Brains of Agnathans, Volume 1, p. 16

STURGEON

Physiology of Sturgeon *SI Doroshov and JJ Cech Jr.* 1800

CHONDRICHTHYES

Physiology of Sharks, Skates, and Rays *JS Ballantyne and JW Robinson* 1807
see Functional Morphology of the Brains of Cartilaginous Fishes, Volume 1, p. 26
see The Ear and Hearing in Sharks, Skates, and Rays, Volume 1, p. 262

BONY FISHES

Zebrafish *JG Richards* 1819
Crucian Carp *GE Nilsson* 1824
Life in Hot Water: The Desert Pupfish *SD Hillyard* 1831
Blind Cavefish *Y Yamamoto and WR Jeffery* 1843
see Eel Migrations, Volume 3, p. 1937

AIR-BREATHING FISHES

The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction *JB Graham* 1850
Respiratory Adaptations for Air-Breathing Fishes *JB Graham* 1861
Circulatory Adaptations for Air-Breathing Fishes *JB Graham* 1875

PELAGIC FISHES

An Introduction to the Biology of Pelagic Fishes *D Bernal* 1887
Physiology of Tuna *KA Dickson* 1903
Endothermy in Tunas, Billfishes, and Sharks *BA Block* 1914
see Tracking Oceanic Fish, Volume 3, p. 1928

FISH MIGRATIONS

The Biology of Fish Migration *TR Binder, SJ Cook, and SG Hinch* 1921
Tracking Oceanic Fish *BA Block* 1928
Eel Migrations *D Righton and J Metcalfe* 1937

Pacific Salmon Migration: Completing the Cycle *SJ Cooke, GT Crossin, and SG Hinch* 1945
see Spatial Orientation: How do Fish Find their Way Around?, Volume 1, p. 720

DEEP-SEA FISHES

Deep-Sea Fishes *AP Farrell* 1953

INTERTIDAL FISHES

Intertidal Habitats *PM Schulte* 1959

APPLIED ASPECTS OF FISH PHYSIOLOGY

CELLULAR, MOLECULAR, GENOMICS, AND BIOMEDICAL APPROACHES

Culture of Fish Cell Lines *NC Bols, A Kawano, and LEJ Lee* 1965
Pigment Genes and Cancer Genes *I Braasch and D Liedtke* 1971
Evolution of Fish Genomes *J-N Volff, F Brunet, A Böhne, and D Galiana-Arnoux* 1980
Fish as Model Organisms for Medical Research *JN Cech and LI Zon* 1986
Transgenesis and Chromosome Manipulation in Fish *N Maclean* 1998
Physiology of Triploid Fish *TJ Benfey* 2009
Growth Hormone Overexpression in Transgenic Fish *RH Devlin* 2016
Molecular and Chromosomal Aspects of Sex Determination *KR Siegfried* 2025
Molecular Fish Pathology *CJW Scott, PC Morris, and B Austin* 2032
Germ Cell Migration and Trans Sex *M Kinoshita* 2046
Global Gene Expression Profiling in Fish *AY Gracey* 2055
see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Erythropoiesis in Fishes, Volume 2, p. 992
see Blind Cavefish, Volume 3, p. 1843
see Zebrafish, Volume 3, p. 1819

TOXICOLOGY

The Toxicology of Metals in Fishes *CJ Kennedy* 2061
The Toxicology of Organics in Fishes *CJ Kennedy* 2069
The Effects of Toxicants on Olfaction in Fishes *KB Tierney* 2078
see Anthropogenic Influences on Fish Behavior, Volume 1, p. 783

AQUACULTURE

Physiology of Fish in Culture Environments *CA Myrick* 2084
see Dietary Requirements of Fish Under Culture Conditions, Volume 3, p. 1617
see Bioenergetics in Aquaculture Settings, Volume 3, p. 1664
see Physiology of Triploid Fish, Volume 3, p. 2009
see Manipulation of Fish Phenotype by Parasites, Volume 1, p. 777

INDEX 2091

PREFACE

The *Encyclopedia of Fish Physiology: From Genome to Environment* is the first of its kind. It follows in the strong tradition built by the book series *Fish Physiology* that was pioneered in the late 1960s by Bill Hoar and Dave Randall and is still ongoing; however, the Encyclopedia differs markedly from this book series in three important respects.

Foremost, the Encyclopedia targets a much broader audience, not just the research community. My hope is that the Encyclopedia will serve as a general educational tool for biologists, as well as being of general use as an information resource for those interested in fishes. After all, the Encyclopedia is replete with magnificent examples of how fishes are marvelously adapted to almost every water body on the Earth, no matter how deep, how hot, how cold, how salty, or, in some cases, how dry!

Second, the articles span well beyond just the physiology of fishes. The intent here is to connect physiological functions, environmental factors, behavior, and the genome. This is the case even though the main focus here is on physiology.

Third, and as a result, the breadth of topics covered by the Encyclopedia far exceeds that of any focused volume of the *Fish Physiology* book series. Thus, the Encyclopedia is ambitious in both its content and its target audience.

The Encyclopedia contains a very impressive array of entries, each in the form of a discrete, self-standing article that is typically 3000–4000 words in length, not counting the ancillary material contained in figures, glossary, further reading, etc. In total, there are 252 articles written by over 200 international experts from over 20 countries.

The articles are arranged thematically. The larger thematic sections have an introductory article, written by a section editor, who explains contributions in that theme and places them in a broader and often historical context. Tributes are deservedly given to those who made key scientific advances.

The electronic format of the Encyclopedia was deliberate and a crucial requirement before this project even started. I have to thank Andy Richford for garnering Elsevier's support of this idea, as well as initiating this project when he was an acquisition editor. The electronic format, through 'hot keys', provides an easy and almost instant access to the glossary and related articles. Each article is generously linked to many others in this manner, offering the reader easy digressions or more detailed information and explanations. The 'Further Reading' section associated with each article provides additional resources.

This ambitious opus could not have been possible without the dedication and commitment of a great number of individuals. I had an idea that I have steered to completion, but others did most of the hard work. I am especially indebted to my associate editors, Don Stevens, Joe Cech, and Jeffrey Richards. They embraced my dream early on and stayed with me until completion. Without their wisdom, breadth of knowledge, and hard work, the Encyclopedia could not have come to fruition. I think we worked well together, and, on my part, it was a distinct pleasure to have such enthusiastic support.

I am equally indebted to the section editors: Colin Brauner, Ann Butler, Bruce Carlson, Brandon M Casper, Greg Goss, Jeff Graham, Toshiaki Hara, Sue Holmgren, Norman Maclean, Jay Nelson, Ken Olson, Catharina Olsson, Arthur Popper, Trish Schulte, Bob Shadwick, Kath Sloman, Adam Summers, Matt Vijayan, and Hans-Joachim Wagner. They shaped their section and carried the core of the editorial responsibilities for the contributed articles during the many rounds of review and revisions.

Finally, and importantly, there are over 200 authors and their collaborators, who contributed the articles. My hope is that your rewards go well beyond the recompense, which is a pittance for the time that I know you devoted to generating such marvelous articles. You are thanked for your dedication and trust in the project, and are congratulated for your respective skills and knowledge.

There is another side to the production of this Encyclopedia, and that is the Elsevier team, with whom the editors and authors interacted. This team was headed by Kristi Gomez, the acquisitions editor in San Diego. Milo Perkins, the

development editor in the Oxford office, was our go-to-man after Esmond Collins had set the ball rolling. Milo endured the rough and the smooth, and his rapid responses, as well as his flexible deadlines, were greatly appreciated. Kate Mittell worked quietly and efficiently in the background supporting Milo and was responsible for keeping me on track with the enormous glossary of terms. Nicky Carter, our project manager, superbly rounded out the team. We relied on her for the professional appearance and content of the final copy.

As Editor-in-Chief, I am ultimately responsible for any errors contained herein. If you find any, please let me know so that they can be corrected; likewise, if you feel there are important omissions, please let me know of these. They might be incorporated in future updates.

Bringing this Encyclopedia to its conclusion means one thing for me personally: Miriam, Dustin, Naomi, Nicole and Natalie will all be seeing a lot more of me over the next while! Miriam, your patience throughout was astounding, although it was pushed to the limit at times.

Tony Farrell
Vancouver

SENSING THE ENVIRONMENT

Brain and Nervous System

Contents

Functional Morphology of the Nervous System: An Introduction
Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern
Functional Morphology of the Brains of Agnathans
Functional Morphology of the Brains of Cartilaginous Fishes
Functional Morphology of the Brains of Ray-Finned Fishes
Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria
Motor Control Systems of Fish
Physiology of the Mauthner Cell: Discovery and Properties
Physiology of the Mauthner Cell: Function
Autonomic Nervous System of Fishes
Opioids and Opioid Receptors in Fishes

Functional Morphology of the Nervous System: An Introduction

AB Butler, George Mason University, Fairfax, VA, USA

© 2011 Elsevier Inc. All rights reserved.

Overview of Brain Organization
Historical Perspective
Variation in Brain Structure across the Taxa
Further Reading

Glossary

Afferent Activity conducted toward the brain, normally sensory. Incoming, e.g., in the case of nucleus A projecting to nucleus B, the neurons in nucleus B would receive an afferent input from nucleus A.

Alar plate The dorsal, or upper, part of the developing neural tube. The alar plate gives rise to sensory structures, motor feedback structures (such as the cerebellum), and, except for the hypothalamus, the entire forebrain (telencephalon and diencephalon).

Basal plate The ventral, or lower, part of the developing neural tube. The basal plate gives rise to the hypothalamus and to the motor structures of the brainstem, such as the cranial and spinal nerve motor nuclei.

Caudal Toward the tail.

Cortex (pl., cortices) A neural structure in which several different populations of neurons are arranged in layers, or laminae. The dendrites of some of the neuron cell bodies extend across several laminae and thus can receive somewhat different inputs (afferent projections) that are segregated along the length of the dendrite. Likewise, outputs (efferent projections) to different targets can arise from different layers.

Diencephalon The more caudal of the two divisions of the forebrain. Caudal part of the prosencephalon (forebrain) associated with the third ventricle. The general subdivisions are (from dorsal to ventral): epithalamus, dorsal thalamus, subthalamus, and hypothalamus.

Efferent Outgoing, e.g., in the case of nucleus A projecting to nucleus B, the axons that arise from neuron cell bodies in nucleus A would be efferent projections of that nucleus. Note that the adjectives efferent and afferent can apply to the same set of axons; the terms are used in reference to the nucleus from which they arise vs. the nucleus to which they project, respectively.

Ganglion A collection of nerve cell bodies of similar function. A ganglion is a type of nucleus but often refers to structures in the peripheral nervous system. An exception is the basal ganglia that are nuclei of the telencephalic subpallium.

Mesencephalon Midbrain. It consists of a dorsal tectal region and a more ventral tegmentum.

Neural crest A transient developmental tissue at the interface between ectoderm and neuroectoderm found in craniates which gives rise to numerous adult tissue, for example, peripheral sensory neurons, melanocytes, and skeletal cells.

Neural tube The developing central nervous system, consisting of a tube formed by invagination (in-pocketing) of the dorsal part (in vertebrates) of the ectoderm (the outer body layer). It gives rise to the brain and the spinal cord.

Neuraxis The rostrocaudal length of the neural tube in both its developing and adult form, including the brain and the spinal cord.

Nissl stained The histological procedure that utilizes a Nissl stain results in the darkening of neuron cell bodies, making them visible against a pale background when viewed through a microscope. This stain does not darken most neuron cell processes, so most of the dendritic tree and the fiber pathways composed of groups of axons are not stained.

Nucleus (pl., clei) In neuroanatomy, this term refers to a discrete group of similarly connected neuron cell bodies that are not cortically laminated (see cortex). In a nucleus, the dendritic arborizations of all of the neurons, their receptive portion, are confined within the boundary of the nucleus.

Pallium Dorsal part of the telencephalon. The projection neurons of the pallium are glutamatergic.

Rhombencephalon The hindbrain; in jawed fish, it includes the cerebellum and the medulla.

Rostral The front end of a structure, opposite of caudal. Rostral refers to the beak or snout.

Subpallium Ventral part of the telencephalon. The projection neurons of the subpallium are GABAergic.

Telencephalon The more rostral of the two divisions of the forebrain.

Ventricles Fluid-filled cavities within the brain that are formed when the neural tube tissue invaginates, resulting in a cavity at the center of the newly formed tube. As the brain develops, each part of the ventricular system acquires its own dimensions in relation to the surrounding neural tissue. Each telencephalic hemisphere has a ventricular space (referred to as lateral ventricles in nonactinopterygian vertebrates), and these ventricles are continuous with the third ventricle, which runs vertically along the medial surfaces of each side of the diencephalon. The third ventricle is continuous with the ventricle of the midbrain, which, in turn, is continuous with the ventricle of the hindbrain, the fourth ventricle. (In fish, the midbrain ventricular space is enlarged laterally to include tectal ventricles, whereas in mammals, it is elongated and very small in diameter and is called the cerebral aqueduct.) The fourth ventricle is continuous with the central canal of the spinal cord. The ventricles are filled with cerebrospinal fluid, which is secreted by the choroid plexus, a structure composed of layers of ependymal cells and vasculature.

Overview of Brain Organization

In all vertebrates, the brain develops by the generation of neurons in the ventricular zone, which lies along the ventricular surface of the developing neural tube (**Figure 1**(**a**)), and, in some cases, a neighboring subventricular zone. The cell bodies of these neurons migrate away from the ventricular region to varying degrees and in varying numbers. Different patterns of neuron-cell migration result in the formation of either cortex or nuclei, which are important to distinguish for a number of functional reasons.

A cortex (**Figure 1**(**b**)) consists of several different populations of neurons that lie in layers and that thus can receive somewhat different inputs (afferent connections) and give rise to somewhat different outputs (efferent projections). In a cortex, unlike in a nucleus, the receptive portions of some or all of the neurons of one layer, their dendrites, can extend into the territory of another population, and thus sample the inputs arriving there as well.

Nuclei (**Figure 1**(**c**)) are discrete groups of similarly connected neuron cell bodies that are not cortically laminated, the crucial distinction being that in a nucleus, the dendritic arborizations of all of the neurons, their receptive portion, are confined within the boundary of the nucleus. Some nuclei are laminated, but in that case, the dendritic arborizations of a neuron cell body in a specific lamina remain within the boundaries of that lamina. The neurons of both nuclei and cortex can have local, internal connectivity via the axons of their local circuit neurons, which are usually inhibitory, as well as long, efferent projections to other neuron cell groups, that lie some distance away, either nuclear or cortical in structure.

During embryological development, patterning genes that are active in different regions of the neuraxis, the rostrocaudal axis of the neural tube, result in differentiation of various regions in that dimension. Likewise, other patterning genes specify dorsoventral differentiation into a dorsally lying alar plate and a ventrally lying basal plate, which each give rise to a set of structures in the adult brain. Among the derivatives of the basal plate are the efferent motor neurons of the nervous system, particularly the motor nuclei of cranial nerves in the brainstem and spinal

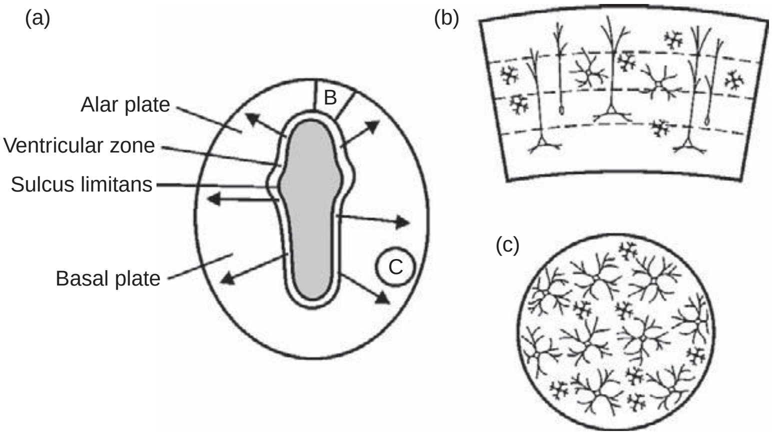


Figure 1 (a) Drawing of the developing neural tube to show the ventricular zone, which surrounds the ventricular space (gray shading). Neuron cell bodies generated in the ventricular zone migrate radially, as indicated by arrows (or, in some cases, tangentially). They form either laminated cortices, as shown in (b), or nuclei, as in (c). In (b), the glutamatergic pyramidal neurons, which give rise to long projections, are shown with vertically extending apical dendrites and basal dendrites arising from their triangular-shaped cell bodies. Multipolar larger and smaller local-circuit (locally projecting), GABAergic inhibitory neurons are also shown. In (c), large, multipolar, glutamatergic projection neurons are intermingled with small, local-circuit, GABAergic interneurons.

cord that innervate muscles or are the preganglionic neurons (first in a two-neuron chain) of the autonomic nervous system, while the alar plate is more sensory in nature, including feedback information from motor system actions.

Much of the variability across taxa, whether among fishes or across all vertebrates, involves the number of nuclei present, the degree to which they migrate away from their germinal zone along the ventricular surface in a radial (or, in some cases, tangential) direction, and the degree to which they develop in size. Variation in the morphological features of the dendritic tree of various neurons also occurs, giving rise to many diverse phenotypes, and, likewise, major variation occurs in the differentiation into multiple different neurotransmitter and neuromodulator phenotypes. The number of afferent and efferent projections of nuclei and cortical areas at various locations along the neuraxis and how elaborate the cortical areas are, also contribute to the variability. Most of this variation arises from variations in the degree and elaborateness of development of alar plate-derived structures, while basal plate-derived structures vary to a substantially lesser degree. While both the basal and alar plates give rise to nuclei, all cortical structures are alar plate derivatives.

Like the brains of other vertebrates, the brains of fishes comprise a caudal hindbrain region, an intermediate region called the midbrain, and a rostrally lying forebrain. The major parts of the brain of a gar (*Lepisosteus osseus*) are shown in **Figure 2**(**A**). The hindbrain contains the caudal part of the brainstem, the medulla, and the cerebellum; the midbrain contains the more rostral part of the brainstem and a roof region, the tectum; and the forebrain consists of the more caudally lying diencephalon and the more rostrally lying telencephalon, which comprises a dorsally lying pallium and a ventrally lying subpallium (**Figure 2**(**B**)) as well as the olfactory bulbs. The hindbrain and midbrain contain the basal plate-derived motor neurons for the various motor cranial nerves that innervate the muscles of the head and the preganglionic neurons that form part of the parasympathetic system for both head and body, as well as a number of alar plate-derived sensory nuclei in which peripheral sensory nerves synapse. They also both have alar plate-derived cortical regions, such as the cerebellum of the hindbrain and the tectal region of the midbrain, which includes the visually dominated optic tectum (**Figure 2**(**C**)) and the auditory tectum called the torus semicircularis. The forebrain also contains sensory-receptive nuclei as well as many nuclei that are part of multisynaptic ascending and descending pathways. Across fishes, the upper (i.e., dorsal) part of the telencephalon, the pallium, varies in its degree of development from a relatively simple lamina of neurons that remain near the ventricular surface to having multiple migrated nuclear areas, as is the case for the gar (**Figure 2**(**C**)). In some species, such as squirrelfish, among the ray-finned fishes, the pallium is elaborated to the extent that some cortical, or at least cortical-like, regions are formed. The lower (i.e., ventral) part of the telencephalon, the subpallium, contains only nuclei.

Historical Perspective

For decades and through the first half of the twentieth century, it was thought that fish brains were merely smell brains, dominated by the sense of smell to the exclusion of other sensory systems. While the anatomical organization of fish brains had been studied by the early comparative neuroanatomists, little was understood that had functional

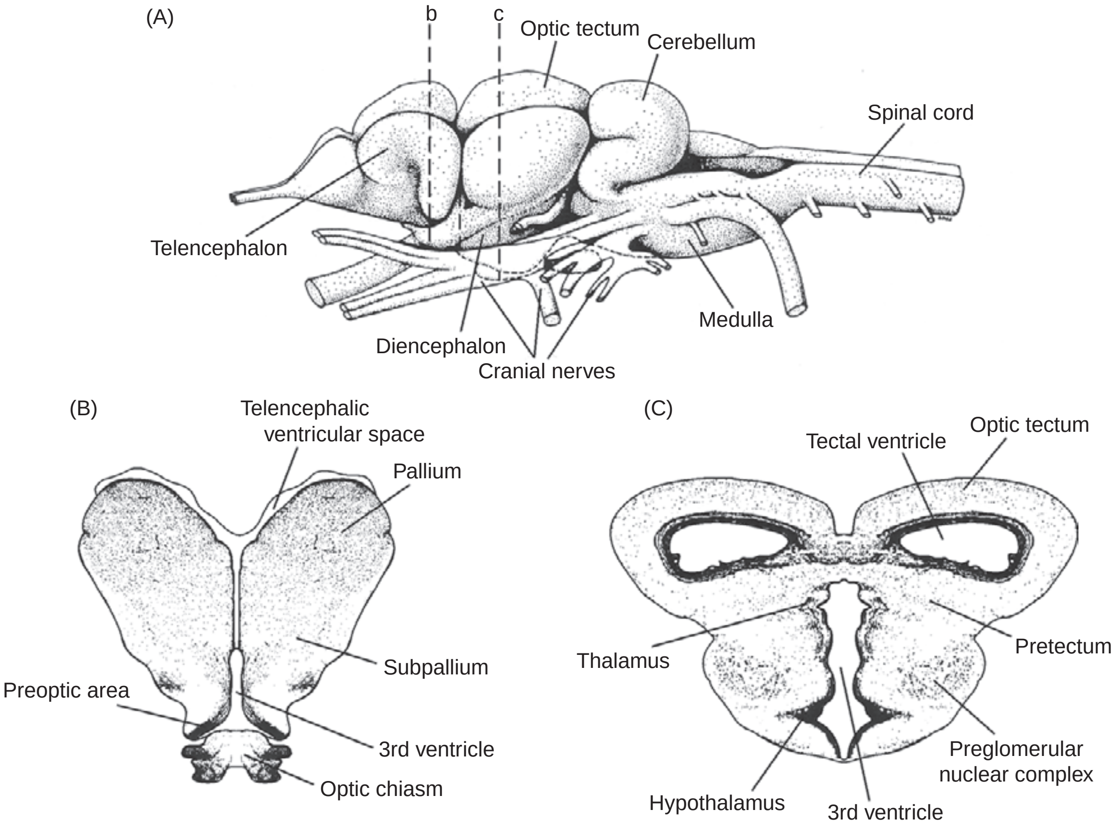


Figure 2 (A) Side view of the brain of a gar, with some of the various major parts lebeled. The dashed lines b and c indicate the levels at which the sections shown in parts (B) and (C) are taken. The section in (B) is through the telencephalon, showing the pallium dorsally and the subpallium ventral to it. The section in (C) is through the midbrain roof, showing the tectum, which is cortical in structure, dorsally and the various nuclei of the caudal part of the diencephalon, ventrally.

relevance. Beginning in the early 1960s, the Dutch neuroscientist Rudolf Nieuwenhuys provided new insights into the organization of fish brains, including seminal observations on the topology of the pallial areas in ray-finned fishes, which develops in a rather unique manner as compared with most other fishes and land vertebrates, and thus has been particularly challenging to understand.

A second breakthrough came with the work of the Swedish-American neuroscientists Sven Ebbesson and Lennart Heimer, who showed that the olfactory projections in sharks are restricted to only a small part of the telencephalon and that visual and other sensory information was relayed to other, separate areas of the telencephalon. These seminal findings engendered interest in the overall organization of fish brains and ushered in a new era of research activity. The organization of the various sensory systems, including the mechanosensory lateral line, electrosensory lateral line (where present), auditory, visual, gustatory, and olfactory systems and, to some extent, the descending motor pathways, have been elucidated over the past several decades. The remarkable degree of variation in brain structure and in the degree of elaboration of the various systems is addressed across the major taxa of fishes in this section.

Variation in Brain Structure across the Taxa

In the article **Brain and Nervous System**: Functional Morphology of the Brains of Agnathans, the marked differences in brain structure between lampreys and hagfishes are considered. While pathway tracing and histochemical methods allow identification of many of the major brain regions, many of the homologous relationships with the brains of jawed fishes have yet to be established, particularly concerning the very divergent morphology evinced in hagfishes. The article **Brain and Nervous System**: Functional Morphology of the Brains of Cartilaginous Fishes details the major brain regions in members of this diverse taxon. In many respects, including pallial organization, cartilaginous fishes have brains that are more like those of land vertebrates than any other extant fish taxon. The article **Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned

Fishes deals with the extreme variability in brain structure across this very large and highly diverse taxon. As noted above, pallial organization differs in marked respects from that in other vertebrate taxon even though comparable ascending sensory-system pathways can be identified. Additional information on the major pallial component of the midbrain, the optic tectum, in ray-finned fishes can be found in the article **Vision**: Optic Tectum. The article **Brain and Nervous System**: Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria turns to the sarcopterygian radiation. Recent histochemical work has provided new insights into brain organization in this taxon and allowed for more detailed comparison with tetrapods than previously possible.

Five additional chapters cover other evolutionary aspects of nervous-system organization in fishes. The article **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern details the evolution of the nerves in the context of the components present in invertebrate deuterostomes. The article **Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties highlights the use of this unique motor neuron as a model neuron system for general physiological properties, while the companion article **Brain and Nervous System**: Physiology of the Mauthner Cell: Function details the specific physiological and behavioral aspects of the system. The article **Brain and Nervous System**: Motor Control Systems of Fish discusses the Mauthner cell system, but in the context of other aspects of motor behaviors. Finally, the autonomic nervous system is discussed in the article **Brain and Nervous System**: Autonomic Nervous System of Fishes.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes; Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Functional Morphology of the Brains of Agnathans; Functional Morphology of the Brains of Cartilaginous Fishes; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria; Motor Control Systems of Fish; Physiology of the Mauthner Cell: Discovery and Properties; Physiology of the Mauthner Cell: Function. **Vision**: Optic Tectum.

Further Reading

Butler AB (2000) Nervous system: Gross functional anatomy. In: Ostrander GK (ed.) *Handbook of Laboratory Animals: Fish*, pp. 129–149. San Diego, CA: Academic Press.

Butler AB (2000) Nervous system: Microscopic functional anatomy. In: Ostrander GK (ed.) *Handbook of Laboratory Animals: Fish*, pp. 331–355. San Diego, CA: Academic Press.

Butler AB and Hodos W (2005) *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. Hoboken, NJ: Wiley-Liss.

Ebbesson SOE (ed.) (1980) On the organization of the telencephalon in elasmobranchs. In: *Comparative Neurology of the Telencephalon*, pp. 1–16. New York: Plenum.

Huesa G, Anadón R, Folgueira M, and Yáñez J (2009) Evolution of the pallium in fishes. In: Binder MD, Hirokawa N, and Windhorst U (eds.) *Encyclopedia of Neuroscience,* vol. 2, pp. 1400–1404. Berlin, Heidelberg: Springer.

Nieuwenhuys R, ten Donkelaar HJ, and Nicholson C (1998) *The Central Nervous System of Vertebrates*, vol. 2 Berlin: Springer.

Saidel WM (2009) Evolution of the optic tectum in anamniotes. In: Binder MD, Hirokawa N, and Windhorst U (eds.) *Encyclopedia of Neuroscience,* vol. 2, pp. 1380–1387. Berlin, Heidelberg: Springer.

Wullimann MF and Vernier P (2007) Evolution of the nervous system in fishes. In: Kaas JH (ed.) *Evolution of Nervous Systems. A Comprehensive Reference,* vol. 2, pp. 39–60. San Diego, CA: Elsevier.

Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern

B Fritzsch and DC de Caprona, University of Iowa, Iowa City, IA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Acorn Worm CNS and the Molecular Basis for Its Development
Inducing a CNS and Suppressing Neuronal Capacity in General Ectoderm: A Common Theme Shared among Chordates but Not With All Hemichordates?
Motor Neuron(s), The Ancient Efferent Communication of All Nerves to Induce Muscle Movements for Locomotion
Summary and Conclusion
Further Reading

Glossary

Basic helix–loop–helix (bHLH) proteins They bind as heterodimers to specific promoter-binding sites (so-called M-box) to induce expression or repression of specific genes involved in cell differentiation. Important members of this family are Neurogenin, atonal/Atoh1, and Neurod.

Bone morphogenetic protein (BMP) A group of diffusible proteins that bind to the BMP receptor to elicit intracellular signals that are important for brain, heart, cartilage, and bone development.

Central nervous system (CNS) An internal aggregation of neurons involved in processing information obtained from sensors and eliciting appropriate motor responses. Depending on the systematic relationships, the CNS has been suggested to have evolved only once in bilaterians (nephrozoa) or, a position taken here, multiple times out of a diffuse nerve net that is shared among diploblasts and basal triploblasts.

Decapentaplegic (Dpp) The fruit fly homolog of BMP with a somewhat similar function, except for bone and cartilage.

Fibroblast growth factors (Fgfs) Diffusible factors that signal through their Fgf receptors and are involved not only in proliferation regulation but also in differentiation, such as legs and CNS.

Forkhead genes (Foxg1) They play major roles in proliferation regulation. For example, forebrain growth and cellular diversity is extremely stunted in Foxg1 null mice.

Homology Indicates similarities due to inheritance, is used for homologous structures (e.g., limbs) or homologous gene sequences.

Hox genes A set of genes which contain the homeodomain sequence and are involved in patterning, and in some species in patterning of specific antero-posterior compartments of the body (segments) or brain (rhombomeres).

Metamere and metameric Repeated units (compartments) and their formation.

Micro-RNA (miR) A set of small single-stranded RNA molecules which can bind to specific messenger RNAs and essentially remove them from being translated into proteins. Many miRs co-evolved with increasing complexity of multicellular organisms and are currently perceived as mediating in part this complexity through differential regulation of gene-product translation. Specific miRs have been identified which co-evolve with neurons and sensory systems and are crucial for their development.

Neural crest A transient developmental tissue at the interface between ectoderm and neuroectoderm found in craniates which gives rise to numerous adult tissues, for example, peripheral sensory neurons, melanocytes, and skeletal cells.

Neurogenic placodes Embryonic ectodermal thickenings that give rise to neurons and sensory cells such as the ear, lateral line and olfactory organs, and, in some cases, cranial nerves.

Olfactores A grouping of chordates comprising urochordates and craniates but excluding cephalochordates.

Orthodenticle-like 1 (Otx1) Like Foxg1, orthodenticle genes play a major role in the differentiation of forebrain and sensory organs.

Placode A plate-like thickened area of embryonic ectoderm which gives rise to sensory organs.

Peripheral nervous system (PNS) A set of nerve tracks with or without neurons which connect the centralized CNS with sensory input and effector organs.

Rhombomere Transverse compartments of the hindbrain.

Synapomorph A shared derived (apomorphic) feature found only in this group, for example, a lower jaw is a synapomorphy of all jawed vertebrates.

Introduction

Understanding how the vertebrate's body and head, with its nervous system and craniate senses, evolved requires a broad perspective taking into account the evolution of multicellular animals. A brief overview of animal evolution is therefore needed here to set the stage for our discussion of how the original, diffuse nervous systems, organized as nerve nets with no centralized nervous or sensory component, differentiated over time into complex brains interconnected to the peripheral sensory organs and effector systems with peripheral sensory and motor nerves.

A new theory has recently emerged based on the insight that all multicellular organisms were derived from single-celled organisms called choanoflagellates, so named for their single flagellum surrounded by a collar of microvilli. The flagellum propels food caught by and ingested at the base of the microvilli. Aside from sponges (Porifera) and an odd species of an amorphous colony-like aggregation of single-celled organisms (Placozoa), multicellular animals can be divided into two great clades (**Figure 1**), which are based on the type of body symmetry and the number of primary tissue layers:

1. Radially symmetrical animals are diploblasts. They have two primary tissue layers, the outer ectoderm and the inner endoderm. They include jellyfish and their relatives (Cnidaria) and the comb jellies (Ctenophora).
2. Bilaterally symmetrical animals are triploblasts. They have three primary tissue layers (intermediate layer of mesoderm in addition to the ectoderm and endoderm), and include all bilaterally symmetrical invertebrates and vertebrates. Among triploblasts, a group of flatworms lacking a gut are referred to as acoelomate flatworms (Acoela, **Figure 1**). These animals are no longer considered to be true flatworms (the phylum Platyhelminthes) but rather are viewed as basal triploblasts. The rest of the bilaterally symmetrical animals, the Nephrozoa (animals with kidneys), comprise the Protostomes and Deuterostomes. The former include most species of invertebrates (including the 'true' flatworms, Platyhelminthes), while the latter include the vertebrates and their deuterostome invertebrate relatives. The most basal extant deuterostomes (**Figure 1**) are of the genus *Xenoturbella*, which comprises two species of marine worms, and the newly recognized clade Ambulacraria, which contains the echinoderms (starfish, sea urchins, etc.) and the hemichordates (acorn worms, with cylindrical bodies, and pterobranchs, with vase-shaped bodies). Chordates comprise those animals which have a notochord at some point in their lifecycle and are divided into three major extant clades: the cephalochordates (amphioxus), the urochordates (sea squirts or tunicates), and the craniates (vertebrates) (**Figure 1**).

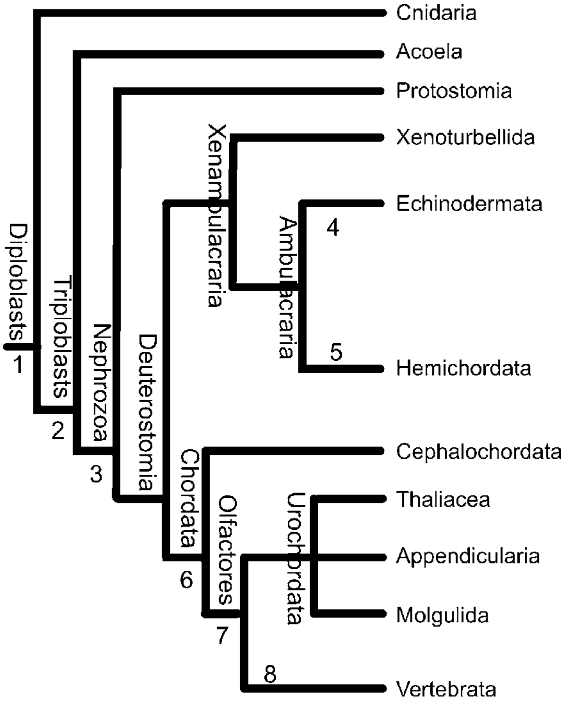


Figure 1 The simplified cladistic relationships of animals and some critical events concerning the evolution of neurons and sensory systems: (1) diffuse epithelial nerve net; (2) miR-124 specific for neurons appear; (3) miR-183 specific for sensory cells appear; (4) formation of a pentameric nerve net with rudimentary sensory organs; (5) partial dorsal invagination of the epithelial plexus and appearance of localized sensory cells; (6) neurons are concentrated in a dorsal neural tube and composite sensory organs appear. Sensory neurons are inside the neural tube and motor neuron(s) are ventral in the neural tube. Possible precursors of neural crest, branchial motor neuron(s), and somatic motor neuron(s) appear; (7) branchial motor neuron(s) are present, first appearance of neural crest and possibly placodes; (8) placode-derived sensory systems, cranial branchial motor neuron(s), and spinal somatic motor neuron(s) appear. Eyes, ears, nose, taste buds, lateral line, electroreceptive ampullary organs, and ocular muscles appear.

Of the numerous theories about the origin of the craniate head, brain, and peripheral nerves which have been proposed over the past 150 years, most assumed a transformation of either an amphioxus-like (cephalochordate) organism into a craniate with a clearly delineated head (the new head hypothesis), or that tunicates (urochordates) form the outgroup of craniates and consequently, many head organs, jaws, and the craniate spinal cord had to evolve anew. Ideas that the last common bilaterian ancestor of nephrozoans had a highly developed central nervous system (CNS) and was segmentally organized require the assumption that many basal protostomes and deuterostomes have secondarily lost all of these features and devolved into a more ancestral-like organization.

As all multicellular organisms derive from single-celled choanoflagellates, they share molecular synapomorphies at the level of diploblasts and triploblasts, indicating that the ectodermal nerve net found in diploblasts may also have been the primitive state in basal triploblasts. In fact, within basal deuterostomes, the marine worm in the genus *Xenoturbella* has an epithelial nerve plexus with no recognizable sensory system. Among Ambulacralia, acorn worms (hemichordates, **Figure 1**) have some centralization of nervous elements without formation of a true CNS and single sensory cells instead of sensory organs. Likewise, what appears to be basal protostomes, the arrow worms (Chaetognathans), mostly have an epidermal nerve plexus with little concentration into a ganglion, similar to basal triploblasts, the acoel worms. The wide phylogenetic distribution of these shared features indicates that the common triploblast ancestor, and therefore likely also the common deuterostome ancestor, may have had an epithelial nerve plexus with little, if any, metameric (segmental) organization. If the morphology of the nervous system in *Xenoturbella* is indeed close to the basal deuterostome organization and not secondarily derived through regressive evolution, the ancestor of craniates could have had a nerve net, avoiding the need to transform an already existing CNS, as in amphioxus or tunicate larvae, into a craniate-like CNS (**Figure 1**).

For this article, we assume that the deuterostome and craniate ancestor had a nerve net very much like the *Xenoturbella's* unorganized nerve net with little to no metameric organization. It seems unlikely that a metameric organization of a well-developed CNS devolved to form the epithelial nerve nets now found in diploblasts and basal triploblasts.

Following already well-entrenched arguments for the evolution of eyes and ears, the different patterns of nervous system found in deuterostomes are considered here to be independently derived forms reflecting either different and independent steps in forming a CNS (acorn worms, cephalochordates, urochordates, and craniates) or a transformation into a pentameric (five-portioned) nerve net (echinoderms). A consequence of this hypothesis is that many problems in establishing homology of peripheral nerves and sensory organs across deuterostomes become obsolete, leading to the more parsimonious idea that the formation of a CNS comes about by aggregating a subepidermal nerve net and condensation of distributed sensory cells into sensory organs. Such centralization may have happened at least twice in deuterostomes (acorn worms, chordates) and at least twice in protostomes (insects and related species; mollusks, and related species).

A consequence of this condensation is the formation of connecting strands of the emerging CNS with effector and sensory organs, referred to here as 'nerves'. Obviously, in order to communicate with the remaining parts of the body, such nerves have to carry both afferents (for sensory input from the periphery) and efferents (for motor output to the periphery). A by-product of this condensation of the CNS out of a diffuse nerve net is the condensation of various sensory cells with more or less discrete segregation of sensory input into organs dedicated to a specific set of stimuli such as photic, mechanical, or chemical stimulation as well as the evolution of the neural crest (embryonic tissue giving rise to most of the peripheral nervous system (PNS).

For this idea to be true, each of the deuterostomes having a CNS will have to show certain similarities in its overall development and its molecular basis which in part will reflect the patterning already found in the more or less centralized nerve plexus of acorn worms. In particular, genes related to neurulation should be common among all deuterostomes with a CNS, but may reflect common co-option or a prototypical partial aggregation into a CNS that does not reach the level of obvious homology. Apparently, only craniates achieved the aggregation of all neurons into a CNS, aggregated all peripheral sensory systems into discrete sensory organs, and developed a unique set of innervations via neural-crest-derived sensory neurons and neurogenic-placode-derived sensory organs. Neurogenic placodes, like neural crest, are ectodermally derived structures but are present only in the head; along with neural crest, they produce the sensory neurons of the PNS in the head region. The formation of these special embryonic tissues – neurogenic placodes and neural crest – is the newly evolved developmental mechanism which achieves this aggregation of sensory cells in craniates' embryos.

Whereas much work has concentrated on the specific gene networks promoting embryonic formation of placodes or neural crest, few studies have been focusing on the molecular mechanism of suppression of neuronal fate determination in the remaining ectoderm. In other words, the ectoderm in a developing embryo will become nervous-system tissue unless this fate is repressed; in the parts of the ectoderm where this repression occurs, the ectoderm forms epidermis, the outer layer of the skin. It is now clear that fibroblast growth factor (Fgf) upregulation, combined with limited to no expression of bone morphogenetic proteins (BMPs) induce a neuronal fate in the ectoderm of chordates (i.e., it becomes neuroectoderm) but apparently not in *Xenoturbella.* That the default state of ectoderm for chordates is indeed neurogenic has been demonstrated by overexpressing proneuronal basic helix-loop-helix (bHLH) genes in the ectoderm, revealing a transformation into neurons.

Another emerging concept is the importance of microRNA (miR) for the development of neurons and sensory cells. Recent data have shown that specific miRs (miR-124) are evolutionary conserved and evolved first with triploblasts. MiR-124 has been shown to be essential for neuronal development through the regulation of chromatin remodeling and absence of miR-124 (and other miR species) causes rapid degeneration of developing neurons. Another set of miR, miR-183,182, and 96, is evolutionary conserved among nephrozoa (i.e., bilaterians excluding acorn worms). It has been shown to be associated with mature sensory cells and is crucial for their development. In fact, even single-base mutations in miR-96 lead to deafness. Obviously, the ability of miR to regulate large sets of transcription factors could be perceived as a prerequisite for the evolution of a more complex neuronal and sensory system.

This article provides an overview of the evolutionary steps and their underlying developmental transformations toward the formation of a PNS such as the spinal and cranial sensory nerves, molecular mechanisms of suppressing neurogenesis in the ectoderm, invagination of the neuroectoderm, and induction and initial differentiation of placodes and of neural crest. It is organized to reflect the currently accepted phylogenetic relationships among deuterostomes, with *Xenoturbella* (**Figure 1**) as a likely ancestor type for all deuterostomes as far as its nerve organization is concerned – it has a simple epithelial nerve net with no evidence of concentration of neurons or sensory cells. Unfortunately, beyond the knowledge of an unusual Hox code, no molecular data exist on these animals to illustrate what might be the original molecular code for the development of a nerve net.

The Acorn Worm CNS and the Molecular Basis for Its Development

C. Lowe has demonstrated that many patterning genes thought to be associated with craniates' CNS neuromeres (repetitive compartments of the developing brain) exist in the ectoderm of these animals and may play a distinct role in developmental patterning. Comparative expression data provide strong molecular evidence indicating that the basic organization of repetitive neuronal elements, as well as the evolution of neuromeric boundaries which can be molecularly dissected, are discrete steps in deuterostome and chordate evolution and that these elements progress from a more basic, non-neuromeric organization to a fully developed craniate organization with clear association with compartmental boundaries. For example, lampreys have three paralogous Hox-groups while jawed vertebrates have four. In addition, the trigeminal motor neurons extend from r2 to the first half of r4 in lampreys while it extends from r2 to r3 in jawed vertebrates. While the overall similarities in expression domains and neuronal organization between flies and gnathostomes have long been understood and used to indicate a possible homology at the molecular and structural level across phyla, it appears more likely in light of these more substantial data that the limited molecular toolbox used across phyla provides for a molecularly homologous basis for anatomically analogous organs and their organization. In fact, detailed analysis of cellular elements contributing to what have been viewed by some as possibly homologous peripheral nerves has long pointed out this problem at the cellular identity level. Clearly, basal protostomia and deuterostomia do not have metamerically organized 'nerves' radiating out in a specific pattern from the basically epithelial nerve plexus (the CNS of these animals). In fact, the asymmetric but metameric organization of nerves in cephalochordates and basal chordates may reflect as much an independently derived peripheral nerve reorganization as the molecular basis of boundary formation in the CNS reflects a step-wise evolution of neuromeric boundaries and their underlying molecular basis. Likewise, the dorso-ventral patterning differences now recognized in flies, acorn worms, and craniates may reflect different anatomic solutions driven largely by similar molecules and distributions.

Inducing a CNS and Suppressing Neuronal Capacity in General Ectoderm: A Common Theme Shared among Chordates but Not With All Hemichordates?

It is now well known that key early steps in CNS and ectodermal placode formation involve the transformation of ectoderm into neuroectoderm. A key player across phyla is the decapentaplegic (Dpp)/BMP pathway. However, chordates require the additional action of Fgfs, which apparently play little role in this process in arthropods and hemichordates. It remains unclear if this Fgf dependence is linked to the apparent dorso-ventral patterning difference in nervous-system induction which has so long been a source of a general body-plan-reversal ideas. These ideas have been questioned by several lines of evidence – the neural tube is ventral in protostomes but dorsal in craniates, and while the acorn worms shows both a dorsal collar region and ventral trunk nerve cord. The most parsimonious interpretation is to assume that the dorsal and ventral position of the CNS in protostomes and deuterostomes is an independent condensation of the ancestral nerve net making obsolete the apparent need for a dorsal–ventral position reversal.

In the absence of understanding all the genes involved in these processes and their interactions, it appears most parsimonious to assume that the already known differences are likely to indicate a nonhomologous origin of a

CNS based on partially homologous transcription-factor actions to transform an epidermal plexus-like PNS, as was initially proposed for the acorn worm. Other known similarities such as the use of different and similar sets of bHLH genes, the ubiquitous use of the delta-notch system (a signaling pathway involved in cellular differentiation through lateral inhibition), and certain molecular aspects of proliferation regulation could reflect their similar functions not in the common triploblast ancestor but in the common diploblast ancestor. What needs to be clarified now is how the diffuse nerve net of a diploblast ancestor, most likely also present in the common triploblast ancestor, has been tied into the emerging and the differing general embryonic-patterning mechanisms to elicit a local upregulation of specific neural inducers resulting in a dorsal or ventral CNS. The currently available data suggest that hemichordates and chordates differ in that respect.

Motor Neuron(s), The Ancient Efferent Communication of All Nerves to Induce Muscle Movements for Locomotion

The nervous system derives from the ectoderm and has to get access to the mesoderm-derived muscle fibers to execute movements. Interestingly, diploblastic organisms have bHLH genes with both a proneural and promyogenic capacity, indicating that the developmental lines giving rise to muscle and neuronal cells in these organisms might be shared, as implied by the existence of neuromuscular cells which are both receptor and effector. However, with the formation of triploblastic organisms and the evolution of a developmental novelty, the mesoderm, muscle fibers, and neurons derive from different germ layers. This, therefore, requires that motor neuron(s) have the capability of accessing those muscle cells through the basement membrane. Apparently, at an early stage in triploblast evolution, muscle fibers acquired cholinergic receptors and most motor neuron(s) became cholinergic.

With the progressive concentration of the nerve net toward the dorsal side of the animal in deuterostomes, motor neuron(s) or muscle fibers had to develop elongated processes to reach each other for function. As those processes extended in development from or toward the mesoderm-derived target, initially random tracks formed carrying the majority of those processes. The assumption here is that the evolutionary-stabilized appearance of such tracks eventually encoded for discrete tracks – the repetitive peripheral nerves now recognized among craniates. These tracks of motorneuron axons could either have contained only motorneuron axons or could have been associated with sensory fibers, either derived from intraspinal sensory cells or consisting of the axons from peripheral sensory cells. The various hypothetical mixes can be found in various nerves among chordates and hemichordates with different arrangements with respect to the motorneuron exit from the nerve cord as either ventral (most cases) or dorsal (some cases). An additional change found among chordates is that motorneuron axonal tracks can run alone or associate at a certain distance away from the nerve chord with sensory fibers. With this theoretical concept in mind, we can now investigate the organization and content of various nerves found in deuterostomes.

Acorn Worms (Hemichordates)

There is no clear formation of aggregated peripheral nerves in acorn worms, but there is an occasional aggregation of the net to form strands of fibers. As expected by the network-like organization of the epithelial nerve plexus, information might flow through the net to reach the effector organs possibly via short nerves containing motorneuron axons. However, this possibility is highly speculative and based on data dating back to the era prior to modern tracing and neurochemical staining techniques and it requires more work to be solidified. Most interesting here is that the general organization of the dorsal nerve chord seems to resemble very much the craniate spinal-cord organization with the neurons grouped near a central lumen, surrounded by a peripheral neuropil. As with craniates, motor neuron(s) appear to be ventral in the dorsal nerve cord. Clearly, a detailed analysis using modern tracing techniques, *in situ* hybridization, and immunohistochemistry is needed to evaluate the organization of the PNS and its relationship to the partial formation of a CNS in these animals.

Amphioxus (Cephalochordates)

At least three kinds of peripheral nerves (very loosely defined) are found in amphioxus, associated with three different types of motor neuron(s). Amphioxus has an unusual notochord consisting of muscle plates. Processes of the muscle fibers of these notochord muscle plates extend as short ‘ventral roots’ to the spinal cord to form cholinergic synapses on the notochord with a subset of poorly characterized motor neuron(s). The second set of peripheral nerves are the muscle processes of the metameric muscle packages reaching toward the intramedullary motor neuron(s), which have axons that extend rostrocaudally along the nerve chord but do not exit the CNS. Among deuterostomes, this situation is highly specialized but it is also found in nematodes within the protostomes. Whether this situation is indeed uniquely derived or reflects a general property of basic triploblasts requires additional analysis in more basal triploblasts, paying attention to the emerging cladistic relationships. Despite the unusual morphology, the synaptic transmitter appears to be acetylcholine, indicating that motor neuron(s) with acetylcholine as a transmitter are at least deuterostome commonalities and are shared with many protostomes. Of note, the muscle-process bundles of both sets of motor nerves, which formally can be compared to ventral roots of craniates, do not contain sensory fibers.

The dorsal root nerve fibers are composed of processes of multiple intraspinal sensory neurons, of various single or aggregated sensory cells, and of one specific type of motorneuron. There are certain resemblances in the content of these dorsal nerves with the cranial nerves of basal chordates (dorsal exit of motor neuron(s), fibers of intramedullary sensory neurons, and placode-derived nerves to sensory cells), but it is unclear if these similarities are a case of convergent evolution with the nerves of craniates or reflect an ancestral organization of this basal chordate.

Tunicates (Urochordates)

The overall organization of both peripheral nerves and the CNS differs dramatically among the various tunicate lineages reflecting their deeply rooted divergence. In one lineage, the spinal cord contains almost no neurons and is a transient larval structure that is lost during metamorphosis when the larva turns into a sessile adult. motor neuron(s) are only present in what will become the central ganglion of the sessile adult, and their fibers project along the spinal cord to form either pure motor nerves or mixed nerves as they exit. These motor neuron(s) have been homologized based on their organization with the branchial (gill arches) motor neuron(s) of craniates. Interestingly, recent molecular data have confirmed and extended this suggestion by showing that a unique set of genes (Phox2) associated only with craniate oculomotor and branchial/visceral motor neuron(s) show expression in these motor neuron(s). In addition, these motor neuron(s) persist past metamorphosis and thus identify the origin of branchial motor neuron(s) already in urochordates. It remains to be seen whether this approach can be expanded to cephalochordates and hemichordates. In appendicularians (a class of free-swimming tunicates which retains the larval body form into adulthood), the spinal cord contains motor neuron(s) which project first along the spinal cord but eventually exit the spinal cord through ventral roots containing only the motor neuron axons adjacent to the muscle cells they innervate. The latter organization resembles that of the ventral roots in craniates.

The origin of sensory nerve components in chordates is also yet to be fully understood. Despite the presence of certain molecular structures indicative of a neural crest and the presence of most genes now identified with the formation of the neural crest, the entire molecular cascade used to generate neural crest in craniates is not clearly assembled as yet. Likewise, while specific components indicative of neurogenic placodes have been identified and associated with putative placodal structures, it is clear that the entire molecular basis of placode induction has not been elucidated in noncraniate chordates. It is very possible that the final assessment will show an incomplete assembly of the molecular pathways comparable to those of neuromeric boundaries in chordates, in particular the midbrain/hindbrain boundary, rendering molecular homology as tentative as cellular homology and patterning signal homology. In fact, a recent analysis of sensory components in developing *Ciona* (a tunicate with a sessile adult form) shows cells and their distribution which are difficult to reconcile with those found in cephalochordates or craniates.

Craniate (Vertebrate) Chordates

Both jawless and jawed craniates have three different kinds of motor neuron(s): (1) the ventrally exiting somatic motor neuron(s) of the spinal cord; (2) the dorsally exiting branchial motor neuron(s) of the hindbrain targeted for muscle of the branchial arches (hence the name branchial motor neuron(s)); and (3) a unique set of motor neuron(s) innervating the ocular muscles which are present in all craniates except the hagfish, either as primitive absence or due to secondary loss (**Figure 2**). The ocular motor neuron(s) can change their axonal trajectory to exit either dorsally or ventrally, thus resembling somewhat somatic motor neuron(s) associated with the spinal cord. The branchial and oculomotor motor neuron(s) are unique in that they express a specific combination of Phox2 genes not found in the spinal cord. In addition, craniates have a unique set of inner-ear efferent neurons which have been shown to derive from the facial (cranial nerve VII) branchial motor neuron(s). Due to the absence of a recognizable ear in noncraniate chordates, this motorneuron population must be considered as a uniquely derived feature of chordates which coevolved with the evolution of the craniate ear.

All craniates have cranial and spinal sensory nerves which differ in their composition. For example, the lampreys' cranial nerves seem to have only placodal-derived neurons – neural crest does not contribute to them. In addition, those nerves contain axons of intramedullary neurons which appear to be functionally equivalent to the mesencephalic trigeminal sensory neurons of jawed craniates. In contrast to the latter, all cranial nerves in lampreys seem to have some contribution of these intramedullary neurons. Limited embryological evidence suggests that at least the mesencephalic trigeminal sensory neurons are neural crest derived and the intramedullary sensory neurons of lampreys and hagfish may represent such a neural-crest precursor. In the spinal cord,

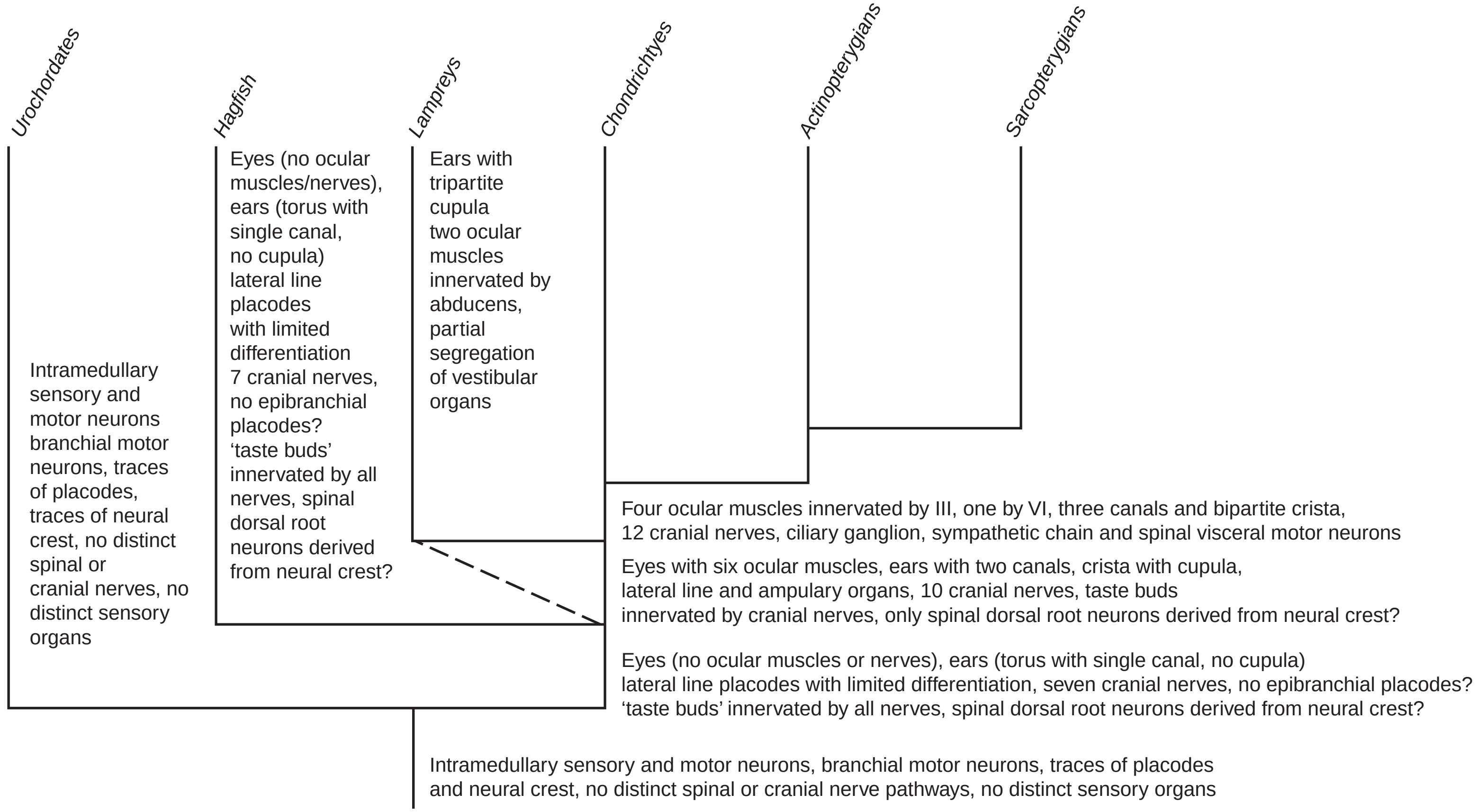

Urochordates
Hagfish
Lampreys
Chondrichtyes
Actinopterygians
Sarcopterygians
Intramedullary sensory and motor neurons branchial motor neurons, traces of placodes, traces of neural crest, no distinct spinal or cranial nerves, no distinct sensory organs
Eyes (no ocular muscles/nerves), ears (torus with single canal, no cupula) lateral line placodes with limited differentiation 7 cranial nerves, no epibranchial placodes? 'taste buds' innervated by all nerves, spinal dorsal root neurons derived from neural crest?
Ears with tripartite cupula two ocular muscles innervated by abducens, partial segregation of vestibular organs
Four ocular muscles innervated by III, one by VI, three canals and bipartite crista, 12 cranial nerves, ciliary ganglion, sympathetic chain and spinal visceral motor neurons
Eyes with six ocular muscles, ears with two canals, crista with cupula, lateral line and ampulary organs, 10 cranial nerves, taste buds innervated by cranial nerves, only spinal dorsal root neurons derived from neural crest?
Eyes (no ocular muscles or nerves), ears (torus with single canal, no cupula) lateral line placodes with limited differentiation, seven cranial nerves, no epibranchial placodes? 'taste buds' innervated by all nerves, spinal dorsal root neurons derived from neural crest?
Intramedullary sensory and motor neurons, branchial motor neurons, traces of placodes and neural crest, no distinct spinal or cranial nerve pathways, no distinct sensory organs

Figure 2 The cladogram shows some of the major changes in the nerve organization and sensory systems in the clade olfactores as discussed in the text. Note that the phylogenetic position of hagfish tends to fall together with lampreys to form a monophyletic taxon based on molecular data. However, most morphological data suggest that hagfish are distinct from lampreys. If indeed hagfish and lampreys are monophyletic, then hagfish have to be extremely derived beyond any such chances encountered in any other vertebrates showing regressive evolution.

jawless craniates differ in their ventral root/dorsal root organization and show asymmetry of dorsal and ventral root exits. In fact, the fusion of dorsal and ventral roots into a common root in hagfish is considered to be independently derived. Most importantly, neither hagfish nor lampreys have spinal or cranial visceral motor neurons and lack both sympathetic and parasympathetic ganglia, the latter being neural-crest derivatives. Interestingly, in hagfish and possibly unique to them, is the innervation of the widespread taste-bud-like Schreiner's organs by all cranial and spinal ganglia. This is in stark contrast to lampreys' taste buds restricted to the branchial arches and innervated only by cranial nerves and jawed craniates whose taste buds are always innervated by epibranchial placode-derived neurons (i.e., placodes forming at the gill arches) requiring Neurogenin2 for their development. Clearly, spinal dorsal root ganglia use both Neurogenin 1 and 2 for development, but it is unclear how widespread this dual use extends among craniates and whether or not the innervations of the hagfish Schreiner's organ is coming from Neurogenin-2-specified dorsal root ganglia, thus presenting a unifying theme of taste-bud innervation with an apparent shift between epibranchial placode-derived cells to neural-crest-derived cells. Whether or not the hagfish spinal dorsal root ganglia develop via placodes like lamprey's cranial ganglia or develop from neural crest as in jawed craniates remains to be demonstrated in hagfish embryos.

Jawed Craniates (Vertebrates)

All jawed craniates are characterized by a set of apomorphies (evolutionary specializations) in their peripheral nervous system:

1. They all have in the caudal midbrain, a set of sensory neurons innervating the muscle of mastication, the mesencephalic trigeminal nucleus. How and why the medullary sensory neurons of lampreys and hagfish were replaced by mesencephalic sensory neurons remains unclear. It can only be speculated that this event somehow correlates with the molecular specification of the gnathostome (jawed craniate) midbrain–hindbrain boundary and the evolution of the cerebellum.
2. They all have spinal sympathetic ganglia innervating the viscera and have somatomotor-derived sympathetic visceral motor neuron(s) which segregate through expression of specific Lim-domain genes from somatic motor neuron(s) and innervate the neural-crest-derived sympathetic and parasympathetic ganglia.
3. They all have dorsal and ventral roots exiting at intersegmental spaces and fusing to form a mixed peripheral nerve. It is likely that the molecular basis of development for these sensory components (the various bHLH genes) is conserved in expression and cell-fate decision, but more basal-jawed craniates as well as jawless outgroups need to be investigated to solidify this assumption.
4. Trigeminal motor neuron(s) (cranial nerve V) project exclusively through the mandibular branch of the trigeminal nerves whereas they also contribute to the maxillary division in lampreys, and this issue remains to be explored in hagfish.
5. All jawed craniates have a vagus (cranial nerve X), accessory nerve (XI), and hypoglossal nerve (XII). However, it is unclear how the lampreys' exclusively spinal somatic motor neuron(s) became reorganized to form the intramedullary hypoglossal motor neuron(s), how vagal motor neuron(s) gained access to the heart and bowel for parasympathetic control, and how the accessory motor neuron(s) evolved from caudal intramedullary branchial motor neuron(s) to form the rostral spinal branchiomotor-like motor neuron(s) of jawed craniates.

Despite this high level of conservation, there were other surprising reorganizations of existing, functional systems into a new pattern at the transition from jawless to jawed craniates and within jawed craniates. Foremost is the reorganization of the ocular motor and vestibular system. Lampreys have 6 eye (ocular) muscles but the pattern of innervation by ocular motor neuron(s) is different from that of jawed craniates: two ocular muscles are innervated by the abducens (cranial nerve VI) in lampreys whereas only one ocular muscle is innervated by the abducens in jawed craniates. This difference suggests either a reorganization of the ocular muscles from a functional eye-movement system into an equally functional system with a different organization combined with a concomitant reorganization of the input mechanisms or a parallel evolution from a possibly seven-ocular muscle ancestor. Instead of reorganizing a lamprey-like into a gnathostome-like six-ocular muscle pattern, a more parsimonious but somewhat speculative idea would be to assume a hypothetical seven-ocular muscle ancestor. If such an ancestor exists, this would not require reorganization of one existing and functional system into another one. Instead, the two lineages would have lost one oculomotor and one abducens innervated muscle in the lamprey and gnathostome lineage, respectively, converting a seven-ocular muscle ancestral pattern into the two derived systems now found in vertebrates. Interestingly, one of those inputs, the vestibular system, evolved from a two-semicircular canal system (lampreys and hagfish) into a three canal system (jawed craniates) through the evolutionary addition of the horizontal (lateral) canal. More recent data suggest that at least two molecular changes underlie this reorganization: orthodenticle-like 1 (Otx1) is needed to form the horizontal canal proper whereas Foxg1 is needed for the formation of the horizontal canal crista containing the sensory part of the horizontal canal system. Another major change is the evolution of cranial and caudal parasympathetic ganglia and their specialized parasympathetic visceral motor neuron(s) which are apparently found only in jawed craniates. Consequently, there is no ciliary ganglion-mediating lens movements in lampreys, which have evolved a different accommodation mechanism. Thus, the eye underwent numerous changes in terms of its ocular muscles, intraocular muscles, and the retina (change in position of the retinal ganglion cells) at the transition from jawless to jawed craniates. Molecular data for the formation of parasympathetic ganglia are beginning to appear in the literature and may help to understand the evolutionary history of this complex system.

While neurogenic placodes are the basis for all craniate sensory systems and their molecular basis is beginning to emerge, it remains unclear how the neurogenic placodes have been molecularly changed to produce the various craniate ears, the various taste-bud neurons, lateral line and electroreceptive organs, and neurons. Most importantly, there is clear experimental evidence indicating that at least the ampullary electroreceptors of Axolotl (a salamander) are derived from the same lateral-line placodes generating the mechanosensory lateral-line organs. Another molecularly unresolved problem of craniate sensory evolution is the generation of the novel auditory sense for which we are just beginning to understand the molecular basis, and the loss of the mechanosensory lateral line and electroreceptive ampullary organs and their placodes in land vertebrates, leaving numerous open issues both for vertebrate neurosensory progressive and regressive evolution of neurosensory systems, including gain and loss of entire sensory epithelia such as the auditory system (amphibian papilla, basilar papilla/cochlea) and the lagena.

The view presented here assumes that the original deuterostome PNS was a nerve plexus closely resembling that of modern diploblasts and basal triploblasts, without aggregation of sensory cells into discrete organs. The evolution of the PNSs and sensory organs in deuterostomes shows certain similarities at the level of single genes and even cells embedded in an overall changing system. The nervous system of deuterostomes may have evolved, hypothetically, from initial peripheral nerve ramifications and rudimentary sensory-cell aggregations into molecularly precise specified systems which, once established, limited evolutionary changes among gnathostomes. Still, while the overall pattern is conserved, even the gnathostome PNS shows remarkable system and cellular evolution for which we do not yet understand the molecular basis. In conclusion, it appears that evolutionary developmental transformation of a nerve net retains peripheral motor nerves which can be modified by various sensory additions derived from intramedullary neurons, neural crest, or placodes to evolve into the almost stereotyped patterns found in all vertebrates.

Summary and Conclusion

Projecting crown-group organization, such as mammalian cranial and spinal nerve organization, onto a putative ancestor biases any attempt to understand the evolution of neural systems. Modern molecular analysis, instead, has demonstrated the expression of homologous molecules and cellular resemblances in analogous parts of peripheral nervous and sensory system such as eyes and ears, organs which are anatomically rather different. This cellular and molecular homology, which can occasionally be established across a wide diversity of phyla, suggests that cellular and molecular identity predates the evolution of complex sensory systems. Different sensory and nervous systems may present examples of convergent evolution with homologous cellular and molecular bases in an analogous organ.

See also: **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy; Auditory System Morphology.

Further Reading

Bertrand V, Hudson C, Caillol D, Popovici C, and Lemaire P (2003) Neural tissue in ascidian embryos is induced by FGF9/16/20, acting via a combination of maternal GATA and Ets transcription factors. *Cell* 115: 615–627.

Bourlat SJ, Juliusdottir T, Lowe CJ, *et al.* (2006) Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* 444: 85–88.

Briscoe J and Ericson J (2001) Specification of neuronal fates in the ventral neural tube. *Current Opinion in Neurobiology* 11: 43–49.

Dufour HD, Chettouh Z, Deyts C, *et al.* (2006) Precraniate origin of cranial motorneurons. *Proceedings of the National Academy of Sciences of the United States of America* 103: 8727–8732.

Fritzsch B (1998) Evolution of the vestibulo-ocular system. *Otolaryngology – Head and Neck Surgery* 119: 182–192.

Fritzsch B, Beisel KW, and Hansen LA (2006) The molecular basis of neurosensory cell formation in ear development: A blueprint for hair cell and sensory neuron regeneration? *Bioessays* 28: 1181–1193.

Fritzsch B and Glover JC (2006) Evolution of the deuterostome central nervous system: An intercalation of developmental patterning processes with cellular specification processes. In: Kaas JH (ed.) *Evolution of the Nervous System* vol. 2 pp. 1–24. Oxford: Academic Press.

Fritzsch B and Northcutt RG (1993) Cranial and spinal nerve organization in amphioxus and lampreys: Evidence for an ancestral craniate pattern. *Acta Anatomica (Basel)* 148: 96–109.

Lacalli TC (2004) Sensory systems in amphioxus: A window on the ancestral chordate condition. *Brain, Behavior and Evolution* 64: 148–162.

Lowe CJ, Terasaki M, Wu M, *et al.* (2006) Dorsoventral patterning in hemichordates: Insights into early chordate evolution. *PLoS Biology* 4: e291.

Lowe CJ, Wu M, Salic A, *et al.* (2003) Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* 113: 853–865.

Murakami Y, Uchida K, Rijli FM, and Kuratani S (2005) Evolution of the brain developmental plan: Insights from agnathans. *Developmental Biology* 280: 249–259.

Nieuwenhuys R (2002) Deuterostome brains: Synopsis and commentary. *Brain Research Bulletin* 57: 257–270.

Northcutt RG and Gans C (1983) The genesis of neural crest and epidermal placodes: A reinterpretation of vertebrate origins. *Quarterly Review of Biology* 58: 1–28.

O’Neill P, McCole RB, and Baker CV (2006) A molecular analysis of neurogenic placode and cranial sensory ganglion development in the shark, *Scyliorhinus canicula*. *Developmental Biology* 304: 156–181.

Peterson KJ, Dietrich MR, and McPeek MA (2009) MicroRNAs and metazoan macroevolution: Insights into canalization, complexity, and the Cambrian explosion. *Bioessays* 31: 736–747.

Pierce ML, Weston MD, Fritzsch B, *et al.* (2008) MicroRNA-183 family conservation and ciliated neurosensory organ expression. *Evolution and Development* 10: 106–113.

Satoh N (2008) An aboral-dorsalization hypothesis for chordate origin. *Genesis* 46: 614–622.

Sauka-Spengler T and Bronner-Fraser M (2008) Evolution of the neural crest viewed from a gene regulatory perspective. *Genesis* 46: 673–682.

Functional Morphology of the Brains of Agnathans

MA Pombal and M Megías, University of Vigo, Vigo, Spain

© 2011 Elsevier Inc. All rights reserved.

Introduction
Hindbrain
Midbrain
Forebrain
Further Reading

Glossary

Agnathans Vertebrates (fishes) without jaws (a = without; gnathos = jaw).

Ammocoete Alternate name for the larval stage of the lamprey life cycle.

Cyclostomes Animals with round mouth (cyclo = round; stoma = mouth). Synonym of agnathans.

Monorhinic Animals having a single nasal sac, such as agnathans.

Neuromeres Segments or transversal bands of the developing neural tube exhibiting specific properties, such as prosomeres in the forebrain (prosencephalon) or rhombomeres in the hindbrain (rhombencephalon).

Neuromeric model Morphological interpretation of the brain based on the restricted expression pattern of regulatory genes and a segmental conception of brain organization, besides considering four longitudinal subdivisions (floor, basal, alar, and roof plates) throughout the entire longitudinal axis.

Vertebrates As used here, a synonym of craniates, the clade that includes agnathans and gnathostomes; alternately defined by some authors as excluding hagfishes, reserving the term craniates for all agnathans and jawed vertebrates.

Introduction

Lampreys, together with hagfishes, are the only living jawless fishes included in one of the two major taxa of the craniate lineage: the agnathans or cyclostomes; they arose about 500 million years ago and, since then, have evolved independently. The species of this group are of interest for studying the shared characters with gnathostomes and for reconstructing the anatomy of the brain of the common ancestor of craniates. Therefore, the study of their central nervous system (CNS) offers an excellent starting point to understand the development, structure, and function of the craniate neuraxis.

Lampreys (Petromyzontoidea or petromyzontids) belong to the cephalaspidomorphi class that comprises a single family Petromyzontidae (including 9 genera and 41 species), whereas hagfish (Myxinoidea or myxinoids) are included in the Myxini class that comprises two families: Eptatretidae and Myxinidae (with 5 genera and about 60 species). These two classes of craniates share many features such as the absence of jaws, scales, or paired fins (**Figures 1**(**a**) and **1**(**b**)). In addition, they have an elongated eel-like body, a cartilaginous skeleton (without bones), a single nostril (monorhinic), a tongue-like structure bearing sharp denticles, pore-like gill openings, and the ability to exude copious quantities of slime or mucus. However, there are some other features that differ considerably between them, such as the presence of seven pairs of gill openings, a round sucking mouth with many teeth, and two dorsal fins in lampreys. By contrast, hagfishes have a variable number of gill openings, ranging from 2 to 14 pairs, and posses several tentacle-like barbels located around the mouth and the nostril. Another marked difference between the two groups relates to their lifecycles: lampreys have a complex lifecycle that comprises a very long freshwater larval phase (3–5 or more years), followed by a metamorphosis. During the adult stage many of them, but not all, are predators of other fishes. Those that are predacious can complete their lifecycle in freshwater or migrate to the sea and return to freshwater streams for reproduction before dying. Hagfishes are benthic marine organisms that live in relatively cold water at depths ranging from ten to several hundred meters and have no larval stage (**see also Hagfishes and Lamprey**: Hagfishes; Lampreys: Energetics and Development; **Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines).

At the brain level, lampreys and hagfishes have remarkable differences but also many similarities. In

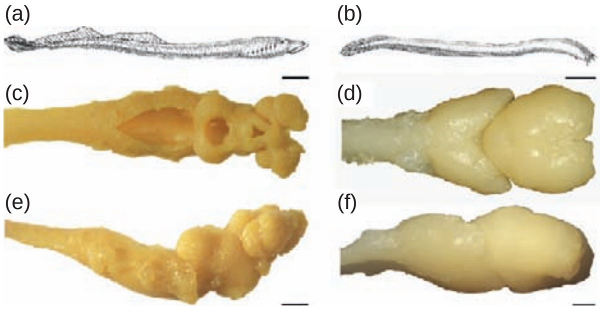


Figure 1 Drawings of lateral views of a lamprey, *Lampetra fluviatilis* (a) and a hagfish, *Myxine glutinosa* (b), as well as dorsal (c, d) and lateral (e, f) views of their brains. Scale bars = 35 mm (a), 90 mm (b), and 1 mm (c–f).

contrast to amphioxus (a cephalochordate), they have a number of special sense organs that convey information over the cranial nerves toward specific centers of the CNS. As in all craniates, their brains consist of a hindbrain or rombencephalon caudally, a midbrain or mesencephalon, and a forebrain or prosencephalon rostrally, with the latter classically thought to consist of a telencephalon and a diencephalon. The adult neural tube of agnathans has a minimal morphogenetic deformation (cephalic flexure) that occurs at the level of the rostral end of the notochord (which persists in the adult stage). This anatomical feature helps establishing particular transversal boundaries and understanding its topology better.

The adult lamprey brain is slender, elongated, and very small (8 mm in length and 2.3 mm width, with the smallest proportion in relation to the body mass among the vertebrates), with an ample mesencephalic ventricle (**Figures 1**(**c**) and **1**(**e**)). The adult hagfish brain is larger (8.4 mm in length and 3 mm width) and unusually compact (**Figures 1**(**d**) and **1**(**f**)). There are no choroidal plexuses in hagfishes, but they cover most of the dorsal part of the brain in lampreys and, together with the ample ventricular cavities, make up for the absence of vascularization in the larval brain and spinal cord as well as in the adult spinal cord.

The eyes in hagfishes are degenerate in that they are not externally visible, and a lens and extraocular muscles are absent; therefore, they have no somatomotor nerves (III, IV, and VI) innervating the eye orbit. Nonetheless, hagfishes do have small optic nerves that arise from the eyes and project centrally. Lampreys, like jawed vertebrates, have fully developed eyes and extrinsic musculature. Some of the lamprey extraocular muscles, however, are differently arranged than their gnathostome counterparts. From a histological point of view, there are also striking differences between lamprey and hagfish brains; in hagfishes, most neurons migrate away from the ventricular surface (where they originated) and are highly organized and particularly arranged in some areas, whereas in lampreys the majority of cells are densely packed in a periventricular position.

Some specific differences with gnathostomes are that the dura mater, arachnoid, and pia mater are absent in the cyclostomes, where the CNS is protected by one undifferentiated meninx, the meninx primitive, which is covered by perimeningeal tissue formed of mucoid cells. In addition, agnathans apparently lack a true cerebellum and have no oligodendroglia; therefore, myelin-sheaths are not developed.

Research to understand the organization and evolutionary development of the agnathan brain has intensified over the last decades. As lampreys have a relatively simple nervous system, with a basic structural organization similar to that of the rest of vertebrates, there are many functional studies. This research has benefitted from the ability to maintain an *in vitro* preparation of its CNS for several days, with attached peripheral nerves and muscles if needed. Many pioneering studies involved the largest reticulospinal neurons, which can be seen without histological preparation because the walls of the brain are translucent. However, the availability of embryonic stages of the various species of hagfishes has hampered progress.

Concerning the nomenclature of the brain, a new paradigm of prosencephalic organization in vertebrates, including lampreys, has been developed in the last two decades. This implies the redistribution of some nuclei and the definition of morphological landmarks between different zones that allow for establishing new homologies.

Hindbrain

The hindbrain or rhombencephalon represents about half of the length of the whole brain and is well developed. It continues rostrally with the midbrain and caudally with the spinal cord by a transition zone or obex that is located at the level of the first spinal root. In hagfishes, its rostral boundary is delineated dorsally by the isthmic fissure and ventrally by the plica encephali ventralis. In lampreys, only the dorsal limit is externally marked by a deep sulcus.

The hindbrain can be subdivided into four longitudinal regions: roof and alar plates dorsally and basal and floor plates ventrally. The internal cavity or fourth ventricle is much more ample in lampreys, where it is covered dorsally by an extensive and richly folded choroidal plexus (roof plate, **Figure 2**(**a**)), whereas in hagfishes it is rather narrow but slightly enlarged in its rostral part. Most of the agnathan alar plate is formed by the octavolateral area, which can be subdivided into dorsal, medial, and ventral nuclei (**Figures 2**(**a**)–**2**(**d**), **2**(**h**), and **2**(**i**)). The dorsal nucleus in lampreys, as in other craniates, receives electroreceptive inputs; however, in hagfishes, which have no electroreceptive lateral line system, this nucleus receives ascending spinal

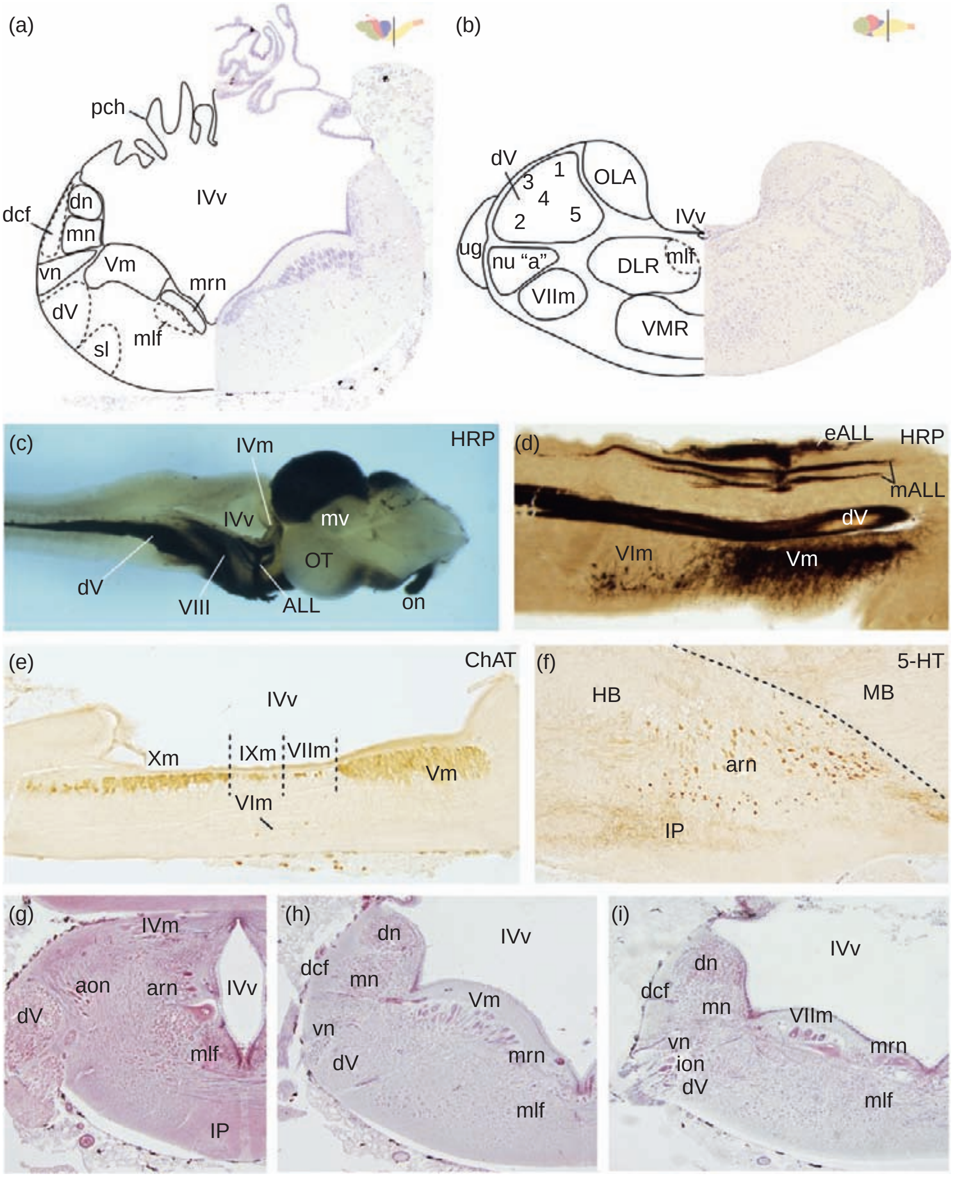


Figure 2 Representative transverse sections through the hindbrain of *L. fluviatilis* (a) and *M. glutinosa* (b). Hematoxylin-stained sections are shown in the right side; the identified areas and nuclei (solid lines), and major tracts (dashed lines) are shown in the left side. (c) Dorsal view of the brain of a postmetamorphic lamprey showing some horseradish peroxidase (HRP)-labeled structures after injection of the tracer into the otic capsule and the eye orbit. (d) HRP-labeled structures from the eye orbit in a sagittal section of a larval lamprey brain. (e) Choline acetyl transferase (ChAT) immunoreactive motoneurons in a sagittal section of the lamprey hindbrain. (f) Serotonin (5-HT) immunoreactive cells in a sagittal section through the anterior rhombencephalic reticular nucleus. (g–i) Bodian-stained transverse sections through the isthmus (g) and the trigeminal (h) and facial (i) motor nuclei. 1–5, 1–5 subdivisions of the sensory trigeminal nucleus; ALL, anterior lateral line; aon, anterior octavomotor nucleus; arn, anterior rhombencephalic reticular nucleus; dcf, dorsal column fibers; DLR, dorsolateral reticular nucleus; dn, dorsal nucleus of the octavolateral area; dV, descending trigeminal tract; eALL, electro-receptive anterior lateral line; HB, hindbrain; ion, intermediate octavomotor nucleus; IP, interpeduncular nucleus; IVm, trochlear nucleus; IVv, fourth (rhombencephalic) ventricle; IXm, glossopharyngeal motor nucleus; mALL, mechano-receptive anterior lateral line; MB, midbrain; mlf, medial longitudinal fasciculus; mn, medial nucleus of the octavolateral area; mrn, medial rhombencephalic reticular nucleus; mv, mesencephalic ventricle; nu "a," nucleus "a" of Kusunoki; OLA, octavolateral area; on, optic nerve; OT, optic tectum; pch, choroidal plexus; sl, spinal lemniscus; ug, utricular ganglion (VIII nerve); VIII, octaval projections; VIIm, facial motor nucleus; VIm, abducens motor nucleus; Vm, trigeminal motor nucleus; VMR, ventromedial reticular nucleus; vn, ventral nucleus of the octavolateral area; Xm, vagal motor nucleus.

projections through the dorsal column pathway. The medial nucleus is innervated by mechanoreceptive lateral line fibers. The ventral nucleus receives vestibular afferents through the octaval (VIII) nerve. In lampreys, three (anterior, intermediate, and posterior) octavomotor nuclei are readily distinguished by their cell body size (**Figures 2(g)** and **2(i)**) and their vestibulo-ocular and vestibulo-spinal connections. In hagfishes, an octaval region is present but is more diffusely organized. A small efferent projection to the labyrinth is also present in lampreys.

The sensory complex of the trigeminal nerve (**Figure 2**(**b**)) and its area of termination are prominent in hagfishes. In lampreys, this sensory system is also well developed (**Figures 2**(**a**), **2**(**c**), **2**(**d**), and **2**(**g**)–**2**(**i**)), with most of its fibers reaching the rostral portion of the spinal cord. The sensory fibers are topographically arranged, and in hagfishes they are segregated into five columns surrounded by cell bodies. The second-order neurons of the sensory trigeminal nucleus have crossed axons projecting to different targets. Remarkably, agnathans have no ascending primary trigeminal fibers, and a principal and a mesencephalic sensory nuclei are apparently lacking. However, lampreys and *Eptatretus stouti* have a number of large intramedullary sensory cells (identified as dorsal cells) with peripheral branches associated with the trigeminal nerve, which are regarded as homologs of the mesencephalic trigeminal nucleus of gnathostomes. In *E. stouti*, these cells are located in the ventral portion of the sensory trigeminal nucleus. In lampreys, they are located periventricularly in the octavolateral area. At the caudal end of the rhombencephalic alar plate, there is another common element of the somatosensory zone, the dorsal column nuclei, which project to the contralateral optic tectum.

In lampreys, a narrow viscerosensory zone is located medial to the descending trigeminal tract and, based on its chemical properties, a nucleus of the solitary tract has been recently identified. In hagfishes, this column is mostly represented by the sensory nucleus of the vagus, which occupies a particular submeningeal location. They also have a nucleus located between the sensory and motor zone called nucleus ‘a’ of Kusunoki (**Figure 2**(**b**)) that cannot yet be homologized with any other craniate nucleus.

The cerebellum has not been convincingly defined in agnathans. Classically, a small and simple ridge or plate between the right and left octavolateral area at the isthmus level is considered as the agnathan cerebellum in lampreys. However, recent experimental results question its existence. Of interest is that the main nuclei related to the gnathostome cerebellum, such as those derived from the rhombic lip (pontine nuclei and the olivary complex) or a nucleus ruber (red nucleus), have not been identified in agnathans.

In the lamprey isthmus, a locus coeruleus has been tentatively identified based on the presence of some catecholaminergic cells projecting rostrally to the striatum. In addition, a nucleus isthmus was identified rostral to the trochlear nucleus by its reciprocal connections with the optic tectum and by the cholinergic nature of its cells. Caudal to this nucleus, there is another radially oriented cholinergic cell population whose periventricular component was considered homologous to the pedunculopontine tegmental nucleus; the superficial portion could correspond to the laterodorsal tegmental nucleus of amniotes. Recent studies suggest that the region of the pedunculopontine tegmental nucleus is the counterpart of the mammalian mesencephalic locomotor region (MLR). Although the MLR is frequently ascribed to the mammalian midbrain, it is located topologically caudal to the trochlear motor nucleus and thus clearly corresponds to the isthmus. None of these isthmic nuclei have been investigated in hagfishes.

The motor nuclei are the most conspicuous nuclei of the rhombencephalic basal plate, and their topographical distribution is relatively well known in agnathans, where they mostly constitute a prominent and rather continuous column of cells along the hindbrain (**Figures 2**(**a**)–**2**(**e**), and **2**(**g**)–**2**(**i**)). In lampreys, the branchiomotor nuclei are located periventricularly, whereas in hagfishes they migrate to the ventrolateral part of the rhombencephalon. These include the nuclei of the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves, whose motoneurons are located at the same dorsoventral level.

The trigeminal nerve innervates all the periphery of the mouth, and its motor nucleus is strongly developed in lampreys, where the very large motoneurons protrude toward the fourth ventricle. Contrary to the situation in gnathostomes, the facial complex lacks internal mandibular and pharyngeal branches, whose targets are innervated instead by the trigeminal nerve, whereas the glossopharyngeal and vagal nerves are thought to innervate motor and sensory components of the pharyngeal region and viscera. An unresolved issue in agnathans is the distinction of branchiomeric from visceral (preganglionic parasympathetic) cell populations. Assuming that the facial, glossopharyngeal, and vagal nerves carry both components, they cannot be distinguished from each other.

Concerning the lamprey somatomotor column, the abducens motoneurons are located caudal to the trigeminal motor nucleus and adjacent to the floor plate (**Figures 2**(**d**) and **2**(**e**)). In contrast to gnathostomes, these motoneurons innervate two muscles, the ipsilateral inferior and posterior rectus muscles. Their axons course rostrally and exit the brain in an atypical lateral position, together with the trigeminal roots. The motoneurons of the nucleus of the trochlear (IV) nerve innervate the caudal oblique extraocular muscle; most of these motoneurons are located quite dorsally at the isthmus level (**Figures 2**(**c**) and **2**(**g**)), where the majority of their axons cross the midline at the velum medullare anterius to exit the brain dorsally. In addition, agnathans have a spino-occipital motor nucleus, which is considered to be the counterpart of the hypoglossal nucleus of tetrapods. This nucleus innervates the floor of the pharynx and the posterior region of the buccal funnel.

The lamprey reticular system is composed of some very large cells (identified as Múller and Mauthner cells) that are not present in hagfishes. The seven pairs of Múller cells have conserved positions: three rostral to the hindbrain (see below), one in the isthmus, and the remaining three pairs in the rhombencephalon proper. They possess uncrossed axons that course in the medial longitudinal fasciculus to the spinal cord. In addition, lampreys have one or two pairs of Mauthner cells, characterized by their position near the vestibular nerve and by their crossed spinal-projecting axons. The agnathan reticular system is usually subdivided into three portions, namely anterior (superior), intermediate (medius), and posterior (inferior) rhombencephalic reticular nuclei (**Figures 2**(**a**) and **2**(**f**)–**2**(**i**)), and it is mostly composed of glutamatergic cells, but possesses numerous serotonin-immunoreactive cells as well (**Figure 2**(**f**)). These nuclei receive inputs from several systems and project to the spinal cord. Spinal-projecting raphe nuclei are also present, but in hagfishes are only two in number.

An interpeduncular nucleus exists in both hagfishes and lampreys (**Figures 2**(**f**) and **2**(**g**)) based on a prominent input from the habenula through the fasciculus retroflexus. In addition, there are some prominent ascending and descending fiber tracts. The dorsal column tract is located in the alar plate, whereas three main fiber bundles can be distinguished in the basal plate: the medial longitudinal fasciculus, the tecto-bulbo-spinal tract, and the spinal lemniscal fasciculus (**Figures 2**(**a**), **2**(**b**), and **2**(**g**)–**2**(**i**)).

Midbrain

The agnathan midbrain or mesencephalon extends dorsally in the rostro-caudal direction from the posterior commissure to the isthmic region, but ventrally it is very narrow. A unique feature of the lamprey mesencephalon is the presence of an ample ventricular cavity, covered dorsally by the corresponding choroid plexuses. It is subdivided in two main areas: one dorsal, constituted by the optic tectum (OT) and torus semicircularis, and other ventral, called the mesencephalic tegmentum (**Figure 3**).

In lampreys, most of the dorsal portion of the mesencephalic wall corresponds to a well-developed and stratified OT that is subdivided into four major layers: superficial, optic, central, and periventricular strata, with

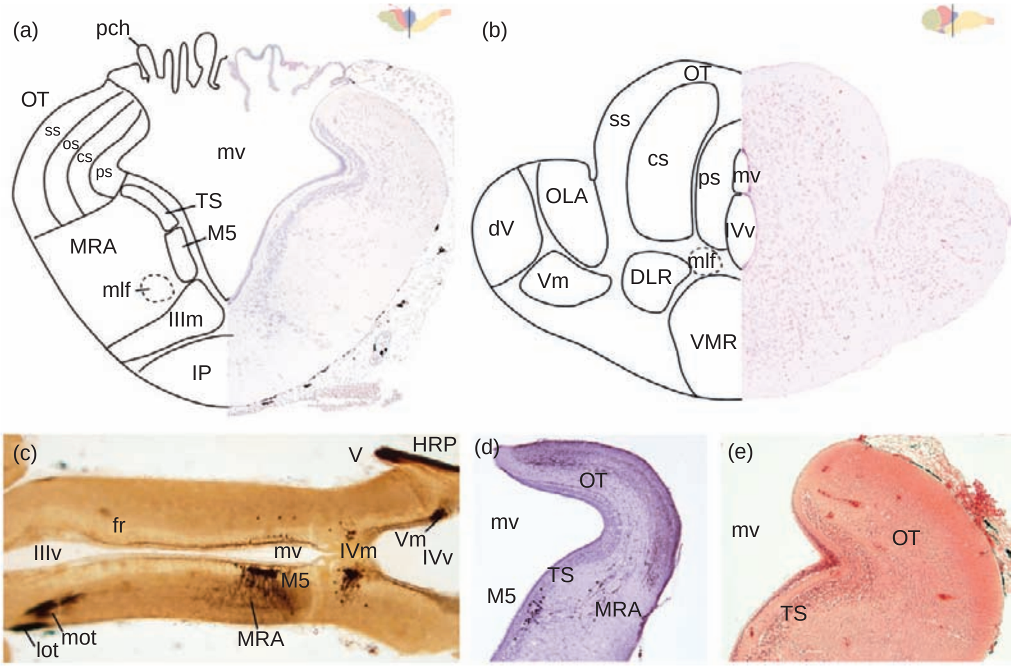


Figure 3 Representative transverse sections through the midbrain and rostral hindbrain of *L. fluviatilis* (a) and *M. glutinosa* (b). Hematoxylin-stained sections are shown in the right side; the identified areas and nuclei (solid lines), and major tracts (dashed lines) are shown in the left side. Some horseradish peroxidase staining (HRP)-labeled structures from the eye orbit are shown in a horizontal section of a larva (c) and a transverse section of an adult (d) lamprey brain. (e) Bodian-stained transverse section of the optic tectum (OT) and the torus semicircularis (TS). cs, central stratum (optic tectum); DLR, dorsolateral reticular nucleus; dV, descending trigeminal tract; fr, fasciculus retroflexus; IIIm, oculomotor nucleus; IIIv, third (diencephalic) ventricle; IP, interpeduncular nucleus; IVm, trochlear nucleus; IVv, fourth (rhombencephalic) ventricle; lot, lateral optic tract; M5, nucleus M5 of Schóber; mlf, medial longitudinal fasciculus; mot, medial optic tract; MRA, mesencephalic reticular area; mv, mesencephalic ventricle; OLA, octavolateral area; os, optic stratum (optic tectum); pch, choroidal plexus; ps, periventricular stratum (optic tectum); ss, superficial stratum (optic tectum); V, trigeminal nerve; Vm, trigeminal motor nucleus; VMR, ventromedial reticular nucleus.

most cell bodies located periventricularly. Although externally visible, the hagfish midbrain is very small and poorly developed. The OT is less developed than in lampreys and generally subdivided in three main layers: superficial (molecular), central, and periventricular cell layers. The major input to the agnathan OT arises from the contralateral retina, but many other sources have also been described.

Ventral to the lamprey OT is a torus semicircularis with ample number of connections, such as ascending lateral line and octaval fibers. This nucleus appears to be absent in hagfishes. In the alar plate of lampreys there are two prominent retinopetal nuclei, that is, the M5 nucleus of Schóber (periventricularly) and the mesencephalic reticular area (displaced laterally and dorsally) (**Figure 3**). Similar retinopetal cells have also been reported in the rostral mesencephalon of hagfishes but identified as the nucleus of the posterior commissure, suggesting that it may actually be diencephalic. The mesencephalic tegmentum of hagfishes is usually subdivided into a dorsal and a ventral tegmental nucleus. On the other hand, the lamprey mesencephalic tegmentum contains the mesencephalic reticular nucleus (which includes the third pair of Múller cells) and the oculomotor (somatomotor) motoneurons. In these animals, only three extraocular muscles (dorsal rectus contralaterally, and rostral rectus and rostral oblique ipsilaterally) are innervated by the third cranial nerve.

An area homologous to the mesencephalic substantia nigra of other vertebrates has been described in lampreys based on its immunoreactivity for dopamine and its projections to the striatum, but it is located farther rostrally in the diencephalon (nucleus of the posterior tuberculum). Such nucleus could also be present in hagfishes.

Forebrain

The forebrain or prosencephalon is the rostral part of the brain. Following the prosomeric model, the forebrain of vertebrates consists of two main parts, the diencephalon and the secondary prosencephalon. This model has been successfully applied in lampreys where, similar to other craniates, the diencephalon can be further divided into three prosomeres (p1, p2, and p3), which are numbered in a caudal to rostral order (**Figure 4**). In addition, two hypothalamic subdivisions (hp1 or prepeduncular and hp2 or peduncular) have recently been proposed in the secondary prosencephalon. As this model is not yet tested in hagfishes, the organization of the forebrain will be commented on following the general lamprey schema, with reference to hagfishes when appropriate. The hagfish diencephalon is dorsally separated from the midbrain by the di-mesencephalic sulcus and from the telencephalon by the sulcus telo-diencephalicus. Inside the hagfish forebrain, there is a complex and prominent central prosencephalic nucleus that can be subdivided into several subnuclei of uncertain homologies with amniotes.

Diencephalon

The pretectal region or pretectum represents the alar plate of the first prosomere (p1) (**Figures 4**(**c**) and **4**(**d**)). In lampreys it is subdivided into a rostral precommissural, an intermediate juxtacommissural, and a caudal commissural region. In agnathans, the most important nuclei of this region are the pretectal nuclei, which receive a substantial retinal projection and are involved in visuomotor behaviors, and the nuclei of the posterior commissure. Below the rostral part of the posterior commissure, there is a well-developed subcommissural organ that is paired in lampreys. The secretory material of this organ forms the so-called Reissner's fiber that runs caudally through the ventricular system to the end of the spinal cord.

The alar plate of the second prosomere (p2) is constituted by the epithalamus and the thalamus (the latter corresponding to the classic dorsal thalamus). The epithalamus consists of the epiphysis (or pineal complex that is only present in lampreys) and the habenular complex. The lamprey pineal complex is well developed and consists of two photosensitive organs (pineal and parapineal) (**Figures 4**(**e**) and **5**(**a**)). They have ample connections with the fore- and midbrain, and some parapineal efferent fibers reach the left habenula. The agnathan habenular complex is well developed and possesses a massive commissure. As in other craniates, it is highly asymmetric with the right habenula being much larger than the left (they are fused in hagfishes) (**Figures 4**(**a**), **4**(**b**), and **4**(**d**)). Its major efferent tract is the habenulo-interpeduncular tract or fasciculus retroflexus that is also asymmetric and mostly courses to the interpeduncular nucleus of the rostral hindbrain. Most of the habenular afferents originate rostrally in the secondary prosencephalon and course via the stria medullaris.

In the lamprey thalamus, which is characterized by the presence of many calretinin immunoreactive cells (**Figure 4**(**f**)), two zones dominate, a periventricular compact gray substance and an external (lateral) zone with dispersed cells that correspond to the primordium of the lateral geniculate nucleus.

In the third prosomere (p3), the main component of the alar plate is the prethalamus (which includes the classic ventral thalamus). Furthermore, the prethalamus is divided into dorsal (including the Bellonci nucleus), medial (including the ventral geniculate nucleus), and ventral portions. A prethalamic nucleus involved in the control of locomotion exists in lampreys, which corresponds to the diencephalic locomotor region of amniote vertebrates.

The hagfish thalamus is relatively small, but contains several nuclei: anterior, internal, external, and subhabenular

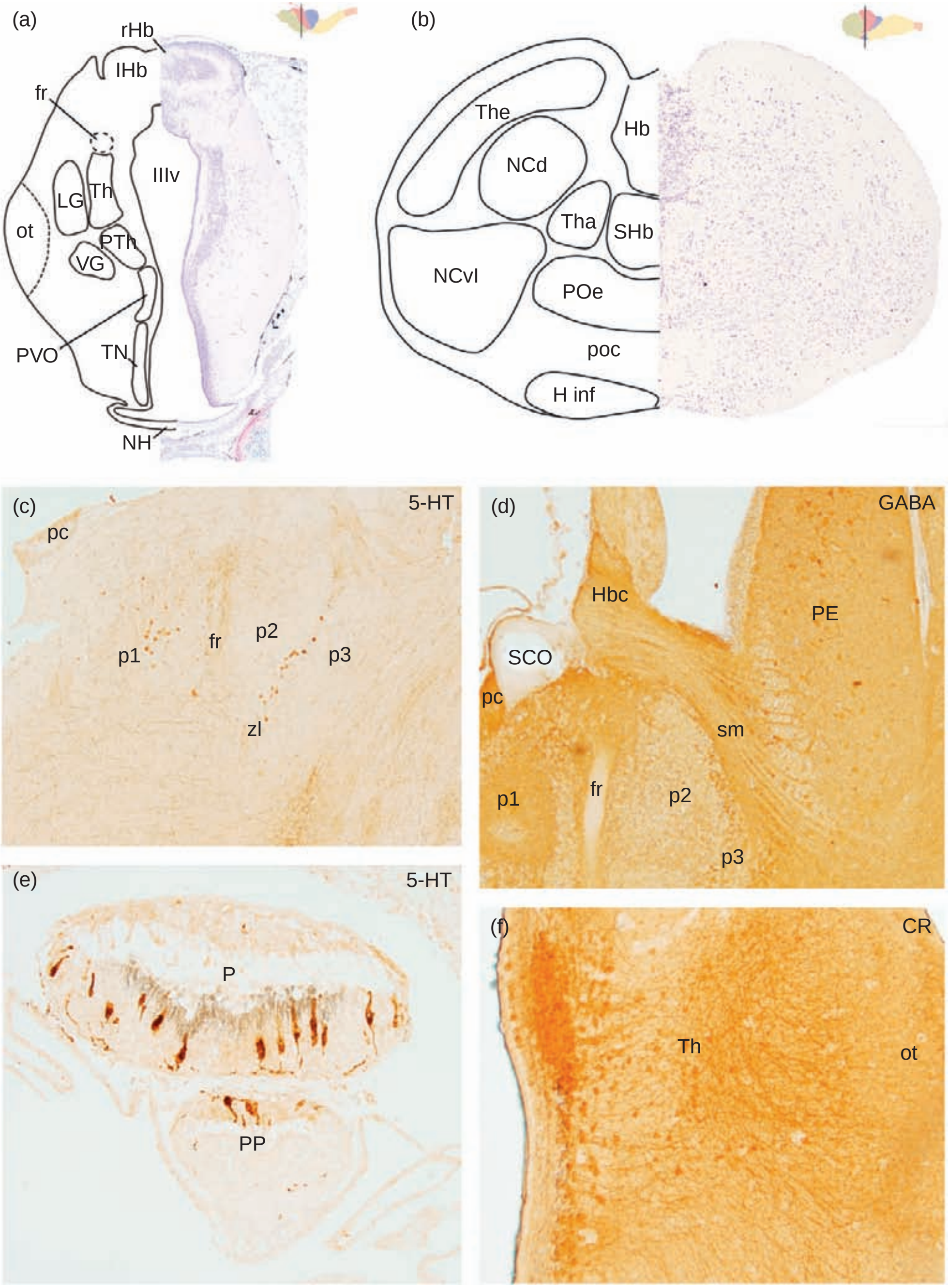


Figure 4 Representative transverse sections through the forebrain (diencephalon) of *L. fluviatilis* (a) and *M. glutinosa* (b). Hematoxylin-stained sections are shown in the right side; the identified areas and nuclei (solid lines), and major tracts (dashed lines) are shown in the left side. (c) Sagittal section showing serotonin (5-HT) immunoreactive cells in the pretectum (p1) and the zona limitans intrathalamica (zl). (d) Sagittal section showing gamma aminobutyric acid (GABA) immunoreactive cells in the prethalamus (p3) and the prethalamic eminence (PE). (e) 5-HT immunoreactive cells in a transverse section through the pineal and parapineal organs. (f) Calretinin (CR) immunoreactive cells in a transverse section through the thalamus. fr, fasciculus retroflexus; H inf, infundibular hypothalamus; Hb, habenula; Hbc, habenular commissure; IIIv, third (diencephalic) ventricle; LG, lateral geniculate nucleus; lHb, left habenula; NCd, dorsal nucleus of the central prosencephalic nucleus; NCvl, ventrolateral nucleus of the central prosencephalic nucleus; NH, neurohypophysis; ot, optic tract; P, pineal organ; p1–p3, prosomeres 1–3; pc, posterior commissure; PE, prethalamic eminence; poc, postoptic commissure; POe, external nucleus of the preoptic area; PP, parapineal organ; PTh, prethalamus; PVO, hypothalamic periventricular organ; rHb, right habenula; SCO, subcommisural organ; SHb, subhabenular nucleus; sm, stria medullaris; Th, thalamus; Tha, anterior thalamic nucleus; The, external thalamic nucleus; TN, tuberal nucleus; VG, ventral geniculate nucleus.

thalamic nuclei (**Figure 4**(**b**)), triangular thalamic nucleus, intracommissural thalamic nucleus, paracommissural thalamic nucleus, and diffuse nucleus of the thalamus, with the first receiving retinal projections. Whether these nuclei are components of the thalamus or the prethalamus is uncertain.

The basal diencephalic tegmentum is poorly developed in agnathans. The nucleus of the medial longitudinal fascicle in the subpretectal tegmentum has ipsilateral descending projections to the spinal cord. The huge M1 and M2 Múller cells of lampreys, previously included in the mesencephalic reticular nucleus, are now considered to lie within the subpretectal tegmentum. The agnathan rostral (p2/p3) diencephalic basal plate is formed by the nucleus of the tuberculum posterior, where some catecholaminergic cells with ascending telencephalic projections are located. In fact, the dopaminergic striatal innervation in lampreys originates in this region.

Secondary Prosencephalon

The alar plate of the secondary prosencephalon contains the telencephalon, with the preoptic area included in the molecularly defined subpallium, and part of the hypothalamus, whereas the remaining parts of the hypothalamus constitute its basal plate component.

The agnathan telencephalon comprises two well-developed olfactory bulbs, two relatively large cerebral hemispheres (more prominent in hagfishes), and a nonevaginated portion (**Figure 5**). The two latter parts are subdivided into pallial and subpallial regions. In the evaginated portion, the olfactory lobe is externally demarcated from the telencephalic hemisphere by a circular fissura. In lampreys, the telencephalic ventricle expands laterally from the third ventricle with which it is connected through the interventricular foramen; then, it divides into two portions (Y-shaped cavity): a rostral portion that continues forward into the olfactory bulb (forming its ventricle), and a caudal portion that extends backward into the telencephalic hemisphere (thus corresponding to the lateral ventricle). In hagfishes, the rostral ventricular system has two or three (preoptic, infundibular, and subhabenular) isolated cavities, depending on the species and partial obliteration during development.

The agnathans median, unpaired olfactory organ, located in an internal duct that opens to the exterior by a single nostril, is an exceptional feature among craniates. Paired olfactory nerves enter the olfactory bulb in lampreys, but they are numerous (approximately ten per side) in hagfishes. The proportionately fairly large olfactory bulbs of agnathans are sessile and are interconnected by an interbulbar commissure. The following cytoarchitectonic layers can be discerned in lampreys: a superficial fiber layer with primary olfactory afferents that ramify in relation to the terminals of the mitral cells inside the olfactory glomeruli of the glomerular layer. Close to the glomeruli, there is a noncontinuous row of large mitral cells. Internally, there is a thick granular layer composed of intermingled medium-sized and smaller cells. In hagfishes, the superficial primary olfactory afferents terminate within the deeper glomeruli. Numerous periglomerular cells, including some glomerular cells, invade the glomerular layer. Below, a cell-poor molecular layer is followed caudally by a region of large mitral cells that merges with the internal granular layer. In addition, primary fibers from the accessory olfactory organ project to the ventromedial part of the lamprey olfactory bulb, whereas a dorsal accessory olfactory bulb has been suggested in hagfishes.

The pallial region in lampreys is classically subdivided into four zones without general agreement regarding their correspondence with those identified in other vertebrates. Some authors recognize a lateral pallium, including a primordium pallii dorsalis and a primordium piriforme, a subhipocampal lobe, and a primordium hippocampi. Others identify a lateral pallium, including dorsal and ventral parts, a dorsal pallium, and a medial pallium. Finally, the unevaginated dorso-medial portion has been variously regarded as part of the pallium, thus representing the hippocampal primordium, or as part of the diencephalic prethalamic eminentia (**Figure 5**(**a**)). The pallium of hagfishes is very large, consists of five layers, and is highly differentiated from a cytoarchitectural point of view (**Figure 5**(**b**)). In addition to abundant olfactory inputs, it possesses reciprocal connections with the thalamus; however, no direct correspondence has been established with the pallial subdivisions of gnathostomes.

The agnathan subpallium contains a medial septum, a striatum, and a preoptic region. A putative pallidum has been recognized lateroventral to the striatum. The lamprey striatum (**Figures 5**(**c**) and **5**(**d**)) is basically constituted by a cell layer, which is partially evaginated, and a prominent periventricular neuropil. The hagfish subpallium is poorly differentiated and contains an unpaired septum and a putative striatal region associated with the lateral forebrain bundle. The lamprey preoptic area is constituted by a compact layer of cells located close to the surface of the ventricle. As in hagfishes, it consists of several nuclei. The optic chiasm, where some retinopetal and most of retinofugal axons cross the midline, is larger in lampreys. Slightly caudally to it, there is a conspicuous postoptic commissure.

The hypothalamus, which is classically considered as the basal plate of the diencephalon, is currently interpreted as the ventral portion (including the whole basal and part of the alar pate) of the secondary prosencephalon. This region is slightly differentiated in hagfishes, where the infundibular part of the third

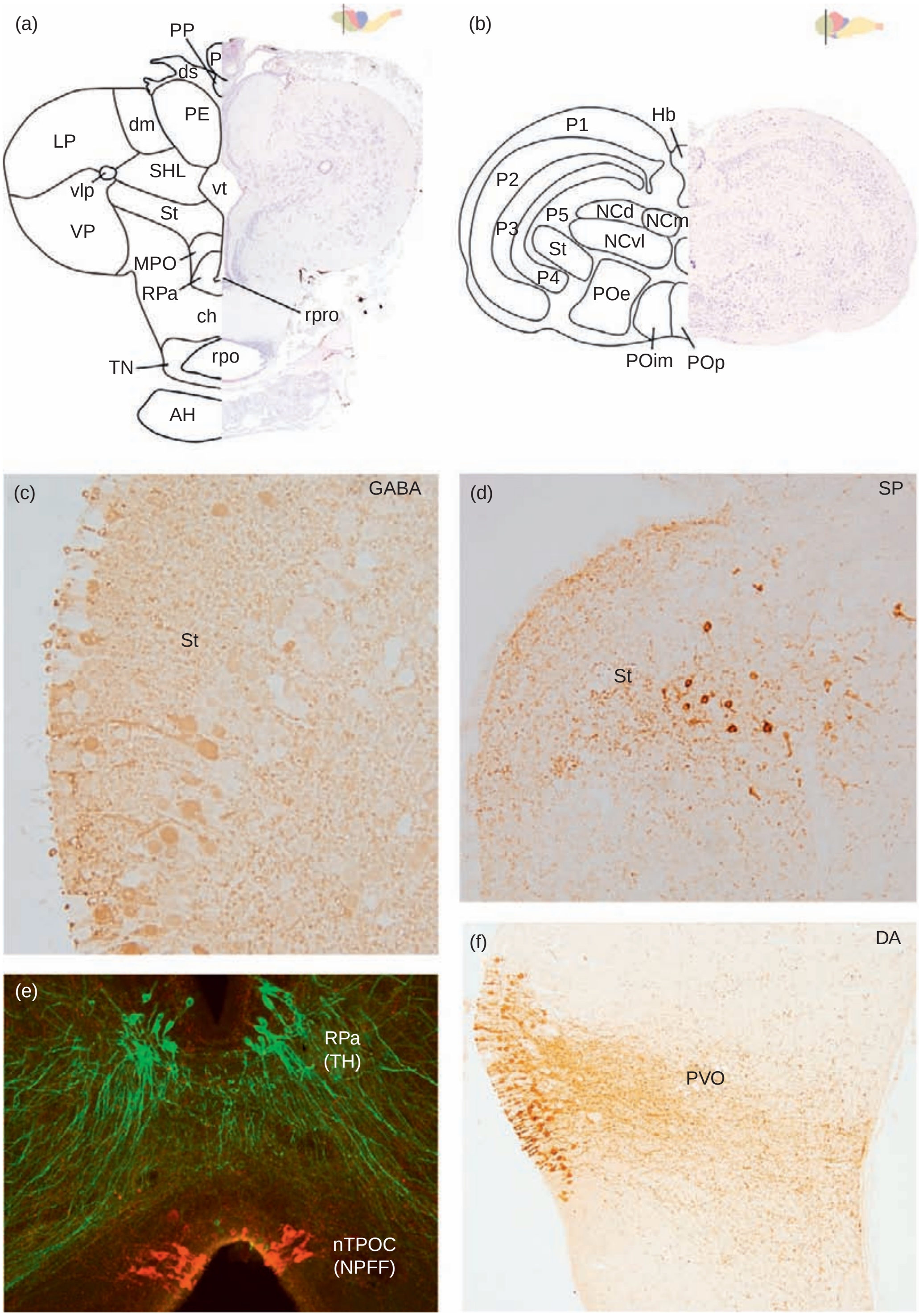


Figure 5 Representative transverse sections through the forebrain (secondary prosencephalon) of *L. fluviatilis* (a) and *M. glutinosa* (b). Hematoxylin-stained sections are shown in the right side; the identified areas and nuclei (solid lines), and major tracts (dashed lines) are shown in the left side. Gamma aminobutyric acid (GABA) (c) and substance P (SP) (d) immunoreactive cells in transverse sections through the lamprey striatum (St). (e) Tyrosine hydroxylase (TH) (green) and neuropeptide FF (NPFF) (red) immunoreactive cells in a transverse section through the lamprey hypothalamus. (f) Dopaminergic cerebrospinal fluid-contacting cells in a transverse section through the periventricular hypothalamic organ (PVO). AH, adenohypophysis; ch, optic chiasma; DA, dopamine; dm, dorsomedial telencephalic neuropil; ds, dorsal sac; Hb, habenula; LP, lateral pallium; MPO, medial preoptic nucleus; NCd, dorsal nucleus of the central prosencephalic nucleus; NCm, medial nucleus of the central prosencephalic nucleus; NCvl, ventrolateral nucleus of the central prosencephalic nucleus; nTPOC, nucleus of the tract of the postoptic commissure; P, pineal organ; P1–P5, pallial layers 1–5; PE, prethalamic eminence; POe, external nucleus of the preoptic area; POim, intermediate nucleus of the preoptic area; POp, periventricular nucleus of the preoptic area; PP, parapineal organ; RPa, rostral paraventricular area; rpo, postoptic recess; rpro, preoptic recess; SHL, subhippocampal lobe; TN, tuberal nucleus; vlp, posterior lateral telencephalic ventricle; VP, ventral pallium; vt, impar telencephalic ventricle.

ventricle has a medial hypophyseal recess and two caudal and lateral recesses. Cytoarchitectonically, some cerebrospinal fluid-contacting (CSF-c) neurons are present in the preoptic and infundibular ventricular cavities, as well as a lateral migrated infundibular hypothalamic nucleus. In lampreys, there are many subventricular CSF-c neurons covering most of the infundibular walls and the mammillary recess (**Figures 5**(**e**) and **5**(**f**)). Some of these cells are present in the neurosecretory nuclei of the alar hypothalamus that projects to the neurohypophysis. The periventricular hypothalamic organ also possesses numerous CSF-c cells that are immunoreactive to dopamine, serotonin, and other substances. Some other nuclei were tentatively identified in the basal hypothalamus, of which we emphasized the nucleus of the tract of the postoptic commissure, the tuberal nucleus, and the mammillary region. The most ventral part of the secondary prosencephalon is the neurohypophysis. An interesting feature of the agnathan hypothalamus is that there is no saccus vasculosus.

See also: **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Functional Morphology of the Brains of Cartilaginous Fishes; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria; Motor Control Systems of Fishes. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Energetics and Development.

Further Reading

Braun CB (1996) The sensory biology of the living jawless fishes: A phylogenetic assessment. *Brain Behavior and Evolution* 48: 262–276.

Butler AB and Hodos W (2005) *Comparative Vertebrate Neuroanatomy. Evolution and Adaptation*, 2nd edn. New York: Wiley-Liss.

Fritzsch B and Northcutt RG (1993) Cranial and spinal nerve organization in amphioxus and lampreys: Evidence for an ancestral craniate pattern. *Acta Anatomica (Basel)* 148: 96–109.

Nieuwenhuys R and Nicholson C (1998) Lampreys, Petromyzontoidea. In: Nieuwenhuys R, ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates,* vol. 1, pp. 397–495. Berlin: Springer.

Osorio J and Retaux S (2008) The lamprey in evolutionary studies. *Developmental Genes and Evolution* 218: 221–235.

Pombal MA, El Manira A, and Grillner S (1997) Afferents of the lamprey striatum with special reference to the dopaminergic system: A combined tracing and immunohistochemical study. *Journal of Comparative Neurology* 386: 71–91.

Pombal MA, Megías M, Bardet SM, and Puelles L (2009) New and old thoughts on the segmental organization of the forebrain in lampreys. *Brain Behavior and Evolution* 74: 7–19.

Pombal MA and Puelles L (1999) Prosomeric map of the lamprey forebrain based on calretinin immunocytochemistry, Nissl stain, and ancillary markers. *Journal of Comparative Neurology* 414: 391–422.

Ronan M and Northcutt RG (1998) The central nervous system of hagfishes. In: Jorgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 452–479. London: Chapman and Hall.

Weigle C and Northcutt RG (1999) The chemoarchitecture of the forebrain of lampreys: Evolutionary implications by comparisons with gnathostomes. *European Journal of Morphology* 37: 122–125.

Wicht H (1996) The brains of lampreys and hagfishes: Characteristics, characters, and comparisons. *Brain Behavior and Evolution* 48: 248–261.

Wicht H and Nieuwenhuys R (1998) Hagfishes (Myxinoidea). In: Nieuwenhuys R, ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates,* vol. 1, pp. 497–549. Berlin: Springer.

Functional Morphology of the Brains of Cartilaginous Fishes

I Rodríguez-Moldes, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

© 2011 Elsevier Inc. All rights reserved.

Introduction
Hindbrain
Midbrain
Forebrain
Further Reading

Glossary

Amniotes Vertebrates whose embryonic development takes place in a fluid-filled sac called amnion that is responsible for keeping the embryo moist. Reptiles, birds, and mammals are amniotes.

Anamniotes Vertebrates that do not form an amnion in the embryonic stage. Fishes and amphibians are anamniotes.

Brainstem A variably defined term that includes the brain regions where most cranial nerves have their origin and/or termination, i.e., the hindbrain and midbrain. Alternatively, the base of the brain extending forward from the spinal cord and including the medulla oblongata and midbrain.

Cerebrospinal fluid (CSF)-contacting neurons Periventricular neurons that contact the CSF by a dendritic process ending in a bulbous protrusion bearing sensory-type cilia.

Circumventricular organs Specialized ventricular regions that contain CSF-contacting neurons among the glial cells. In these regions, the blood–brain barrier (i.e., the cellular barrier that restricts the passage of substances between the blood and the neuronal tissue) is incomplete.

Gnathostomes Vertebrates with true jaws (cartilaginous fish, bony fish, and tetrapods).

Neuromeres Segments or transversal bands of the developing neural tube exhibiting specific properties, such as prosomeres in the forebrain (prosencephalon) or rhombomeres in the hindbrain (rhombencephalon).

Vertebrates As used here, a synonym of craniates, the clade that includes agnathans (hagfishes and lampreys) and gnathostomes; alternately defined by some as excluding hagfishes, reserving the term craniates for all agnathans and jawed vertebrates.

Introduction

Cartilaginous fishes (chondrichthyans) represent an ancient radiation of the gnathostome vertebrates characterized by having calcified cartilage but little or no bone internally, and rarely any in their scales. This group includes sharks, skates, and rays (Elasmobranchs), and chimaeras (Holocephali). Skates and rays show characteristic flat bodies and are grouped together as batoids. Most sharks have a streamlined body and are divided into two groups, squalomorphs (the most primitive group of living sharks) and galeomorphs (includes most of living sharks – advanced sharks), while sharks with flattened bodies are squatinomorphs or angel sharks. Chondrichthyans are currently considered the sister group of the Teleostomi, that is, the group of gnathostomes with a bony skeleton that gave rise to land vertebrates, including mammals. This position as an out-group to all other living jawed vertebrates makes chondrichthyans essential in assessing the ancestral vertebrate condition of brain organization. However, this does not mean they have a more primitive or unspecialized brain. On the contrary, they have well-developed, large brains whose size is comparable to those of birds and mammals with equivalent body weights, and they also have relatively larger brain sizes than the other major taxa of fishes, agnathans (**see also Brain and Nervous System**: Functional Morphology of the Brains of Agnathans) and ray-finned fishes (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes), as well as the sarcopterygian fishes (**see also Brain and Nervous System**: Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria).

Differences are noted among chondrichthyans regarding the organization of mature/differentiated neuronal populations with respect to the periventricular matrix. Squatinomorph and squalomorph sharks, together with holocephala, have laminar (type I) brains, a pattern of brain organization characterized by the location of most of the neuronal cell bodies close to the region where they originate, the periventricular region (most neurons remain periventricular, i.e., close to the place along the ventricular surface where they proliferated), which implies the scarce migration of these neurons. In contrast, galeomorph sharks, skates, and rays (similar to those of teleosts and amniotes) show elaborated (type II) brains, that is, brains in which the neuronal cell bodies migrate away from the periventricular matrix during development and form more or less distinct cell groups.

During the early development of the elasmobranch brain, as in other vertebrates, two prominent external constrictions divide the brain into three main vesicles related to the basic structure of the adult brain, which represent the fundamental anteroposterior (i.e., rostrocaudal) vertebrate subdivisions: hindbrain (or rhombencephalon), midbrain (or mesencephalon), and forebrain (or prosencephalon) (**Figure 1**). These brain vesicles become subdivided into more or less evident transverse bands called neuromeres, which have fixed relations with structures of the head and the peripheral nervous system, being very useful as reference points in comparative studies. Simultaneously with the formation of transverse bands, four longitudinal zones with clear morphological and functional identity are seen along the brain axis. The hindbrain presents four longitudinal columns: ventral and ventrolateral columns (together forming the basal plate), and dorsolateral and dorsal columns (together forming the alar plate), which represent four functional longitudinal subzones: alar-plate-derived somato- and viscerosensory columns and basal-plate-derived viscero- and somatomotor columns, as prototypically present in the spinal cord. The ventral column does not extend rostrally beyond the midbrain, and the dorsal column is limited to the hindbrain. Bearing in mind the developmental patterns in longitudinal bands and transverse subunits, the anatomical structure of the adults may be classified not only according to the longitudinal bands but also in terms of their segmentary units. The hindbrain and midbrain tegmenti, the area of the nucleus of the medial longitudinal fascicle, the posterior tubercle, and the hypothalamus have been considered to be derived from the ventral and ventrolateral columns. In contrast, the cerebellum, optic tectum, pretectal area, thalamus (also called dorsal thalamus), prethalamus (or ventral thalamus), preoptic region, and telencephalon have been considered to derive from the dorsolateral and dorsal columns. The understanding of the segmentation of the brain has become essential for comparative neuroanatomical studies. Two flexures, the cervical between the spinal cord and hindbrain and the cephalic between midbrain and forebrain, result in an early bending of the anteroposterior axis and thus distort some anatomical relationships as development proceeds.

Hindbrain

The organization of the hindbrain is very similar in all elasmobranchs except for the existence of well-developed electric lobes in electric rays, most of them members of the genus *Torpedo* (**Figure 2**). These lobes contain electromotoneurons whose axons innervate the electric organs located in the head disk. These axons form electromotor nerves, which are special branches of the facial, glossopharyngeal, and vagal nerves (**see also Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern). Neurons within a nucleus located in the hindbrain tegmentum that is called the 'oval nucleus' are responsible for the rhythmic discharge of the electric lobes and electric organ.

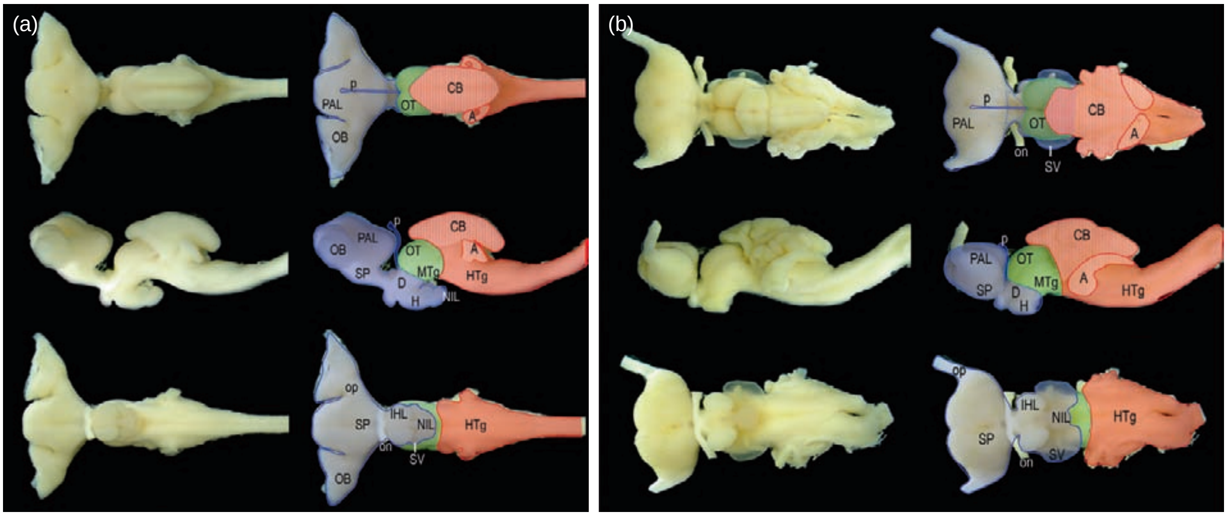


Figure 1 From top to bottom, dorsal, lateral, and ventral views of the brain of the small shark *Scyliorhinus canicula* (a) and of the skate *Raja undulata* (b). Right column shows in color the extension of hindbrain (red), midbrain (green), and forebrain (blue). For abbreviations, see **Figure 2**.

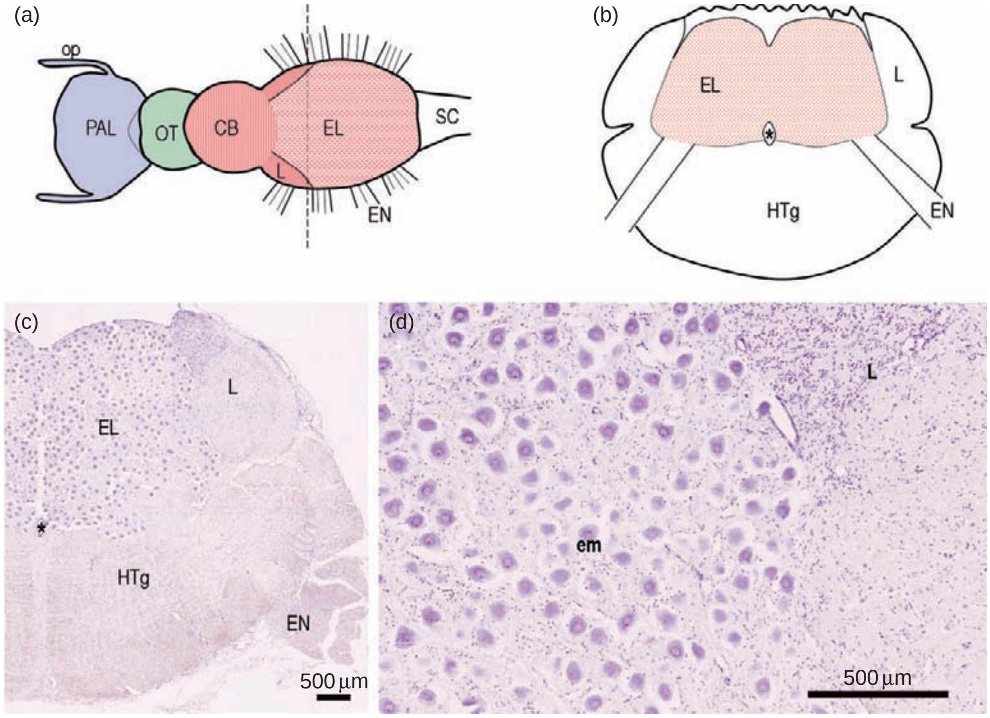


Figure 2 Electric lobes of the electric ray *Torpedo marmorata*. (a) Drawing of a dorsal view; and (b) drawing of a transverse section at the level indicated by the dashed line in (a). (c, d) Panoramic and detail of a transverse section stained with hematoxilin–eosin. Asterisk, fourth ventricle; A, auricles; Ad, adenohypophysis; asb, area superficialis basalis; CB, cerebellar body; cbcr, cerebellar crest; D, diencephalon; DON, dorsal octavolateral nucleus; EL, electric lobes; em, electromotoneurons; EN, electric nerves; GR, granular layer (eminences) of the cerebellum; H, hypothalamus; HTg, hindbrain tegmentum; i, infundibulum; IHL, inferior hypothalamic lobes; L, lateral line lobe; LAL, lower auricular leaf; lln, lateral line nerve; LP, lateral pallium; MOL, molecular layer; MON, medial octavolateral nucleus; MTg, midbrain tegmentum; NIL, neurointermediate lobe; OB, olfactory bulb; OLA, octavolateral area; on, optic nerve; op, olfactory peduncle; OT, optic tectum; p, pineal organ; PAL, pallium; PL, Purkinje cell layer; PTu, posterior tubercle; PVO, paraventricular organ; PZ, proliferating zone; SC, spinal cord; SP, subpallium; Str, striatum; SV, saccus vasculosus; Sy, synencephalon; tc, tela choroidea; Th, thalamus; UAL, upper auricular leaf; VIIIn, octaval nerve.

The caudal limit of the hindbrain is in a transition area corresponding to the obex (the point where the spinal canal ends and the hindbrain ventricle begins), but a clear landmark is not recognized. The rostral limit is the isthmus, the hindbrain region with the smallest ventricle which forms the boundary between hindbrain and midbrain. The roof of the ventricular cavity of the hindbrain (the fourth ventricle) is covered caudally by the choroid plexus, a thin membranous structure, and rostrally by the cerebellum. The ventral parts of the hindbrain derive from the basal plate and contain efferent ('motor') centers (**see also Brain and Nervous System**: Motor Control Systems of Fish and Physiology of the Mauthner Cell: Discovery and Properties), whereas the lateral walls of the ventricle are made up of the alar plate and contain afferent ('sensory') centers. A conspicuous ventricular groove called the sulcus limitans of His (a nineteenth-century embryologist and neuroanatomist) is currently considered the landmark between the basal and alar plates. Visceral and somatic areas are recognized both in alar and basal plates, which appear, to some extent, as longitudinal columns (somatomotor, visceromotor, viscerosensory, and somatosensory).

The basal plate contains a ventral (medial) area with somatomotor centers and an intermedioventral (lateral) area where visceromotor centers are located. In addition, during development, prominent ventricular bulges and sulci divide the hindbrain into transverse segmental units called rhombomeres. In adults, the rhombomeres are no longer visible, but the segmental disposition remains in some hindbrain centers as the motor nuclei and reticular formation. The ventral (medial) area (somatomotor) region contains the fasciculus longitudinalis medialis, a large fiber bundle which carries fibers that connect anterior and posterior regions of the brain. It is one of the earliest longitudinal pathways that appear during development. Caudally in the ventral (medial) area, the neurons of the spino-occipital nerves (i.e., the hypoglossal nerve homologs) form a continuous column with the motoneurons of the ventral horn of the spinal cord (spinal motor column). The motoneurons of the abducens nerve (somatomotor) occupy an intermediate medial location in the ventral (medial) area of the hindbrain (**see also Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern).

Also related to somatomotor function is the reticular formation, a set of scattered cell populations that forms superior and inferior groups, each containing several subgroups located medially (raphe groups) and laterally (reticular groups). Large neurons of the reticular formation project to somatomotor centers in the spinal cord via the fasciculus longitudinalis medialis. The cholinergic nature of these large reticulospinal cells has been reported. Cells of the reticular formation also project to the cerebellum and tectum mesencephali and receive inputs from these regions as well as from the spinal cord and the telencephalon. The superior and inferior groups are organized in an apparent segmental pattern in relation to the segmental disposition of the hindbrain neuromeres (called rhombomeres). The reticular formation contains abundant serotoninergic cells and, to a lesser extent, enkephalinergic, catecholaminergic, and cholinergic cells. The serotoninergic cells arise close to the floor plate, and most populations migrate ventrally and mediolaterally to organize distinct medial (raphe) and laterally migrated (reticular) groups. The ventral (medial) area is also occupied by nuclei/centers not related to somatomotor function, such as the inferior olive, which projects to the cerebellum. At rostral levels, the ventral (medial) area continues with the medial parts of the isthmic, midbrain, and synencephalic tegmenti (basal plate). The intermedioventral area contains the visceromotor column formed by the motor nuclei of the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) nerves. While traditionally designated as 'visceral' in classification, it should be noted that the muscles of the head that are innervated by components of V, VII, IX, and X are in fact striated somatic muscles, derived from the same embryological source (the epimere component of the mesoderm) as the external eye and tongue muscles, which are correctly classified as somatic.

In the alar plate, two longitudinally arranged cell zones that form elongated intraventricular protrusions can be recognized, the area intermediodorsalis and the area dorsalis. The area intermediodorsalis (viscerosensory zone) is the center of termination for visceral afferent fibers which enter the brain by means of the nerves VII, IX, and X. This center of fiber termination forms a longitudinal protrusion of the lateral wall of the fourth ventricle called the viscerosensory column or vagal lobe. In cartilaginous fishes, there is no clear separation between nuclei specific for taste or general visceral sensibility, as in other fishes. Some of the primary viscerosensory fibers do not terminate in the lobe but course caudally forming the fasciculus solitarius. The general somatosensory nucleus of the trigeminal nerve is found in the superficial part of the intermediodorsal zone. The area intermediodorsalis contains centers which are not related to viscerosensory function, such as the nucleus octavomagnocellularis, a special somatosensory center that receives direct afferents fibers of the octaval nerve (for hearing and vestibular senses). This nucleus octavomagnocellularis contains efferent cells that project directly to somatic efferent centers in the spinal cord. These efferent cells of the octavolateral system are located near the fourth ventricle associated with the median fascicle of Stieda (a nineteenth-century German neuroanatomist).

The area dorsalis is entirely occupied by two large lateral line centers that are unique to chondrichthyans. They are cerebellar-like structures that form two longitudinal nuclear columns: the dorsal and medial octavolateral nuclei that receive primary afferents from the lateral line nerves and may be designated as a special somatosensory zone. The two longitudinal nuclear columns are covered by a thick layer called the cerebellar crest, because it resembles the molecular layer of the cerebellum. Actually, the cerebellar crest is continuous over the two longitudinal columns and extends rostrally toward the cerebellar auricles, which also are continuous with the molecular layer of the cerebellar body. Characteristically, the cerebellar crest contains large cells with spiny dendrites that are called Purkinje-like cells. These spiny dendrites are contacted by a large number of parallel fibers that originate in the granule cells of the auricles (see below).

The isthmic tegmentum contains the motoneurons of the somatomotor nucleus of the trochlear (IV) nerve, which innervate the extrinsic superior oblique muscle of the eye, and other centers not related to somatomotor function, such as the interpeduncular nucleus and the griseum centralis whose efferent connections are unknown.

Cerebellum

The cerebellum is an evolutionary innovation of gnathostomes that is unambiguously recognized in chondrichthyans. The cerebellum of these fishes contains the same kind of elements as the mammalian cerebellum and has similar interactions, being also involved in the modulation of motor control. It has four types of neurons (Purkinje cells, granule cells, Golgi cells, and stellate cells), and it exhibits the basic cerebellar circuitry typical of vertebrates. Its large cavity represents an expansion of the fourth ventricle. It is relatively larger than that of most teleosts and has been related to the need of coordination of more complex movements during swimming. The elasmobranch cerebellum is formed by a cerebellar body and the auricles (**Figure 3**). The cerebellar body is well developed and in squalomorphs is smooth, without fissures, but in batoids and galeomorphs it varies from slight to considerably convoluted, respectively (**Figure 1**). The

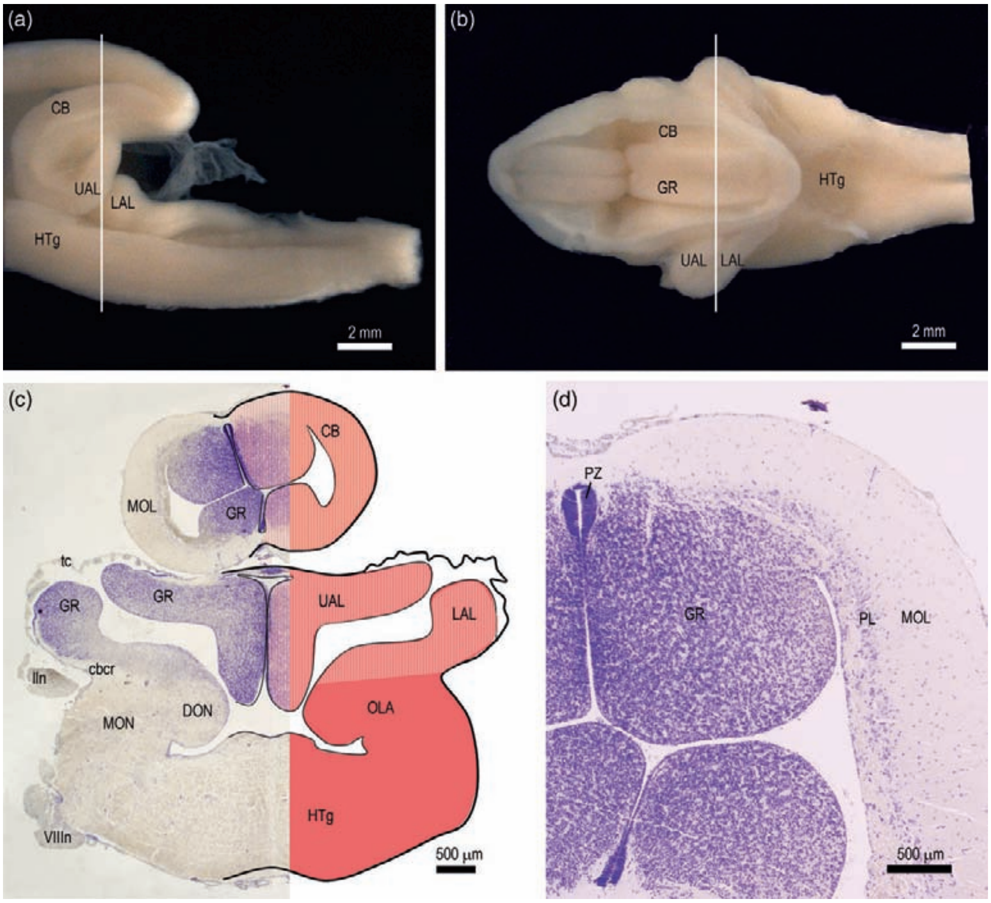


Figure 3 Hindbrain of the galeomorph shark *Scyliorhinus canicula* sectioned longitudinally (a), horizontally (b), and transversally (c) at the level indicated by the white line in (a) and (b); the dorsal part of the cerebellar body has been removed in (b). (d) Detail of the cerebellar body; (c) (left part) and (d) are hematoxilin–eosin stained sections. For abbreviations, see **Figure 2**.

auricles are paired and form large ear-shaped lobes closely related to the lateral line centers, which have a cerebellar-like organization. The basal part of the cerebellar body forms a distinct stalk, the peduncle, which joins rostrally with the tectum of the midbrain by the velum medullare anterius and caudally with the auricles. A cerebellar nucleus located within the cerebellar peduncle is considered the equivalent to the deep cerebellar nuclei of mammals.

The cerebellar body is a well-recognized cortical structure, because there is a clear cellular segregation that allows distinguishing the outer molecular layer, which contains stellate cells, the layer of Purkinje cells, and the deep layer that contains granule cells. In elasmobranchs, this granular layer forms two characteristic paramedian longitudinal ridges called the granular eminences, which protrude into the ventricle of the cerebellar body. During the cerebellar development, a granule cell layer is recognized at the lateral walls of the cerebellar body, as in other vertebrates, but in adults these cells are congregated in the paramedian eminences. Besides granule cells, there are other types of neurons in the granular eminences, such as the Golgi cells that are similar to those of mammals but simpler in their morphology. In the cerebellum of sharks, the proliferative activity remains active during the whole lifespan. This proliferative activity in adult sharks is located in a ventricular zone located medially within the longitudinal granular eminences.

The auricular lobes are covered by the tela choroidea of the fourth ventricle and are formed by upper and lower leaves. The lower leaf is contiguous caudally with the area octavolateralis, while the upper leaves unite medially to bridge the fourth ventricle. These regions differ in their internal organization and connections. The lower lip shows a typical cerebellar organization, with the granule cells concentrated in the paramedian eminences that are continuous with those of the cerebellar body and with the granule cells of the upper leaves. The lateral portion of the upper leaf is composed exclusively of granule cells and perhaps Golgi cells, and it is not covered by a molecular layer. Only a few Purkinje cells are found in the lower leaf, but in the lower lip they form a well-defined layer.

Midbrain

The alar plate of the midbrain is strongly developed and it consists of a pair of bilateral hemispherical lobes that enclose the midbrain ventricle (**Figures 4(a)–4(c)**), which is largely reduced in batoids. It is named optic tectum because it is the main center of termination for retinofugal fibers and the primary center for the perception of vision, but it also receives fibers from centers in the telencephalon (dorsal pallium), diencephalon (pretectum, thalamus, and prethalamus), hindbrain (octavolateral and vestibular areas), and spinal cord. There are tectal efferent pathways ascending to the pretectum and thalamus and descending to the reticular formation. The organization pattern of the elasmobranch retinal projections displays a high degree of complexity similar to that found in some actinopterygians and amniote vertebrates. It is thought that the evolution of the primary visual system in cartilaginous fishes has occurred due to architectonic modifications of the projection zones related to an increase of neuronal density rather than an increase in the number of projection zones. The tectum is organized in at least five layers or strata, distinguished by the different distribution of cells and fibers (**Figures 4(b)** and **4(c)**). The superficial layers are the

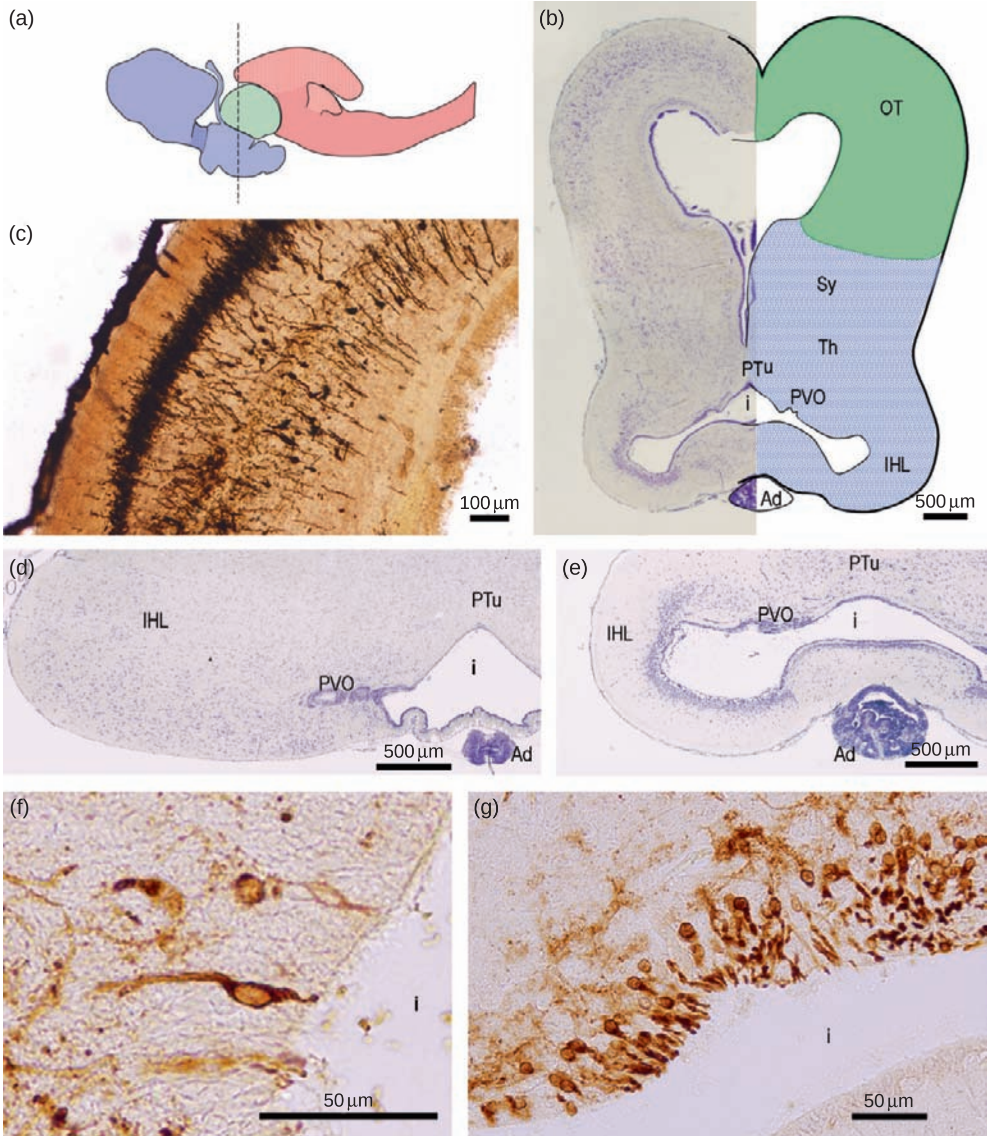


Figure 4 Panoramic view (b) and details of transverse sections through the midbrain and caudal forebrain at the level indicated by the dashed line in (a). (c) Optic tectum of *S. canicula* with some neurons labeled with the Golgi method at different tectal layers; (d, e) inferior hypothalamic lobes of the skate *Raya undulata* (d) and of the shark *S. canicula* (e). (f, g) Details of the paraventricular organ of *Raya undulata* (f) and *S. canicula* (g) with catecholaminergic (f) and serotoninergic (g) cerebrospinal fluid (CSF)-contacting neurons. For abbreviations, see **Figure 2**.

main recipients of visual inputs, whereas the deep tectal layers receive nonvisual information. Tectal neurons not only respond to light but also to mechanical and acoustic stimuli, as well as to electric currents. Retinotectal fibers and electrosense are spatiotopically organized. Ventral to the optic tectum, but also derived from the midbrain alar plate, are several nuclei that correspond to the torus semicircularis of other anamniotes; they receive ascending auditory and lateral-line inputs.

In the elasmobranch tectum, there is a band of the large primary sensory neurons that form the mesencephalic trigeminal nucleus. These cells form a strip in the periventricular region of the tectum. Ventral to the tectum is the midbrain tegmentum, which is continuous caudally with the hindbrain tegmentum. The midbrain tegmentum does not exhibit a clear zonal pattern, but there is a motor zone containing the neurons that innervate the extrinsic muscles of the eye, that is, the nucleus of the oculomotor (III) nerve (somatomotor). This motor nucleus is located in the medial part of the tegmentum. Also at a medial level, but located more dorsally, is the Edinger-Westphal nucleus, which is the rostral-most component of the visceromotor column; it is the origin of the cholinergic visceromotor fibers that course along nerve III to innervate the iris and ciliary body of the eye. In contrast to the situation in bony fish, two distinct cell populations have been recognized in the midbrain tegmentum of batoids and sharks, a dense medial group (ventral tegmental area) and a scarcer lateral one (substantia nigra), with neurons that contain the catecholaminergic neurotransmitter dopamine. These cell masses extend rostrally along the basal part of the caudal diencephalon (see below).

Forebrain

The forebrain is divided into diencephalon and a secondary prosencephalon that includes the hypothalamus, preoptic region, and telencephalon.

Diencephalon

Along the diencephalon, the limit between alar and basal territories is less conspicuous than in the midbrain and hindbrain, and some transverse boundaries are also recognizable. The exit of the oculomotor nerve sets the ventral limit between midbrain and forebrain. Dorsally, the midbrain is separated from the forebrain by a constriction at the level of posterior commissure. The cell masses of the diencephalon have a more diffuse arrangement and contain neurons of more uniform size than those of the brainstem. In the alar plate, the epithalamus (which comprises the habenula and the epiphysis or pineal organ), the thalamus (also called dorsal thalamus), and the prethalamus (or ventral thalamus) are distinguished. The most caudal segment of the diencephalon is called the synencephalon. Its dorsal/alar part is occupied by the pretectum, which encloses a retinorecipient center, the pretectal nucleus. It lies lateral to the posterior commissure.

The pineal organ is well developed in elasmobranchs, although it is lacking in some electric rays. It is a tubular structure that opens into the third ventricle between the habenular and posterior commissures. Its distal part remains under a dorsal window of the cartilaginous skull that covers the telencephalon. It consists of receptor cells, supporting cells, and ganglion cells. GABAergic intrinsic neurons and an extensive GABAergic innervation have been observed in the pineal of some elasmobranchs. The ganglion cells give rise to the pineal tract that courses to the thalamus, pretectal area, posterior tubercle, and midbrain tegmentum. The pineal organ also receives afferent fibers. Pineal neural circuits are involved in the photic control of brain functions. Similarly to retinal photoreceptors, the pineal receptor (sensory) cells have well-developed outer segments related to the photoreceptive capacity of the organ. These pineal photoreceptors are cholinergic, which appears to be a primitive feature of vertebrates. A parapineal organ similar to that observed in other fishes has not been observed in elasmobranchs.

The habenular nuclei are well developed in all chondrichthyans. There is a marked asymmetry as for their size, with the left habenular ganglion being larger than the right one. There are also considerable differences in the arrangement of the neurons and in their most important efferent connection, the fasciculus retroflexus, which terminates in the interpeduncular nucleus. The right and left bundles are asymmetrically developed, with the left bundle containing more myelinated fibers. The afferent pathway to the habenula is the stria medullaris, which carries fibers of telencephalic origin. In addition, afferent connections from tectal and tegmental midbrain regions have been reported.

According to the now widely accepted concept of segmentary subdivision of the caudal forebrain in transverse subunits or prosomeres, the thalamus and prethalamus represent transverse and not longitudinal elements along the anteroposterior brain axis. This means that the thalamus is caudal to the prethalamus and not dorsal. Both regions have a medial part with densely arranged cells, as well as a lateral part with larger scattered cells. The thalamus and prethalamus receive retinal inputs and ascending projections from the spinal cord, cerebellum, and tectum, and also project to the telencephalon. Thus, the thalamic and prethalamic regions represent relay centers for ascending sensory pathways to the telencephalon.

The basal part of the diencephalon is formed caudally by the tegmental part of the synencephalon, and rostrally by the posterior tubercle. The synencephalon contains the nucleus of the fasciculus longitudinalis medialis, which is related to the somatomotor coordinating apparatus. As in other fishes, many catecholaminergic cells occupy the posterior tubercle of elasmobranchs, but unlike cyclostomes and actinopterigyans, catecholaminergic cells also occupy the basal part of the caudal diencephalon and midbrain. It has been considered that this may represent the ancestral vertebrate condition even though these synencephalic/mesencephalic catecholaminergic cell groups are lacking in holocephala (the sister group of elasmobranchs).

Hypothalamus

The hypothalamus is a large forebrain region formed by the walls of the infundibular recess of the infundibulum, which is a ventrocaudal expansion of the third ventricle. Two main regions are distinguished: the medial or tuberal region, intimately related to the hypophysis (the pituitary gland), and the caudal area, which contains the broadest circumventricular organ of the elasmobranch brain. The tuberal hypothalamus contains the hypothalamic-hypophyseal tract along which the axons of classical neurosecretory system and other fibers of aminergic and peptidergic nature course. As in other fishes, the walls of the infundibular recess expand laterally to form the characteristic inferior hypothalamic lobes, related to the control of feeding behavior. In sharks, the walls of these lobes surround wide lateral expansions of the infundibular walls, but they are further reduced or even obliterated in batoids (**Figures 4(d)** and **4(e)**). The caudal hypothalamus is mostly occupied by functionally integrated structures that form two continuous circumventricular organs: the posterior recess organ, caudally, and its rostrolateral continuation, the paraventricular organ. The walls of both organs are characteristically folded and formed by a high density of cerebrospinal fluid (CSF)-contacting neurons of catecholaminergic, serotoninergic, and peptidergic nature (**Figures 4(f)** and **4(g)**). At the caudal hypothalamus is located the saccus vasculosus (SV), an enigmatic specialized circumventricular organ which is also observed in many bony fishes. It is a highly vascularized neuroepithelium formed by a specialized and exclusive cell type, the coronet cells (**Figure 5**). It also contains CSF-contacting neurons and supporting cells. Coronet cells are glial cells characterized by an apical crown of globule-tipped cilia protruding into the ventricle. They are considered responsible for the function(s) of the SV, which include pressure

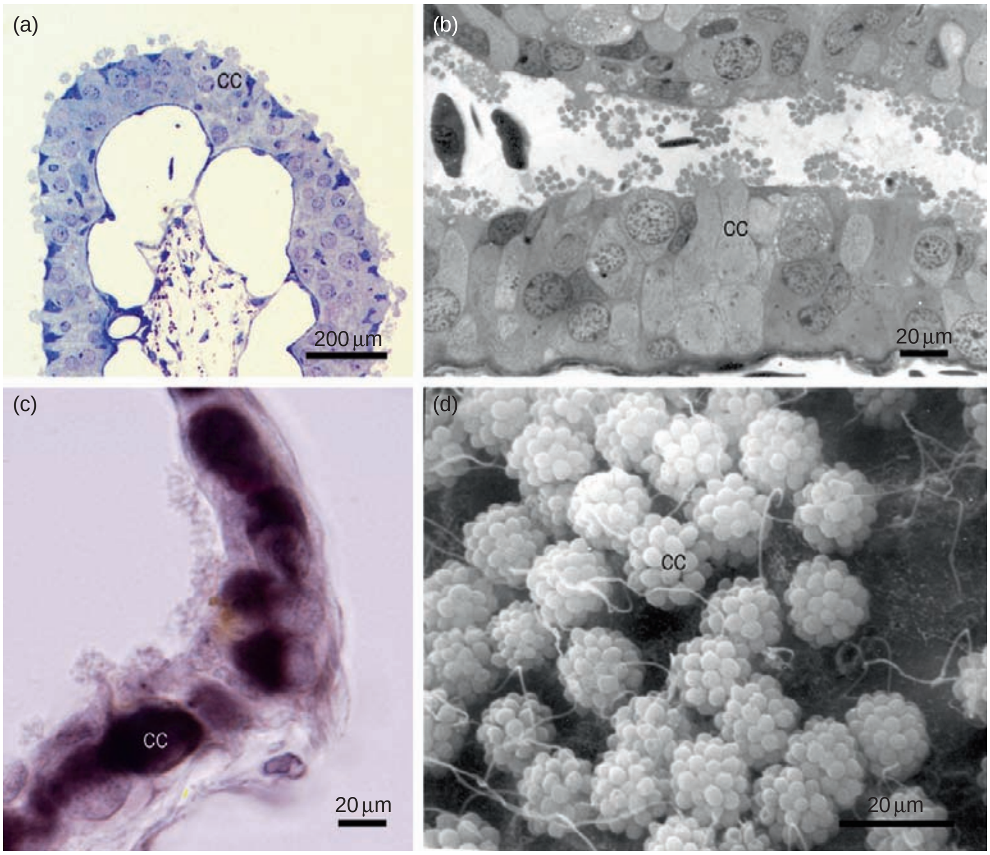


Figure 5 *Saccus vasculosus*. (a, b) Semithin (1-μm thick) sections stained with toluidine blue; (c) coronet cells labeled for immunohistochemistry against the glial fibrillary acidic protein (GFAP); (d) scanning electron microscopy of the ventricular surface of the *S. vasculosus* showing their characteristic crown of globules. CC, coronet cells.

reception, chemoreception, glucose loading, and ionic regulation of the CSF.

Preoptic Area

The preoptic area is the forebrain region that borders the preoptic recess, that is, a rostroventral expansion of the III ventricle. Morphologically and embryologically, it belongs to the telencephalon, but functionally it is part of the hypothalamus as it contains a neurosecretory nucleus, the magnocellular preoptic nucleus. The axons of this nucleus form a tract (the hypothalamus–hypophyseal tract) that courses along the hypothalamic floor to end in the neurointermediate lobe formed by the association of the hypothalamic neural lobe and the pars intermedia of the hypophysis. The magnocellular preoptic nucleus, the hypothalamus–hypophyseal tract, and the neurointermediate lobe form the classic neurosecretory system. Along its course, the neurosecretory tract shows large dilatations called Herring bodies. Extrahypophysial projections of the magnocellular preoptic nucleus have also been reported: some neurosecretory fibers ascend to the telencephalon while others descend to the spinal cord forming terminal fields in the midbrain tegmentum, isthmus, and the obex region. Besides the cells of the classical neurosecretory system, in the preoptic region there are also neurons containing neurotransmitters such as dopamine and serotonin and neuromodulators that are peptidergic in nature. Most of these cells are CSF-contacting neurons that form a circumventricular organ in the walls of the preoptic recess called the preoptic recess organ. Cells of this organ may regulate the adenohypophyseal functions as some of their axons terminate on portal capillaries that drain toward the adenophypophysis. This neurohemal structure is arranged in the region of the hypothalamic floor named the median eminence.

Telencephalon

The development of the telencephalon in elasmobranchs is very different from that in bony fishes (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes). In elasmobranchs, as in tetrapods, the telencephalon develops by evagination, a morphogenetic process which implies protrusion toward the outside and expansion of the lateral telencephalic walls. The telencephalon is subdivided into telencephalic hemispheres and olfactory bulbs. The telencephalic hemispheres are joined in the midline and share a common ventricle. Among elasmobranchs, the telencephalon differs in the shape and insertion of the olfactory bulb, in the length of olfactory peduncle, and in the extent of the ventricular cavities and hemispheric lobe fusion.

The telencephalic hemispheres are subdivided in a dorsal part, or pallium, and a ventral part, or subpallium, but the limit between both regions is not conspicuous (**Figure 6**). In the pallium, dorsal, medial, and lateral

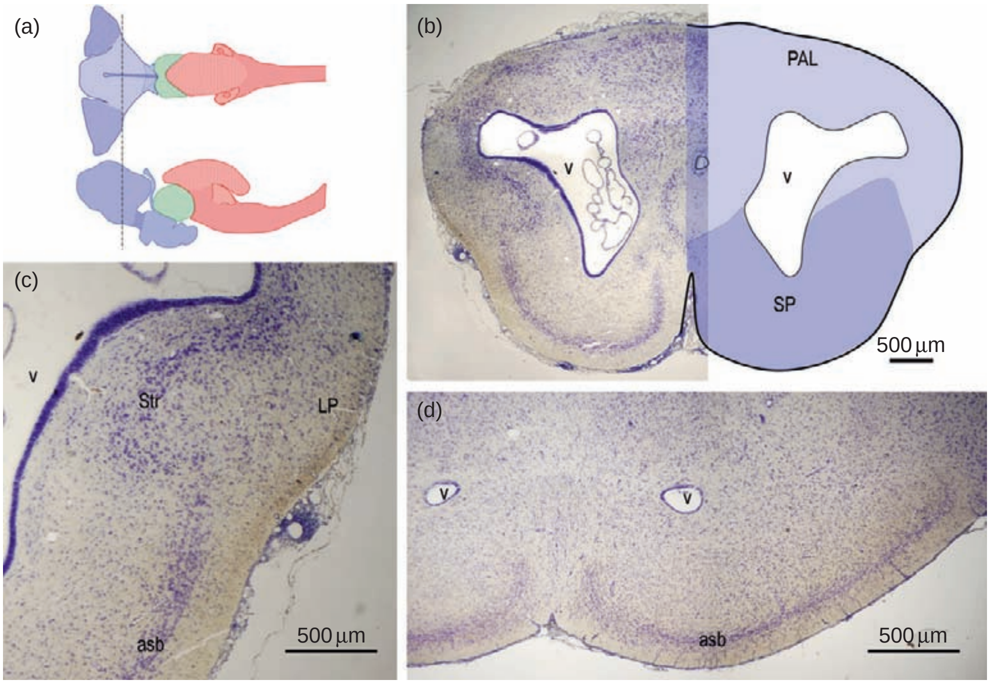


Figure 6 Panoramic view (b) and detail (c) of transverse sections through the telencephalon of *S. canicula* at the level indicated by the dashed line in (a). (d) Subpallium of *Raja undulata*. Note lower dispersion of the cell masses and higher extent of the ventricular cavity in shark (b, c) than in batoid (d). v, telencephalic ventricle. For other abbreviations, see **Figure 2**.

territories are distinguished. The dorsal pallium is a cortical structure that forms the roof of telencephalic hemispheres that presents a more or less stratified organization, with an external superficial part, a central part and an internal periventricular part. The medial pallium is considered the counterpart of the hippocampus of amniotes and it is recognized as the protrusion of the dorsomedial wall into the telencephalic ventricle. The lateral pallium, which receives the main olfactory input, lies dorsolateral to the area superficialis basalis. In batoids, these pallial subdivisions are hardly recognized because of the high dispersion of the cell masses (**Figure 6**). The subpallium is subdivided into a medial part that contains the septal nucleus, and a lateral part occupied by the area superficialis basalis, the striatum, and the area periventricularis ventrolateralis, structures that are related to the striatal and pallidal subdivisions of the basal ganglia of mammals. The area superficialis basalis is a region of densely packed neurons with a laminar organization that may be the equivalent to the pallidum of tetrapods. The striatum, located laterally, and the more medial area periventricularis ventrolateralis may represent the striatal subdivision, probably related to the striatum proper and the nucleus accumbens, respectively. These striatal subdivisions are not identified with certainty in batoids.

Olfactory bulbs are large, well-developed structures that enclose extensions of the lateral ventricles. Irrespective of their shape, four layers are distinguished: the layer of the primary olfactory fibers; the glomerular layer (formed by numerous glomeruli which contain arborizations of primary olfactory fibers), the layer of mitral cells, and the broad granular cell layer. Most of the neuronal elements found in the mammalian olfactory bulb are present in elasmobranchs, but, unlike in mammals, the mitral cells lack basal dendrites and the periglomerular cells are very scarce. In addition, the granular cells, as in lampreys, have axons that contribute to form secondary olfactory fibers together with those of the mitral cells. These structural distinctions imply differences in the circuitry and processing of the olfactory information with respect to mammals.

Classically, the elasmobranch telencephalon was considered simple in organization and exclusively involved in olfaction. This conception has been revised after the finding that the olfactory information reaches only to a restricted part of the telencephalon and that it also receives inputs from visual, auditory, mechanoreceptive, and electroreceptive centers.

Unlike the situation in bony fishes in which the lateral pallium projects directly to the dorsal and/or medial pallium, the lateral pallium of elasmobranchs projects to the subpallium (area superficialis basalis), and from there the information is sent to the dorsal pallium. The dorsomedial pallium of batoids, which receives a strong olfactory input, appears to be the most important relay center of information leaving the telencephalon. At least part of this projection reaches octavolateral centers, which means that olfactory information may reach centers with electrosensory and mechanosensory lateral-line information, perhaps used to detect prey in the substrate.

See also: **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Functional Morphology of the Brains of Agnathans; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria.

Further Reading

Anadón R, Molist P, Rodríguez-Moldes I, *et al.* (2000) Distribution of choline acetyltransferase (ChAT) immunoreactivity in the brain of an elasmobranch, the lesser-spotted dogfish (*Scyliorhinus canicula*). *Journal of Comparative Neurology* 420: 139–170.

Bell CC, Han V, and Sawtell NB (2008) Cerebellum-like structures and their implications for cerebellar function. *Annual Review of Neuroscience* 31: 1–24.

Bone Q and Moore RH (2008) *Biology of Fish*, 3rd edn. New York: Taylor and Francis.

Butler AB and Hodos W (2005) *Comparative Vertebrate Neuroanatomy. Evolution and Adaptation*, 2nd edn. New York: Wiley-Liss.

Carrera I, Molist P, Anadón R, and Rodríguez-Moldes I (2008) Development of the serotoninergic system in the central nervous system of the dogfish *Scyliorhinus canicula* (Elasmobranchs). An immunohistochemical study. *Journal of Comparative Neurology* 511: 804–831.

Coolen M, Menuet A, Chassoux D, *et al.* (2009) The dogfish *Scyliorhinus canicula*, a reference in jawed vertebrates. In: Behringer RR, Johnson AD, and Krumlauf RE (eds.) *Emerging Model Organisms. A Laboratory Manual,* vol. 1, pp. 431–446. New York: Cold Spring Harbor Laboratory Press.

Gilland E and Baker R (2005) Evolutionary patterns of cranial nerve efferent nuclei in vertebrates. *Brain, Behavior and Evolution* 66: 234–254.

Hofmann MH (1999) Nervous system. In: Hamlett WC (ed.) *Sharks, Skates and Rays. Biology of Elasmobranch Fishes*, pp. 273–299. Baltimore, MD: Johns Hopkins University Press.

Hofmann MH and Northcutt RG (2008) Organization of major telencephalic pathways in an elasmobranch, the thornback ray *Platyrhinoidis triseriata. Brain, Behavior and Evolution* 72: 307–325.

Lisney TJ, Yopak KE, Montgomery JC, and Collin SP (2008) Variation in brain organization and cerebellar foliation in chondrichthyans: Batoids. *Brain, Behavior and Evolution* 72: 262–282.

Northcutt RG (1977) Elasmobranch central nervous system organization and its possible evolutionary significance. *American Zoologist* 17: 411–429.

Northcutt RG (1978) Brain organization in the cartilaginous fishes. In: Hodgson ES and Mathewson RF (eds.) *Sensory Biology of Sharks, Skates, and Rays*, pp. 117–193. Arlington, VA: Office of Naval Research, Department of the Navy.

Northcutt RG (1989) Brain variation and phylogenetic trends in elasmobranch fishes. *Journal of Experimental Zoology* 252(S2): 83–100.

Sawtell NB and Bell CC (2008) Adaptive processing in electrosensory systems: Links to cerebellar plasticity and learning. *Journal of Physiology (Paris)* 102: 223–232.

Smeets WJAJ (1990) The telencephalon of cartilaginous fishes. In: Jones EG and Peters A (eds.) *Cerebral Cortex, Vol. 8A: Comparative Structure and Evolution of Cerebral Cortex, Part I*, pp. 3–30. New York: Plenum.

Smeets WJAJ (1998) Cartilaginous fish. In: Nieuwenhuys R, Ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates*, vol. 1, pp. 551–654. Berlin: Springer.

Smeets WJ, Nieuwenhuys R, and Roberts BL (1983) *The Central Nervous System of Cartilaginous Fishes: Structure and Functional Correlations*. Berlin: Springer.

Stuesse SL, Cruce WL, and Northcutt RG (1994) Localization of catecholamines in the brains of chondrichthyes (cartilaginous fishes). In: Smeets WJAJ and Reiner A (eds.) *Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates*, pp. 21–47. Cambridge: Cambridge University Press.

Sueiro C, Carrera I, Ferreiro S, *et al.* (2007) New insights on *Saccus vasculosus* evolution: A developmental and immunohistochemical study in elasmobranchs. *Brain, Behavior and Evolution* 70: 187–204.

Wullimann MF and Vernier P (2007) Evolution of the nervous system in fishes. In: Kaas JH (ed.) *Evolution of Nervous Systems. A Comprehensive Reference*, vol. 2, pp. 355–406. San Diego, CA: Elsevier.

Functional Morphology of the Brains of Ray-Finned Fishes

AB Butler, George Mason University, Fairfax, VA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Hindbrain
Midbrain
Forebrain
Further Reading

Glossary

Collothalamic Refers to the set of dorsal thalamic nuclei that receive their predominant input from the midbrain roof (optic tectum or torus semicircularis) and, in turn, project to the pallium and/or subpallium. In ray-finned fish, the collothalamus comprises two nuclei, nucleus centralis posterior and dorsalis posterior. Due to their afferent and efferent connections, the preglomerular nuclei also qualify for the designation of collothalamic.

Eversion Process by which the pallium develops in ray-finned fish, wherein the originally medial-most part of the pallium turns outward and thus comes to lie in the most lateral position topographically in the adult, and the originally lateral-most part of the pallium comes to occupy the medial-most aspect of the pallium in the adult. This is the reverse topography from that seen in most other fish and in land vertebrates.

Lemnothalamic Refers to the set of dorsal thalamic nuclei that receive their predominant input not from the midbrain roof but rather more directly from sensory system centers, such as the retina or, in land vertebrates, the dorsal column nuclei. This direct type of projection can be referred to as lemno-, derived from the word 'lemniscal', which means ribbon like. In ray-finned fish, only one dorsal thalamic nucleus, nucleus anterior, constitutes the lemnothalamus. The projections of nucleus anterior are not fully elucidated in ray-finned fish; in most vertebrate taxa, the lemnothalamus projects to the pallium.

Pallium The more dorsal part of the telencephalon. The projection neurons of the pallium are glutamatergic.

Preglomerular nuclear complex A set of nuclei that lie within the caudal part of the telencephalon and relay sensory information rostrally to the pallium. They resemble dorsal thalamic, sensory relay nuclei in this regard but are located in a more caudal position within the neuraxis. They are developmentally derived from a region called the posterior tuberculum.

Subpallium Ventral part of the telencephalon. The projection neurons of the subpallium are GABAergic.

Vertebrates As used here, the clade that includes agnathans and jawed vertebrates (gnathostomes), alternately defined by some as limited to lampreys and jawed vertebrates, excluding hagfish, with the term craniates used for agnathans and jawed vertebrates.

Introduction

Ray-finned fish (actinopterygians) comprise over 20 000 species. This group includes five major taxa (**Figure 1(a)**): reedfish (Cladistia), sturgeons and paddlefish (Chondrichthyes), gars (Ginglymodi), the bowfin *Amia calva* (Halecomorphi), and the huge radiation of bony fish (Teleostei). The ray-finned fish occupy an extreme diversity of niches (*sensu* George Gaylord Simpson for the entire way of life and relationship to the environment of a species) and exhibit similarly high degrees of diversity in their behavior and in the structure of their brains. Further, they are distinguished from most other vertebrate groups (the term vertebrate is used here to include agnathans and jawed vertebrates) by the way in which the most rostral part of the brain is formed during embryological development. Due to this divergent embryological development and their morphological diversity, ray-finned fish present a large and mostly untapped resource for studying the relationship of neural structure to function.

Hindbrain

As in other vertebrates, the hindbrain of ray-finned fish contains most of the motor and sensory cranial nerve nuclei and the reticular formation. Sometimes included in the latter are the serotoninergic nuclei of the raphe.

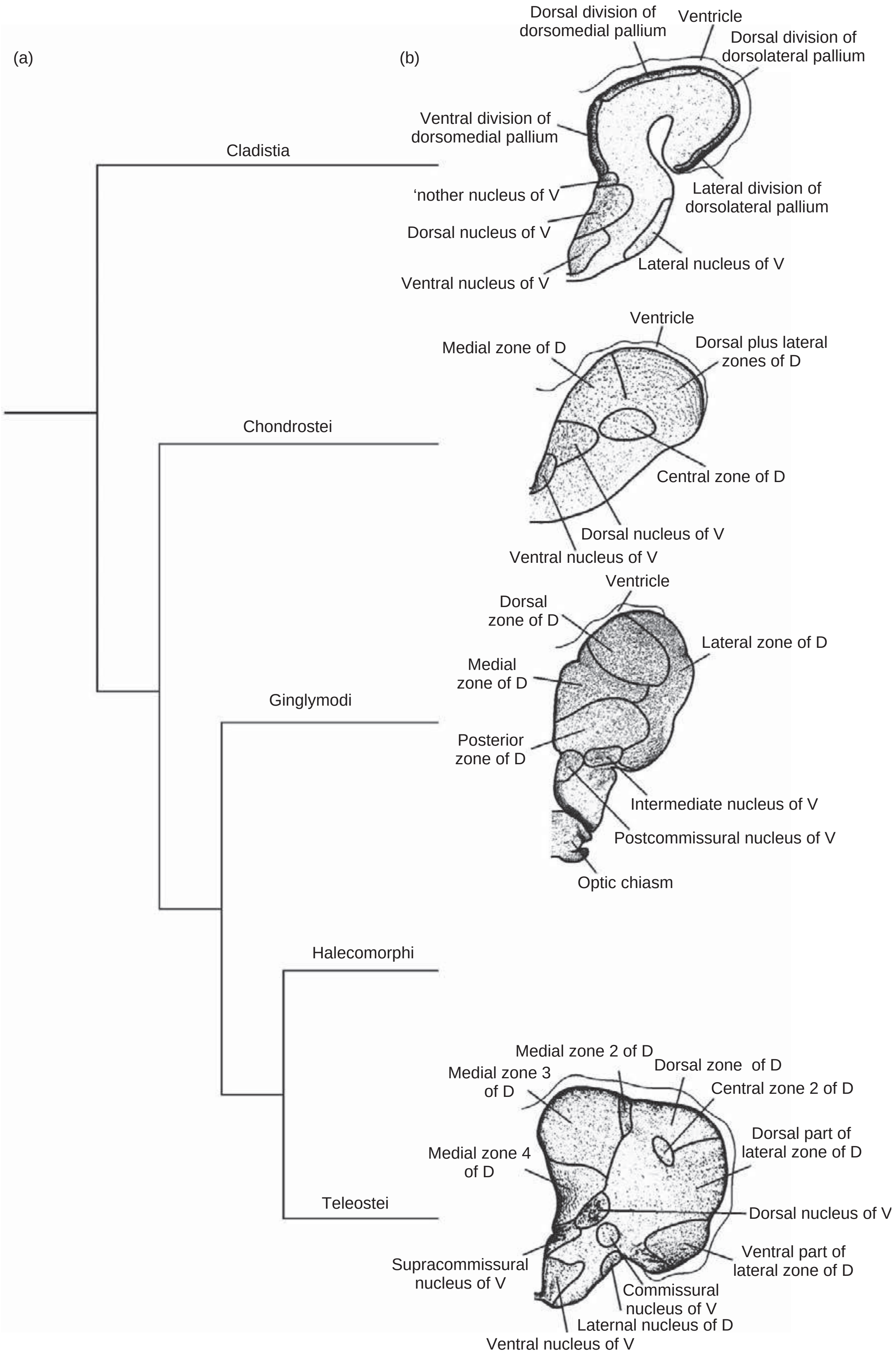


Figure 1 (Continued)

The hindbrain motor nuclei, which all are basal plate derivatives, include the abducens (VI) nucleus, which innervates some of the extraocular musculature, and the branchiomeric (gill arch) series for cranial nerves V (trigeminal), VII (facial), IX (glossopharyngeal), and X (vagus); the latter can be seen in **Figure 2(g)**. In some ray-finned fish species, as in the other aquatic vertebrate groups and in land vertebrates, the abducens nucleus typically lies in a dorsomedial position. An unusual condition for the abducens nucleus has been documented in goldfish (*Carassius auratus*); however, its neuron cell bodies migrate to occupy a ventromedial position. The number of other ray-finned fish species in which this occurs has not been explored. The branchiomeric motor nuclei in ray-finned fish migrate somewhat ventrolaterally, as is also the case in the other vertebrate taxa. A second variation occurs with the mesencephalic nucleus of the trigeminal nerve (MesV) in tarpons, which are large fish with powerful jaws. In this species, MesV is exceptionally large, consisting of a very large population of neural crest-derived neurons that (as in other vertebrate taxa) migrate into the central nervous system and lie on the outer border of the periventricular (deep) layers of the optic tectum.

The alar-plate-derived sensory structures of the hindbrain include the octaval (VIIIth) cranial nerve for the auditory and vestibular senses (**Figure 2(f)**) as well as several examples of sensory specializations in ray-finned fish. Teleosts that are electroreceptive include notopterids (featherbacks and knifefish) and mormyrids (freshwater elephantfish), both of which are osteoglossomorph teleosts, and siluriforms (catfish) and gymnotids (electric eel and electric knifefish) (**see also Detection and Generation of Electric Signals**: Generation of Electric Signals and Detection and Generation of Electric Signals in Fishes: An Introduction). These teleosts have an electrosensory lateral line lobe that is highly laminated and cytoarchitectonically complex, with up to 15 cell and fiber layers, which differentially receive afferent information and give rise to efferent projections. Likewise, the gustatory system, which is most elaborately developed in catfish and in the nonelectroreceptive cyprinids, such as goldfish, has afferents that terminate in three laminated lobes, the facial, glossopharyngeal, and vagal lobes, rather than in a simple gustatory nucleus as in most vertebrates, including mammals (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction and Neurophysiology of Gustation). Both the electrosensory lateral line lobe and the gustatory lobes are cortical in their cytoarchitecture, indicating that highly complex processing of the sensory information is being carried out within them. There are also ascending pathways that relay this information to the pallium, as is the usual case for sensory systems. In teleosts, most of this information is relayed through part of the diencephalon to the medially lying part of the pallium, an area referred to as Dm (as discussed below).

The cerebellum is also an alar plate derivative and shows considerable variation in the extent to which it is developed across the different groups of ray-finned fish. As is the case in cartilaginous fish, ray-finned fish have a corpus cerebelli (**Figure 2(f)**) with an outer molecular layer, granule cells, and Purkinje cells and a related structure, the cerebellar crest. The corpus cerebelli receives inputs from the electrosensory system in those species with that sense as well as mechanosensory lateral line and octaval (auditory/vestibular) inputs and descending inputs relayed from the telencephalon (**see also Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy). The outputs of the cerebellum are via Purkinje cells, as in other vertebrate taxa, but rather than projecting to deep cerebellar nuclei, the Purkinje cells project to eurydendroid cells that lie within the cerebellar cortex. The eurydendroid cells project to a wide range of targets, such as the reticular formation, red nucleus, and various diencephalic nuclei. An additional cerebellar structure unique to teleosts is the valvula cerebelli, which is an extended part of the corpus cerebelli. In the electroreceptive mormyrids, the valvula is hypertrophied to the extent that it covers the entire dorsal surface of the brain, covering not only the corpus cerebelli but also the tectal roof and the pallial hemispheres.

Mormyrids and other fish that use electrosensory communication also have descending pathways through the brainstem that are involved in the generation of the electric signals (**see also Detection and Generation of Electric Signals**: Generation of Electric Signals). In

---

Figure 1 (a) Cladogram of ray-finned fish showing the phylogenetic relationships of Teleostei (bony fish), Halecomorphi (the bowfin *Amia*), Ginglymodi (gars), Chondrostei (sturgeons and paddlefish), and Cladistia (reedfish). (b) Nissl-stained photomicrographs of transverse hemisections through the right telencephalon of a cladistian, the bichir *Polypterus senegalus* (top), the sturgeon *Scaphirhynchus platorynchus* (second from top), the gar *Lepisosteus osseus* (third from top), and the sunfish *Lepomis cyanellus* (bottom). The Nissl stain stains the cell bodies of neurons but not their myelinated processes. Thus, each tiny black dot in the photomicrographs represents a neuron cell body. In instances where the cell bodies are very densely packed along the ventricular surface, the staining creates the appearance of a thick black line, as seen in *Polypterus*. Due to the eversion process during embryological development (discussed in the text), the ventricular space overlies the dorsal surface of the pallial areas. D, area dorsalis (= pallium); V, area ventralis (= subpallium). The author thanks R. Glenn Northcutt for generously providing the original photomicrographs shown in this figure and in **Figure 2**.

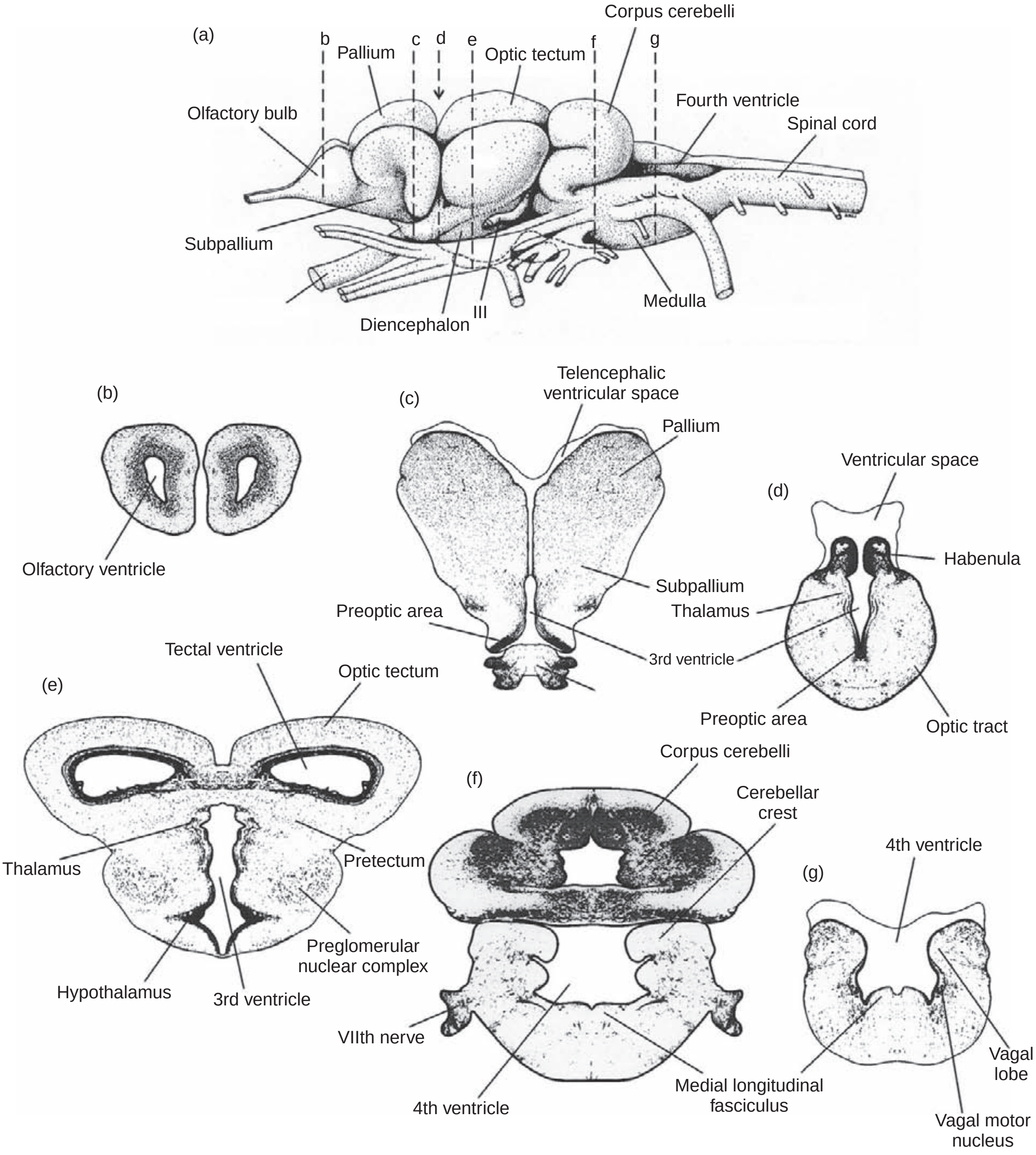


Figure 2 (a) Drawing of a side view of the brain of a gar (*Lepisosteus osseus*), with rostral toward the left. The levels of Nissl-stained sections below are indicated by dashed lines and labeled with corresponding letters (b–g). As seen in (a), the forebrain consists of the diencephalon, to which the optic nerve projects, and the telencephalon; the latter consists of olfactory bulbs rostrally and hemispheres that contain the pallium dorsally and the subpallium ventrally. The midbrain consists of the optic tectum, which also receives optic nerve projections, and the tegmentum (the latter is not visible from the side view). The oculomotor nerve (III), which originates in the midbrain can be seen exiting the brainstem just ventral to the optic tectum. The hindbrain consists of the cerebellum dorsally and the medulla ventrally, which is continuous caudally with the spinal cord. The transverse hemisections are through the olfactory bulbs (b); the telencephalic hemispheres (c), with the ventricular space, equivalent to the lateral ventricles of other vertebrates, between and dorsal to them and the optic chiasm (the crossing, or decussation, of the optic nerves) lying in the ventral midline at this level; the rostral part of the diencephalon (d), with its ventricular space, equivalent to the third ventricle of other vertebrates, between and dorsal to it; the midbrain (e) with the optic tectum dorsally and the caudal parts of the diencephalon present at this level ventrally; the rostral part of the hindbrain (f) with the corpus cerebelli and cerebellar crests dorsal to the fourth ventricle; and the caudal part of the medulla (g).

gymnotids, a prepacemaker nucleus in the diencephalon projects to a pacemaker nucleus in the hindbrain, which in turn relays its outputs to electromotor neurons in the spinal cord that control discharges from the animal's electric organ. A similar pathway in mormyrids involves projections from a pre-command nucleus in the midbrain to a command nucleus and the medullary electromotor relay nucleus in the hindbrain and thence to the spinal cord electromotor relay nucleus, which controls the electric organ discharges.

Midbrain

The hindbrain transitions into the midbrain proper through a region called the isthmus. Rostral to the isthmus is the midbrain tegmentum located ventrally with the tectum, the alar-plate roof structures, dorsal to it. The isthmus and tegmentum contain a number of ascending and descending tracts, like the hindbrain, and also a number of nuclei specific to them. An interpeduncular nucleus is present in the ventromedial aspect of the isthmal midbrain; it is named for its position between the cerebral peduncles of the midbrain in mammals, structures that are absent or less well developed (depending on how one defines them) in fish. It receives projections from the habenula (**Figure 2(d)**), as discussed below. The nuclei of the reticular formation and raphe extend into the midbrain, which also houses the norepinephrine-containing neurons of the locus coeruleus and the motor nuclei for cranial nerves IV (trochlear) and III (oculomotor). These two oculomotor nuclei are interconnected with each other and with the abducens nucleus and vestibular nuclei of the hindbrain via a long tract called the medial longitudinal fasciculus (**Figures 2(f)** and **2(g)**). The oculomotor nuclear complex of III includes somatic motor neurons that innervate the extraocular muscles and also the visceral motor neurons of the Edinger–Westphal nucleus that supply parasympathetic innervation to eye structures. The oculomotor system displays a rather unique specialization related to the electromotor system in the stargazer *Astroscopus* that has an electric organ formed from modified extraocular muscles innervated by a pool of the oculomotor nucleus neurons.

The tectal structures that form the midbrain roof include the optic tectum (**Figure 2(e)**) (the superior colliculus of mammals), which is dominated by visual input but which also receives multisensory (auditory and somatosensory) inputs in its deeper layers, and the torus semicircularis (the inferior colliculus of mammals), which is dominated by auditory inputs and, in some cases, electrosensory inputs. The optic tectum projects rostrally to a nucleus in the dorsal thalamus (**Figures 2(d)** and **2(e)**), the dorsal posterior nucleus, while the torus semicircularis similarly projects to the central posterior nucleus. Of these two structures, the optic tectum has the most elaborate cytoarchitecture, consisting of cortical layers that group into three zones: superficial, central, and periventricular. The visual input is confined to the superficial layers and the somatosensory and auditory inputs are confined to the two deeper zones; the latter also give rise to efferent tectal projections, which, in addition to the ascending input to the dorsal thalamus, also include descending pathways to the reticular formation and other brainstem sites. The optic tectum has a structure unique to ray-finned fish at its medial-most aspect, the torus longitudinalis, with which it is reciprocally connected and which may be interconnected with the cerebellum. It also has reciprocal, topographical connections with a prominent nucleus in the isthmal region, nucleus isthmi, which contains cholinergic neurons. This nucleus interconnects the optic tectum with the pretectum, a caudal part of the diencephalon involved in the control of eye movements. For a more detailed treatment of the optic tectum, the torus longitudinalis, and nucleus isthmi **see also Vision**: Optic Tectum.

Forebrain

The forebrain comprises the diencephalon and the telencephalon. The diencephalon contains the epithalamus, dorsal and ventral thalamic nuclei, hypothalamus, pretectum, and a set of laterally migrated nuclei known as the preglomerular nuclear complex. The telencephalon consists of the pallium in its dorsal part, which mainly receives and integrates sensory information, and the subpallium ventrally, which is involved in motor control and related functions. Except for the hypothalamus, all of the forebrain is derived from the alar plate.

Diencephalon

The pretectum (**Figure 2(e)**) is a caudal part of the diencephalon. It contains multiple nuclei, many of which receive retinal projections. However, unlike the dorsal thalamus (**Figures 2(d)** and **2(e)**) and the preglomerular nucleus (**Figure 2(e)**, discussed below), pretectal nuclei do not project rostrally to telencephalic sites. Instead, they are interconnected with the optic tectum and are involved in multiple functions, including the control of eye movements. There is considerable variation across ray-finned fish in the extent to which the pretectal region is developed. In most ray-finned fish, the nuclei lie in superficial, central, and periventricular zones that are continuous with the same zones of the optic tectum, and several nuclei, including a rather prominent nucleus, the superficial small-celled (parvocellular) pretectal nucleus (PSp), receive direct retinal input. In non-euteleost teleosts, PSp projects to a nucleus of variable size, the posterior pretectal nucleus (PO), which in turn projects to the inferior lobe of the hypothalamus. The latter is involved in feeding behavior. In euteleosts, PO is replaced by two nuclei, which may represent a separation of the neurons that comprise PO, the intermediate superficial nucleus (PSi) and a large, cytoarchitectonically complex nucleus called nucleus glomerulosus (NG). In this case, PSp receives the retinal input but projects to PSi, which in turn projects to NG, and NG then projects to the inferior lobe of the hypothalamus. This circuitry is an example of an increase of complexity in both the number of relay nuclei and their internal cytoarchitecture in a particular system across the phylogenetic spectrum.

The epithalamus generally comprises the pineal gland (and/or other dorsal diencephalic structure) and the habenula. A single dorsal structure, the pineal gland, is present in most ray-finned fish and has rather widespread projections, including to the habenula and to sites within the dorsal and ventral thalamus, pretectum, hypothalamus, and tegmentum. Trout additionally have a parapineal organ, which projects to the habenula. The habenula itself (**Figure 2(d)**) is asymmetrical in size (not apparent in the figure), as in other vertebrates. In addition to pineal (and parapineal) afferent connections, it receives inputs from a subpallial part of the telencephalon, called Vd (the dorsal nucleus of area ventralis) that appears to be homologous to the striatum of other vertebrates. The main efferent projection of the habenula is via the fasciculus retroflexus tract, which is universally present across vertebrates. This tract is alternatively known as the habenulointerpeduncular tract, defining its target as the interpeduncular nucleus, which lies in the medial aspect of the isthmal region of the midbrain tegmentum. In mammals, the interpeduncular nucleus has at least some serotoninergic neuronal components and is known to project to the hippocampus; it may in fact be a rostral component of the raphe system. Its projections in ray-finned fish have not yet been determined, however.

An additional nucleus, nucleus rostrolateralis, which appears to be a component of the epithalamus – possibly a sort of supernumerary habenula – is present in some fish but only in a phylogenetic distribution that is very sporadic, appearing here and there across the phylogeny, being absent or undetectable in many species in between. It receives retinal and tectal afferent projections, is apparently high in its metabolic activity (as evinced by high cytochrome oxidase staining), and may be involved in circuitry for feeding behaviors. Its far-flung phylogenetic distribution but multiple points of similarity may be due to its being produced by the same genetic pathway, inherited from a deep common ancestor, which is only occasionally expressed in the phenotype.

The thalamus and preglomerular nuclear complex are involved in sensory relay pathways to the telencephalic pallium (**Figures 2(a)** and **2(c)**). In tetrapods, the dorsal thalamic nuclei are the only relay sites for ascending sensory pathways, but in ray-finned fish, multiple diencephalic components provide such relay sites. Several ventral thalamic nuclei are present in ray-finned fish and most of them receive retinal as well as other inputs. Unlike the situation in most other vertebrates, at least one of the ventral thalamic nuclei has been reported to project to the telencephalon. They contain inhibitory γ-aminobutyric acid (GABA)ergic neurons as in other vertebrates, but their other connections and their functions have not been studied. The major relay sites involve components of the preglomerular nuclear complex and two of the three dorsal thalamic nuclei (dorsalis posterior and centralis posterior) that receive their inputs predominantly from the midbrain roof and project to the telencephalic pallium, particularly targeting its medial zone (the medial zone of area dorsalis, Dm, see **Figure 1(b)**, bottom). In its circuitry, the preglomerular nuclear complex resembles the dorsal thalamic nuclei of tetrapods and may be a serial (iterative) homolog of these nuclei, that is, a similar structure occurring at a more caudal segmental level. These preglomerular and dorsal thalamic ascending pathways carry visual and multimodal information from the optic tectum and auditory information from the torus semicircularis. Due to the receipt of major projections from the midbrain roof, these groups can be classified as collothalamic ('collo' referring to the mammalian term for the midbrain roof, the 'colliculi'). The preglomerular nuclear complex (**Figure 2(e)**) relays ascending lateral line and gustatory information and appears to be present in cartilaginous fish also. The connections of the remaining dorsal thalamic nucleus, nucleus anterior, which lies at a more rostral level, are less well understood. This nucleus does not receive major midbrain roof projections, instead receiving visual input directly from the retina in a lemniscal (or straight, ribbon-like) fashion, thus classifying this nucleus as the lemnothalamic part of the dorsal thalamus. In cladistians, it does not project directly to the pallium but rather to another diencephalic nucleus (nucleus medianus of the posterior tuberculum), which in turn projects to the dorsal and lateral parts of the pallium. Nucleus anterior has been reported but not confirmed to project to part of the lateral pallium in goldfish. The afferent projections to the lateral pallium also have been difficult to identify, although its dorsal part is metabolically active for visual system inputs in goldfish.

The hypothalamus (**Figure 2(e)**) and its rostral extension, the preoptic area (**Figure 2(d)**), contain a number of distinct nuclear groups. In the preoptic region are anterior and posterior parvocellular (small-celled) nuclei, a magnocellular (large-celled) nucleus, and the suprachiasmatic nucleus. Caudal to these nuclei lies the hypothalamus proper and consists of dorsal and ventral portions that border the third ventricle as well as some more laterally lying nuclei. Most ray-finned fish have an enlarged part of the hypothalamus that lies ventrally and is known as the inferior lobe. The inferior lobe receives descending projections from the telencephalon and from brainstem gustatory nuclei. As noted above, it also receives projections from the pretectal region – the posterior pretectal nucleus in most non-euteleosts and nucleus glomerulosus in teleosts – and has been functionally implicated in feeding behaviors and some other actions, such as circling and chasing, which may be invoked for establishing territorial boundaries. The preoptic area and hypothalamus also give rise to major descending pathways, which, as in other vertebrates, affect the parasympathetic (general visceral motor) centers of the brainstem, as well as to reticular formation sites that relay information to spinal cord sympathetic centers. It should also be noted that the retinal part of the eyes is derived from the central nervous system; the eye stalk that divides to form them during embryological development buds off from the most rostral part of the neural tube in the preoptic region of the hypothalamus.

The hypothalamus and preoptic area also play major roles in endocrine regulation. Various preoptic and hypothalamic nuclei project to the pars nervosa and/or pars intermedia of pituitary gland and regulate the production and/or release of multiple hormones, including melanocyte-stimulating hormone, which controls skin pigmentation and consequently plays a crucial role in vertical banding displays, arginine vasotocin, which functions as a diuretic and to regulate blood pressure, and isotocin, which also contributes to blood pressure regulation and additionally plays a role in the release of eggs or live birth (**see also The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis). The adenohypophysis also receives hypothalamic innervation and is involved in the release of other hormones, including prolactin, corticotropin, gonadotropin, growth hormone, and thyrotropin. Overall, the hypothalamus is crucial for many different behaviors that range from endocrine control to courtship (nest building and courting rituals), reproductive behaviors (sperm and egg release), feeding behaviors (especially the inferior lobe as noted above), sound production in some species, and the control of respiration and heart rate.

Telencephalon

As in other vertebrates, the telencephalon consists of olfactory bulbs (**Figures 2**(**a**) and **2**(**b**)) and the telencephalic hemispheres, which can be divided into two regions, a ventrally lying subpallium and, dorsal to it, the pallium (**Figures 2**(**a**) and **2**(**c**)). One major distinguishing characteristic of these two regions across all vertebrates is the difference in the neurotransmitter of their projection neurons: pallial projection neurons are glutamatergic, whereas those of the subpallium are GABAergic.

The subpallium contains septal and striatopallidal components. In contrast to other major vertebrate taxa, the striatopallidal component lies in a relatively medial position and both striatal and pallidal cell populations intermingle within a single nucleus, the dorsal nucleus of V (the ventral zone of the telencephalon), as shown in the bichir (Cladistian), sturgeon (Chondrostei), and sunfish (Teleostei) in **Figure 1**. The ventral nucleus of V (shown in the bichir, sturgeon, and sunfish in **Figure 1**) receives gustatory information and may be homologous to part of the septum or other medial subpallial regions of other vertebrates, while a more laterally situated nucleus, the lateral nucleus of V (shown in the bichir and sunfish in **Figure 1**), which contains cholinergic neurons, may be homologous to the medial septal nucleus or the basal nucleus of Meynert.

The pallium in ray-finned fish undergoes a different developmental process than the pallium in most other vertebrates; however, this results in a reversal of the medial-to-lateral topography of its component parts. In most vertebrates – including agnathans, cartilaginous fish, amphibians, and amniotes – pallial development follows a process of evagination in which the two hemispheres expand outwardly, rather like puffing out one's cheeks. In contrast, in ray-finned fish, the process is one of eversion. This process is diagrammed in **Figure 3**. After the initial formation of the neural tube, shown at the left, the dorsal part of the tube thins to form a thin ependymal roof of non-neural, epithelial tissue. The two dorsal parts of the neural tube then turn outward, that is, laterally, and expand in that direction as the ependymal roof extends over them. This process results in having the originally medial part of the neural tube attain the lateral-most position in the adult and vice versa.

Further development of the pallial region varies to a marked degree across the different major clades of ray-finned fish. As also shown in **Figure 3**, the degree to which the eversion process proceeds varies. The outline of the telencephalon of a basal cladistian, in this case the bichir *Polypterus senegalus* (compare with the section shown in **Figure 1**(**b**), top) is shown at the upper right of the figure. In this clade, eversion continues until the origin of the ependymal roof is at approximately 5:00 on the right side. Eversion is less extensive in sturgeons, as in *Scaphirhynchus platorynchus* shown at the lower right (compare to the section shown in **Figure 1**(**b**), second from top), however, reaching to about 3:00 on that side. As can be seen in **Figure 1**(**b**) (third from top), eversion in gars, *Lepisosteus osseus*, is strikingly less than in either cladistians or sturgeons, reaching only to about 1:00 on the right side. Again, in contrast, in some teleosts, such as the sunfish *Lepomis cyanellus*, also shown in **Figure 1**(**b**) (bottom), eversion is more extensive. Thus, the degree of eversion does not fall

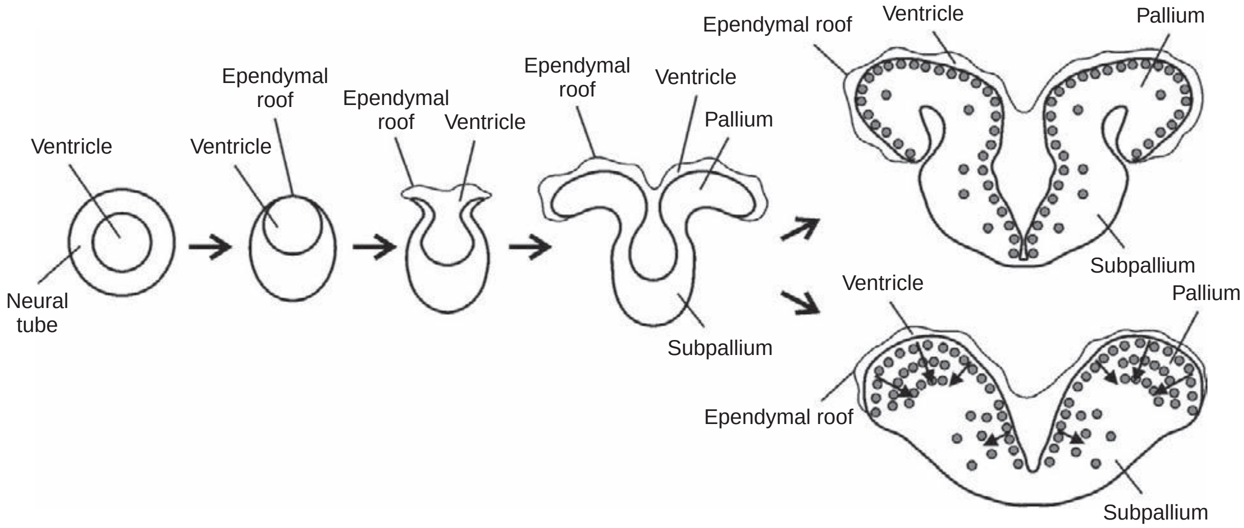


Figure 3 Diagrammatic representation of the eversion process that occurs in telencephalic development in ray-finned fish. As the diagrams show from left to right, the roof of the original round neural tube thins to form a non-neural, ependymal roof, and the upper ends of the neural tube turn outward. Thus, the ventricular space extends between the ependymal roof and the dorsal surface of the pallium. In some ray-finned fish, such as cladistians (*Polypterus senegalus*), shown at the upper right (to compare with its Nissl section in **Figure 1**(**b**), top), there is very little migration of the neuron cell bodies (indicated by large gray dots) away from the proliferative zone along the ventricular surface. In others, there is more proliferation and migration, as shown to a moderate degree here at the lower right in the sturgeon *Scaphirhynchus platorynchus* (to compare with its Nissl section in **Figure 1**(**b**), second from top). In other fish, such as the gar and sunfish shown in **Figure 1**(**b**) (third from top and bottom, respectively), there is much more extensive proliferation and migration. Also, note that the degree of the eversion process, indicated by the lateral point of attachment of the ependyma, varies, being most extensive in some teleosts, such as *Lepomis* (but not in others, such as *Salmo*, not shown), cladistians, and in the halecomorph *Amia* (not shown) and much less in ginglymodi and chondrosteans.

onto a morphocline (a graded series) from basal to crown taxa but rather varies independently across the major clades.

Because the zone of cell proliferation is along the ventricular surface, as is also the case in other vertebrate groups, variation in the degree of eversion results in differing patterns of migration of the newly formed neuronal cell bodies. The extent to which the proliferation and migration processes continue is yet another variable both across and within the different clades. As shown in **Figure 1**(**b**) (and diagrammatically in **Figure 3**), most neuron cell bodies remain very near the ventricular surface in cladistians and show only some migration in wave-like lines in sturgeons but are in much greater abundance and substantially migrated in teleosts, with gars and the bowfin *Amia* having intermediate conditions. These patterns form a continuum of variation, allowing one to somewhat arbitrarily divide the taxa by their type I, or laminar, brains, as in nonteleosts, and the teleost condition of type II, or elaborated brains. (Gars and *Amia* have been classified as type I, but an argument for type II could also be made.) As a result of all these factors – the eversion process itself, the variation in the degree of eversion, and marked variation in the degree of cell proliferation and migration – the identification of comparable pallial areas across ray-finned fish and in comparison to other vertebrate groups is extremely challenging, and consensus on this issue is still far from being achieved. The differential pallial evolution in ray-finned fish in fact presents a natural laboratory of immense opportunity that has only begun to be exploited.

Far from being simple ‘smell brains’, as erroneously thought for decades, some of the true functional capabilities of the actinopterygian pallium have been recently discovered, and the generally larger brain–body ratios of cartilaginous fish imply that such capabilities apply to them as well. In both cartilaginous and ray-finned fish, the olfactory projections have been shown to be limited to a circumscribed region of the pallium, and multiple other ascending sensory inputs to other pallial regions have been traced. Despite the difficulties in identifying pallial areas, some hypotheses have been formulated that are reasonably supported by existing data. In ray-finned fish, the medial zone of D (area dorsalis, which is the pallium) (see **Figure 1**(**b**), bottom), originally lateral in topographical position and which is in receipt of caudal dorsal thalamic and preglomerular projections, has been identified as homologous to the mammalian lateral amygdala, which lies within the lateral-most part of the mammalian pallium and also is in receipt of collothalamic projections relayed from the superior colliculus through the dorsal thalamus. The ventral part of the lateral zone of D (see

Figure 1(b), bottom), originally medial topographically, has been proposed as a homolog of the amniote hippocampus, which can be identified in both mammals and reptiles (including birds) with certainty. Interestingly, while not a criterion for homology, the functional role of this part of the ray-finned fish pallium is intriguingly like the amniote hippocampus in terms of memory and spatial mapping abilities, while the functional role of the medial zone is amygdala-like for fear-conditioning, as discovered by Cosme Salas and his co-workers in goldfish (**see also Sensory Systems, Perception, and Learning**: Fish Learning and Memory). Thus, remarkably, the basic functions of different pallial regions appear to be preserved to some extent despite marked differences in embryological development and also drastic differences in the cytoarchitecture (the lack of specific cell types and their lamination as opposed to the amniote condition). Study of the neuron cellular capacities that underlie these functions in fish has the potential to provide insightful advances in understanding the functions of the comparable regions of mammalian brains.

A recent study in Russ Fernald's laboratory has opened the door to intriguing new questions about the cognitive abilities of fish (**see also Sensory Systems, Perception, and Learning**: Fish Learning and Memory). Male cichlids (*Astatotilapia burtoni*), which have relatively elaborate brains among their euteleost peers, can deduce their individual position in the social hierarchy by serial observation of the behavior of other pairs of males. Such an inductive process has been thought to require working memory – an indication of high-level consciousness subserved by the prefrontal cortex in mammals and previously thought to be unique to them. However, it has now been demonstrated convincingly in birds, and this recent study now also suggests that it may be present in at least some fish. The extreme degree of variation across the brains of extant ray-finned fish, including the high degree of pallial elaboration in some euteleosts and the recent observations of surprising behavioral similarities, at least in general terms, to amniotes, requires reevaluation of fish brain structure and functional capacities.

See also: **Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction; Generation of Electric Signals. **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy. **Sensory Systems, Perception, and Learning**: Fish Learning and Memory. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction; Neurophysiology of Gustation. **The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis. **Vision**: Optic Tectum.

Further Reading

Butler AB (2000) Nervous system: Gross functional anatomy; and nervous system: Microscopic functional anatomy. In: Ostrander GK (ed.) *Handbook of Laboratory Animals: Fish*, pp. 129–149; 331–355. San Diego, CA: Academic Press.

Butler AB and Hodos W (2005) *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. Hoboken, NJ: Wiley-Liss.

Grosenick L, Clement TS, and Fernald RD (2007) Fish can infer social rank by observation alone. *Nature* 445: 429–432.

Holmes PH and Northcutt RG (2003) Connections of the pallial telencephalon in the Senegal bichir, *Polypterus*. *Brain, Behavior and Evolution* 61: 113–147.

Huesa G, Anadón R, Folgueira M, and Yáñez J (2009) Evolution of the pallium in fish. In: Binder MD, Hirokawa N, and Windhorst U (eds.) *Encyclopedia of Neuroscience*, vol. 2, pp. 1400–1404. Berlin; Heidelberg: Springer.

Ito H, Ishikawa Y, Yoshimoto M, and Yamamoto N (2007) Diversity of brain morphology in teleosts: Brain and ecological niche. *Brain, Behavior and Evolution* 69: 76–86.

McCormick CA and Braford MR,Jr. (2009) Evolution of auditory system in anamniotes. In: Binder MD, Hirokawa N, and Windhorst U (eds.) *Encyclopedia of Neuroscience*, vol. 2, pp. 1231–1236. Berlin; Heidelberg: Springer.

Mueller T and Wullimann MF (2009) An evolutionary interpretation of teleostean forebrain anatomy. *Brain*, *Behavior and Evolution* 74: 30–42.

Nieuwenhuys R (2009) The forebrain of actinopterygians revisited. *Brain, Behavior and Evolution* 73: 229–252.

Nieuwenhuys R, ten Donkelaar HJ, and Nicholson C (1998) *The Central Nervous System of Vertebrates*, vol. 2. Berlin: Springer.

Northcutt RG (2006) Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *Journal of Comparative Neurology* 494: 903–943.

Saidel WM (2009) Evolution of the optic tectum in anamniotes. In: Binder MD, Hirokawa N, and Windhorst U (eds.) *Encyclopedia of Neuroscience*, vol. 2, pp. 1380–1387. Berlin; Heidelberg: Springer.

Salas C, Broglio C, Durán E, *et al.* (2006) Neuropsychology of learning and memory in teleost fish. *Zebrafish* 3: 157–171.

Wullimann MF and Vernier P (2007) Evolution of the nervous system in fish. In: Kaas JH (ed.) *Evolution of Nervous Systems: A Comprehensive Reference*, vol. 2, pp. 39–60. San Diego, CA: Elsevier.

Yamamoto N, Ishikawa Y, Yoshimoto M, *et al.* (2007) A new interpretation on the homology of the teleostean telencephalon based on hodology and a new eversion model. *Brain, Behavior and Evolution* 69: 96–104.

Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and *Latimeria*

A González, University Complutense of Madrid, Madrid, Spain

RG Northcutt, University of California, San Diego, La Jolla, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Macroscopical Anatomy of the Brain in Lungfishes and *Latimeria*
Histological Features of the Brain in Lungfishes
The Brain of the Coelacanth *Latimeria chalumnae*
Further Reading

Glossary

Area octavolateralis This is the area where the VIII cranial nerve and the lateral line nerves terminate in the dorsal alar plate of the rhombencephalon.

Forebrain The forebrain (or prosencephalon) is the rostral-most portion of the brain in vertebrates. During development it separates into the diencephalon and the secondary prosencephalon, which includes the hypothalamus, the preoptic area, and the telencephalon.

Hindbrain The hindbrain is the caudal-most portion of the brain. It includes the large rhombencephalon, which early in development gives rise to the cerebellum rostrally.

Hodological Adjective referring to pathways (axonal connections) in the central nervous system, derived from *hodos*, the Greek word for road or way.

Midbrain The midbrain (or mesencephalon) forms a roof, which consists of an optic tectum and a torus semicircularis, and a floor, which forms the midbrain tegmentum.

Sarcopterygians The Sarcopterygii, or lobe-finned fishes + terrestrial vertebrates, are characterized by having an axial skeleton connecting the pectoral and pelvic regions. Tetrapods comprise the largest living group of sarcopterygians, but two extant groups of fishes are most commonly referred to as the sarcopterygians: the Actinistia or coelacanths (*Latimeria*) and the Dipnoi or lungfishes (*Lepidosiren*, *Neoceratodus*, and *Protopterus*).

Introduction

The lobe-finned fishes (Sarcopterygii) are currently represented by six species of lungfishes (Dipnoi) and two species of coelacanths (Actinistia). The lobe-finned fishes diverged from the ray-finned fishes about 450 million years ago (mya) and were highly successful during the Devonian, when hundreds of species lived in the oceans and rivers of most continents. By the end of the Devonian, 360 mya, the first tetrapods had diverged from the Rhipidistia, an extinct family of lobe-finned fishes, and colonized land. The diversity of all lobe-finned fishes began to decline after the mass extinction in the Permian (290–245 mya). Most recent paleontological analyses support a Dipnoi + Rhipidistia sister group relationship, although Actinistia + Rhipidistia or Dipnoi + Actinistia sister group relationships have also been proposed. Based on molecular data, rather than phenotypic data, however, lungfishes, and not the coelacanths, appear to be the closest living relatives of tetrapods.

Nowadays, lungfishes live in the rivers of Africa (four species of the genus *Protopterus*), South America (*Lepidosiren paradoxa*), and Australia (*Neoceratodus forsteri*). For many years they were considered the only extant lobe-finned fishes until 1938, when a surviving member of the coelacanths, *Latimeria chalumnae*, was caught off the Comoro archipelago near the eastern coast of Africa in the Indian Ocean. A second species, *Latimeria menadoensis*, was discovered in 1999 in Indonesia.

Because they appear to be the closest living relatives to the ancestors of all tetrapods, information on the anatomy and physiology of lobe-finned fishes is critical to any explanation of the origin of land vertebrates. However, the availability of these animals for experimental research has been limited. *Latimeria*, especially, is extremely rare and critically endangered; its population is estimated to consist of fewer than 400 animals alive today. Lungfishes are somewhat more plentiful but are not always easily obtained. In the following sections, we describe the organization of the brain in lungfishes and

Latimeria, based on the information available. For lungfishes, the data from normal histology and experimental hodological techniques have been supplemented with data from immunohistochemistry for the localization of neurotransmitters, neurotransmitter-related molecules, or neuroregulators. For *Latimeria*, the description is necessarily based only on normal histological material.

Macroscopical Anatomy of the Brain in Lungfishes and *Latimeria*

The gross morphology of the brain of a lungfish (*Protopterus*) and the brain of *Latimeria* is shown in **Figure 1**. In both cases the brain is elongated and possesses only small flexures, compared to those seen in most bony fishes. Generally speaking, the brain in lungfishes is similar to that in urodele amphibians. The brain of *Latimeria* is similar to that of lungfishes but is smaller in relation to body size. It occupies only 1% (approximately) of the total cranial volume, being confined to the most caudal division of the skull. As in other vertebrates, lungfishes and *Latimeria* both have three major brain parts, corresponding to the three encephalic vesicles formed during development: the hindbrain, the midbrain, and the forebrain, from caudal to rostral. The hindbrain, with the rhombencephalon and cerebellum, is similar to the hindbrain in other vertebrates, and it shows paired cranial nerves organized in a pattern similar to that seen in most groups of vertebrates. In both lungfishes and *Latimeria*, there are up to six pairs of lateral line nerves. In lungfishes the cerebellum is less well developed than in other fishes, and it has laterally located auriculae that are relatively larger than the medial corpus cerebelli. In *Latimeria* the cerebellum is larger than that in lungfishes. It comprises two extremely large auriculae and an unpaired, evaginated corpus cerebelli. The midbrain develops from the mesencephalic vesicle. In lungfish, it is represented by a small, slender portion of the brain, which caps the rostral rhombencephalon and cerebellum caudally and shows a poorly developed bilateral optic tectum. This region appears better developed in *Latimeria.* The forebrain comprises the diencephalon, hypothalamus, and telencephalon. The diencephalon is small and tubular in both groups of fishes and shows prominent bulges corresponding to the dorsal thalamus. A well-developed tela choroidea forms a particularly large dorsal sac in lungfishes. The hypothalamus shows macroscopical differences among the lungfishes and between them and *Latimeria. Protopterus* and *Lepidosiren* have a caudally directed, long infundibular hypothalamus, whereas this structure is almost vertically oriented in *Neoceratodus.* In contrast, the whole hypothalamus of *Latimeria* bends rostrally. The telencephalon comprises caudal, intermediate, and rostral subdivisions. The caudal portion is a small region known as the telencephalon impar, from which the intermediate telencephalic hemispheres emerge. These are large and evaginated in lungfishes, with a floor (subpallium) that is remarkably bigger than the dorsal (pallial) regions, which in some cases (*Neoceratodus*) are partially reduced to an ependymal tela. In *Latimeria*, the subpallium is thin walled and clearly evaginated, whereas the pallium is a thickened, solid body that is partially covered by a membranous roof, which constitutes an ependymal septum in the median plane. The rostral telencephalic region is formed by the secondarily evaginated olfactory bulbs, which, in lungfishes, can be sessile on the rostral tip of the hemispheres, as in *Protopterus*, or separated for a short distance and connected by hollow peduncles, as in *Neoceratodus.* In *Latimeria*, the olfactory bulbs are directly apposed to the nasal cavity, and very long peduncles connect them to a particular pair of swellings (the rostral bodies) at the rostral tip of the hemispheres.

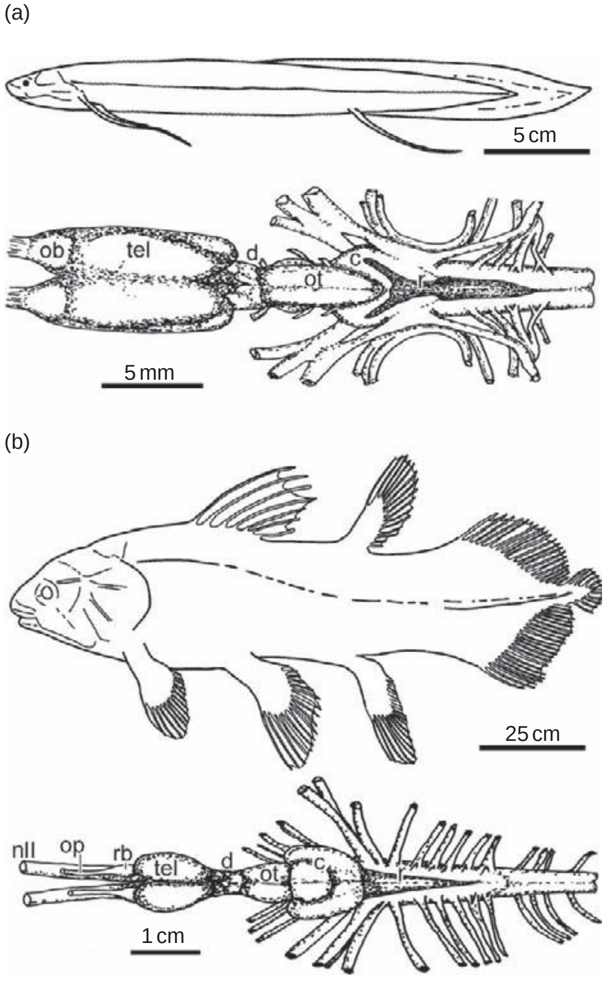


Figure 1 Line drawings of *Protopterus* (a) and *Latimeria* (b) in lateral view and their respective brains in dorsal view. Abbreviations: c, cerebellum; d, diencephalon; nII, optic nerve; ob, olfactory bulb; op, olfactory peduncle; ot, optic tectum; r, rhombencephalon; rb, rostral body; tel, telencephalon.

Histological Features of the Brain in Lungfishes

Hindbrain

As in most brain regions, the cell populations of the hindbrain retain an essentially periventricular arrangement (**Figure 2**), and particular neuronal groups can be distinguished only by experimental techniques, such as tract tracing or immunohistochemistry (**Figure 3**). The wide rhombencephalon shows a largely horizontal basal plate and a vertically oriented alar plate around the fourth ventricle, which is mostly covered by a

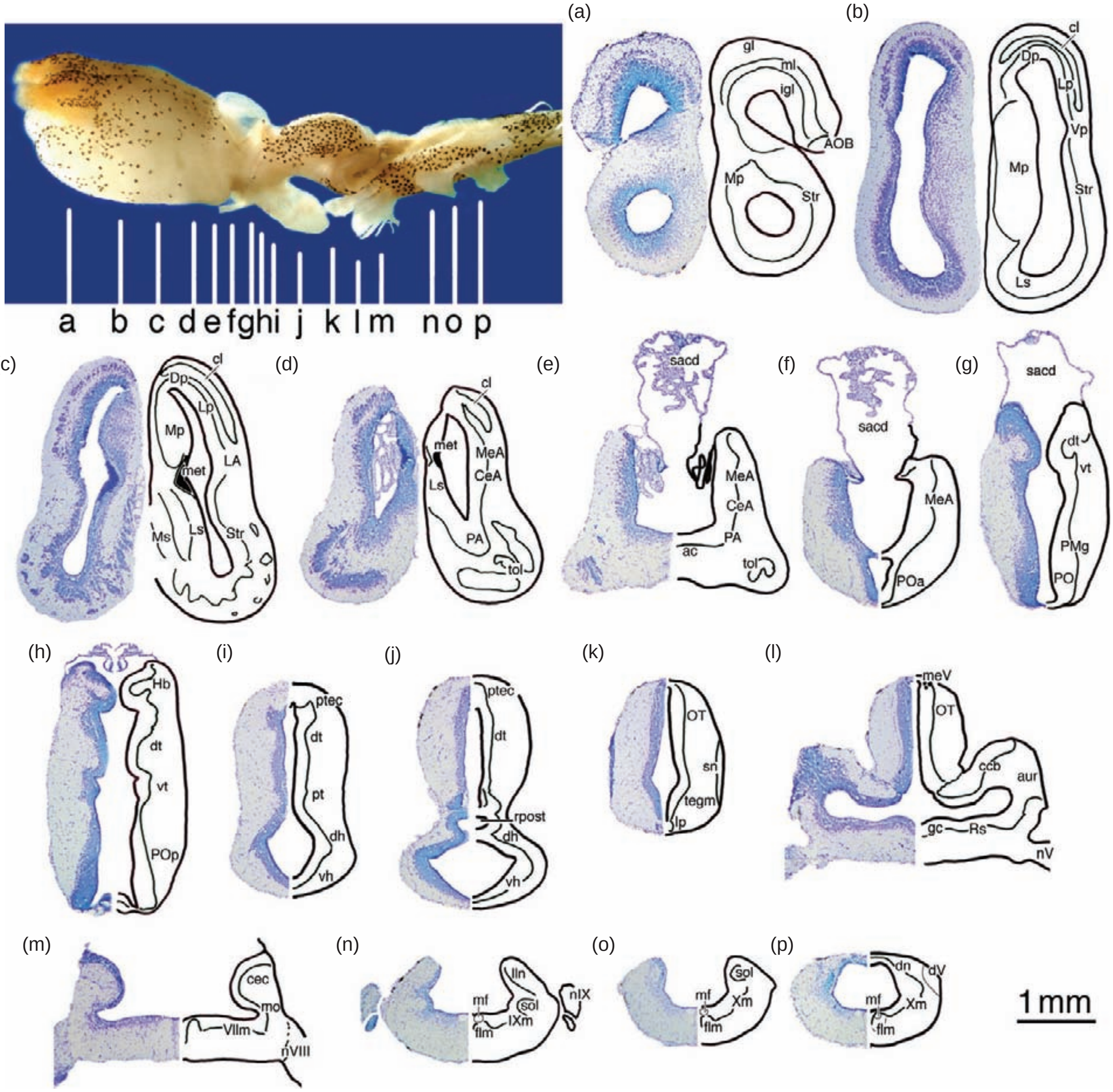


Figure 2 Photomicrographs of transverse hemisections through the brain of *Protopterus dolloi,* stained with cresyl violet, showing the cytoarchitecture (left) and corresponding line drawings indicating the primary structures (right) at the rostrocaudal levels (a–p) indicated on the photograph of the brain in lateral view. Abbreviations: ac, anterior commissure; AOB, accessory olfactory bulb; aur, auricula cerebelli; ccb, corpus cerebelli; CeA, central amygdala; cec, cerebellar crest; cl, cortical layer; dh, dorsal hypothalamus; dn, dorsal column nucleus; Dp, dorsal pallium; dt, dorsal thalamus; dV, descending trigeminal tract; flm, fasciculus longitudinalis medialis; gc, central gray; gl, glomerular layer; Hb, habenula; igl, internal granular layer; Ip, interpeduncular nucleus; IXm, glossopharyngeal motor nucleus; LA, lateral amygdala; lln, lateral line nucleus; Lp, lateral pallium; Ls, lateral septum; MeA, medial amygdala; met, medial ependymal thickening; mf, Mauthner cell fiber; ml, mitral cell layer; mo, median octavolateral nucleus; Mp, medial pallium; Ms, medial septum; nIX, glossopharyngeal nerve; nV, trigeminal nerve; nVIII, octaval nerve; OT, optic tectum; PA, pallidum; PMg, magnocellular preoptic nucleus; PO, preoptic area; POa, anterior preoptic area; Pop, posterior preoptic area; pt, posterior tubercle; ptec, pretectum; rpost, posterior recess; Rs, nucleus reticularis superior; sacd, saccus dorsalis; sn, superficial nucleus; sol, solitary tract; Str, striatum; tegm, tegmentum; tol, tuberculum olfactorium; vh, ventral hypothalamus; VIIm, facial motor nucleus; Vp, ventral pallium; vt, ventral thalamus; Xm, vagal motor nucleus.

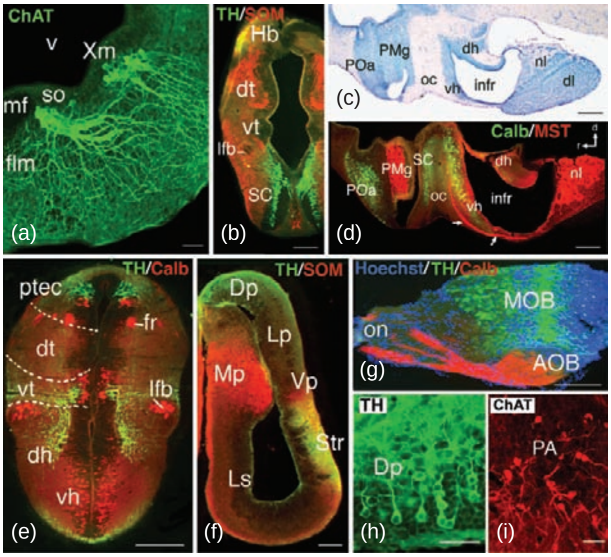


Figure 3 Photomicrographs of sections reacted for immunohistochemistry at different levels of a lungfish brain (*Protopterus dolloi*). (a) Transverse section through the caudal rhombencephalon showing motor nuclei revealed with choline acetyltransferase. (b) Transverse section through the diencephalon and hypothalamus showing distinct structures revealed with somatostatin and tyrosine hydroxylase. (c, d) Adjacent sagittal sections through the hypothalamus and the preoptic area showing the cytoarchitecture revealed by cresyl violet staining (c) and the specific distribution of calbindin and mesotocin (arrows point to the hypophyseal tract). (e) Transverse section through the diencephalon and hypothalamus showing distinct tyrosine hydroxylase and calbindin positive structures. (f) Transverse section through the telencephalic hemisphere showing distinct somatostatin and tyrosine hydroxylase positive structures. (g) Sagittal section through the olfactory bulbs showing structures positive for tyrosine hydroxylase, and the unique calbindin labeling of the putative accessory olfactory bulbs. (h) Cells positive for tyrosine hydroxylase in the dorsal pallium. (i) Cells immunoreactive to choline acetyltransferase in the region of the pallidum. Scale $=100\,\mu$m (a, g), $400\,\mu$m (b–f), $50\,\mu$m (h, i). Abbreviations: AOB, accessory olfactory bulb; dh, dorsal hypothalamus; dl, distal lobe of the hypophysis; Dp, dorsal pallium; dt, dorsal thalamus; flm, fasciculus longitudinalis medialis; fr, fasciculus retroflexus; Hb, habenula; infr, infundibular recess; lfb, lateral forebrain bundle; Lp, lateral pallium; Ls, lateral septum; mf, Mauthner cell fiber; MOB, main olfactory bulb; Mp, medial pallium; nl, neural lobe of the hypophysis; oc, optic chiasm; on, olfactory nerve; PA, pallidum; PMg, magnocellular preoptic nucleus; POa, anterior preoptic area; ptec, pretectum; SC, suprachiasmatic nucleus; so, spino-occipital nucleus; Str, striatum; v, ventricle; vh, ventral hypothalamus; Vp, ventral pallium; vt, ventral thalamus; Xm, vagal motor nucleus.

tela choroidea except for the rostral portion, which is occupied by the cerebellum. Three main longitudinal sulci divide the hindbrain into four longitudinal zones that are imposed over the transversal segments (rhombomeres) formed during development. Traditionally, each column has been considered to serve a specific function, but in many cases cell groups of different functions may occupy positions within a single longitudinal column. We will comment on functional grouping under subdivisions of the motor and sensory modalities.

The rhombencephalon includes most of the motor cell groups that directly innervate the muscles of the head (and upper part of the trunk). The major component of this system is formed by what are classically viewed as the special visceral motor nuclei of the trigeminal (cranial nerve V), facial (VII), glossopharyngeal (IX), and vagal (or vagus X) nerves, which innervate the jaw muscles and muscles of the branchial (gill) arches. However, because this set of muscles has been demonstrated to arise from paraxial mesoderm, the hindbrain motor centers that innervate them are better considered special motor centers. They constitute a column in the lateral half of the rhombencephalic basal plate (**Figures 2**(**m**)–**2**(**p**)), in which the trigeminal motor nucleus is the most rostrally located and separated from the rest of the column by a small gap. Choline acetyltransferase immunohistochemistry reveals that the morphology of these motor cells is characterized by profuse dendritic arborizations (**Figure 3**(**a**)). Close to the rostral motor neurons of the facial nerve, a pair of large Mauthner cells (**see also Brain and Nervous System**: Motor Control Systems of Fish and Physiology of the Mauthner Cell: Discovery and Properties) is located in the reticular formation, adjacent to the entry of the octaval nerve into the rhombencephalon.

Located medially in the basal plate, slightly caudal to the facial nerve root, are the somatic motor neurons that innervate the external rectus eye muscle and constitute a small abducent (or abducens, cranial nerve VI) motor nucleus. In a position that is similar but in the caudal portion of the rhombencephalon are the somatic motoneurons whose axons form two pairs of spino-occipital nerves (**Figure 3**(**a**)) that innervate the hypobranchial muscles of the head.

A third motor modality identified in the rhombencephalon of vertebrates is represented by general visceral motor nuclei of the parasympathetic nervous system. These neurons are generally associated with the facial, glossopharyngeal, and vagal motoneurons, but in lungfishes they are difficult to identify. It is likely, however, that the small cholinergic cells revealed by immunohistochemistry at the lateral aspect of the motor column may represent these neurons.

Peripheral information regarding pain, temperature, and touch reaches the rhombencephalon and contacts somatic sensory centers. This kind of sensory information ascends through the dorsal funiculus of the spinal cord and contacts cells of an incipient dorsal column nucleus located close to the obex (**Figure 2**(**p**)). Sensory fibers of the trigeminal nerve carry similar information from the skin and deeper tissues of the head and reach sensory cells in the rhombencephalon. In lungfishes, as in most vertebrates, fibers carrying touch information would terminate in the principal sensory nucleus, located dorsolateral to the trigeminal motor nucleus. The fibers carrying pain and temperature information would enter the rhombencephalon in the trigeminal nerve and form a long descending tract in the lateral margin of the alar plate. Throughout its course, fibers from the tract would contact scattered cells that, as a group, represent the descending trigeminal nucleus. A third constituent of the trigeminal sensory system is formed by proprioceptive information from the extrinsic eye muscles and jaw muscles conveyed by primary fibers of the mesencephalic trigeminal nucleus that enter the hindbrain in the trigeminal nerve and course toward the mesencephalic tectum, where the large ganglionic somata that constitute the mesencephalic trigeminal nucleus are distributed dorsally along the tectum (**Figure 2**(**l**)).

Visceral sensory information is carried by facial, glossopharyngeal, and vagal fibers, which reach the rhombencephalic alar plate and form a descending tract known as the solitary tract (**Figures 2**(**n**) and **2**(**o**)). In relation to this tract, the nucleus of the solitary tract is formed by a column of cells that extends from the level of the facial nerve root to the obex. At the level of the vagal roots, the solitary tract and nucleus are more conspicuous and form a bulge into the ventricle termed the vagal lobe. Part of the information carried by this tract is gustatory, coming from taste buds that are located in the oropharynx and also externally on the skin of the head.

Most of the dorsal portion of the rhombencephalic alar plate is devoted to octavolateral sensory information. Lungfishes possess a complex inner ear, which is innervated by the octaval cranial nerve (VIII). They also have a series of mechanoreceptive neuromasts and electroreceptive ampullary organs that are innervated by up to six pairs of lateral line nerves. The octaval fibers enter the rhombencephalon together with the facial motor root and terminate in the ventral zone of the octavolateral area. As in most fishes, lungfishes have four nuclei: anterior nucleus, magnocellular nucleus, descending nucleus, and posterior nucleus. Only the magnocellular nucleus is easily recognized, as the other three are less distinct. The lateral line fibers enter the hindbrain in the dorsal portion of the alar plate. The dorsal roots of the anterodorsal and anteroventral lateral line nerves carry electroreceptive information, enter the dorsal portion of the octavolateral area, and form a dense neuropil, lateral to the dorsal nucleus. The ventral roots of the anterodorsal and anteroventral lateral line nerves, and the roots of the posterior lateral line nerve, carry mechanoreceptive information from neuromasts, enter the intermediate region of the alar plate, and end on cells of the medial (or intermediate) nucleus. This nucleus forms a cell column that extends throughout most of the rhombencephalon, and its caudal pole is dorsal to the solitary tract. The caudal part of the medial nucleus receives fibers of the posterior lateral line root, which enters just rostral to the glossopharyngeal root. The rostral two-thirds of the dorsal alar plate contains a typical crista cerebellaris, formed by a fiber layer similar to the molecular layer of the cerebellum, with which it is directly continuous rostrally (**Figures 2**(**l**) and **2**(**m**)).

Cerebellum

In lungfishes, the cerebellum is relatively simple compared to that in other groups of fishes, and it resembles the cerebellum in amphibians more. It comprises a pair of large auriculae that possess upper and lower leaves, the latter in continuation with the octavolateral area. These auriculae contain numerous granule cells and are particularly small in *Neoceratodus*. They are medially joined over the rostral rhombencephalon by the transverse plate of the corpus cerebelli. This region is clearly layered into molecular and granular layers, but the Purkinje cells are irregularly distributed and do not form a continuous layer. The rostroventral part of the cerebellum protrudes into the IV ventricle, forming an eminentia that has been regarded as the cerebellar nucleus, although its connections are unknown. Notably, the corpus cerebelli is particularly small in *Protopterus* and *Lepidosiren,* resembling that in urodele amphibians, and is covered by the midbrain tectum.

Isthmus

As in many vertebrates, the boundary between the hindbrain and midbrain in lungfishes is oblique, and the region between the two is generally known as the isthmic region. Actually, this region constitutes the rostral part of the rhombencephalon, and it contains a series of nuclei with different functions. Immunohistochemistry has revealed a noradrenergic locus coeruleus and cholinergic laterotegmental and isthmic nuclei (with their connections to the optic tectum and the forebrain) in lungfishes, as in amphibians. The ventral tegmentum of the isthmus possesses the rostral part of the serotoninergic raphe column, which continues throughout the rhombencephalon. A small trochlear (IV) motor nucleus, which innervates the superior oblique eye muscle, is also located ventrally in the isthmus. In the midline, just ventral to the trochlear nucleus, there is an interpeduncular nucleus (**Figure 2**(**k**)), which receives information from the habenula via the fasciculus retroflexus. A peculiar cell group found laterally in the isthmus, just in a subpial position, is termed the superficial isthmic nucleus (**Figure 2**(**k**)). Its nature and function are unknown. At least part of it was proposed to be a homolog to the isthmic nucleus of other vertebrates, but its lack of cholinergic cells does not support this contention.

Midbrain

This region of the brain in lungfishes is tubular in shape, and the walls are thick around a vertically oriented ventricle (**Figures 2**(**k**) and **2**(**l**)). The dorsal part, or tectum, is similar to that in urodele amphibians, as it constitutes only a periventricular cell layer and a superficial fiber zone. Recent experimental research, however, has shown that in spite of its simple cytoarchitecture, the tectum in lungfishes has the same heterogeneous cells as in other vertebrates. Tectal neurons containing serotonin, different calcium binding proteins, or nitric oxide synthase all have been demonstrated. In addition, fibers of retinal origin are organized in the superficial zone, whereas other tectal afferents and the efferent axons of tectal neurons are organized in the deeper fiber zones. As noted above, the conspicuous cells of the mesencephalic trigeminal nucleus occupy a long narrow strip on the dorsal portion of the tectum. A small and poorly differentiated torus semicircularis is located beneath the optic tectum and is better developed in *Neoceratodus*. Most likely, it receives mechanosensory information from the octaval and lateral line systems, as in other groups of fishes. Within the midbrain tegmentum, there is a rostral portion of the superficial isthmic cell group, and, medially, the oculomotor (III) nucleus. The latter is difficult to distinguish in normal material but has been demonstrated with experimental techniques. It should be noted that lungfishes, as do chondrichthyans, possess a large dopaminergic cell group in the midbrain tegmentum that is readily comparable to the substantia nigra-ventral tegmental cell groups in amniotes.

Forebrain

As noted above, the diencephalon comprises the caudal portion of the forebrain. Embryologically, this region is constituted by three caudorostral segments, or prosomeres, whose alar plates give rise to the pretectal area, dorsal thalamus (or thalamus), and ventral thalamus (or prethalamus), and whose basal plates form different tegmental regions that are generally smaller than the alar regions. All regions are poorly differentiated cytoarchitectonically, showing only a continuous internal zone of periventricular gray and an external zone of fibers and neuropils (**Figures 2**(**g**)–**2**(**i**)). Immunohistochemical data have highlighted a distinct diencephalic organization, however. In the first (i.e., most caudal) diencephalic segment (also called the synencephalon), several cell groups in the pretectal region have been revealed with specific markers (**Figure 3**(**e**)). As in tetrapods, these cell groups are termed according to their relationship with the posterior commissure: subcommissural, juxtacommissural, and postcommissural. They receive direct retinal information, and are better developed in *Neoceratodus* than in the other lungfishes, as is the rest of the visual system. The tegmental part of this segment houses the large neurons of nucleus fasciculus longitudinalis medialis, whose axons descend to the spinal cord.

The second diencephalic segment (posterior parencephalon) contains the large thalamus, which protrudes into the third ventricle. No subnuclei can be distinguished, although immunohistochemical reactions for different neuropeptides have demonstrated the heterogeneity of this region (**Figure 3**(**b**)). Dorsally in this segment is the large habenula, which contains abundant cholinergic cells in its dorsal part, which give rise to the habenular–interpeduncular tract. Ventrally in this segment, the posterior tubercle shows abundant dopaminergic cells. The third segment of the diencephalon (anterior parencephalon) is narrow and contains distinct cell populations within a poorly differentiated ventral thalamus (or prethalamus) in the alar plate (**Figure 3**(**e**)). The ventral portion of this segment is caudally continuous with the hypothalamic mammillary region and is called the retromammillary zone; in *Protopterus*, this zone shows a unique recess of the III ventricle, also called the posterior recess (**Figure 2**(**j**)).

Hypothalamus and preoptic area

Classically, the hypothalamus was included in the diencephalon, but contemporary neuromeric models, based on developmental data, indicate that the hypothalamus, preoptic area, and telencephalon form a single unit within the forebrain, known as the secondary prosencephalon. The hypothalamus consists of a tuberal (infundibular) portion (which is derived from the basal plate and located caudal (topologically ventral) to the optic chiasm) and an alar portion (which is formed by a suprachiasmatic region). The basal region is traditionally divided into dorsal and ventral hypothalamic parts, with respect to the infundibular ventricle (**Figures 2**(**i**), **2**(**j**), and **3**(**c**)–**3**(**e**)), and both contain immunohistochemically distinct cell populations that are similar to those described in amphibians (**Figures 3**(**c**)–**3**(**e**)). The alar hypothalamic region is mainly comprised of the suprachiasmatic nucleus, an extremely heterogeneous cell group containing distinct populations of catecholaminergic and peptidergic cells with strong projections to the intermediate lobe of the hypophysis.

The preoptic area is located topographically rostral to the suprachiasmatic nucleus. It contains a preoptic recess of the III ventricle, and in its walls cells of the magnocellular nucleus are recognized by their content of the neurohormones vasotocin and mesotocin (**Figure 3**(**d**)). These cells issue a well-developed tract that borders the chiasmatic ridge and courses in the ventral hypothalamus toward the neural lobe of the hypophysis. The rest of the preoptic area is comprised of small cells that form subgroups, distinguished only by immunohistochemistry, such as that for specific calcium-binding proteins (**Figures 3**(**c**) and **3**(**d**)).

Telencephalon

As described above, the olfactory bulbs, the pallial and subpallial regions of the hemispheres, and the telencephalon impar are all considered part of this large forebrain region. The olfactory bulbs are well developed in all lungfishes and show the cell types present in most vertebrates, as well as the organization of granular, mitral, and glomerular cell layers separated by plexiform layers. A much-debated issue regarding the olfactory system in lungfishes has been the possible presence of an accessory olfactory bulb that is cytoarchitectonically indistinct. Only recently have immunohistochemical data, based on specific labeling for calbindin (**Figure 3**(**g**)) and other olfactory markers, clearly indicated the existence of an accessory olfactory system, which may represent an incipiently segregated vomeronasal system in lungfishes, as exists in tetrapods.

The actual extent of the pallial regions in lungfishes has also been the basis for long debate. The telencephalic hemispheres are unusual in Neoceratodus, as a part of the medial wall is reduced to an ependymal membrane. The structures in the medial wall located ventral to this ependymal membrane in *Neoceratodus* and most of the thick medial wall in *Protopterus* and *Lepidosiren* have been believed by some researchers to be homologous to the septum in other vertebrates. Current data on telencephalic organization, however, strongly indicate that the medial telencephalic wall in lungfishes consists of a dorsal medial pallium and a ventral septum, as in other vertebrates. Specific immunohistochemical labeling has identified medial, dorsal, and lateral pallial regions, and, in addition, a new ventral pallial region, as defined in tetrapods (**Figure 3**(**f**)). The medial pallium extends rostrocaudally throughout most of the length of the hemisphere; it is large at rostral levels but progressively reduced at caudal levels, as the ventrally located septum becomes enlarged (**Figures 2**(**b**)–**2**(**d**)). Several immunohistochemical features allow the distinction of ventral, intermediate, and dorsal parts within the medial pallium, as in tetrapods (**Figure 3**(**f**)). Both the dorsal and lateral pallial regions are considerably small and have a periventricular cell layer of nonmigrated neurons and a cortical-like layer of cells detached from the periventricular layer (**Figures 2**(**b**)–**2**(**d**)). A peculiarity of these pallial regions is the large number of catecholaminergic neurons that they possess (**Figure 3**(**h**)). At the boundary between the lateral pallium and the subpallium, a portion of the lateral hemispheric wall has been recognized as the ventral pallium in lungfishes, due to its neurochemical similarity to that area in tetrapods (**Figures 2**(**b**), **2**(**c**), and **3**(**f**)). In amphibians, this pallial region contains the lateral amygdala (the only pallial amygdaloid component), and this is likely true in lungfishes as well.

The striatal and pallidal structures of the basal ganglia are major components of the subpallium in lungfishes. The striatum is located in the ventrolateral part of the telencephalic hemisphere; although its boundaries are unclear, it can be identified by the presence of an abundant neuropil, formed by dopaminergic fibers originating in a mesencephalic tegmental cell group (**Figure 3**(**f**)). It has a dense periventricular cell plate, but in *Protopterus* a ventral lobe is formed at caudal levels by migrated islands of cells (**Figures 2**(**b**)–**2**(**d**)). Classically, these were considered to comprise the olfactory tubercle in lungfishes, but they are at least in part a component of the dorsal striatum, and they project to the mesencephalic tegmentum. The large striatal regions in lungfishes can be divided into dorsal and ventral parts, based on cytoarchitecture. In addition, developmental markers have identified a pallidal structure in the caudoventral telencephalon, and cholinergic neurons of a basal forebrain system are also found among its cells, as in amniotes and amphibians (**Figures 2**(**d**), **2**(**e**), and **3**(**i**)).

The septal complex in lungfishes is restricted to the ventromedial wall of the hemispheres, as in terrestrial vertebrates (**Figures 2**(**c**) and **2**(**d**)). The expression of several developmental markers reveals subregions within the septum, including a large lateral septum, a small medial septum, and a specific ventral septum.

Homologs of the amygdaloid complex in amniotes have been proposed in the telencephalon of lungfishes, mainly on the basis of immunohistochemical features and some hodological data. Three distinct amygdaloid regions are characterized: (1) the lateral amygdala within the ventral pallium, which is related to secondary olfactory fibers; (2) the central amygdala, a striatal component of the amygdala in the subpallium that is mainly related to autonomic brain centers; and (3) the medial amygdala, a region in the caudal telencephalon that includes the dorsal portion of the telencephalon impar above the preoptic area; it receives a subset of olfactory information that may be comparable to that received by the vomeronasal amygdala of tetrapods (**Figures 2**(**c**)–**2**(**f**)).

The Brain of the Coelacanth *Latimeria chalumnae*

Our current knowledge of the organization of the brain in *Latimeria* is based exclusively on nonexperimental histological material, and, as *Latimeria* is both rare and endangered, it is highly unlikely that hodological or other experimental studies will be ever conducted. The general organization of the brain is very conservative, and many cell masses do not require further comment, as they are similar in location and structure to their homologs in

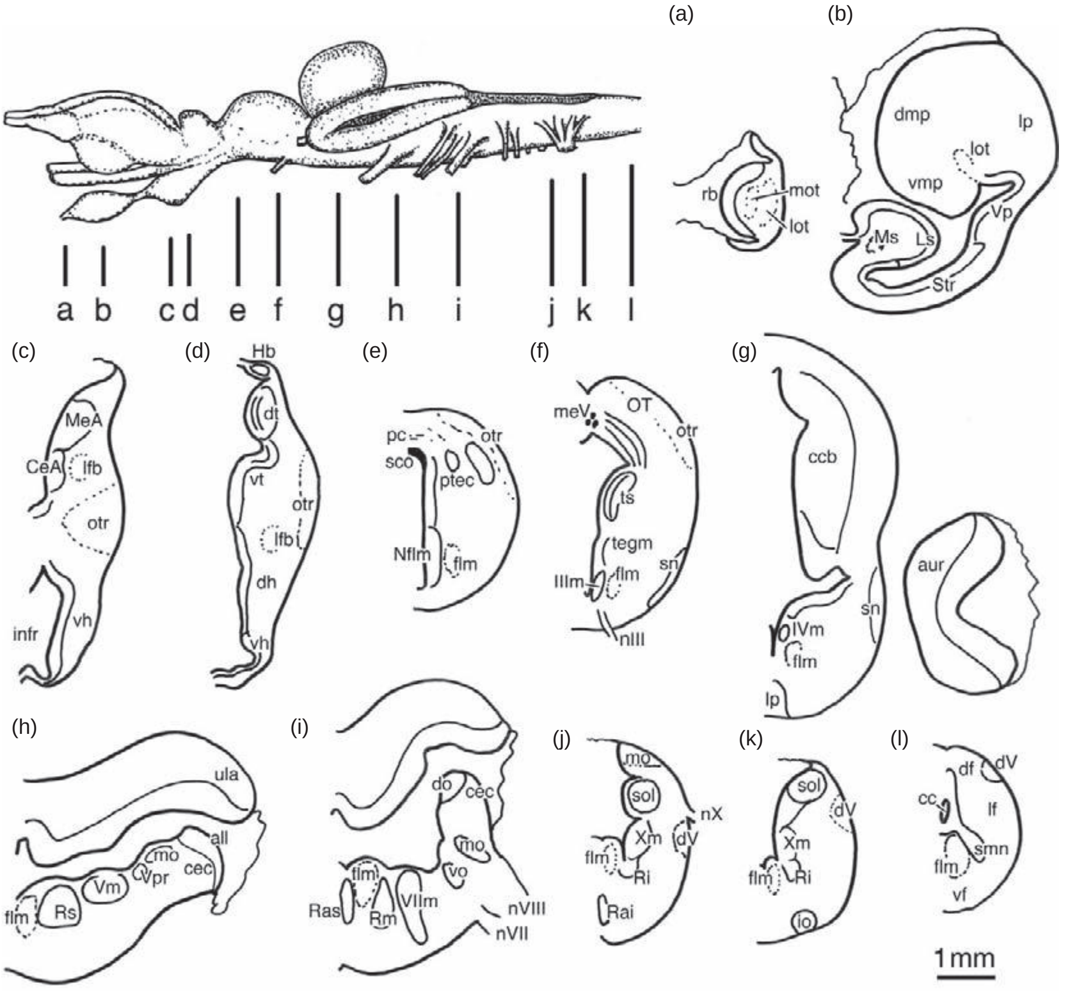


Figure 4 Diagrams of transverse left hemisections, rostral to caudal (a–l), through the brain of *Latimeria chalumnae* at levels indicated in the drawing of the brain in lateral view. Abbreviations: all, lower leaf of the auricle; aur, auricula cerebelli; cc, central canal; ccb, corpus cerebelli; CeA, central amygdala; cec, cerebellar crest; df, dorsal funiculus; dh, dorsal hypothalamus; dmp, dorsal part of medial pallium; do, dorsal octavolateral nucleus; dt, dorsal thalamus; dV, descending trigeminal tract; flm, fasciculus longitudinalis medialis; Hb, habenula; IIIm, oculomotor nucleus; infr, infundibular recess; io, inferior olive; Ip, interpeduncular nucleus; IVm, trochlear nucleus; lf, lateral funiculus; lfb, lateral forebrain bundle; lot, lateral olfactory tract; Ls, lateral septum; MeA, medial amygdala; meV, mesencephalic trigeminal nucleus; mo, median octavolateral nucleus; mot, medial olfactory tract; Ms, medial septum; Nflm, nucleus of the fasciculus longitudinalis medialis; nIII, oculomotor nerve; nX, vagal nerve; OT, optic tectum; otr, optic tract; pc, posterior commissure; ptec, pretectum; Rai, inferior raphe nucleus; Ras, superior raphe nucleus; rb, rostral body; Ri, nucleus reticularis inferior; Rm, nucleus reticularis medius; Rs, nucleus reticularis superior; sco, subcommissural organ; smn, somatomotor neurons; sn, superficial nucleus; sol, solitary tract; Str, striatum; tegm, tegmentum; ts, torus semicircularis; ula, upper leaf of the auricle; vf, ventral funiculus; vh, ventral hypothalamus; VIIm, facial motor nucleus; Vm, trigeminal motor nucleus; vmp, ventral part of medial pallium; vo, ventral octavolateral nucleus; Vp, ventral pallium; Vpr, principal sensory trigeminal nucleus; vt, ventral thalamus; Xm, vagal motor nucleus.

lungfishes and other groups of fishes (**Figure 4**). A peculiar feature of the brain in *Latimeria*, as compared to that in lungfishes, is reflected in its overall histology: in addition to periventricularly arranged nuclei, there are a number of neurons and neuronal groups that occupy peripheral (migrated) positions (**Figure 5**).

Starting from the spinal cord, *Latimeria* shows distinct dorsal and ventral horns, with intermediate gray between them. Large, scattered motoneurons are located in the ventral tip of the ventral horn (**Figures 4**(**l**) and **5**(**a**)). Notably, the spinal cord shows slight pectoral and pelvic enlargements associated with the innervation of the extraordinary paired fins.

The rhombencephalon is characterized by conspicuously developed longitudinal ventricular sulci (**Figures 4**(**j**), **4**(**k**), and **5**(**b**)). Thus, prominent ridges are formed, mainly by the development of large branchiomotor nuclei that form an almost uninterrupted series (**Figures 4**(**h**)–**4**(**k**), 5(**b**), and **5**(**c**)). A peculiarity of *Latimeria* is that the abducent rootlets emerge as ventral roots, caudal to the level of the glossopharyngeal root, and innervate a powerful basicranial muscle present in the well-developed

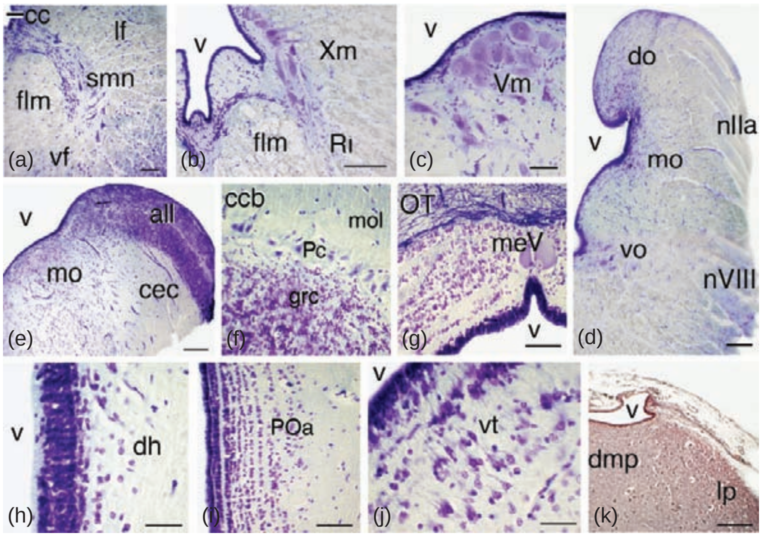


Figure 5 Photomicrographs of transverse sections, caudal to rostral (a–k), through the brain of *Latimeria chalumnae*. (a) The ventral horn of the spinal cord. (b) Detail of the vagal motor nucleus and medial longitudinal fascicle. (c) Trigeminal motor nucleus. (d) Octavolateral area and octaval and anterior lateral line nerve roots. (e) Lower leaf of the auricle and cerebellar crista. (f) Detail of the three cerebellar layers in the corpus of the cerebellum. (g) Laminated organization of the optic tectum and cells of the mesencephalic trigeminal nucleus. (h) Periventricular cell layer in the dorsal hypothalamus. (i) Layered cells around the preoptic recess. (j) Cell layers in the ventral thalamus. (k) Dorsal aspect of the pallium showing the insertion of the membranous roof. Scale = 100 μm (a, c, d, f, g, i), 200 μm (b, e, k), 50 μm (j, h). Abbreviations: cc, central canal; dh, dorsal hypothalamus; dmp, dorsal part of medial pallium; do, dorsal octavolateral nucleus; flm, fasciculus longitudinalis medialis; lp, interpeduncular nucleus; lf, lateral funiculus; mo, median octavolateral nucleus; nlla, anterior lateral line nerve; nVIII, octaval nerve; POa, anterior preoptic area; Ri, nucleus reticularis inferior; smn, somatomotor neurons; v, ventricle; vf, ventral funiculus; Vm, trigeminal motor nucleus; vo, ventral octavolateral nucleus; vt, ventral thalamus; Xm, vagal motor nucleus.

intracranial joint that divides the neurocranium. As part of the somatic motor column of the caudal rhombencephalon, a conspicuous spinooccipital nucleus gives rise to three pairs of nerves, the first pair emerging as ventral roots approximately at the level of the last vagal rootlet. These somatic motor neurons are located just lateral to the very conspicuous fasciculus longitudinalis medialis, which constitutes the most important system involved in supraspinal control, because Mauthner cells – which are present in lungfishes and most actinopterygians – are lacking in *Latimeria*.

The octavolateral area consists of three zones, similar to those described for lungfishes: dorsal, intermediate, and ventral (**Figures 4**(**h**), 4(**i**), and **5**(d)). The first two receive their input exclusively from the lateral line nerves. The anterodorsal and anteroventral lateral line nerves have dorsal roots that end only in the dorsal nucleus and carry information from an enigmatic rostral electroreceptive organ, which actually represents a collection of ampullary organs. The intermediate (median) nucleus receives mechanosensory fibers from neuromasts innervated by the ventral roots of the anterodorsal and anteroventral lateral line nerves, as well as the roots of the remaining lateral line nerves. The ventral nucleus comprises three separate cell masses: the anterior, magnocellular, and descending octaval nuclei. In the caudal part of the alar plate, the gustatory afferent fibers form small vagal lobes; together with the lack of a distinct secondary visceral nucleus, this denotes that the gustatory system is poorly developed in *Latimeria*.

The cerebellum is formed by a dome-like corpus and a large vestibulolateral lobe, the lateral parts of which constitute huge auricles with upper and lower leaves (**Figures 4**(**g**)–**4**(**i**)). While the corpus and upper auricular leaves contain the usual three cerebellar layers, the lower leaves contain mainly granule cells (**Figures 5**(**e**) and **5**(**f**)). A superficial isthmic nucleus, obviously a homolog to that in lungfishes, is also found in *Latimeria* (**Figures 4**(**f**) and **4**(**g**)).

In the midbrain, the optic tectum is relatively large and bilobed. Compared to that in lungfishes, it is better laminated, and the large perikarya of the mesencephalic trigeminal nucleus forms a dorsomedian strip in the caudal two-thirds of the tectum (**Figures 4**(**f**) and **5**(**g**)). Also better developed and better laminated is the torus semicircularis (**Figure 4**(**f**)).

A characteristic feature of the caudal forebrain in *Latimeria* is a distortion of the infundibulum, which is directed rostrally. Thus, the postoptic hypothalamus (basal hypothalamus) becomes topographically rostrobasal, located ventral to the chiasmatic ridge and extending below the telencephalon. Similarly, most of the ventricular sulci and cytoarchitectural boundaries in the diencephalon are altered, as evidenced by the rather caudal position of the asymmetrical habenulae, for example. The dorsal thalamus resembles that of *Neoceratodus* in the way it protrudes into the III ventricle and in the way its cell bodies are arranged into distinct laminae. This laminar arrangement continues, and is better specified, in the narrow ventral thalamus (**Figure 5**(**j**)). This contrasts with the ventricular lining of the large hypothalamus, which shows a thick periventricular cell layer (**Figure 5**(**h**)), clearly differing from the layered arrangement seen in the preoptic region (**Figure 5**(**i**)). Apart from the distortion of the infundibulum, the hypothalamic organization in *Latimeria* appears conservative, except for two peculiarities: the location of the eminentia media in the rostrodorsal part in the infundibular wall and the presence of a small sacus vasculosus in its ventrocaudal wall.

The telencephalon is by far the most striking portion of the brain in *Latimeria*. Because of the lack of embryonic material and experimental data, much of its organization is interpreted speculatively. Within the telencephalon impar, central and medial amygdalar nuclei (**Figure 4**(**c**)) can be identified, based on their similarity to the same-named structures recently established in lungfishes and amphibians. The hemispheres consist of a greatly thickened dorsal part (pallium) and a thin-walled ventral part (subpallium). The subpallium is the product of simple evagination, as in lungfishes and amphibians, but the pallium does not participate in this evagination, and it instead suggests the everted pallium in actinopterygians (**Figure 4**(**b**)). The subpallium shows a very simple cytoarchitecture in all its parts; a narrow layer of periventricular cells and a peripheral fiber zone are common features. The lateral part of the subpallium has been considered the striatum, and a distinct change in cell density at its dorsal border may indicate the presence of a ventral pallidum, as in lungfishes and tetrapods (**Figure 4**(**b**)). The medial portion of the subpallium comprises the septum and shows migrated cells from the ventricle that constitutes a medial septal nucleus.

The cell bodies of the pallium are evenly scattered throughout the entire thickness of this solid structure (**Figure 5**(**k**)), and three main fields have been recognized: dorsomedial, ventromedial, and lateral (**Figure 4**(**b**)). Their putative homologies with pallial fields in lungfishes or tetrapods cannot be proposed with any confidence, because of the lack of developmental or neurochemical data. Different hypotheses have been advanced, however, depending on the proposer's interpretation of a total or partial eversion process. Only the ventromedial field that surrounds the large central olfactory tract is directly comparable to the lateral pallium of tetrapods.

As mentioned above, the olfactory bulbs are small. They are located at the posterior extreme of the nasal cavity and connected by long, thin olfactory stalks or peduncles (15–20 cm long) to the so-called rostral bodies (**Figure 4**(**a**)), a pallial formation in *Latimeria* that represents the retrobulbar area in other vertebrates. Overall, given the rather small olfactory bulbs and the limited histological differentiation of the olfactory centers (rostral bodies and ventromedial pallial field), the olfactory system in *Latimeria* would appear to be only moderately developed.

See also: **Brain and Nervous System**: Motor Control Systems of Fish, Physiology of the Mauthner Cell: Discovery and Properties.

Further Reading

Butler AB and Hodos W (2005) *Comparative Vertebrate Neuroanatomy. Evolution and Adaptation*, 2nd edn. Hoboken, NJ: Wiley-Liss.

González A and Northcutt RG (2009) An immunohistochemical approach to lungfish telencephalic organization. *Brain Behavior and Evolution* 74(1): 43–55.

Moreno N and González A (2007) Evolution of the amygdaloid complex in vertebrates, with special reference to the anamnio-amniotic transition. *Journal of Anatomy* 211: 151–163.

Nieuwenhuys R (1998) Lungfishes. In: Nieuwenhuys R, Ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates,* vol. 2, pp. 939–1006. Berlin: Springer.

Nieuwenhuys R (1998) The coelacanth *Latimeria chalumnae*. In: Nieuwenhuys R, Ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates, vol. 2*, pp. 1007–1043. pp. 1007–1043. Berlin: Springer.

Northcutt RG (1987) Lungfish neural characters and their bearing on sarcopterygian phylogeny. *Journal of Morphology Supplement* 1: 277–297.

Northcutt RG (2008) Forebrain evolution in bony fishes. *Brain Research Bulletin* 75: 191–205.

Northcutt RG (2010) The central nervous system of lungfishes. In: Jørgensen JM and Joss J (eds.) *Biology of Lungfishes*, pp. 389–444. Enfield, NH: Science Publishers.

Reiner A and Northcutt RG (1987) An immunohistochemical study of the telencephalon of the African lungfish, *Protopterus annectens*. *Journal of Comparative Neurology* 256: 463–481.

Wullimann MF and Vernier P (2007) Evolution of the nervous system in fishes. In: Kaas JH and Bullock TH (eds.) *Evolution of Nervous Systems*, pp. 39–60. Amsterdam: Elsevier.

Motor Control Systems of Fish

S Grillner, Karolinska Institutet, Stockholm, Sweden

© 2011 Elsevier Inc. All rights reserved.

Goal-Directed Locomotion – Overview of Neural Control System
Concluding Remarks
Further Reading

Glossary

Basal ganglia Subcortical forebrain nuclei involved in the control of movements.

Brainstem The interface between the forebrain and the spinal cord.

Calcium-activated potassium channels Potassium channels that open when the intracellular calcium levels increase.

Central pattern generator Neuronal circuits that generate rhythmic motor output for coordination without the aid of sensory feedback.

Cerebellum Structure above the brainstem that is involved in the control of movement.

Climbing fibers Afferent fibers in the cerebellum that originate from the inferior olivary nucleus and may serve as error detectors.

Command signals A signal that can initiate activity in a CPG.

Diencephalon The more caudal of the two divisions of the forebrain. Caudal part of the prosencephalon (forebrain) associated with the third ventricle. The general subdivisions are (from dorsal to ventral): epithalamus, dorsal thalamus, subthalamus, and hypothalamus.

Disinhibition Activation that occurs as a result of a decrease in the level of inhibition.

Eurydendroid cells Efferent cells in the teleost cerebellar cortex, homologous to the deep cerebellar nuclei in mammals, which receive input from Purkinje cells and project to the brainstem and spinal cord.

Glutamatergic Neuron that uses glutamate as transmitter.

Interneurons Neurons with local axonal arborization.

Intrinsic intersegmental connectivity Synaptic interaction within a segment.

Mauthner cells Two giant neurons located in the hindbrain of most fish species. Mauthner cells usually control escape responses. However, non-Mauthner cell escapes also have been reported, and the Mauthner cells may be involved in other behaviors such as post-feeding turns.

Mesopontine Brainstem structures at the border between the mesencephalon and the pons.

Metabotropic Receptors on the postsynaptic membrane that act through G-proteins.

Mossy fibers The major afferent input to the cerebellum, originating from the brainstem and spinal cord.

Motor learning Different movements can be modified through learning.

Optic tectum Structure in the dorsal part of the mesencephalon in which a retinotopic map is formed by afferents from the eye.

Periaqueductal The canal in the mesencephalon that joins the third and the fourth ventricle is called the aqueduct. Periaqueductal refers to the location of cells around the aqueduct.

Premotor The term premotor is used to label interneurons that terminate on motor neurons.

Purkinje cells GABAergic neurons in the cerebellar cortex that constitute the sole output of motor coordination in the cerebellar cortex.

Reticulospinal neurons One of the most important descending pathways in the vertebrate nervous system. They integrate sensory inputs and higher motor commands in the brain to ultimately regulate motor functions in the spinal cord.

Goal-Directed Locomotion – Overview of Neural Control System

The nervous system coordinates different motor acts through a number of dedicated networks or modules that are specialized to coordinate specific motor patterns, be it locomotion, respiration, or eye movements. The locations of the different networks are illustrated in **Figure 1**. Most jawed fish and cyclostomes swim with an undulatory wave of muscle contraction transmitted along the body. The caudally directed movement is generated by a network that activates motor neurons in successive segments with a delay that is proportional to the duration of each movement cycle. Usually, the delay along the entire body

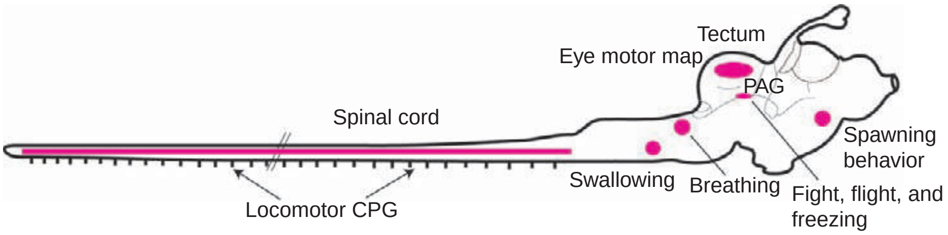


Figure 1 Motor infrastructure in cyclostomes and fish. Along the neuraxis, different motor programs/CPG networks are located that can be recruited when needed, from protective reflexes and locomotor CPG networks in the spinal cord to respiration and the saccadic eye motor map at the brainstem level. Each motor program can be recruited into action by neural mechanisms that determine when a given motor program is selected. More integrated motor patterns can be induced as fight, flight, or freezing behavior from the periaqueductal gray (PAG) and spawning behavior from part of the preopticus nuclei.

corresponds to around one undulatory cycle (**Figure 2**, left). The lag between each segment is described as a phase lag, because it is proportional to the cycle duration, whether it is 0.5 s or 100 ms (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops).

This motor pattern is generated by a central pattern-generating (CPG) network, that is, a spinal network extending throughout the spinal cord (**Figure 1**). The spinal CPG network is controlled by descending reticulospinal neurons from the brainstem, which, in turn, are activated from locomotor command regions in the diencephalon (diencephalic locomotor region (DLR)) and the mesopontine area (mesencephalic locomotor region (MLR)). The command signals issued from the MLR or DLR represent a continuous signal

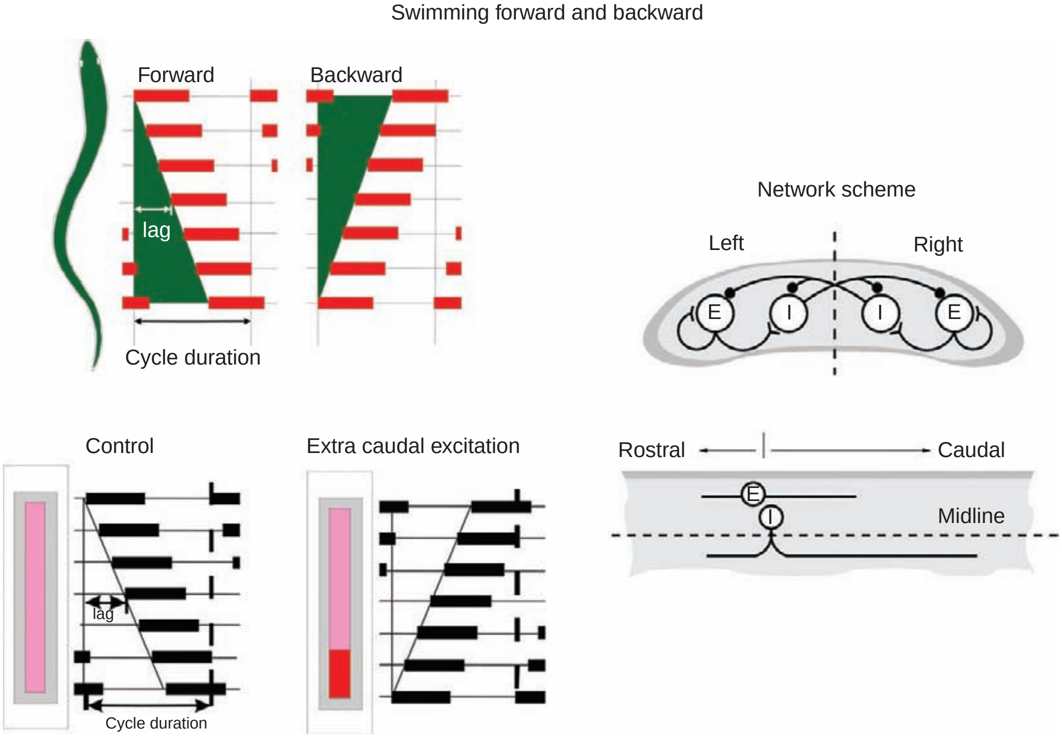


Figure 2 Intersegmental coordination through phase coupling. Cyclostomes, jawed fish, and some amphibians and reptiles swim by producing a mechanical wave that is transmitted along the body. As illustrated during forward locomotion, there is a lag between consecutive segments in the spinal cord. This lag is always a certain proportion of the cycle duration and is therefore referred to as a constant phase lag. During backward locomotion, the wave is instead propagated from tail to head. Below in the isolated spinal cord (control) a rostro-caudal phase lag also can be produced and thus the ability to generate a constant phase lag is inherent within the spinal cord. The pattern can be reversed if extra excitation is added to the caudal spinal cord (right). This is explained by the fact that the segments of the caudal part then get a higher excitability and generate a somewhat higher burst rate that is able to entrain the segmental networks in the rostral part of the spinal cord. The rostral segments have a lower inherent burst rate and are therefore entrained but with a certain lag. The schemes to the right show the connectivity of populations of excitatory (E) and inhibitory (I) interneurons at the segmental level (upper graph), and below the intersegmental connectivity of E and commissural I interneurons.

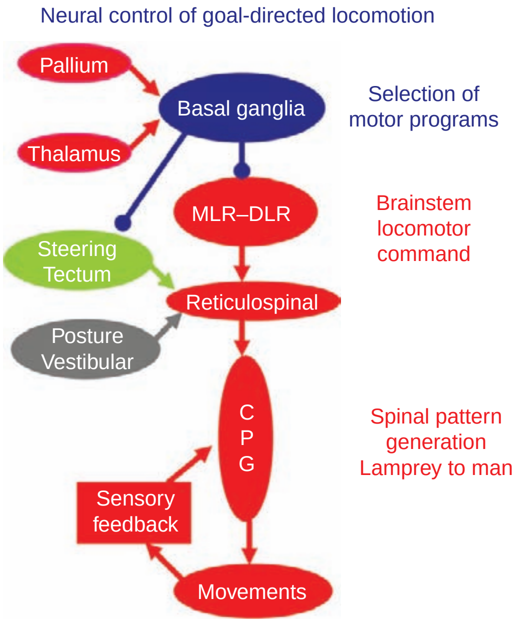

Neural control of goal-directed locomotion
Pallium
Basal ganglia
Selection of motor programs
Thalamus
MLR–DLR
Brainstem locomotor command
Steering Tectum
Reticulospinal
Posture Vestibular
C P G
Spinal pattern generation Lamprey to man
Sensory feedback
Movements

Figure 3 Goal-directed locomotion – components of the control system. Subsystems are involved in the control of goal-directed locomotion. Selection of a motor program is performed in the basal ganglia, which receives inputs from the cortex (pallium) and the thalamus. The basal ganglia output stage (pallidum) inhibits command centers in the diencephalic locomotor region (DLR) and mesencephalic locomotor region (MLR) during resting conditions. Through a well-controlled inhibition of pallidal regions, the spinal CPG network for locomotion can be activated via the reticulospinal (RS) neurons. In the brainstem, information is further integrated based on visual, sensory, and vestibular inputs to control both steering and posture. In all vertebrates, the spinal cord CPG neurons are modulated by local sensory feedback.

that turns on the network. The level of activity in the command centers determines the degree of activation of the CPG network and thus determines swimming speed (**Figures 3** and **4**). The complex of MLR/DLR, reticulospinal neurons, and the spinal CPG network constitutes the circuit controlling the propulsion itself (**Figure 4**). To be behaviorally meaningful, the locomotor movements also need to be steered toward the goals of different types. The different components of the neural control system are detailed in the following.

Spinal CPG Network and the Locomotor Pattern Generation

Trunk movements

The motor pattern produced by the spinal CPG network during forward locomotion (**Figure 2**) is generated by a network composed of a population of excitatory glutamatergic premotor neurons and inhibitory commissural interneurons (**Figure 4**, left). The intrinsic connections within the segmental pools of excitatory (E) interneurons (red) give rise to the burst activity in each segment, if in addition they are activated from supraspinal sources. The termination of the burst depends on the intrinsic membrane properties of these cells and particularly one subtype of ion channel, the calcium-activated potassium channels that become progressively activated by the calcium entry occurring during each burst (**Figure 4**, right). The coordination of the alternating activity in each segment depends on the commissural inhibitory neurons, which use glycine as their transmitter (inhibitory (I) interneurons (blue), **Figure 4**). When there is burst activity on one side, the commissural inhibitory neurons inhibit the contralateral side, but as the burst terminates, the other side becomes disinhibited and thus starts bursting.

The coordination along the spinal cord results in the progressive activation of successive segments during locomotion and is generated by the intrinsic intersegmental connectivity (**Figure 2**, right) in that both excitatory and inhibitory interneurons have axons projecting over several segments. The isolated spinal cord generates forward locomotor coordination that has been detailed in modeling experiments.

Although the spinal network generates the coordination underlying forward locomotion under ordinary conditions, it can instantaneously switch to backward locomotion with an undulatory wave instead propagated from tail to head (**Figure 2**). How can this be explained? It turns out that the flexibility can be explained by a simple control mechanism; the active spinal cord receives some inhibition of the most rostral segments. Their lower excitability makes them become activated after the segments with higher excitability located in a more caudal direction. Because the spinal cord network functions as a unit, this reverses the phase lag along the entire spinal cord.

Dorsal fins

The dorsal fins increase the lateral surface of the body during swimming, and thereby provide stability but at the expense of increasing drag (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability). Their position in relation to the body during movement is stabilized by an exclusive set of fin motor neurons that are activated in antiphase with the motor neurons controlling the trunk muscles during locomotion. This is required to stabilize the vertical position of the dorsal fins in relation to the body movement.

In some teleost species, the dorsal fin extends along most of the body (e.g., *Gymnarchus niloticus*), and an undulatory wave can actually be transmitted along the entire dorsal fin, in either a backward or a forward direction, moving the fish in the corresponding direction. Instead of having only one undulatory wave, as in trunk locomotion, there are many small amplitude waves transmitted along the fin.

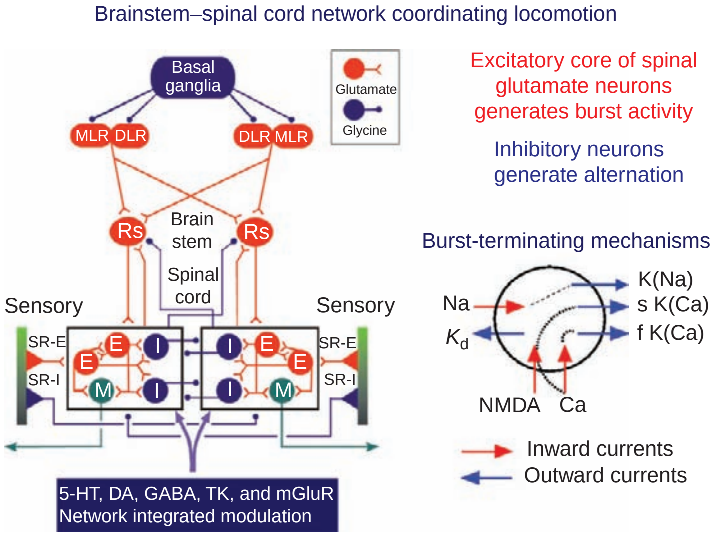

Brainstem–spinal cord network coordinating locomotion
Basal ganglia
MLR
DLR
DLR
MLR
Glutamate
Glycine
Excitatory core of spinal glutamate neurons generates burst activity
Inhibitory neurons generate alternation
Rs
Brain stem
Rs
Spinal cord
Sensory
Sensory
SR-E
SR-I
E
E
I
M
I
I
E
E
I
M
SR-E
SR-I
5-HT, DA, GABA, TK, and mGluR
Network integrated modulation
Burst-terminating mechanisms
Na
K_d
K(Na)
s K(Ca)
f K(Ca)
NMDA
Ca
Inward currents
Outward currents

Figure 4 Locomotor network of the lamprey: schematic representation of the segmental neural circuitry that generates rhythmic locomotor activity. All neuron symbols denote populations rather than single cells. The reticulospinal (RS), glutamatergic neurons excite all classes of spinal interneurons and motor neurons. The excitatory interneurons (E; red) excite all types of spinal neurons, that is, the inhibitory glycinergic interneurons (I: blue) that cross the midline to inhibit all neuron types on the contralateral side, including motor neurons (M). The stretch receptor neurons are of two types – one excitatory (SR-E), which excites ipsilateral neurons, and the other inhibitory (SR-I), which crosses the midline to inhibit contralateral neurons. RS neurons receive excitatory synaptic input from the diencephalic and the mesencephalic locomotor regions (DLR and MLR), which in turn receive input from the basal ganglia as well as visual and olfactory input. In addition, metabotropic receptors are also activated during locomotion and are an integral part of the network (5-HT, GABA, tachykinins (TK), and mGluR). (Right) Several factors contribute to burst termination. Calcium entering via voltage-gated calcium channels during the action potential activates calcium-dependent potassium channels (f KCa) that underlie the postspike after-hyperpolarization (AHP). During the burst, AHP summation leads to frequency adaptation and contributes to burst termination. During *N*-methy-D-aspartic acid (NMDA) receptor activation, KCa channels are also activated by calcium entering through NMDA channels and contribute to NMDA plateau termination. In addition, sodium entry activates sodium-dependent potassium channels (KNa) that may also contribute to burst termination.

Pectoral fins

Whereas the lampreys have no paired fins, elasmobranchs such as sharks and dogfish use their large extended fins to maneuver. By changing the position of the left and right pectoral fins respectively, they can change the instantaneous direction of locomotion and also stabilize their body position. In contrast, rays have pectoral fins enlarged along a large part of the body and swim by propagating an undulatory wave along each fin.

Teleosts have fins that are much more flexible and can be used for very slow locomotion or for positioning themselves in relation to different objects, as when, for instance, they nibble on a piece of seaweed or a snail on a rock. When swimming rapidly, teleost fish generally keep their fins close to the body to reduce drag. The design of the neural networks controlling the pectoral fins has not, as yet, been investigated in any detail, although it should be of major interest as they are a precursor of tetrapod forelimbs (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability).

Escape swimming

In lamprey, visual stimuli can elicit a rapid change in swimming direction (escape swimming). This rapid response does not depend on processing in the optic tectum, but requires intact pretectal nuclei (see also the dorsal light response below) that, in turn, activate brainstem reticulospinal neurons and Mauthner cells. Escape swimming elicited through other stimuli, such as vibration in the water, is mediated directly at the brainstem level to the very large cells (i.e., the Mauthner cells) with a contralateral axon that projects down the spinal cord to activate contralateral motor neurons along the spinal cord (**see also Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties and Physiology of the Mauthner Cell: Function). This system provides the fastest avenue to generate a rapid escape response, which is a fast turning to the opposite side combined with fast locomotor movements utilizing the spinal CPG network.

Steering and the Related Orienting and Eye Motor Control

In order for locomotion to be behaviorally meaningful, it must also be steered toward different points of interest. In order to steer the body to the left or the right, the motor activity must be asymmetric, so that the body is bent for a

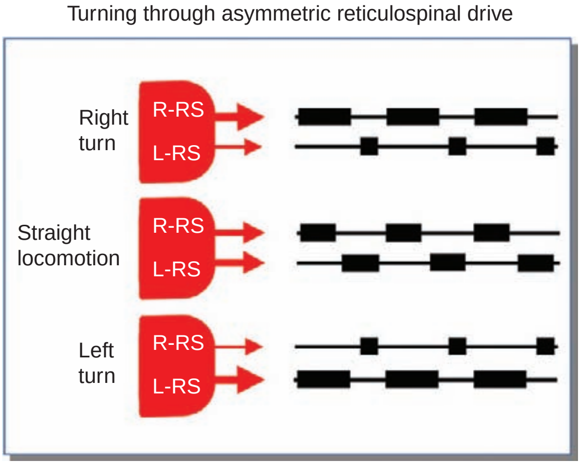


Figure 5 Neural mechanism for steering – asymmetric activation of reticulospinal command. The locomotor command system elicits a symmetric reticulospinal activity on the right (R-RS) and left (L-RS) side (middle trace – straight). A steering command to the right is produced by any signal that enhances the reticulospinal activity on the right side and/or lowers it on the left side (right turn). The converse changes would produce a turn to the left (left turn).

short while to the left or the right side. This is achieved through an asymmetric activation of the reticulospinal neurons, which then in turn activate motor neurons of one side more than the other (**Figure 5**).

If a fish sees something that calls for action such as a prey, there needs to be an interface to the motor system to generate a steering command appropriate to reach the point of interest. In this context, not only vision but also other senses are important (electroreception, input from the lateral line, olfaction, etc.). A structure that is of critical importance in this context is tectum mesencephali (**see also Vision**: Optic Tectum) and the retinotopic map formed in it by the afferents from the optic nerve projecting onto its contralateral superficial layers (**Figure 6**). This mapping is achieved through direct projection from the retinal ganglion cells.

Aligned with the sensory map, there is a motor map in the deeper layers of tectum with efferent neurons projecting out of tectum in the form of a motor map. In the goldfish, important output targets are the vertical and horizontal gaze centers, which are evolutionary conserved structures in all vertebrates. They are activated through interneurons in the cranial eye motor nuclei (no. III, IV, and VI) of both the eyes in a coordinated fashion to produce a movement of the eye directed to any point in the visual space and also the accompanying orienting movements of the trunk mediated via an asymmetric activation of reticulospinal neurons. The latter represent movements of the head and trunk that orient the body toward the point of interest, similar to the human head and neck movements. Orienting movements occurring during ongoing swimming result in turning that steers the body toward the point of interest, that is, it is goal-directed behavior.

If the tectum and the parts of the locomotor system that we just have discussed constituted the entire control system, the fish would automatically swim toward any

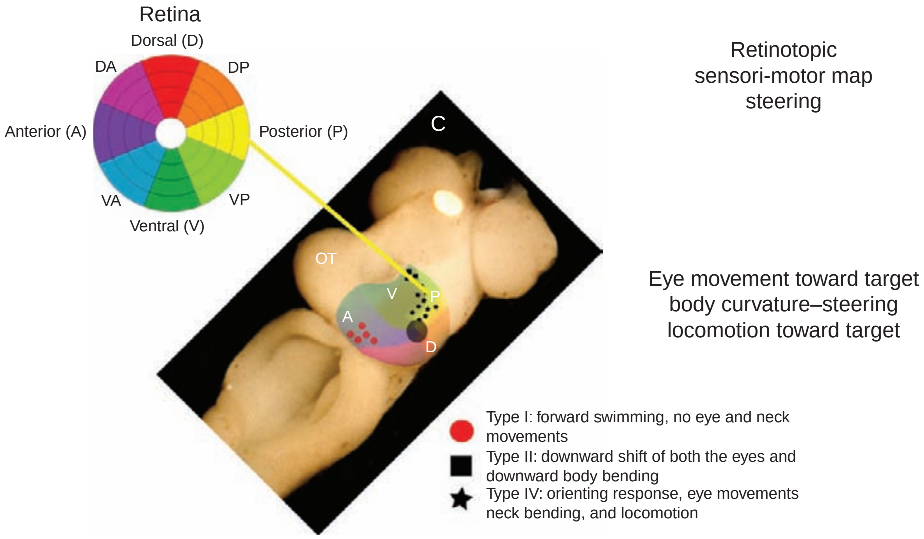

Retina
Dorsal (D)
DA
DP
Anterior (A)
Posterior (P)
VA
VP
Ventral (V)
C
OT
V
P
A
D
Retinotopic sensori-motor map steering
Eye movement toward target body curvature–steering locomotion toward target
● Type I: forward swimming, no eye and neck movements
■ Type II: downward shift of both the eyes and downward body bending
★ Type IV: orienting response, eye movements neck bending, and locomotion

Figure 6 Retinotopic sensori-motor map – relation to steering. The different parts of retina (color coded) project onto different parts of tectum as indicated in color. The image of the lamprey brainstem is from above with the fourth ventricle in the lower left. The different symbols superimposed on the retinotopic map indicate different motor responses that can be elicited from the different regions as also indicated in the figure. Activation of a certain part of retina results in eye movement and the accompanying orienting movement. If this occurs during locomotion, this steers the movements toward the object of interest. Modified from Jones M, Grillner S, and Robertson B (2009) Selective projection patterns from subtypes of retinal ganglion cells to tectum and pretectum: Distribution and relation to behaviour. *Journal of Comparative Neurology* 517: 257–275.

target that would appear in the retina. This is clearly not the case. One structure that takes part in selecting the appropriate motor behavior in a given instant is the basal ganglia, as discussed separately below.

Control of Body Orientation

All vertebrates from lamprey to man have the ability to control body orientation during movement, mostly with the back maintained in a dorsal position. In contrast, the vertebrate-like amphioxus (Cephalochordate), which has a spinal cord but a poorly evolved brain, is unable to control the position of its body, and swims in a spiral-like fashion, because it lacks the most important sensor in this context, the vestibular apparatus (**see also Hearing and Lateral Line**: Vestibular System Anatomy and Physiology).

The vestibular apparatus compares inputs from the two sides of the body in relation to gravity. The vestibular afferents project to the vestibular nuclei of the brainstem that contain vestibular interneurons projecting to neurons on the ipsi- and contralateral sides. They, in turn, activate reticulospinal neurons that project to spinal motor neurons (**Figure** 7, right). If the body is tilted to one side, the vestibular input from the two sides becomes asymmetric and signal this (via interneurons) to affect the level of activity of the reticulospinal neurons, so that their activity increases on one side and decreases on the other side, as shown by the recordings from reticulospinal cells and the left and right side (**Figure** 7, uppermost). As the body is tilted, the activity of the neurons on one side increases, whereas it decreases on the other side. A tilt to one side leads to a compensatory movement that returns the body to a position with the dorsal side upward. **Figure** 7 (lower right) illustrates the balance between the two sides.

The reticulospinal cells are in fact not one entity, but have four distinct nuclei located in the mesencephalon and the anterior, middle, and posterior nuclei of the rhombencephalon (**Figure** 7, upper left). These four nuclei are activated maximally by different degrees of tilt. Some nuclei are activated at 45° and others at 90° of tilt, while the anterior rhombocephalic nucleus actually is activated when the fish is upside down (180°). This illustrates that these different nuclei are specialized to correct for different tilt angles.

Dorsal light response

Although fish normally stabilize the body with dorsal side up, under particular conditions they stabilize at an angle. Behaviorally this can be explained by the color of the fish body. Predators such as birds find it more difficult to see

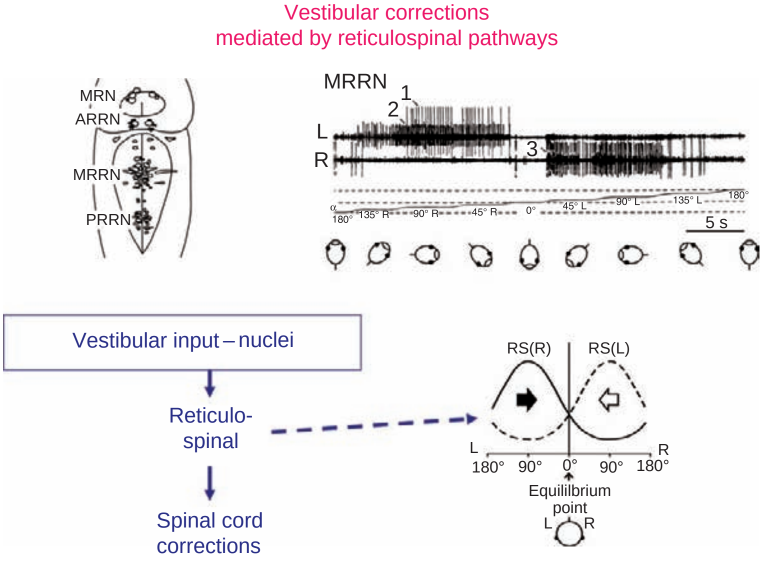


Figure 7 Vestibular corrections mediated by reticulospinal pathways. Schematic representation of the control systems for control of body orientation. The vestibular system plays a key role in the control of body orientation during locomotion. A dorsal view of the brainstem is shown (upper left) with the reticular nuclei (MRN (mesencephalic reticular nuclei), anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei). (Right) Simultaneous extracellular recordings from MRRN reticulospinal neurons on the left (L) and right (R), while the brainstem is rotated (full cycle, 360°). Note that when the dorsal side is upward (0), the activity on both sides is similar (low). The gravistatic sensors sense the orientation of the head and thereby detect any deviation of the position of the head from the dorsal side up position. Via vestibular interneurons, they activate reticulospinal neurons. With the dorsal side up, the input from the vestibular receptors is symmetric and thereby the activity of the reticulospinal system. In the diagram, the activity of the reticulospinal populations on the right (RS(R)) and on the left side (RS(L)) are represented. Any deviation to the left or right side leads to an asymmetric activity of the left and right reticulospinal populations. This asymmetry leads to a correction of the body position. Modified from Deliagina TG, Beloozerova IN, Zelenin PV, and Orlovsky GN (2008) Spinal and supraspinal postural networks. *Brain Research Review* 57: 212–221.

fish when viewed from above due to its dark color, while fish predators have the same difficulty from below due to the whitish abdomen (**see also Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish). However, during sunrise or sunset when light comes from the side, these safety mechanisms are inappropriate, and it is advantageous to rotate the body so that the back faces the sun. This is achieved by changing the balance between the two sides, so that the equilibrium point is stabilized at an angle different from vertical.

The visual input that detects the asymmetry of the incoming light is projected to the pretectum, a small group of nuclei in front of tectum that convey the asymmetry signal to the reticulospinal cells, thereby creating a bias to one side or the other. Most likely, the dorsal light response is a dynamic motor response, because the fish needs to continuously change its orientation as it swims around at sunset or sunrise.

Respiration

The respiratory centers in the brainstem activate motor neurons controlling the respiratory muscles that act primarily via the cranial nerves (no. VII, IX, and X). The respiratory movements differ between groups, such as the gill movements in teleost fish, the water passage through gill slits in elasmobranchs, and movements of water in and out of the seven gill pouches of the lamprey (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish).

In lampreys, an enhanced respiratory activity occurs in anticipation of a bout of locomotor activity – the increased respiratory activity in conjunction with the locomotor activity is thus preprogrammed rather than occurring in response to increased metabolic demand.

Details of the control of respiration are covered in a separate article (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish).

Jaw Control, Sucker Mouth, and Feeding

Jawed fish can pick up small and large objects, and some fish use their jaws to bite smaller or larger parts out of a prey and bring them into the mouth. Many fish such as sharks can generate exceptionally strong power in their jaw-closer muscles (**see also The Muscles**: Cartilaginous Fishes Cranial Muscles). However, fish, in contrast to mammals, have not developed the ability to chew food. The rich taste receptor innervation in the mouth cavity allows the fish to sense whether the object brought into the mouth is edible, and, if so, it is swallowed but otherwise rejected (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior). Some fish, such as carp and goldfish that feed from detritus and mud in ponds, have developed an intricate intraoral system to filter out inedible particles, while retaining and swallowing the edible ones.

The adult form of some lampreys is parasitic, and they develop the ability to actively suck by applying negative pressure with their sucker mouth, which is equipped with several layers of teeth (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and Lampreys: Environmental Physiology). In the middle of the mouth there is a stiff and rough tongue that can be moved rhythmically to make a hole in fish skin. The lamprey applies rhythmic suction, resulting in fluid and tissue movements from the prey into its mouth and esophagus. The mechanisms underlying attachment and suction depend on the trigeminal motor system, but the details of the brainstem neural control system remain to be elucidated.

Migration and Spawning

Some fish remain in the same general area throughout life, whereas fish such as eels and salmon migrate thousands of kilometers (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle, Eel Migrations, and Tracking Oceanic Fish). They start from where they hatch to move to feeding grounds far away, and after many years they return to the their birth place. Similarly, many species of lamprey are born in small brooks and spend their first years of life as filter feeders buried in the mud except for the head. After metamorphosis, they migrate down to the mouth of the river and the ocean. Little is known regarding the neural control of these behaviors, but it is well documented that lamprey identify suitable rivers to migrate upward by the detection of species-specific bile acids in the water secreted by young lampreys upstream (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior; **Hagfishes and Lamprey**: Lampreys: Energetics and Development; and Lampreys: Environmental Physiology). These bile acids are detected at very low concentrations, and may also serve to identify the location of potential mates.

Different species of fish display different characteristics and stereotyped spawning behavior (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish). Electric stimulation in the ventral telencephalon and the preoptic nucleus of sexually primed individuals can instantaneously trigger different steps of the spawning behavior in both male and female salmon. Male and female position themselves in the nest close to each other while vibrating the body at a high frequency – followed by oviposition and sperm release. The neural structures that trigger sexual patterns of behavior when stimulated electrically are rich in gonadotropin-releasing hormone.

Periaqueductal Gray – Fight, Flight, and Freezing Responses

Stimulation of the periaqueductal gray (PAG), which is located in mesencephalon around the cerebral aqueduct (the ventricular canal that joins the third and fourth ventricles), appears to trigger specific patterns of motor behavior. For instance, stimulation of different parts of the PAG in zebrafish can trigger fight, flight, or freezing responses. Neurons in these different PAG compartments can activate different specific motor patterns (e.g., goal-directed locomotion): in the case of fight, a set of aggressive motor patterns must be recruited that need to be adapted to the foe. The PAG areas probably represent downstream command centers that can be used by higher-order control from telencephalic nuclei such as the amygdala, a structure known to be involved in fear (from fish to humans), and hypothalamic involvement can similarly be implied.

Neural Structures of Particular Relevance for the Motor System

In passing, we have noted the importance of different structures in the spinal cord and brainstem, where most of the different motor programs are elicited. What we have not dealt with so far are the mechanisms by which the brain can decide to select, and thereby recruit a given motor program. Here, the basal ganglia appear to play a critical role. We will therefore deal with this structure separately. The second issue that we have not considered is motor learning, which can occur at two levels – learning to calibrate integrated processes such as the vestibulo-ocular reflex and learning specific new motor sequences or associations with what may be required for feeding in a particular location. For the former type, the cerebellum is often involved, and for the second type of motor learning, both the cerebellum and the basal ganglia usually are involved **(see also Sensory Systems, Perception, and Learning**: Fish Learning and Memory**)**.

Basal ganglia

The structure and function of the basal ganglia, located in the forebrain, appear very similar throughout the vertebrates from lamprey to mammals. For the sake of simplicity we consider only the input level, the striatum, and the output level, the pallidum (see **Figure 8**; in reality, each can be subdivided into different components). The output level is unusual in that it consists of tonically active inhibitory neurons (gamma-aminobutyric acid (GABA)) that project to all the different command centers in the brainstem that control different motor programs, such as tectum, DLR, and MLR. The rationale for this is that the different motor programs need to be controlled so that they are not activated accidentally. A precondition for a motor program to be activated is thus to remove the pallidal inhibition. This is the task of striatum, which contains GABAergic neurons that instead are silent at rest. When a group of striatal neurons are activated, however, they will inhibit a subpopulation of pallidal neurons, thereby effectively removing the inhibition from a given motor center, which then becomes free to operate.

What activates the striatum? It receives fast synaptic input from the pallium (analogous to the layered cortex in mammals) and directly from thalamus. These two

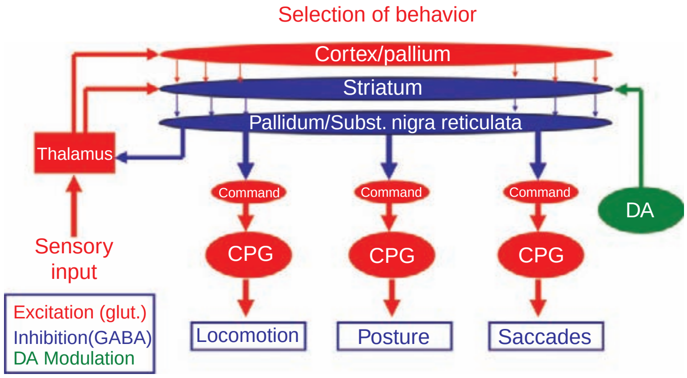


Figure 8 The basal ganglia control CPG activity – schematic diagram. The cerebral cortex in mammals and the corresponding structures in lower vertebrates (pallium) have an excitatory (red) output to striatum, the input layer of the basal ganglia, that is also directly activated by the thalamus. Striatum consists of inhibitory (blue) GABAergic neurons (95% of the extrinsically projecting medium spiny neurons), which have a very high threshold for activation. They are therefore difficult to activate, and may be regarded as a filter for cortical input. The striatum is composed of different subpopulations of neurons. They inhibit the output layer of the basal ganglia, the pallidum, which instead consists of GABAergic neurons with a very high level of resting activity keeping different CPG networks under tonic inhibition. The CPG activity is only released when the appropriate striatal subpopulation is activated and inhibits pallidum, resulting in a disinhibition of the CPG networks. The dopamine (DA) input to striatum has a very powerful effect in controlling the responsiveness of striatal neurons. The basal ganglia thus have a very important role in determining which CPG networks are active at a given instant. This diagram only includes what often is referred to as the indirect loop that the dopamine input inhibits.

excitatory inputs converge onto the striatal output neurons and determine whether they will become activated or not. There is a third factor, however, the dopamine neurons that also innervate striatum, and their effect is to determine the responsiveness of the striatal output neurons. If the dopamine innervation is deficient, it becomes more difficult to activate them and then also to activate the different motor programs. In humans, this condition is known as Parkinson's disease, and similar symptoms occur in fish and lamprey. Both with regard to structure and function, the basal ganglia are thus conserved throughout the vertebrate evolution. All evidences suggest that the striatum plays a prominent role in the selection of which motor programs are turned on at a given instant.

Cerebellum

In lampreys, the cerebellum is very small, whereas it is well developed in jawed fish (**see also Brain and Nervous System**: Functional Morphology of the Brains of Agnathans and Functional Morphology of the Brains of Ray-Finned Fishes). The critical cellular elements are arranged in a similar way in jawed fish as in mammals. Briefly, the input from the spinal cord and brainstem carries information about ongoing movements and activity in central motor circuits. In the general case, the input is of two types – one via mossy fibers that terminate on granular cells that in turn project to the output cells of cerebellum, the Purkinje cells, and the other via climbing fibers that project directly to the Purkinje cell dendrites. The Purkinje cells are inhibitory (GABAergic) and project to the efferent cerebellar nuclei. They, in turn, are excitatory and project to the spinal cord and brainstem nuclei that affect movement. In ray-finned fish, the deep cerebellar nuclei are absent. Cells that may be homologous to them are located within the cortex of the cerebellum and are called eurydendroid cells; they receive input from Purkinje cells and project to spinal cord and brainstem sites.

In many movements such as locomotion, the microcircuit from mossy fibers via Purkinje cells to motor nuclei operates during each movement cycle. This circuit can thus modify the motor pattern. The strength by which this circuit can modify a given movement is changed through synaptic plasticity. An error signal results if a movement is not well calibrated (e.g., if the vision become blurred due to the inappropriate eye movements). A separate set of cerebellar afferents, the climbing fibers, serve as error detectors. Each climbing fiber projects to a small number of Purkinje cells, and a climbing fiber activation affects the entire Purkinje cell and leads to changes in the efficacy of the synaptic input via the mossy fiber pathway (on the granular cell synapse on Purkinje cells). This constitutes the cellular basis of learning in cerebellar cortex; in addition, synaptic plasticity also operates at the level of the cerebellar nuclei. One function of cerebellum appears to take part in the fine tuning (learning) of sensory motor pathways dynamically activated during locomotion and other motor systems.

Concluding Remarks

This brief overview has focused on the neural basis of motor behavior in lamprey and jawed fish. Needless to say, there are a number of adaptations of the motor repertoire in different species, and in this brief article only the most general aspects are included.

See also: **Brain and Nervous System**: Functional Morphology of the Brains of Agnathans; Functional Morphology of the Brains of Ray-Finned Fishes; Physiology of the Mauthner Cell: Discovery and Properties; Physiology of the Mauthner Cell: Function. **Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops; Maneuverability. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish. **Fish Migrations**: Eel Migrations; Pacific Salmon Migration: Completing the Cycle; Tracking Oceanic Fish. **Hagfishes and Lamprey**: Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Hearing and Lateral Line**: Vestibular System Anatomy and Physiology. **Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish; Fish Learning and Memory. **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior. **Social and Reproductive Behaviors**: Sexual Behavior in Fish. **The Muscles**: Cartilaginous Fishes Cranial Muscles; **Vision**: Optic Tectum.

Further Reading

Deliagina TG, Beloozerova IN, Zelenin PV, and Orlovsky GN (2008) Spinal and supraspinal postural networks. *Brain Research Review* 57: 212–221.

Dubuc R, Brocard F, Antri M, *et al*. (2008) Initiation of locomotion in lampreys. *Brain Research Review* 57: 172–82.

Fetcho JR (2008) Zebrafish and motor control over the last decade. *Brain Research Review* 57: 86–93.

Finger TE (2008) Sorting food from stones: The vagal taste system in Goldfish, *Carassius auratus. Journal of Comparative Physiology* 194: 135–143.

Gabriel JP, Mahmood R, Walter AM, *et al*. (2008) Locomotor pattern in the adult zebrafish spinal cord *in vitro*. *Journal of Neurophysiology* 99: 37–48.

Grillner S (2003) The motor infrastructure: From ion channels to neuronal networks. *Nature Reviews Neuroscience* 4: 573–586.

Grillner S, Hellgren J, Ménard A, Saitoh K, and Wikström M (2005) Mechanisms for selection of basic motor programs – roles for the striatum and pallidum. *Trends in Neurosciences* 28: 364–370.

Grillner S, Wallén P, Saitoh K, Kozlov A, and Robertson B (2008) Neural bases of goal-directed locomotion in vertebrates – an overview. *Brain Research Reviews* 57: 2–12.

Jones M, Grillner S, and Robertson B (2009) Selective projection patterns from subtypes of retinal ganglion cells to tectum and pretectum: Distribution and relation to behaviour. *Journal of Comparative Neurology* 517: 257–275.

Kozlov A, Huss M, Lansner A, *et al*. (2009) Simple cellular and network control principles govern complex patterns of motor behavior. *Proceedings of the National Academy of Sciences of the United States of America* 106: 20027–20032.

Martel B, Guimond JC, Gariépy JF, *et al*. (2007) Respiratory rhythms generated in the lamprey rhombencephalon. *Neuroscience* 148: 279–293.

Mentel T, Cangiano L, Grillner S, and Buschges A (2008) Neuronal substrates for state-dependent changes in coordination between motoneuron pools during fictive locomotion in the lamprey spinal cord. *Journal of Neuroscience* 28: 868–879.

Rovainen CM (1979) Neurobiology of lampreys. *Physiological Reviews* 59: 1007–1077.

Wang G, Shen L, and Wu Y (2007) Research on swimming by undulatory long dorsal fin propulsion. *Frontiers of Mechanical Engineering in China* 2: 77–81.

Physiology of the Mauthner Cell: Discovery and Properties

AE Pereda and DS Faber, Albert Einstein College of Medicine, Bronx, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Mauthner Cell
The Mauthner Cell As Model Vertebrate Neuron
Summary
Further Reading

Glossary

Antidromic Propagation of an impulse along an axon in a direction that is the reverse of normal. Electrical stimulation either of peripheral nerves or of white matter tracts has long served as a mechanism for electrophysiologically identifying the projections of recorded neurons.

Chemical synapse Modality of synaptic transmission whereby transfer of information from presynaptic neurons is mediated by depolarization-induced release of a neurotransmitter, which in turn activates specific receptors located on the postsynaptic membrane.

Cholinergic A general term pertaining to processes or structures related to acetylcholine. For example, neurons that release acetylcholine as transmitter. Nicotinic and muscarinic receptors are cholinergic receptors because they bind acetylcholine. Cholinergic synapses are found in both the central and peripheral nervous systems.

Electrical synapse Modality of synaptic transmission whereby transfer of information is mediated by intercellular channels known as gap junctions, which provide a pathway of low resistance for the spread of presynaptic electrical currents.

Ephaptic Functional interaction between neighboring neurons where the electrical field generated by one neuron influences the excitability of the second.

Glycinergic Synapse that uses glycine as its neurotransmitter, one of the major inhibitory neurotransmitters in the spinal cord and the brainstem.

Reticulospinal neurons One of the most important descending pathways in the vertebrate nervous system. They integrate sensory inputs and higher motor commands in the brain to ultimately regulate motor functions in the spinal cord.

Rhombomeres Distinct segments of the embryonic neural tube in the area that will eventually become the rhombencephalon, or hindbrain. The neural crest cells from each rhombomere do not intermingle and give rise to different ganglia or groups of neurons.

Time constant Index that expresses how fast the membrane potential of a neuron changes in response to changes in an applied transmembrane current. The time constant (*t*) can be calculated as the product between membrane resistance and membrane capacitance. As with other RC circuits, it expresses the time needed to charge the capacitor through the resistor to ~63% of full charge, or to discharge it to ~37% of its initial voltage (values derived from the mathematical constant *e*). Thus, due to the presence of the membrane capacitance, the voltage across the membrane usually lags behind current injections. Time constants are usually fast in neurons that require high temporal precision.

Introduction

During the nineteenth century, comparative anatomical analyses in fish and amphibians led to major breakthroughs in the understanding of the vertebrate nervous system. As part of this effort, the young Austrian medical student Ludwig Mauthner (**Figure 1(a)**) stumbled upon the presence of two large, in fact colossal, axons in the fish spinal cord (**Figure 1(b)**). He was at the time an associate in the laboratory of Ernst Brucke, who, together with Carl Ludwig, Emil Du Bois-Reymond, and Hermann Helmholtz, constituted an influential group of scientists that provided the grounds for the modern understanding of the mechanisms underlying physiological processes. In opposition to the then prevalent dogma of vitalism (the life force which gave vitality to all living organisms), they adopted the view that all physiological processes were potentially understandable in terms of ordinary physical and chemical principles (the doctrine of mechanism).

Honoring perhaps the context of its discovery, Mauthner's seminal observation endured over the years by providing the opportunity to examine the physiological properties of a single vertebrate neuron and to correlate them with its structure, leading to seminal discoveries in

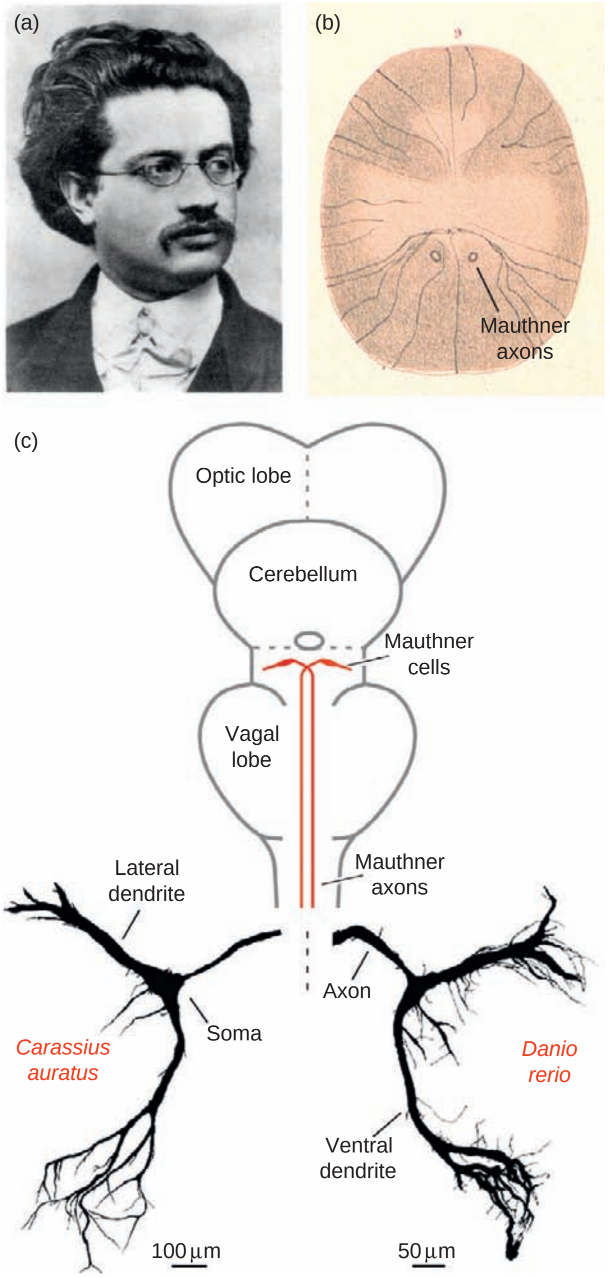


Figure 1 Description of the Mauthner cells. (a) Portrait of Ludwig Mauthner, the discoverer of these giant reticulospinal neurons, which have taken his name. (b) Koestler's 1898 hand-drawn cross section of pike (*Esox lucius*) spinal cord, based on microscopic observations, and illustrating the prominent Mauthner axons. (c) Schematic of a dorsal view of the teleost brain, with the paired Mauthner cells superimposed. Note that the axons cross the midline and descend to the spinal cord on the contralateral side. With this perspective, only the lateral dendrites are represented. Below are camera lucida drawings of goldfish (left) and zebrafish (right) Mauthner cells, projected onto the saggital plane and highlighting the two dominant dendrites. Modified from Lee RK, Eaton RC, and Zottoli SJ (1993) Segmental arrangement of reticulospinal neurons in the goldfish hindbrain. *Journal of Comparative Neurology* 329: 539–556; and Lee RK and Eaton RC (1991) Identifiable reticulospinal neurons of the adult zebrafish, *Brachydanio rerio*. *Journal of Comparative Neurology* 304: 34–52.

the understanding of the complexities of mechanisms underlying synaptic transmission. Today, this model vertebrate neuron provides a catalog of cellular and synaptic specializations that are repeatedly found to be relevant to neurons and their networks in general and for a wide range of species, including mammals.

The added attraction of this neuron is that it also has a well-defined function, as its physiological properties are critical to the appropriate expression of a signal behavior, the tail flip or escape response. Yet, the full extent of the functional role of this enigmatic colossal neuron remains undetermined. The physiological properties of the Mauthner cell, emphasizing its use as a model system to investigate cellular and synaptic properties, are reviewed in this article. The physiological role of the Mauthner cell in organizing reflex and voluntary behaviors and the cellular properties of the Mauthner cell in the context of its functional role are discussed elsewhere in this encyclopedia.

The Mauthner Cell

While Ludwig Mauthner later became a prominent ophthalmologist, his observation led to the identification by others of the origin of these large axons: a pair of large cells in the medulla of teleost and other fish (**Figure 1(c)**). Due to their uncommon size and shape, these cells are anatomically identifiable. The cell bodies are large (~100 µm in diameter) and characteristically exhibit, along with a number of much smaller dendritic processes, two large main dendrites: the lateral and the ventral dendrites (**Figure 1(c)**). Their prominent myelinated axons cross the midline to descend the length of the spinal cord, issuing axon collaterals that massively activate cranial and spinal motor systems. Such an anatomical arrangement allows a single action potential in this cell to initiate an escape response by producing a tail flip and coordinated movements of the jaw and eyes (see below). These anatomical features seem to be fairly constant among various species of fish, allowing the unequivocal identification of these cells.

We know now that the Mauthner cells are two bilaterally paired, cholinergic, reticulospinal neurons located in rhombomere 4. As with most reticulospinal neurons, the Mauthner cells receive multiple sensory inputs (**Figure 2**). Some of these inputs are clearly segregated: while the lateral dendrite receives information from the octavolateralis system (vestibular, lateral line, and, prominently, auditory modalities), the ventral dendrite receives visual and somesthetic information (**Figure 2**). Interestingly, a group of reticulospinal neurons in mammals is thought to mediate a comparable function, the startle response. The presence of a single, albeit larger,

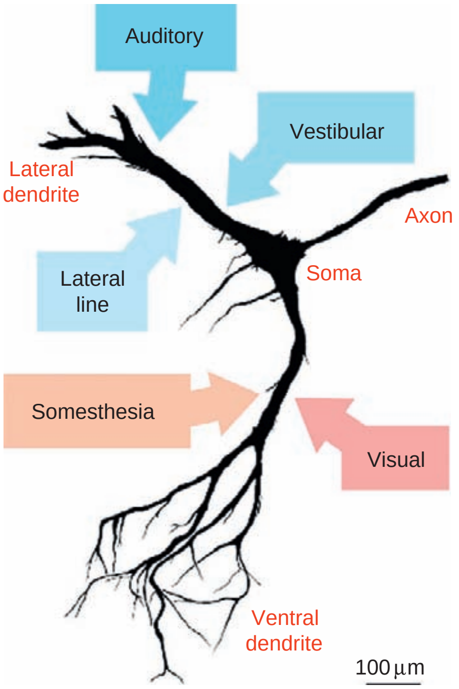


Figure 2 The projections of sensory afferents to the Mauthner cell dendrites are highly specified, as revealed by electrophysiological mapping experiments. The lateral dendrite processes stato-acoustic information, with the terminal fields of the auditory, vestibular, and lateral line systems localized to different dendritic regions. In contrast, the ventral dendrite is sensitive to visual and somatosensory stimuli. Goldfish Mauthner cell modified from Lee RK, Eaton RC, and Zottoli SJ (1993) Segmental arrangement of reticulospinal neurons in the goldfish hindbrain. *Journal of Comparative Neurology* 329: 539–556.

cell in rhombomere 4 seems to be functionally beneficial for the escape responses of fish, enabling a more efficient combination of speed and acceleration under water. This notion of a functional advantage, dependent in part on anatomical specializations, is emphasized by the finding that the amphibian Mauthner cells are identifiable only at the tadpole stage, and are either lost or significantly reduced in size during metamorphosis, when they become terrestrial.

The Mauthner Cell As Model Vertebrate Neuron

These uncommonly large cells are not only anatomically but also physiologically identifiable (see below) and have historically constituted a valuable experimental preparation. The large size of the Mauthner cell, the characteristic morphology of its soma and main dendrites, and its physiological identifiability and accessibility for intracellular microelectrode recordings combined to provide one of the first possibilities of exploring cellular and synaptic properties in a vertebrate neuron. While slice techniques now allow direct exploration of cellular properties in nearly every vertebrate brain structure, the Mauthner cells still provide the uncommon opportunity of correlating structure with function to study detailed cellular mechanisms. In particular, the use of the Mauthner cells and their identifiable synaptic inputs led to many insights into the mechanisms of synaptic transmission, including mechanisms of inhibition, transmitter release, and electrical transmission.

Inhibitory Mechanisms

As already noted, the Mauthner cell has served as a model for the discovery and elucidation of basic synaptic mechanisms. This advantage is derived from its *in vivo* identifiability, linked to a unique property of the axon cap, which is a sophisticated anatomical specialization restricted to the initial segment of the axon and amplifies the extracellular field produced by the action potential of the cell. In fact, this feature also underlies a unique form of inhibition that has made it possible to identify presynaptic inhibitory cells and to study their synapses in exquisite detail. Based on this property, Mauthner cells can be distinguished from thousands of other cells in the brain of fishes. This implies that from fish to fish, recordings can be obtained from the same neuron that was genetically determined to be the Mauthner cell, thereby providing a unique comparative advantage.

The special property of the axon cap is that it has a very high extracellular resistance, such that the Mauthner cell action potential, which can be antidromically evoked experimentally by a stimulating electrode on the spinal cord, is as large as 30 mV in the center of the cap (**Figure 3(a)**). The current associated with this field directly hyperpolarizes the membrane of a special class of interneurons, called ‘passive hyperpolarizing potential’ (PHP) cells; their impulses in turn electrically inhibit the Mauthner cell. These impulses are associated with a positive extracellular field, and both electrical inhibitions are mediated by field effects. That is, the electrical field produced by one neuron can modify the excitability of a nearby neuron without the existence of cellular contacts. In the case of the Mauthner cell, the positive extracellular electrical field produced by the synchronization of many PHP cells generates a mirror hyperpolarization in the initial segment, the region of the cell in which action potentials originate (**Figure 4(b)**). While field effects (also known as ephaptic interactions) have been observed to occur in some pathological conditions (i.e., hippocampus during seizures), the axon cap of the Mauthner cell truly represents a synaptic specialization, and thus this mechanism is considered the best example of electrical inhibition. Similar mechanisms are thought to operate in the cerebellum.

The electrical inhibition of the Mauthner cell was discovered first, by Furukawa and Furshpan, as

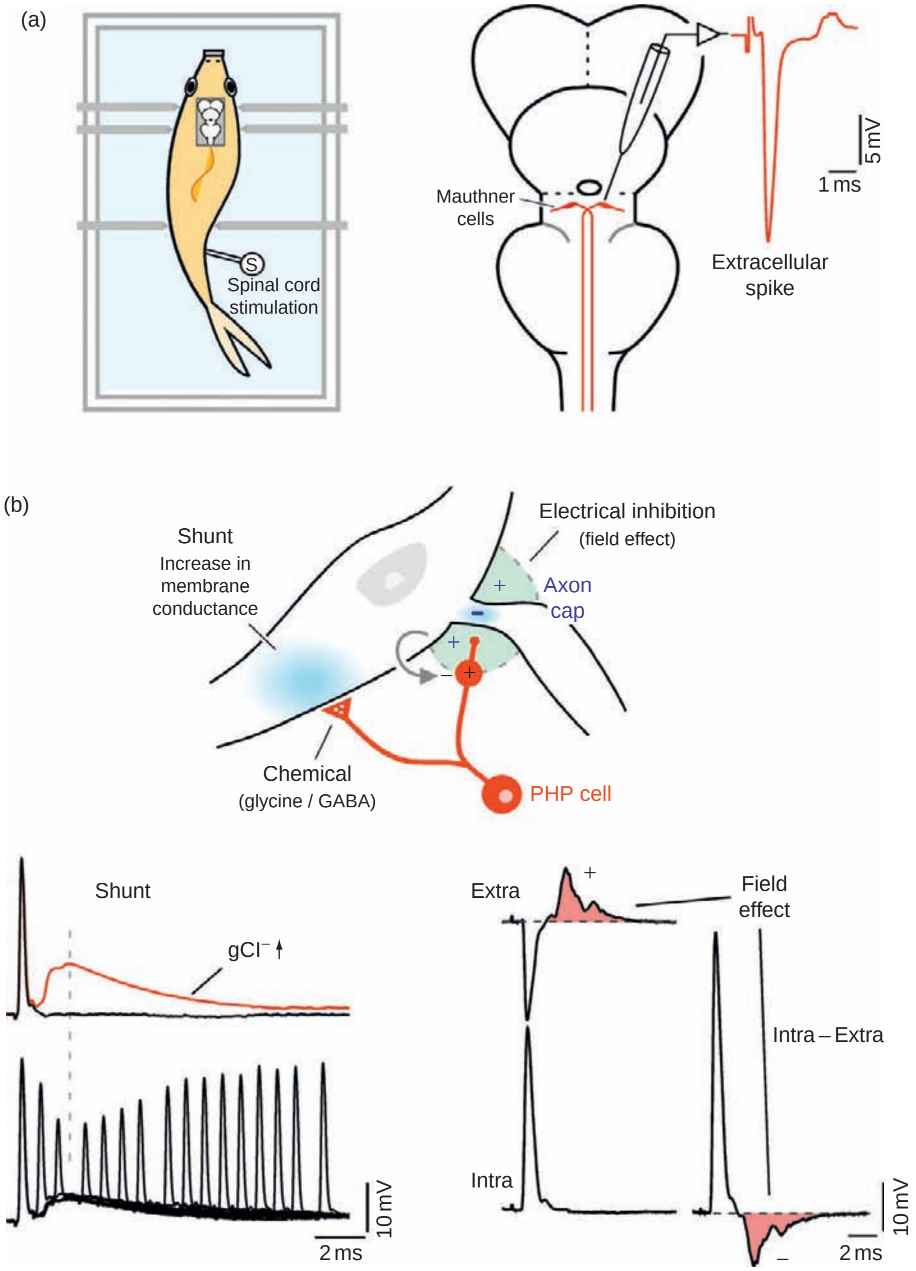


Figure 3 Hallmarks of Mauthner cell electrophysiology: inhibitory mechanisms. (a) Schematic of *in vivo* recording arrangement (left panel), with an immobilized and anesthetized fish in air. A bipolar electrode on the exposed vertebral column is used to antidromically activate the Mauthner axon while recording extra- or intracellularly from defined regions of the cell. Right panel shows the Mauthner cell electrophysiological signature that allows this localization, namely an antidromic action potential as large as -40 mV in the center of the axon cap that surrounds the cell's axon hillock and initial segment. This locus serves as a reference for all the other recording loci. (b) A special class of inhibitory interneurons, the PHP cells, mediates both electrical and chemical (glycinergic) inhibition of the Mauthner cell soma. Electrical inhibition is a consequence of failure of action potentials in PHP cells to actively propagate beyond the border of the axon cap and on the high electrical resistance of this region. It is signaled by an extracellular positivity in the center of the cap. Chemical inhibition, due to release of glycine, and possibly γ-aminobutyric acid activated (GABA) receptors, is the consequence of an increase in Cl^- conductance that shunts any currents generated in the Mauthner cell. Records below illustrate the electrical (right) and chemical (left) inhibitions evoked experimentally by antidromic stimulation of the Mauthner axon. The former is a frank hyperpolarization that follows the Mauthner spike and is revealed by computing the true transmembrane potential change, that is, the difference between the responses recorded intra- and extracellularly. The time course of the shunt produced by recurrent inhibition is manifested by the reduction in the amplitude of a second antidromic action potential, evoked at different intervals in the lower left panel. Note that the shunt mirrors the underlying increase in Cl^- conductance, which appear as a depolarization after Cl^- injections (red trace).

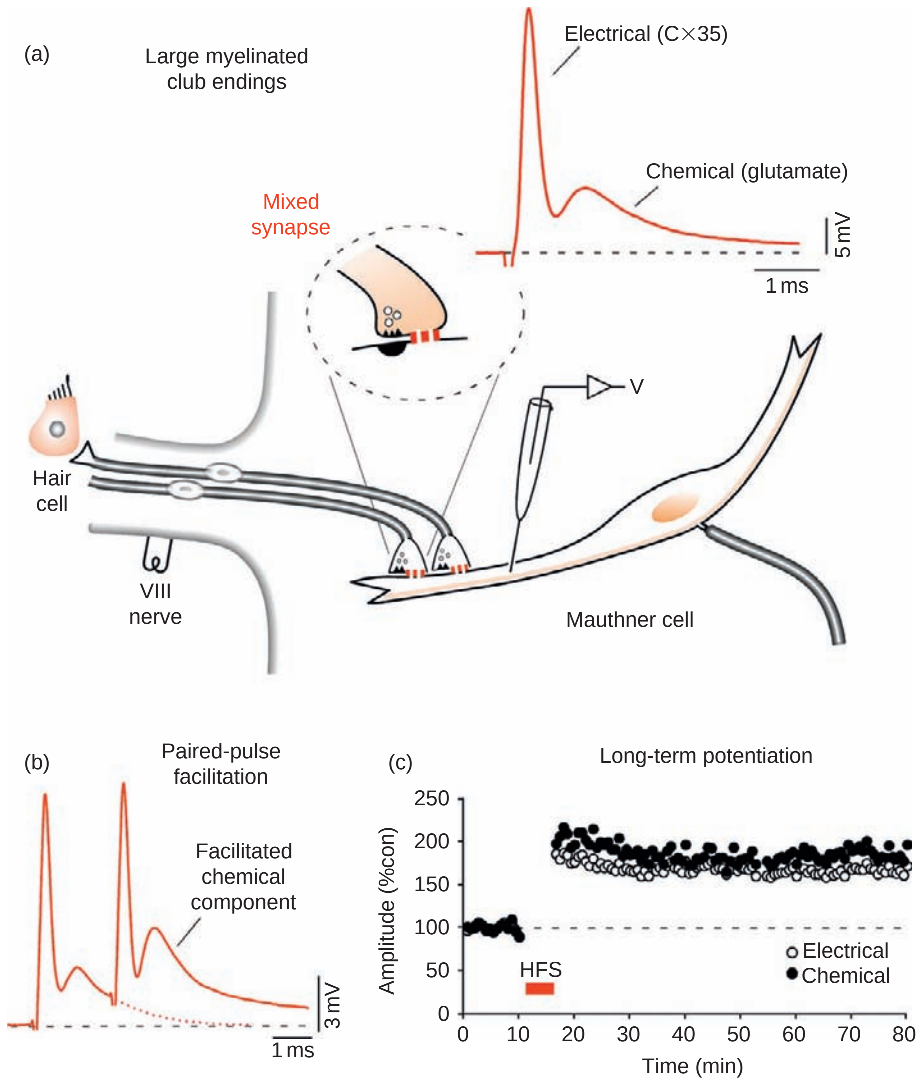


Figure 4 Club endings mediate mixed synaptic transmission. (a) Experimental arrangement showing VIII nerve auditory primary afferents (from saccular hair cells) terminating as club endings on the Mauthner cell lateral dendrite. (Inset) Both mechanisms of synaptic transmission, electrical (gap junction), and chemical (glutamatergic), coexist. VIII nerve stimulation evokes graded, mixed electrical and chemical synaptic potential. (b) Glutamatergic synapses at club endings exhibit paired-pulse facilitation. (c) High-frequency stimulation (HFS) of club endings (VIII nerve stimulation) leads to long-term potentiation of both the electrical and chemical components of the mixed synaptic response.

antidromic stimulation triggers a feedback activation of about 40 PHP cells, and their synchronous activity hyperpolarizes the Mauthner cell by about 10–15 mV. However, a major advance came when these cells were identified by the field effect from Mauthner cell activation, and it became apparent that their impulses also mediated glycinergic inhibition of the Mauthner cell. Thus, as shown in **Figure 3(b)**, action potentials in terminals of PHP cells produce a two-component inhibition of the Mauthner cell: a fast and brief electrical component followed by a slower and longer-lasting chemical component. The latter is due to the opening of Cl^- channels, and because the Cl^- equilibrium potential is close to the Mauthner cell resting potential, this inhibition does not itself produce a significant membrane potential change. Rather, the inhibition works by producing a shunting effect (i.e., synaptic conductance short-circuits the currents that are generated at adjacent excitatory synapses), which can be clearly appreciated by comparing the time course of the synaptic potential with that of the underlying conductance change (**Figure 3(b)**), evidenced in this case by a reduction in the amplitude of consecutive antidromic spikes obtained at different intervals. The Mauthner cell action potential, generated in the initial segment, is a convenient indicator of the cell's conductance. Because it propagates passively through the somatodendritic membrane, changes in the conductance of the cell alter its amplitude. As a footnote, the two electrical inhibitions are associated with fields of opposing polarities. This observation has driven home the point that the sign of a field effect can only be determined by computing the true transmembrane potential change, which is the difference between the potentials recorded intracellularly and extracellularly (**Figure 3**(**b**)).

Mechanisms of Transmitter Release

There followed a decade of research in which the properties of central inhibitory synapses were studied with paired presynaptic and postsynaptic intracellular recordings, as well as dye injections to expose fundamental properties of synaptic transmission and their morphological correlates. This breakthrough was achieved by combining physiological and ultrastructural data that were possible to collect due to the anatomical and physiological identifiability of the Mauthner cell and the afferent synapses of the PHP cells. Some basic properties discovered in this system include: (1) the quantal and probabilistic nature of transmitter release in the CNS, with the realization that at most one or two synaptic vesicles undergo exocytosis at a single synaptic site; (2) the first support for the concept that there is crosstalk between neighboring central synapses due to the lateral diffusion of transmitter; and (3) the evidence for activity-dependent plasticity of inhibition.

Mixed Electrical and Chemical Synapses at Primary Auditory Afferents

Because of their large size, characteristic myelinization, and dendritic localization, a group of auditory afferents constitutes the most recognizable synaptic input to the Mauthner cells. Such anatomical characteristics support high-speed impulse conduction and suggest that they likely provide the Mauthner cells with critical auditory information for the initiation of escape responses. First described by Bartelmez in 1915 in the catfish (*Ameiurus*), (**Figure 4**(**a**)), this population of ~90 large afferents (8–15 μm in diameter) originates in the rostral portion of the saccular macula (the main auditory component of fish ear) and runs in the posterior branch of the VIII nerve. Although each afferent issues a few substantially thinner branches, likely targeting other neurons in the hindbrain, the primary axon characteristically terminates as a large single terminal (about the same diameter as that of the parent axon) on the lateral dendrite of the Mauthner cell. These endings are known as large myelinated club endings or simply club endings.

A wealth of anatomical and electrophysiological data shows that club endings support both chemical and electrical modalities of transmission (**Figure 4**(**a**)). In fact, these contacts provided one of the first demonstrations of gap junction plaques and electrical transmission in the vertebrate central nervous system and, because of their accessibility, constitute a valuable model for exploring the properties of this modality of transmission. These gap junctions are formed by connexin 35, the fish ortholog of the widely expressed mammalian connexin 36, which is responsible for electrical coupling between many cell types, including neocortical inhibitory interneurons and inferior olivary cells.

From the electrophysiological point of view, a presynaptic impulse generates a mixed excitatory response in the Mauthner cell. The stimulation of the posterior branch of the VIII nerve, where these fibers run, evokes a gap junction-mediated electrical synaptic potential followed by a chemically mediated excitatory postsynaptic potential (**Figure 4**(**a**)). Due to the fast time constant of the Mauthner cell (~400 μs), the two components can be easily distinguished.

Chemical transmission is mediated by the release of glutamate, which activates both *N*-methyl-D-aspartate (NMDA)-activated glutamate receptors and non-NMDA receptors. Even at its relatively hyperpolarized resting potential (~−80 mV), the chemical component of the mixed synaptic response of the club endings on the Mauthner cell is normally mediated by the activation of both NMDA and non-NMDA receptors and, surprisingly, the NMDA component is almost as fast as the non-NMDA component. In contrast to most primary auditory afferent synapses, which are known to depress in response to multiple stimuli, glutamatergic synapses at club endings characteristically exhibit frequency-dependent facilitation (**Figure 4**(**b**)), suggesting the existence of unusual synaptic specializations in these teleost afferents.

While mixed synapses are often found in lower vertebrates, the combination of these two forms of transmission matches the functional role of club ending afferents in the Mauthner cell system. Electrical transmission provides speed and reliability of transmission (i.e., absence of synaptic delay and associated probabilistic mechanisms), and the presence of chemical transmission, with a relatively longer duration, allows temporal summation during repetitive responses in a cell in which the membrane time constant is unusually brief (**Figure 4**(**b**)). In fact, electrical component of the response predominates when naturalistic acoustic stimuli are used, suggesting chemical transmission might also have another functional role. Indeed, the advantage gained by combining electrical and chemical synapses is emphasized by the existence of important functional interactions between these two modalities of transmission. The most remarkable of these interactions is the induction by glutamatergic synapses of long-term activity-dependent changes in the efficacy of both chemical and electrical synaptic transmission. That is, not only the glutamatergic component but also the gap junction-mediated component of the mixed synaptic response undergo activity-dependent potentiation of their synaptic strengths in response to high-frequency stimulation of the VIII nerve (**Figure 4(c)**). This phenomenon provided the first example of activity-dependent plasticity of electrical synapses and the opportunity to explore its mechanisms in an accessible experimental model. The induction of long-term activity-dependent synaptic plasticity requires stimulation with brief trains, a protocol inspired by the natural bursting properties of these afferents. Induction also requires activation of NMDA receptors, leading in turn to a localized increase in the intracellular concentration of Ca^{2+} that activates the kinase Ca^{2+}-calmodulin-dependent kinase II (CaM-KII), which is necessary for the potentiations. Interestingly, another activity-dependent protocol has also been shown to lead to potentiation of the mixed synaptic response, suggesting that multiple mechanisms converge for the regulation of the strength of these important synapses. In this second case, the mechanism involves the production of endocannabinoids by the Mauthner cell and is indirectly mediated via the release of dopamine from nearby varicosities that in turn leads to potentiation of the synaptic response via a cyclic adenosine monophosphate (cAMP)-dependent protein kinase-mediated (PKA) postsynaptic mechanism. The increased synaptic gain of these auditory nerve synapses should sensitize a vital escape response, lowering its threshold to acoustic stimuli, and it likely represents a mechanism for modulating sensory-motor processing.

Summary

Because of their experimental accessibility, the Mauthner cells have provided important insights into basic mechanisms of cellular excitability and synaptic transmission. Despite the availability of modern techniques, which now allow direct exploration of cellular properties in slices of brain tissue, these cells will still provide the uncommon opportunity of directly correlating structure with function to study detailed cellular mechanisms. Moreover, the rich and singular physiological properties of the Mauthner cells will continue to provide a unique, fascinating window for the discovery of basic mechanisms of brain function.

See also: **Brain and Nervous System**: Physiology of the Mauthner Cell: Function. **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Intracardiac Neurons and Neurotransmitters in Fish.

Further Reading

Cachope R, Mackie K, Triller A, O'Brien J, and Pereda A (2007) Potentiation of electrical and glutamatergic synaptic transmission mediated by endocannabinoids. *Neuron* 56: 1034–1047.

Curti S and Pereda AE (2009) Functional specializations of primary auditory afferents on the Mauthner cells: Interactions between membrane and synaptic properties. *Journal of Physiology – Paris* 104: 203–214.

Faber DS, Fetcho JR, and Korn H (1989) Neuronal networks underlying the escape response in goldfish: General implications for motor control. *Annals of the New York Academy of Sciences* 563: 11–33.

Faber DS and Korn H (1978) Electrophysiology of the Mauthner cell, basic properties, synaptic mechanisms, and associated networks. In: Faber DS and Korn H (eds.) *Neurobiology of the Mauthner Cell*, pp. 47–131. New York: Raven.

Furshpan EJ and Furukawa T (1962) Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *Journal of Neurophysiology* 25: 732–71.

Korn H and Faber DS (2005) The Mauthner cell half a century later: A neurobiological model for decision-making?. *Neuron* 47: 13–28.

Lee RK and Eaton RC (1991) Identifiable reticulospinal neurons of the adult zebrafish, *Brachydanio rerio*. *Journal of Comparative Neurology* 304: 34–52.

Lee RK, Eaton RC, and Zottoli SJ (1993) Segmental arrangement of reticulospinal neurons in the goldfish hindbrain. *Journal of Comparative Neurology* 329: 539–556.

Pereda AE, Rash JE, Nagy JI, and Bennett MVL (2004) Dynamics of electrical transmission at club endings on the Mauthner cells. *Brain Research Reviews* 47: 227–244.

Seyfarth EA and Zottoli SJ (1991) Ludwig Mauthner (1840–1894): Neuroanatomist and noted ophthalmologist in Fin-de-Siècle Vienna. *Brain Behavior and Evolution* 37: 252–259.

Physiology of the Mauthner Cell: Function

DS Faber, and AE Pereda, Albert Einstein College of Medicine, Bronx, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Functions of the Mauthner Cell
Cellular Properties as Functional Specializations
Summary
Further Reading

Glossary

Command neuron A single neuron whose activation evokes an endogenous, naturally occurring behavior. Because they often receive a convergence of integrative and sensory inputs and are connected to specific efferent systems, command neurons have the property of acting as decision-making cells.

Directional hearing Ability to identify the location or origin of sound in space.

Electrical resonance Ability of neurons to respond selectively to inputs at preferred frequencies, supporting subthreshold oscillatory behaviors. Oscillatory properties of neurons can serve as substrates for coordinating network activity around a particular frequency and are generated by a variety of ionic mechanisms.

Escape response A sudden acceleration maneuver (i.e., a fast start) as a response to a threatening stimulus.

Excitatory input Synaptic input that increases the probability of initiating an action potential in the postsynaptic neuron.

GABAergic Synapse that uses GABA as its neurotransmitter, the major inhibitory neurotransmitter across the central nervous system.

Glycinergic Synapse that uses glycine as its neurotransmitter, one of the major inhibitory neurotransmitters in the spinal cord and the brainstem.

Inhibitory input Synaptic input that decreases the probability of initiating an action potential in the postsynaptic neuron.

Sensory–motor processing Aspects of vertebrate behavior indicate that both motor actions and sensory processing are inextricably linked. Alterations in sensory–motor processing are thought to underlie various cognitive dysfunctions.

Time constant Index that expresses how fast the membrane potential of a neuron changes in response to changes in an applied transmembrane current. The time constant (τ) can be calculated as the product between membrane resistance and membrane capacitance. As with other RC circuits, it expresses the time needed to charge the capacitor through the resistor to ~63% of full charge, or to discharge it ~37% of its initial voltage (values derived from the mathematical constant *e*). Thus, due to the presence of the membrane capacitance, the voltage across the membrane usually lags behind current injections. Time constants are usually fast in neurons that require high temporal precision.

Introduction

Together with its use as an experimentally accessible model neuron for the study of cellular mechanisms (**see also Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties), the Mauthner cell also constitutes a model for the study of cellular determinants of vertebrate behavior. While activity of the Mauthner cell has been correlated with a number of behaviors, its role as the principal neuron in the organization of escape responses has been documented in detail (**see also Buoyancy, Locomotion, and Movement in Fishes**: Fast Start). The evidence supporting the participation of the Mauthner cell in escape and voluntary responses will be reviewed here, and the shared aspects of the neural network that make these behaviors possible will be described.

Functions of the Mauthner Cell

Escape Responses

Initial speculations about the behavioral function of the Mauthner cell centered around the likelihood that it controlled or coordinated swimming activities, in part because its axon extended the length of the spinal cord. However, Zottoli provided strong evidence in 1977, subsequently reinforced by Eaton and his colleagues, for its central role in the auditory-evoked escape, by recording the Mauthner cell action potential extracellularly from the axon cap in free-swimming goldfish (*Carassius auratus*): the recorded Mauthner cell never fired during swimming, but always fired when a loud and abrupt sound was rapidly followed by a fast body turn to the side, contralateral to that cell. This escape response is referred

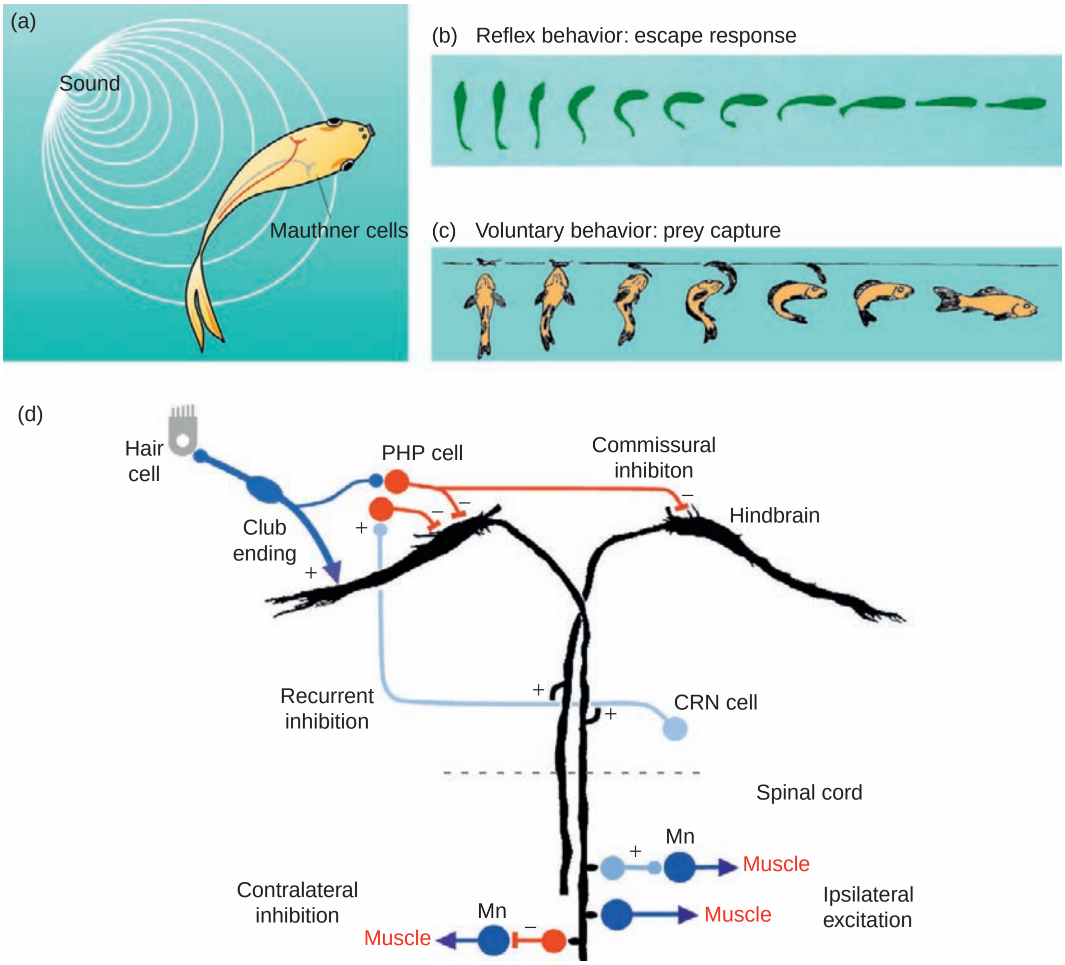


Figure 1 Functional role of unilateral Mauthner cell activation. (a) Acoustic startle response is directional, due to excitation of the ipsilateral Mauthner cell (left). (b) The resulting escape response takes the form of a C-bend and propels the fish away from the threatening stimulus (silhouettes captured at 5 ms intervals). (c) The same output circuits can be used for prey capture, or feeding (middle five drawings at 16.6 ms intervals. (d) Backbone of the input and output networks brought into play by a sound from the left. Excitation of hair cells in the left inner ear activates feedforward excitation and inhibition of the ipsilateral Mauthner cell, as well as feedforward inhibition of the contralateral cell. At the spinal level, the resulting pattern of contralateral activation and ipsilateral deactivation of motoneurons (Mn) causes contraction of contralateral trunk musculature and relaxation of the ipsilateral musculature (goldfish Mauthner cells). (b) Modified from Eaton RC, Bombardieri RA, and Meyer DL (1977) The Mauthner-initiated startle response in teleost fish. *Journal of Experimental Biology* 66: 65–81. (c) Modified from Canfield JG and Rose GJ (1993) Activation of Mauthner neurons during prey capture. *Journal of Comparative Physiology A* 172: 611–618. (d) Modified from Lee RK, Eaton RC, and Zottoli SJ (1993) Segmental arrangement of reticulospinal neurons in the goldfish hindbrain. *Journal of Comparative Neurology* 329: 539–556.

to as a‘C-start’, as the body of the fish takes the form of that letter (**Figures 1**(**a**) and **1**(**b**)), and it can be easily examined in any household by simply tapping on the wall of a fish tank. However, it was found that the recorded cell did not fire when the C-start was in the ipsilateral direction. These correlations are consistent with the trajectory of the Mauthner axon, which crosses the midline before descending in the spinal cord. Hence, the conclusion was reached that only one Mauthner cell fires once before an escape, and which cell fires determines the initial direction of the behavior. As described in the following section, the auditory-evoked escape is typically directional, that is, directed away from the location of the aversive stimulus.

By combining extracellular Mauthner cell recordings with electromyography and high-speed video, it has been possible to apportion the timing of the auditory-evoked escape to the successive steps in the Mauthner cell circuits. Here we stress its speed by noting that at room temperature the total time from sound onset to the first detectable movement of body musculature is ∼15 ms, and this timing has been important in addressing a question fundamental to neuroscience. Specifically, it has long been postulated that there exist command neurons, cells that are both necessary and sufficient for a behavior. The Mauthner cell comes closest to fitting that definition: a single action potential in one Mauthner cell can trigger a C-start with initial kinetics comparable to that evoked with natural stimuli. That observation might support the requirement for sufficiency, but in fact, later components of the escape evoked by directly activating one Mauthner cell are less complex than those produced by the natural stimulus. Also, the results of testing for necessity are open to debate, as deleting one Mauthner cell does not block the C-start but does delay the response and decreases the maximal angular velocity, both of which could have catastrophic consequences for the survival value of an escape response. The appropriate perspective might be that there is a parallel (perhaps multiple) system(s) that can both compensate for the Mauthner cell, if absent, and also have a role in the execution of the Mauthner-initiated escapes.

The considerations above have led to the notion of a brainstem escape network that is more extensive than the Mauthner cell and its associated circuitry. Indeed, experiments in zebrafish (genus species) embryos, where it is possible to record noninvasively from individual identified neurons and to selectively laser ablate them, have focused on the role of the Mauthner cell and of its identifiable homologs in adjacent rhombomeres, the MiD2cm, and MiD3cm neurons. These studies have shown that specific homologs are differentially active depending on whether the aversive stimulus is directed toward the head or tail of the fish. While stimuli coming from the head activate all three neurons, those coming from the tail only activate the Mauthner cell, supporting the notion of their organization into functional groups. These findings provided a basis for understanding some variable aspects of the escape behavior that are expressed after the initial C-start. For example, the final direction taken by the fish depends upon the initial angle between the stimulus source and body orientation. Also, escapes evoked by stimuli originating from behind the fish include a mid-course direction change, so that the fish can escape by forward propulsion rather than continue to turn toward the predator.

In addition to its role in auditory-evoked behaviors, the Mauthner cell is also central to other types of escapes, a feature that is consistent with its identification as a reticulospinal neuron responsive to multiple modalities of sensory information. For example, the ventral dendrite is responsive to visual inputs relayed to it from the retina via the pretectum and optic tectum. While there is minimal support for the notion of short latency visual-evoked escapes in fish, looming stimuli that mimic the shadows cast by the approach of diving birds, natural stimuli, are very effective in triggering Mauthner-mediated C-starts. Looming auditory stimuli, which would be associated with predation from other fish, are also effective. In contrast to C-starts evoked by abrupt acoustic stimuli, the latency of the behavior triggered by the looming stimuli is of the order of hundreds of milliseconds. Yet, it has the same kinematics once it is initiated. It can be postulated that noxious tactile and olfactory inputs may also trigger the escapes. Finally, it should be emphasized that although specific sensory modalities predominate in evoking certain types of escape behaviors, most escape-triggering stimuli are likely to be multisensory in nature and include the participation of other modalities.

Prey Capture and Voluntary Behavior

As mentioned above, the Mauthner cells are known to initiate the characteristic C-start escape response of many fish. There exists, on the other hand, an increasing amount of evidence indicating that in species that exhibit C-start escapes, the Mauthner cell might play a role in other behaviors.

When the activity of the Mauthner cell was monitored using chronically implanted electrodes while fish were surface feeding on live crickets, it was observed that this cell became active when the fish performed a C-shaped flexion in association with the terminal phase of prey capture (**Figure 1**(**c**)). Mauthner-initiated flexions during feeding rapidly served to remove the prey from the water's surface, also minimizing the fish's own susceptibility to surface predation. Other behaviors that might involve the contribution of the Mauthner cells include hatching, social interactions, feeding, intercepting, and capturing targets shot down from above, airborne behaviors in flying fish, and upstream swimming in salmon. None of these behaviors, however, have yet been directly correlated with the activation of this cell. Interestingly, goldfish were observed to strike an object while executing voluntary C-bends that are similar to their C-start escape responses, suggesting that the Mauthner cells can be activated voluntarily, even in the absence of predator or feeding-associated stimuli. Thus, the Mauthner cell is known to be active in the context of at least two general types of behaviors, involuntary and voluntary (predatory escape and prey capture), which have a significant amount of circuitry in common.

The Mauthner Cell Network and Its Functional Properties

The escape behaviors that have been studied most extensively are those evoked by abrupt sounds in goldfish or vibratory tactile stimuli in zebrafish and, therefore, our knowledge of the functional structure of the underlying network is primarily based on these modalities. The organization of the escape circuit supports the notion of two parallel competing systems, represented by the right and left Mauthner cell and their respective inputs, capable of triggering escapes in opposite directions. As previously mentioned, large primary auditory afferents, known as Club endings, provide each Mauthner cell with essential excitatory information for the initiation of auditory-evoked escape responses (**Figure 1**(**d**)). These afferents activate a feedforward inhibitory network of passive hyperpolarizing potential (PHP) interneurons. PHP cells (**see also Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties) represent a population of neurons that regulate the excitability of the Mauthner cell. There are two classes of PHP cells. One class, the 'commissural' PHP neurons, provides the substrate for a feedforward inhibitory network. The interplay between excitatory and inhibitory inputs to the Mauthner cell is thought to influence both the threshold and direction of the escape responses, where feedforward inhibition plays a determinant functional role. Only powerful excitatory inputs that overcome feedforward inhibition are thought to be capable of firing the Mauthner cell. In the case of directionality, the characteristic bilateral distribution of feedforward inhibition, with its contralateral commissural projections, allows these neurons to influence the excitability of both cells (**Figure 1**(**d**)). The activation of this feedforward inhibitory network by other sensory inputs remains unexplored.

In contrast to sensory inputs of the Mauthner cell, which have a high degree of variability in terms of connectivity (di- vs. multisynaptic), the output network of the Mauthner cell is likely to be common to all C-shape behaviors. At the cranial level, activation of cranial relay neurons (CRNs) activates contralateral inhibitory neurons called 'collateral' PHP cells, the second class of PHP cells, providing the substrate for feedback inhibition. These cells form feedback networks with each Mauthner cell, as well as the reciprocal inhibitory networks. Thus, when one Mauthner cell is activated, the other is inhibited by the PHP feedback network (**Figure 1**(**d**)), which also inhibits the active cell to prevent further discharges. In addition, CRN cells activate circuits that lead to bilateral closure of the opercula, rendering the fish more hydrodynamic during the execution of the escape. At the spinal level, because the crossed Mauthner axon reliably activates its follower neurons, the activation of the Mauthner cell results in a massive unilateral excitation of interneurons and motoneurons on the opposite side of the body, which, combined with a simultaneous inhibition of ipsilateral motorneurons, leads to a tail flip of the fish, representing the initial stage of the so-called C-start (**Figure 1**(**a**)).

The properties of escape behaviors suggest that this neural network does not support simple reflexes. For example, in the goldfish auditory evokes response, both Mauthner cells are brought close to threshold in only a few milliseconds, yet the response typically is appropriately directional in 75–85% of the trials, if the fish is in an open field. That is, the initial direction of movement is to the side opposite the sound source. However, directional hearing in fish is poorly understood, in that fish are transparent to sound pressure. More specifically, because the fish body and water have the same acoustic density, bilateral external ears would not detect changes in pressure. The receptor organs are thus encased within the head of the animal and accessible to sounds from all directions. Furthermore, the presence of a single pressure-detecting organ that connects to both inner ears, such as the swimbladder in hearing specialists, also does not seem to support directional hearing (**see also Hearing and Lateral Line**: Auditory System Morphology and Biomechanics of the Inner Ear in Fishes). The apparent lack of directionality in the acoustic information prevents the existence of interaural time differences, such as those underlying directional hearing in mammals (**see also Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes). Thus, the question arises of how the Mauthner cells detect stimulus direction. The two models that might explain this puzzle, at least in the context of the C-start, both invoke a critical role for the feedforward inhibitory pathway. In one, the PHP cells are part of logic gates that compare sound pressure and acceleration signals from the left and right inner ears, and in the second, phase locking of PHP cell activity accomplishes the necessary discrimination.

Another remarkable and perhaps underappreciated feature of the escape behavior is the degree of uncertainty in the decisions reached by the Mauthner cell networks, in that the response is not always appropriately directional and there is no guarantee that even a strong threatening stimulus will always evoke a C-start. Interestingly, these uncertainties are presumably adaptive, as predictability would allow predators to anticipate the fish's response to a threat and to then reliably correct their attack trajectories. This adaptive property is best illustrated by the scaling function that is computed by the Mauthner cell for visual looming stimuli and determines the timing of the C-start. This function peaks and then declines while the stimulus is still approaching, providing the basis for a significant number of trials when the fish does not respond. This feature, coupled with evidence for bilateral Mauthner cell excitation for all naturalistic stimuli studied so far, stresses the complexity of this supposedly simple system and further supports the notion of two parallel and competing Mauthner cell systems, right and left.

Cellular Properties as Functional Specializations

The best-known function of the Mauthner system is its role in organizing sensory-evoked escape responses, most notoriously those triggered by sound. The morphological characteristics of the Mauthner cell as well as the circuit in which it is embedded (i.e., sensory inputs and motor outputs) seems highly appropriate to this function. Together with these network factors, a number of relevant cellular properties constitute important determinants of this behavioral response. These properties, often called 'specializations', denote the adaptation of neurons to the requirements of their particular functions. In this case, the synaptic and membrane properties of the cells involved in the Mauthner cell network match the functional requirements not only of the sound-evoked escape response but also of escapes evoked by visual looming stimuli, as well as for prey capture behaviors, particularly speed, reliability combined with an element of uncertainty, and a high behavioral threshold (**Figure 2**).

As a first example, the escape response has a characteristic high threshold, presumably because of its dramatic behavioral consequences, and this feature is supported by the cellular properties of the Mauthner cells. Specifically, the low input resistance, short-time constant, and hyperpolarized membrane potential (~–83 mV) of the Mauthner cell act in concert to prevent the spontaneous occurrence of this behavior as a consequence of weak, environmental noise. That is, they guarantee a high behavioral threshold. This property also derives from the strong afferent or feed-forward inhibition of the Mauthner cell that is evoked by the same sensory stimuli that drive excitation of this neuron.

Another functional adaptation is that Mauthner cells are unable to fire repetitively, an otherwise inconvenient feature for a cell in which a single action potential initiates an escape response that lasts several hundreds of milliseconds. In other words, a second C-start would be likely to defeat the purpose of the initial behavioral reaction. It has been proposed that this intrinsic membrane property relies on the presence of K^+ channels of the K_v1 family, known to antagonize repetitive firing. Again, specialized membrane properties are complemented by a powerful feedback synaptic inhibition that also blocks the firing of the other Mauthner cell after the escape is triggered. Further, the large diameter and heavily myelinated Mauthner axons, which span the length of the spinal cord, guarantee fast conduction of action potentials and are connected to their output targets via high-safety factor synapses, that is, synapses with a high probability of generating a postsynaptic action potential in response to a presynaptic one. Interestingly, these strong output synapses are mediated by acetylcholine, which acts on a combination of post-synaptic cholinergic receptors.

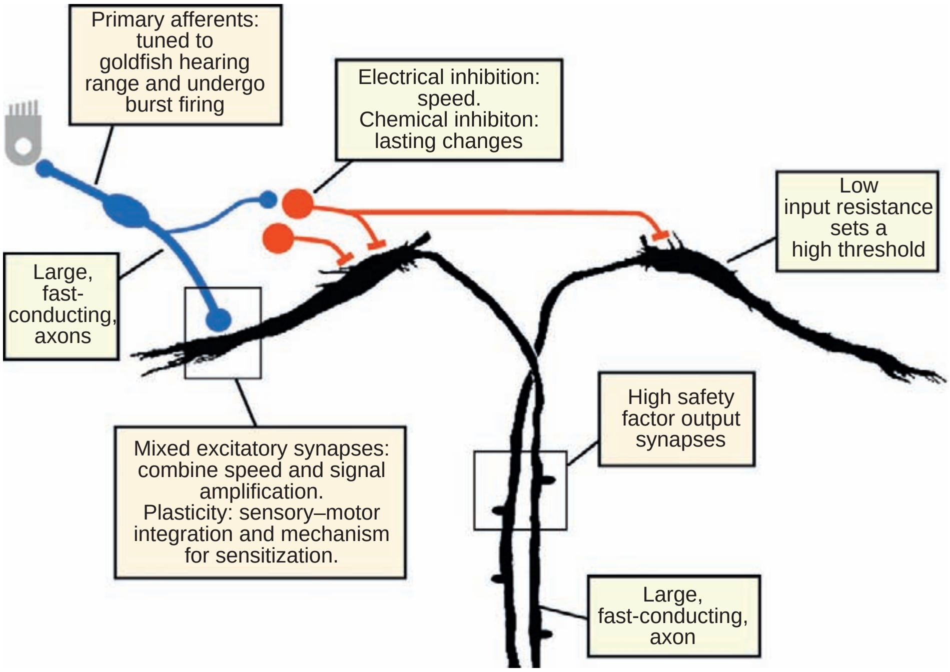

Primary afferents: tuned to goldfish hearing range and undergo burst firing
Electrical inhibition: speed. Chemical inhibiton: lasting changes
Low input resistance sets a high threshold
Large, fast-conducting, axons
Mixed excitatory synapses: combine speed and signal amplification. Plasticity: sensory–motor integration and mechanism for sensitization.
High safety factor output synapses
Large, fast-conducting, axon

Figure 2 Schematic of Mauthner cell connections, annotated to indicate some of the synaptic and membrane properties that match the functional requirements of both the sound-evoked escape and the prey capture behaviors, particularly speed, reliability, and a high but modifiable behavioral threshold (goldfish Mauthner cells). Modified from Lee *et al*. (1993).

The most important inputs to the Mauthner cell, such as those carrying auditory information for the initiation of the escape response, also have special cellular specializations (**Figure 2**). The existence of a single terminal in the large diameter and heavy myelinated auditory afferents (the Club endings) constitutes by itself a functional specialization. The lack of terminal branching provides safe impulse conduction, eliminating the possibility of branch point failures and of slower conduction through thinner processes, thus guaranteeing faster transmission and higher temporal fidelity. Consistent with these anatomical features, the dendritic arborization of these afferents at the saccular macula is also significantly simpler and with thicker processes than those of other afferent types. Thus, the relatively simpler anatomical features of Club ending afferents are likely to represent a functional specialization advantageous to their vital behavioral role.

In addition to these anatomical features, both intrinsic synaptic and membrane properties contribute to shaping the function of this relevant input. As previously mentioned, the combination of electrical and chemical synapses is particularly beneficial, as electrical transmission provides speed and reliability of information transfer while the relatively longer-lasting chemical component allows temporal summation during repetitive responses and provide the means for induction of long-term activity-dependent potentiation in both forms of transmission. Such plastic properties are unusual for synapses of a primary auditory afferent, and they likely represent a mechanism for modulating sensory–motor processing and therefore a clear cellular specialization of these contacts, whose function is to provide a decision-making neuron such as the Mauthner cell with critical sensory information.

Finally, the intrinsic membrane properties of these afferents seem also adapted to their function. Specifically, Club ending afferents are endowed with electrophysiological properties that favor the generation of high frequency (200–600 Hz) bursts in response to strong depolarization. Because the Mauthner cells are not capable of repetitive firing (see above), rather than relaying timing information via one-to-one spiking correlations, the ability to generate high-frequency bursts most likely represents a functional specialization of these afferents that provides the Mauthner cell with adequate (phase-locked) patterns of synaptic activation for the initiation of an escape response. Bursts, which are often generated by the interaction of synaptic inputs with intrinsic membrane properties, are thought to represent reliable neural codes during information processing, and they play special roles in the induction of synaptic plasticity. The generation of bursts in response to abrupt strong acoustic stimuli seems particularly advantageous to the Mauthner cell system since, in contrast to a single action potential, they produce a prolonged synaptic response that can efficiently depolarize the large and unusually low input resistance Mauthner cell. In addition, bursts are required for the induction of activity-dependent potentiation of the synaptic strength of these endings.

The bursting properties critically rely on the interplay between a persistent Na^+ current and an A-type K^+ current. Furthermore, the interaction between these conductances with the membrane's passive properties supports the presence of electrical resonance, whose frequency preference is consistent with both the effective range of hearing in goldfish and the firing frequencies required for synaptic facilitation, the latter being obligatory for the induction of activity-dependent changes. Thus, membrane properties allow these afferents to translate behaviorally relevant, naturally occurring auditory signals into patterns of activity that match the requirements of their fast and highly modifiable synapses.

Inhibitory inputs also present remarkable specializations that are adapted to their function. Inhibitory interneurons are capable of exerting powerful inhibition of the Mauthner cell via a combination of mechanisms (**Figure 2**). The unusual electrical inhibition, based on field effects, produces fast and short-lasting inhibition of the Mauthner cell by targeting the initial segment of this cell, where action potentials are initiated, providing speed and adequate temporal resolution. In addition, glycinergic and GABAergic synapse contacts produce longer-lasting inhibitory action by producing a more profound reduction in the excitability of the cell, via an increase in conductance of chemically gated Cl^- channels. The probability of release from the glycinergic terminals is relatively high, maximizing the initial strength of both the feedforward and feedback inhibitions, thereby supporting the high behavioral threshold and helping to prevent repetitive Mauthner cell firing, respectively. In addition, feedforward inhibition depresses in the same frequency range that afferent excitation facilitates, properties that aid in reaching threshold and evoking the escape.

Summary

Our current knowledge indicates that the Mauthner cells are involved in escape behaviors that are essential for the survival of fish. These escape responses can be triggered by a variety of sensory inputs, most prominently auditory. Based on the connectivity of the Mauthner cell axon at cranial and spinal cord levels, the activation of this cell produces a tail flip (C-start), which constitutes the initial phase of the escape response. The properties of the escape network are supported by singular cellular and synaptic properties that endow the system with speed and functional flexibility. However, a full understanding of their functional role remains undetermined. A growing body of evidence suggests that the Mauthner cells are involved in a number of voluntary behaviors. Furthermore, the fact that fish often execute motor behaviors that are reminiscent of the C-start raises the possibility that these cells could be involved in a wide range of functions. The use of zebrafish, where it is possible to combine physiology with sophisticated methods of behavioral analysis and genetic manipulations, promises to bring our understanding of the function of the Mauthner cells and its use as cellular model to new, exciting, levels.

See also: **Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties. **Buoyancy, Locomotion, and Movement in Fishes**: Fast Start. **Hearing and Lateral Line**: Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Sound Source Localization and Directional Hearing in Fishes.

Further Reading

Canfield JG and Rose GJ (1993) Activation of Mauthner neurons during prey capture. *Journal of Comparative Physiology A* 172: 611–618.

Eaton RC, Guzik AL, and Casagrand JL (1997) Mauthner system discrimination of stimulus direction from the acceleration and pressure components at sound onset. *Biological Bulletin* 192: 146–149.

Faber DS, Fetcho JR, and Korn H (1989) Neuronal networks underlying the escape response in goldfish. General implications for motor control. *Annals of the New York Academy of Sciences* 563: 11–33.

Korn H and Faber DS (2005) The Mauthner cell half a century later: A neurobiological model for decision-making? *Neuron* 47: 13–28.

Liu KS and Fetcho JR (1999) Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. *Neuron* 23: 325–335.

Liu KS, Gray M, Otto SJ, Fetcho JR, and Beattie CE (2003) Mutations in deadly seven/notch1a reveal developmental plasticity in the escape response circuit. *Journal of Neuroscience* 23: 8159–8166.

Lorent K, Liu KS, Fetcho JR, and Granato M (2001) The zebrafish space cadet gene controls axonal pathfinding of neurons that modulate fast turning movements. *Development* 128: 2131–2142.

O'Malley DM, Kao YH, and Fetcho JR (1996) Imaging the functional organization of zebrafish hindbrain segments during escape behaviors. *Neuron* 17: 1145–1155.

Preuss T, Osei-Bonsu PE, Weiss SA, Wang C, and Faber DS (2006) Neural representation of object approach in a decision-making motor circuit. *Journal of Neuroscience* 26: 3454–3464.

Autonomic Nervous System of Fishes

S Nilsson, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
The ANS and Chromaffin Tissue
The ANS of Different Fish Groups
Functional Aspects of the Fish ANS
Further Reading

Glossary

Adrenergic A general term pertaining to processes or structures related to catecholamines. For example, neurons that release noradrenaline as transmitter; catecholamines binding to adrenergic receptors (adrenoceptors), and adrenergically activated (catecholamine-activated) physiological processes.

Autonomic nervous system A collection of motor neurons (ganglia) ordered segmentally from cranial to caudal levels. The system controls the inner organs (viscera) through reflexes without conscious control.

Catecholamine Any of a class of amines derived from a catechol that acts as chemical messangers (a hormone or neurotransmitter). The main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Cholinergic A general term pertaining to processes or structures related to acetylcholine. For example, neurons that release acetylcholine as transmitter. Nicotinic and muscarinic receptors are cholinergic receptors because they bind acetylcholine. Cholinergic synapses are found in both the central and peripheral nervous systems.

Cranial autonomic nerve Autonomic nerve starting in the cranial (head) region of the CNS.

Enteric nervous system The part of the autonomic nervous system contained within the gastrointestinal wall.

Ganglion A collection of nerve cell bodies of similar function. A ganglion is a type of nucleus but often refers to structures in the peripheral nervous system. An exception is the basal ganglia that are nuclei of the telencephalic subpallium.

Parasympathetic nervous system Autonomic pathways in the cranial and sacral nerves.

Peptidergic Neurons that release a peptide as transmitter.

Spinal autonomic nerve Autonomic nerve initiating from the spinal cord region of the CNS.

Sympathetic chains Paired strings of ganglia, more or less connected longitudinally, that run parallel to the spinal column.

Sympathetic nervous system Autonomic pathways from the thoracic and lumbar segments of the (mammalian) spinal cord.

Introduction

The term autonomic nervous system (ANS) was proposed by John Newport Langley near the end of the nineteenth century, and the suggested terminology has well withstood the ravages of time – at least for mammals. Langley subdivided the ANS into three parts: (1) the sympathetic nervous system, where the pathways leave the thoracic and lumbar segments of the spinal cord, (2) the parasympathetic nervous system, where the pathways leave in cranial nerves III, VII, IX and X, and also in the sacral nerves, and (3) the enteric nervous system (ENS), which is the local nervous system within the wall of the gut. (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut).

In his original subdivision, Langley noted that for the sympathetic and parasympathetic systems, each autonomic pathway consists of two neurons known as preganglionic and postganglionic. At the connection between the two neurons of each pathway, the nerve terminals of the preganglionic neuron release a transmitter substance that excites the soma (cell body) of the postganglionic neuron. The postganglionic somata normally form clusters; much like bunches of grapes, called ganglia – hence the terminology that simply means before the ganglion and after the ganglion.

Langley also noted that the axons of the preganglionic neurons in the sympathetic system were short, while the axons of the postganglionic neurons were long. The reverse is true for the parasympathetic pathways, where the ganglia lie close to, or within the walls of the target organs. This is true for both the cranial and the sacral pathways, which is the reason why the pathways from the most anterior and the most posterior part of the CNS are lumped together as parasympathetic.

In the nonmammalian vertebrates, however, the original subdivision made by Langley is imperfect, because it is generally impossible to separate a lumbar from a sacral region along the spinal cord. This is certainly true for the fish from different groups. Therefore, it is better to refer to cranial autonomic pathways, which are identical to the cranial part of the parasympathetic system in Langley's terminology, and spinal autonomic pathways for all pathways leaving the spinal cord – that is, the sympathetic system, and the sacral parasympathetic system in Langley's terms.

The most advanced – or tetrapod-like – features of the ANSs of the different groups of fish are found in the (few) teleost species that have been examined. The descriptions of the basic anatomy of the ANS in cyclostomes, elasmobranchs, teleosts, and dipnoans are supplemented by brief presentations of autonomic nerve function in the control of two systems only: the heart and the iris of the eye (see later). For detailed accounts of the anatomy and functions of the autonomic control systems in fish, the reader is referred to the 'Further reading' section at the end of this article.

The ANS and Chromaffin Tissue

Closely related to the ANS is the chromaffin tissue, which consists of catecholamine-storing cells with many of the characteristics of adrenergic neurons of the ANS. Just like the adrenergic neurons of the ANS, these cells can synthesize, store, and release noradrenaline and/or adrenaline. The release is not from specialized nerve endings directly on top of the target cells as with the autonomic neurons, but instead into the bloodstream. This makes the chromaffin cells formally endocrine in function.

The similarity between the chromaffin cells and the adrenergic neurons runs further. In both cases the cells are controlled by preganglionic neurons that release acetylcholine as transmitter substance (**Figure 1**). In some cases, it is also possible to discern a third type of adrenergic cell: chromaffin cells intermingled with cells of the target tissue, for example, heart muscle cells (see later). These chromaffin cells release their amines to the neighboring tissue, and are therefore neither truly neurocrine (i.e., neurons) nor endocrine, but are often referred to as paracrine.

The ANS of Different Fish Groups

There are great similarities in the anatomical arrangement of the ANS in all vertebrates except, possibly, the cyclostomes where the structure and function of the system need further elucidation. The ANS of teleosts is

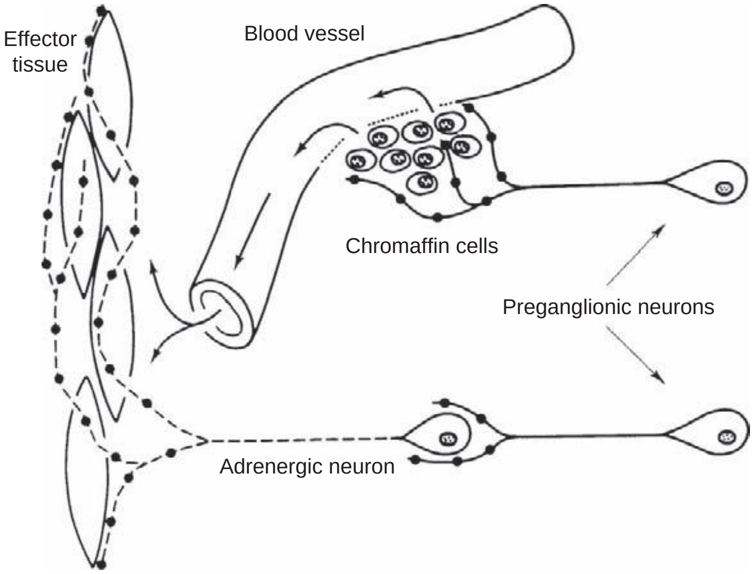


Figure 1 An adrenergic control of tissues and organs by the ANS can occur either by an innervation of catecholamine-containing chromaffin cells, or by a traditional autonomic nervous pathway, where the postganglionic neurons are adrenergic. The chromaffin cells and the adrenergic neurons share many features in catecholamine synthesis, storage, and release. Reproduced from Fig. 1 in Nilsson S (1984) Adrenergic control systems in fish. *Marine Biology Letters* 5: 127–146, with permission from Elsevier Science Publishers BV.

very similar to that in the tetrapods, while elasmobranchs show some less advanced features. The ANS of other fish, such as ganoids, is not well known, and it is worth remembering that the general view of the structure and function of the ANS in the different fish groups rests on more or less intense studies of only a handful of species. The much generalized patterns presented here, therefore, should be best enjoyed with a pinch of salt. Of the three divisions of the ANS, the ENS of fishes is well described in **Integrated Function and Control of the Gut**: Nervous System of the Gut, and will not be discussed further here.

Cyclostomes

As already mentioned, the ANS of the two cyclostome groups, hagfish (myxinoids) and lampreys (lampetroids), is not well known. At least one main reason for this is probably that the organization is less coherent than in the other fish groups. The normal pattern in the vertebrates is that autonomic pathways from the spinal cord leave via the ventral roots of the spinal nerves, but in the cyclostomes there is also a dorsal root outflow.

In hagfish, there is no extrinsic innervation of the heart, although cardiac control via intrinsic chromaffin cells has been well documented (see later). In fact, the best and clearest evidence for any control via the ANS in hagfish is a well-developed cholinergic, vagal control of the gallbladder! (**see also Hagfishes and Lamprey**: Hagfishes).

Lampetroids possess an autonomic innervation of the heart via the vagus but, interestingly, the effect of vagal stimulation is a stimulation of the heart – quite the opposite to vagal effects on the heart of all other vertebrates (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development, Lampreys: Environmental Physiology).

Elasmobranchs

An ANS that more or less follows the plan of the other vertebrates is found in the elasmobranchs. The seminal work of John Z Young in the early 1930s included both anatomical and functional studies in teleosts and elasmobranchs. In this work, Young made use of pharmacological tools – drugs that were used to elucidate the nature of the autonomic transmitter substances involved and consequently the functional patterns of the innervation.

Cranial autonomic pathways in the cranial nerves III (oculomotor) and X (vagus). Although tear glands and salivary glands are absent in elasmobranchs, pathways in cranial nerve VII (facial) and IX (glossopharyngeal) have been postulated and may control the pharynx. Oculomotor pathways synapse in the ciliary ganglion and postganglionic fibers control the iris (more about this later), and the vagal pathways control the heart and the gut. The spinal autonomic system (the sympathetic) shows paired segmental ganglia that are not always longitudinally connected to form sympathetic chains, as is the case in teleosts and most tetrapods. Preganglionic fibers from the spinal cord synapse with postganglionic neurons in the segmental ganglia, and several sets of splanchnic nerves (anterior, mid-, and posterior) run to the viscera. The most anterior sympathetic ganglia are relatively large, and comprise both cell bodies of postganglionic autonomic neurons and clusters of chromaffin cells. These anterior ganglia are known as axillary bodies, originally described as Axillar-Herz. They lie within the dorsal venous sinuses, and catecholamines released from the chromaffin cells will rapidly reach the heart, and subsequently the gills.

The general design of the ANS in elasmobranchs is shown in **Figure 2**.

Teleosts

The teleost ANS follows relatively closely the general vertebrate plan, as shown in most textbooks on mammalian and, especially, human anatomy/physiology (see also **Figure 2**). One of the most striking deviations from this plan is the lack of autonomic pathways in the facial (VII) and glossopharyngeal (IX) cranial nerves – a deficiency most likely related to the lack of tear glands and salivary glands. Autonomic fibers do, however, run in the oculomotor (III) nerve to the ciliary ganglion and the eye and in the vagus nerve (X) to the gills, heart, gut, and swimbladder (in fish that possess a swimbladder).

A second discrepancy from the plan is the cephalic portion of the sympathetic chains. These run forward into the head region, with sympathetic chain ganglia in close contact with the cranial nerves. The autonomic pathways in the cranial nerves are thus mixed, with both cranial autonomic (parasympathetic) and spinal autonomic (sympathetic) pathways running together. For this reason, the vagus nerve (X) is sometimes referred to as a vagosympathetic trunk.

An innervation of chromaffin tissue in the walls of the posterior cardinal veins by preganglionic spinal autonomic fibers has been demonstrated. Electrical stimulation of these nerve fibers does, indeed, produce a release of adrenaline from the chromaffin stores in the Atlantic cod (*Gadus morhua*). Similar to the situation in the elasmobranchs, the catecholamines released would immediately reach the heart via the blood stream, and shortly after the gills.

It was argued earlier that the distinction between the classical sympathetic and sacral parasympathetic portions makes little sense, and the mix of the two portions in the posterior parts of the teleost sympathetic chains can be illustrated in **Figure 3**. Relatively small cell bodies occur in the chain ganglia, while a bit more peripherally in the posterior splanchnic nerve along the ureter, a vesicular ganglion with somewhat larger nerve cell bodies can be found. The small cells in the chain ganglia contain

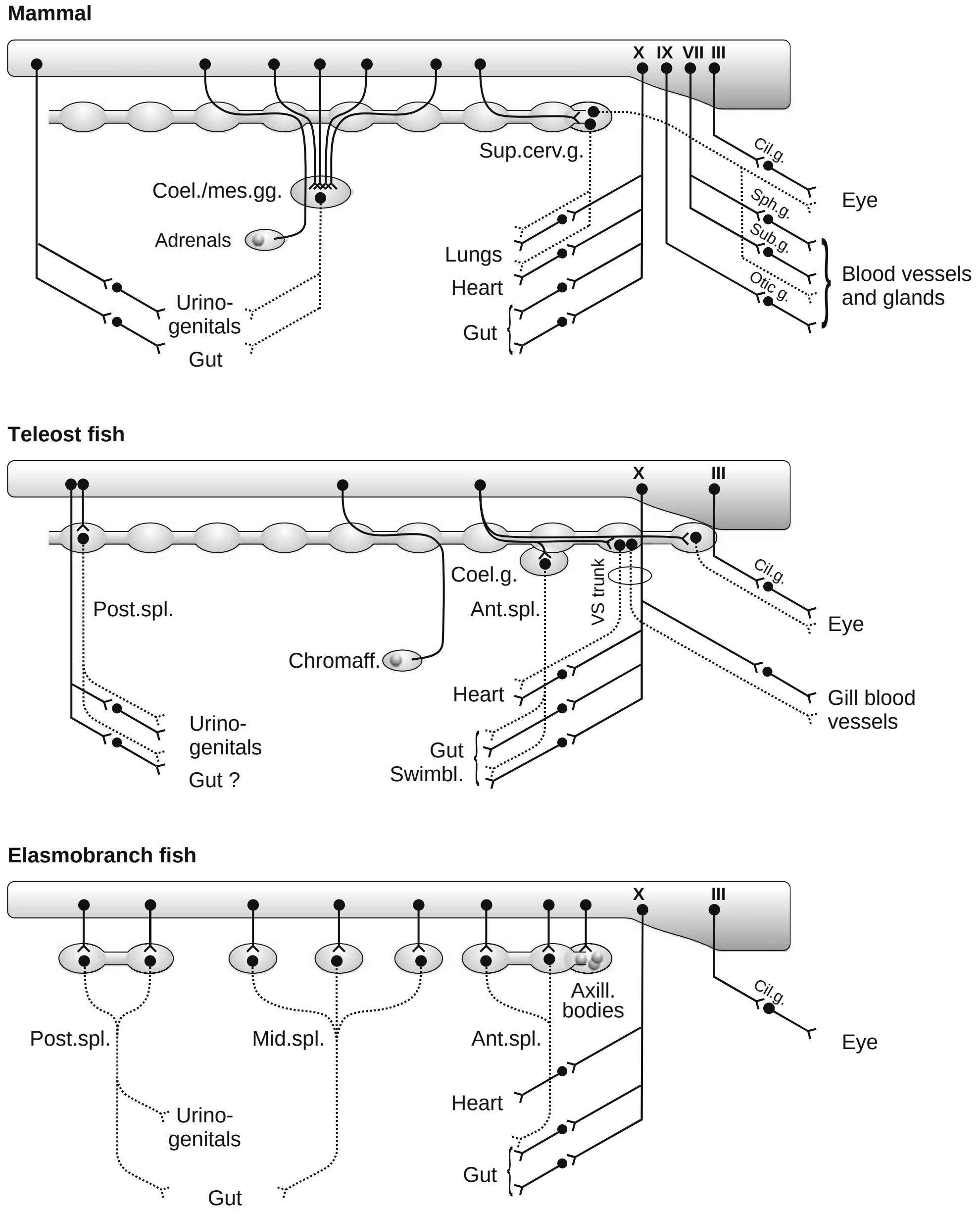


Figure 2 Simplified summary of the anatomy of the ANS in elasmobranch and teleost fish, compared to the mammalian system. Abbreviations: Ant.spl., anterior splanchnic nerve; Axill. bodies, axillary bodies (chromaffin cells); Cil.g., ciliary ganglion; Coel.g., coeliac ganglion; Coel./mes.gg., celiac and mesenteric ganglia; Mid.spl.; middle splanchnic nerve; Otic g., otic ganglion; Post.spl., posterior splanchnic nerve; Sph.g., sphenopalatine ganglion; Sub.g., submandibular ganglion; Sup.serv.g., superior cervical ganglion; VS trunk, vago-sympathetic nerve trunk; Roman numerals refer to cranial nerves III (oculomotor), VII (facial), IX (glossopharyngeal), and X (vagus). Reproduced from Fig. 1 in Nilsson S (1997) The autonomic nervous system of the dog and the dogfish: A comparative approach. *Acta Physiologica Scandinavica* 161(supplementum 638): 39–46.

catecholamines, while those in the vesicular ganglion do not. It is very tempting to regard the neurons of the chain ganglia as classically sympathetic/adrenergic, and those of the vesicular ganglion as parasympathetic (possibly cholinergic). However, a mix up of anatomical and functional terminology is not useful.

The full wiring diagram of the teleost ANS, albeit simplified, is presented in **Figure 2**.

Dipnoans

The ANS and its function in the lungfish have received comparatively scant attention. Sympathetic chains were described by E Giacomini in 1906, who was pleased (one assumes) to quote verbatim texts in German and English by workers who failed to find the chains. In fact, the sympathetic chains are flimsy and indeed hard to spot.

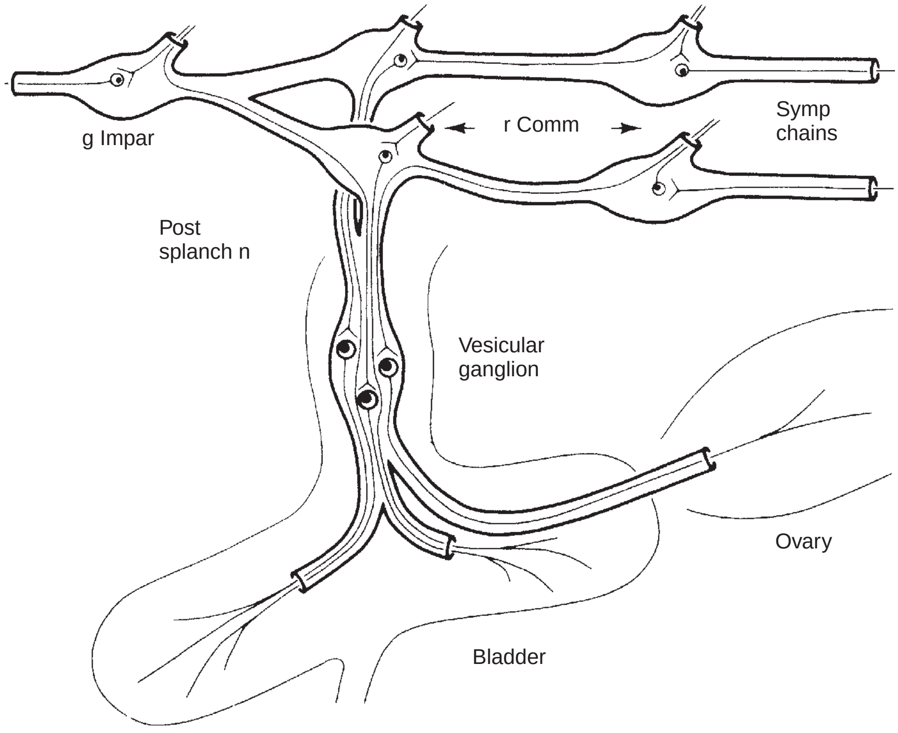


Figure 3 Arrangement of the ANS in the posterior part of the sympathetic chains in the Atlantic cod, *G. morhua*. Nerve fibers from the spinal cord enter the chain ganglia as white (i.e., myelinated) rami communicantes (r comm) and synapse either on smaller ganglion cells within the sympathetic ganglia, or on larger ganglion cells in the vesicular ganglion along the ureter. A double antagonistic innervation of the urinary bladder has been demonstrated, and it is possible that the smaller, catecholamine-containing cells in the ganglia represent the sympathetic/adrenergic control, while the larger nonadrenergic cells in the vesicular ganglion represent postganglionic parasympathetic/cholinergic control. Reproduced from Fig. 2 in Holmgren S and Nilsson S (1982) Neuropharmacology of adrenergic neurons in teleost fish. *Comparative Biochemistry and Physiology* 72C: 289–302.

They run parallel to the spinal column, just as in other vertebrates, and form loops (annuli) around the intercostal arteries. Histochemical studies have so far failed to provide solid evidence for adrenergic nerve fibers in lungfishes, but there is an extensive system of chromaffin tissue that may, and perhaps does, service as an adrenergic control system.

This chromaffin system resides in three places: in the wall of the atrium of the heart, in the wall of the left cardinal (azygos) vein, and in the walls of the intercostals arteries where they exit from the dorsal aorta. The control of the chromaffin tissue in these sites is not well understood.

Cranial autonomic components occur in the vagus (X) nerve and, in the Australian lungfish (*Neoceratodus*), probably also in the oculomotor (III) nerve. A brief comment on the cardiac control in dipnoans is given later. Some information about control of the very intriguing shunting of blood between the lung and the gill is available.

Functional Aspects of the Fish ANS

The number of fish groups multiplied by the number of organs and organ systems that are controlled by the ANS makes for a formidable variation in wiring diagrams, transmitter systems, and physiological adaptations. All this cannot be covered here; the interested reader is referred to the 'Further reading' section.

There are two systems that could, maybe, serve to illustrate on the one hand some kind of phylogenetic development and, on the other hand, a much less organized albeit still functional control. The systems chosen here are the patterns of cardiac control – from cyclostomes to elasmobranchs, dipnoans, and teleosts, and the autonomic nervous control of the iris of the eye.

Control of the Fish Heart

The systemic heart of fish, like in all vertebrates, serves as a pump to propel blood through the body (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). The ANS is an important system for controlling cardiac performance, to regulate blood flow and blood pressure. Cardiac control in tetrapods is performed by an inhibitory innervation by vagal, cholinergic nerve fibers that slow the heart rate, and decrease stroke volume. In addition, there is an excitatory innervation by adrenergic fibers from the sympathetic chains. This double antagonistic innervation is a common pattern in the ANS.

In the cyclostomes, there may be no autonomic cardiac innervation at all (hagfish), or the vagal innervation is excitatory (lampreys). Some influence from catecholamines released from intra-cardiac chromaffin cells can be surmised, since drugs that block β-adrenoceptors cause a slowing, and eventually a stop, of the heart in hagfish.

In the elasmobranchs there is no solid evidence for an adrenergic nervous control of the heart, although a cholinergic inhibitory vagal innervation (as in all other vertebrates) is present. A contribution to cardiac control by circulating catecholamines released from the axillary bodies is possible, although definite evidence is required. The main question being: Is the blood concentration of the amines high enough to affect the heart?

In the lungfish, not much is known about the cardiac control beyond the vagal inhibitory innervation, but the presence of chromaffin tissue in the atrium certainly suggests some kind of catecholaminergic contribution to the control of cardiac performance. As in the elasmobranchs, catecholamine release from chromaffin tissue in the large veins running to the heart is likely. The role of these circulating catecholamines in direct cardiac control does, however, need further attention.

In the teleosts, finally, cardiac control by the ANS resembles that in the tetrapods, with a cholinergic, vagal inhibitory control forming a double antagonistic innervation together with an adrenergic excitatory innervation from the spinal autonomic portion of the ANS. A stimulation of the heart performance in the Atlantic cod (*G. morhua*) has been shown in response to electrical stimulation of the preganglionic autonomic nerve supply to the chromaffin tissue of the posterior cardinal veins. The importance of the circulating catecholamines in cardiac control *in vivo* needs further elucidation similar to the situation in the other fish.

The cardiac control patterns in fish are summarized in **Figure 4**.

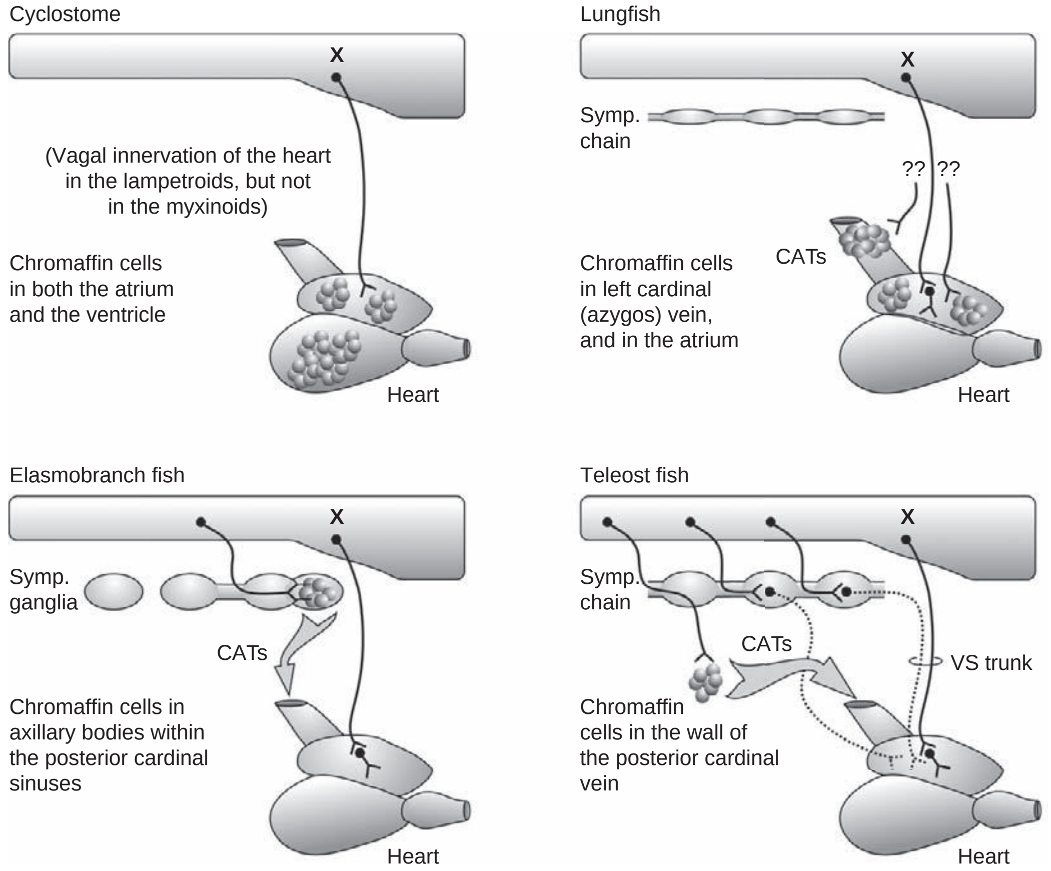

Cyclostome
X
(Vagal innervation of the heart in the lampetroids, but not in the myxinoids)
Chromaffin cells in both the atrium and the ventricle
Heart
Lungfish
X
Symp. chain
?? ??
CATs
Chromaffin cells in left cardinal (azygos) vein, and in the atrium
Heart
Elasmobranch fish
X
Symp. ganglia
CATs
Chromaffin cells in axillary bodies within the posterior cardinal sinuses
Heart
Teleost fish
X
Symp. chain
CATs
VS trunk
Chromaffin cells in the wall of the posterior cardinal vein
Heart

Figure 4 Cardiac control in four groups of fish, showing vagal nervous control (cholinergic – solid lines) and control by spinal autonomic pathways (adrenergic – dotted lines) via postganglionic neurons from the sympathetic chains, and endogenous or exogenous chromaffin cells. The vagal control of the heart is absent in hagfishes, excitatory in lampreys and inhibitory in the other fish groups (and also in tetrapods). It may be possible to discern a phylogenetic trend in the adrenergic control: from endogenous catecholamine-releasing cells with unclear control, via exogenous chromaffin cells releasing catecholamines (CATs) to the blood, to the direct innervation of the heart seen in teleosts (and, again, also in the tetrapods). Reproduced from Fig. 2 in Nilsson S (1997) The autonomic nervous system of the dog and the dogfish: A comparative approach. *Acta Physiologica Scandinavica* 161(supplementum 638): 39–46.

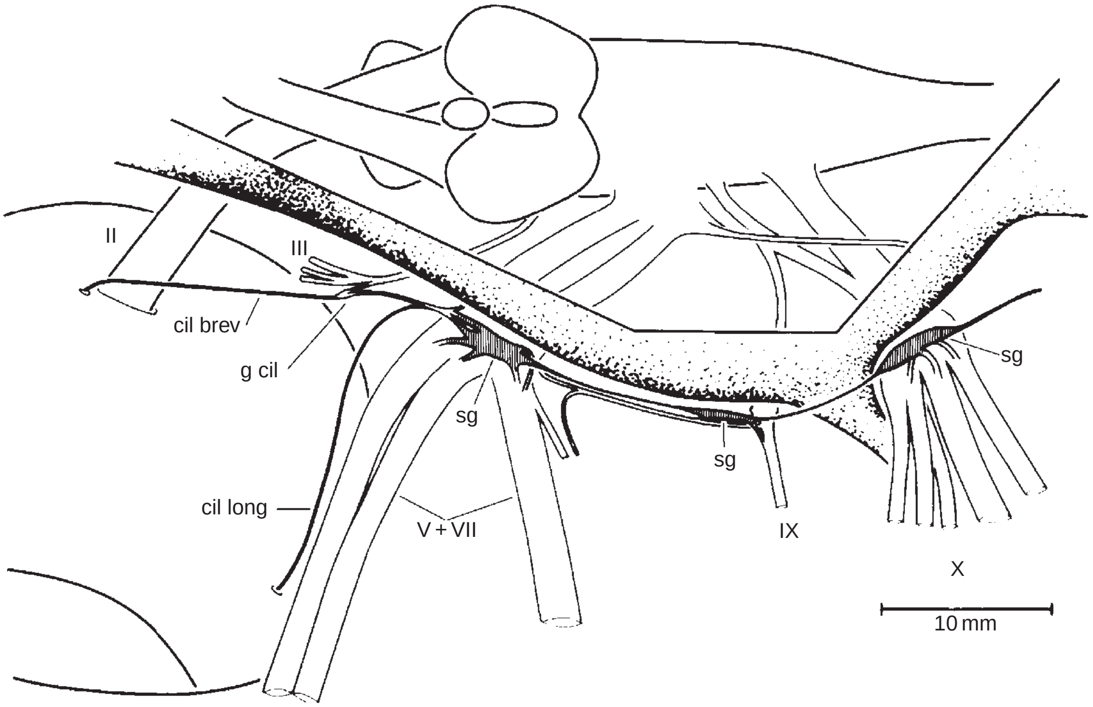


Figure 5 ANS anatomy in the head of the Atlantic cod, *Gadus morhua*, outlining the cephalic portion of the sympathetic chain and the ciliary nerves. Legend: cil brev, short ciliary nerve; cil long, long ciliary nerve; g cil, ciliary ganglion; sg, sympathetic ganglion; II, optic nerve; III, oculomotor nerve; V + VII, trigeminus-facialis nerve complex; IX, glossopharyngeal nerve; X, vagus nerve. Reproduced with kind permission from Fig. 1 in Nilsson S (1980) Sympathetic nervous control of the iris sphincter of the atlantic cod, *Gadus morhua. Journal of Comparative Physiology A*, 138: 149–155.

Control of the Iris

If it is possible to see some phylogenetic trend in the adrenergic control systems of the heart of fish and other vertebrates (cf. **Figure 4**), this is certainly not so for the ANS control of the iris in those few vertebrates that have been studied. There are two sets of muscle that act to alter the size of the pupil: the circular sphincter around the edge of the iris and the radial arrangement of muscle from the edge, much like the spokes of a bicycle wheel. Contraction of the sphincter causes miosis (narrowing of the pupil), while contraction of the radial muscle causes mydriasis (widening of the pupil). The smooth muscle cells of the iris are of ectodermal origin, originating from retinal cells. An indication of this can be found in the direct constrictor effect of light on the isolated iris muscle in some fish and amphibians (**see also Vision**: Vision in Fishes: An Introduction).

The pattern of ANS influence on the two sets of smooth muscle varies between the fish studied, and also further into the tetrapods. The structure of the cephalic sympathetic chains in teleost fish is somewhat aberrant from other vertebrate groups, in that chain ganglia are present in contact with the cranial nerves as they emerge from the cranium (**Figure 5**).

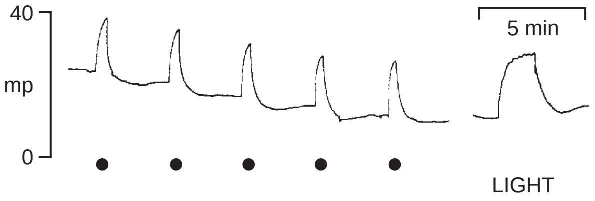


Figure 6 Contractions of the iris sphincter of the Atlantic cod, *Gadus morhua*, caused by electrical stimulation of the sympathetic chain posterior to cranial nerves V + VII with trains of pulses for 20 s every 4 min. The contraction on the right (LIGHT) is caused by 2 min of illumination of the iris by bright light. Reproduced with kind permission from Fig. 7 in Nilsson S (1980) Sympathetic nervous control of the iris sphincter of the atlantic cod, *Gadus morhua. Journal of Comparative Physiology A*, 138: 149–155.

A control via the oculomotor nerve (III) appears to be absent in *Gadus*, but present in other fish. This effect is cholinergic, and affects the radial muscle and not, as in for instance mammals, the sphincter. At least in the teleost *Uranoscopus*, an innervation of the sphincter by fibers from the spinal autonomic system has been described; these fibers are also cholinergic. Thus, in this species, a double antagonistic innervation of sorts is present – spinal

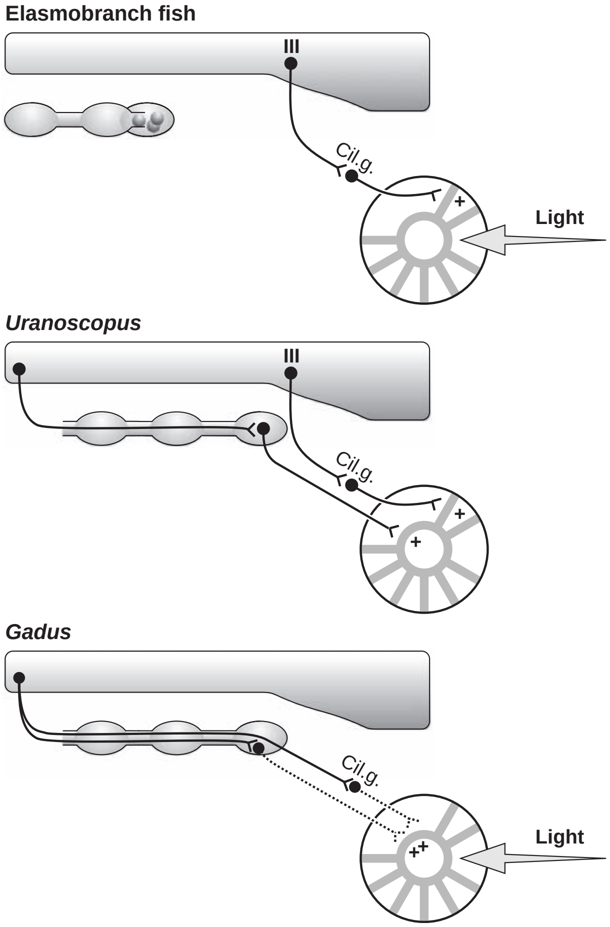


Figure 7 Control of the fish iris by autonomic innervation of the sphincter and the radially arranged dilator muscle. In addition, there is a direct pupillo-constrictor effect of light on the iris sphincter in both elasmobranchs and in *Gadus*. In the elasmobranchs, an excitatory oculomotor innervation of the radially arranged muscle antagonizes the direct pupillo-constrictor effect of light on the sphincter. In *Uranoscopus*, there are cholinergic fibers (solid lines) to both the sphincter (spinal autonomic in origin) and the dilator muscle (from the oculomotor nerve) that serve a double antagonistic control of the pupil. There may, however, be no direct effect of light on the sphincter. In *Gadus*, finally, a third pattern can be seen, in which there appears to be an innervation of the iris sphincter by excitatory nerve fibers from the spinal autonomic system that are adrenergic (dotted lines). These produce pupillo-constriction, as does direct light on the sphincter. Any double antagonistic control, therefore, appears to be lacking. Reproduced with kind permission from Fig. 3 in Nilsson S (1997) The autonomic nervous system of the dog and the dogfish: A comparative approach. *Acta Physiologica Scandinavica* 161(supplementum 638): 39–46.

autonomic pathways cause miosis, while the cranial autonomic fibers cause mydriasis.

In contrast to the elasmobranchs studied and *Uranoscopus*, the nervous control of the sphincter in the cod (*G. morhua*) is solely adrenergic, with postganglionic fibers running from the sympathetic chain ganglia or even from as far anterior as the ciliary ganglion. The adrenergic control is excitatory, acting via α-adrenoceptors. Again, this is a different arrangement from mammals (and the amphibian *Bufo marinus*) where spinal autonomic adrenergic innervation produces a relaxation of the sphincter muscle via β-adrenoceptors. As in many fish and amphibians, the iris sphincter of the cod responds directly to light with no need for an involvement of nerves (**Figure 6**).

To further complicate the view of pupillary control patterns in the vertebrates, the iris muscle of reptiles and birds is largely composed of striated muscle fibers. However, further discussion of this does not fit into the scope of the present text. However, it can be concluded (**Figure 7**) that already in the few fish that have been investigated, there are marked variations in the nervous control patterns of the iris.

See also: **Integrated Function and Control of the Gut**: Nervous System of the Gut. **Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes.

Further Reading

Burnstock G (1969) Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacological Review* 21: 247–324.

Campbell G (1970) Autonomic nervous system. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. IV, pp. 109–132. New York: Academic Press.

Fänge R, Johnels AG, and Enger PS (1963) The autonomic nervous system. In: Brodal A and Fänge R (eds.) *The Biology of Myxine*, pp. 124–136. Oslo: Universitetsforlaget.

Gibbins I (1994) Comparative anatomy and evolution of the autonomic nervous system canal. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 1–67. Chur, Switzerland: Harwood Academic Publishers.

Holmgren S and Nilsson S (1982) Neuropharmacology of adrenergic neurons in teleost fish. *Comparative Biochemistry and Physiology* 72C: 289–302.

Nilsson S (1980) Sympathetic nervous control of the iris sphincter of the atlantic cod, *Gadus morhua*. *Journal of Comparative Physiology A,* 138: 149–155.

Nilsson S (1983) *Autonomic Nerve Function in the Vertebrates*. Berlin: Springer.

Nilsson S (1984) Adrenergic control systems in fish. *Marine Biology Letters* 5: 127–146.

Nilsson S (1997) The autonomic nervous system of the dog and the dogfish: A comparative approach. *Acta Physiologica Scandinavica* 161(supplementum 638): 39–46.

Nilsson S (2009) Nervous control of fish swimbladders. *Acta Histochemica* 111: 176–184.

Nilsson S (2010) On the autonomic nervous and chromaffin control systems of lungfish. *Australian Zoologist* 35(2): 363–368.

Stannius H (1849) *Das Peripherische Nervensystem der Fische*. Rostock: Stiller.

Young JZ (1931) On the autonomic nervous system of the teleostean fish *Uranoscopus scaber*. *Quarterly Journal of Microscopical Science* 74: 491–535.

Young JZ (1933) The autonomic nervous system of selachians. *Quarterly Journal of Microscopical Science* 75: 571–624.

Opioids and Opioid Receptors in Fishes

MJ Herrero-Turrión, FM Sánchez-Simón, and RE Rodríguez, Institute of Neurosciences of Castilla y León, Salamanca, Spain

© 2011 Elsevier Inc. All rights reserved.

Endogenous Opioid Peptides
Opioid Receptors
Further Reading

Glossary

G-protein-coupled receptors (GPCRs) Comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways. Also known as seven-transmembrane domain receptors (7TM receptors), heptahelical receptors, serpentine receptor, and G-protein-linked receptors (GPLRs).

Opioid peptides Short sequences of amino acids that activate opioid receptors.

Opioid receptors A group of G-protein-coupled receptors that are activated by opioids ligands.

Phylogenetic analysis The use of the structure of molecules to gain information about evolutionary relationship between different organisms, the result of which is expressed in a phylogenetic tree.

Endogenous Opioid Peptides

Endogenous opioid peptides are primarily synthesized by distinct neurons in the central nervous system. Some peptides are also synthesized in regions of the pituitary gland in both fishes and mammals. Typical endogenous opioid peptides are agonists of opioid receptors and behave like inhibitory neuropeptides. They are derived from four precursors: proenkephalin (PENK), prodynorphin (PDYN), proopiomelanocortin (POMC), and pronociceptin/orphanin FQ (PNOC). The most representative opioid peptides are the enkephalins (ENKs), which are derived from the precursors PENKs and PDYNs.

It is possible that the ancestral opioid gene evolved in a chordate, as opioid precursor genes have not been detected in bacterial or protostome genomes. Thus, the proenkephalin hypothesis suggests the successive duplication of a *PENK*-like gene of an ancestral chordate. The sequential duplication of this gene gave rise to *PENK, POMC, PNOC*, and, subsequently, to *PDYN*.

The genomic organization of opioid precursor genes has been conserved. In brief, one to two small exons encode a portion of the 5′untranslated region (UTR) of the messenger RNA (mRNA), and another small exon encodes the rest of the 5′UTR, the signal sequence, and a portion of the N-terminal region. One large exon encodes the remainder of the open reading frame and the 3′UTR of the transcript.

Opioid peptides influence a great variety of physiological processes when bound to opioid receptors, including analgesia, respiratory function, cardiovascular function, and thermoregulation.

Proenkephalin

In mammals, the *PENK* classically codes for seven ENKs: four Met (M)-ENKs, one Leu (L)-ENK, the octapeptide M-ENK-Arg-Gly-Leu (MERGL), and the heptapeptide M-ENK-Arg-Phe (MERF). In contrast, in fishes, the *PENK* codes for six ENKs (**Table 1**). The number and spatial distribution of opioid sequences in these precursors have been well conserved within the vertebrates. However, there are different groups of amino acids in the C-terminal domain of the MERGL and the MERF sequences of mammals, which are species specific. In addition, a heptapeptide M-ENK-Gly-Tyr (MEGY) sequence in PENKs has been characterized in zebrafish (*Danio rerio*), Neopterygian fishes, and lungfishes. In addition, other sequences, such as M-ENK-Arg-Ser-Val/Ile/Leu (MERS(V/I/L)), have been identified in PENKs from Neopterygian fishes and lungfishes, and two hexapeptide M-ENK-Ile/Asp sequences (MEI/MED) have been detected in zebrafish PENKs.

MEGY, a newly discovered endogenous peptide from fishes, as well as two of its analogs (D-Ala^2)-MEGY (Y-D-Ala-GFMGY) and (D-Ala^2, Val^5)-MEGY (Y-D-Ala-GFVGY), have been identified in zebrafish and other fishes (**Table 1**). These peptides, homologous to the mammalian MERF, bind differentially and with high affinity (a K_D in the nanomolar range) to the two duplicate

Table 1 Proenkephalins (PENK) identified in fishes by different authors

Taxonomy group/ species				*Common name*	*Precursor*	*Endogenous peptide*	*References/Genbank accession N°*
Cartilaginous fish	Actinopterygii	Polypteridae	*Polypterus senegalus*	Gray bichir	PENK	M-ENK (4), F-ENK, MERSI, MEGY	Khalap *et al*., 2005 AF538845
		Chondrostei	*Acipenser transmontanus*	White sturgeon	PENK[a]	MEGY	Danielson and Dores, 1999 AF084454
			Heterodontus portusjacksoni	Galeoid shark	PENK	M-ENK (4), L-ENK, MERI, MENGF	Khalap *et al*., 2005 AY699827
		Neopterygii	*Lepisosteus platyrhynchus*	Florida gar	PENK[a]	M-ENK (3[a]), L-ENK, MEGY, MERSV	Khalap *et al*., 2005 AY699826
			Amia calva	Bowfin	PENK	M-ENK (4), L-ENK, MEGY, MERSI	Khalap *et al*., 2005
Ray-finned fish, Modern bony fish		Teleostei Euteleostei	*Salmo salar*	Atlantic salmon	PENK	M-ENK (4), L-ENK, MEGY, MERSA	BT046858
		Teleostei Otocephala	*Danio rerio*	Zebrafish	PENKs PENK-I PENK-II	PENK-I: M-ENK (4), L-ENK, MEGY PENK-II: M-ENK (4), MEI, MED, MERSV	Gonzalez-Nuñez *et al*., 2003a, 2005, 2007b NM_182883 NM_200083
Lobe-finned fish	Sarcopterygii	Dipnoi	*Protopterus annectens*	African lungfish	PENK	M-ENK (5), MEGY, MERSL	Dores *et al*., 2000 AF232670
			Neoceratodus forsteri	Australian lungfish	PENK	M-ENK (5), MEGY, MERSL	Sollars *et al*., 2000 Af232671

[a] Partial sequence.

δ opioid receptors (DORs) present in zebrafish (DOR1 and DOR2) and other opioid receptors in rat (*Rattus norvegicus*) brain.

Prodynorphin

PDYN and *PENK* precursor genes may have arisen from a common ancestral gene as paralog genes nearly 400 million years ago (Ma). In particular, the *PDYN* gene could have been present in the ancestral Gnathostomes.

Interestingly, in comparison with PDYNs from mammals, fish PDYNs contain opioid peptides different from mammalian M-ENK. The Sarcopterygian Australian lungfish (*Neoceratodus forsteri*) has an L-ENK, Actinopterygian fishes have an Ile (I)-ENK, and another Sarcopterygian fish, African lungfish, *Protopterus annectens* has a Phe (F)-ENK (**Table 2**). The presence of these types of opioid sequences (L-, I-, or F-ENK) is similar to the structure of PENKs from Actinopterygians.

The pharmacological profiles of two zebrafish dynorphins (DYN A and DYN B) and mammalian DYN A indicate similarities. Both mammalian DYN A and zebrafish DYN A bind to the four classical opioid receptors from zebrafish (μ or MOR, κ or KOR, DOR1, and DOR2), although with different affinities. The differences in receptor distribution, functional regulation, and the ratio of each receptor *in vivo* also may modify the pharmacological profile of zebrafish DYN A. The zebrafish DORs display a relative preference for the M-ENK found in zebrafish DYN A than for the L-ENK present in the mammalian DYN A, although, interestingly, zebrafish MOR shows higher affinity for the mammalian DYN A than for its own DYN A.

Pronociceptin

Nociceptin (NOC) is the endogenous ligand of the NOP receptor and it is derived from the cleavage of a larger precursor named PNOC. In fishes, PNOCs have been fully cloned in the white sturgeon (*Acipenser transmontanus*) and zebrafish. Two partial sequences exist for Japanese pufferfish (*Takifugu rubripes*) (PNOC1 and PNOC2) probably reflecting the genome duplication that took place in teleosts.

The amino acid sequences of fish PNOCs are slightly longer than the sequence of mammalian PNOCs. The mammalian precursor includes only one NOC, while the cleavage of fish *PNOCs* can produce two possible NOCs and a C-terminal peptide. Moreover, fish PNOCs do not contain the nocistatin peptide, an endogenous molecule with rather controversial functions in mammals. The fact that one NOC from zebrafish contains a Tyr residue (vs. Phe) as the first amino acid at the N-terminus domain, as occurs in DYN A, suggests that zebrafish NOC could bind to opioid receptors, especially to KOR, and as a consequence, it could have different roles than those observed for the mammalian NOC.

The expression of zebrafish *PNOC* gene in CNS was expected, but it is surprising to find PNOC in intestine, since in mammals, *NOC* is expressed in brain and in lower levels in kidney, spleen, ovary, and peripheral blood leukocytes, but not in the digestive tract. It is interesting to note that the expression in intestine is more similar to that displayed by opioid precursors, such as *PENK* and *PDYN* in mammals.

Proopiomelanocortin

POMC is the most complex precursor in the opioid gene family. This gene is the most common precursor of an endorphin opioid peptide (β-END), in addition to the α-neo-END encoded by *PDYNs*. *POMC* also encodes nonopioid peptides such as melanocortins and is involved in stress, pain, energy balance, and food intake. In addition, in fishes and amphibians, it is implicated in the adaptation to background color.

In fishes, *POMC* mRNA is expressed in two types of cells of the pituitary gland. In the corticotrophs in the *rostral pars distalis* (RPD), *POMC* is processed into ACTH (adrenocorticotropic hormone) and β-lipotropin. In the melanotrophs in the *pars intermedia* (PI), *POMC* gives rise to alpha-melanocyte-stimulating hormone (α-MSH) and β-END. Moreover, *POMC* transcripts can also be detected in a subset of neurons in the CNS such as in the *nucleus lateralis tuberis* (in the ventral hypothalamus) and in the *preoptic nucleus area*; both regions are considered to be the topological and functional equivalent to the *arcuate nucleus* and the *paraventricular* and *supraoptic nuclei* of the mammalian *hypothalamus*, respectively.

The POMC-derived peptides have undergone specialization in different fishes (**Table 3**). For instance, cartilaginous fishes have an extra melanocortin, δ-MSH, and the teleosts that existed before the divergence of eels (*Anguilla japonica*, Japanese eel and *A. rostrata*, American eel) lack the segment that encodes γ-MSH.

Furthermore, the higher teleosts have two *POMC* paralog genes (*POMC*α and *POMC*β) that originated after the ancient teleost whole-genome duplication. This duplication apparently occurred in the teleost lineage after the branching of the longnose gar lineage (*Lepisosteus osseus*, Neopterygii) and before the branching of Elopomorpha Anguilliformes (Japanese and American eels) and, within the Clupeocephala, the separation of the superorders Ostariophyssi, Portacanthopterygii, and Acanthopterygii. In the Agnathan sea lamprey (*Petromyzon marinus*), two *POMC-related* genes (*POC*-procortin and *POM*-promelanocortin) have also been found. They are expressed in the pituitary and may be the result of a genome duplication.

Table 2 Prodynorphins (PDYN) identified in fishes by different authors

Taxonomy group/ species				*Common name*	*Precursor*	*Endogenous peptide*	*References/Genbank accession N°*
Cartilaginous fish	Actinopterygii	Polypteridae	*Polypterus senegalus*	Gray bichir	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B	Alrubaian *et al*., 2006 DQ141340
Ray-finned fish Modern bony fish		Teleostei Elopomorpha Anguilliformes	*Anguilla rostrata*	American eel	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B	Alrubaian *et al*., 2006 AY445636
		Teleostei Clupeocephala Euteleostei Protacanthopterygii	*Oncorhynchus salmon*	Masu salmon	PDYN[a]	DYN A and DYN B	Dores *et al*., 2002 AF525291
			Salmo salar	Atlantic salmon	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B	NM_001140923
		Teleostei Clupeocephala Euteleostei Acanthomorpha	*Takifugu rubripes*	Japanese pufferfish	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B	FuguGenscan 15833
			Oreochromis niloticus	Nile tilapia	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B	Alrubaian *et al*., 2006 DQ141339
		Teleostei Clupeocephala Otocephala Ostariophysi	*Danio rerio*	Zebrafish	PDYN	Ile-ENK, α-neoEND, DYN A, DYN B.	Gonzalez-Nuñez *et al*., 2007[a] NM_182883
Lobe-finned fish	Sarcopterygii	Dipnoi	*Protopterus annectens*	African lungfish	PDYN[a]	L-ENK, F-ENK, α-neoEND, DYN A.	Dores *et al*., 2004 AY445638
			Neoceratodus forsteri	Australian lungfish	PDYN	L-ENKs (2), Neo-ENDs, DYN A, DYN B.	Dores *et al*., 2004 AY 445637

[a] Partial sequences.

Table 3 Proopiomelanocortins (POMCs) identified in fishes by different authors

Taxonomy group/species				*Common name*	*Precursor*	*Endogenous peptide*	*References/Genbank accession N°*
Jawless fish	Agnatha		*Petromyzon marinus*	Sea lamprey	POMCs-like POM and POC	POM: α-, β-MSH, β-END_1 POC: β-END_2, ACTH	Heining et al., 1995; Takahashi et al., 1995
Cartilaginous fish	Actinopterygii	Polypteridae	*Polypterus senegalus*	Gray bichir	POMC	α-, β-, γ-, δ-MSH β-END	Bagrosky et al., 2003 AF465781
		Chondrostei	*Acipenser transmontanus*	White sturgeon	POMCs POMC-A POMC-B	α-, β-, γ-, δ-MSH β-END	Amemiya et al., 1997; Alrubaian et al., 1999
			Squalus acanthias	Dogfish	POMC	α-, β-, γ-, δ-MSH β-END	Amemiya et al., 1999b
			Dasyatis akajei	Stingray	POMC	α-, β-, γ-, δ-MSH β-END	Amemiya et al., 2000
			Polyodon spathula	Mississippi paddlefish	POMCs POMC-A POMC-B	α-, β-, γ-, δ-MSH β-END	AF117302 AF117303
			Heterodontus portusjacksoni	Galeoid shark	POMC	α-, β-, γ-, δ-MSH β-END	Dores et al., 2003
			Chimaera phantasma	Ratfish	POMC	α-, β-, γ-, δ-MSH β-END	Takahashi et al., 2004
		Neopterygii	*Lepisosteus osseus*	Longnose gar	POMC	α-, β-, γ-, δ-MSH β-END	Dores et al., 1997
		Teleostei Elopomorpha Anguilliformes	*Anguilla japonica*	Japanese eel	POMC	α-, β-MSH, β-END	AY158010
			Anguilla rostrata	American eel	POMC	α-, β-MSH, β-END	AF194969
		Clupeocephala Euteleostei Protacanthopterygii	*Oncorhynchus nerka*	Sockeye salmon	POMCs	POMC-Aα-, β-MSH	Okuta et al., 1996
Ray-finned fish, Modern bony fish			*Oncorhynchus mykiss*	Rainbow trout	POMC	POMC-A (α) POMC-B (β)	Salbert et al., 1992 X69808 X69809
			Oncorhynchus keta	Chum salmon	POMC	POMCβ	X01122
		Clupeocephala Euteleostei Acanthomorpha	*Verasper moseri*	Barfin flounder	POMCs	POMC-A (α) POMC-B (α) POMC-C (β)	Takahashi et al., 2005 AB051424 AB051425 AB051426

(*Continued*)

Table 3 (Continued)

Taxonomy group/species				*Common name*	*Precursor*	*Endogenous peptide*	*References/Genbank accession N°*
			Paralichthys olivaceus	Bastard halibut	POMCs	POMC-I (α) POMC-II (α)	AF184066 AF191593
			Tetraodon nigroviridis	Black pufferfish	POMCs	POMCα POMCβ	De Souza et al., 2005
			Tetraodon fluviatilis	Green pufferfish	POMCs	POMCα POMCβ	De Souza et al., 2005
			Takifugu rubripes	Japanese pufferfish	POMCs	POMCα POMCβ	Klovins et al., 2004 De Souza et al., 2005
		Clupeocephala Otocephala Ostariophysi	*Danio rerio*	zebrafish	POMCs	POMCα POMCβ	Hansen et al., 2003 Liu et al., 2003 Gonzalez-Nuñez et al., 2003c AL590149 / NM_181438 AL845420 / NM_181438
			Cyprinus carpio	Common carp	POMCs	POMC-I (α) POMC-II (α)	Y14618 Y14617
			Carassius auratus	Goldfish	POMC	POMCα	AJ431209
			Ictalurus punctatus	Channel catfish	POMC	POMCα	AY174050
Lobe-finned fish	Sarcopterygii	Dipnoi	*Protopterus annectens*	African lungfish	POMC	α-, β-, γ-MSH β-END	Amemiya et al., 1999a; Dores et al., 1999; Lee et al., 1999 AF100164

Opioid Receptors

Opioid receptors are members of the Rhodopsin family, formed by four closely related protein subfamilies, which belongs to the large superfamily of G-protein-coupled receptors (GPCRs). Opioid receptors are the targets for endogenous opioid peptides and opiate drugs (morphine and its synthetic derivatives). Using pharmacological criteria, these receptors have been classified into four types: MOR, DOR, KOR, and an orphanin FQ receptor or nociceptin opioid receptor (NOP). The vertebrate opioid system has always been a complex system, even before the radiation of jawed vertebrates (≈450 Ma). Different comparative studies have suggested that the opioid receptor gene family arose early in vertebrate evolution by tetraploidization and has been highly conserved over time. Evolutionary analysis of vertebrate opioid receptors suggests that early in animal evolution, there was a single opioid receptor gene, which was present in the first jawed vertebrates. The first round (1R) of genome duplication early in chordate evolution produced the ancestral *DOR/MOR* and *NOP/KOR* genes. A second round (2R) of genome duplication led to the specification of the four opioid receptors present in all extant vertebrates today. In addition, there is evidence of a third round (3R) of genome-wide duplication in fishes that took place early in ray-finned fish evolution and it led to the lineage of bony fishes (teleosts).

Opioid receptors from fishes have been implicated in a broad range of physiological roles, including nociception and cognition, as well as affective, endocrine, cardiovascular, gastrointestinal, immune, metabolic, and respiratory functions, stress, and autonomic regulation. The first studies on the analgesic effects of opioids in fish models, carried out in goldfish (*Carassius auratus*), Derjugin codfish (*Gadus morhuamarisalbi*), rainbow trout (*Oncorhynchus mykiss*), and common carp (*Cyprinus carpio*), demonstrated that these species showed a dose-dependent anti-nociceptive effect when treated with morphine-like compounds (β-casomorphine or dermorphin) or the opioid analgesic tramadol. The effects produced by some of these compounds are partially antagonized by naloxone, as observed in mammals.

All opioid receptors from vertebrates, studied to date, show high constitutive expression in the nervous system. In fishes, opioid receptors are found in the nucleus preopticus of the hypothalamus and pituitary gland. A much lower constitutive expression of opioid receptors occurs in peripheral tissues such as kidney and head kidney (the anatomical equivalent of the mammalian adrenal gland), and all systemic immune organs, including thymus and spleen. Furthermore, pharmacological analysis of opioid-binding sites in brain homogenates from fishes indicate three types of classical opioid-binding sites (μ, δ, and κ), similar to those characterized in mammalian tissues, but with significant differences in the binding of selective opioid ligands.

μ Opioid Receptor

MOR is the opioid receptor that shows higher affinity for morphine, the most used analgesic. Several *MOR* genes have been cloned from teleost species (zebrafish, common carp, and white sucker (*Catostomus commersoni*)). Moreover, partial sequences of *MORs* have been identified in other two fish species (Euteleostean teleost: *Morone saxatilis,* striped bass, and the cartilaginous fish, chondricthyes, *Alopias vulpinus,* thresher shark) and one jawless fish (Agnatha: *Eptatretus stoutii,* Pacific hagfish). Recently, the genomes of many animals have been completely or nearly completely sequenced, which facilitates the identification of *MOR* genes in different vertebrate species. In this sense, *in silico* determinations have identified the putative MORs of four teleost species (*Tetraodon nigroviridis,* black pufferfish; Japanese pufferfish; *Oryzias latipes,* Japanese medaka; and *Gasterosteus aculeatus,* three-spine stickleback).

The genomic organization of all vertebrate *MOR* genes, including fishes, presents interesting homologies. In all vertebrates, only one copy of the *MOR* gene has been detected, but at least three different genomic organizations have been identified. Most *MOR* genes are composed of three main coding exons. In fishes, the *MOR* genes from Euteleostei teleosts (black pufferfish, Japanese pufferfish, three-spine stickleback, and Japanese medaka) display a different genomic organization. They have five exons; the equivalent of the third mammalian exon is subdivided into three exons. Mammalian *MOR* genes could have lost two introns between exons 3–4 and 4–5 during evolution, although it is also possible that these two introns were acquired by the Euteleost lineage. Different studies have also identified several variants that encode truncated proteins of mammalian MORs, and although the functional significance of these truncated receptors is not clear, other research on different receptor systems have reported that truncated proteins modulate the activity of other receptors.

The amphibian and fish MORs express fewer GCPR and MOR-specific motifs than the tetrapod species, despite their position in the MOR clade in phylogenetic analysis. Analysis of functional mapping indicates that MORs from teleosts (and several amphibians) may not function as classical MORs, suggesting that opioid ligands may be less selective in nonmammalian species. In particular, the functional characterization of zebrafish MOR, by competition, saturation, [^{35}S]-GTPγS binding experiments, and intracellular cyclic adenosine monophosphate (cAMP)-level determinations using both peptidergic and nonpeptidergic opioid ligands, shows a pharmacological profile similar to that of the mammalian MOR. Besides, the internalization process of MOR from zebrafish after opiate agonist treatment (morphine, [D-Ala^2, N-Me-Phe^4, Gly5-ol]-enkephalin (DAMGO), etorphine) resembles the pattern observed in its mammalian counterpart.

δ Opioid Receptor

Analysis of *DOR* genes from fishes has been fully cloned and characterized in only one teleost, the zebrafish, although recently a new *DOR* from other cyprinid, the common carp, has been identified. *DOR1* and *DOR2* in zebrafish are also named ZFOR1 and ZFOR4.

As mentioned above, the degree of homology is not uniform along the opioid receptor sequences. Hence, in DORs of fishes identified up to now, the most divergent area is the second extracellular loop, which has more positive and negative charges than tetrapods. This may explain why the highly selective δ ligands from mammals show lower or no affinity for some DORs from fishes, such as zebrafish DOR2. In addition, zebrafish DORs have some divergences in their amino-acid sequences that may explain their pharmacological differences. *DOR1* and *DOR2* are co-expressed in the same brain areas and, like the mammalian *DOR,* are detected in olfactory bulb regions. However, *DOR1* is uniquely detected in the supracommisural part of the ventral area of the zebrafish telencephalon, which is considered to be homologous to the mammalian amygdaloid complex. The expression of two *DORs* from zebrafish in neuroendocrine regions, including the preoptic area and the hypothalamus, is likely involved in control of gonadotrophin-releasing hormone (GnRH) release. Moreover, zebrafish DORs may be involved in analgesic mechanisms because both mRNAs are present in the rostrocaudal extension of the reticular formation. Finally, the moderate expression of *DOR2* in other brain areas from zebrafish, such as the pretectum and posterior tuberculum, suggests that this receptor might be influenced by adaptive mechanisms also related to behavioral responses basic for survival.

On the other hand, pharmacological analysis of zebrafish DORs shows specific and saturable binding with nonspecific antagonist [^{3}H]diprenorphine, displaying one binding site with an affinity constant in the same range (nM) to that previously observed for mammalian DORs. However, the δ-selective radioligand [^{3}H] [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) does not bind to DOR2 and has low affinity for DOR1. The selectivity of this ligand is gradually lost when moving down in the evolutionary scale, which suggests that it may not be suitable for understanding the δ-binding profile in nonmammalian vertebrates.

κ Opioid Receptor

The analysis of *KOR* genes from fishes has been characterized in zebrafish and common carp. The distribution of zebrafish KOR is throughout the dorsal telencephalic areas, epithalamus, hypothalamic recess nuclei, periventricular layer of the optic tectum, granular layer of the cerebellum, reticular formation, facial lobe, and with lower intensity in other regions such as the olfactory bulb, vagal lobe, octavolateral areas, and spinal cord.

Pharmacological analysis of KOR from zebrafish shows specific and saturable binding using the nonspecific antagonist [^{3}H]diprenorphine, displaying one binding site. The selectivity of this κ ligand is also gradually lost when moving down in the evolutionary scale, which suggests that it may not be suitable for determining the κ-binding profile in nonmammalian vertebrates. Although the κ-selective agonist U69,593 ([5α,7α,8β)-(+)-*N*-methyl-*N*-[7(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide]) failed to show effective displacement, the antagonist naloxone, the nonspecific antagonist bremazocine, morphine, and DYN-related peptides displaced the specifically bound [^{3}H]diprenorphine, but with different affinities.

Nociceptin Opioid Receptor

NOP exhibits a significant degree of sequence identity with the three classical opioid receptors. Nevertheless, the pharmacological implications of these receptors are different, since NOP does not recognize the classical opioid ligands such as morphine or naloxone. Analysis of *NOP* genes from fishes has been exclusively characterized in one teleost (zebrafish), with two *NOP* cDNAs that could be the result of the extra genome duplication that took place in teleosts.

See also: **Vision**: Adaptations of Photoreceptors and Visual Pigments; Photoreceptors and Visual Pigments.

Further Reading

Bodnar RJ (2008) Endogenous opiates and behavior: 2007. *Peptides* 29: 2292–2375.

De Souza FS, Bumaschny VF, Low MJ, and Rubinstein M (2005) Subfunctionalization of expression and peptide domains following the ancient duplication of the proopiomelanocortin gene in teleost fishes. *Molecular Biology and Evolution* 22: 2417–2427.

Dores RM and Lecaude S (2005). Trends in the evolution of the proopiomelanocortin gene. *General and Comparative Endocrinology* 142: 81–93.

Dreborg S, Sundstrom G, Larsson TA, and Larhammar D (2008) Evolution of vertebrate opioid receptors. *Proceedings of the National Academy of Sciences of the United States of America* 105: 15487–15492.

Herrero-Turrion MJ and Rodríguez RE (2008) Bioinformatic analysis of the origin, sequence and diversification of mu opioid receptors in vertebrates. *Molecular Phylogenetics and Evolution* 49: 877–892.

Janecka A, Fichna J, and Janecki T (2004) Opioid receptors and their ligands. *Current Topics in Medicinal Chemistry* 4: 1–17.

Khalap A, Bagrosky B, Lecaude S, *et al.* (2005) Trends in the evolution of the proenkephalin and prodynorphin genes in gnathostomes. *Annals of the New York Academy of Sciences* 1040: 22–37.

Larhammar D, Dreborg S, Larsson TA, and Sundstrom G (2009) Early duplications of opioid receptor and peptide genes in vertebrate evolution. *Annals of the New York Academy of Sciences* 1163: 451–453.

Stevens CW (2009) The evolution of vertebrate opioid receptors. *Frontiers in Bioscience* 14: 1247–1269.

Relevant Websites

http://www.fishbase.org – FishBase.

http://www.ncbi.nlm.nih.gov – National Center for Biotechnology Information.

SENSING THE ENVIRONMENT

Vision

Contents

Vision in Fishes: An Introduction
Physiological Optics in Fishes
Photoreceptors and Visual Pigments
Adaptations of Photoreceptors and Visual Pigments
Inner Retina and Ganglion Cells
Optic Tectum
Behavioral Assessment of the Visual Capabilities of Fish
Color vision and Color Communication in Reef Fish
Extraretinal Photoreception
Visual Adaptations to the Deep Sea

Vision in Fishes: An Introduction

H-J Wagner, Universität Tübingen, Tübingen, Germany

© 2011 Elsevier Inc. All rights reserved.

Further Reading

Glossary

Benthic Organisms living on, or near, the sea floor; lowest level of a body of water.

Bioluminescence Light produced by living organisms by physiological processes and without heat.

Chromophore Part or moiety of a molecule responsible for its color. In visual pigments the chromophore is retinal, the aldehyde of vitamin A, combined with the protein, opsin.

Circadian rhythm An internal rhythm, free running under constant conditions with a period slightly differing from the environmental 24 h cycle.

Cone The sensory cell type in the eye (photoreceptor) involved in color vision and bright light conditions.

Diverticulum An out-pocketing, or blind-ending sac, branching out from a hollow anatomical structure, here used to refer to ventrolaterally facing 'accessory eye' of some mesopelagic fish with dorsally directed tubular eyes.

Electroretinogram (ERG) A recording of the retina's electrical activity in response to light, usually recorded either on the cornea or on the retinal surface.

Endosymbiont An organism that lives symbiotically within the body of another.

G-protein A large class of intracellular, membrane-associated, proteins that act as second messengers for a wide range of receptors (including visual pigments) and transduce receptor signals by activating a chain of cellular events resulting in amplification and cell signaling.

Hadal Biogeographic region of the ocean bottom below the abyssal zone, typically ocean trenches deeper than about 6500 m depth.

Irradiance The amount of light or electromagnetic radiation at a surface. Spectral irradiance is used here to describe the light incident on the reef available for vision.

Melatonin Neurohormone synthesized and released in the dark by photoreceptor cells (or their derivatives, pinealocytes) in the retina and the pineal gland (epiphysis cerebri).

Mesopelagic A biogeographic region of open oceans between 200 and 1000 m depth and not near the ocean floor.

Microspectrophotometry A technique in which a narrow beam of light is passed through an isolated rod or cone outer segment in order to measure the absorbance of the visual pigment.

Myctophidae A family of teleosts known as lantern fish whose name originates from their conspicuous bioluminescent photophores. They account for up to 65% of the deep-sea fish biomass and are among the most widely distributed and populous of all vertebrates.

Opsin Protein component of the rhodopsin photopigment.

Optical aberration The deviation of light originating from a point in space from a single point in the image. There are monochromatic and chromatic aberrations that affect image quality and there is no perfect optical system that is entirely free of aberrations. Longitudinal spherical aberration and longitudinal chromatic aberration are the major aberrations in fish eyes.

Photic environment The distribution of light, in terms of brightness and spectral composition, within the environment.

Photopigment Labile molecules that change conformation upon absorbing light, thereby triggering various secondary reaction cascades, with transducin and arrestin as intermediate enzymes. In photoreceptive cells photopigments are composed of a protein moiety (generally called opsin) and a nonprotein chromophore part called retinal. Retinal-based opsins are called rhodopsins. The wavelength of the absorption maximum can be tuned by modifications of the amino acid sequence of the opsin molecule, and/or substituting retinal by dehydroretinal.

Pineal gland Part of the diencephalic epithalamus with cells specialized in the production of melatonin. In fish, it is composed of a stalk and a hollow vesicle located underneath a transparent window in the skull. The cellular components include photoreceptor and nerve cells.

Porphyropsin All visual pigments based on vitamin A_2.

Radiance A radiometry term to describe the amount of light, emitted from a particular area and from a defined solid angle or direction.

Receptive field The area of retina within which stimulation effects a response from a given neuron.

Refractive index The speed of light in vacuum divided by the speed of light in the medium in question. Because the speed of light is highest in vacuum, the refractive index of any other medium is always greater than 1. Light changes its direction of propagation according to Snell’s law if it passes across the interface between two media of different refractive indices.

Retinal ganglion cells The output neurons of the retina. Their axons, called ‘optic fibers’, terminate in the tectum and nuclei of the preoptic–thalamic–pretectal (PTP) area.

Retinomotor movements The myoids (parts of the inner segments) of rod and cone photoreceptors contract and elongate in response to changes in light levels and circadian endogenous signals. In the retinal pigment epithelium, melanosomes disperse and aggregate, which may be accompanied by changes in cell shape. Retinomotor movements occur in fishes, amphibia, reptiles, and birds.

Retinotopic map, organization, or projection Found in brain areas, such as tectum, that receive a spatially ordered set of input fibers from the retina, thereby forming a representation of the retina, and the visual field of the eye. A particular case of ‘topographic map’. The retinotectal map is an instance.

Rhodopsin All visual pigments based on vitamin A_1, but commonly used to refer only to rod visual pigments.

Rod A rod-shaped photoreceptor type in the eyes of vertebrates that functions in dim light conditions so may allow night vision. The photoreceptor molecule within rods generally absorbs light in the middle of the spectrum giving it a spectral sensitivity around 500 nm.

Spectral reflectance Objects reflect and absorb wavelengths of light differentially according to their spectral reflection and defined by the pigmentary or physical structure of their surface. The color of the object is also a product of the visual system’s spectral sensitivity and this varies among animals substantially.

Spectral sensitivity At least two cones, each with a different spectral sensitivity, are required for color vision.

Tetrachromatic color vision Color vision based on four spectrally distinct cone classes, normally maximally sensitive in the red, green, blue, and violet or ultraviolet.

Transmitter A chemical released by one neuron that causes a change in the polarization of target neurons with which it makes a synapse.

The visual system of fish has fascinated scientists for many different reasons. It has been used as a model system not only in visual science, but also in neuroscience and cell biology in general, behavioral sciences, embryology, and even for evolutionary ecology, as shown in the following few examples. Sticklebacks (*Gasterosteus aculeatus*) and cichlids were used to demonstrate the importance and specificity of visual stimuli as triggers of defined patterns of innate behavior. Cichlids and their visual system play an important role for the observation of the unexpected dynamics of evolution by ongoing speciation and occupation of new ecological niches in the African rift lakes. The marked morphological and physiological changes associated with light and dark adaptation in the fish retina have contributed to knowledge in synaptic plasticity and cell motility (retinomotor movements). Most of these cold-blooded animals are available in large numbers and their retina is easy to experiment on physiologically – these are good reasons for the great popularity of the teleost visual system. The first intracellular recordings from retinal neurons were obtained by Svaetichin as early as 1953; subsequently, the retinal circuitry for color vision was established. Many of these experiments were based on cyprinids such as the goldfish which were often regarded as the standard model species comparable to the lab rat. More recently, the zebrafish visual system has replaced that of the goldfish in a similar way the mouse has largely replaced the rat for genetic and molecular work (**see also Deep-Sea Fishes**).

However, as important as the insight from these studies undoubtedly is, in the author's view, they miss the most important point about fish as a model system, because, essentially, there is no such thing as the fish visual system. Rather, there is a tremendous diversity of fish and their adaptive radiation, a term used for vertebrate eyes, in general, in the title of G Wall's 1942 seminal book. According to recent numbers in FishBase, there are 31 500 different fish species and these live in habitats as diverse as coral reefs illuminated by the brightest tropical sun; the abyssal depths of the deep sea to which no sunlight penetrates; clear freshwater lakes; murky rivers; and dark caves. Obviously, the entire organism reflects the special constraints imposed by these divergent environments, but the visual system plays a key role. Size and orientation of the visual field are reflected in the design and motility of the eye; the average intensity is reflected by the complement of photoreceptor types; the spectral environments and even their seasonal changes may be reflected by the diversity of visual pigments; and the lifestyle as a predator or vegetarian is reflected by the topography of photoreceptors and retinal ganglion cells. J Lythgoe has characterized this aspect as visual ecology.

The present section on the fish visual system takes a double approach in an attempt to account for these issues.

In the first part, we follow the path of light into the eye onto the retina, investigate its effect on the visual pigments, and watch the processing of the neural signals within the retina and the brain, that is, the optic tectum. R Kroger (**see also Vision**: Physiological Optics in Fishes) outlines the rules of optics to explain the constraints underlying the shape of the teleost eye and lens. More specifically, he deals with the mechanisms that limit, or even overcome, the effects of longitudinal and chromatic aberration. When light enters the photoreceptor outer segments, it may be absorbed by as many as four different cone visual pigments in addition to a rod rhodopsin. Thus, some teleosts are capable of seeing a wider range of color than humans. J Bowmaker (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments) explains the evolution of these pigments and demonstrates how an exchange of a very limited number of amino acids in the opsin protein and/or the presence of a vitamin A_1 or A_2-based chromophore lead to a discrete shift in absorbance that allows fish to perfectly adapt to their spectral environment. With their spectral identities established and attributed, the morphological diversity of photoreceptor types helps to trace the synaptic connectivity of the chromatic channels with the bipolar and horizontal cells. M Levine (**see also Vision**: Inner Retina and Ganglion Cells) unravels the intraretinal network and shows how the photoreceptor signals are transformed into the various types of receptive field at the ganglion cell level. These constitute the codes that are transmitted via the optic nerves to the targets in the brain, foremost among them is the optic tectum. In this complex neural processor, the visual input is integrated with signals from other sensory systems, and D Northmore (**see also Vision**: Optic Tectum) outlines how the tectum arrives at discrimination and decision making preceding motor responses.

The blueprint of the visual system in teleosts outlined in this first group of articles is subject to, sometimes, extreme changes during the adaptation to the diverse habitats fish have conquered. Starting with the very shape of the eye, deep-sea fish arguably show the profoundest deviations from the common plan. Tubular eyes, occasionally associated with highly complex diverticula, are found in many deep-sea fishes. R Douglas and J Partridge (**see also Vision**: Visual Adaptations to the Deep Sea) take the reader on a guided tour of this vast habitat and demonstrate that in spite of the absence of sunlight, vision plays an important role for the majority of fish. Tuning of the spectral sensitivity to the prevailing wavelengths as a general phenomenon and dual-band, not color, vision as an amazing strategy by stomiids to make them invisible to (most) other fish are additional fascinating examples from the deep sea. S Schuster (**see also Vision**: Behavioral Assessment of the Visual Capabilities of Fish) examines freshwater fish and studies mechanisms by which archer fish (*Toxotes jaculatrix*) achieve the high degree of spatial precision in visuo-motor coordination. On the other hand, he also shows that vision plays an important role even in the weakly electric elephant fish. The widest potential of the visual system is undoubtedly encountered in coral reefs fish. J Marshall and K Cheney (**see also Vision**: Color Vision and Color Communication in Reef Fish) focus on the role of color in this habitat and give vivid examples how color is used both for camouflage and for advertising behavior. Importantly, the multicolored pictures of reefs and their inhabitants that are so familiar and appealing to the human eye may appear significantly different when imaged through the visual system (i.e., optical filters and spectral channels) of most reef fish.

See also: **Vision**: Adaptations of Photoreceptors and Visual Pigments; Behavioral Assessment of the Visual Capabilities of Fish; Color Vision and Color Communication in Reef Fish; Extraretinal Photoreception; Inner Retina and Ganglion Cells; Optic Tectum; Photoreceptors and Visual Pigments; Physiological Optics in Fishes; Visual Adaptations to the Deep Sea.

Further Reading

Bowmaker JK (1995) The visual pigments of fish. *Progress in Retinal and Eye Research* 15: 1–31.

Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

Denton EJ (1990) Light and vision at depths greater than 200 metres. In: Herring PJ, Campbell AK, Whitfield M, and Maddock L (eds.) *Light and Life in the Sea*, pp. 127–148. Cambridge: Cambridge University Press.

Douglas RH, Partridge JC, and Marshall NJ (1998) The eyes of deep-sea fish I: Lens pigmentation, tapeta and visual pigments. *Progress in Retinal and Eye Research* 17(4): 597–636.

Foster RG and Kreitzman L (2004) *Rhythms of Life the Biological Clocks that Control the Daily Lives of Every Living Thing*. London: Profile Books.

Kaneko A (1971) Physiological studies of single retinal cells and their morphological identification. *Vision Research* Supplement 3: 17–26.

Land MF and Nilsson D-E (2002) *Animal Eyes*. Oxford: Oxford University Press.

Lorenz K (1962) The function of color in coral reef fishes. *Proceedings of the Royal Institute of Great Britain* 39: 282–296.

Lythgoe JN (1979) *The Ecology of Vision*. Oxford: Clarendon Press.

Marshall NJ (2000) Conspicuousness and camouflage with the same 'bright' colors in reef fishes. *Philosophical Transactions of the Royal Society of London B* 355: 1243–1248.

Marshall NJ and Vorobyev M (2003) The design of color signals and color vision in fishes. In: Marshall NJ and Collin SP (eds.) *Sensory Processing in the Aquatic Environment*, pp. 194–222. New York: Springer.

Meek J (1983) Functional anatomy of the tectum mesencephali of the goldfish. *Brain Research Reviews* 6: 247–297.

Svaetichin G (1953) The cone action potential. *Acta Physiologica Scandinavica* 29: 565–600.

Tinbergen N (1951) *The Study of Instinct*. Oxford: Oxford University Press.

Vanegas H and Ito H (1983) Morphological aspects of the teleostean visual system: A review. *Brain Research Reviews* 6: 117–137.

Wagner H-J (1990) Retinal structure of fishes. In: Douglas R and Djamgoz M (eds.) *The Visual System of Fish*, pp. 109–157. London: Chapman and Hall.

Wagner H-J, Douglas RH, Frank TM, Roberts NW, and Partridge JC (2009) A novel vertebrate eye using both refractive and reflective optics. *Current Biology* 19(2): 108–114.

Wagner H-J, Fröhlich E, Negishi K, and Collin SP (1998) The eyes of deep-sea fish. II. Functional morphology of the retina. *Progress in Retinal Eye Research* 17: 637–685.

Walls G (1942) *The Vertebrate Eye and its Adaptive Radiation*. New York: Hafner.

Warrant EJ and Locket NA (2004) Vision in the deep sea. *Biological Reviews* 79(3): 671–712.

Relevant Website

http://www.fishbase.org/search.php – FishBase: FishBase Consortium.

Physiological Optics in Fishes

RHH Kröger, Lund University, Lund, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
Optical Elements of Fish Eyes
Some Special Optical Adaptations
Further Reading

Glossary

Aphakic aperture Part of the pupillary aperture through which light can enter the eye without passing through the crystalline lens; such light is not focused on the retina and degrades image contrast.

Basement membrane Extracellular component attached to the epithelial layer, consisting of connective tissue made up of a complex of short filamentous proteins, e.g., collagens. Epithelial cells adhere to the extracellular matrix by expressing cell adhesion molecules on their basal surface.

Chaperone A protein that assists the biochemical noncovalent folding and unfolding, as well as the assembly and disassembly of macromolecular structures such as other proteins.

Constructive interference Waves of the same wavelength and phase add up in amplitude; such light waves can occur by partial reflections at interfaces between media of different refractive indices. If several such interfaces are stacked at wavelength intervals, the partial reflections at the interfaces add up by constructive interference, such that light incident on the stack is reflected. The wavelength being reflected and the efficiency of the interference mirror depend on the distances between the interfaces, their number, and the angle of incidence.

Diffraction Every point on a wave front of light can be considered as a point source of light from which a secondary radial wave emanates. The radial waves originating from a wave front passing through an opening spread in all directions and, depending on the angle and distance to the opening, add up if they are in phase (constructive interference) or take out each other if they are in counterphase (destructive interference). This leads to a typical pattern with a central light patch and radially alternating dark and light rings. The smaller the opening (aperture) is, the larger and more diffuse is the central light patch. The spreading of light behind an aperture, such as the pupil of an eye, may thus limit the sharpness of the image.

Optical aberration The deviation of light originating from a point in space from a single point in the image. There are monochromatic and chromatic aberrations that affect image quality, and there is no perfect optical system that is entirely free of aberrations. Longitudinal spherical aberration and longitudinal chromatic aberration are the major aberrations in fish eyes.

Ora retinalis The edge of the retina in the ciliary region, which is also called ora ciliaris retinae. In the human eye, the ora retinalis is serrated in appearance and therefore called 'ora serrata'.

Refractive index The speed of light in vacuum divided by the speed of light in the medium in question. Because the speed of light is highest in vacuum, the refractive index of any other medium is always larger than 1. Light changes its direction of propagation according to Snell's law, if it passes across the interface between two media of different refractive indices.

Retinomotor movements The myoids (parts of the inner segments) of rod and cone photoreceptors contract and elongate in response to changes in light levels and endogenous circadian signals. In the retinal pigment epithelium, melanosomes disperse and aggregate, which may be accompanied by changes in cell shape. Retinomotor movements occur in fishes, amphibians, reptiles, and birds, but not in mammals.

Introduction

One of the earliest known vertebrates, *Haikouichthys ercaicunensis*, was a lamprey-like creature that lived about 540 million years ago, that is, during the early Cambrian period. *Haikouichthys* had already a well-developed eye, most likely with an optical apparatus. From such early eyes, vertebrate vision has evolved into highly sophisticated sensory systems (**see also Vision**: Adaptations of Photoreceptors and Visual Pigments). The evolution of man-made optical systems began at least 3000 years ago. The Nimrud lens, a crudely ground quartz lens found in the ruins of Niniveh, is the oldest known optical instrument. Today, we are surrounded by countless optical devices. However, although the basic physical phenomena are the same, biological and manufactured optical solutions took different routes right from their beginnings, mainly because different materials are used.

Optical Principles

The simplest optical system capable of creating an image is the camera obscura or pinhole camera. Light is allowed to pass through a small opening into an otherwise light-tight chamber. Because light propagates along straight lines, each point in space is imaged at a corresponding location on the back surface of the chamber, creating an image of the surroundings that is rotated 180° (**Figure 1**(**a**)). The detail and the brightness of the image are directly and inversely, respectively, proportional to the size of the pinhole, unless the pinhole is so small that diffraction because of the wave-like nature of light limits resolution.

The light-gathering area can be increased by using a lens that collects light from an angle in space and focuses it on the back of the chamber (**Figure 1**(**b**)). Even in this case, the image is rotated 180°. The brightness of the image is proportional to the size of the light-gathering aperture, while the detail is limited by the quality of the lens, except for small apertures where diffraction plays a role.

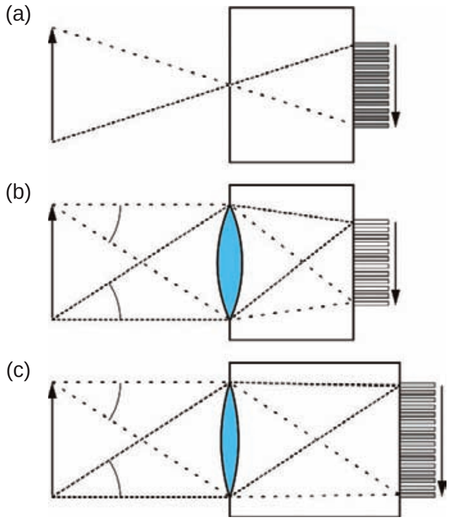


Figure 1 Schematic illustration of basic optical principles. (a) In a pinhole camera, light enters an otherwise light-tight chamber through a small aperture to the rear wall of the chamber. Photoreceptors located at that position (gray bars) would be stimulated with an image of an object that is rotated 180°. Each photoreceptor would receive little light from each corresponding point in space. (b) The amount of light reaching each photoreceptor is increased by a lens that collects light from an angle in space. (c) Image magnification and brightness are directly and inversely, respectively, proportional to the focal length of the lens.

The brightness of the image also depends on the focal length of the lens. If focal length is long, image size is large such that the light passing through the entrance aperture is distributed over a large area (compare the sizes of the images of the arrow in **Figures 1**(**b**) and **1**(**c**)).

In an eye, the image is sampled and translated into neural signals by photoreceptors in the retina (**see also Vision**: Photoreceptors and Visual Pigments and Inner Retina and Ganglion Cells). This means that the sampling density in the retina is limited and that leads to a notable exception from the general rule that image brightness is a function of the focal length of the optical system. The brightness of an imaged point source of light depends only on the size of the light-gathering aperture if all light originating from the point source and passing through the aperture is focused on a single photoreceptor. In this case, optical sensitivity is independent of the focal length and depends only on the size of the aperture. This also applies to groups of photoreceptors if their signals are summed by optical or neural mechanisms.

Constraints on the Evolution of Fish Eyes

Compared with air, water is a dense and viscous medium. An animal therefore has to have a streamlined body if it wants to move swiftly through the water column. This has had an effect on eye evolution because large protruding eyes would hamper a fish's locomotion. Evolution has therefore favored small eyes with a maximum of information capacity in many species. Furthermore, most underwater environments are relatively dark and large pupils are necessary for gathering light.

Small eye size calls for maximizing the refractive power of the optical system. The refractive power of a lens depends on the shape of the lens and the refractive indices (RIs) of the lens material and the surrounding medium. The crystalline lenses of many fishes are spherical (ball-shaped) and such lenses have the shortest possible focal lengths relative to the aperture that collects light. The basic building blocks of the optical elements in fish eyes are water and proteins, and the RI of pure, dried protein is about 1.54. The surrounding intraocular fluids are basically water with an RI of about 1.34. This is the span of RIs with which natural evolution could work, in contrast to glass lenses (RI: 1.46–1.81) in air (RI: 1.0) used in manufactured optical systems.

The information content of an image is limited by diffraction and/or the quality of the optical system. Of many possible optical imperfections, the so-called aberrations, two are most important with regard to fish vision:

- *Longitudinal spherical aberration (LSA).* A spherical lens made of homogeneous material focuses light that passes through its periphery closer to the lens than light that

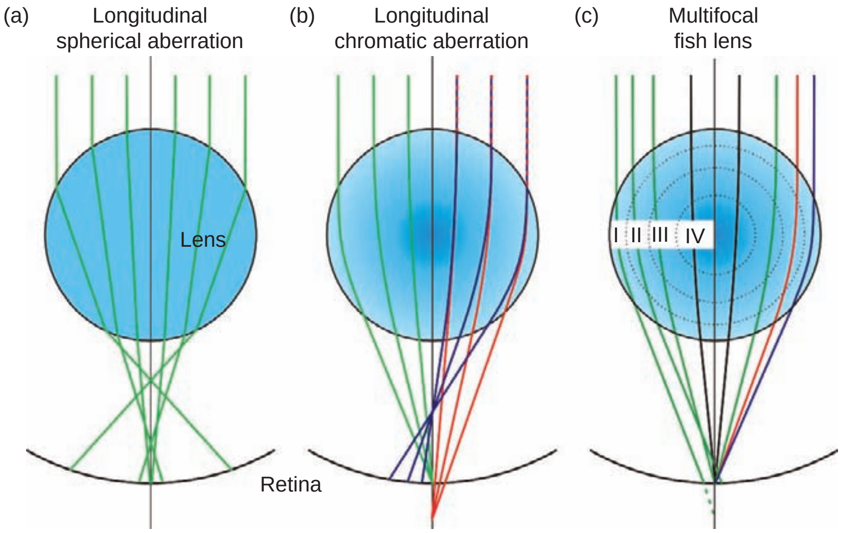

(a)
Longitudinal spherical aberration
Lens
Retina
(b)
Longitudinal chromatic aberration
(c)
Multifocal fish lens
I II III IV

Figure 2 Main optical aberrations in fish eyes and the principle of operation of a multifocal fish lens. (a) Longitudinal spherical aberration (LSA) of a spherical lens of homogenous refractive index. Rays of monochromatic light passing through the periphery of the lens are focused closer to the lens than rays passing through the lens close to the optical axis (axis of symmetry). (b) Gradient-index lenses may be corrected for LSA, but suffer from longitudinal chromatic aberration (LCA). Light of short wavelength (blue) is focused closer to the lens than light of intermediate (green) and long (red) wavelengths. On the retina, only a narrow range of wavelengths is in focus, while the image is defocused for other wavelengths. (c) Multifocal fish lenses are gradient-index lenses with carefully tuned residual LSA. Note that the lens has several focal lengths for monochromatic light (left half of illustration). Because of LCA, different zones (I–III) focus different spectral ranges on the retina (right half of illustration). The central zone (IV) has a small effective aperture and thus long depth of focus, such that all wavelengths are sufficiently well focused on the retina. Note that multifocal lenses create well-focused color images on a background of defocused light that has passed through zones of inappropriate focal lengths, such as the green light that has passed through zones I and III (left half of the illustration).

takes a more central path (**Figure 2**(**a**)). LSA leads to blur and the loss of light that either cannot be captured by the photoreceptors because of exceedingly large angles of incidence or is not directed on any photoreceptor at all.

- *Longitudinal chromatic aberration (LCA).* The RI and thus the refractive power of a lens is a function of the wavelength of light. Lens power decreases from short wavelengths (blue end of spectrum) to long wavelengths (red end of spectrum). LCA leads to chromatic defocus (**Figure 2**(**b**)) that is relevant for color vision.

The biological solution to LSA and LCA are multifocal gradient-index lenses, while optical systems consisting of several lenses are the most common solution in manufactured devices.

Optical Elements of Fish Eyes

Light on its way from the environment to the light-sensitive outer segments of the photoreceptors in the retina passes through several transparent elements that may alter its path (**Figure 3**; **see also Vision**: Photoreceptors and Visual Pigments, Inner Retina and Ganglion Cells, and Adaptations of Photoreceptors and Visual Pigments). The cornea, the crystalline lens, and also the retina may contribute to image formation because they are higher in RI than the surrounding media, which are water (outside the eye), the liquid aqueous humor (between cornea and lens), and the gelatinous vitreous humor or body (between lens and retina; **Figure 3**). The vitreous humor may be highly viscous, almost solid in some species. However, its extracellular matrix has extremely high water-binding capacity such that the RI of the vitreous is only slightly higher than that of water.

The amount of light reaching the retina is limited by the iris that acts as a stop in the optical system. Some fishes have skin flaps that can be extended to cover parts of the pupil. These structures have similar functions as the iris. The spectrum of wavelengths that are allowed to reach the photoreceptor outer segments may be limited by filter pigments that may reside in the cornea, lens, and/or inner segments of the photoreceptors. Long-pass cutoff filters that block short wavelengths are common in the cornea and/or lens and protect the retina from potentially dangerous ultraviolet (UV) light. There are, however, numerous fish species that are sensitive to near-UV light. In the mesopelagic zone that is barely reached by downwelling sunlight, short-wave absorbing pigments may enhance the visibility of bioluminescent signals that contain more long-wave radiation than the

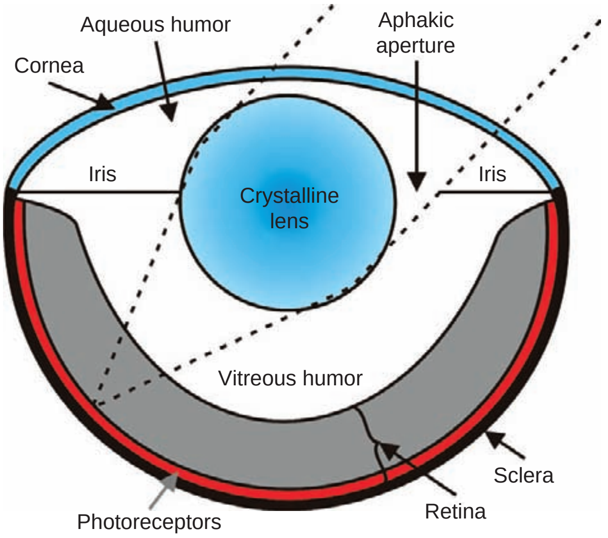


Figure 3 Sketch of a typical fish eye illustrating relevant structures. Note that the aphakic aperture allows for use of the full width of the lens for light that is incident at an angle (dashed lines) and focused on an off-axis area of highest retinal resolution. In small eyes of fish larvae, the neural retina (gray) may fill the entire volume posterior to the iris and lens.

residual sunlight (**see also The Skin**: Bioluminescence in Fishes and **Vision**: Visual Adaptations to the Deep Sea).

Cornea

The cornea is a multilayered sheet of cells and dense extracellular matrix that comes about by the fusion of a transparent patch of skin over the eye (the dermal cornea or primary spectacle) and the primary cornea that is derived, like the sclera, from the dura mater. The separation between dermal and primary corneas is conserved in some groups of fish. In a typical fish eye (**Figure 3**), the cornea's contribution to the total refractive power of the eye is negligible. The cornea is thin and its anterior and posterior surfaces are parallel to each other. Furthermore, the cornea is surrounded by media (water and aqueous humor) that are very similar in RI. In consequence, any refraction of light that occurs at the anterior surface of the cornea is canceled out by a refraction of the same magnitude, but of opposite sign at the posterior surface.

Iridescent layers reflect light by constructive interference and occur mainly in the corneas of shallow-water marine teleosts. If the fish is illuminated from above, the iridescent cornea reflects the light laterally away from the animal. This reduces intraocular glare by preventing strong downwelling light from entering the eye and camouflages the otherwise conspicuously dark pupillary opening for an observer viewing the animal from the side. Light incident on the eye axially or from below is unobstructed because of the orientation of the reflective structures in the iridescent layer.

Iris

The iris is a thin sheet of tissue that is usually richly vascularized on its posterior surface. It stretches from the ora retinalis toward the lens and acts as a stop that limits the amount of light passing through the lens. The anterior surface of the iris is usually pigmented to match the coloration of the body or to be part of a disruptive pattern, while the posterior surface is black such that light astray within the eye is absorbed. In species with contractile pupils, the inner rim of the iris glides on the anterior surface of the lens (**see also The Skin**: Coloration and Chromatophores in Fishes).

In many teleost species, the iris is unresponsive to changing light levels and the pupillary opening may be somewhat larger than the lens, such that there is an aphakic aperture through which light can enter the eye without being focused by the lens. This seems to be an adaptation that allows a maximum of light to be focused on a temporal or dorso-temporal region of highest retinal resolution that exists in a number of fish species (**Figure 3**).

A drop-shaped pupil with an aphakic aperture in the nasal or ventro-nasal region is typical for shallow-water teleosts, but there is a large variety of other pupil shapes among fishes. Irises and extendable skin flaps of complicated shapes, such as in flatheads (Platycephalidae), may camouflage the eye by disrupting the shape of the dark pupillary opening. Shading dorsal flaps are present in a number of fishes that are exposed to large differences in brightness between the dorsal and ventral visual fields. In the Loricariidae among the catfishes (Siluriformes), the pupil is almost circular when dark-adapted. In bright light, an operculum extends from the dorsal rim of the iris and fills the center of the pupil, such that a narrow crescent-like and almost circular slit remains open. Similar ring-shaped apertures are used in astronomical telescopes to increase the contrast of small objects at the expense of some reduction in contrast for large objects.

Lens Structure and Optical Properties

The crystalline lens is derived from an inverted epithelium that develops by invagination of the ectoderm during early embryogenesis. The lens is surrounded by a particularly strong basement membrane, the lens capsule. Directly interior to the lens capsule there is a monolayer of cells, the lens epithelium. These cells are the metabolically most active cells in the lens, but of little optical relevance. The lens epithelium covers the anterior surface of the lens and ends somewhat posterior to the lens's equator, where there is a proliferation zone that remains active throughout life in all vertebrates with well-developed eyes. Here, new cells arise continuously and enter the developmental path toward mature lens fiber cells. The cells stretch over existing cell layers and, when their ends have reached the anterior and posterior poles of the lens, they connect with other cells in the new layer. The areas of interconnecting lens fiber cells are called 'lens sutures' and may be visible in fresh lenses as they scatter some light. The final step of lens fiber cell differentiation is the degradation of all organelles including the nucleus, which leads to the exceptional transparency of the tissue, but also permanently removes the cells from the cell cycle. A mature lens fiber cell has to last for the entire remaining lifetime of the animal.

The main proteins present in lens fiber cells are called 'crystallins' that are synthesized by peripheral cells with full complements of organelles. The crystallins are a heterogeneous group of water-soluble proteins and, during the evolution of vertebrates (and other animals), a variety of different proteins has been recruited for the purpose of refracting light. Many of these proteins have in common that they are exceptionally stable and some of them have enzymatic or chaperone activities.

James C. Maxwell was the first to notice that fish lenses, despite being spherical, are almost free of LSA. Maxwell postulated that these lenses must be gradient-index lenses, with highest RI in the center and lowest RI at the surface (**Figure 2**(**b**)). If such a radial or concentric gradient of RI has the correct shape, a spherical lens can be free of LSA. Today it is clear that Maxwell was right, although the search for the exact RI profiles of fish lenses is still ongoing.

A complication arises from the fact that many fishes have color vision. Surprisingly at first sight, the single crystalline lenses of fishes have the capacity to create well-focused color images despite LCA. In manufactured optical applications, chromatic aberration is accounted for by composite optical systems consisting of several lenses made of different materials. This method leads to sharp color images, and also to bulky optical systems with limited visual fields. The biological solution is based on multifocal lenses that are small and may not only have the same optical properties for all angles of incidence of light, but also have a counterintuitive mode of operation. Well-focused color images are overlaid by defocused light (**Figure 2**(**c**)) that should reduce the information transfer capacity of the lens.

Multifocal lenses are nevertheless common in all vertebrate groups studied to date, ranging from lampreys to primates. This illustrates the power and general applicability of the multifocal principle. There is, however, no single multifocal solution that suits all animals. At least in fishes, the optical properties of the lens are specifically tuned to the needs of the animals.

Furthermore, the optical properties of fish lenses show circadian and developmental plasticity. In many shallow-water fishes, dark adaptation leads to profound changes in retinal function. In a process called 'retinomotor movements', the cone photoreceptors, of which there are up to four different spectral types, are moved out of the presumptive focal plane of the optical system, while the rods, of which there is only one type, are brought into this plane. The switch from polychromatic color vision to monochromatic grayscale vision may be reflected in the optical properties of the lens. The lens of *Aequidens pulcher*, a South American cichlid with trichromatic cone-based color vision, is clearly multifocal during daytime, but significantly less so during nighttime. This means that the optical properties of *A. pulcher* lenses adapt to the changing demands of the retina on a short-term basis. Because retinomotor movements are common in fishes that are regularly exposed to widely changing light levels, adapting lenses may be common as well.

Long-term optical plasticity has been induced experimentally by rearing fishes in various spectral environments, dim light, and continuous darkness. Differences in the optical properties of the lenses have also been observed in a teleost species that has undergone a Lessepsian migration (i.e., migration through the Suez Canal) from the Red Sea to the Mediterranean Sea. In comparison to the Red Sea at comparable depth, the visual environment in the Mediterranean Sea is darker and red-shifted due to a higher load of organic material. Correspondingly, the lenses of *Siganus rivulatus*, a diurnal herbivorous rabbitfish (Siganidae), have shorter focal lengths in the Mediterranean than in the Red Sea.

Computational models of the optical performance and structure of fish lenses have shown that the RI gradient is stringently controlled, because even tiny deviations from the optimal gradient lead to profound reductions in optical quality. The regulatory mechanisms are still unknown, but it is clear that they involve mature lens fiber cells that are devoid of organelles. As the lens grows throughout life by addition of new fiber cell layers in the periphery, the RIs in older, more central cell layers increase such that the correct RI gradient within the lens is maintained. This occurs in parallel with post-translation modifications of the crystallins in lens fiber cells devoid of organelles.

Lens Suspension and Accommodation

A lens of high optical quality is an essential precondition for detailed vision, but not the only one. The lens has also to be exactly positioned, in particular in fishes as their lenses have short depth of focus because of short focal length in relation to the aperture diameter. In agile species, the lens has to be held in place firmly during rapid movements of the animal. It has long been believed that lens suspension in teleosts was rather simple, consisting only of a dorsal ligament and a ventral retractor lentis muscle. A recent analysis revealed, however, a system of several ligaments in addition to the previously described dorsal ligament. The retractor lentis muscle moves the lens toward the temporal (caudal) retina for accommodative adjustment of focus for distance vision.

Lens suspension in chondrichthyes consists of a dorsal ligament and a ventral papilla. In this papilla, there seems to be a muscle that protracts the lens for accommodation to near vision. Interestingly, in sturgeons that are usually regarded as ray-finned fishes, lens suspension is much more chondrichthyes-like than teleost-like. In lampreys, the most basal group of vertebrates, the lens is held in place by a thin fibrous membrane that stretches from the equator of the lens to the region of the ora retinalis. Intraocular muscles seem to be absent and it has been speculated that an extraocular muscle might pull on the dermal cornea to deform the entire eye for accommodation. Electrical stimulation of this muscle, however, failed to induce accommodative changes of focus. Surprisingly, African lungfishes (*Protopterus* spp.) have the same type of lens suspension as lampreys. It is an open question whether or not lungfishes are capable of accommodative adjustments of focus. The tetrapod mode of accommodation by deformation of the lens is unknown in fishes.

Retina

As a result of the invaginating type of eye formation in vertebrates, the neural retina is placed between the incoming light and the photoreceptors' outer segments, that is, the photosensitive layer (**Figure 3**; **see also Vision**: Photoreceptors and Visual Pigments, Inner Retina and Ganglion Cells, and Adaptations of Photoreceptors and Visual Pigments). The retinal nerve cells scatter some light, which leads to loss of light and blur. The inverse retina has therefore long been considered as mainly disadvantageous. There is, however, an optical rationale that identifies a potential advantage of the inverse retina.

The shortest reliably reported focal lengths of fish lenses are $2.2R$ (R = lens radius) or slightly longer. This seems to be the shortest possible focal length for a spherically well-corrected lens consisting mainly of proteins and water, and functioning in an aqueous environment. Consequently, there has to be some space between the lens and the light-sensitive outer segments of the photoreceptors in order to create well-focused images. Some distance between the optical center of the eye and the retina is also necessary for image magnification (**Figure 1**). In fish eyes, some or all of the space between the lens and the photoreceptors is occupied by the neural retina (**Figure 3**). The inverse retinas of fishes, in fact, of all vertebrates, thus allow for using the space between the lens and the outer segments for retinal neurons at no extra cost in terms of weight and volume. In consequence, even small animals can have relatively large eyes.

The inner segments of the photoreceptors have RI gradients that may contribute to the capture and containment of light. The photopigment-containing outer segments of photoreceptors are usually light guides that confine captured light within their volume. Distal to the photoreceptors there may be a reflecting sheet (tapetum lucidum) that reflects unabsorbed light back into the outer segments for a second passage and thus a second chance for absorption by the photopigment.

Fish cone photoreceptors occur often in partially fused pairs. These double cones may consist of morphologically identical partners expressing the same photopigment (twin cones) or partners that differ in one or both of these parameters. An optical reason for the occurrence of double cones may be that the cell membranes between the partners can make the cells sensitive to the polarization of light by polarization-selective reflection and transmission of light.

Some Special Optical Adaptations

Fish vision has evolved to meet very different needs because fish eyes are used in widely varying visual environments, for instance, brightly lit and colorful coral reefs in contrast to the almost completely dark deep sea (**see also Vision**: Visual Adaptations to the Deep Sea and **The Skin**: Bioluminescence in Fishes). Accordingly, there are numerous interesting deviations from the typical fish eye as shown in **Figure 3**.

Lens Focal Length in Relation to Body Size and Feeding Habits

Image magnification is a function of the focal length of the optical system. Large fishes can, in general, support large eyes and have lenses with long absolute focal lengths to see larger, more detailed images. However, many large fishes such as billfishes (Istiophoridae and Xiphiidae) have eyes that are small in relation to body size. Such species tend to have long normalized focal lengths of the lens (i.e., focal length expressed in units of lens radius), probably because even if the eye is large in relation to the lens, it is still small in relation to the body. Another correlation exists between long normalized focal length of the lens and feeding habits. The long and slender coral-reef-associated cornetfish (*Fistularia commersonii*) attacks in short bursts of fast swimming and captures prey with elongated mandibles (**see also Vision**: Color Vision and Color Communication in Reef Fish). Suitably for the long working distance of the eye, its lens has long normalized focal length.

Telescopic Vision

The sandlance (*Limnichthytes fasciatus*) is a small teleost species that lives in shallow, brightly lit waters with sandy bottoms. It buries in the sand with only the eyes exposed and dashes out to capture planktonic prey. The eye has a thick cornea with considerable positive refractive power (converging lens) and an optically weak, flattened crystalline lens (**Figure 4**(**a**)). The optical center of this two-lens optical system is more anterior than the center of the crystalline lens, such that image magnification is higher than in a typical fish eye.

The inner surface of the fish retina is usually smooth and largely perpendicular to incoming light, such that there is little refraction. The sandlance, however, has a so-called deep fovea with a steeply curved inner surface of the retina (**Figure 4**(**a**)). Similar deep foveas are present in some birds of prey and are assumed to increase image magnification locally in the foveal region. The thick cornea and deep fovea of the sandlance lead to telescopic vision that may be necessary to detect small planktonic prey items when they come into the striking distance of the animal.

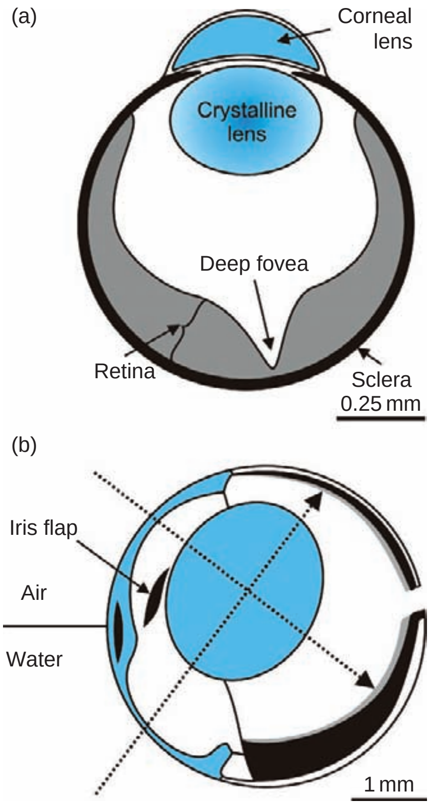


Figure 4 Examples of special optical adaptations in fishes. (a) In the sandlance (*Limnichthytes fasciatus*), the cornea is thick and has considerable positive refractive power, while the crystalline lens is flattened axially and optically weak. This moves the optical center of the eye anteriorly, in comparison to a typical fish eye as illustrated in **Figure 3**, and thus increases image magnification. Further magnification may be achieved locally by the curved inner surface of the retina in the deep fovea. (b) The eye and lens of the four-eyed fish (*Anableps anableps*) are highly asymmetric and there are separate optical paths (dotted lines) for well-focused aerial and aquatic vision. (a) Modified from Pettigrew JD and Collin SP (1995) Terrestrial optics in an aquatic eye: The sandlance, *Limnichthytes fasciatus* (Creediidae, Teleostei). *Journal of Comparative Physiology* 177: 397–408. (b) Modified from Sivak JG (1976) Optics of the eye of the "four-eyed fish" (*Anableps anableps*). *Vision Research* 16: 531–534.

Amphibious Vision

The typical fish cornea is curved (**Figure 3**) and has thus positive refractive power in air, which renders the eye myopic (short-sighted) for aerial vision. However, some fish use their eyes also in air. Atlantic flying fish (*Cypselurus heterurus*) use aerial vision for landing in windrows of Sargassum weed. The cornea is pyramid shaped with three flat zones. The intertidal fishes, foureye rockskipper (*Dialommus macrocephalus*) and Galapagos four-eyed blenny (*Dialommus fuscus*), move considerable distances out of the water and have prism-shaped corneas with two flat zones. In these species, the flat corneal zones do not contribute to the refractive power of the eye in neither water nor air. In both media, the lens is the only focusing optical element. The four-eyed fish (*Anableps anableps*) of South America swims at the water surface with the upper half of the eye exposed to air and the lower half to water. The crystalline lens and the entire eye are highly asymmetric and each half of the eye creates a well-focused image in its respective medium (**Figure 4**(**b**)).

Monofocal Lenses in Deep-Diving Species

Because of the wavelength-selective absorption and scattering of light by water, the available spectrum narrows with increasing depth (**see also Vision**: Visual Adaptations to the Deep Sea and **The Skin**: Bioluminescence in Fishes). Wavelengths around 485 nm penetrate the deepest in clear-blue ocean water. Some fishes make extended foraging excursions from warm and well-lit surface waters to cold and dim depths. At least some of these species have color vision. Within the narrow spectrum at depth, however, LCA leads to little chromatic blur. In accordance with this, a number of deep-diving fish species have monofocal lenses. Some of these lenses are perfectly corrected for LSA within the accuracy of measurement.

Vision in the Deep Sea

Vision in deep-sea fishes may be adapted to the detection of shadows against faint downwelling light or bioluminescence emitted by other organisms (**see also Vision**: Visual Adaptations to the Deep Sea and **The Skin:** Bioluminescence in Fishes). Various astonishing optical adaptations, such as tubular eyes and imaging systems based on mirrors, have evolved and are treated elsewhere in the encyclopedia (**see also Vision**: Visual Adaptations to the Deep Sea).

See also: **The Skin**: Bioluminescence in Fishes; Coloration and Chromatophores in Fishes. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Color Vision and Color Communication in Reef Fish; Inner Retina and Ganglion Cells; Photoreceptors and Visual Pigments; Visual Adaptations to the Deep Sea.

Further Reading

Bassnett S (2009) On the mechanism of organelle degradation in the vertebrate lens. *Experimental Eye Research* 88: 133–139.
Beer T (1894) Die Accommodation des Fischauges. *Pflügers Archiv der Gesammelten Physiologie* 58: 523–649.
Bowmaker J (1995) The visual pigments of fish. *Progress in Retinal and Eye Research* 15: 1–31.
Burnside B (2001) Light and circadian regulation of retinomotor movement. *Progress in Brain Research* 131: 477–485.
Douglas RH (1982) The function of photomechanical movements in the retina of the rainbow trout *(Salmo gairdneri). Journal of Experimental Biology* 96: 389–403.
Duke-Elder S (ed) (1958) *System of Ophthalmology, Vol. 1: The Eye in Evolution*. London: Klimpton.
Hawryshyn CW (1992) Polarization vision in fish. *American Scientist* 80: 164–175.
Jerlov NG (1968) *Optical Oceanography*. New York: Elsevier.
Khorramshahi O, Schartau JM, and Kröger RHH (2008) A complex system of ligaments and a muscle keep the crystalline lens in place in the eyes of bony fishes (teleosts). *Vision Research* 48: 1503–1508.
Kröger RHH and Biehlmaier O (2009) Space-saving advantage of an inverse retina. *Vision Research* 49: 2318–2321.
Kröger RHH, Campbell MCW, Fernald RD, and Wagner H-J (1999) Multifocal lenses compensate for chromatic defocus in vertebrate eyes. *Journal of Comparative Physiology A* 184: 361–369.
Lamb TD, Collin SP, and Pugh EN, Jr. (2007) Evolution of the vertebrate eye: Opsins, photoreceptors, retina and eye cup. *Nature Reviews Neuroscience* 8: 960–975.
Land MF and Nilsson D-E (2002) *Animal Eyes*. Oxford: Oxford University Press.
Matthiessen L (1886) Ueber den physikalisch-optischen Bau des Auges der Cetaceen und der Fische. *Pflügers Archiv der Gesammelten Physiologie* 38: 521–528.
Rochon-Duvigneaud A (1943) *Les Yeux et la Vision des Vertébrés*. Paris: Masson.
Schartau JM, Sjögreen B, Gagnon YL, and Kröger RHH (2009) Optical plasticity in fish crystalline lenses. *Current Biology* 19: 122–126.
Shu D-G, Morris SC, and Han J (2003) Head and backbone of the Early Cambrian vertebrate *Haikouichthys. Nature* 421: 526–530.
Thorpe A and Douglas RH (1993) Spectral transmission and short-wave absorbing pigments in the fish lens. 2. Effects of age. *Vision Research* 33: 301–307.
Walls GL (1942) *The Vertebrate Eye and Its Adaptive Radiation*. New York: McGraw-Hill.
Warrant EJ and Locket NA (2004) Vision in the deep sea. *Biological Reviews* 79: 671–712.

Photoreceptors and Visual Pigments

JK Bowmaker, University College London, London, UK

© 2011 Elsevier Inc. All rights reserved.

Visual Pigment Structure
Evolution of Visual Pigments
Cone Types and Retinal Mosaics
Further Reading

Glossary

Agnaths (agnathostomes) Primitive jawless (a=without; gnathos=jaw) fish dominant in the early Paleozoic, and represented today by hagfish and lamprey.

Chromophore Part or moiety of a molecule responsible for its color. In visual pigments the chromophore is retinal, the aldehyde of vitamin A, combined with the protein, opsin.

Ionone ring A cyclic ring configuration of carbon atoms linked by single and double bonds.

Microspectrophotometry A technique in which a narrow beam of light is passed through an isolated rod or cone outer segment in order to measure the absorbance of the visual pigment.

Opsin The membrane-bound G-protein-coupled receptor protein found in photoreceptors in the retina, which, when combined to the chromophore retinal, forms a visual pigment.

Orthologous genes Genes in different species that are similar to each other because they originated from a common ancestor.

Photic environment The distribution of light, in terms of brightness and spectral composition, within the environment.

Porphyropsin All visual pigments based on vitamin A_2.

Rhodopsin All visual pigments based on vitamin A_1, but commonly used to refer only to rod visual pigments.

Schiff base A functional group that consists of a carbon–nitrogen double bond. In visual pigments, a nitrogen atom of amino acid lysine 296 in opsin is connected to the terminal alkyl group (CH_3) of the chromophore, retinal.

Teleosts A bony fish of the subclass Teleostei, having rayed fins and a swimbladder.

Tetrachromatic color vision Color vision based on four spectrally distinct cone classes, normally maximally sensitive in the red, green, blue, and violet or ultraviolet.

The aquatic environment, both marine and freshwater, provides a wide array of photic environments, ranging from surface waters exposed to almost full daylight, with a broad spectrum available for vision extending from the ultraviolet (UV) to the far red, to deep waters where brightness is severely attenuated and the spectrum is reduced to a narrow band centered in the blue, around a wavelength of 460–480 nm. Different regions of the aquatic environment may show almost any variation between these two extremes, from the clearest oceans to coastal waters, lakes and rivers which may carry high levels of suspended particles and/or be heavily stained with organic run-off from the land and/or with dissolved minerals. These photic environments provide a major evolutionary driving force for the visual systems of fish, and indeed any aquatic animal, leading to dramatic modifications in the basic structure of the vertebrate eye. This article considers specifically the retinal photoreceptors and photosensitive visual pigments concerned with visual imaging.

Visual Pigment Structure

Vertebrates evolved about 550 million years ago (mya) and are clearly recognizable in the primitive jawless fish, agnaths, of the early Paleozoic. These fish swam in shallow seas with the luxury of the full daylight spectrum and rapidly evolved a set of photoreceptors and visual pigments to maximize their visual input. Vertebrate retinas contain two types of photoreceptor cells, cones used in bright light conditions and which form the basis of color vision, and more specialized, highly sensitive rods that function at low light levels and provide only monochromatic vision. Both rods and cones contain visual pigments that are tuned to be most sensitive to a particular spectral region. The pigments are composed of a protein, opsin, combined with a chromophore, the aldehyde of vitamin A, retinal. Opsins, like most proteins, absorb light in the far UV around 280 nm, whereas retinal absorbs in the near UV at about 370 nm. When combined to form a visual pigment, there is an opsin shift, mostly to longer wavelengths, so that visual pigments have maximum absorbances ($\lambda_{\max}$) anywhere from the near UV, around 350 nm, to the red, over 600 nm. The size of the opsin shift and the specific $\lambda_{\max}$ depends on the structure of the opsin, determined by the precise sequence of its constituent amino acids.

Vertebrate opsins are membrane-bound G-protein-coupled receptors composed of about 350 amino acids that form seven transmembrane α-helices connected by straight chain loops (**Figure 1**). The helices form a palisade surrounding the chromophore retinal, which is bound via a Schiff base link to a lysine residue in the seventh helix. Retinal consists of an ionone ring with a conjugated carbon chain and it is the interaction of amino acids lying close to either the ionone ring or the Schiff base which are primarily involved in tuning the pigment to a specific wavelength. In addition to opsin tuning, pigments may also shift their $\lambda_{\max}$ by exchanging their chromophore. Vitamin A exists in two forms, A_1 and A_2, giving rise to two forms of retinal, retinal, based on vitamin A_1 and 3-dehydroretinal, derived from vitamin A_2. Visual pigments based on retinal are termed rhodopsins (*rhodon*, Greek for rose), whereas those based on 3-dehydroretinal are termed porphyropsins (*porphyra*, Greek for purple). Somewhat confusingly, rhodopsin is often used as a term for visual pigments contained only in rods, and not for those in cones. Porphyropsins have an additional double bond in their ionone ring (**Figure 1**) and this causes a long wave shift in the absorbance spectrum of the pigment when compared to the equivalent rhodopsin. The long wave shift is wavelength dependent, being much greater at longer wavelengths, so that a rhodopsin with $\lambda_{\max}$ at 560 nm has a paired porphyropsin with $\lambda_{\max}$ at about 620 nm. The spectral shift reduces at shorter wavelengths and is only a few nanometers for pigments with maxima in the blue.

As a general rule, but with notable exceptions, marine fish, living in a relatively blue environment, possess rhodopsins, whereas freshwater fish, living in a more long-wave green environment, have more longer-wave porphyropsins.

Evolution of Visual Pigments

Ancestrally, there are four spectral classes of cone visual pigments, but only a single class of rod pigment. From studies of mutation rates of genes and of the opsins present in extant species of agnaths, lampreys, it is apparent that the four classes of cone pigment evolved very early in vertebrate evolution, about 450–500 mya (**Figure 2**). The four classes include long-wave-sensitive (LWS) (red/green), with $\lambda_{\max}$ ranging from about 495 to 570 nm; RH2; middle-wave-sensitive (green/blue), with $\lambda_{\max}$ from about 460 to 530 nm; and short-wave-sensitive 2 (SWS2) (blue/violet) with $\lambda_{\max}$ from about 415 to 480 nm and SWS1 (violet/UV) with $\lambda_{\max}$ from about 355 to 450 nm ($\lambda_{\max}$ values for rhodopsins). There is some overlap between these spectral ranges, so that knowing the $\lambda_{\max}$ of a pigment does not necessarily define its opsin class. Rods, RH1, the last class to appear, evolved from the RH2 cone class, and have a spectral range from about 460 to 530 nm, almost identical to that of the RH2 cones.

From an evolutionary position, modern extant species of agnaths, lampreys, have the potential to offer a glimpse of the visual system of early agnaths from the late Cambrian. In the Southern Hemisphere lamprey, *Geotria australis*, there are multiple spectral classes of cone and rods. Microspectrophotometry (MSP), a technique that measures the absorbance spectrum of individual photoreceptor outer segments, has identified two spectrally distinct classes of cone containing porphyropsins with $\lambda_{\max}$ at about 610 and 515 nm, along with rods with $\lambda_{\max}$ at about 505 nm. In addition, a study of the genetic complement of visual pigment opsins in *Geotria* has identified five opsin genes. Three of these are orthologous to the LWS, SWS2, and SWS1 opsin genes of jawed vertebrates (gnathostomes), but the remaining two, RHA and RHB, appear to be equally distantly related to the gnathostome RH1 and RH2 gene families. Four of the *Geotria* pigments, SWS1, SWS2, RHB, and RHA, have been regenerated with retinal, yielding pigments (rhodopsins) with $\lambda_{\max}$ at 358, 439, 492 and 497 nm, respectively, and although the LWS gene has not been regenerated, it represents the 610-nm porphyropsin measured by MSP. The RHA opsin would appear to be that expressed in the rods, whereas the RHB is presumably expressed in middle-wave-sensitive (MWS) cones. From these data, it is clear that the cone opsin genes originated before the major evolutionary event of the appearance of jaws, perhaps as early as 540 mya. The implication is that primitive jawless fish of the shallow late Cambrian and Ordovician seas, exposed to the full daylight spectrum, possessed four spectrally distinct cones classes, including sensitivity to UV and thus had the potential for a sophisticated tetrachromatic color vision system.

With this complex of opsins and chromophores, fish have a number of mechanisms for varying their spectral sensitivity and color vision. The basic plan of four spectral classes of cone can be modified in a number of ways. There may be an evolutionary loss of one or more of the four opsin genes or, in contrast, a duplication of a gene within a class and subsequent gene mutations to separate spectrally the two expressed cone pigment subclasses, such as RH2A and RH2B (shorter wavelength) and SWS2A and SWS2B (shorter wavelength). In addition, gene mutations can occur that spectrally shift pigments within each class or subclass. Finally, spectral variation can be achieved, not by opsin mutations, but by either

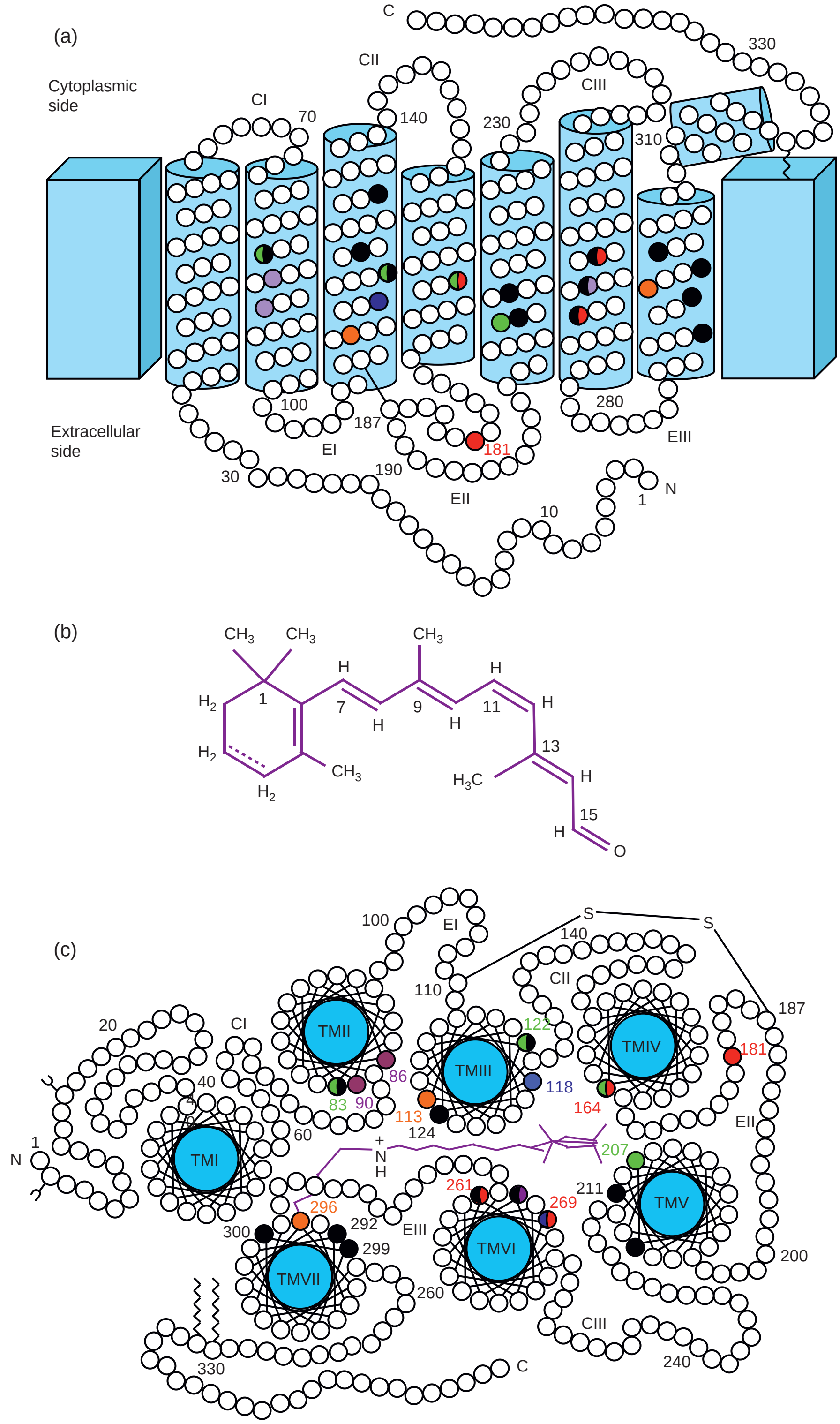


Figure 1 Schematic diagrams of a visual pigment molecule. (a) Two-dimensional diagram illustrating the seven transmembrane α-helices. (b) Molecular structure of retinal as an 11-*cis* isomer. The additional double bond (dashed line in the ionone ring) is found in 3-dehydroretional. (c) View showing the arrangement of the helices around the chromophore, retinal, shown in purple. Although the helices are of different lengths, for simplicity, each helix is shown with only the central 18 amino acids. The numbering is based on mammalian rhodopsin. Lysine 296 (orange) is the binding site of retinal and glutamate 113 (orange) provides the Schiff base counter-ion. Major sites involved in spectral tuning are coded with opsin class: LWS red, RH2 green, SWS2 blue, SWS1 violet, and RH1 black. Splits indicate sites involved in tuning in more than one opsin class. Note how sites tend to cluster around either the Schiff base linkage or the ionone ring of retinal. TMI to TMVII – the seven transmembrane helices of opsin; EI, EII, and EIII – extracellular straight chains of opsin; CI, CII, and CIII – cytoplasmic straight chains of opsin; C and N (carbon and nitrogen) – the terminals of the opsin molecule (common terminology for proteins), the numbering of amino acids starts from the N terminal; CN+, represents the protonated Schiff base. Modified from Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

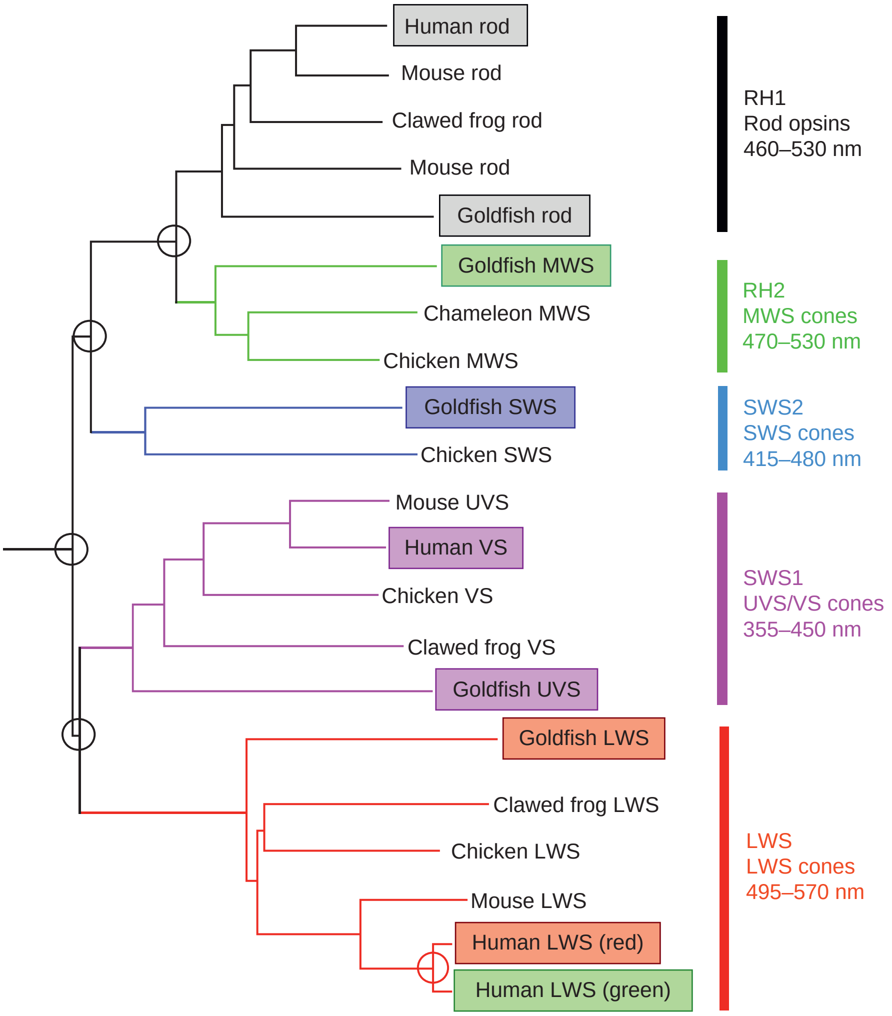


Figure 2 Simplified schematic phylogenetic tree of vertebrate visual pigment opsins with representative opsins from fish, amphibians, birds, and mammalians. Circles indicate gene duplications, four early in evolution about 500 mya, resulting in the four cone opsins and the rod opsins, and the recent duplication, about 35 mya, of the primate LWS opsin to produce the human L and M cone pigments. Note that mammals have lost the RH2 and SWS2 cone opsins.

switching the chromophore completely or varying the percentage of each chromophore incorporated into the opsin. None of these mechanisms are exclusive and the spectral sensitivity of a fish may vary during development, with selective temporal gene expression and by variations in chromophore, both developmentally and/or seasonally. For a more detailed discussion (**see also Vision**: Adaptations of Photoreceptors and Visual Pigments).

Cone Types and Retinal Mosaics

The different classes of cone visual pigments are expressed in a variety of morphologically distinct photoreceptors. Fish possess both single and double cones. In double cones the two halves may be adjacent, but not firmly attached, or they may be tightly bound with direct electrical connectivity. Generally, double cones tend to be relatively large and express the more LWS opsins, whereas single cones tend to be smaller and express more SWS pigments. Moreover, the nomenclature of double cones is confusing, but they can be divided into double cones in which the two halves are morphologically distinct, and twin cones in which the two halves are morphologically identical. Both of these types can be subdivided into identical and nonidentical forms. In nonidentical double or twin cones, the two halves contain spectrally different pigments, but in identical double or twin cones the two halves possess the same visual pigment (**Figure 3**). The precise function of double cones is not immediately apparent, but nonidentical double cones may offer good wavelength discrimination with high chromatic acuity, since the two halves occupy almost the same space within the visual field. By contrast, identical twin cones, probably

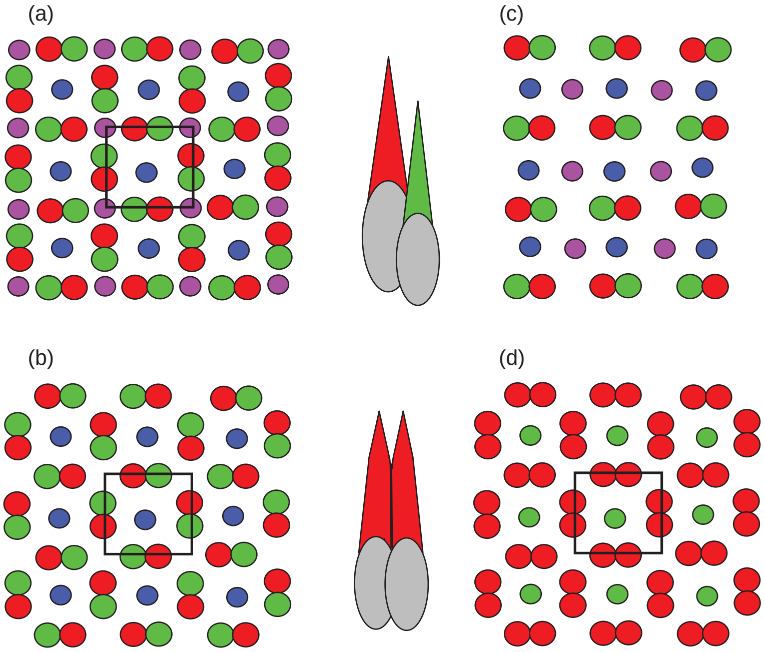


Figure 3 Schematic diagrams of retinal cone mosaics present in some teleost species. (a) The complete square mosaic found for example in some juvenile salmonids and cyprinids, with four longer-wave-sensitive double cones forming the sides of the square, with a central shorter-wave blue-sensitive single cone and UV-sensitive single corner cones. (b) A reduced mosaic with the absence of corner cones as found in some adult salmonids and cichlids. (c) A row mosaic as found in some cyprinids such as zebrafish (*Danio rerio*). (d) A square mosaic with LWS identical twin double cones and a central middle-wave-sensitive single cone, as found for example in pike (*Esox lucius*). The central diagrams illustrate a nonidentical double cone with a larger long-wave-sensitive member, and an identical twin cone.

electrically coupled, may act as large photon catchers designed to give photopic vision at relatively low light levels. In some species triple or even quadruple cones have been identified, in which the members may contain different visual pigments or all express the same pigment.

A striking feature of the retina of many fish is that the photoreceptors are not randomly distributed across the retina, but form a distinct array or mosaic. This normally involves either a row or square pattern with the double cones alternating with single cones (**Figure 3**). In the extreme form of a square mosaic, double cones form the sides of a square, with a central single cone, usually SWS, and four additional single cones occupying the corners of the square, often UV sensitive. In a number of species, the mosaic may not be complete, with some or all of the corner cones absent or the central single cone missing. These patterns can vary spatially across the retina and may also change temporally during development. The functional significance of these ordered arrays, not found outside of fish, is not immediately apparent. Suggestions have been made that they could improve movement detection, but there is only limited evidence to support such a theory.

The wide diversity and arrangement of photoreceptors and visual pigments found in fish can, perhaps somewhat simplistically, be related to differences in the photic environment of the various species and to distinct changes in environment and behavior experienced during development from larva to adult. There may be subtle influences that determine the differences in photoreceptor populations and spectral sensitivities across species. The bright colors and sexual dimorphism exhibited by many species are likely to be significant in species recognition and mate choice (**see also Vision**: Behavioral Assessment of the Visual Capabilities of Fish). Similarly, the feeding behavior of fish varies greatly and has certainly influenced anatomical features such as the mouth and jaws, and may be significant in determining retinal organization. The aquatic environment, unlike the terrestrial scene, is dominated by the direction of the downwelling light, daylight penetrating through the water column, so that the visual field above a fish is markedly different from the lateral view and from the visual field below the fish. These differences clearly will affect retinal organization so that variations in mosaic arrangements and cone complements may well differ between retinal regions that are viewing different visual fields. To understand the significance of all of these more detailed features of fish vision, extensive behavioral studies of individual species and fish communities are needed, which may then be related to the more easily obtained anatomy and physiology of the visual system.

See also: **Vision**: Adaptations of Photoreceptors and Visual Pigments; Behavioral Assessment of the Visual Capabilities of Fish; Physiological Optics in Fishes.

Further Reading

Archer SN, Djamgoz MDA, Loew ER, Partridge JC, and Valerga S (eds.) (1999) *Adaptive Mechanisms in the Ecology of Vision*. Dordrecht: Kluwer.

Bowmaker JK (1995) The visual pigments of fish. *Progress in Retinal and Eye Research* 15: 1–31.

Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

Bowmaker JK and Loew ER (2008) Vision in fish. In: Masland RH and Albright TD (eds.) *The Senses: A Comprehensive Reference, 1 Vision*, pp. 53–76. Oxford: Elsevier.

Douglas RH (2001) The ecology of teleost fish visual pigments: A good example of sensory adaptation to the environment? In: Barth FG and Schmid A (eds.) *Ecology of Sensing*, pp. 215–235. Berlin: Springer.

Douglas RH and Djamgoz MBA (eds.) (1990) *The Visual System of Fish*. London: Chapman and Hall.

Hunt DM, Dulai KS, Partridge JC, Cottrill P, and Bowmaker JK (2001) The molecular basis for spectral tuning of rod visual pigments in deep-sea fish. *Journal of Experimental Biology* 204(19): 3333–3344.

Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) (2006) *Communication in Fishes,* vol. 2. Enfield, NH: Science Publishers.

Lythgoe JN (1979) *The Ecology of Vision*. Oxford: Oxford University Press.

Yokoyama S (2000) Molecular evolution of vertebrate visual pigments. *Progress in Retinal and Eye Research* 19(4): 385–419.

Adaptations of Photoreceptors and Visual Pigments

JK Bowmaker, University College London, London, UK

© 2011 Elsevier Inc. All rights reserved.

Adaptations to the Photic Environment
Further Reading

Glossary

Apoptosis The process of programmed cell death.

Chromophore Part or moiety of a molecule responsible for its color. In visual pigments, the chromophore is retinal, the aldehyde of vitamin A combined with the protein opsin.

Microspectrophotometry A technique in which a narrow beam of light is passed through an isolated rod or cone outer segment in order to measure the absorbance of the visual pigment.

Opsin The membrane-bound G-protein-coupled receptor protein found in photoreceptors in the retina, which, when combined to the chromophore retinal, forms a visual pigment.

Photic environment The distribution of light, in terms of brightness and spectral composition, within the environment.

Porphyropsin All visual pigments based on vitamin A_2.

Rhodopsin All visual pigments based on vitamin A_1, but commonly used to refer only to rod visual pigments.

Teleost The most recently evolved of all the bony fishes (Osteichthyes). Teleosts have rayed fins and a swimbladder, and include everything from goldfish to tuna to salmon.

Tetrachromatic color vision Color vision based on four spectrally distinct cone classes, normally maximally sensitive in the red, green, blue, and violet or ultraviolet.

Different types of photoreceptors and their visual pigments are discussed elsewhere in this encyclopedia. This article considers how the retinal photoreceptors and photosensitive visual pigments have adapted to different photic environments and how, in some fish, they may change during development.

The aquatic ecosystem provides a wide array of photic environments, ranging from surface waters exposed to almost full daylight, with a broad spectrum available for vision extending from the ultraviolet (UV) to the far red, to deep waters where brightness is severely attenuated and the spectrum is reduced to a narrow band centered in the blue around 460–480 nm. Different regions of the aquatic environment may show almost any variation between these two extremes, from the clearest oceans to coastal waters, lakes, and rivers which may carry high levels of suspended particles and/or be heavily stained with organic run off from the land and/or with dissolved minerals.

In the evolution of vertebrates, four spectral classes of cone photoreceptors evolved early and have representatives in all extant groups of fish, from lampreys to teleosts. The four classes can be defined as: LWS, long-wave-sensitive (red/green), with λ_{max} ranging from about 495 to 570 nm; RH2, middle-wave-sensitive (green/blue), with λ_{max} from about 460 to 530 nm; SWS2 (blue/violet), short-wave sensitive, with λ_{max} from about 415 to 480 nm; and SWS1 (violet/UV), with λ_{max} from about 355 to 450 nm (λ_{max} values for rhodopsins). There is some overlap between these spectral ranges, so that knowing the λ_{max} of a pigment does not necessarily define its opsin class. Rods, RH1, the last class to appear, evolved from the RH2 cone class, and have a spectral range from about 460 to 530 nm, almost identical to that of the RH2 cones (**Figure 1**).

Adaptations to the Photic Environment

Present-day bony fish, teleosts, arose about 150 million years ago (MYA) in the Cretaceous, and now comprise about 96% of all living fish species. They are found in all aquatic environments and show some of the greatest ranges in visual capacity, extending from pure rod vision in many deep-sea species to tetrachromacy in a number of shallow-living species. The filtering effect of the water column first attenuates longer wavelengths in the red, followed by the shorter UV and, as a consequence, epipelagic fish living in the top 100 m or so of the ocean tend to be trichromatic or dichromatic, no longer expressing

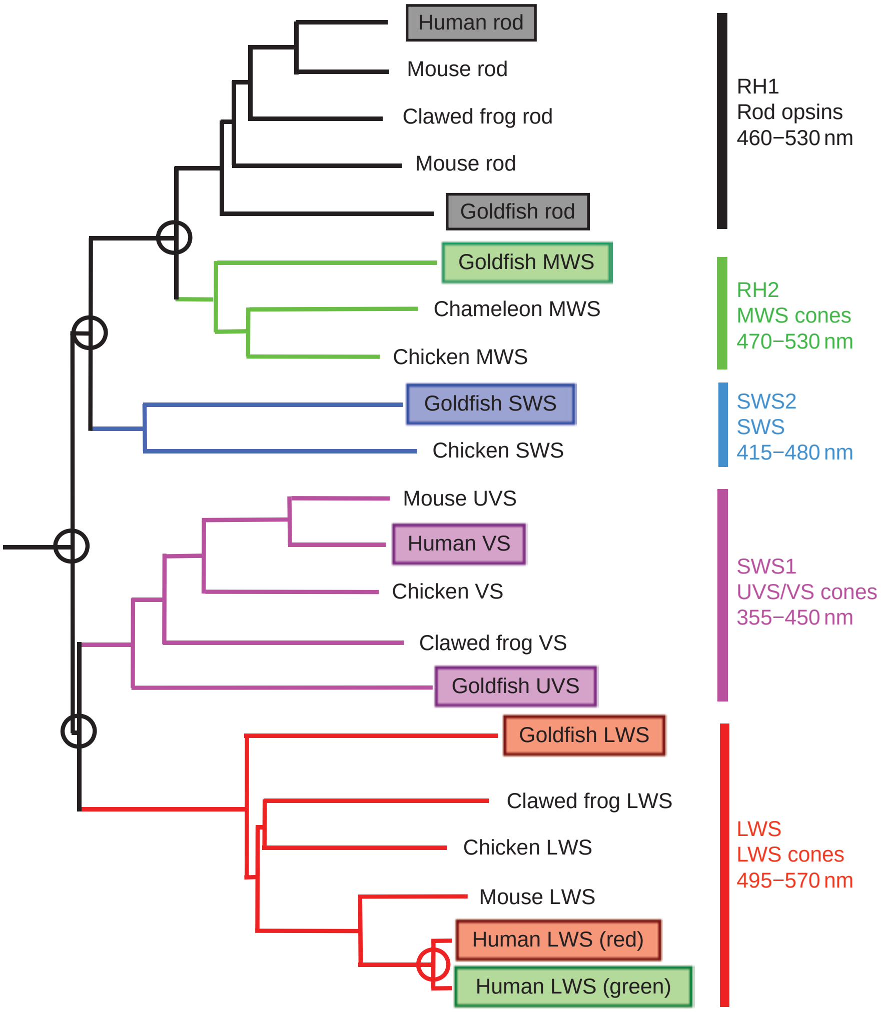


Figure 1 Simplified schematic phylogenetic tree of vertebrate visual pigment opsins with representative opsins from fish, amphibians, birds, and mammalians. Circles indicate gene duplications, four early in evolution about 500 MYA, resulting in the four cone opsins and the rod opsins, and the recent duplication, about 35 MYA, of the primate LWS opsin to produce the human L and M cone pigments. Note that mammals have lost the RH2 and SWS2 cone opsins.

either the LWS or the SWS1 opsins or both. In contrast, fish living in highly turbid or deeply stained waters, which strongly absorb and scatter short wavelengths, tend to lose or not express the shorter-wave opsins, but retain the LWS and RH2 opsin genes. In more extreme conditions at low-light levels, because of either increased depth or turbidity, fish may become cone monochromats expressing only the RH2 or LWS genes.

Adaptations to Increasing Water Depth

A striking illustration of the effects of the changing photic environment with depth on visual pigments and photoreceptors is provided by the distinctive environment of Lake Baikal. The lake, in central Siberia, is the deepest lake in the world at over 1600 m and is unique in that its waters are fully oxygenated even in the deepest regions. This has allowed a flock of over 20 species of sculpins, cottoid fish, to evolve endemic to the lake and which can be divided into groups that occupy progressively deeper habitats. Against the general rule for freshwaters, their visual pigments are all rhodopsins. Species living in the surface waters, exposed to the full daylight spectrum, have relatively cone-rich retinas with three spectral classes of cone, comprising red/green-sensitive double cones, green-sensitive identical twin cones, and single blue-sensitive cones (**Figure 2**). These cones are all relatively small and tightly packed presumably providing high visual acuity. Species occupying deeper waters, where daylight is restricted to a narrower spectral band centered in the blue/green region, have lost the LWS cones, retaining only the green-sensitive twin cones and blue-sensitive single cones. With increasing depth, the species show a series of short-wave shifts in the λ_{max} of

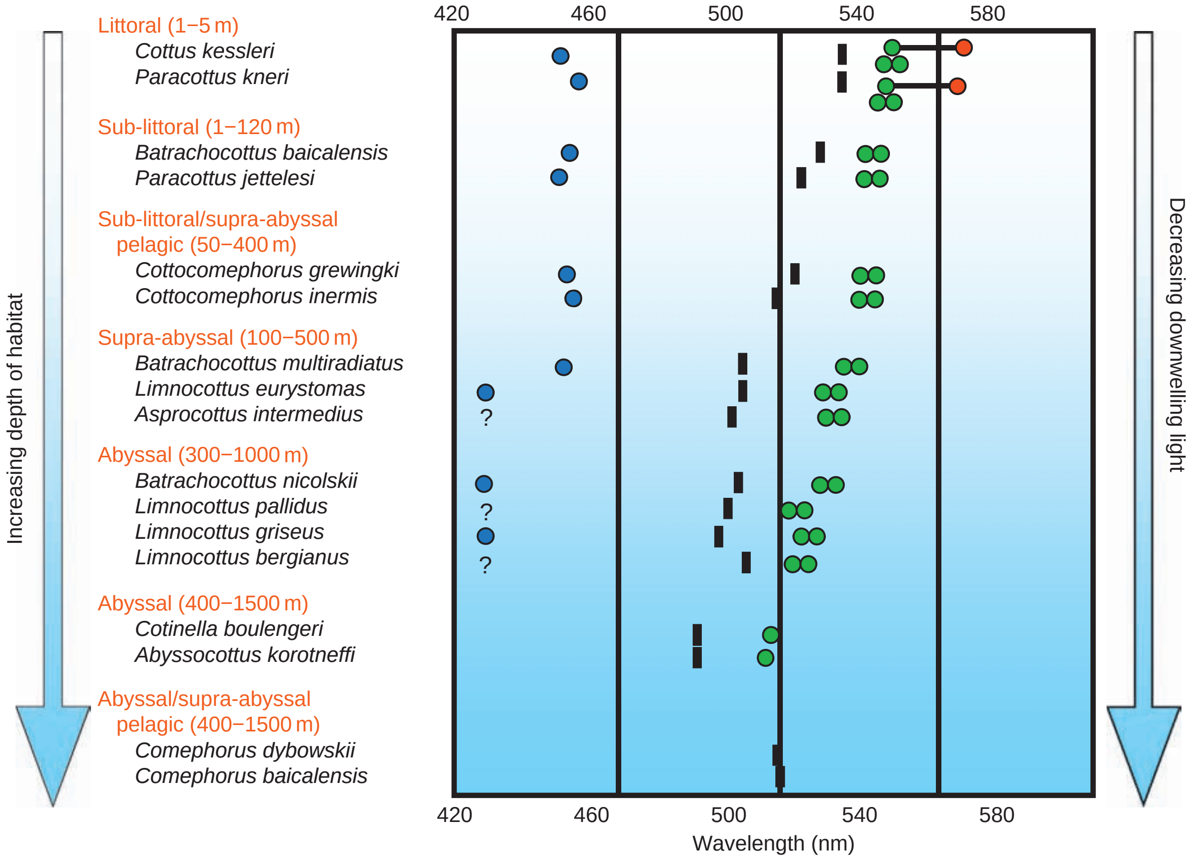


Figure 2 The distribution of visual pigments in the cottoid species flock of Lake Baikal. Spectral locations of the LWS, RH2, SWS2, and RH1 pigments from a range of species living at different depths in the lake. Red and green linked circles, LWS/RH2 double cones; twin green circles, RH2 double cones; blue circles, SWS2 single cones; black symbols, rods. Note the spectral shift to shorter wavelengths in all pigment classes with increasing depth of habitat. The LWS opsins are present only in surface-living species. Spectral tuning of the rod pigments is achieved through only three amino acid sites: 83, 261, and 292. From Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

both the green-sensitive cones and the rods. The middle-wave cones (RH2) fall into four clusters with λ_{max} ranging from about 525 to 500 nm, and the rods similarly show distinct spectral steps, with λ_{max} shifting from about 516 nm in the more surface species down to 484 nm in abyssal species. In addition, with increasing depth, the twin cones become much larger, both in diameter and in length, becoming large photon-capturing devices maintaining photopic vision at depth, but with a concomitant loss in acuity. In abyssal species, where light levels are below the limits of photopic vision, cones have been lost completely, leaving pure rod retinas with maximum sensitivity around 480–500 nm (**Figure 2**).

Comparisons of the derived amino acid sequences of the opsin genes of the rod pigments from these sculpins have identified just four amino acid substitutions that account for the spectral shifts to shorter wavelengths (**Figure 2**). These same substitutions, along with a small number of additional substitutions, are also found in deep-sea fish rod pigments that show the same spectral shifts toward shorter wavelengths. Surveys of large numbers of deep-sea species suggest that the peak sensitivities of rod pigments cluster at specific spectral locations between about 450 and 500 nm the clusters being separated by 5–10 nm, with the vast majority of pigments centered at 477 and 483 nm. The effect of each amino acid substitution on the λ_{max} is approximately additive, and these mutations, which must have occurred independently within each group, are clear examples of parallel evolution. Sensitivity to these shorter wavelengths not only matches the wavelength of maximal spectral transmission of water at depth, but, in the case of oceanic fish, also matches the blue bioluminescence emitted by photophores present on many species of deep-sea fish. Bioluminescence is not present in Lake Baikal.

Gene Duplication in Shallow-Living Species

In contrast to the loss of opsin genes, many teleost families have duplicated their cone opsin genes to produce a range of functional opsins within each opsin gene class. The evolution of gene families is an active process, in which gene duplication is accompanied by the subsequent mutation of genes, leading either to the decay of some genes into pseudogenes and eventually junk DNA or to a divergent gene with a new function. In teleosts, such as cichlids, the dominant species in many tropical and subtropical lakes and rivers, and cyprinids, the equivalent to cichlids, but in more temperate regions, mutations in the duplicated genes have led to additional functional genes which are expressed as visual pigments spectrally displaced from one another.

Lake Malawi, one of the Great Lakes in central Africa, has more than 700 species of cichlids that have evolved from a common ancestor within the past million years, and the species flock provides a notable example of opsin gene duplication. Malawi cichlids are remarkable for their diversity, particularly in their color patterns, and most species are sexually dimorphic with visual communication crucial for mate choice. African cichlids typically possess three cone visual pigments, with nonidentical double cones maximally sensitive at longer wavelengths and shorter wavelength-sensitive single cones, with the precise λ_{max} of the cones seemingly related to the clarity of the water in their specific habitats. Species living in clear water above a rocky substrate tend to have double cones with maximum sensitivities at about 535 and 488 nm and UV- or violet-sensitive single cones with λ_{max} at about 370 or 420 nm, whereas species living in a more sandy environment may be less sensitive to shorter wavelengths, possessing double cones with maximum sensitivities at about 570 and 535 nm and blue-sensitive single cones with λ_{max} at about 450 nm (**Figure 3**).

In some species, however, rare additional spectrally distinct cones have been identified by microspectrophotometry, suggesting that at least seven spectral types of cone pigment are expressed in the retina. Analysis of the opsin gene complement of these cichlids has confirmed the presence of seven functional cone opsin genes, three

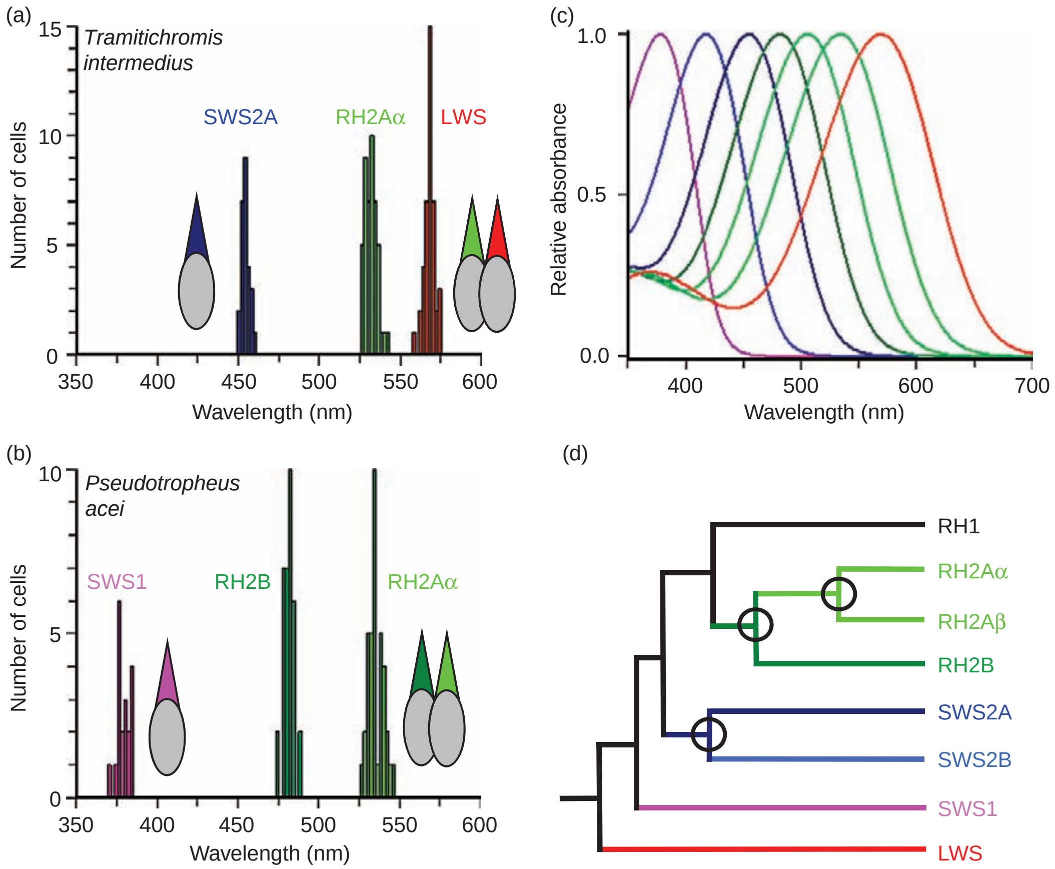


Figure 3 The organization of the seven cone opsin genes present in the species flocks of African cichlid fish. (a, b) The distribution of the dominant spectral cone classes in two different species of Malawi cichlids as measured by microspectrophotometry. The two species illustrate differential expression of three of the seven cone opsins present in the cichlids. (c) Absorbance spectra of the seven cone pigments color coded with opsin class. (d) Schematic of the phylogenetic arrangement of the opsins illustrating the gene duplications (circles) that have occurred within the SWS2 and RH2 opsin classes. The recent duplication within the RH2A class may be restricted to African cichlids. Modified from Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

from the RH2 gene class, two from the SWS2 class, with single representatives of the LWS and SWS1 gene classes. The three green-sensitive cone pigments suggest that duplication in the RH2 gene has occurred on at least two occasions (**Figure 3**). It is most unlikely that all seven cone pigments are functional at one and the same time, but are selectively expressed during development. This has been demonstrated in at least one species, the Nile tilapia (*Oreochromis niloticus*), considered equivalent to the riverine ancestor of the lake cichlids, which expresses primarily four of the seven cone opsins in the larval form, including sensitivity to UV, but on maturity switches opsin gene expression by downregulating the shorter-wave opsin genes and upregulating the longer, giving the fish sensitivity to longer wavelengths, but with the loss of UV sensitivity (**Figure 4**).

Adaptations through Chromophore Exchange

A further group of teleosts that alter their spectral sensitivity by selective gene expression includes a number of migratory fish such as the anguilliform eels and the salmonids. In addition to switching opsin gene expression, these species also change chromophores during development and migration. The European eel, *Anguilla anguilla*, has a complex life history undergoing two distinct metamorphoses (**see also Fish Migrations**: Eel Migrations). As young yellow eels, the fish spend between 6 and 20 years maturing in the rivers of Western Europe and are somewhat typical of freshwater teleosts. Although rod dominated, the retina contains two spectrally distinct classes of single cone with λ_{max} at about 545 nm (RH2) and 440 nm (SWS2), with the rods having λ_{max} at 523 nm (**Figure 5**). All the pigments are porphyropsins, and, unusually for teleosts, double cones are not present. During autumnal downstream migration, the eel undergoes a metamorphosis, becoming a silver eel, showing dramatic changes in body structure and eye size. The eel becomes a truly oceanic mesopelagic fish, reaching sexual maturity, and is thought to breed at depths of up to 400 m in the Sargasso Sea.

As part of the metamorphosis, there is a substantial addition of new rods to the retina with probably a reduction in the number of cones. At the same time, the chromophore of the visual pigments switches, over a number of weeks, from 3-dehydroretinal to retinal, with a

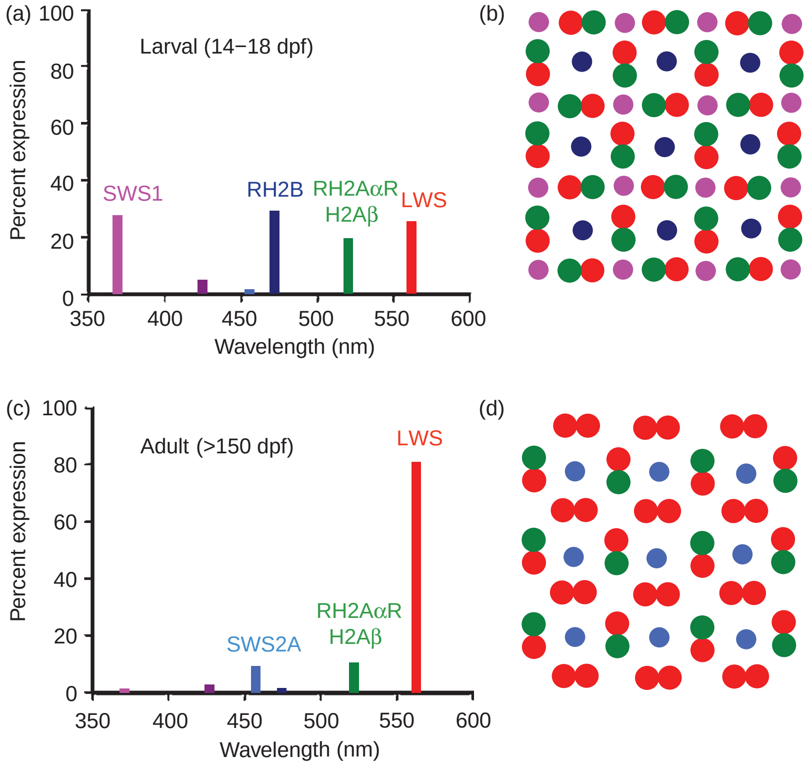


Figure 4 Differential expression of cone opsins during development in an African cichlid, the Nile tilapia. (a) Expression levels of the seven cone opsin genes in larval fish 14–18 days post fertilization (dpf). Relatively similar levels of expression of the LWS, RH2A, RH2B, and SWS1 opsins suggest the potential for tetrachromatic color vision. (b) Schematic of the possible cone array in these larval fish. (c) Expression levels of the cone opsins in adult tilapia. Expression is dominated by the LWS opsin with lower levels of expression of the RH2A and SWS2A suggesting the potential for trichromacy with the loss of UV sensitivity and the addition of identical LWS double cones, as shown in (d).

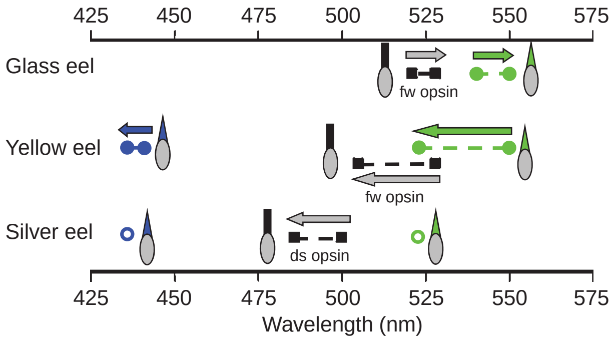


Figure 5 Variations in the peak sensitivities of the rods and cones of the European eel, *Anguilla anguilla,* at different stages during its life history. Circles indicate single cones and black squares indicate rods, as shown by the cartoons. Shifts in sensitivity are indicated by the arrows. At the juvenile glass eel stage, on entering freshwater, only rods and green-sensitive (RH2) cones are present and there is a shift from a rhodopsin-dominated system to porphyropsin-dominated pigments. At the yellow eel stage, both blue (SWS2)- and green sensitive cones are present and as metamorphosis progresses toward the silver eel stage there is a spectral shift toward shorter wavelengths with the reintroduction of rhodopsins. In addition, there is downregulation of the freshwater rod pigment (fw opsin) and upregulation of the deep-sea rod pigment (ds opsin). Cones may be lost at the silver eel stage (open circles).

displacement of the $\lambda_{\max}$ to shorter wavelengths: 525 and 435 nm in the cones and 501 nm in the rods. There is also a change in opsin expression in the rods, with a downregulation of a freshwater opsin and the upregulation of a new deep-sea opsin (**Figure 5**). The deep-sea opsin is expressed as a rhodopsin with $\lambda_{\max}$ at about 482, typical of deep-sea fish. During evolution of the anguilliforms, the rod opsin has been duplicated in order to achieve more short-wave sensitivity in an oceanic environment, enhanced by the change in chromophore to provide more short-wave rhodopsins. After breeding, the eel larvae, called 'leptocephali', return to Europe on the North Atlantic Drift over a period of a few years when they undergo a second metamorphosis changing into glass eels or elvers and entering into freshwater to develop into yellow eels. During the early stages of this transition, the glass eels initially express only the freshwater rod opsin and the RH2 cone opsin, with the SWS2 blue-sensitive opsin being expressed later (**Figure 5**).

A further group of teleosts, the salmonids, also illustrate a feature perhaps relatively common among fish and that is the loss of UV sensitivity during development. Salmonids, including migratory Pacific and Atlantic salmon (*Oncorhynchus* spp. and *Salmo salar*, respectively), as well as land-locked trout (e.g., *Salmo trutta*), exhibit at early juvenile stages the typical teleost square mosaic with red/green double cones forming the sides of the square, and single cones located in the center and at the corners of the array. Initially, it is likely that all the single cones express an UV-sensitive pigment (SWS1), but during development two distinct changes occur. First, the SWS1 opsin gene is downregulated and most of the UV-sensitive cones start to express a blue-sensitive SWS2 opsin. At the same time, the corner cones are gradually lost through apoptosis, so that after a few weeks or months (depending on species) the retinal mosaic changes from having central cones primarily expressing the SWS2 pigment and the corner cones primarily expressing the SWS1 pigment, to a simpler pattern with the loss of corner cones leaving only the central blue-sensitive single cone. The double cones and rods do not show changes in opsin expression. The developmental pattern in the single cones is further complicated by regional variations in expression and loss, with both progressing from ventral to dorsal across the retina.

The significance of these changes has traditionally been related to a number of environmental and behavioral aspects. UV sensitivity has been suggested as a means of improved detection of planktonic food, since plankton often absorb short wavelengths and UV sensitivity would increase visual contrast. In addition, it is thought that UV-sensitive cones are involved in the ability of fish to detect the plane of polarization of underwater light, thought to be involved in orientation. However, such theories have been questioned, since the transition from UV sensitivity to more blue sensitivity occurs at very different times during development across salmonid species – in some species occurring at the very early larval stages, but in others not until smoltification approaching 1 year of development. The early appearance of single cones, in many cases UV-sensitive, may be a more general feature important in retinal development, and may play a fundamental role in the establishment of the retinal photoreceptor mosaic, such a striking feature of many teleosts. Developmental opsin transitions have been reported not only in salmonids, but also in many other teleost groups, notably in cyprinids, such as goldfish (*Carassius auratus*) and zebrafish (*Danio rerio*).

In salmonids, as in eels, the chromophore also switches during migrations, with rhodopsins dominating in oceanic environments and porphyropsins becoming more prominent in freshwater. In land-locked species, there can also be chromophore variations that may be seasonal in temperate climates, and/or reflect the color and turbidity of the water. In dystrophic lakes where the water may be heavily stained yellow or brown with organic run off from the land, visual pigments have a higher percentage of porphyropsins than in clearer eutrophic lakes where more short-wave light can penetrate and rhodopsins predominate. In general, there may also be an increase in porphyropsins during the winter months, seen not only in salmonids, but also in some cyprinids, such as rudd (*Scardinius erythrophthalmus*), and again this is thought to relate to increases in turbidity and run off during the wetter winter season, but may also reflect seasonal changes in feeding behavior.

These examples of variations in visual capacity in a range of teleosts illustrate the great diversity of photoreceptors and visual pigments found in fish. Broadly, and perhaps somewhat simplistically, these can be related to differences in the photic environment of the various species and to distinct changes in environment and behavior experienced during development from larva to adult. However, there may be much more subtle influences that determine the differences in photoreceptor populations and spectral sensitivities across species. The bright colors and sexual dimorphism exhibited by many species are likely to be significant in species recognition and mate choice, but the degree to which this has governed spectral sensitivity and color vision is not well understood. Similarly, the feeding behavior of fish varies greatly and has certainly influenced anatomical features, such as the mouth and jaws, but how significant these behaviors are in determining retinal organization is again not well understood. The aquatic environment, unlike the terrestrial scene, is dominated by the direction of the downwelling light, so that the visual field above a fish is markedly different from the lateral view and from the visual field below the fish. These differences clearly affect retinal organization so that variations in mosaic arrangements and cone complements may well differ between retinal regions that are viewing different visual fields. To understand the significance of all of these more detailed features of fish vision, extensive behavioral studies of individual species and of fish communities are needed, which may then be related to the more easily obtained anatomy and physiology of the visual system.

See also: **Fish Migrations**: Eel Migrations. **Vision**: Behavioral Assessment of the Visual Capabilities of Fish; Photoreceptors and Visual Pigments; Visual Adaptations to the Deep Sea.

Further Reading

Archer SN, Djamgoz MDA, Loew ER, Partridge JC, and Valerga S (eds.) (1999) *Adaptive Mechanisms in the Ecology of Vision*. Dordrecht: Kluwer.

Bowmaker JK (1995) The visual pigments of fish. *Progress in Retinal and Eye Research* 15: 1–31.

Bowmaker JK and Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16(13): R484–R489.

Bowmaker JK and Loew ER (2008) Vision in fish. In: Masland RH and Albright TD (eds.) *The Senses: A Comprehensive Reference, Vol. 1: Vision* Vol. 1 pp. 53–76. Oxford: Elsevier.

Deutschlander ME, Greaves DK, Haimberger TJ, and Hawryshyn CW (2001) Functional mapping of ultraviolet photosensitivity during metamorphic transitions in a salmonid fish, *Oncorhynchus mykiss*. *Journal of Experimental Biology* 204(14): 2401–2413.

Douglas RH (2001) The ecology of teleost fish visual pigments: A good example of sensory adaptation to the environment? In: Barth FG and Schmid A (eds.) *Ecology of Sensing*, pp. 215–235. Berlin: Springer.

Douglas RH and Djamgoz MBA (eds.) (1990) *The Visual System of Fish*. London: Chapman and Hall.

Hunt DM, Dulai KS, Partridge JC, Cottrill P, and Bowmaker JK (2001) The molecular basis for spectral tuning of rod visual pigments in deep-sea fish. *Journal of Experimental Biology* 204(19): 3333–3344.

Hunt DM, Fitzgibbon J, Slobodyanyuk SJ, and Bowmaker JK (1996) Spectral tuning and molecular evolution of rod visual pigments in the species flock of cottoid fish in Lake Baikal. *Vision Research* 36: 1217–1224.

Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) (2006) *Communication in Fishes,* vol. 2. Enfield, NH: Science Publishers.

Lythgoe JN (1979) *The Ecology of Vision*. Oxford: Oxford University Press.

Parry JWL, Carleton KL, Spady T, *et al.* (2005) Mix and match color vision: Tuning spectral sensitivity by differential opsin gene expression in Lake Malawi cichlids. *Current Biology* 15(19): 1734–1739.

Yokoyama S (2000) Molecular evolution of vertebrate visual pigments. *Progress in Retinal and Eye Research* 19(4): 385–419.

Inner Retina and Ganglion Cells

MW Levine, University of Illinois at Chicago, Chicago, IL, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Major Cells of the Inner Retina
Feedback
Ganglion Cells
Summary and Conclusions
Further Reading

Glossary

Axon The long extension of a nerve cell that carries spikes (action potentials, or neural impulses) to distant target cells away from the cell body where the nucleus lies (also called the nerve fiber).

Cross-correlation A measure of the relatedness of the firing of pairs of neurons. Typically, a cross-correlogram is created in which the number of occurrences of spikes in one neuron is tallied as a function of the time before or after each spike in the other neuron.

Counterphase A contrast alternating in time; dark areas become light and light areas become dark.

Distal The furthest from the central nervous system, or in the direction away from the central brain. Receptors are the most distal retinal neurons.

Electrotonic The passive spread of an electrical signal.

Gap junction A connection between two neurons that allows electrical signals to pass directly between them.

Maintained discharge The ongoing spiking activity of a neuron in the absence of changing stimulation.

Myelinated axon An axon that is coated with a fatty sheath that promotes faster conduction of spikes.

Nuclear layer The layer of the retina in which the cell bodies of neurons are found.

Plexiform layer The layer of the retina in which neurons synapse with other neurons.

Proximal Nearest to the central nervous system, or in the direction toward the central brain. Ganglion cells are the most proximal retinal neurons.

Receptive field The area of retina within which stimulation effects a response from a given neuron.

Sign-conserving A synapse in which depolarization of the presynaptic neuron leads to depolarization of the postsynaptic neuron; also called excitatory.

Sign-inverting A synapse in which depolarization of the presynaptic neuron leads to hyperpolarization of the postsynaptic neuron; also called inhibitory.

Spike (also called action potential or neural impulse) All-or-nothing brief depolarization that propagates along an axon, typically encoding a signal by the rate at which they occur.

Syncytium A large cell-like structure containing many nuclei derived from the fusion of many individual cells.

Transmitter A chemical released by one neuron that causes a change in polarization of target neurons with which it makes a synapse.

Introduction

Rods and cones of the retina respond to light and the optic nerve carries the information to the brain. However, there are nerve cells within the retina itself that analyze and integrate the signal. The cells involved in this pre-processing are arranged in layers at the back of the retina and are the focus of this article.

From the earliest days of electrophysiology, the fish retina has been an attractive preparation. It shares the major features and organization of all vertebrate retinas, with a level of complexity approximating that of the mammalian retina. This is in contrast to most amphibian and reptilian retinas, which apparently perform more complex processing that mammals relegate to the cerebral cortex.

The fish retina offers the simplicity of a cold-blooded preparation. The retina can be removed from the eyeball, providing a mechanically stable preparation devoid of feedback from higher centers. Lying on a glass slip, it is readily accessible for stimulation, recording, and pharmacological manipulation. The preparation is, of course, slowly dying, but it can be maintained in a relatively stable state for several hours.

As a result, several general principles of retinal processing were first described in fish retinas. These include the functional layering of the plexiform (synaptic) layers, many of the details of synaptic transmission, and even a new cell type (the interplexiform cell) later noted in mammalian retinas. Other important principles first described in anuran, reptilian, and mammalian retinas have been confirmed in fish. Thus, a description of the fish retina is, in many ways, a general review of the retinas of all vertebrate species.

The Major Cells of the Inner Retina

The cells of the retina present a variety of shapes and functions. This article considers the major types and most common categorizations. It does not include the glial cells (Müller cells), although they may also play a role in retinal processing. The anatomical configurations and arrangement of these neurons are illustrated schematically in **Figure 1**.

Horizontal Cells

Horizontal cells form the first layers of cells proximal to the receptors. As suggested by their name, horizontal cells are lateral elements that serve a relatively large area of retina. In fact, electrotonic connections (gap junctions) between horizontal cells allow each to share information over a larger area than its own anatomical extent. This electrically joined complex of cells is referred to as a 'syncytium'. Although the response of a horizontal cell is greatest directly under the receptors that are illuminated, a gradually diminishing response may be observed in horizontal cells over much of the retina. Some of this spread is through a long, tubular axon-like process that extends through the inner nuclear layer of the retina. The responses of horizontal cells were originally believed to be the responses of cones; when this was corrected, they were called S-potentials in honor of G. Svaetichin, who first recorded these responses.

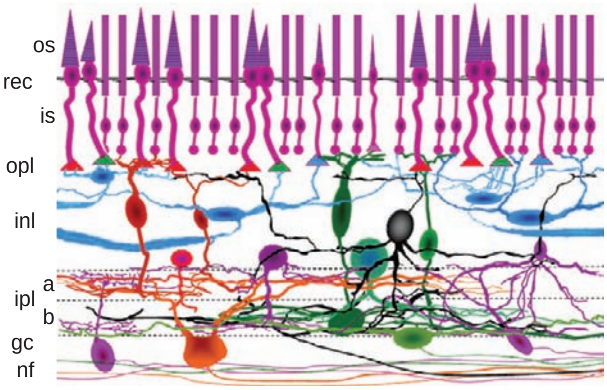


Figure 1 General schematic of the fish retina. Back of the eyeball is at the top; vitreous humor at the bottom (light enters from the bottom). Receptor layer (rec) at the top, with striped outer segments (os) above connecting inner segments (is; including somas, cone pedicles, and rod spherules) shown in purple. Cone pedicles are colored to indicate wavelength preferences. Horizontal (H) cells are blue. Small H1 is in the outer part of the outer plexiform layer (opl), with an exemplar at the left. An H2 is at the right, with a rod horizontal cell below it; an H3 is left of center. Bipolar cells span the inner nuclear layer (inl); hyperpolarizing bipolars shown in brown, and depolarizing bipolars in green. Various amacrine cells with their somas in the inl and processes branching in the inner plexiform layer (ipl) are colored according to type (orange for OFF-center, green for ON-center, and purple for ON–OFF). Ganglion cells (gc) are similarly color coded, as are their axons in the nerve fiber layer (nf). Interplexiform cells are shown in black, as are the centrifugal fibers. Glial cells are not shown.

Horizontal cells are presynaptic to both cones and bipolar cells. These are synergistic effects, antagonistic to the effects of light on individual cones and the bipolar cells that those cones influence directly. As horizontal cells collect information from a large area of retina, encompassing many receptors, the effect is to mitigate the effects of local illumination according to the general level of illumination. In effect, this is the first step in the process of extracting spatial contrast, rather than responding to the absolute level of illumination.

The synapses from receptors to horizontal cells are aminergic; glutamate is the transmitter released by cones, and aspartate by the rods. These transmitters produce what are commonly considered excitatory postsynaptic potentials; that is, the transmitter (released by depolarization of the receptor) causes depolarization of the postsynaptic membrane. It is often preferable to consider these as sign-conserving synapses, meaning the postsynaptic cell potentials mimic the presynaptic potentials.

Horizontal cells release the transmitter gamma amino butyric acid (GABA), which is generally inhibitory (hyperpolarizing the postsynaptic membrane; sign inverting). The release mechanisms seem to be through both a calcium-dependent vesicular release and a slower nonvesicular transporter release. The feedback to cones, however, seems not to be GABAergic.

Most fish have four layers of horizontal (H) cells. H1 cells form the outermost layer. The input to these cells is from cones, almost exclusively the long-wavelength-sensitive cones. H2 cells in the next layer contact the middle-wavelength-sensitive cones; they also receive indirect inhibition from the H1 cells. This inhibition is through feedback to the middle- and short-wavelength sensitive cones. As a result, H2 cells are biphasic with respect to wavelength; they are hyperpolarized by middle-wavelength lights and depolarized by long-wavelength lights. The feedback from H1 cells also enhances the temporal tuning of the cones.

The H3 cells at the next deeper level are postsynaptic to the short-wavelength-sensitive cones. They also receive indirect inhibition from horizontal cell feedback to the cones. As a result, they exhibit chromatically triphasic responses: hyperpolarizing for middle wavelengths, and depolarizing for long and short wavelengths.

H1, H2, and H3 are purely cone-driven horizontal cells; a deeper layer of horizontal cells that resemble H3 but lack an axon receive purely rod input. This is distinct from the mammalian horizontal cells, which have both rod and cone inputs. Note that this description refers to fish that live in shallow water and have color vision; deep-sea fish generally lack color vision, have rods but only one (or no) cone type, and may have only a single type of horizontal cell.

Bipolar cells

Bipolar cells convey the processing of the outer plexiform layer to the inner plexiform layer. There are at least two major types of bipolar cell: hyperpolarizing and depolarizing (also referred to colloquially as 'OFF' and 'ON', respectively). These two types represent the initiation of one set of parallel pathways carrying distinct or, in this case, complementary, information through the retina.

How is it possible for one bipolar cell to respond to light with hyperpolarization, while a neighboring bipolar cell, contacting the same photoreceptors, responds with depolarization? The answer is that the receptor molecules in the dendrites of hyperpolarizing bipolar cells respond by opening channels for sodium or closing channels for potassium and chloride; these are sign-conserving synapses in which the transmitter binds to a receptor molecule that opens or closes an ion channel (ionotropic or ligand-gated receptors). The receptors of depolarizing bipolar cells, on the other hand, act through a second messenger (metabotropic receptors) to open a chloride channel. This results in hyperpolarization when the transmitter is present, so it is a sign-inverting synapse.

In most species, the depolarizing bipolar cells have dendrites that form the central element of the invaginations in receptors, while the hyperpolarizing bipolar cells make more traditional synapses outside the invaginations. The depolarizing bipolar cells terminate in the deepest (innermost or proximal) portion of the inner plexiform layer, the sublamina b (also referred to as layers 4 and 5). The hyperpolarizing bipolar cells, on the other hand, terminate in the outer part of the inner plexiform layer, the sublamina a (layers 1–3). Thus, the parallel 'ON' and 'OFF' pathways are anatomically segregated in the inner plexiform layer.

Bipolar cells may display either transient or sustained responses. Transient bipolar cells, which respond maximally when a light is first presented and then relax to a level nearer their polarization in the dark, may have exclusively rod inputs, or mixed rod and cone inputs. The sustained type, which produces a nearly rectangular response to a step in illumination, may have exclusively cone inputs or mixed rod and cone inputs. The effect of rod inputs seems to be a change in sodium conductance, while cones affect both chloride and potassium conductances. Direct rod and cone inputs to the same bipolar cell are a feature of fish retinas that is not found in mammalian retinas.

About 25% of the bipolar cells in carp (*Cyprinus carpio*) have color-opponent cone inputs. That is, a cell might hyperpolarize in red light, but depolarize in green light. The opposite response is obtained from annular light in the receptive field surround. Other bipolar cells of this type have the complementary arrangement: depolarizing in red light in the center and hyperpolarizing in green, while hyperpolarizing in red light and depolarizing in green in the surround. This arrangement, called 'double opponent', is prevalent among ganglion cells of shallow-water fish.

As was noted for photoreceptors and horizontal cells, bipolar cells are electrically coupled with near neighbors of the same type. It might seem that this electrotonic spread would degrade images and undercut lateral inhibition, but it provides redundancy and noise reduction as well. It is also possible that correlation across the retina plays a role in joining parts of the images of single objects.

Amacrine Cells

Amacrine cells form a diverse group of neurons that often interconnect bipolar cells with ganglion cells. There are two major subgroups: transient amacrine cells that respond to changes in time, and sustained amacrine cells that have spatial and chromatic organization.

The transient amacrine cells typically have dendritic trees that either span the entire inner plexiform layer, or that have separate branches within sublamina a and sublamina b. The transient cells apparently receive depolarizing inputs from both hyperpolarizing and depolarizing bipolar cells, and respond with depolarization at both onset and offset of a stimulus (an ON–OFF response). Often, the response includes a series of brief depolarizations; some cells may actually produce a train of spikes, although others apparently produce a regenerated but scaled brief depolarization or pair of depolarizations riding on a slower depolarizing wave. In continuing illumination, the cell potential returns to its level in the dark.

The sustained amacrine cells show responses more like those of bipolar cells, although they may also produce graded brief depolarizations. These amacrine cells generally exhibit a center-surround organization, and may also have chromatic selectivity. Their dendrites are typically confined to a single sublamina of the inner plexiform layer. Among amacrine cells, there is usually electrotonic coupling.

Ganglion Cells

Ganglion cells are the major output cells of the retina. Their axons gather at the optic disk, where they become myelinated and form the optic nerve. As their message is conveyed over a significant distance, it is encoded as trains of spikes (also known as 'action potentials', or 'neural impulses').

Ganglion cell dendrites branch in the inner plexiform layer. Many arborize within one sublamina, depending on whether the cell is ON-center or OFF-center. Many are ON–OFF cells, which extend their dendrites through both levels of the inner plexiform layer. Some of these have diffuse dendrites, while some have distinct sets of dendrites arborizing in distinct sublaminae (see **Figure 1**).

In fish, most ganglion cells receive inputs from both bipolar and amacrine cells. There are also synapses and gap junctions between ganglion cells. The various response types and responses of ganglion cells are discussed later.

Feedback

One feature of visual processing is that higher centers influence the lower centers from which they obtain their information. This is also true within the retina of fish as well as those of mammals.

One example has already been discussed: horizontal cells are presynaptic to receptors, providing an area-wide adjustment of their membrane potentials. Similarly, many amacrine cells are presynaptic to the bipolar cells that convey information from the outer plexiform layer. There are also feedback loops that extend across layers of cells.

Interplexiform Cells

Interplexiform cells were first described in goldfish (*Carassius auratus*), before being observed in mammals. These are cells that appear much like amacrine cells in the inner plexiform layer, but sport a process extending into the outer plexiform layer.

There are at least two basic pharmacological types of interplexiform cell in goldfish: the dopaminergic interplexiform cell and the glycinergic interplexiform cell. The dopaminergic type was reported first, and best fits the usual concept of an interplexiform cell. It has a cell body among the amacrine cells, where it is both pre- and postsynaptic. Its dendrites have processes that ascend to the outer plexiform layer, where they are presynaptic to horizontal cells and bipolar cells. They also affect receptors, controlling their retinomotor movements. The effect of dopamine seems to be to uncouple the electrical synapses of the horizontal cells (and perhaps also the bipolar cells), thus shrinking the surround of the bipolar-cell receptive fields. Dopamine also depolarizes the horizontal cells and reduces the hyperpolarization due to light. Dopamine is apparently necessary for horizontal cell spinule formation, an anatomical change that can be observed over a relatively short time span. In this manner, interplexiform cells reduce the sensitivity of the outer retina during stimulation, which is a form of adaptation.

Centrifugal Fibers

Another feedback pathway arises in a nucleus at the lower border of the telencephalon at the olfactory bulbs (the nucleus olfactoretinalis). Axons of the cells in this nucleus join the optic nerve and terminate in the inner plexiform layer. These fibers apparently affect dopaminergic interplexiform cells, and, possibly, also the ganglion cells, amacrine cells, and bipolar cells.

The effect of the centrifugal fibers (i.e., from the brain to the retina) upon interplexiform cells is apparently to promote light adaptation in the retina. The centrifugal fibers also show rhythmic variations in their activity, which is correlated with other rhythmic firing patterns in the central nervous system. They can thereby provide the mechanism for coordinating retinal rhythms with those of the rest of the central nervous system.

Ganglion Cells

Receptive Fields

The term receptive field refers to that region of retina within which illumination affects the responses of a particular neuron. In many cases, there may be subtle effects of light over a wide region of the retina, but this discussion only considers the classical field within which light has a direct effect upon the firing rate.

Most ganglion-cell receptive fields in fish have been characterized as circularly symmetric, concentric (center and surround) fields. There are some cells that respond to motion and direction of movement, and many of the fields are rather more elongated than circular. The sensitivity across the field can be quite uneven, rather than forming a smooth profile.

There are three main types of receptive fields, with subtypes representing color coding: the ON-center, the OFF-center, and the ON–OFF fields. The fields are classified according to the type of response initiated by a small spot of light centered upon the receptive field. The response to light in the surrounding region is typically of the opposite type from that of light in the center region. As the surround region is relatively large, a small spot is often insufficient to elicit a response from it, so an annulus (ring) of light is generally used.

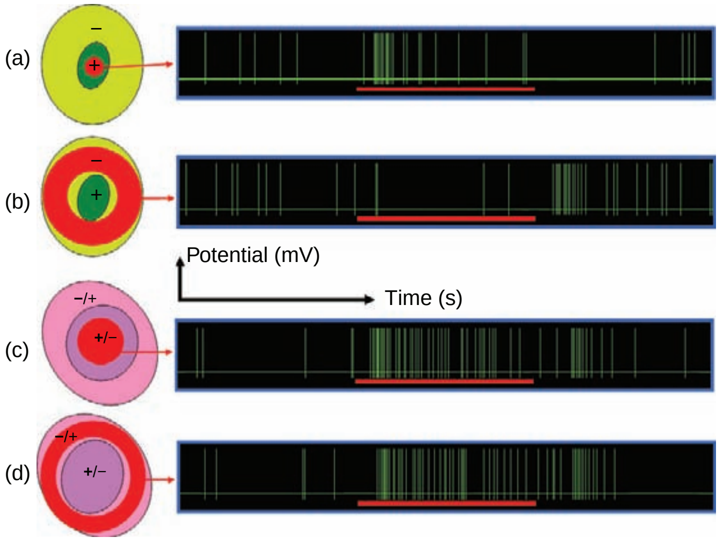


Figure 2 Responses of two ganglion cells. Receptive field sketched to the left, with '+' indicating an ON response, '–' an OFF response. Oscilloscope-like traces to the right indicate responses to the stimuli superimposed on the field sketch as a red spot (a, c) or annulus (b, d). Each vertical line is an idealized spike; the stimulus is present at the time indicated by the red bar under each trace. The cell shown in (a) and (b) is ON-center; the cell in (c) and (d) is of the ON–OFF type.

Responses of an ON-center ganglion cell in a goldfish are shown in **Figures 2(a)** and **2(b)**. (Responses of an OFF-center cell would be reversed.) The receptive field is represented by the concentric circles on the left; the plus sign in the inner circle represents the ON-responding center, while the minus sign in the outer ring represents the OFF-responding surround region. A small, centered spot of deep red light was presented at the time shown by the red bar under the upper trace, corresponding to the stimulus spot superimposed on the receptive field center in **Figure 1(a)**. The response is shown to the right as a train of spikes, as if this were an oscilloscope trace. Notice that the cell was firing irregularly in the dark before presentation of the spot (the maintained discharge, discussed later); the spot caused a burst of firing, which declined to a lower rate slightly above the firing rate in the dark. When the spot was extinguished, the firing was depressed, gradually recovering to its maintained rate in the dark. This is an ON response.

The response of the same cell to an annulus of red light is shown in **Figure 2(b)**. The onset of the annulus elicited a brief pair of spikes, followed by a suppression of firing. The offset of the stimulus caused a robust burst of firing. The slight burst preceding depression of firing is relatively common in fish ganglion cells; nevertheless, because of the major effect of depressed firing during the stimulus, this is considered an OFF response.

The opposite responses at offset of the light may reflect two factors. To some extent, it is a rebound from the previous level during stimulation; if the cell had been firing above its maintained level, removal of the excitatory signal could allow a recovery to below the normal resting level. Conversely, removal of an inhibitory signal could allow a rebound above the normal level. However, there is also statistical evidence indicating that the opposite response at light offset is actively generated. That is, there is a push–pull arrangement, in which one set of inputs is responsible for the onset response, and a different set contributes to the offset response. Anatomical considerations indicate that it is not likely that the two types of bipolar cells (hyperpolarizing and depolarizing) are directly the source of this antagonism, as is the case in cat. Rather, it appears amacrine cells contribute to the offset response.

An ON–OFF response is characterized by an increase in firing at both onset and offset of a stimulus. Cells that produce such a response typically do not distinguish a clear center and surround region, although the relative sizes of the two responses (onset and offset) may vary with stimulus configuration. Responses of an ON–OFF cell are shown in **Figures 2(c)** and **2(d)**. The distinction between traditional ON or OFF responses and the ON–OFF response is not always unambiguous.

Color Coding

Color specificity of ganglion cells was first studied in the goldfish. Some cells are not color opponent, such as the cell illustrated in **Figures 2(a)** and **2(b)**, having center and surround opponency driven mainly by the long-wavelength-sensitive cones. Note that the red light used to stimulate the ON-center cell described in the previous section (and **Figure 2**) was of

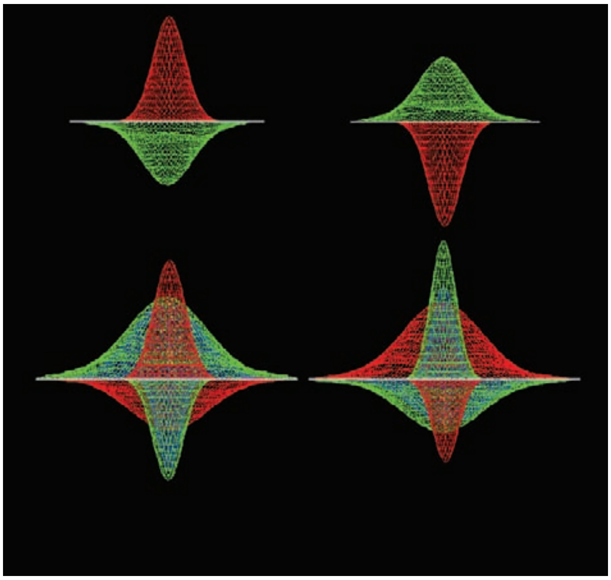


Figure 3 Color-coding of goldfish ganglion cells. Excitatory regions (ON regions) are shown as Gaussians above the gray line; inhibitory regions (OFF regions) extend below the gray line. Colors indicate the spectral sensitivities: blue for short wavelengths, green for middle wavelengths, and red for long wavelengths. Note that although the sensitivity profiles are shown as Gaussians, they are actually quite irregular. Top row: type P cells, lower row: type O cells.

sufficiently long wavelength (650 nm) such that only the long-wavelength-sensitive cones would detect it. Since goldfish have porphyropsin-based pigments, their long-wavelength-sensitive cones have a maximum spectral sensitivity close to 620 nm.

Some ganglion cells, called 'type P' (for previous), have color-opponent responses in the receptive field center, but not in the surround. Thus, a cell might produce ON responses to red light in the receptive field center, and OFF responses to green light in either the center or the surround (the reverse situation, OFF for red and ON for green, is also found; see **Figure 3**, top row). In effect, the area affected by middle-wavelength-sensitive cones is simply larger than that affected by the long-wavelength-sensitive cones.

Nearly 50% of goldfish ganglion cells are type O (for ordinary), the double-opponent cells. As for the type P cells, red and green (or blue) lights in the center region have opposite effects, but the responses are reversed for all colors in the surround (see **Figure 3**, bottom row). Such a cell displays center-surround antagonism for any particular colored light (although of the opposite types for red or green lights), and color opponency for small, centered spots. Notice that the strongest responses would arise from a red spot in a green surround (or vice versa). This is the arrangement that would best enhance simultaneous color contrast.

Another category is called type Q (for queer). These appear to be type O cells that have a very weak middle-wavelength-sensitive cone component, which can only be demonstrated by intense stimulation or chromatic adaptation to red lights (desensitizing the long-wavelength-sensitive cones). This reduction of middle-wavelength-sensitive cones may be a consequence of the physiological preparation; dissection in room light tends preferentially to desensitize all but the long-wavelength-sensitive cones.

Parallel Paths

In addition to ON-center and OFF-center and the various combinations of wavelength sensitivities, ganglion cells in goldfish may be categorized into the linear and nonlinear classes described for mammals. There are distinctly X-, Y-, and W-ganglion cells, each forming its own mosaic with complete coverage of the retina. Each of these types displays the various color-coding schemes outlined above.

The X-, Y-, and W-like cells have spatial summation properties similar to those of the X, Y, and W cells of the cat. X-like cells exhibit linear spatial summation; that is, a counterphase grating in the null position (spatial sine phase relative to the receptive field middle) elicits no response. Y-like cells do not sum signals linearly; their responses are frequency-doubled for counterphase gratings in sine phase. Unlike the situation in cat, the overall receptive fields of the X- and Y-like cells in goldfish are about the same sizes. Because of the nonlinear subunits, however, the Y-like cells respond to higher spatial frequencies with the frequency-doubled component. Many Y-like cells also show direction tuning, preferring particular directions of motion of a stimulus. W-like cells display spatial summation that is between that of the X- and Y-like types, and always show orientation and direction tuning. The latter two types originally were called 'not-X-like' cells.

Maintained Discharges

The maintained discharges of ganglion cells (sometimes referred to as spontaneous firing) are a general feature across species. In the dark or in steady uniform illumination, ganglion cells in fish fire at rates that vary (across cells) from a few spikes per second to 30 or more per second. There are two aspects of maintained discharge to consider: the simple fact that there is ongoing activity in the absence of stimulation, and the variability of that firing.

Source of maintained discharges

The fact that the ganglion cell fires in the absence of stimulation makes teleological sense: without a level against which to measure changes, decreases could not be represented. However, there remains the question of the source of that mean firing.

The main source of the maintained discharge is bombardment of the ganglion cell by presynaptic neurons (bipolar and amacrine cells, possibly also other ganglion cells). When synaptic transmission is disabled (by adding cobalt to a superfusate to block vesicular release), the maintained discharge is completely or very nearly completely abolished. There is the possibility that the surviving discharge is due to nonvesicular release (or leakage) of transmitters, but it seems more likely that a very small component of the discharge is intrinsic to the ganglion cell itself.

Variability of maintained discharges

The ganglion cell does not fire in a regular, metronome rhythm like a pacemaker cell. The intervals between successive spikes are stochastically variable. They are drawn from a positively skewed distribution that is generally more extreme than the gamma distribution that has been used to describe the distribution in mammalian ganglion cells. The distribution is often bi- or even multimodal, with a concentration of very short intervals (spikes occurring in close pairs or triplets) and another mode (or more) at longer intervals (the intervals between pairs, triplets, and solo spikes).

While the order in which intervals are drawn from this distribution during stationary maintained discharge is nearly random, a subtle ordering representing high-pass filtering is demonstrable. There are fewer strings of similar duration intervals than might be expected by chance; as a result, the average firing rate in periods of moderate duration (0.2–0.5 s) is less variable than one would expect from the distribution of the intervals. This filtering, while subtle, is less subtle than is observed in cats.

Cross-correlations within the maintained discharges

In fish, as in mammals, the firing of a ganglion cell is not entirely independent of the firing of its neighbors. Obviously, since neighbors are responding to the same stimuli, there is a coarse relationship in that they increase or decrease firing at essentially the same time. However, there is a more precise cross-correlation that is observable in maintained discharges as well as during responses.

This cross-correlation is generally not precise. The probability of one cell producing a spike at a given time increases (or decreases) within a window of 5–30 ms starting up to 15 ms after (or before) each spike in a neighboring ganglion cell. This delay and imprecision are incompatible with simple electrotonic coupling with the neighbor (as has been reported in salamanders), but is most likely the result of synaptic influence by another neuron affecting both of the cells. The common influence is not simply additive, but rather has a multiplicative effect upon the firing mechanism of each ganglion cell.

As in mammals, cross-correlation is positive between cells of the same response type, and negative for pairs of cells of opposite type. An ON-center cell is more likely to fire in near coincidence with the spikes of a neighboring ON-center cell than at other times, but less likely to fire in near coincidence with the spikes of a neighboring OFF-center cell.

The function of this cross-correlation in the mature retina is not clear. In the developing retinas (of mammals), correlated firing is essential for the proper collation of fibers in the optic nerve as they select their targets in the thalamus. Disruption of spike activity with the drug tetrodotoxin, even before there are bipolar cells to convey visual information from the receptors, prevents the proper anatomical mapping from developing in the brain. Fish eyes continue growing as the fish grows throughout life; therefore, such a process might contribute to the alignment of axons of the newly formed cells. However, most of these cells are born at the growing periphery of the retina, while cross-correlation is evident throughout the retina.

A putative function for cross-correlation is to provide additional multiplexed channels of information. That is, in addition to each cell conveying information about the stimulus attributes to which it is tuned, the coincident firing of two (or more) cells could carry information about some other attribute of the visual scene. Decoding this message would require a higher-level cell that receives input from both of the ganglion cells, and only fires when its inputs both fire within a short time window (a logical AND). However, no attempt to find what information is conveyed, or a way in which this signal might improve upon the information present in the better-tuned individual cell, has been successful. A similar failure to demonstrate usefulness has been reported for mouse.

Summary and Conclusions

The fish retina, like the retinas of other vertebrates, performs the first steps of visual processing. By the somewhat phasic nature of its responses, it begins the process of temporal tuning. The lateral antagonism of center versus surround of the receptive fields begins the process of responding to contrast rather than absolute luminance. In effect, the retina (and the visual system) is most responsive to changes in the stimulus: changes in time and space.

Two additional principles that are typical of retinas and of visual systems as a whole are also evident in the fish retina. The first is the division into parallel pathways. Cells operate in parallel to extract different aspects of the stimulus. These aspects may be temporal properties, scale (size), direction of motion, color, or contrast.

The second principle is that of feedback. Horizontal cells affect the receptors that synapse upon them; amacrine cells affect the bipolar cells that carry information from the outer plexiform layer. Interplexiform cells in the inner plexiform layer affect the state of adaptation in the outer plexiform layer, and themselves are affected by activity in more central structures via the centrifugal fibers.

Finally, fish ganglion cells display a maintained discharge against which their responses are impressed. The function of the stochastic nature of this maintained discharge is not clear.

What is the value of this convergence, processing, and encoding? The interconnection of cells comes at the expense of some precision, but with gains in efficiency. A single goldfish retina contains nearly 1.5 million cones and about 10 times as many rods, but only about 350 000 ganglion cells. If each cone enjoyed a private line to the central nervous system, the optic nerve would contain about 5 times as many axons, and would be more than twice the diameter (at least double that when including pathways for rods). The costs in energy, physical flexibility, and volume would be prohibitive. Moreover, the processing performed in the retina would have to be accomplished in the brain. The processing and encoding executed in the retina provide an economy for the fish, and a window into visual processing for the vision scientist.

See also: **Vision**: Adaptations of Photoreceptors and Visual Pigments; Behavioral Assessment of the Visual Capabilities of Fish; Photoreceptors and Visual Pigments; Visual Adaptations to the Deep Sea.

Further Reading

Connaughton VP (2001) Organization of ON- and OFF-pathways in the zebrafish retina: Neurotransmitter localization, electrophysiological responses of bipolar cells, and patterns of axon terminal stratification. *Progress in Brain Research* 131: 161–176.

Djamgoz MBA and Wagner H-J (1987) Intracellular staining of retinal neurons: Applications to studies of functional organization. In: Osborne N and Chader G (eds.) *Progress in Retinal Research,* vol. 6, pp. 85–150. Oxford: Pergamon.

Djamgoz MBA and Yamada M (1990) Electrophysiological characteristics of retinal neurons: Synaptic interactions and functional outputs. In: Douglas R and Djamgoz M (eds.) *The Visual System of Fish*, pp. 159–210. London: Chapman and Hall.

Kamermans M and Spekreijse H (1999) The feedback pathway from horizontal cells to cones: A mini review with a look ahead. *Vision Research* 39: 2449–2468.

Kaneko A (1971) Physiological studies of single retinal cells and their morphological identification. *Vision Research Supplement* 3: 17–26.

Kato S, Negishi K, Teranishi T, and Ishita S (1991) The use of the carp retina in neurobiology: Its uniqueness and application for neural network analyses of the inner retina. *Progress in Neurobiology* 37: 287–327.

Levine MW (2007) Variability in the firing of retinal ganglion cells of goldfish: A review. *Visual Neuroscience* 24: 239–246.

Negishi K, Teranishi T, and Kato S (1990) The dopamine system of the teleost fish retina. *Progress in Retinal Research* 9: 1–48.

Twig G, Levy H, and Perlman I (2003) Color opponency in horizontal cells of the vertebrate retina. *Progress in Retinal Eye Research* 22: 31–68.

Wagner H-J (1990) Retinal structure of fishes. In: Douglas R and Djamgoz M (eds.) *The Visual System of Fish*, pp. 109–157. London: Chapman and Hall.

Wagner HJ, Fröhlich E, Negishi K, and Collin SP (1998) The eyes of deep-sea fish. II. Functional morphology of the retina. *Progress in Retinal Eye Research* 17: 637–685.

Wu S (1994) Synaptic transmission in the outer retina. *Annual Review of Physiology* 56: 141–168.

Optic Tectum

D Northmore, University of Delaware, Newark, DE, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Anatomical Overview
The Retinotectal Projection and Visual Processing
Tectum–TL Subsystem
Tectum–NI Subsystem
Multimodal Processing and Torus Semicircularis
Ascending Connections – via PTP
Descending Connections and Motor Control
Conclusions
Further Reading

Glossary

Motoneurons Neurons in the central nervous system that send their axons to muscles, controlling their contraction.

Plasticity of neural connections The alteration of synaptic connections. Injury to neurons, especially in fishes, prompts regeneration of axons which may grow into normal and abnormal target areas. Developmental and learning processes involve changes in synaptic arrangement and efficacy.

Receptive field Region of retina or visual field within which stimulation alters the activity of a neuron. An electrode that picks up activity from more than one neuron or receptor cell has a multiunit receptive field (MURF).

Reticular formation Diffusely organized neuronal groups around the core of the midbrain and brainstem involved in a wide variety of functions. Most relevant function here is as premotor intermediary between tectum and motoneurons.

Retinal ganglion cells The output neurons of the retina. Their axons, called optic fibers, terminate in tectum and nuclei of the preoptic–thalamic–pretectal (PTP) area of the brain.

Retinotopic map, organization or projection Found in brain areas, like tectum, that receive a spatially ordered set of input fibers from retina, thereby forming a representation of the retina, and the visual field of the eye. It is a particular case of topographic map. The retinotectal map is an instance.

Topographic map, organization or projection Found in brain areas that receives a spatially ordered set of input fibers from another brain structure (e.g., the connections from tectum to nucleus isthmi (NI)).

Visual looming The pattern of motion on the retina created by approaching objects. A symmetrical expansion of the retinal image presages collision.

Introduction

Looking down upon the brain of a sunfish (*Lepomis* sp.), the optic tectum appears as a pair of rounded lobes between the forebrain and the cerebellum (**Figure 1**). A side view shows how the optic nerves from the eyes cross over one another, becoming the optic tracts, each of which enters the front of its corresponding tectal lobe. This simple anatomical examination shows that the information from each eye travels directly to the tectal lobe on the opposite side. Indeed, the great majority of the optic nerve fibers, the axons of retinal ganglion cells, terminate in the tectum, making it the primary visual center in the brain.

This article focuses on the functional aspects of tectum – its role as an information processor and controller of behavior – bearing in mind that the view presented will be limited in scope. There are more than 25 000 fish species occurring in widely diverse forms and in every aquatic habitat. While the optic tectum is present in all species, it is developed to different extents. Our knowledge of tectal function derives almost entirely from a handful of teleost species, mainly perciforms (e.g., sunfish) or cyprinids (e.g., the goldfish, *Carassius auratus*), with much less information available on elasmobranchs, the cartilaginous fishes. To fill some of the gaps in our understanding, we must also look at tectum in other vertebrate groups.

The importance of tectum to a teleost fish can be shown by damaging it. Complete ablation renders the animal effectively blind. However, a partial ablation or localized lesion of one tectal lobe produces a loss of responsiveness to visual stimuli over part of the visual field of the opposite eye, showing that visual space is mapped topographically across the tectum. Damage to the tectum in teleosts also results in losses that go beyond blindness, being impaired in their ability to navigate the environment using nonvisual senses, bumping into objects in a way that eyeless fish would not because the tectum

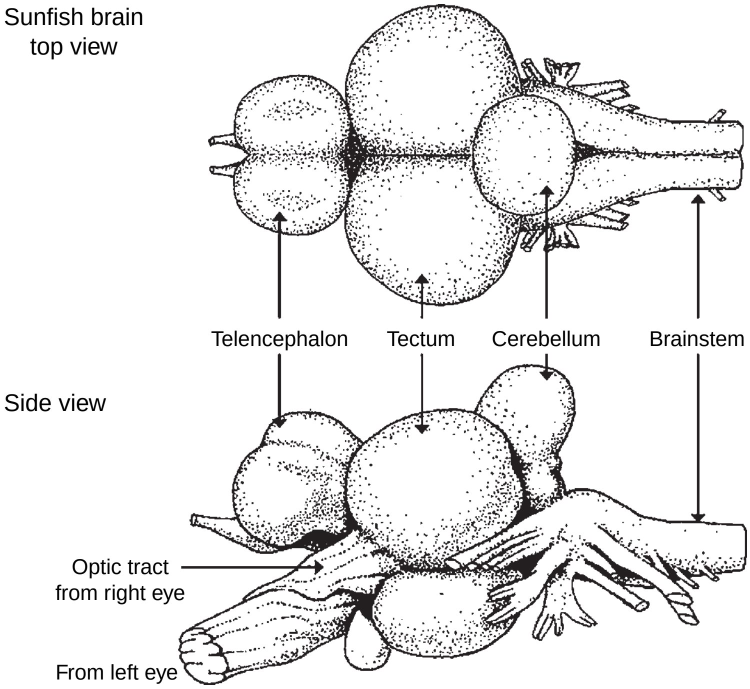


Figure 1 Top and side views of a sunfish brain (*Lepomis* sp.). Adapted from S. Karger AG, Basel from Wullimann and Northcutt (1988) Connections of the corpus cerebelli in the green sunfish and the common goldfish; A comparison of perciform and cyprinform teleosts. *Brain Behavior and Evolution* 32: 293–316.

serves other sensory modalities besides vision. In other vertebrate species, it is known that the tectum, or the mammalian equivalent, the superior colliculus, contains neurons that respond to sound and touch. Animals with exotic senses such as infrared sensitivity (e.g., rattlesnakes) and electroreception (e.g., skates and rays) possess tectal neurons that respond to these modalities too. Fishes have a well-developed system of sensitive mechanoreceptors, the lateral line organs, that detect minute water currents, enabling them to localize prey, avoid objects, and school with other fish. This valuable source of spatial information underwater is almost certainly represented in tectum too, but like many aspects of tectal function in fishes has not been investigated.

The varieties of sensory information converging upon the tectum may evoke behavioral responses via its efferent connections to motor centers of the reticular formation. The tectum, in conjunction with other brain areas, integrates sensory signals of different modalities, and, in ways not yet understood, arrives at a decision to react positively with orientation and approach, negatively with avoidance, or not to react at all.

Anatomical Overview

The tectum, the Latin for roof, covers the midbrain. In teleost fishes, it is a twin-lobed canopy of neural tissue inflated over a fluid-filled ventricle. The two lobes are connected at the midline by the tectal commissure, and at their bottom edges merge with the tegmentum, the floor of the midbrain (see **Figure 4**(**b**)). A well-developed tectum in a highly visual teleost may be 0.8 mm thick with a clearly layered appearance when examined under the microscope. The layering scheme shown in **Figure 2** represents the most common interpretation of tectal structure (tectal layers in teleosts – listed from outermost to innermost: stratum marginale (SM), stratum opticum (SO), stratum fibrosum et griseum superficiale (SFGS), stratum griseum centrale (SGC), stratum album centrale (SAC), and stratum periventriculare (SPV)). Functionally, the most important distinction is between the superficial layers (SM, SO, and SFGS) which are mainly visual, and the deep layers (SGC, SAC, and SPV) which are multimodal and motor. Some layers contain many fibers (SM, SO, and SAC), others contain some neuronal cell bodies, fibers, and synapses (SFGS and SGC), while the innermost layer (SPV) is composed of densely packed neuronal cell bodies. Around 15 morphologically distinct neuron types have been described in goldfish, a few of which are illustrated in **Figure 2**. In elasmobranch fishes, the tectum is less prominent than it is in a typical teleost, being smaller than the telencephalon or cerebellum, and its structure is less well differentiated into distinct cell types and layers. Nevertheless, the same functional distinction between superficial and deep layers seems to apply as it does in teleosts.

Dominant among the neural connections with the tectum are the optic fibers coming from the retinas, but

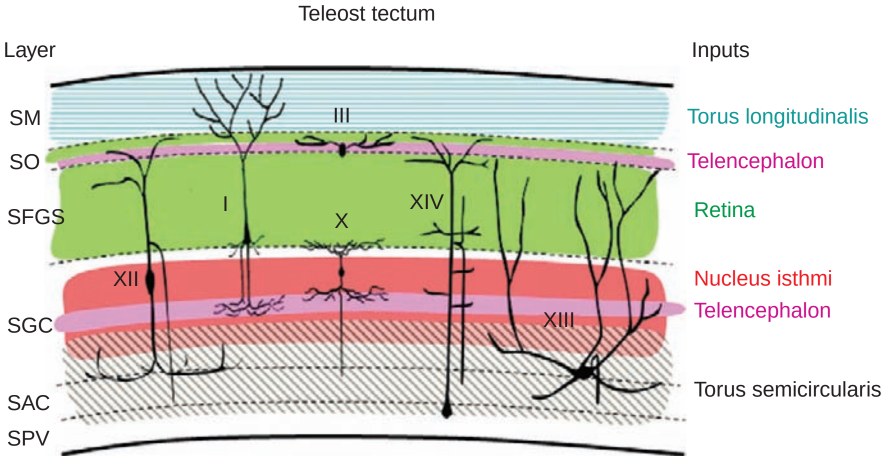


Figure 2 Laminar structure of teleost tectum, showing layer nomenclature and a few representative cell types. Roman numerals are Meek's types. Colored bands show principal termination layers of different inputs to tectum. SM, stratum marginale; SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; and SPV, stratum periventriculare.

the tectum is also richly interconnected with many other brain structures. These include: (1) upstream structures, the telencephalon and the preoptic, thalamic, and pretectal (PTP) areas; (2) midbrain structures, notably the opposite tectal lobe, the torus longitudinalis (TL), nucleus isthmi (NI), torus semicircularis (TS); and (3) downstream structures: the reticular nuclei of the midbrain and brainstem that interface with motor systems. The following sections deal with these structures as part of functional subsystems in which tectum plays a central role.

The Retinotectal Projection and Visual Processing

The Retinotectal Map

Anatomical fiber tracing methods show that the great majority of the optic nerve fibers from one eye cross the midline, enter the opposite lobe of the tectum, and form terminal branches within certain tectal layers where they synapse upon tectal neurons. Optic terminals are densest in the SFGS and SO, with some being found in deeper layers, and are distributed in an orderly fashion over the tectum so as to make a map of the retina. This retinotectal map is organized so that the front, back, top, and bottom of the visual field seen by one eye are represented at the front, back, top, and bottom of the opposite tectal lobe.

The retinotectal map can also be investigated by electrophysiological recordings from tectum. A microelectrode inserted into the superficial layers of tectum (principally SFGS) picks up spikes that are evoked by visual stimulation in a particular region of the visual field of the opposite eye, called the receptive field. The receptive field is usually delineated by flashing small spots of light or moving objects against a contrasting background. **Figure 3(a)** shows the roughly circular receptive fields

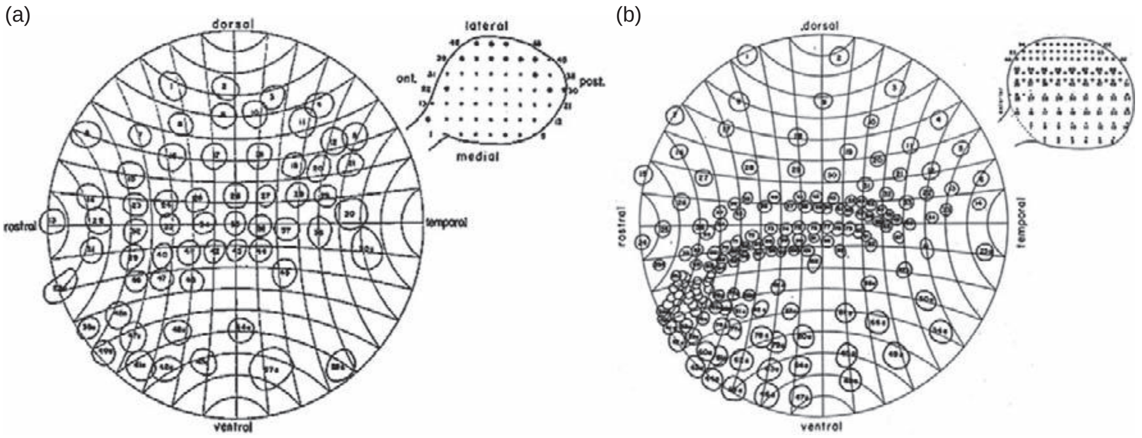


Figure 3 Retinotectal maps: (a) sunfish – uniform retina and (b) spotted sand bass (*Paralabrax maculofasciatus*) – foveate retina. Each large circle represents the visual field of the left eye superimposed with the boundaries of MURFs corresponding to each tectal recording site in the right tectal lobe (insets). Reproduced from Schwassmann HO and Kruger L (1965) Organization of the visual projection upon the optic tectum of some freshwater fish. *Journal of Comparative Neurology* 124: 113–126.

from each of a number of electrode penetrations in the tectum of a sunfish. In this animal, the receptive fields are of fairly similar size and each part of the visual field is represented by a roughly equal area of tectal surface. Thus, the tectum in sunfish, as in goldfish, receives a uniform representation of the entire visual scene. Some species, such as the rock basses, have retinas with a fovea, a region of high photoreceptor and ganglion cell density positioned in the eye for detailed forward vision. In these fish, the part of the retinotectal map corresponding to the fovea exhibits smaller receptive fields and a fivefold magnification of frontal vision compared to the rest of the visual field (see **Figure 3**(**b**)).

Retinotectal Map Development and Plasticity

The formation of the retinotectal map during development is of great interest because it provides a model for how ordered connections develop in brains generally. The retinotectal system of fishes has been a fertile field of research since the 1940s when R.W. Sperry, taking advantage of the discovery that optic nerves in fish and amphibians regenerate when severed, showed that optic axons grow back into the tectum restoring the retinotectal map, along with apparently normal visual behaviors. The chemoaffinity theory that Sperry propounded, held that individual optic fibers are labeled chemically and grow into tectum to terminate on correspondingly labeled tectal neurons. Much research has since been devoted to discovering the molecular and genetic bases for chemoaffinity underlying pathfinding and synapse formation in the retinotectal system. The facility with which optic axons regrow, and the remarkable neural plasticity that their terminals exhibit within tectum is part and parcel of the continual growth that fish undergo throughout life. Indeed, plasticity is essential to maintain a regular retinotectal map in the face of different patterns of growth in retina and tectum – the retina grows by addition of cells at its circumference, whereas the tectum grows by elongation. Of special relevance to this review is that plasticity of the retinotectal projection when experimentally manipulated can provide fresh opportunities to understand tectal function.

Visual Processing in Tectum

In the retinotectal mapping experiments of the kind shown in **Figure 3**, the spiking activity picked up by an extracellular microelectrode in superficial tectum (SO and SFGS) is not generally resolvable into action potentials of constant waveform that would be indicative of the firing of an individual neuron (or single unit), but instead consists of bursts of action potentials of different amplitudes called multiunit activity. It is evoked by stimuli, such as small spots of light flashed on or off, in roughly circular areas (5–10° across in goldfish) termed the multiunit receptive field (MURF). This activity used to be thought to come from the terminals of optic fibers. However, it can be largely eliminated by drugs that block synapses suggesting that it comes from the dendrites of tectal cells where optic fibers make synapses.

Microelectrodes may also pick up action potentials indicative of individual neurons in tectum. If such units are visually responsive, their receptive fields generally differ from the relatively small, retinotopically ordered MURFs of superficial tectum, instead taking a bewildering variety of forms. In the various cyprinid and perciform species investigated, tectal cell receptive fields probed with flashed or moving spots of light vary greatly in size, ranging from roughly circular fields 2–5° across to complex fields extending some 160°. The latter may have elongated or irregular shapes, with multiple subregions with differing stimulus preferences, such as the onset or offset of light. Experiments that scan spots of light across receptive fields have shown spikes being elicited from band-shaped regions of space, suggesting that these cells may be selective for complex shapes, perhaps the body markings of conspecifics. While most tectal cell receptive fields are centered appropriately with respect to the retinotectal map, a high proportion (22% in the perch, *Perca fluviatilis*) are displaced 45° or more, showing that tectal neurons can perform a large-scale integration of visual pattern.

In the foregoing observations, the cell types being recorded from were unidentified. A few studies recording with intracellular micropipettes have allowed the tectal cells to be stained for identification, but only the simplest characterizations of visual trigger properties have been made, adding little to our understanding of processing by tectum. The properties of some of the identified cell types shown in **Figure 2** will be dealt with in subsequent sections on tectal subsystems. In summary, most tectal cell types respond in some way to light flashes or movement, typically with large receptive fields. Tectal cell responses are also complex in the time domain, typically firing to light stimuli with successive periods of excitation and inhibition.

Little can be learned about the processing of location, motion, color, and shape of visual stimuli by making gross lesions of tectum, as these have a profoundly blinding effect. Happily, the plasticity of the retinotectal system allows more subtle interventions to be made, throwing some new light on tectal processing. When optic nerve fibers are severed by crushing, they grow back into the tectum and eventually reform a correctly oriented retinotectal map with nearly normal MURFs. The ability of a sunfish to localize a brief light flash by orienting to it is fully restored, at least where visual space is represented by an orderly retinotectal map; where space is represented by a disorderly map as a result of regenerating optic fibers going astray, orientation movements are misdirected. Map orderliness is also important to pattern processing because after optic nerve regeneration, when optic terminals are disorganized and sparser than normal, fish can discriminate coarse bar patterns, but not the fine patterns that they can normally discriminate.

The ability of goldfish to discriminate colors is also restored, apparently to normal, after optic nerve regeneration but this observation alone does not mean that tectum is involved – the PTP nuclei (see below) also receive regenerated optic fibers that could be responsible. However, recordings of tectal activity strongly suggest that tectum is involved in color processing because its neuronal responses evoked by different wavelengths of light cannot be reproduced by varying the intensity of a single wavelength, as would be the case in a colorblind system. TL (see below), which is closely interconnected with tectum, is such a colorblind system. Moreover, some tectal cells can be excited by one range of wavelengths while being inhibited by another. Such qualitative response differences indicate a color discriminating system. As in mammalian visual cortex, color processing is likely bound up with pattern and shape processing.

Motion is an important stimulus parameter for exciting tectal neurons in all vertebrates studied. In fish, recordings of neuronal activity in tectum have demonstrated directional preferences: activity is evoked most vigorously by a visual stimulus moving in one direction through a receptive field compared to all other directions tested. Most of the data suggest that directional selectivity is elaborated in tectum, although retinal units may exhibit it as well. Visual motion is a valuable source of information assisting the detection and recognition of objects and providing cues to distance, as will be seen in the discussion of the tectum–NI system.

Because the fields of view of the two eyes overlap in the front, some of frontal space is represented in both tectal lobes. A small number of optic fibers serving this part of the visual field, appropriately enough, do not cross over, but terminate in frontal tectum on the same side. This arrangement suggests that the tectum should be able to process images binocularly to help determine the distance of objects such as prey, which fish have been shown to do. Neurons that can be driven via either eye have been found in the tectum of goldfish and trout, but their large and diffuse receptive fields seem unsuited to the demands of stereoscopic depth perception.

From what is known about visual processing in the fish tectum, it seems clear that it does not work in the hierarchical fashion of mammalian visual cortex, where the visual stream ascends from one cortical region to another with a progressive enlargement of receptive fields and a progressive elaboration of trigger features. Structurally, the tectum in fishes is rather uniform across its surface, although some anatomists have seen evidence of a repeating, columnar organization. Pattern processing underlying the complex visual discriminations that fish are capable of most likely depends on tectum, at least in teleosts, because it is the only high-resolution visual center. Evidently, spatial information from the retina, such as object shape and location which is preserved by the orderly topography of the retinotectal map, is immediately broken up in tectum and recoded in ways yet to be deciphered.

Tectum–TL Subsystem

The TL, which occurs only in teleost fishes, consists of a pair of elongated cell masses connected to the medial margins of the tectum, lying just below the intertectal commissure, a thin band of fibers that connects the two tectal lobes (**Figures 4**(**a**) and **4**(**b**)). Large numbers of small neurons in each TL (100 000 in goldfish) send an abundance of fine, unmyelinated axons into the SM, the most superficial layer of the adjacent tectum, where they course in parallel over the tectal lobe (see **Figures 4**(**c**) and **5**). Indeed, SM is filled with these TL axons, called marginal fibers, together with the dendrites of tectal neurons (mainly type I) onto which they synapse. The synaptic contacts made by the marginal fibers may outnumber all other inputs to tectum. The type I neurons, with cell bodies in SFGS, have extensively branched dendritic trees that receive marginal fiber synapses. This arrangement is strongly reminiscent of cerebellum where Purkinje cells receive synapses from the parallel fiber axons of granule cells. Type I cells also receive retinal input on their basal dendrites in SFGS and send a short axon into SGC. A return pathway from tectum to TL is made by type X cells in SGC whose dendrites ramify at the SFGS/SGC boundary and in central SGC where the type I axons also terminate.

Anatomical studies in various species show that TL is composed of two main divisions which are called here dorsomedial and ventrolateral that have different connectivity and function. Electrophysiological studies have confirmed the existence of a precisely ordered loop between dorsomedial TL, tectum, and back again, and have also shown that dorsomedial TL deals with a specific kind of visual information. A microelectrode inserted into dorsomedial TL picks up sustained firing activity that is related in an inverse way to the illumination of the opposite eye, such that activity is high in the dark and is reduced by illumination. This sustained dimming activity also can be evoked by dark objects. It shows a clear retinotopic character so that an electrode in the front of TL picks up activity to dimming in the front of the visual field; positions farther back in TL have dimming receptive fields farther back in the visual field (see **Figures 4**(**c**) and **4**(**d**)). Dorsomedial TL responds to dimming with a considerably longer latency than does superficial tectum.

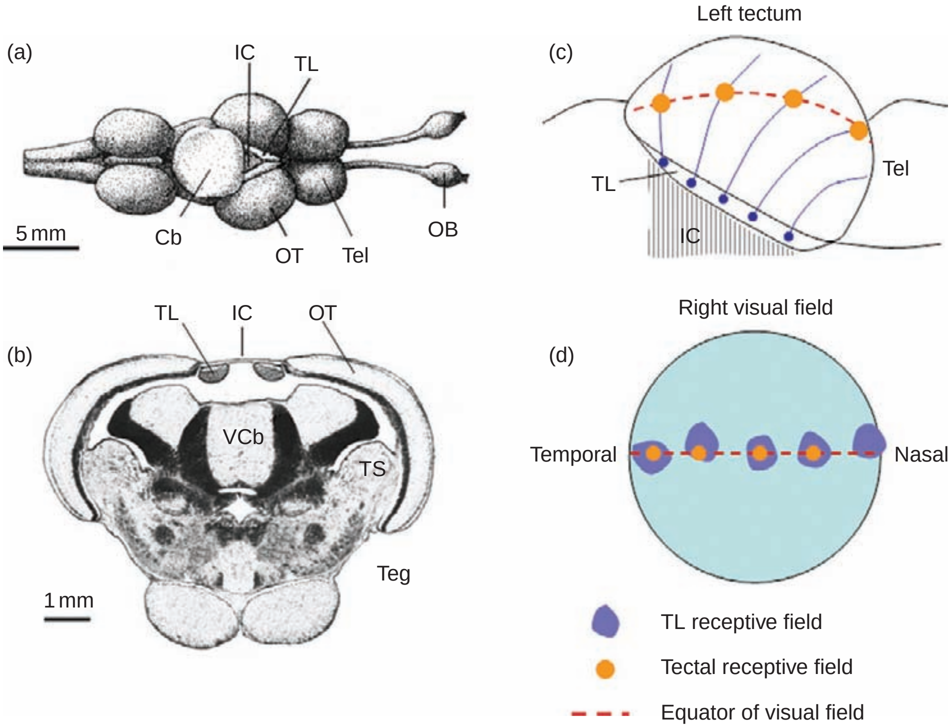


Figure 4 (a) Dorsal view of goldfish brain. (b) Goldfish brain cross section. (c) Left tectum and TL showing paths of marginal fibers from five points in TL. Dashed curve on tectum represents visual field equator. (d) Right visual field showing equator (dashed line), tectal MURFs (small circles), TL receptive fields (larger areas). Cb, cerebellum; IC, intertectal commissure; OB, olfactory bulb; OT, optic tectum; Teg, tegmentum; Tel, telencephalon; TL, torus longitudinalis; TS, torus semicircularis; VCb, valvula of the cerebellum.
(a) Reproduced by M.A. Gibbs. (b) Adapted from Meek (1983) Functional anatomy of the tectum mesencephali of the goldfish. *Brain Research Reviews* 6: 247–297.

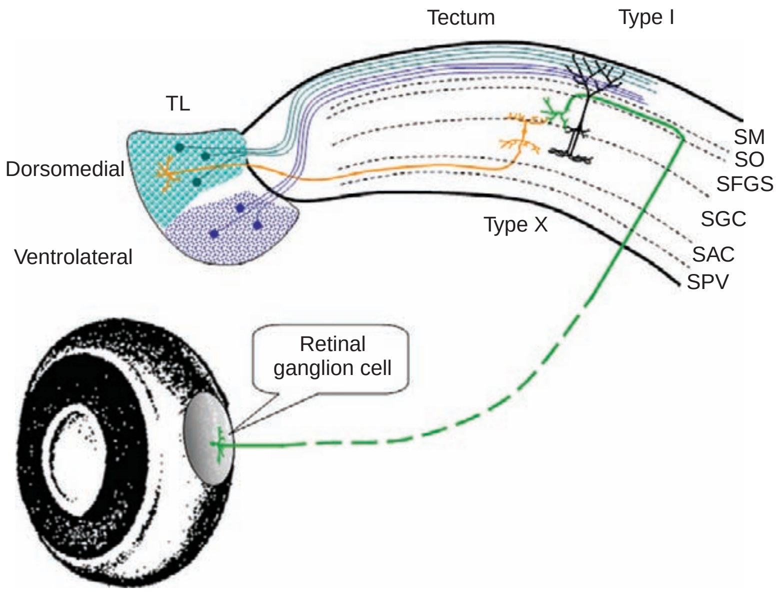


Figure 5 Tectum–TL circuitry, showing a cross section through one TL and part of adjacent tectum. A retinal ganglion cell transmits to the main visual layer of tectum, SFGS. A type X cell receives visual signals and relays to the dorsomedial TL, which transmits luminance information over tectum in SM. This is received by the dendrites of type I neurons, which are also activated by retinal input. Corollary discharge of eye movements is relayed via the ventrolateral TL in SM and also received by type I dendrites. SM, stratum marginale; SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; and SPV, stratum periventriculare.

Electrical stimulation of a point in TL sends impulses along a beam of marginal fibers across tectum (see **Figure 4**(**c**)). This beam passes over a point in SFGS with a MURF that overlaps the receptive field of the TL point that was stimulated (**Figure 4**(**d**)). To complete the loop from tectum back to TL, type X cells connect topographically to dorsomedial TL. It is noteworthy that this tectum–dorsomedial–TL loop appears to be concerned only with dimming and with retinal location of a light stimulus; it is totally unconcerned with stimulus color, shape, or motion. That dimming is a separate visual submodality is underlined by the existence of a class of dimming retinal ganglion cells. Moreover, when the optic nerve regenerates after being cut in goldfish, dimming responses are the first to reappear in tectum and simultaneously in TL, followed significantly later by other kinds of visual responding (e.g., light onset).

Microelectrode recordings from ventrolateral TL yield distinctly different neural signals: bursts of firing that are synchronized with saccadic eye movements. There is evidence that TL is signaling the direction and extent of every eye movement, and presumably relaying this information to tectum via a different and deeper set of marginal fibers than those carrying the dimming signal from dorsomedial TL. These saccadic bursts are corollary discharge signals, meaning that they derive from eye movement commands; they are not actual eye movement commands because ablation of TL has no effect on the eye movements that a fish makes spontaneously. The source of the saccadic information is not tectum, but other inputs to TL, possibly nuclei in the midbrain near the midline where oculomotor nuclei reside, or nuclei connected with the cerebellum.

The role of the TL–tectum loop can only be guessed at. Comparative studies suggest that TL is highly developed in species that live in turbulent environments. One such species, the squirrel fish (*Holocentrus ascensionis*), has a marginal layer that makes up nearly half the thickness of the tectum. It lives in and around coral reefs, moving between brightly lighted and dim recesses in surging water. The TL–tectal loop may be involved in adjusting the visual processing of tectum under these circumstances.

A further clue to function is the association of marginal fibers with the type I cells, an arrangement that strikingly resembles parallel fiber systems elsewhere in the central nervous system, notably the cerebellum, and the electrosensory lobe of mormyrid electric fish. In the latter case, the neural circuitry learns to predict the consequences of the fish's own movements on the electroreceptor signals and enables the brain to discount them, perhaps thereby improving the detection of objects in the environment using its electric sense. The TL–tectal system may be doing something similar for vision according to the following scenario. Neural activity in dorsomedial TL can be thought of as a picture of the environment seen through the opposite eye. This picture is monochromatic; it is without motion enhancement; it overrepresents the visual horizon and is transmitted continuously, but with some delay, via the marginal fibers over the tectum. When the fish makes an eye movement, a corollary discharge is generated along the length of the ventrolateral TL and transmitted across tectum via the deep marginal fibers. At the same time, the eye movement shifts the luminance distribution in the scene across the retina, which, in turn, shifts the activity pattern within the main retinal termination layer, the SFGS. This activity change is picked up by the dendrites in SFGS of type I cells which fire in response; simultaneously, the dendrites of type I cells in SM are excited by a pattern of (1) marginal fiber activity representing the visual scene before eye movement and (2) the corollary discharge representing eye movement direction and amplitude. Learning takes place by strengthening and/or weakening of the marginal fiber synapses so that after a period of experience, the activity of all the type I cells constitutes a prediction of how the scene will look after the eye movement, based on what it looked like before. Subtraction of the predicted pattern from the current pattern would highlight changes in the scene that are not self-produced, and are therefore significant.

Tectum–NI Subsystem

A cross section through the brain of a teleost fish, just in front of the corpus of the cerebellum, reveals a cell mass below the TS, surrounded by fibers exiting the tectum. This is the NI. It is well developed in highly visual fish, but is still present even in blind cavefish (*Astyanax mexicanus*); however, it has not been found in elasmobranchs. It consists of a shell of tightly packed neurons surrounding a core containing dendrites, incoming fibers, and synapses (see **Figure 6**). The incoming fibers are mainly from the tectal lobe on the same side and make excitatory synapses within the core on the dendrites of the neurons whose cell bodies lie in the shell. The latter send their axons back to tectum on the same side. Anatomical tracer studies have shown that tectal neurons project to NI in topographic fashion, and that NI neurons project back to tectum topographically.

Introducing a microelectrode into NI immediately confirms that tectal input confers visual responsiveness upon NI. Extracellular microelectrodes pick up bursts of high-amplitude spiking activity that occur spontaneously or are evoked by visual stimuli shown to the opposite eye. Flashed spots of light can evoke spike burst responses but they habituate with repeated presentation. Movement is a much more effective stimulus, but its effects also habituate. NI, therefore, is excited by change or novelty. It is

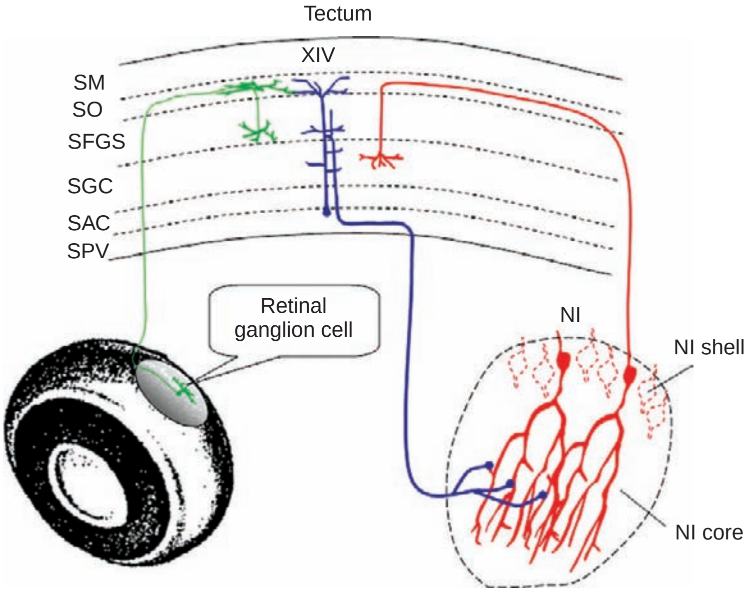


Figure 6 Tectum–NI circuitry, showing a cross section through tectum and the nucleus isthmi (NI). A retinal ganglion cell transmits to the visual layers of tectum, SO and SFGS. A type XIV cell receives visual input and relays to the core of NI. Neurons in the shell of NI send axons back to tectum, primarily SGC. SM, stratum marginale; SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; and SPV, stratum periventriculare.

interesting that although the anatomical connections from tectum to NI are topographically organized, the visual responses are not: visual stimuli anywhere in the field of the opposite eye can elicit NI bursts.

The movements that especially excite NI in goldfish and sunfish are approaching objects. A checkered ball stimulus that approaches the fish evokes bursts of activity that steadily increase in vigor. When the ball stops, the activity drops off, and when it moves away, there is a brief burst of activity followed by relative silence. Averaging over several approach trials reveals a ramp-up in neural activity; faster approach gives a correspondingly faster ramp-up, suggesting that NI activity signals the closeness of the ball. The ramp-up is somewhat independent of the size of the ball, showing that the response is not simply a function of retinal image size. On the face of it, the NI-tectum circuit appears to be signaling the closeness of objects derived from monocular motion cues. NI also responds to stimulation via nonvisual stimuli (somatosensory, auditory, and lateral line), modalities that tectum also processes.

The neural circuitry of the NI–tectum subsystem involves the type XIV cells of tectum, the source of tectal input to NI (see **Figure 6**). These complex neurons which are the most numerous of the tectal cell types have cell bodies in the SPV and a long dendritic trunk that ascends to the superficial layers (SO and SFGS) where they detect retinal input. They also have dendritic branches that ramify in deeper layers (SGC), probably picking up nonvisual inputs that arrive there. The type XIV output axons originate at the SFGS/SGC boundary and then split, with one axonal branch running upward, eventually to a nucleus of the pretectal area, the other axonal branch running downward, branching again to make terminals in SGC, and then leaving tectum to make excitatory synapses on the dendrites of NI cells in the core of the nucleus. The loop is completed by the NI neurons that send axons back into the SGC of tectum.

Two factors seem to be responsible for the complete absence of any topography in the visual responses of NI, despite anatomical evidence to the contrary. The first is the likely existence of electrical synapses within the core of NI, providing low-resistance pathways between neighboring NI neurons that tend to synchronize activity across the nucleus. Experimentally, such synchronization is observed: two well-spaced electrodes within NI pick up almost identical signals, showing that NI behaves as an integrated unit. The second factor is a layer of possible connectivity between type XIV cells in the upper SGC formed by type XIV dendrites and type XIV axon branches that seem to be arranged to spread activity laterally across tectum.

The feedback from NI to tectum has interesting effects on activity in deep tectum, the significance of which is still unclear. Spiking activity recorded extracellulary at any one point in SGC correlates highly with NI spiking; it also correlates highly with any other point in SGC because of the connectivities just discussed that nullify the topography. Even more remarkable is that when NI and SGC activity is correlated, as occurs in bouts of several hundreds of milliseconds, NI and SGC spikes synchronize together within a fraction of a millisecond.

While the relationship of this synchrony to stimulation or to behavior is unknown, it appears that precise timing of neural events is critical to processing in deep tectum, whereas precise spatial topography is not.

The function of the tectum–NI loop is still a matter of surmise. The fact that NI's responses, which are evoked by novelty, motion, and especially approaching objects, are broadcast throughout tectum, suggests an alerting or attentional role. This is supported by experiments showing that the response of one NI to stimuli seen by the opposite eye can be reduced or distracted by a more salient stimulus presented to the other eye. There is evidence in fish and frogs that NI may modulate optic nerve input in the upper layers of tectum, accentuating the response to visual stimuli. In birds, too, NI has been proposed to play an attentional role. In teleosts, NI's influence is strongly manifest throughout deep tectum, perhaps allowing an assessment of particularly salient events by means of a comparison of NI signals with all current sensory representations in tectum, whatever their sensory modality, and selecting one as an object for attention or behavioral response.

Multimodal Processing and Torus Semicircularis

When the optic tectum is peeled away, one sees lying along the midline, the valvula of the cerebellum, a tongue-like protrusion of the cerebellum, and flanking it, two crescent-shaped bodies, the TS (see **Figure** 4(**b**)). Its corresponding structure in mammals is the inferior colliculus, an auditory center. On anatomic grounds, TS is another potentially important partner with the optic tectum, having reciprocal connections with it. Each TS sends axons to both tectal lobes, but each tectal lobe sends axons to TS only on the same side. TS is a large nucleus, but only a relatively small proportion of TS cells provide input to the deep layers (SGC, SAC, and SPV) of tectum. TS has a diverse set of inputs, some of which are sensory in nature: auditory, lateral line, somatosensory. In electric fishes (gymnotids and mormyrids), TS is large and well differentiated into lamina or subnuclei to process the information provided by the electrosensory receptors about surrounding objects and the electric signals of conspecifics. Other inputs to TS come from the cerebellum, the forebrain, and the opposite TS. Inputs to TS from the optic tectum arise on the same side from type XII and type XIII cells whose cell bodies lie in the deep layers (SGC, SAC).

Microelectrode recordings in TS reveal neuronal responses representing its various sensory inputs. Many neurons in TS of goldfish and trout are visually responsive, presumably as a result of tectal input. Indeed, the response characteristics of these neurons – very large receptive fields; unreliable responses to light flashes; preference for motion but without directional preference; and a propensity to habituate – reflect the visual responses recorded in deep tectum that are so closely correlated with NI activity. Other TS neurons are responsive to acoustic and/or lateral line stimulation, and some combine this acousticolateral responsiveness with the kind of visual responsiveness just described. In such bimodal neurons, the two modalities can combine in different ways – in some, responding to stimuli of either modality, in others to both in an additive fashion; sometimes the interaction is nonlinear, requiring that both stimulus modalities be present for a response. The feedback pathway from TS to tectum is therefore a conduit for introducing multimodal responsivity into deep tectal circuits. So far, there is very little physiological data on multimodal processing in tectum of fishes, although it has been studied in amphibians where visual and lateral line inputs combine to activate deep tectum. The benefit of combining different modalities within tectum may be that events and objects such as prey or conspecifics can be more easily detected and more rapidly responded to, if the mappings of the different modalities overlap in tectum allowing them access to common motor mechanisms.

TS in teleost fishes, in its role as auditory center, contains directionally selective neurons that respond to underwater sound vibrating in a particular direction. TS also contains neurons that respond to the much lower frequencies of water movement that stimulate the lateral line organs. There is a rough topography in TS so that an object causing a water disturbance near the animal will activate a population of TS cells that is specific to the location of the object. In fishes that sense objects such as prey through the small electric fields that they generate, different populations of TS cells will be activated by different positions of the field source. Finally, somatosensory signals that convey touch and pain arrive from the spinal cord, and also go directly to tectum. In each of these modalities, TS elaborates and refines differential sensitivities to the important stimulus dimensions (frequency, direction, position, etc.), relaying to the deep layers of the tectum on both sides, and potentially providing information on the whereabouts of events or objects. When these objects can be simultaneously visualized, there will be correlations between the visually evoked activation in tectum and the TS signals. Plasticity of the TS connections in tectum could then generate a topographic map that is in register with the visual map, which is what is found in other vertebrate groups, but yet to be properly demonstrated in fish tectum.

Ascending Connections – via PTP

Between the optic tectum and the forebrain are a number of nuclei forming the brain regions called the PTP (see **Figure** 7). It is inappropriate to detail all of these

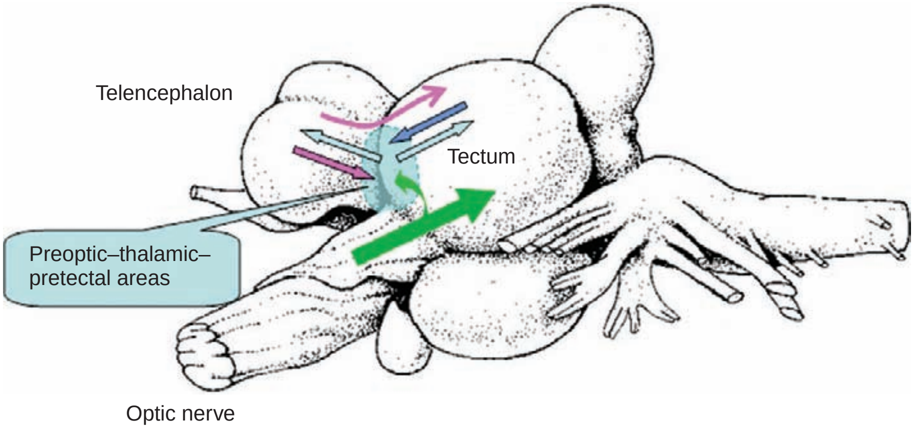


Figure 7 Teleost brain showing the preoptic–thalamic–pretectal (PTP) areas forming an interface between retina, tectum, and telencephalon. Adapted from S. Karger AG, Basel from Wullimann MF and Northcutt RG (1988) Connections of the corpus cerebelli in the green sunfish and the common goldfish: A comparison of perciform and cypriniform teleosts. *Brain Behaviour and Evolution* 32: 293–316.

here (there are 10 or more), especially as there is considerable variation in nuclear composition of the region between species, and in the nomenclature used to describe them. The importance of this region for present purposes is that some nuclei receive direct retinal input, some relay visual information on to the telencephalon or on to the tectum. In addition, some of the nuclei receive tectal input and relay to the telencephalon, many with reciprocal connections back from tectum and telencephalon. The region can therefore be seen as a way station for retinal information traveling to telencephalon, and for tectally processed information, probably multimodal, traveling to telencephalon. Finally, telencephalic regions provide an input to tectum, both directly and via the PTP. The direct telencephalic input is concentrated in SO and in the middle of the SGC of tectum where it could contact the dendritic branches of intrinsic neurons such as the type I, as well as several types with output axons (X, XII, XIII, XIV) (see **Figure 2**).

The fact that our own sensory abilities of recognition and localization are performed by telencephalic cortical areas raises questions about the relative importance of the telencephalon versus the tectum in fishes. In teleost fishes, the ablation of the entire telencephalon has little effect on behavior, at least to the casual observer. Goldfish, for example, after telencephalic ablation successfully identify prey objects and pursue them, so their vision is not obviously impaired. However, after total tectal ablation, they appear completely blind, indeed more disabled than eyeless fish because their use of nonvisual senses for navigating their environment is also impaired.

The very few physiological studies on the PTP nuclei in teleosts show that most of the visually responsive neurons have large receptive fields. Some PTP nuclei do have small receptive fields but contain relatively few cells and these nuclei possess nothing like the detailed retinotopic map that tectum boasts. Evidently, the extratectal pathways via PTP to telencephalon lack the necessary bandwidth for detailed vision and may lack access to motor systems for sensorimotor behavior. The sensory information sent to telencephalon by tectum is probably important for the higher-order tasks that telencephalon performs, such as spatial place learning or the recognition of conspecifics and their displays. This tectally processed information is likely to be highly abstracted, rather than a detailed point-by-point representation of the scene, given what we know about the diffuse character of deep tectal activity, the source of telencephalic input.

The situation in elasmobranchs may well be different. It has been reported that sharks after tectal ablation are still capable of behaviorally discriminating between visually presented shapes. There are also other indicators of the relatively greater importance of telencephalon versus tectum in elasmobranchs. Species of shark that depend heavily on vision, as judged by their mode of hunting in well-lit habitats, have tilted their brain development more toward telencephalon than tectum; highly visual teleosts, on the other hand, seem to have done the reverse.

Descending Connections and Motor Control

The behaviors that depend upon tectum are initiated or guided by sensory input and require a fair degree of spatial resolution. These behaviors include food and prey seeking, navigating around obstacles in the environment, avoidance of approaching objects, schooling, and the ability to swim with a moving textured background (the optomotor response). The tectal outputs mediating these behaviors stem from neurons such as the type XIII with cell bodies in the SGC and dendrites distributed in SFGS and SGC. These neurons send their axons down into the reticular formation of the midbrain and brainstem on both sides, making synapses upon cell groups that activate motoneurons, either in the oculomotor nuclei for eye movements, or in the spinal cord for body movements.

In the absence of direct recordings of the tectal output neurons, the transformations that take place between topographic sensory mappings and muscle movements can only be inferred, mostly from experiments involving electrical stimulation. Trains of electrical shocks evoke behavioral responses most easily in the deep layers of tectum (SGC, SAC). Despite the artificiality of electrical stimulation delivered to a point in tectum, the behaviors elicited in a free-swimming fish may look natural, coordinated, and even purposeful. Stimulation of rostral tectum causes convergence of the eyes and forward swimming in a pattern resembling food searching. Stimulation at sites farther back produce contraversive turning (opposite to the side of stimulation) of eyes and body, similar to a strike at a fly cast, and at high stimulus intensities, movements resembling escape.

By electrically stimulating the tectum of restrained goldfish and measuring the evoked eye movements, it has been possible to draw a detailed vector map showing the direction and amplitude of eye movements evoked from different regions of one tectal lobe (**Figure 8**). The largest, medial zone of tectum elicits movements of both eyes in a contraversive direction. As the stimulation point is moved toward the midline, a greater vertical eye movement component appears; as it is moved caudally, a greater horizontal component appears. This is what would be expected if stimulation is directing movement toward corresponding points in the retinotectal map, where medial tectal positions represent upper field and caudal tectal positions represent caudal field. Stimulation in the posterior zone gives eye movements that are almost entirely horizontal; in the anteromedial zone eye movements do not follow a fixed vector but move toward a goal in frontal space, and in the most anterior region, the two eyes converge inward. In should be pointed out that because goldfish, like most other fishes, do not possess a retinal fovea, these eye movements are not fixating in the sense that our eyes do when scrutinizing something, but are part of a coordinated eye–body movement that orients the whole fish.

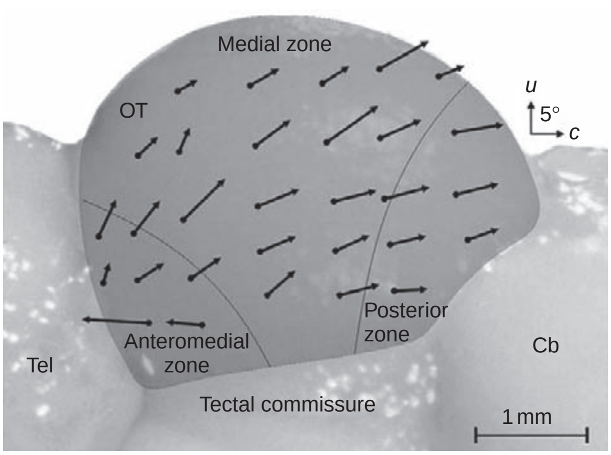


Figure 8 Eye movement vector map on right tectal lobe in goldfish. Arrows show the direction of eye movements evoked by electrical stimulation of different points in tectum. Tel, telencephalon; OT, tectum; Cb, cerebellum. Reproduced from Salas *et al.* (1997) *Neuroscience* 78: 271–288.

While these experiments show conclusively that it is the place of tectal stimulation that determines the direction of the fish's orientation and motion, it is not understood how place might be translated into an intensive neural code, that is, one that specifies the force and duration of muscular contractions. The tectal output fibers do not project to the reticular nuclei with any obvious topography, except that rostral tectal fibers terminate preferentially in rostral reticular areas of the midbrain, a region that seems to contain vertical eye movement generators, while caudal tectal fibers terminate in caudal reticular areas containing horizontal movement generators.

The translation from place to intensive coding appears to be modifiable, perhaps through experience. This is shown by experiments involving plastic rewiring of the retinotectal system. When the optic nerve is crushed and allowed to regenerate into a tectal lobe whose caudal half has been removed, the visual field eventually comes to be represented in compressed form on the remaining rostral half. Sunfish after such surgical rearrangement oriented with normal accuracy to flashed lights in the parts of the visual field that were represented in a compressed retinotectal map. Accurate orienting could also be performed with an expanded retinotectal map produced by removing half the retina and allowing the remaining half to spread its connections across an intact tectal lobe. Evidently, a vector map like that in **Figure 8** is not fixed in the tectum but can be expanded or compressed in compensatory fashion, perhaps by changing the gain of the intensive code. A continual adjustment of this kind through trial-and-error experience would be valuable for keeping prey catching and other motor behaviors well calibrated to the position of objects in space.

The tectum in fishes, as in other vertebrate groups, also plays a part in initiating avoidance and escape behaviors. Moving shadows, especially overhead, and looming objects elicit flight reactions that can be abolished by tectal lesions. Similar defensive reactions can be produced by strong electrical stimulation of tectum. Visual looming stimuli, detected by tectum, probably enabled by NI, activate the giant Mauthner cells in the brainstem that trigger a very rapid and vigorous body flexion, moving the fish out of harm's way.

Conclusions

The basic function of tectum in fishes, as in most other vertebrates groups, is to form moment-to-moment representations of the immediate surroundings, using primarily the visual modality, with back-up from the somatosensory, auditory, lateral line, and electrosensory modalities. These body-centered representations – as distinct from the world-centered (allocentric) representations that telencephalon develops – connect with motor centers, enabling fish to react swiftly in an appropriate direction, whether it be in prey capture, avoidance of objects or predators, or in complex social maneuvers. Interactions among tectum, its satellite nuclei, PTP, and telencephalon help pick out salient objects or events for a possible response. Retinal input provides a detailed and panoramic image of the surroundings to the superficial layers of tectum, making tectum a likely site for pattern recognition. There are units in tectum with oddly shaped receptive fields that may represent selectivity for important image shapes, but no evidence so far of a systematic parsing of features in a hierarchy of specialized regions such as is found in mammalian visual cortex. Virtually nothing is known of the learning capabilities of tectum in fishes, although it has the requisite machinery such as long-term potentiation of synaptic strengths and *N*-methyl-D-aspartic acid (NMDA) receptors. It does, however, demonstrate plasticity of its retinal input connections and possibly its motor output connections, which may be vital for keeping sensorimotor functions in good calibration, especially as the bodies and brains of fishes continue to grow throughout life.

See also: **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Motor Control Systems of Fish; Physiology of the Mauthner Cell: Discovery and Properties. **Detection and Generation of Electric Signals**: Active Electrolocation. **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy; Lateral Line Neuroethology; Sound Source Localization and Directional Hearing in Fishes. **Sensory Systems, Perception, and Learning**: Fish Learning and Memory; Spatial Orientation: How do Fish Find their Way Around?. **Vision**: Behavioral Assessment of the Visual Capabilities of Fish; Inner Retina and Ganglion Cells.

Further Reading

Calvert GA, Spence C, and Stein BE (2004) *The Handbook of Multisensory Processes*. Cambridge, MA: MIT Press.

Dean P, Redgrave P, and Westby GWM (1989) Event or emergency? Two response systems in the mammalian superior colliculus. *Trends in Neuroscience* 12: 137–147.

Debski EA and Cline HT (2002) Activity-dependent mapping in the retinotectal projection. *Current Opinion in Neurobiology* 12: 93–99.

Demski LS and Beaver JA (2001) Brain and cognitive function in teleost fishes. In: Roth G and Wullimann MF (eds.) *Brain Evolution and Cognition*, pp. 297–332. New York: Wiley.

Douglas R and Djamgoz M (eds.) (1990) *The Visual System of Fish*. London: Chapman and Hall.

Gallagher SP and Northmore DPM (2006) Responses of the teleostean nucleus isthmi to looming objects and other moving stimuli. *Visual Neuroscience* 23: 209–219.

Gruberg E, Dudkin E, Wang Y, *et al.* (2006) Influencing and interpreting visual input: The role of a visual feedback system. *Journal of Neuroscience* 26: 10368–10371.

Hueter RE (1991) Adaptations for spatial vision in sharks. *Journal of Experimental Zoology* 5: S130–S141.

Kinoshita M and Ito E (2006) Roles of periventricular neurons in retinotectal transmission in the optic tectum. *Progress in Neurobiology* 79: 112–121.

Lisney TJ and Collin SP (2006) Brain morphology in large pelagic fishes: A comparison between sharks and teleosts. *Journal of Fish Biology* 68: 532–554.

Meek J (1983) Functional anatomy of the tectum mesencephali of the goldfish. *Brain Research Reviews* 6: 247–297.

Northcutt RG (2002) Understanding vertebrate brain evolution. *Integrative and Comparative Biology* 42: 743–756.

Northmore DPM (1984) Visual and saccadic activity in the goldfish torus longitudinalis. *Journal of Comparative Physiology A* 155: 333–340.

Rodríguez F, Broglio C, Durán E, Gómez A, and Salas C (2006) Neural mechanisms of learning in teleost fish. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behavior*, pp. 243–277. Oxford: Blackwell.

Sawtell NB and Bell CC (2008) Adaptive processing in electrosensory systems: Links to cerebellar plasticity and learning. *Journal of Physiology – Paris* 102: 223–232.

Schwassmann HO and Kruger L (1965) Organization of the visual projection upon the optic tectum of some freshwater fish. *Journal of Comparative Neurology* 124: 113–126.

Udin SB and Fawcett FW (1988) Formation of topographic maps. *Annual Review of Neuroscience* 11: 289–327.

Vanegas H (ed.) (1984) *Comparative Neurology of the Optic Tectum*. New York: Plenum.

Vanegas H and Ito H (1983) Morphological aspects of the teleostean visual system: A review. *Brain Research Reviews* 6: 117–137.

Yamamoto N, Kinoshita M, and Xue H-G (2007) The nucleus isthmi of teleosts. In: Watanabe S and Hofman MA (eds.) *Integration of Comparative Neuroanatomy and Cognition*, pp. 155–178. Tokyo: Keio University Press.

Behavioral Assessment of the Visual Capabilities of Fish

S Schuster, P Machnik, and W Schulze, University of Bayreuth, Bayreuth, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Recognizing Objects
Detecting Color
Spatial Vision
Detecting and Analyzing Movement
Detecting Polarized Light
Conclusion
Further Reading

Glossary

Color constancy The capability to assign a unique color to an object, regardless of the spectrum of the illuminating light.

Dorsal light reflex A response to light in which fish try to tilt so as to keep the sun upright.

Heart rate conditioning If an electric shock that slows down heart rate is repeatedly paired with a stimulus, the stimulus alone will slow down heart rate.

Optokinetic nystagmus When the eyeball rotates to track a moving extended pattern.

Optomotor response When an extended visual pattern moves, animals often reduce retinal image motion by moving themselves.

Size constancy Assigning the absolute size of an object, irrespective of its distance from the eye.

Template matching A simple recognition strategy that lacks viewpoint invariance.

Two-alternative forced choice A paradigm in which the animal unequivocally selects between two options.

Introduction

With more than 30 000 species inhabiting nearly every aquatic environment on earth, fish provide a rich source for studying fundamental aspects of visual processing (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals and **Vision**: Vision in Fishes: An Introduction). Many fish show highly sophisticated visual behaviors that often allow the analysis of unique visual specializations. Furthermore, most fish can readily be trained. This was masterly used to dismount the claim that lower vertebrates and invertebrates could not see color. Interestingly, later Nobel laureate Karl von Frisch first succeeded to show this in fish, before his famous work on bees. In this article, we will illustrate a number of behavioral approaches that have been used successfully to tackle most aspects of fish vision. We hope that our brief survey is both stimulating and useful for scientists wishing to study the visual capabilities of their particular species.

Many methods can simply be adapted to study vision in the species that have not yet entered the laboratories and are certain to hold great discoveries in store. Some of the methods can easily be adapted to any species of fish and even to their larvae. Others require sophisticated learning capabilities of the fish and even more so of the investigator. Sometimes methods can be tailored to particular species, elegantly making use of sophisticated naturally occurring behavior that relies on vision. Here, we present these methods on the way through a brief overview of the visual capabilities of fish. We are well aware of the many excellent accounts on visual behavior and visual capabilities of fish and have therefore focused on a few examples and more recent discoveries.

Recognizing Objects

The first studies on learning in fish addressed their ability to recognize visual objects. Two-alternative forced-choice tasks were most commonly used. In these, the fish views two different objects (or two-dimensional images of objects) and has to decide which object to approach. Often, the objects are placed outside the tank to provide no other than visual cues for the task. Sometimes a divider between the possible paths is used to really force the choice (see e.g., **Figure 1(a)**). The approach to one of the two objects is rewarded. Many species of fish have been trained this way, providing a great wealth of data not only on the spatial resolution and representation of shape and color, but also on the way fish represent more abstract features such as the texture of a

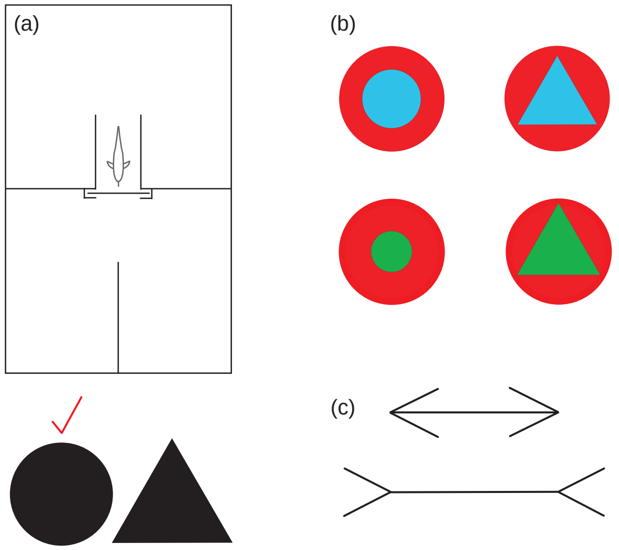


Figure 1 Two-alternative forced-choice tasks can be used to study visual pattern recognition. (a) Setup (top view) used to train the electric fish *Gnathonemus* to select one of the two visual patterns – in this case a circle – from a fixed vantage point. The fish views the patterns from its shelter and is rewarded upon moving to the circle. (b) Evidence suggesting simple template matching in *Gnathonemus*. Although successfully trained to a circle, fish chose both figures (top pair, blue) when they equally matched a putative snapshot (red) taken of the training circle. A triangle was preferred when it better matched the putative snapshot (bottom pair, green). (c) Several fish share the Müller–Lyer illusion with human observers. Though both horizontal lines are of equal length, the lower one is considered longer.

pattern or the number of elements in a pattern (e.g., the number of vertical lines). Some earlier studies even attempted to demonstrate a representation of more abstract topological features such as the closeness of a line.

While fish certainly have highly sophisticated capabilities to represent visual patterns, it is always important to keep Ockham's razor sharp and to look for the simplest explanation. A study on pattern recognition in a nocturnal electric fish, *Gnathonemus petersii*, reminds us that fish can discriminate shapes without necessarily using a concept of shape. In a typical two-alternative forced-choice arrangement, *Gnathonemus* viewed two visual patterns, a set distance away, through a transparent screen from its daytime shelter. When the screen was lifted, the fish swam to one of the patterns to receive a reward or to be driven back into the shelter (**Figure 1(a)**). It was possible to train the fish to discriminate visual patterns but specifically the visual (as opposed to e.g., electrosensory) training worked only at low ambient light intensities of about 10 lx and when the patterns subtended angles larger than about 3°. After the fish were able to discriminate visual patterns, transfer tests were run in which the training patterns were replaced by other patterns (**Figure 1(b)**). This way the features could be identified that the fish had used to discriminate the patterns. Surprisingly, the results of such tests suggested an extremely simple way of storing the rewarded pattern: template matching. In this strategy (whose use is much better documented in insects), the fish stores the view of the rewarded pattern from its fixed vantage point and then chooses that one of the two other patterns whose retinal appearance best matches the stored view. These experiments may illustrate the power of a forced-choice arrangement: with fixing the decision point and suitably arranging transfer tests, much can be learned about the way and accuracy in which fish represent visual patterns.

Nonetheless, most fish use much more sophisticated visual representations and seem even to share certain visual illusions with us. The Müller–Lyer illusion, for instance, has been repeatedly demonstrated by Herter in the 1930s and the 1950s in minnows (*Phoxinus phoxinus*), sunfish (*Lepomis* sp.), and in the Siamese fighting fish (*Betta splendens*). He trained fish, for instance, to select the longer of the two stripes and then tested them with a Müller–Lyer pair of figures that were of equal length but appear to have different lengths for a human observer (**Figure 1(c)**). Herter ran two important controls to support his conclusion: in one the total lengths were equal, whereas in the other the lines were just changed in length so as to make the figures appear of equal length to a human observer. The outcome fully supports the equivalence of humans and fish as far as the Müller–Lyer illusion is concerned. It will be a very interesting future research direction to further probe the degree to which the visual system of fish parallels human performance. To our knowledge no work has specifically been done to disclose the number of images that can be stored over extended periods of time. Early work on acoustically trained fish in the laboratory of

Karl von Frisch suggests that acoustical memories last up to 1 year. Perhaps the visual system of some fish parallels that of the primates also in terms of the number of objects that can be stored during the lifetime of these fish.

Detecting Color

Spectral Sensitivity

The range of wavelengths, that stimulate the eye, can be probed, in principle, by testing which wavelength composition is capable of eliciting a particular visually mediated behavior (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments). An interesting behavior that has been used is the dorsal light reaction of most teleosts (shared also by other swimming or flying animals) – a reflexive tendency to tilt about the longitudinal axis to keep the sun directly overhead. **Figure 2**(**a**) shows how it can be employed as a typical null method in which the animal is made to compare two stimuli. The fish receives light from two sources. One provides reference light, which is kept constant, whereas the other provides a probing light of known spectral range and intensity. This probing light is adjusted in intensity such as to balance the fish: if the probing light is too strong, the fish will tilt its dorsal part toward the probing light; if it is too weak, it will tilt to the reference light. This simple and clever method avoids a large number of problems one would run into if spectral efficiency were measured with only one light source and the angle of tilt being the measure of sensitivity. This approach is instructive as an example of how easy it can be to interrogate a fish about matching stimuli.

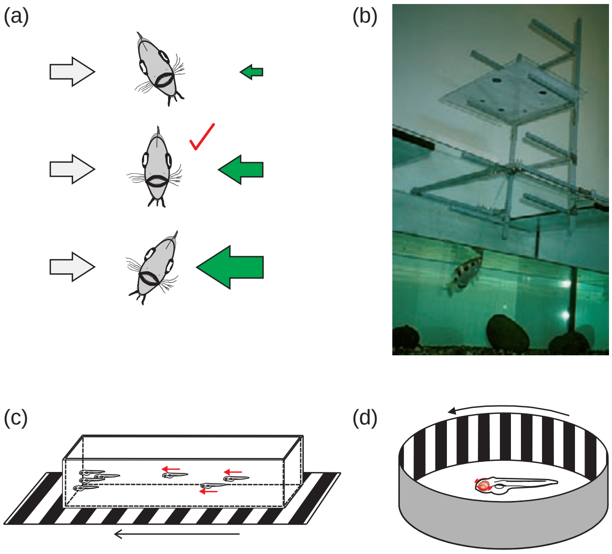


Figure 2 Making use of spontaneously occurring behaviors. (a) Many fish show a dorsal light reflex in which they partly turn their dorsal side toward the light. By shining reference light (constant, gray) from one side and probing monochromatic light (intensity varied, green) of wavelength λ from the other, it is possible to use this response to make the fish tell when both the light stimuli are equal. (b) Size constancy, and how it is acquired, can be analyzed using the natural shooting behavior of archerfish that requires no coupling between reward and target. The fish receives a reward only upon selecting, regardless of presentation height and its own horizontal distance from the target, a disk of fixed absolute size. (c) A way in which the optomotor response can be used for screening zebrafish larvae for visual defects. When a stripe pattern moves on the ground of the tank, fish with intact vision (and locomotion) follow it and can be collected on one side. (d) The optokinetic nystagmus (OKN) has profitably been used in zebrafish larvae. A larva is embedded in agar and follows the movement of a grating with its eyes. (b) Adapted from Fig. 1 in Schuster S, Rossel S, Schmidtmann A, Jäger I, and Poralla J (2004) Archer fish learn to compensate complex optical distortions to determine the absolute size of their aerial prey. *Current Biology* 14: 1565–1568.

Discriminating Colors

Many more methods have been employed to measure the action spectrum of various behaviors, that is, the efficiency of different wavelengths to drive the behavior. None of these *per se* demonstrates the capability of the animal to discriminate color. For color vision, circuitry is needed that compares the output of at least two photoreceptors with different spectral absorption peaks (**see also Vision**: Photoreceptors and Visual Pigments and Optic Tectum). Such circuitry is useful for detecting objects against a differently colored background in very much the same way as circuitry for detecting coherent movement aids in figure–ground discrimination. Perhaps for this reason, all major taxa use color for detecting and discriminating objects. In fish, color vision was demonstrated by von Frisch and ever since many clever behavioral experiments have been performed to test for color vision in fish. In the following, we describe a set of particularly carefully designed experiments by Neumeyer and colleagues that demonstrated tetrachromatic color vision in goldfish (*Carassius auratus*).

In a typical two-alternative forced-choice training, Neumeyer's goldfish first had to choose between the two test fields that were homogeneously illuminated with monochromatic light of different wavelength. For some fish, the task was to select that one of the two test fields which had the shorter wavelength. Other fish had to select the field with the longer wavelength of the two. For a given training wavelength, these experiments yielded the minimum difference, $\Delta\lambda$, the two wavelengths needed to have so that not less then 75% choices were correct. By then shifting the training wavelength in small steps – eventually through the complete spectrum – Neumeyer was able to determine the wavelength dependency of the required difference, $\Delta\lambda$, the so-called $\Delta\lambda$-function. Interestingly, wavelength discrimination was best (i.e., the required difference in wavelengths,

$\Delta\lambda$, was lowest) at 400, 500, and 600 nm. The high discrimination ability in the ultraviolet (UV) range was surprising because only three cone types were known before, with maximum absorption at about 450, 530, and 620 nm, but not in the UV range. The behavioral finding, thus, was compatible either with a new fourth cone type with an absorption maximum in the UV range or with a secondary absorption peak in the sensitivity curve of the long-wave cone type.

Neumeyer settled this question in elegant color-mixing experiments. Suppose the long-wave cone had a (previously unnoticed) secondary UV absorption peak so that UV discrimination could be mediated by an interaction of the long-wave and a short-wave cone. If that is the case, then it should be possible to create a mixture of blue light (absorbed by one of the two players, the short-wavelength cone) and far-red light (absorbed by the other player) with just intensities matched so that the mixture excites the two cones exactly so as presenting monochromatic light of 404 nm would. In other words, there should be a suitable mixture of blue and red light that the goldfish should not be able to discriminate against monochromatic light of wavelength 404 nm. However, no such mixture could be found – at every intensity combination tested, the goldfish always detected the monochromatic light. A second set of color-mixing experiments tested directly if an UV receptor mediated the goldfish's performance. In this case, 404 nm should not be distinguishable from a suitable mixture of blue and UV. This was indeed found to be the case. Therefore, in very simple but well-designed behavioral experiments and with some patience, it was possible to discover a new UV receptor.

Color Constancy

An important challenge in color vision is that the spectrum of the illuminating light can change dramatically. To label an apple and a banana unequivocally with the attribute color requires independence of the label with respect to these changes. The ability of our visual system in achieving this is impressive: when the illumination is changed such that now a banana reflects more red light then was previously reflected by a nearby apple, we basically perceive the colors unchanged. This property is described by the term color constancy. In primates, the visual cortex is thought to mediate color constancy. Nonetheless, in fish – that lack a cortex – color constancy was demonstrated not long after the von Frisch's discovery of color vision in fish. More recently, a thorough study by Neumeyer and colleagues has probed in great detail the full extent to which color constancy of the goldfish parallels that of the humans. Such findings are important and can, taken together, suggest that color constancy is a rather basic trait that does not rely on a visual cortex but can be realized with the circuitry at hand (**see also Vision**: Optic Tectum).

Spatial Vision

Knowing the distance of objects can be decisive in the selection of an adapted behavior: How close is the predator – is it better to hide or to escape? Is the prey close enough to launch an attack (**see also Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?) When food (or being fed on) is involved in an interesting visually guided natural behavior, it often can be used to characterize information about distance that the visual system provides. A good example is the archerfish (*Toxotes* sp.) that is famous for shooting water at aerial prey to dislodge it from its substrate and to catch and devour it at the water surface. Because these fish do not fire from directly below their prey, they cannot simply send their shot straight up. Rather the fish must set angle and speed of the water jet, depending on their vertical and horizontal distance from their target, so that it passes through the target location. Because in well-trained fish the jet is sharp and because the fish can hit from large vertical and horizontal offsets, this requires the fish to have precise distance information. The fish also use information on distance to infer the absolute size of their aerial targets, regardless of their horizontal and vertical distance (**Figure 2(b)**). Archerfish learn this in surprisingly efficient ways and achieve accuracy in estimated absolute size of at least 1 mm for targets presented 80 cm above the water surface, thereby fully accounting for the fact that the judging eye is under water while the target is in air. Training of the fish is easy because their natural feeding behavior requires neither identity of the reward with the object they fire at, nor strict temporal congruency of shot and reward. Furthermore, the fish are opportunistic and can be made to fire at anything that is rewarded. Thus, the spontaneous firing behavior of this fish can readily be used to assay size constancy. For archerfish, having size constancy is very useful to set the caliber of their shot: the maximal attachment force of prey tends to increase linearly with absolute size. Therefore, knowing size is useful for scaling the investment. Size constancy has also been shown in goldfish in a less dramatic setting without the complications introduced by a water–air boundary. Using a two-alternative forced-choice training, it was possible to show that size constancy in goldfish does not even require binocular vision. This suggests that the distance information is already provided by monocular cues (at least when binocular cues are absent).

Detecting and Analyzing Movement

Large Field Motion

When a fish drifts in a stream, the image of its surroundings moves coherently across its retina. Fish can compensate for drift by detecting this coherent motion and adjusting their forward speed so that the image motion vanishes. This is the basis of the so-called optomotor response (OMR) shown by most fish, but widely distributed among animals and is also present in humans. A standard paradigm used to characterize motion vision is to place the fish in the center of a rotating drum. The drum is lined, for instance, with a vertical grating of alternating black and white stripes. Using a suitable projector, the drum can also display a moving computer-generated pattern. If the eyes of the fish do not move themselves, the fish then attempts to move so as to minimize image motion. Ideally, this would make the fish circle at the pattern's angular speed. Many variations of this setting have been used. A very convenient method is, for instance, to project a computer-generated pattern on the bottom of the tank (**Figure 2(c)**). The movement can then be just in one direction or swinging back and forth.

A second useful behavior in response to visual large field motion is the optokinetic nystagmus (OKN; **Figure 2(d)**). Here, the large-field retinal image motion is not compensated by moving the whole body but by following the moving pattern with the eye. Obviously, this cannot go on forever. The typical OKN therefore consists of periods in which the eyeball rotates to track the pattern motion and brief intervals when the eyeball is rapidly rotated back. Both the OMR and the OKN can be done noninvasively, do not require training, and can be probed over extended periods of time. This makes these methods very useful for probing the time course of light adaption, the spectral input to the pathways that drive the OMR/OKN, or the robustness of large-field motion detection with respect to floating particles (visual noise) in the foreground. The OMR has also been useful to probe functional specializations in species such as the four-eyed fish (*Anableps anableps*) that views its aerial and underwater world with different pairs of eyes and in which the OMR is predominantly driven by aerial motion. In light of all its advantages, it should not be forgotten that fish are not reflex machines that are under the strict command of moving stripe patterns. Fish use their OMR as a tool serving an intended movement but not preventing it. In intended locomotion from one location to another, the function of the OMR is to stabilize the chosen path through a stream. It is therefore important to check the readiness of the chosen species to cooperate in the given setup. Our lab has, for instance, failed in demonstrating and using an OMR in seahorses (*Hippocampus reidi*).

Nevertheless, OMR and OKN work well for most fish and, most surprisingly, even in some larvae. In zebrafish larvae (*Danio rerio*), this helps to assay genes important for retinal function and for assembly and maintenance of postretinal processing. With the visual system fully developed already 5 days after fertilization, the larvae already show visually guided behaviors. Because many techniques can readily be applied to larvae but not to adults, it is important to see which features of the fully developed system in adults are already functioning in the larva. Zebrafish larvae respond to visual motion very early on. Surprisingly, they not only detect the so-called first-order motion in which a pattern is defined by a spatial intensity gradient (e.g., in the case of the classic grating). The larvae even detect second-order motion of contours that are defined by changes in texture or flicker but leave the spatial distribution of luminance constant. Detecting such more elaborate ways of movement is – in primates – often considered a task for the visual cortex in which separately processed information on first- and second-order motion is combined. It is an exciting finding that the zebrafish larva is already fully equipped to detect second-order motion. This suggests that the computations can be done much more peripherally and, perhaps, even within the retina.

Small Field Motion: Perceiving the Speed of Objects

Fish can also assess the speed of objects but little is known on how accurate they are. A good alternative to starting a forced-choice training setup is to look for naturally occurring behaviors that rely on information about speed. A particularly rewarding subject in this regard is the archerfish whose unique hunting technique requires it – in at least two ways – to represent the speed of small target objects. Archerfish can learn to hit moving aerial targets (**Figure 3(a)**) that travel considerably during the rise time of their shot. This requires the fish to adjust their shots to target speed. In the respective experiments, target motion was shown only in a small area. Surprisingly, fish that had been trained exclusively with horizontal motion also fired appropriately when the target had a vertical component of speed whose neglect would have been experimentally detectable. This suggests that they can represent three-dimensional speed, at least in ways that can readily be used to set the appropriate combination of shooting angle and horizontal distance from the target that is required to hit.

A different context in which it is important for the fish to judge the speed of small objects is in their predictive start. Archerfish launch a rapid start toward their prey's later impact point as soon as they estimate the direction and speed of their dislodged prey (**Figure 3(b)**). They can estimate speed well enough in an impressively short

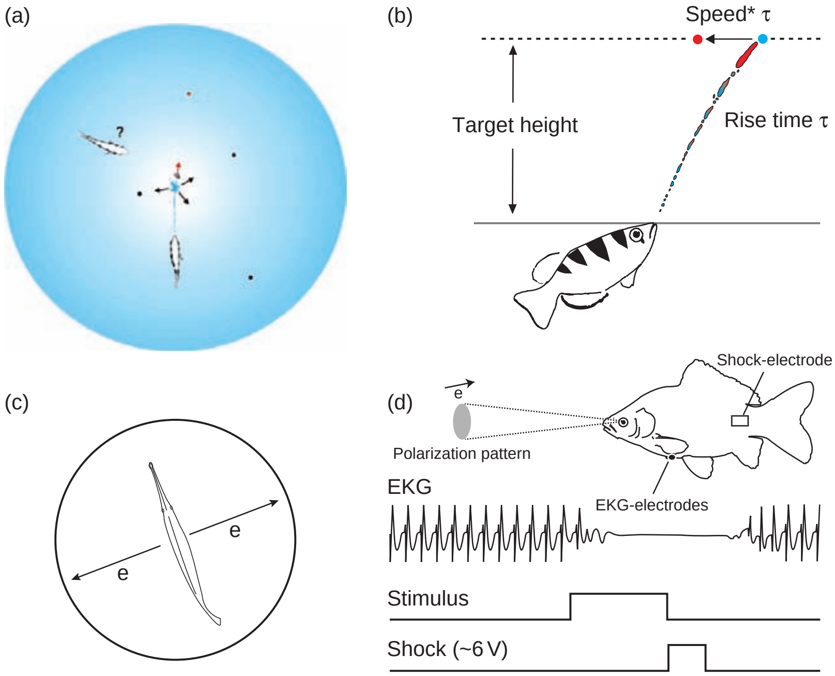


Figure 3 Naturally occurring behavior and classical conditioning. (a), (b) Two naturally occurring behaviors that help to assess how fish represent the motion of small objects. (a) Top view of prey catching in archerfish. The moment a shot is fired the fish do not know at what speed and in which direction its prey is going to move when dislodged. Archerfish therefore monitor initial motion and then turn and start so as to be at the right time at the inferred later point of catch. This behavior involves accurate representation of speed and direction. (b) Representing speed also is needed when the fish learn to hit a target that moves, in a restricted area, at constant speed. Depending on target height the fish must learn to account for the speed-dependent displacement of their target during the rise time of their shot. (c, d) Using behavior to demonstrate that fish detect polarized light. (c) *Zenarchopterus* spontaneously orient their body axes according to the e-vector of linearly polarized light. (d) Heart-rate conditioning: by pairing a visual stimulus with a mild electric shock fish eventually suppress their heart-beat activity when stimulated with polarized light. The conditioned response can then be used to obtain threshold levels and to correlate them with photoreceptor distribution in that spot of the retina that has been stimulated. (a) Adapted from Fig. 1 in Schlegel T and Schuster S (2008) Small circuits for large tasks: High-speed decision-making in archerfish. *Science* 319: 104–106. (b) Adapted from Fig. 1(a) in Schuster S, Wöhl S, Griebsch M, and Klostermeier I (2006) Animal cognition: How archer fish learn to down rapidly moving targets. *Current Biology* 16: 378–383.

interval of time. Experiments in which the fish are confronted with falling objects of novel sizes, in which the background behind the objects has a novel distance and in which novel vertical speed components are added to the movement currently, suggest that archerfish use an estimate of absolute speed to drive their predictive start.

Detecting Polarized Light

Behavioral methods have also demonstrated that fish can detect the polarization of light. This was surprising because in vertebrate eyes the morphological foundation of this sense is less conspicuous than in the compound eyes of the arthropods: chromophores in the disks of vertebrate photoreceptors are not aligned as they are in the microvilli structures of arthropod rhabdoms and can move freely within the disk membranes in the outer segments. The first evidence came from orientation behavior of *Zenarchopterus dispar* and *Z. buffoni* under illumination with linearly polarized light. *Zenarchopterus* preferred heading directions perpendicular or parallel to the e-vector (**Figure 3(c)**). This and other results disclosed the ability of fish to detect the polarization of light and to use this attribute for orientation. Furthermore, contrast enhancement, for example, for prey detection, was suggested as a further advantage of polarization vision in fish (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments).

Behavioral studies also revealed the morphological basis of polarization vision in fish. One successful approach used the so-called heart-rate conditioning. In this classical conditioning method, fish are trained to associate a stimulus, in this case a polarization pattern (conditioned stimulus) with a harmless electric shock (unconditioned stimulus). Fish respond to mild shocks (about 6 V) by a short interruption of heart activity (**Figure 3(d)**), mostly combined with suppression of respiration activity. Monitoring the electrocardiogram of trained fish thus offers the possibility to measure precise thresholds of polarized light detection. By comparing the sensitivity thresholds for polarized light with the absorption spectrum and the arrangement of double cones in the retina of the sunfish (*Lepomis cyanellus*), it became evident that the double cones are responsible for polarization vision in at least some fish.

Conclusion

A rich repertoire of behavioral approaches is ready for use for future discovery. It is important, however, to keep in mind the simple fact that behavioral approaches elucidate those aspects of visual information that are fed into the behavior at hand. To demonstrate color vision, for instance, one must select a behavior that (if the fish has color vision at all) would probably be driven based on color information. For example, using the OMR would be a bad choice here: in goldfish, the OMR is colorblind. However, suitable training of the fish readily reveals its tetrachromatic color vision. Similarly, placing an archerfish into rotating stripe drums would not allow us discovering its many exciting capabilities.

We predict that behavior will continue to play an important role in the future of fish vision:

1. A promising direction is comparing visual capabilities of fish with those of primates. Such studies can show that many apparently complex tasks can be solved quite early by comparatively simple circuitry. Zebrafish larvae, for instance, readily detect second-order motion and may use circuitry right within their retinae to detect this more abstract form of motion.
2. Exploiting the visual behaviors in the larvae of zebrafish will continue to be a promising direction for future research that provides key factors required for the assembly and higher functions of the visual system.
3. The behavioral analysis of visually specialized species will provide further surprises in visual information processing that force us to reconsider our notions on basic physiological processes.
4. Finally, vision is not simply a prerequisite of behavior but is tightly interwoven with it: fish move to obtain visual information, modulate incoming sensory information during intended movements, and integrate vision with other sensory information to most efficiently pursue their goals. In fish, these aspects are well accessible to behavioral dissection.

See also: **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals; Spatial Orientation: How do Fish Find their Way Around?. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Optic Tectum; Photoreceptors and Visual Pigments; Vision in Fishes: An Introduction.

Further Reading

Cameron DA and Pugh EN Jr. (1991) Double cones as a basis for a new type of polarization vision in vertebrates. *Nature* 353: 161–164.

Douglas RH and Hawryshyn CW (1990) Behavioral studies of fish vision: An analysis of visual capabilities. In: Douglas RG and Djamgoz MBA (eds.) *The Visual System of Fish*, pp. 373–418. London: Chapman and Hall.

Herter K (1953) *Die Fischdressuren und ihre sinnesphysiologischen Grundlagen*. Berlin: Akademie.

Horváth G (2004) *Polarized Light in Animal Vision*. Berlin: Springer.

Neuhauss SCF (2003) Behavioral genetic approaches to visual system development and function in zebrafish. *Journal of Neurobiology* 54: 148–160.

Neuhauss SCF, Biehlmaier O, Seeliger MW, *et al.* (1999) Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish. *Journal of Neuroscience* 19: 8603–8615.

Neumeyer C (1992) Tetrachromatic color vision in goldfish. Evidence from color mixture experiments. *Journal of Comparative Physiology A* 171: 639–649.

Neumeyer C (2003) Color vision in fishes and its neural basis. In: Collin SP and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments,*, pp. 223–235. Berlin: Springer.

Powers MK (1978) Light-adapted spectral sensitivity of the goldfish: A reflex measure. *Vision Research* 18: 1131–1136.

Schlegel T and Schuster S (2008) Small circuits for large tasks: High-speed decision-making in archerfish. *Science* 319: 104–106.

Schuster S (2007) Quick guide: Archerfish. *Current Biology* 17: R494–R495.

Schuster S and Amtsfeld S (2002) Template-matching describes visual pattern-recognition tasks in the weakly electric fish *Gnathonemus petersii. Journal of Experimental Biology* 205: 549–557.

Schuster S, Rossel S, Schmidtmann A, Jäger I, and Poralla J (2004) Archer fish learn to compensate complex optical distortions to determine the absolute size of their aerial prey. *Current Biology* 14: 1565–1568.

Schuster S, Wöhl S, Griebsch M, and Klostermeier I (2006) Animal cognition: How archer fish learn to down rapidly moving targets. *Current Biology* 16: 378–383.

Color Vision and Color Communication in Reef Fish

NJ Marshall and K Cheney, The University of Queensland, Brisbane, QLD, Australia

© 2011 Elsevier Inc. All rights reserved.

Introduction
Camouflage and Communication on the Reef
Communication and Deception
Deception and the Use of Your Neighbors Color Message
Conclusion
Further Reading

Glossary

Aposematic Warning signal; aposematic coloration is often bright or conspicuous, warning potential predators that animal is toxic or in some other way unpalatable.

Color An object's color is determined by its spectral reflectance, the spectral sensitivity of the visual system viewing the object, and the way in which these signals are interpreted by the brain. This varies substantially among animals depending on the composition of photoreceptors and their connections.

Cone The sensory cell type in the eye (photoreceptor) involved in color vision and bright light conditions.

Irradiance The amount of light or electromagnetic radiation at a surface. Spectral irradiance is used here to describe the light incident on the reef available for vision.

Radiance A radiometry term to describe the amount of light, emitted from a particular area and from a defined solid angle or direction.

Rod A rod-shaped photoreceptor type in the eyes of vertebrates that functions in dim light conditions so may allow night vision. The photoreceptor molecule within rods generally absorbs light in the middle of the spectrum giving it a spectral sensitivity around 500 nm.

Spectral reflectance The wavelengths of light reflected, and therefore not absorbed or transmitted, through an object, as determined by the pigmentary or physical structure of the objects surface. For visual purposes this often expressed as a curve from ultraviolet to far red (300–700 nm).

Spectral sensitivity The specific spectral region, usually a bell-shaped curve, within which a photoreceptor absorbs light. This includes the absorptive effect of any photostable pigment, such as a yellow lens, on the absorbance of the visual pigment molecule within the photoreceptor.

Introduction

Anyone who has snorkelled or dived on a coral reef always surfaces exclaiming about the beauty of the fish. These jewelled inhabitants of this shallow tropical habitat have been a source of wonder and interest since the first reef was encountered. Both Darwin and Wallace, the grandfathers of the theory of evolution, were so worried by the diversity of what they saw on reefs that both questioned if their new idea could explain the myriad colors of the reef inhabitants. Today, the more we learn about the language of reef fish colors and the design of the eyes that decode their message, the more astonishing this aquatic world becomes.

It is important to realize that in fact there is no such thing as color out in the environment. Objects reflect the spectrum of light that reaches them differently, depending on the physical or pigmentary properties of their surface. Photoreceptors, usually cones for color vision, absorb this light according to the spectral absorption of the visual pigment molecule within them and convert this to an electrical message that is sent to the brain. These steps and the way in which the brain combines and interprets these messages is what gives us the sensation of colors and, as made clear later in this article, this varies substantially between animals.

Few of the color codes on the reef have been explained well, but the basic principles of evolution, natural selection, and sexual selection help us to understand some of the functions of reef color (**Figure 1**). New technology and spectrophotometric (light measuring) methods have also advanced our knowledge of the ways in which fish see and how they use color in the context of their marine environment for survival.

Coral reefs are the densest packed ecosystem on the planet for animal life per cubic meter and this gives one clue to the explanation behind the color diversity of the fish and other animals living there. As recognized by last

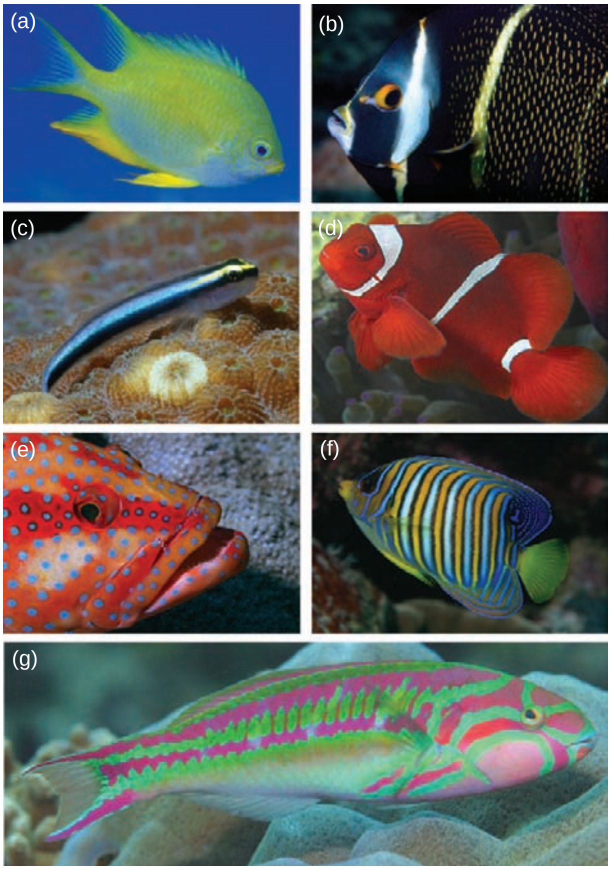


Figure 1 Reef fish and their color patterns. (a) The yellow color of the golden damselfish *Amblyglyphidodon aureus* is highly contrasting against a blue ocean background. (b) French angelfish *Pomacanthus paru* uses strongly contrasting colors to disrupt its body outline. (c) Cleaner goby *Elacatinus evelynae* combines yellow and blue to construct a cleaner fish guild flag. (d) Anemonefish *Premnas biaculeatus* uses strongly contrasting color patterns possibly for disruptive camouflage and for aposematic ('I am distasteful') communication in different circumstances. (e) Coral cod *Cephalopholis miniata* is covered in small spots of color that are highly contrasting at close range but mix additively with the background color of the rest of the fish at a distance for camouflage. (f) The angelfish *Pygoplites diacanthus* uses yellow and blue, contrast-enhanced with white and black stripes for communication and camouflage. (g) The unusual colors of five stripe wrasse *Thalassoma quinquevittatum* are strikingly complementary at close range and blend to match water color at a distance.

century's keen observers of the reef such as Longley, Thayer, Cott, and later on Lorenz, reef inhabitants are in constant communication or anti-communication with each other. Konrad Lorenz, who taught us many of the principles of how to observe animal behavior, said of reefs that "There is in all the world, no other biotope which has produced .... in so closely allied groups of animals, an equal number of extremely specialised forms." He was mostly talking about the fish and their variety of form and color, and this led him to the idea of poster coloration in reef fish, bright colors that enabled fish to sort themselves out into species types and a hierarchical social structure. That is, conspicuous colors and patterns encode species identity enabling close cohabitants to rapidly decide what to do when encountering each other as far as aggression, defense, and the desire to mate are concerned. However, this idea has yet to be explicitly tested.

Anti-communication, an attempt to conceal or camouflage, is intricately woven into the physical nature of light on coral reefs and the eyes that attempt to recognize camouflaged individuals for various reasons. The same close relationship exists between light and color used in deliberate communication and this article gives examples of both communication and camouflage.

Communication methods may be loud and very specific, as noted by Lorenz. Many reef fish are probably colored aposematically, meaning that they use bright coloration to advertise toxicity, or other spiny reasons that make them difficult to swallow. The importance of considering all these possible ways of hiding and conveying information through 'the eyes of the beholder' cannot be overstated and an understanding of the visual capabilities of reef fish, rather than using our own eyes for analyzing particular colors or patterns, is critical.

This article also provides a brief review of the visual ecology of the reef and examines some of the recent advances in our knowledge of the eyes and colors of reef fish. Visual ecology, an area of biology most elegantly explained by John Lythgoe, links the physics of light and color of an environment to the visual system and signaling systems that have evolved there, and lastly examines the visually driven behaviors a species exhibits (**Figure 2**). This must be done not only from the perspective of the visual system observing the color and pattern, but also from the scale of the species under consideration.

The fish we see when we go to a reef represent a small proportion of the biomass of fish that live there. Most fish on the reef are less than 3 cm long and many of them are cryptic (hiding) or shy. However, because we are relatively large animals, we tend to see relatively large fish, perhaps a velvet blue-yellow angelfish defending his territory of sponges and pink-purple parrot fish male with his drab harem of females in tow.

Another subjective problem is partial color blindness because some reef fish have a very different color vision than we do. Humans are primates and our color vision evolved for the tasks of survival in forests and other terrestrial environments looking to find ripe fruit or young red leaves. Put a primate in a mask and snorkel on the reef and the first things they often notice are all banana-colored objects (**Figure 1**(**a**)) at the long-wavelength end of the spectrum, which get our special attention.

In the relatively clear blue waters of the reef, one thing any diver knows is that by the time he gets to 20 m or so, a red snorkel has become greenish-black and blood looks dark green. The reason for this is that the absorption (or more accurately the total attenuation which includes scatter) of water at 20 m has removed red light (**Figure 2**). This would make the red-belly signaling system of a stickleback male ineffective here. Reef fish have evolved communication colors and visual systems set by the physical envelope of light wavelengths that their water transmits best. Their world is very ultraviolet (UV)/blue/green dominated (**Figure 2**). Because their reef visual systems are adapted to see well in this spectral range, yellow, orange, and red may be less conspicuous and indeed match the general reef background.

Camouflage may be achieved by directly matching the color, intensity, and sometimes the texture of the reef substrate (**Figure 1**). As superbly detailed by Cott in his 1940s book *Adaptive Coloration in Animals*, it also pays to have highly contrasting even seemingly ridiculous bold body patterns where the background is bold and highly contrasting (**Figure 3**). The tropical sun coming through the waves, coral branches, coral plates and other reef structures creates a very high contrast environment, and reef fish colors and patterns must be considered in this context. The coffee-table-book flash photography does not achieve this. The colors of the background are deliberately masked or de-focused and the bold colors of the fish picked out with full spectrum light to show them off as best as possible. This topic of color contrast and visual adaptation deserves attention, as many reef inhabitants clearly use this principle of disruptive camouflage, but it is not detailed any further here.

Camouflage and Communication on the Reef

Yellow and Blue, Reef and Water

The underwater visual scene at around 10 m is quite different to land. Directly up to an angle of 43° (Brewster's angle beyond which total internal reflection starts), there is the bright shimmering surface, through which the sky may be more or less visible depending on the waves. Below this is a relatively uniform and dimmer blue, diffuse background body of water into which vision cannot penetrate more than a few tens of meters. The coral and other reef structures appear at a various heights, depending on the ruggedness of the reef and the observer's own size. In shallow water habitats over light reflective sand, looking down may also be very bright, a terrestrial condition experienced only when light reflects off the surface of snow.

The aquatic world is also more three dimensional. Observers and signalers are more dispersed and this is important when considering what angle to view others at and when trying to be camouflaged from all angles. Many reef animals also tend to 'hug the ground' or hide in the reef structure for protection and camouflage. Nonetheless, reef fish may take off into the water column at some stage or indeed some species do spend most of their life there, and this can be dangerous but a good way to advertise your presence. Ultimately, the intrinsic

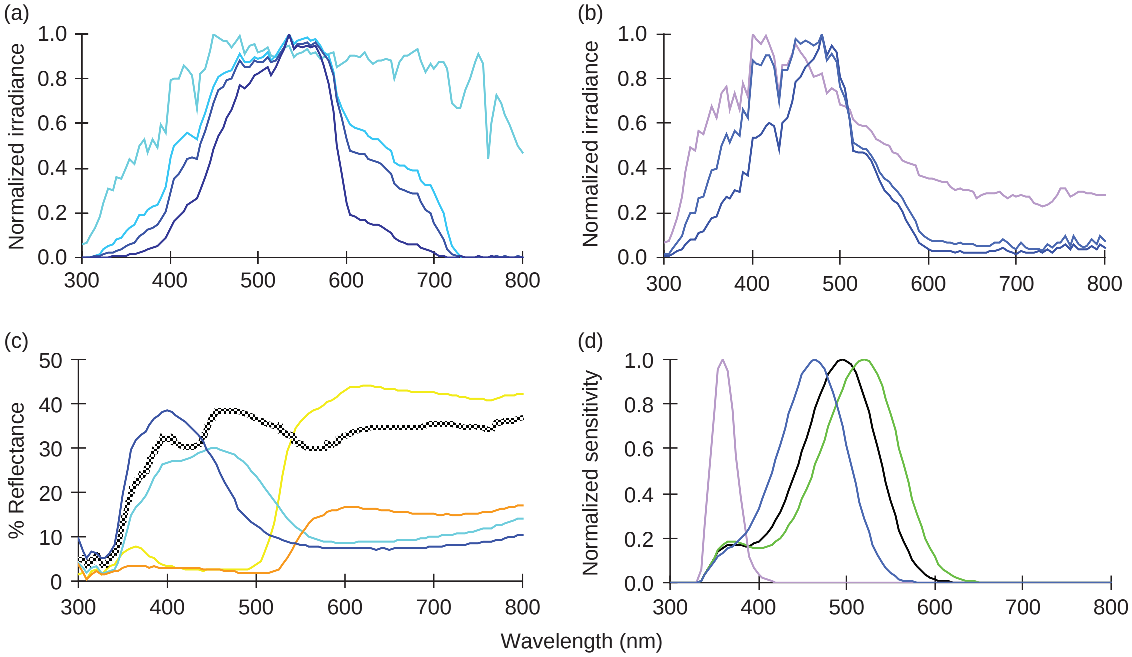


Figure 2 (Continued)

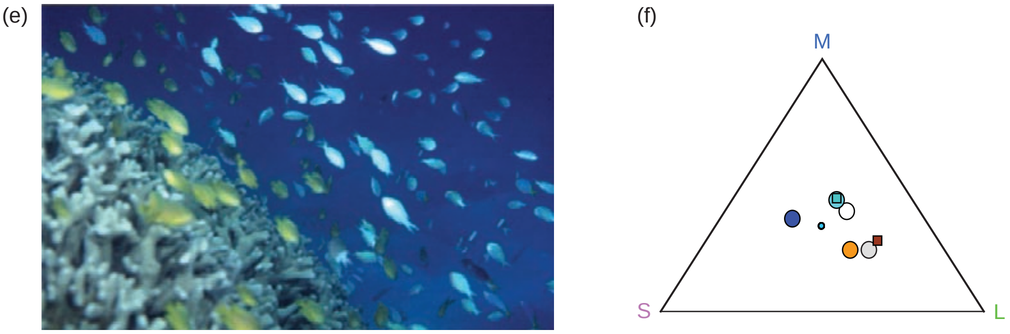


Figure 2 Extended caption – visual ecology. In order to understand and quantify the way in which other animals see the colors of their world, four bits of information are required. (1) The environmental light envelope may be quite different meaning that some parts of the spectrum are not available and therefore not worth investing visual sensitivity in. This is especially so in different water environments. On the coral reef, wavelengths from near UV (350 nm) to yellow (600 nm) are most useful. (2) The colors produced must be measured as their spectral shape can reveal much about how the color is used and combined. Complex colors, that have two or more areas of reflection, may make use of both regions of chromatic reflection or one area may be irrelevant. This seems to be the case for many reef fish colors that contain a long-wavelength component (**Figure 5**). (3) Visual systems on the reef are very different to those on land and indeed than those in freshwater environments. Color vision can be constructed by two or more cones and reef fish show a diversity of color vision types ranging from apparent dichromacy to apparent tetrachromacy. One new observation that has not been explained is the astonishing diversity of spectral sensitivity mechanisms in reef fish. (4) A good understanding of the behavior of the species is essential to appreciate how their visual system and color communication or camouflage systems have evolved. This includes basic observations such as depth range and habitat (does the fish spend its time under a coral ledge, in mid-water, or hidden in holes in the reef) as well as the more complex inter- and intraspecific interactions for predation, defense, and reproduction. The above components and observations can then be used to make predictions and model ideas of how reef fish colors can be used. This sort of calculation is often plotted in a visual space and an example of that is shown here for the colors of angelfish *P. diacanthus* and its possible background colors. (a) Downwelling irradiance or spectral distribution of light on the reef at different depths (0, 3, 5, and 10 m – light blue to dark blue lines). With increasing depth, the filtering and scattering effect of water filters out wavelengths at either end of the spectrum, resulting in a narrowed spectral envelope or light habitat, depending on depth and water type. (b) Radiance or light that might directly reflect from a fish from different angles at a depth of 1 m (purple, from above; light blue, from the side; and dark blue, from below). This directional difference in light background demonstrates the variability of light on reefs, even at a single depth. (c) The spectral reflectance or colors of *P. diacanthus* (**Figure 1(f)**). Reef fish colors can be measured with a reflectance spectrophotometer and the resulting peaks and steps and complex colors enable us to nonsubjectively quantify how colors are used. Line colors are color coded to match approximately the colors of the fish in life (**Figure 1(f)**). (d) The spectral sensitivities of rods (black line) and cones (purple, blue, and green lines) of reef fish must be known before a good understanding of their visual ecology can be built up. This may be achieved through a variety of methods and defines the species color vision range and complexity. (e) Behavioral observation of how colors are used and the context of their use are essential. Here damselfish alerted to the photographer, sort themselves against different backgrounds that provide more effective camouflage for their respective basic body colors. (f) The measurements (a)–(c) above can be combined in visual models to estimate how different colors are used or are effective in the visual world of different species. The Vorobiev/Osorio model is often used and essentially combines light, spectral sensitivity, and spectral reflection in a weighted scatter-plot as seen here. Each data point represents how the fish with visual system in (d) sees the colors of the angelfish colors in (c). The small black circle in the center is the white point and achromatic colors fall close to this point. Note how the white of the fish (gray line in (c)) does not fall near the white point in this visual space as the fish visualizing these colors has UV sensitivity (d). The distance between the data points on this plot is related to how easy each color is to distinguish. Where color vision is potentially trichromatic, with three photoreceptor types or cones, the plot falls into a triangle with each corner representing one of the three spectral sensitivities and labeled here S, M, and L for short, medium, and long wavelength, respectively, and color coded as in (d). The plot shown here is called a Maxwell triangle. The round symbols are the fish colors in (c) and are color coded the same as the colors they represent. This is also their approximate color in life (**Figure 1(f)**). The square symbols are background reef colors shown in **Figure 4**, water background (blue square) and an average of 255 coral and algae found on the reef (brown square). Note how closely the fish yellow matches coral color and how closely one of the fish blues matches water color. This is also demonstrated in **Figure 4**.

turbidity and absorbance of water over a reef precludes the need for long-distance vision and also breaks down fine color patterns at relatively small distances.

Thus, the horizontally divided, distance-truncated three-dimensional habitat of the reef has resulted in a number of specific visual and signaling adaptations. Reef fish lack the high visual acuity of many land animals (most reef fish have resolving power around 10 times worse than humans) and some have evolved relatively simple color vision with only two cone types or color channels. These dichromatic fish are much like many mammals on land and are in some ways similar to a red–green color blind human. These two-channel color vision systems can extract enough information from the world for survival on just one color axis. In behavioral tests for humans, this is called the blue-yellow axis, and for all dichromatic

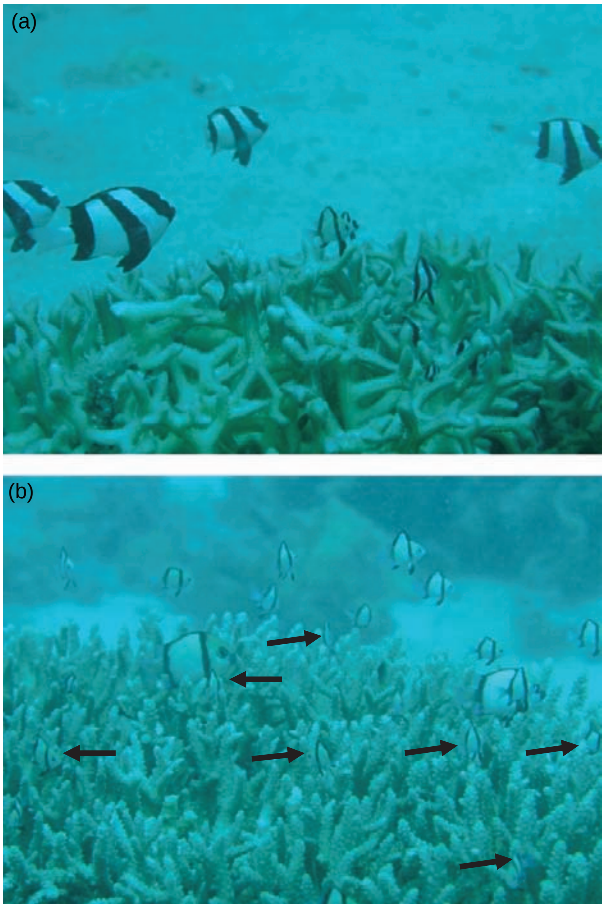


Figure 3 Apparently conspicuous fish in natural surroundings with natural illumination are well camouflaged. Black and white provides the strongest contrast and potential for conspicuousness. (a) Humbugs (*Dascyllus aruanus*) above the coral head in which they live are maximally conspicuous and this may help communication within the school. (b) The damselfish *Dascyllus reticulatus* also inhabits coral heads and when these or *D. aruanus* drop down into the coral branches, they merge with the background through disruptive camouflage. This is especially effective in juvenile *D. reticulatus*, which also mimic the coral fingers. Arrows indicate individual fish.

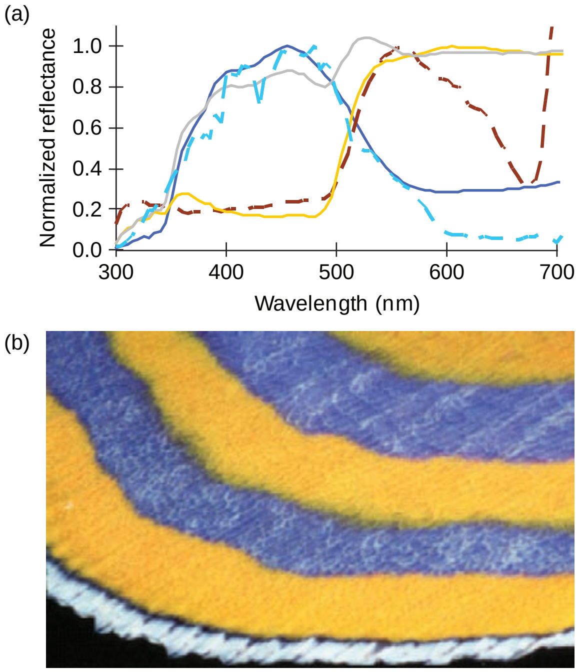


Figure 4 Yellow and blue colors within reef fish body patterns may combine in an additive mixture to reflect gray. This only works when the pattern is constructed of fine stripes or spots which blend at a distance as the resolving power of the eye fails. (a) Reflectance spectra of the yellow/orange and blue anal fin of angelfish *P. diacanthus* (**Figure 1(f)**) shown in (b). Yellow/orange and blue solid lines are color coded to match the body colors in (b). Gray solid line is the additive mix of these two colors. This would appear gray to both fish and humans lacking UV spectral sensitivity as it reflects fairly evenly from 400 to 700 nm. Dotted blue line is side-welling radiance of reef water (**Figure 2(b)**); dotted brown line is average coral and algae reflectance (255 samples). Note the spectrally close match between these colors and the fish colors (**Figure 2(f)**). (b) The anal fin of *P. diacanthus* (**Figure 1(f)**).

species it is constructed from a photoreceptor sensitive to the short wavelength or blue part of the spectrum and one sensitive to longer wavelength around yellow. Color detection or discrimination that requires information from the red–green axis has resulted in the addition of a third color channel or cone mechanism in some primates, allowing, for example, better determination of ripening fruit and other tasks. Some reef fish become trichromatic, and others possess four color channels. At depth, the whole color world is more restricted to wavelengths shorter than yellow (see **Figure 2** and extended caption for further explanation). However, a fish living very close to the reef top will experience close to full spectrum daylight, like that on land. As a result, if their lifestyle demands it, coral reef fishes clearly take advantage of the colors available and increase their color sampling range.

The color code adopted for effective camouflage and communication in this habitat is biased toward yellow and blue (**Figure 4**). Several small reef fish species, notably in the damselfish (Pomacentridae), are colored mostly blue, blue-green, or yellow all over. Other larger fish, such as the angelfish (Pomacanthidae) or surgeonfish (Acanthuridae), use stripes or large blocks of yellow and blue colors (**Figure 1**). These complementary colors are clearly being used to communicate. Complementary colors are ones that contrast strongly with each other, the result of them reflecting in opposing parts of the spectrum (**Figures 4** and **5**). To almost any color vision system, these yellow and blue colors, seen side by side, stand out and it is not just fish such as *P. diacanthus* (**Figures 1** and **4**) that parade this combination in agonistic and sexual displays. Smaller reef fish that are blue all over can get the same effect by positioning themselves against the

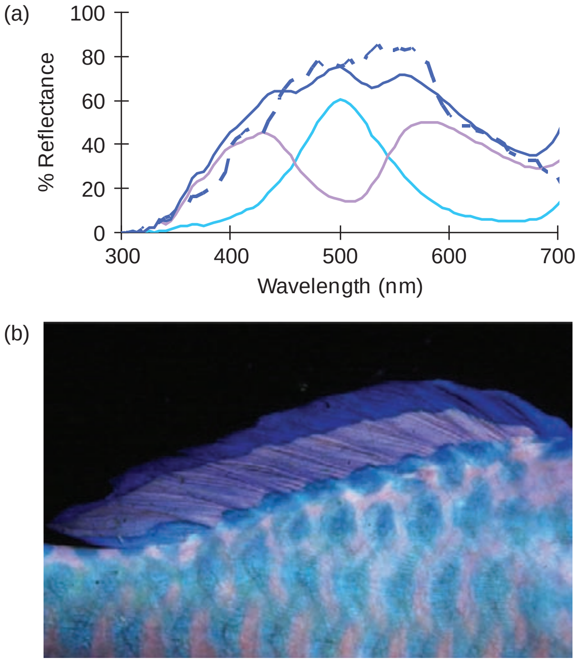


Figure 5 The unusual colors of parrotfish and wrasse can combine to give broadband blue, a good match to background water color. These colors are often intense green and purple/pink (see also **Figure 1**(**g**)) and are complementary as they reflect in different regions of the spectrum. This makes them highly conspicuous at close range. (a) Reflectance spectra of green and purple/pink scales of parrotfish *Chlororus sordidus*. Green, purple/pink solid lines are color coded to match the body colors in (b). Blue solid line is the additive mix of these two colors. Dotted blue line is reef-top water color (radiance) where these fish are often found. Note the spectrally close match between this additive color and the background allowing such fish to be well camouflaged at distance.

yellow-brown of coral and yellow fish can do the same against the blue water background (**Figure 1**).

Lythgoe, Loew, McFarland, and other investigators have also pointed out that yellow and blue colors transmit information a long distance in marine waters, mainly because their regions of largest spectral change fall within the marine environment lighting envelope. The frequently used colors on the reef are also those that can transmit information over a long distance while also remaining good for camouflage in certain circumstances.

Reef fish containing yellow and blue within their body patterns are not doomed to be constant beacons of communication and open to predation. Little fish become instantly well camouflaged, blue-green against blue-green water and yellow against yellow-brown coral. Larger reef fish can take advantage of one of the important scaling factors previously discussed. In an enlarged close-up, many striking patterns are quite finely detailed with spots or stripes (**Figure 1**) that are ideal for close encounters with the opposite sex, or in aggressive displays over mates, food, and territory ownership. From further away, however, this fine detail becomes blurred. Here the complementary nature of the colors is of benefit, as their spectra combine (additive color mixing), and the resulting color is gray and dull because it now reflects over the whole spectrum (**Figure 4**). The relatively poor resolving power of potential predators means that at only a few meters away brilliantly colored angelfish becomes a shadow.

Pointillism in Parrotfish and Other Reef Fish

There is another, more subtle, color mix story found in the parrotfish (Scaridae) and wrasse (Labridae). These fish often possess rather spectrally complex colors with many peaks and troughs (**Figures 2** and **5**) that appear striking pink and green to our eyes. These are also complementary colors and may function differently to yellow and blue. These colors combined do not just render the fish dull and gray, but make it an astonishingly good match to background water color (**Figure 5**). This is again additive color mixing, resulting in a good match to the blue-green water background, which may be a more effective camouflage against the distant eyes of predators and more in line with the lifestyle of these species. Whereas angelfish often lurk in the overhangs and shadows of the reef and may want to appear like a shadow, parrotfish and wrasse patrol over the top of the reef, in the case of the former looking for coral and algae to graze, and, for the latter, looking for crustaceans and other food. As a result, the social function colors of wrasse and parrotfish are unknown, but one function in the parrotfish seems to be to keep the drab female harem together and to signal competitively to other males.

It is worth pointing out that the impressionist painters, particularly the pointillists, secondarily discovered the usefulness of adding small dots and stripes of color to appear one way when observed in close proximity, and another way when observed further away. Seurat and Signac were disappointed with the dullness of the result, as they were searching for ways to make colors more vibrant. Instead, they ended up with dull blues and grays, which eventually they learned were useful to represent the blue-gray cast of shadow, just like an angelfish or a parrotfish at a distance.

Communication and Deception

Cleaner Blue (and Yellow)

Reef fish can be divided into guilds, or color clubs that, according to either behavior or habitat, could explain some of the color diversity. Eibl-Eibesfeldt, Losey, and others have suggested that there is a blue cleaner fish guild. Cleaner fish, often wrasse or gobies, are relatively small and offer a skin, teeth, and gill cleaning services to clients. Fish cannot easily preen themselves of the many invertebrate parasites, such as worms and crustaceans. Cleaner fish exploit the protein banquet by picking off these itchy hitch-hikers and cleaning dead skin and scales from wounds from generally larger reef, or clients. This mutualistic relationship is a delicate one, as the small fish must enter the mouths of predators to pick off parasites and damaged skin. If the predators swallowed the cleaner, or if the cleaner ripped off more than just the required bit, the relationship would never have evolved.

Cleaners may set up a cleaner station on the reef and clients learn where to come to be cleaned. Part of this service involves advertising by the cleaners and there are stereotyped movements and dances cleaner fish perform over their station to show off what they offer. Many cleaner fish from diverse families are yellow and blue, normally in longitudinal stripes and often with black to increase contrast (**Figures 1**(**c**) and **6**). The idea of a cleaner blue guild that arose was untested until recently. Not surprisingly from what we know of yellow and blue on the reef, through a combination of behavioral tests and visual system models, the cleaner fish colors, including blue, were indeed shown to be an effective advertisement color combination, and likely instrumental in setting up the mutualism required. Thus, the cleaner blue guild has given way to a cleaner yellow-blue guild, in many cases emphasized with black.

Deception and the Use of Your Neighbors Color Message

Any relationship, even the cleaner–client mutualism, is open to exploitation. For example, some fangblennies imitate the colors and patterns of cleaner fish. *A. taeniatus* is a remarkable blenny mimic of the cleaner wrasse, *L. dimidiatus*, borrowing the blue-yellow-black stripe uniform of the cleaner (**Figure 6**). This sabre-toothed fish visually deceives unsuspecting clients, biting off chunks of skin as well as feeding on cleaner fish eggs.

Another fangblenny, *Plagiotremus rhinorhynchos*, also closely resembles juvenile *L. dimidiatus* and can rapidly change its body coloration through the neural control of melanophores and possibly other skin cells that produce color. By putting on different colors to prevent detection, the fangblenny effectively camouflages itself against the cleaner fish or within the school, and then darts out to bite unsuspecting victims. This matching and adaptable color change may also help them avoid retaliatory predation.

The UV Communication Channel and the Diversity of Reef Fish Vision

Around half the reef fish species that have the potential to see UV, unlike humans. This ability is often enabled with a cone having a spectral sensitivity between 300 and 400 nm. What is the advantage of UV vision, given the potential disadvantages of UV photodamage to the retina and poor UV transmission in water (**Figure 2**)? Small reef fish may benefit from short-range partially private or covert communication. The extra contrast and patterns are evident in the UV image of a damselfish *Pomacentrus ambionensis* (range 340–400 nm, **Figure 7**) would only be available to other fish with UV sensitivity. Large reef fish, often the predators, lack UV transmitting ocular media (lens and cornea) and cannot see the UV patterns of smaller fish.

UV facial patterns have recently been shown to be important in sexual selection of the UV-detecting

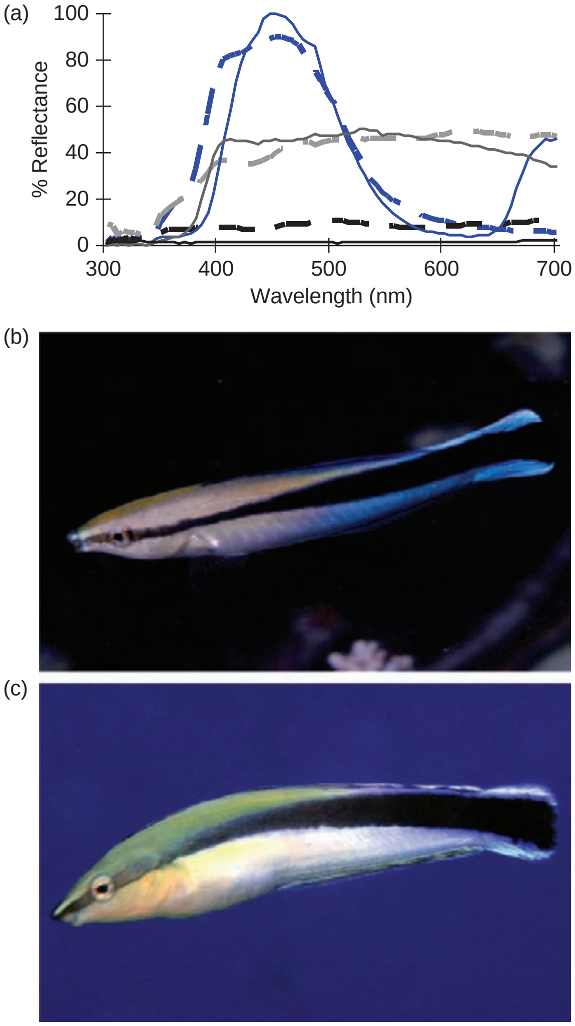


Figure 6 Mimic fangblenny *Aspidontus taeniatus* takes on the color pattern of a cleaner fish *Labroides dimidiatus* to fool clients and cleaner fish allowing them close enough to rip off scales and eat eggs. (a) Reflectance spectra of cleaner fish and fangblenny. Solid lines, cleaner fish colors; dotted lines, fangblenny. Lines are color coded to match the fish colors. (b) Cleaner wrasse *L. dimidiatus*. (c) Fangblenny *A. taeniatus*.

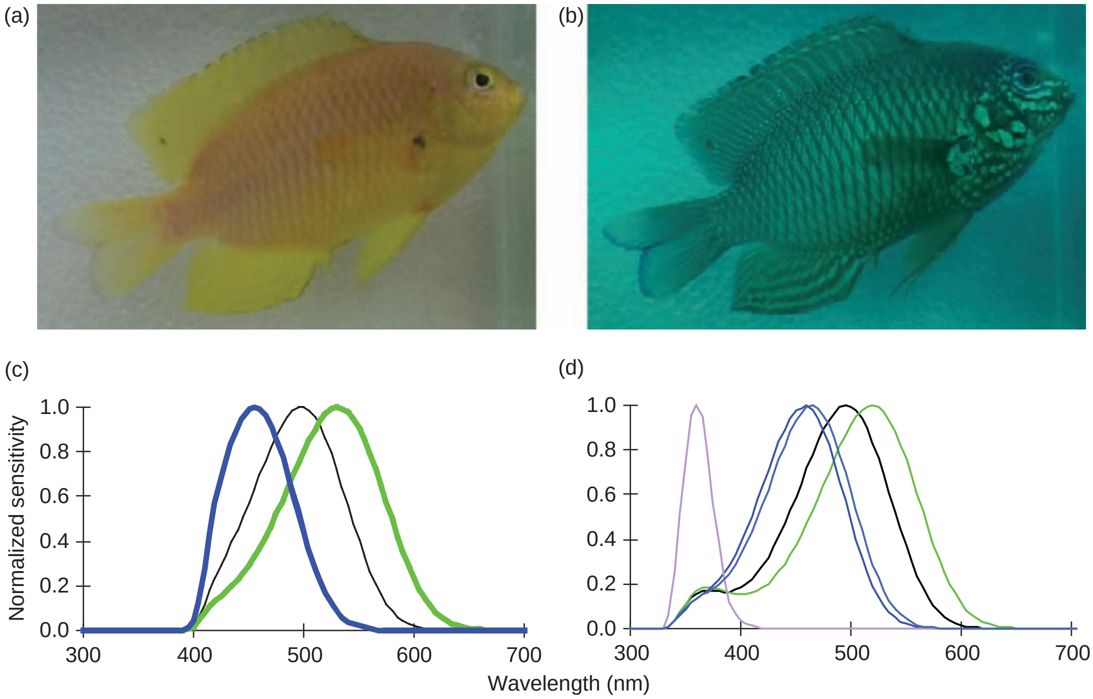


Figure 7 UV colors on damselfish are made visible with a UV camera sensitive to light from 350 to 400 nm. (a) Ambon damselfish *P. ambionensis* as non-UV sensitive humans or fish might see them. (b) Same fish viewed with UV sensitive camera reveals a pattern of high-contrast UV-reflective colors that are available for this and other damselfish species with UV sensitivity. The fish would not appear this way as this frame shows only contrast in the UV. (c) Spectral sensitivity of the barracuda *Sphyraena helleri* which, like humans, lacks UV sensitivity and would not be able to see these UV colors. (d) Spectral sensitivities of damselfish, which includes a dedicated UV cone. This sensitivity would enable these fish to see and communicate in this waveband.

damselfish *P. ambionensis*. UV patterns are effective over the short communication distances of males defending territories from other males. Such signals are therefore an effective close-up signal for little fish whose displays could attract unwanted attention from far away.

Conclusion

Both the number and spectral sensitivity of cone mechanisms are very diverse on the reef and so far we have no clear understanding of what drives this variability. Ideas of basic visual ecology can only explain some of the general trends such as the UV, blue, green bias of reef fish visual systems that broadly matches water transmission characteristics. Phylogenetic trends are very general and are also populated with general loose observations such as 'damselfish often have UV vision'.

The idea of co-evolution of colors and color vision has been proposed for a number of animals. Some correlation does exist between the spectral positioning of reef fish colors and cone sensitivity placement as noted by Marshall, McFarland, Losey, Loew, and others; however, this has many exceptions. There are no cases of fish possessing a color vision system that is designed specifically for its colors, and as reef fish colors are so diverse and there being many tasks that require color vision, this is perhaps no surprise.

We are still left with the mystery of why reef fish possess such apparently diverse color vision types. Aside from those fish in the deeper regions, or those species that are crepuscular or nocturnally active, the general light environment is the same for all species. As a result, to find the answer, we must further study the individual species on the reef and learn more about their specific ways of living.

See also: **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals. **The Skin**: Coloration and Chromatophores in Fishes. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Behavioral Assessment of the Visual Capabilities of Fish; Photoreceptors and Visual Pigments.

Further Reading

Cheney KL, Grutter A, and Marshall NJ (2007) Facultative mimicry: Cues for colour change and colour accuracy in a coral reef fish. *Proceedings of the Royal Society of London B* 275: 117–122.

Cheney KL, Grutter AS, Blomberg SP, and Marshall NJ (2009) Blue and yellow signal cleaning behavior in coral reef fish. *Current Biology* 19: 1283–1287.

Cott HB (1940) *Adaptive Coloration in Animals*. London: Methuen.

Loew ER and McFarland WN (1990) The underwater visual environment. In: Douglas RH and Djamgoz MBA (eds.) *The Visual System of Fishes*, pp.1–43. London: Chapman and Hall.

Longley WH (1918) Haunts and habits of tropical fishes. *American Museum Journal* 18: 79–88.

Lorenz K (1962) The function of colour in coral reef fishes. *Proceedings of the Royal Institute of Great Britain* 39: 282–296.

Losey GS, McFarland WN, Loew ER, *et al*. (2003) Visual biology of Hawaiian coral reef fishes. I. Ocular transmission and visual pigments. *Copeia* 3: 433–454.

Lythgoe JN (1972) The adaptation of visual pigments to the photic environment. In: Autrum H, Jung R, Loewenstein WR, MacKay DM, and Teuber HL (eds.) *Handbook of Sensory Physiology* vol. VII/1 pp. 3–56. New York: Springer.

Lythgoe JN (1979) *The Ecology of Vision*. Oxford: Clarendon.

Marshall NJ (1999) The visual ecology of reef fish colours. In: Espmark Y, Amundsen T, and Rosenqvist G (eds.) *Animal Signals: Signalling and Signal Design in Animal Commumication*, pp. 83–120. Trondheim: Tapir Academic Press.

Marshall NJ (2000) Conspicuousness and camouflage with the same 'bright' colours in reef fishes. *Philosophical Transactions of the Royal Society of London B* 355: 1243–1248.

Marshall NJ, Jennings K, McFarland WN, Loew ER, and Losey GS (2003) Visual biology of Hawaiian coral reef fishes. II. Colors of Hawaiian coral reef fish. *Copeia* 3: 455–466.

Marshall NJ, Jennings K, McFarland WN, Loew ER, and Losey GS (2003) Visual biology of Hawaiian coral reef fishes. III. Environmental light and an integrated approach to the ecology of reef fish vision. *Copeia* 3: 467–480.

Marshall NJ and Vorobyev M (2003) The design of colour signals and colour vision in fishes. In: Marshall NJ and Collin SP (eds.) *Sensory Processing in the Aquatic Environment*, pp. 194–222. New York: Springer.

Marshall NJ, Vorobyev M, and Siebeck UE (2006) What does a reef fish see when it sees a reef fish? Eating 'Nemo'. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes*. Enfield, NH: Science.

McFarland WN (1991) The visual world of coral reef fishes. In: Sale PF (ed.) *The Ecology of Fishes on Coral Reefs*, pp. 16–38. San Diego: Academic Press.

Randall JE, Allen GR, and Steene RC (1997) *Fishes of the Great Barrier Reef and Coral Sea*. Bathurst: Crawford House.

Siebeck UE (2004) Communication in coral reef fish: The role of ultraviolet colour patterns in damselfish territorial behaviour. *Animal Behaviour* 68: 273–282.

Siebeck UE and Marshall NJ (2001) Ocular media transmission of coral reef fish – can coral reef fish see ultraviolet light? *Vision Research* 41: 133–149.

Thayer GH (1909) *Concealing-Coloration in the Animal Kingdom: An Exposition of the Laws of Disguise through Color and Pattern: Being a Summary of Abbott H. Thayer's Discoveries*. New York, NY: Macmillan.

Extraretinal Photoreception

H-J Wagner, Universität Tübingen, Tübingen, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Deep-Brain Photosensitivity
The Pineal Complex
Conclusion
Further Reading

Glossary

Circadian rhythm An internal rhythm, freerunning under constant conditions with a period slightly differing from the environmental 24-h cycle.

Diencephalon The caudal part of the prosencephalon (forebrain) associated with the third ventricle. The general subdivisions are (from dorsal to ventral): epithalamus, dorsal thalamus, subthalamus, and hypothalamus. The epithalamus is composed of the pineal gland and the habenular nuclear complex. The peripheral parts of the visual system including the retina of the lateral eyes are also derived from the diencephalon.

Ependyma The ciliated neuroepithelial cells lining the ventricular system. In the embryonic optic vesicle of the eye, cilia are of the sensory type and give rise to the outer segments of photoreceptor cells, the membranes of which contain the visual pigments.

Epithalamus The dorsal part of the diencephalon comprising the habenulae as part of the paleolimbic system and the pineal complex with associated structures such as the dorsal sac and the parapineal.

Hypothalamus The ventral part of the diencephalon comprising nuclei controlling vegetative functions either by direct neural connections or via the intermediary of the pituitary gland.

Melatonin The neurohormone synthesized and released in the dark by photoreceptor cells (or their derivatives, pinealocytes) in the retina and the pineal gland.

Opsin The membrane-bound G-protein-coupled receptor protein found in photoreceptors in the retina, which when combined to the chromophore retinal, forms a visual pigment.

Photopigment The labile molecules that change conformation upon absorbing light, thereby triggering various secondary-reaction cascades, with transducin and arrestin as intermediate enzymes. In photoreceptive cells, photopigments are composed of a protein moiety (generally called opsin) and a nonprotein chromophore part called retinal. Retinal-based opsins are called rhodopsins. The wavelength of the absorption maximum can be tuned by modifications of the amino acid sequence of the opsin molecule, and/or substituting retinal by dehydroretinal.

Pineal gland Part of the diencephalic epithalamus with cells specialized in the production of melatonin. In fish, it is composed of a stalk and a hollow vesicle located underneath a transparent window in the skull. The cellular components include photoreceptor and nerve cells.

Introduction

Photoreceptors are cells specialized to detect light, for example, the rods and cones of the retina of the eye. However, vertebrates have photoreceptors in addition to those located in the retina, referred to as extraretinal photoreceptors, and these form the focus of this article. These extraretinal photoreceptors have a cell structure similar to that of rods and cones and contain molecules that respond to light that are similar to those in rods and cones. There are extraretinal photoreceptors located in the skin, the cornea, the iris, and in the central nervous system (CNS). The structure and function of these photoreceptors located in the CNS are the subject of the present article. They are located in the brain itself and comprise the pineal gland, with associated structures, and a cluster of diverse nuclei, referred to as the deep-brain photoreceptive system, which is absent in mammals (**Figure 1**). Thus, in addition to vision, photoreception has an additional, and arguably even more fundamental, and phylogenetically more ancient role in all organisms, namely to synchronize them to the various cycles imposed on the earth by its position in the solar system, most importantly the daily and seasonal cycles of the temperate latitudes.

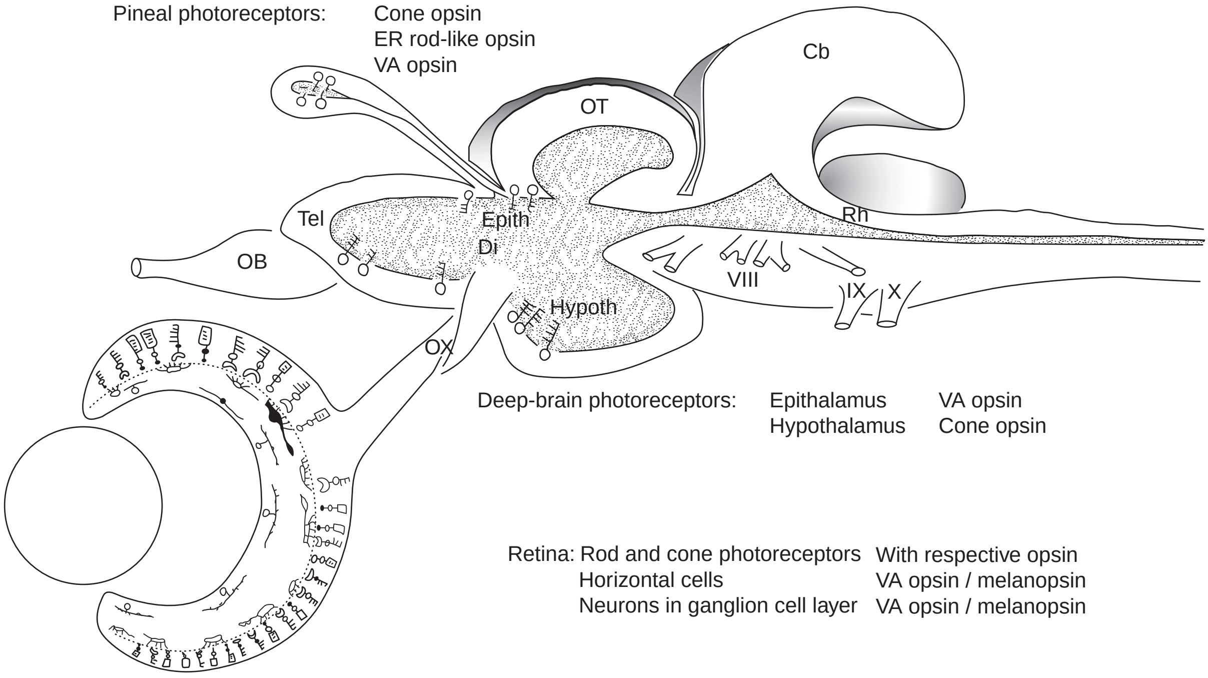


Figure 1 Semi-schematic drawing of a fish brain in midsagittal view including the retina, indicating the location of photoreceptive cells. OB, olfactory bulb; OT, optic tectum; OX, optic chiasm; Cb, cerebellum; Di, diencephalon, Rh, rhombencephalon; Tel, telencephalon; Epith, epithalamus; Hypoth, hypothalamus; VIII, IX, and X, cranial nerves.

The two different photoreceptor systems have been referred to as image forming or cognitive and nonimage forming or vegetative. In the vertebrate CNS, light sensitivity is a property of certain cells located in the part of the brain called the 'diencephalon'. Therefore, both the cognitive and the vegetative photoreceptive systems are derived from the same part of the brain. The forebrain of fishes consists of the more anterior telencephalon and the caudal diencephalon. The diencephalon is further subdivided into four parts: from dorsal to ventral they are the epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus. Deep photosensitivity is found in several areas of the lower diencephalon, a part of the hypothalamus. The photoreceptor cells are modified ependymal cells of the eyecup and the pineal vesicle. In the eye, the retinal primordium induces the formation of a complex image-forming optic apparatus, and the epithalamic pineal vesicle lacks this property; instead, it is associated with a transparent or translucent window in the skull. Light sensitivity in both cases is mediated by visual pigments called 'opsins', the molecular composition and the absorption spectra of which, while pointing to a common ancestral origin, show clear differences.

Deep-Brain Photosensitivity

While the pineal gland and its functional significance have been a matter of speculation and intense debate for several centuries, deep-brain light sensitivity has been recognized only since the beginning of the twentieth century. About 100 years ago, KV Frisch, working with minnows (*Phoxinus laevis*), observed that these fish could change color in response to light and darkness although they had had their eyes and pineal organs removed. Further lesion experiments led him to conclude that photosensitivity was localized in the basal hypothalamus, and he proposed that the ependymal cells lining the third ventricle were the probable functional units. Comparative studies subsequently showed functional light responsiveness in the deep forebrain of many other vertebrates ranging from lampreys to birds. Only after antibodies against various opsins, or transducin and arrestin, became available was it possible to take the search for deep-brain photosensitivity to the cellular level. **Figure 1** shows the location of the extraretinal photoreceptors.

In cyclostomes, representing an ancestral stage of vertebrate evolution, these markers labeled neurons in various regions of the diencephalon, such as the hypothalamus, the epithalamus, and posterior diencephalic areas. Such a heterogeneous and distributed localization might enable the receptor cells to be associated directly with their respective effector system.

Among teleosts, salmonids have been best studied for their organization of the deep-brain photosensory system. The epithalamic habenular region, situated close to the origin of the pineal stalk, contains the most prominent nuclei in this group. In the Atlantic salmon, *Salmo salar*, a member of the photopigment family of vertebrate ancient (VA) opsins is expressed in subependymal cells from the habenulae to the posterior commissure. This indicates a local and autonomous photosensitivity in a region that is the target of both retinal and pineal projections. The habenular region may therefore act as an integration site where the inputs from the retina and the pineal organ are compared with intrinsic signals. The role of this area as a paleolimbic relay center is also confirmed by its afferents and efferents: the habenulae receive afferent inputs from the entopeduncular nucleus in the preoptic region, the hypothalamic tuberculohabenular nucleus, and the preoptic region. Its efferents form the fasciculus retroflexus and target the interpeduncular nucleus, the isthmal raphe, and the central gray. In this manner, the habenulae would be in a position to integrate olfactory and photic stimuli to control feeding and reproductive behavior.

Apart from the habenular region, cells in several nuclei of the anterior hypothalamus, such as the suprachiasmatic nucleus and the magnocellular and parvocellular preopticus nuclei, are also photosensitive. However, they contain a photopigment different from the VA opsin in the habenular area; that is, they showed immunoreactivity against anti-cone opsin.

Yet another kind of photopigment was detected in the ventral telencephalon: anti-transducin antibodies labeled cells within the ventral telencephalon, a region implicated in photoreception in both reptiles and birds.

There appears to be no single and simple explanation for the diversity of photoreceptive systems deep in the fish brain. It has been speculated that various kinds of visual pigments may serve to broaden the range of wavelengths to be captured and also analyze the diurnal changes in the spectral composition of light. The differential receptive stimuli could then bring about specific vegetative and/or behavioral responses.

The Pineal Complex

In nonmammalian vertebrates, the pineal complex develops from the roof of the diencephalic forebrain, that is, the epithalamus. Generally, this is a bipartite intracranial complex composed of the pineal and the parapineal organ. In vertebrates where there is only one part of the complex, it is the pineal organ that is retained. In some vertebrates, there may be an extracranial component, which in frogs is termed as frontal organ or stirnorgan; however, in lizards, it becomes the parietal organ or parietal eye (the third eye). Typically, the pineal complex is located directly underneath the meninges and the skull. In mammals, by contrast, the pineal stalk is short and the large increase of the telencephalic hemispheres hides the pineal organ in the deep recess associated with the cerebellar tentorium and the large cerebral vein.

Parapineal and Pineal Organs in Lamprey

In hagfish, the habenulae form the most prominent epithalamic nuclear complex and a further differentiation into a pineal complex is absent. However, other cyclostomes, such as the river lamprey, show the typical components of the pineal complex. These include the epithelial saccus dorsalis, an ependymal diverticulum of the roof of the third ventricle, the pineal organ situated in the midline dorsal of the saccus dorsalis, and a more ventral parapineal organ (except in *Mordacia praecox*). The pineal and parapineal organs develop from a common precursor, a bilateral evagination from the epithalamus with the pineal forming the right, and the parapineal the left part. Both consist of a hollow terminal vesicle and a stalk, the lumen of which obliterates during development. The pineal vesicle is located directly underneath a melanocyte-free transparent window of the skull and epidermis.

Electrophysiological recordings indicate the presence of at least two classes of photoreceptors, a dominant group with a photopigment of a maximal sensitivity or peak absorption (λ_{max}) between 505 and 545 nm, and a second group with a photopigment of a λ_{max} around 380 nm implying ultraviolet (UV) sensitivity. The broad spectral range of the first group is due to species-specific differences and caused by varying ratios of retinal1/retinal2-based chromophores. These two populations of pineal photoreceptors (short-wave (UV) sensitive, and a middle-wave (green) sensitive) have also been detected by immunocytochemistry. In the underlying neuroepithelium of the pineal vesicle, neurons have been identified that transform the input of the two chromatic types of photoreceptors into a color-opponent response. In addition, there were neurons with an achromatic response.

Molecular work on genomic DNA in lampreys showed the presence of an opsin family that shared only 40% identity with the rod and cone opsins of the lateral eyes. Further analysis revealed that this lamprey opsin belonged to the teleost VA opsin family. In addition, complementary DNA (cDNA) sequencing identified another non-rod, non-cone opsin in the lamprey pineal complex belonging to the parapinopsin family. This photopigment was located in the dorsal region of both the pineal and parapineal organs, whereas rhodopsin was found in the ventral regions. The lamprey parapinopsin has a λ_{max} of about 370 nm and is bistable, because it is converted by UV light into a stable green-sensitive photoproduct with λ_{max} at 515 nm that in turn reverts to the UV pigment on absorption of middle-wave light, a unique case for a vertebrate opsin-based pigment. The behavior of the bistable pigment explains the electrophysiological responses of the chromatic secondary neuron type in lamprey pineal complex, whereas the rhodopsin would account for the achromatic neurons.

The Pineal of Teleosts

In holosteans and teleosts, the general organization of the pineal complex does not differ markedly from that of the lampreys, because we find a pineal and parapineal organ surrounded by the dorsal sac epithelium and situated dorsally underneath a translucent window of the bony or cartilaginous skull. Its role as a photosensory organ was first established in the rainbow trout (*Oncorhynchus mykiss*). Subsequent electron microscopic studies confirmed the presence of photoreceptor cells in the inner lining of the pineal vesicle whose morphology was in many aspects strikingly similar to their retinal counterparts. The discovery in trout pineal of hydroxyindole-*O*-methyl transferase, a key enzyme for the synthesis of melatonin, suggested that the pineal in fish represented a neuroendocrine organ capable of perceiving the photoperiod and using melatonin as the signaling mechanism with systemic targets. At least with respect to this last role, the pineal in fish is equivalent to that in the other vertebrates, but photosensitivity of pineal cells has been lost in the evolution of mammals.

The pineal gland of teleosts is connected to the habenular nuclei by the pineal stalk, which contains mostly efferent projections. Further targets include visual targets in the diencephalon such as the periventricular pretectum, the dorsal thalamus, the ventral thalamus, the posterior periventricular nucleus, and the mesencephalic tegmentum. Like the retina, the teleost pineal also receives afferent innervation via Phe-Met-Arg-Phe-NH2 (FMRFamide) immunoreactive fibers, originating in the nucleus olfactoretinalis (ventral telencephalon). The pineal organ is surrounded by a plexus of small capillaries.

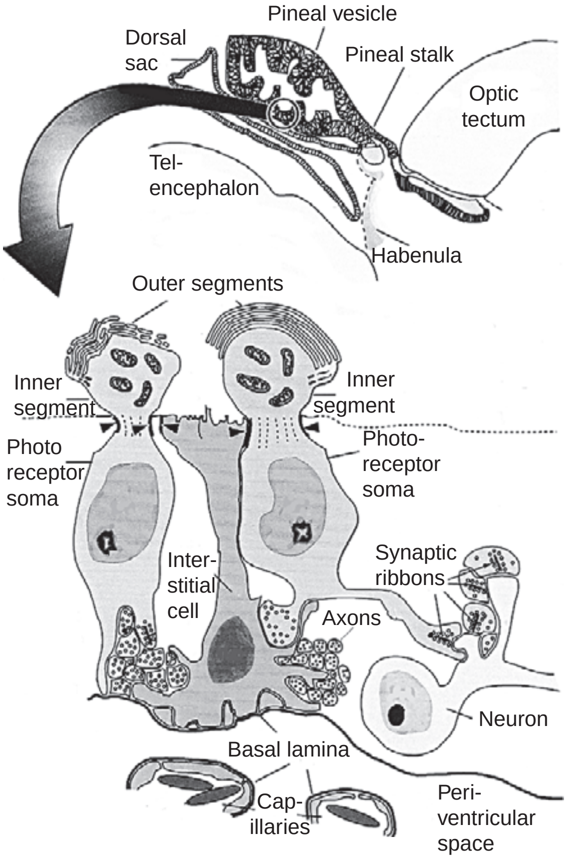


Figure 2 (Top) Schematic midsagittal section through the epithalamic region showing the pineal organ, the dorsal sac, and the habenula. (Bottom) Summary diagram of the cell types in the sensory epithelium of the pineal vesicle; arrowheads indicate tight junctions forming a barrier against the cerebrospinal fluid of the pineal lumen. Modified from Ekström P and Meissl H (1997) The pineal organ of teleost fishes. *Reviews in Fish Biology and Fisheries* 7: 199–284.

Structure and ultrastructure

The general morphology and ultrastructure of the pineal gland (vesicle) is characterized by the following features (**Figure 2**): two types of cells line the central lumen, ependymal-derived photoreceptor cells and radial glia cells spanning the entire width of the vesicle wall. The morphology of photoreceptors is variable and, unlike the retina, it is difficult to identify different types based on their structural appearance. The cell body is situated inside the neural epithelium and contains numerous mitochondria, endoplasmic reticulum, and Golgi apparatus, surrounding the nucleus. Like rod and cone cells, they have an inner and outer segment. The inner segment bulges into the central lumen and gives rise to a short outer segment. In contrast to rods and cones, the shape of the outer segment resembles an inverted cup, resulting in a concentric arrangement of the inner segment surrounded by a ring of outer segment disks in cross sections. Inner and outer segments are joined by the typical sensory cilium containing nine doublets of microtubules. The number of disks that makes up the outer segment varies considerably, between about 10 and 100. The outer segments are thus surrounded by cerebrospinal fluid and not by an epithelial sheath corresponding to the retinal pigment epithelium. The axon of the pineal photoreceptor may contact secondary neurons located in the wall of the pineal vesicle; alternatively, it may terminate against the basal membrane of the external limiting membrane and abut on the capillaries in the surrounding subarachnoidal space, or enter the pineal stalk and terminate in the brain. The wall of the pineal vesicle also contains interstitial neurons, and a neuropil of axonal, and dendritic processes in addition to glial processes. While it has been shown that some of these neurons project to targets in the brain via axons in the pineal stalk, it is as yet unclear whether there are also intrinsic or short axon interneurons, similar to retinal horizontal, bipolar, or amacrine cells that would provide for intrapineal signal processing. Synaptic contacts have been observed mainly between photoreceptors and these neurons. They have the typical hallmark of photoreceptor synapses, 'synaptic ribbons', that is, electron-dense plate-like presynaptic structures, which are surrounded by a dense halo of clear synaptic vesicles. Occasionally, conventional reciprocal synapses have been observed from interstitial neurons back onto photoreceptors.

More than 25 000 species of fish occupy photic environments, ranging from coral reefs exposed to tropical sun to dark caves and the abyssal and hadal depths outside the reach of sunlight. Therefore, it is not surprising that this diversity is reflected in an adaptive radiation of pineal morphology. Pineals of more than 100 species have been studied to date. Differences are found at all structural levels, including the shape (cylinder- to mushroom-like) and sheer volume of the pineal vesicles. Furthermore, the vesicle wall ranges from less than 20 μm to more than 100 μm in thickness and the lumen varies between widely patent to nearly occluded. The density of photoreceptors is as variable as the number of outer segment disks (see above). In a number of studies on deep-sea fish pineals, it has been proposed that the pineal increased in size as the fish inhabit deeper, and, therefore, dimmer environments, implying an increasing sensitivity associated with larger size. Such a correlation was, however, not confirmed in a subsequent comparison of 10 species of abyssal demersal fish, where species with small and large pineal organs were found living at similar depths. A more relevant observation in fish species living in continual darkness concerns the parallel differentiation of eyes and their retina, and the pineal. Whenever fish had large eyes and a highly differentiated retina, a well-developed pineal organ was found. By contrast, in the few species with reduced lateral eyes (spiderfish *Bathypterois dubius* and Southern cavefish *Typhlichthyes subterraneus*), a small and rudimentary pineal was observed. In the Mexican blind cavefish (*Astyanax mexicanus*), a fully functional pineal organ persists in spite of the complete degeneration of the eyes. They have fully developed photoreceptors, which confer a residual photosensitivity to the animal. Interestingly, during ontogeny, the pineal and its photoreceptors clearly precede the emergence of the lateral eyes, further supporting the notion of a considerable independence of the two systems (**Figure 3**).

Physiology: Light-evoked responses

Pineal photoreceptors function as luminance detectors, because the window in the skin and skull as well as the irregular organization and convolutions of the pineal epithelium allow only diffuse light to reach pineal photoreceptors. The absolute sensitivity of pineal photoreceptors and the time course of dark adaptation are in the same range as in retinal rods. However, the overall response characteristic is markedly different: pineal photoreceptors maintain the same response amplitude during the entire illumination period and maintain an intensity-related steady membrane potential. In terms of spectral sensitivity, most isolated photoreceptors from trout pineal showed peak responses between 520 and 530 nm; a minor population of cells had a λ_{max} of about 500 nm indicating a heterogeneity of visual pigments.

Extracellular and intracellular recordings from pineal neurons confirmed the function of the entire organ as a luminance detector. In goldfish (*Carassius auratus*) and pike (*Esox lucius*), spontaneously active cells were found whose discharge frequency was almost an exact mirror image of the intensity of the incident light. Electrotonic coupling of photoreceptors via gap junctions as well as a network of chemical synapses between the secondary pineal neurons provide two independent average systems that ensure a homogenous neural output. In pike and rainbow trout, antagonistic chromatic responses have been recorded from pineal neurons, where short-wavelength light caused inhibition, and long wavelengths resulted in excitation, with reciprocal sign-inverting interaction between the responses. As the spectral composition of the ambient light is different at dawn and dusk from that during the rest of the day, the antagonistic spectral organization would enhance the sensitivity of the pineal gland to perceive the change from scoto- to photophase (i.e., from night to day) and vice versa.

Visual pigments

The electrophysiological experiments on chromatic activity in teleost pineals were complemented by microspectrophotometric analyses on the absorption properties of visual pigments. Two photopigments were identified in trout (λ_{max} 460 and 560 nm), possibly three in pike (λ_{max} 380, 530, and 620 nm), but only one in cyprinids (λ_{max} 530 nm). Immunocytochemical work indicated that these pineal visual pigments were closely related to rod and/or cone-like visual pigments of retinal origin. However, while there was similarity because they were either rhodopsins or porphyropsins, there were also clear differences, because in no case were the absorption maxima of the visual pigments in a given species identical in the retina and in the pineal organ.

More recently, additional opsins have been identified in fish pineals that were either different from their retinal counterparts or not expressed at all in the retina. These include VA opsins and parapinopsin. Furthermore, an extraretinal rod-like opsin (referred to as errlo), first characterized in zebrafish (*Danio rerio*) is now assumed to be ubiquitous in teleost pineal organs. An interesting analogy to retinal opsin is, however, that the pineal errlo shows a similar wide degree of variation. In some shallow-water fish species, such as the goldfish and the blind cavefish, the

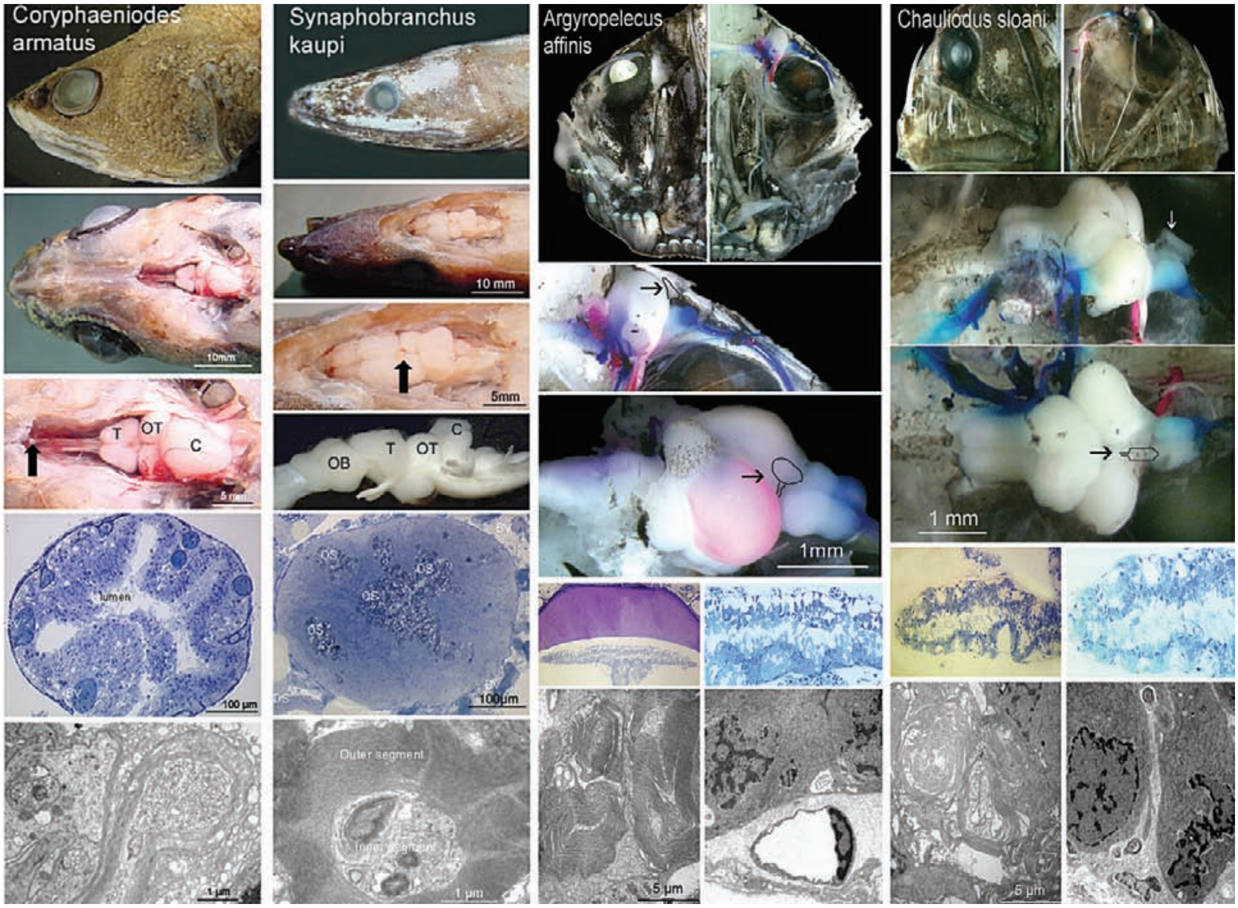


Figure 3 Morphology and fine structure of the pineal organs in four species of deep-sea fish. The columns show grenadier (*Coryphaenoides armatus*), deep-sea eel (*Synaphobranchus kaupii*), hatchetfish (*Argyropelecus affinis*), and viperfish (*Chauliodus sloani*). From top to bottom, each column shows the following panels: head, intact and with dissection of the brain to demonstrate the location of the pineal (arrows) in the cranial cavity. Some of the brains are labeled by carbocyanine dyes for unrelated experiments; light micrographs of cross sections of the pineal vesicles (methylene blue stain); electron micrographs of pineal photoreceptors and neurons. T, telencephalon; OT, optic tectum; C, cerebellum; OB, olfactory bulb.

λ_{max} of pinealocytes was shorter than in retinal rods, whereas in the orfe it was longer. This was mostly due to variations in the ratio of rhodopsin and porphyropsin. While the spectral composition of light reaching the pinealocytes in shallow-water habitats depends on the quality of the water body and the time of day (see above), and thus might account for this variability, photic conditions in the deep sea are much more constant, being centered around 480 nm below 200 m of depth. Surprisingly, however, a similar degree of variability and divergence was found in the errlo visual pigments in a comparative study of mesopelagic fish. While the rod visual pigments had λ_{max} peaks between 478 and 485 nm, the errlo λ_{max} was consistently displaced toward longer wavelengths between 485 and 505 nm, in one species (from below 2000 m) even as far as 515 nm. The great majority of the shallow- and deep-water species only had a single kind of visual pigment; however, the deep-sea hatchetfish had two populations of pinealocyte with visual pigment λ_{max} about 12 nm apart. Consequently, the variability of visual pigments in the pinealocytes of deep-sea fish was considerably greater than in the retinal rods. It is difficult to interpret this observation because there is general agreement that light intensities in the deep sea are not sufficient to penetrate the window in the skull and stimulate the pinealocytes in adult specimens. Possibly, pineal photoreceptivity had been functional during the larval stage, during which many deep-sea fish live at shallower depths. Alternatively, one may speculate that since light appears to be of no importance in deep-sea fish pineal organs, they are no longer subject to adaptational constraints to tune the sensitivity of their visual pigments to the wavelength of the downwelling light, a situation that would lead to the wide spread of λ_{max}. This is different from the situation in the highly specialized and functional eyes, whose rod rhodopsins cluster closely around the emission spectra of bioluminescence and the downwelling light of the sun.

The teleost pineal as a transducer of circadian rhythms

Vertebrate photoreceptor cells use two major neuroactive substances to transmit light-evoked changes of their membrane potential: in the retina, glutamate binds to receptor molecules on bipolar and horizontal cells, whereas melatonin acts as a neuromodulator and is involved in increasing sensitivity during dark adaptation. Although evidence for a role as neurotransmitter for glutamate in pinealocytes is available only in mammals, there is no reason to assume that the situation is different in fish. However, while the targets for melatonin in the retina are local, pineal melatonin takes on a systemic relevance. Through the blood stream, it reaches targets in the entire organism and is thus in a strategic position to synchronize or reset the multiple and individual endogenous oscillators (biological clocks) in the peripheral tissues and organs to the actual photoperiod perceived directly through the window in the skull and the photoreceptors.

Conclusion

Non-image-forming extraretinal photoreception via deep brain cells and/or the pineal organ is crucial for the control of rhythmic processes and behaviors in fish. They synchronize internal molecular clocks to the environment and are therefore involved in the organization of circadian, seasonal, and circannual rhythms. In this manner, they contribute fundamentally to daily feeding and seasonal reproductive behavior, and also to developmental processes associated with seasonal or circannual migration and/or smoltification. Results from this research are also of applied relevance and have contributed to the design of lighting system used for fish farming with the aim of influencing growth processes.

See also: **Vision**: Adaptations of Photoreceptors and Visual Pigments; Inner Retina and Ganglion Cells; Photoreceptors and Visual Pigments.

Further Reading

Ali MA (1992) *Rhythms in Fishes*. New York: Plenum.

Bowmaker JK and Wagner H-J (2004) Pineal organs of deep-sea fish: Photopigments and structure. *Journal of Experimental Biology* 207: 2379–2387.

Ekström P and Meissl H (1997) The pineal organ of teleost fishes. *Reviews in Fish Biology and Fisheries* 7: 199–284.

Foster RG, Grace MS, Provencio I, de Grip WJ, and Garcia-Fernandez JM (1994) Identification of vertebrate deep brain photoreceptors. *Neuroscience and Biobehavioural Review* 18: 541–546.

Foster RG and Kreitzman L (2004) *Rhythms of Life The Biological Clocks That Control the Daily Lives of Every Living Thing*. London: Profile Books.

Foster RG, Wagner H-J, and Bowmaker JK (2006) Non-image-forming photoreception and its role in communication. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes*, pp. 543–575. Enfield, NJ: Science Publishers.

Philip AR, Garcia-Fernandez JM, Soni BG, *et al.* (2000) Vertebrate ancient (VA) opsin and extraretinal photoreception in the Atlantic salmon (*Salmo salar*). *Journal of Experimental Biology* 203: 1925–1936.

Roenneberg T and Foster RG (1997) Twilight times: Light and the circadian system. *Photochemistry and Photobiology* 66: 549–561.

Wagner H-J and Mattheus U (2002) Pineal organs in deep demersal fish. *Cell and Tissue Research* 307: 115–127.

Wicht H and Niuewenhuys R (1998) Hagfishes (*Myxinoidea)*. In: Nieuwenhuys R, ten Donkelaar HJ, and Nicholson C (eds.) *The Central Nervous System of Vertebrates*, pp. 497–549. Berlin: Springer.

Visual Adaptations to the Deep Sea

RH Douglas, City University London, London, UK

JC Partridge, University of Bristol, Bristol, UK

© 2011 Elsevier Inc. All rights reserved.

The Deep-Sea Light Environment
Deep-Sea Eyes Are Adapted to a Photon-Limited Environment
Ocular Anatomy of Deep-Sea Fish
Visual Pigments
Further Reading

Glossary

Benthic On or near, the seafloor.

Bioluminescence Light produced by living organisms by physiological processes and without heat.

Diverticulum An out-pocketing, or blind-ending sac, branching out from a hollow anatomical structure, here used to refer to ventrolaterally facing 'accessory eyes' of some mesopelagic fish with dorsally directed tubular eyes.

Electroretinogram (ERG) A recording of the retina's electrical activity in response to light, usually recorded on either the cornea or retinal surface.

Endosymbiont An organism that lives symbiotically within the body of another.

G protein A large class of intracellular, membrane-associated, proteins that act as second messengers for a wide range of receptors (including visual pigments) and transduce receptor signals by activating a chain of cellular events resulting in amplification and cell signaling.

Hadal Biogeographic region of the ocean bottom below the abyssal zone; typically, ocean trenches deeper than *c.* 6500 m depth.

Hyposchromic Change in the wavelength of maximum absorption, transmission, reflection, etc. of a molecule toward higher frequencies or shorter wavelengths (sometimes called a 'blue shift').

Mesopelagic A biogeographic region of open oceans between 200- and 1000-m depth and not near the ocean floor.

Myctophid A family of teleosts, known as lantern fish, whose name originates from their conspicuous bioluminescent photophores. They account for up to 65% of the deep-sea fish biomass and are among the most widely distributed and populous of all vertebrates.

The deep sea encompasses those parts of the ocean below 200 m, where insufficient sunlight penetrates to allow photosynthesis. Due to its high pressure (0.1 MPa or *c.* 1 atm for every 10 m of depth), low temperature, and relative lack of light (**Figure 1**), it is often regarded as an unusual and extreme environment. However, this is true only when seen from the viewpoint of a terrestrial animal. The often higher temperatures and particularly the intense (and potentially damaging) sunlight to which land animals are exposed could be regarded as equally extreme. As the ocean covers over 70% of the Earth's surface with an average depth of around 4000 m, over 99% of habitable space on the planet is deep sea making it anything but unusual. Neither is the deep sea lifeless. Teleosts have recently been filmed in some of the deepest Hadal trenches at nearly 8000 m (although jawless and cartilaginous fish are absent below 4000 m). Not surprisingly, given its vast volume, the deep sea is the home of the world's most abundant vertebrate genus, *Cyclothone*, and many of the species that live there far outnumber human beings (**see also Deep-Sea Fishes**).

Despite its importance, the deep sea is the least understood environment on Earth. Although animals can be observed *in situ* by remotely operated vehicles, autonomous landers, and submersible, these generally utilize bright lights and generate significant physical disturbance. Also, laboratory observation of deep-sea fish is not feasible as it is impossible to ensure the long-term survival of captured animals. As a consequence, the behavior of deep-sea animals remains relatively unexplored. However, since most animal behaviors are at least influenced by, and often the direct result of, visual stimuli, much can be inferred indirectly about the life of deep-sea creatures by examining their eyes. Volumetric analysis of various sensory brain regions indicates that although chemical and mechanical sensory modalities are important to deep-sea fish, the most important sense for most demersal and mesopelagic species is vision.

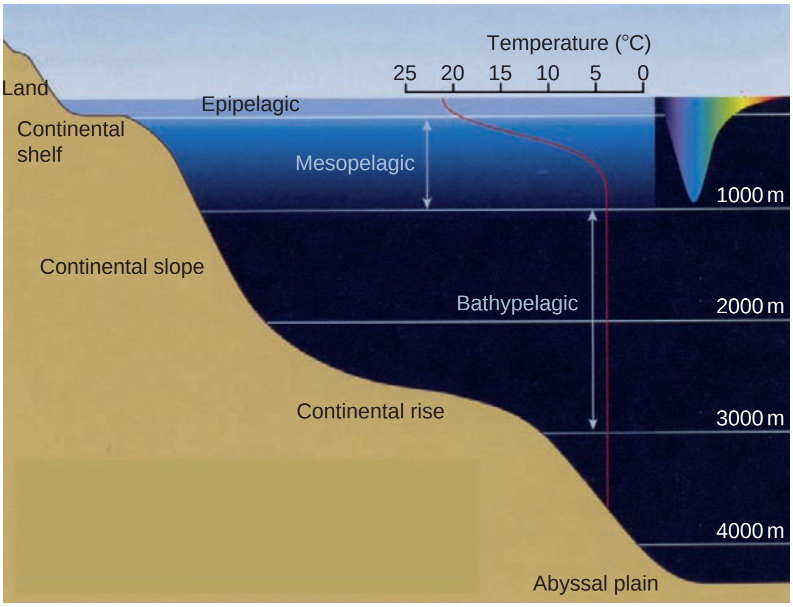


Figure 1 Diagram of the oceanic environment highlighting the amount of light (shades of blue/black), the temperature and the visible spectrum with increasing depth. From Douglas RH (2006) Visions of the deep. *Biological Sciences Review* 19(1): 36–41.

The Deep-Sea Light Environment

The presence of large eyes in the majority of fish inhabiting the deep sea is clear testament that their environment is not the realm of perpetual darkness often imagined. During the day, sunlight illuminates the upper reaches of the water column. However, its intensity rapidly decreases and its spectral composition becomes restricted with depth. By 600–700 m in depth, light levels are approaching those provided by starlight at the water surface and, in even the clearest water, at depths exceeding 1000 m, there is insufficient sunlight energy to stimulate photoreceptors and enable vision. As most waters have a degree of turbidity, vision using sunlight usually becomes impossible in waters shallower than this. Long and, to a lesser extent, short wavelengths are attenuated more by the sea than other wavelengths, so that at depth the predominant part of the light spectrum is narrowed to around 460–480 nm (**Figure 1**).

However, the light produced by most species in the deep sea is arguably a more important source of visual information than sunlight. Although during the day in shallower water the contrast of this bioluminescence is too low for it to be significant, it becomes more important with depth and at night as the veiling sunlight is removed.

In most fish bioluminescence is produced when an enzyme (luciferase) catalyzes the oxidation of a substrate (luciferin) within light organs (photophores) on the animal's body (**Figure 2(c)**), but in a few species it results from endosymbiotic bacteria (**Figure 2(d)**) (**see also The Skin**: Bioluminescence in Fishes). Bioluminescence has many potential functions including: the simple illumination of an otherwise dark world, attracting mates, avoiding predation, and aiding the capture of prey (**Figure 2**). The peak emissions of most bioluminescence, although in a similar part of the spectrum to the residual sunlight, cover a wider range (450–510 nm) and their bandwidth may be broader.

Thus, what animals view changes depending on their depth, the water clarity, and the time of day; from extended sunlit visual scenes near the water surface during the day to bioluminescent point sources in deeper water and at night.

Deep-Sea Eyes Are Adapted to a Photon-Limited Environment

There is not much light in the deep sea; not only does sunlight rapidly decrease in intensity with depth, but also bioluminescence is not as frequent as might be supposed when descending the water column in a submersible. Although one is surrounded by the 'fireworks' emitted by a plethora of animals, they are reacting to the presence of the submersible. When the submersible stops, darkness returns. Since bioluminescence is 'expensive' to produce it is usually only emitted sporadically. The relative paucity of light in the deep sea means that most visual adaptations of the fish living there serve to increase sensitivity.

Visual sensitivity to extended sources is maximized by a large aperture, each photoreceptor sampling a large volume of visual space and absorbing as much light incident on the photoreceptor as possible. The eyes of many mesopelagic deep-sea fish, therefore, have enlarged pupils, a short focal length lens, and long/wide photoreceptors with a high

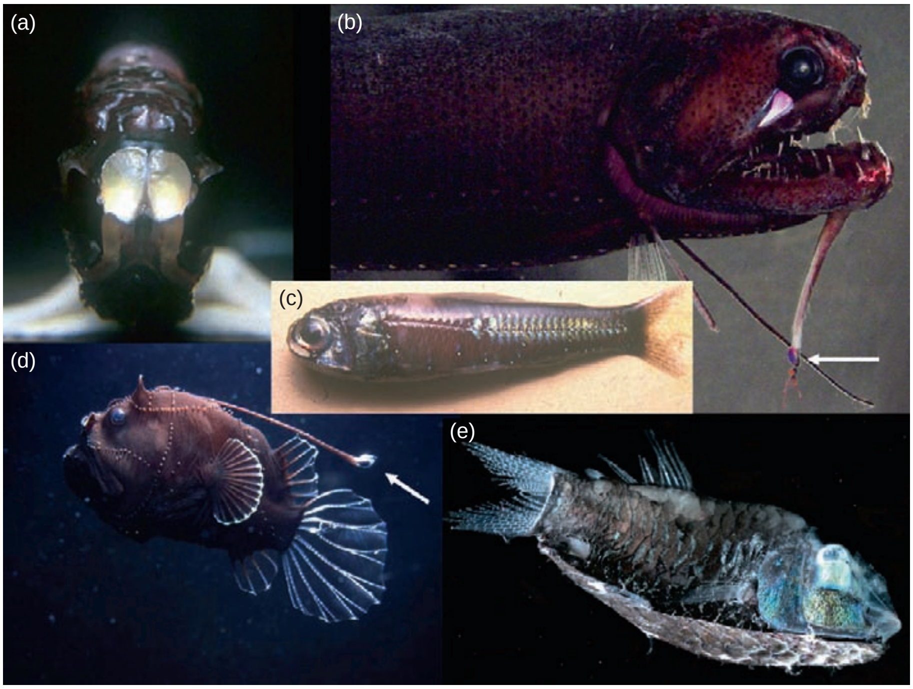


Figure 2 The functions of bioluminescence in deep-sea fish. (a) The 'headlamps' on this myctophid (*Diaphus* sp.) seen from head on, emit diffuse light illuminating the water ahead of the animal. (b) Photophores are often positioned below the eye, as in *Echiostoma barbatum*, so that the object being looked at is illuminated. Such photophores radiate over a wide angle and would be seen from many directions. *Echistoma* also has a bioluminescent barble hanging below its lower jaw (arrow) presumably to act as a lure to attract prey or mates as in the angler fish (see (d)). (c) Researchers often use the pattern of photophores on the body of a fish as a guide to identification. Perhaps fish, such as this myctophid (*Diaphus holti*), can also do this? The majority of the animal's lateral photophores are outside its own visual field and it is tempting to suggest that they are for communication. However, most of the emission is downward, suggesting that communication may be secondary to ventral camouflage (see **Figure 2(e)**). (d) Angler fish, such as this *Phrynichthys wedli*, hang a bioluminescent esca (arrow) in front of their mouth as a lure to attract prey. (e) Seen from below animals will cast a dark silhouette against the residual sunlight. *Opisthoproctus soleatus* obscures the silhouette of its very wide ventral 'sole' with bioluminescence produced by symbiotic bacteria within an anal pouch. Bioluminescence thus serves as a form of counterillumination camouflage. (a) Photo courtesy of S. Collin. (b) Photo courtesy of T. Frank. (c, d) Photo courtesy of J. Marshall. (e) Photo courtesy of E. Widder.

absorption coefficient coupled to a reflective tapetum. Sensitivity can be further enhanced by both temporal and spatial summation of photoreceptor outputs. Consequently, deep-sea fishes are approximately 120 times more sensitive to extended sources than man.

However, a point source will be focused to a similar point on the retina (assuming little scatter and few aberrations). Thus, although wide pupils and large, highly absorbent, photoreceptors will enhance sensitivity whatever the light source, increasing the volume of visual space sampled by each photoreceptor through a short focal length lens and spatial summation of photoreceptor output will not increase sensitivity to bioluminescent point sources.

Unfortunately, adaptations that enhance sensitivity are often detrimental for spatial resolution. However, most deep-sea fish must also be able to resolve some degree of spatial information to, for example, locate bioluminescent point sources in deeper water and at night, or resolve spatial detail within the extended visual scenes produced by sunlight. Thus, while most of the retina in deep-sea fish is indeed adapted to optimize sensitivity, some species also possess regions of increased spatial acuity.

Ocular Anatomy of Deep-Sea Fish

Outline of the Teleost Eye

The eyes of the vast majority of vertebrates have a similar construction with a single cornea and lens forming an image on a light-sensitive retina (**see also Vision**: Physiological Optics in Fishes). While the eyes of a few deep-sea fish are reduced or degenerate, most are broadly similar to those of more familiar fish (**Figure 3**). Such eyes generally have a tough outer sclera composed mainly of cartilage and collagen, which anteriorally, along with a layer derived from the skin, forms a transparent cornea. Throughout much of the eye a reflective argentea lies internal to the sclera, separating it from the vascular uveal tract. This uveal layer is composed posteriorally of the choroid, which

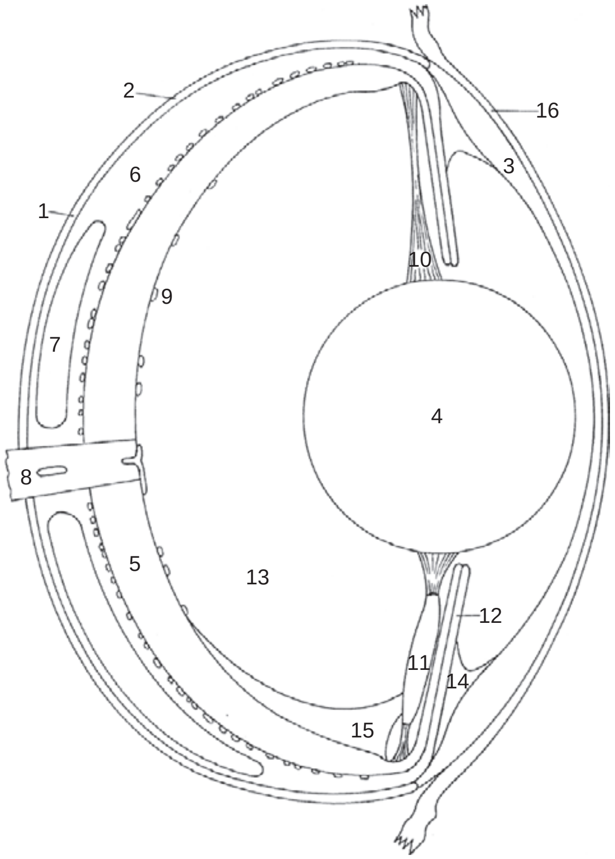


Figure 3 Vertical section through a generalized teleost eye. 1, sclera; 2, scleral cartilage; 3, cornea; 4, lens; 5, retina; 6, choroid; 7, choroidal rete mirabile (**see also Design and Physiology of Arteries and Veins:** The Retia); 8, optic nerve; 9, hyaloid vessel; 10, lens suspensory ligaments; 11, retractor lentis muscle; 12, iris; 13, vitreous; 14, annular ligament; 15, falciform process; 16, dermal component of cornea. From Locket NA (1977) Adaptations to the deep-sea environment. In: Crescitelli F (ed.) *Handbook of Sensory Physiology*, *Vol. VII/5. The Visual System in Vertebrates*, pp. 67–192. Berlin: Springer.

anteriorally is continuous with a small ciliary region and an inward projecting iris forming the pupil, which in most teleosts is immobile. The internal surface of the posterior globe is covered by the light-sensitive retina which receives a focused image formed by a large spherical lens, whose plane of focus can be adjusted (accommodation) by altering its distance from the retina with one or two retractor muscles. However, within this basic framework, the eyes of deep-sea fish show specific adaptations in comparison to their shallower dwelling relatives.

Eye Shape

The eyes of most shallow water fish, with some exceptions such as flatfish, roughly approximate a laterally flattened sphere and are usually situated laterally on the head (**Figure 4**(**a**)). The same is true for most deep-sea fish, although some mesopelagic species have very differently shaped, dorsally positioned eyes (**Figure 4**(**b**)).

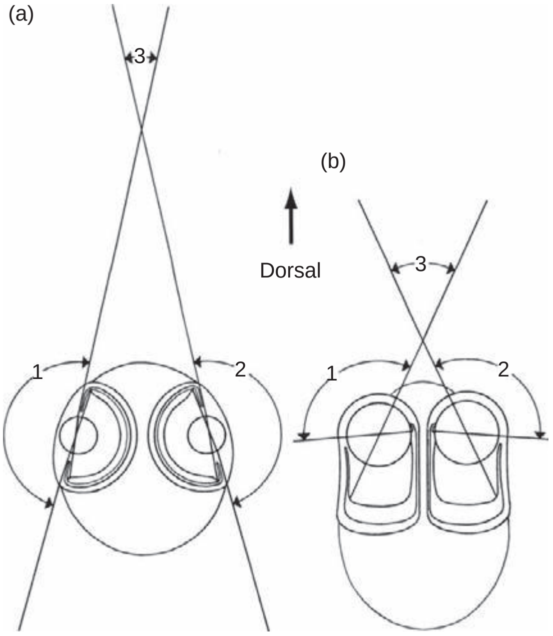


Figure 4 The two major eye ‘designs’ of mesopelagic fish. (a) ‘Typical’ laterally placed eyes with large lateral monocular fields of view (1 and 2) and a small frontal binocular field (3). (b) Dorsally directed ‘tubular eyes’ with restricted lateral monocular field (1 and 2) but a larger dorsally situated area of binocular vision (3). From Warrant EJ, Collin SP, and Locket NA (2003) Eye design and vision in deep-sea fish. In: Collin SP and Marshall NB (eds.) *Sensory Processing in Aquatic Environments*, pp. 303–322. New York, NY: Springer.

Tubular eyes

Sunlight in the open ocean is several hundred times more intense from above than below. In order to maximize sensitivity to the relatively brighter downwelling sunlight, to see in silhouette animals above them, and to increase the binocular overlap of their eyes thus aiding both sensitivity and range finding, some mesopelagic fish have upwardly directed tubular eyes (**Figures 4**(**b**), **5**, and **6**).

Although in most cases such eyes are directed toward the water surface (**Figures 2**(**e**) and **5**(**a**)–**5**(**d**)), their orientation is not always fixed. The barreleye, *Macropinna microstoma*, for instance, is able to rotate its normally dorsally directed tubular eyes rostrally, directing its eyes toward its horizontally orientated mouth. It is possible that other fish with tubular eyes are also able to move their eyes in a similar manner.

The base of a tubular eye is covered by a well-developed ‘main’ retina, which terminates abruptly at the medial wall of the cylinder that is mostly covered by a thinner ‘accessory’ retina (**Figures 6**(**b**), **7**, **8**, and **10**(**b**)).

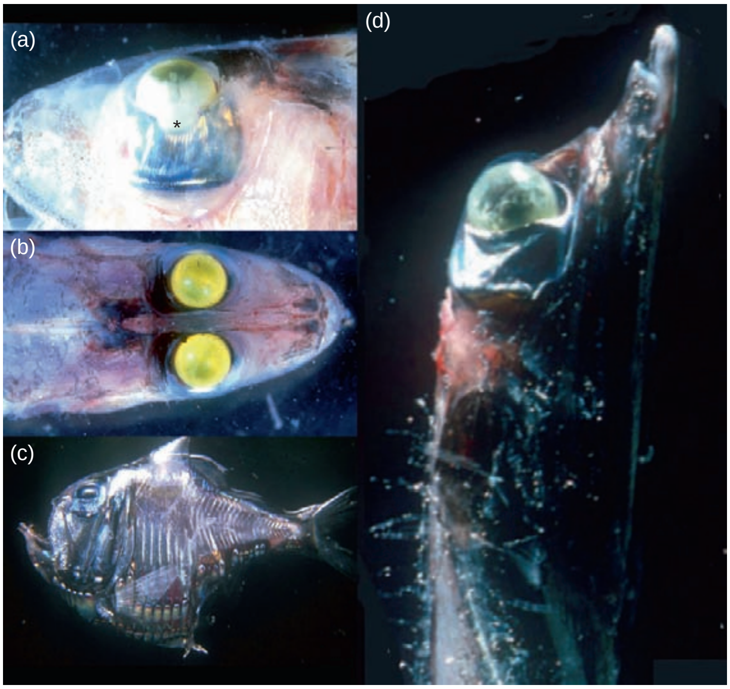


Figure 5 Mesopelagic fish with tubular eyes. (a) *Scopelarchus analis* viewed from the side. The asterisk indicates the lens pad. (b) *Scopelarchus analis* seen from above, highlighting the dorsally directed nature of the tubular eyes. (c) *Argyropelecus aculeatus* side view. (d) *Stylephorus chordatus* side view. This animal hangs vertically in the water column as shown. (a, b) Photo courtesy of J. Marshall. (c, d) Photo courtesy of S. Collin.

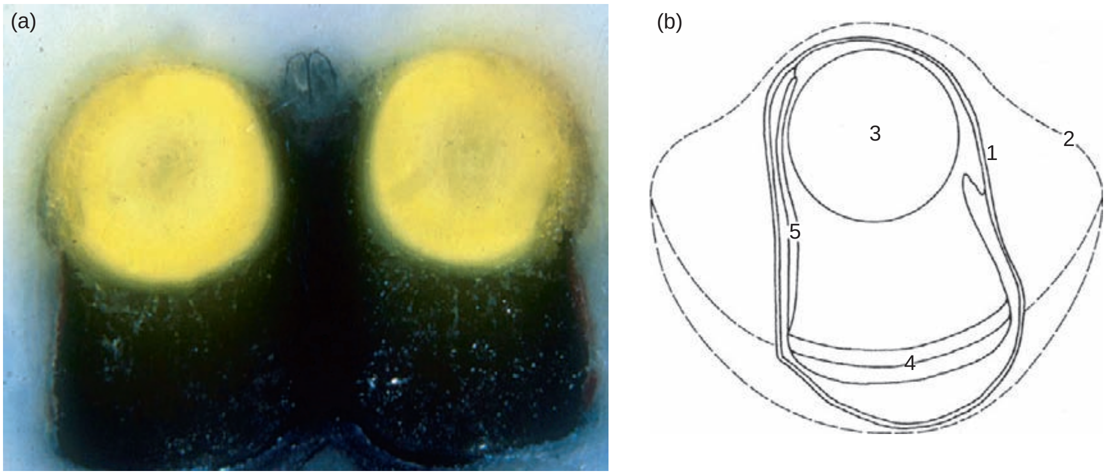


Figure 6 (a) Cryostat section of the head of *Scopelarcus analis* showing the animal's yellow lenses and how their tubular eyes completely fill the head. A conventional eye with a comparable f-number would require a much larger head. (b) Outline of a tubular eye (1) superimposed on that of a normal fish eye (2) with the same size of lens (3). The main retina of the tubular eye (4) corresponds to the central part of the normal eye. 5, Accessory retina of the tubular eye. (a) Photo courtesy of J. Marshall. (b) From Locket NA (1977) Adaptations to the deep-sea environment. In: Crescitelli F (ed.) *Handbook of Sensory Physiology*, *Vol. VII/5. The Visual System in Vertebrates*, pp. 67–192. Berlin: Springer.

The other walls of the cylinder are mainly covered by a thin layer of epithelial, uveal, and argenteal layers that line the sclera.

In fish, a well-focused image is produced when the retina is approximately 2.55 lens radii from the center of the lens (Matthiessen's ratio). In tubular eyes, this is the case only for the main retina. The accessory retina covering the medial wall is too close to the lens to receive a focused image and thus serves simple light detection.

A tubular eye with a given pupil size will take up far less space than a conventional eye with the

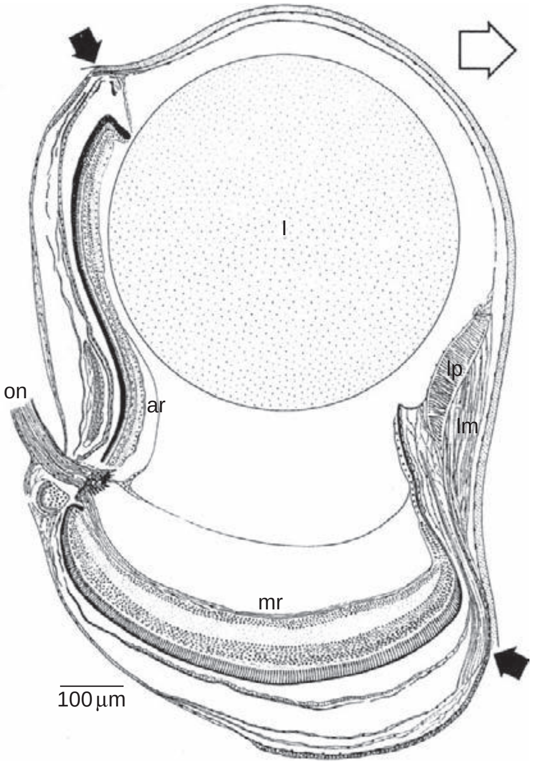


Figure 7 Transverse section through the eye of *Scopelarchus guentheri*. The area between the arrows indicates the cornea covering the lens and the lateral wall of the eye cup. Open arrow points laterally. 'lp' is the lens pad composed of angled, corneally derived, cellular lamellae that directs light onto the dorsal accessory retina. tm, modified corneal tissue; mr, main retina; ar, accessory retina; on, optic nerve; lm, lens muscle. From Munk O (1966) Ocular anatomy of some deep-sea teleosts. *Dana Report* 70: 1–62.

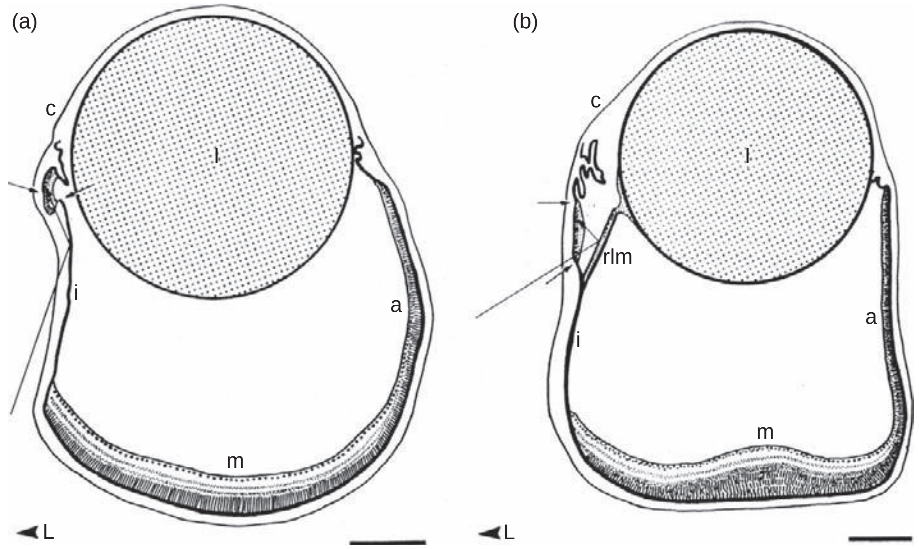


Figure 8 Transverse section of the eyes of (a) *Opisthoproctus grimaldii* and (b) *Stylephorus chordatus*. In *O. grimaldii*, light is reflected by a silvery iris (i) onto diverticular photoreceptors (arrows), whereas in *S. chordatus* the light is reflected by silvery deposits in the retractor lentis muscle (rlm). a and m, accessory and main retina; c, cornea; l, lens; L, lateral. Scale = 0.5 mm (a) and 1.0 mm (b). From Collin SP, Hoskins RV, and Partridge JC (1997) Tubular eyes of deep-sea fishes: A comparative study of retinal topography. *Brain Behavior and Evolution* 50: 335–357.

similarly sized aperture (**Figure 6**), thus avoiding the need for a disproportionately large head when optimizing sensitivity. The penalty, however, is a reduced field of view.

Methods of enlarging the visual field

If the sun was the only light in the deep sea, tubular eyes, with their restricted dorsally facing visual field, would be optimal (**Figure 4(b)**). However, bioluminescence, arguably the most important source of illumination in the deep sea, occurs with equal frequency and intensity all around an animal and, consequently, much of it would remain undetected by fish with upward pointing tubular eyes. Some mesopelagic fish with tubular eyes, therefore, have various devices for extending their visual fields laterally and ventrally.

Members of the Scopelarchidae (**Figure 5(a)**), for example, have a 'lens pad' in the lateral wall of their tubular eye that directs ventro-lateral illumination onto the accessory retina (**Figure 7**). A similar function is achieved by the epidermally derived optical fold in Evermannellids. Alternatively, seven genera have extended their visual fields by diverticula on the eyes' lateral walls, where light enters through an unpigmented outward-facing wall reflecting onto a laterally facing area of retina (**Figure 8**). *Bathylychnops exilis*, although it does not have tubular eyes, also has an extensive diverticulum into which light enters through a scleral lens (**Figure 9**). However, in all these species (except perhaps *Bathylychnops*), these devices only provide unfocused images with little spatial resolution.

Uniquely, *Dolichopteryx longipes* has a bipartite eye composed of a conventional tubular eye and a lateral diverticulum that light enters through a ventro-laterally facing cornea (**Figure 10**). The retina of the diverticulum is positioned laterally and light is reflected onto it by a mirror, derived from the retinal tapetum that separates the diverticulum and the tubular portion of the eye (**Figure 10**). A focused image is generated by thousands of reflective plates whose orientation varies depending on their location within the mirror. This is the only

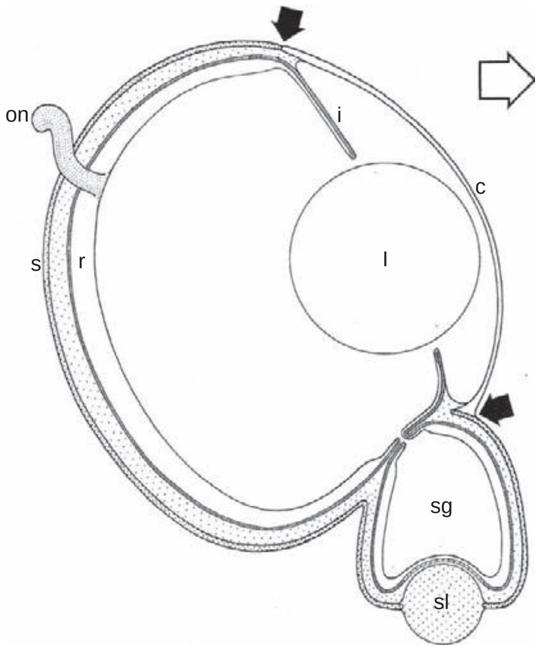


Figure 9 Transverse section of the eye of *Bathlychnops exilis*. The thick arrows indicate the extent of the cornea and the open arrow points laterally. i, iris; c, cornea; l, lens; on, optic nerve; s, sclera; r, retina; sg, secondary globe; sl, scleral lens. From Munk O (1966) Ocular anatomy of some deep-sea teleosts. *Dana Report* 70: 1–62.

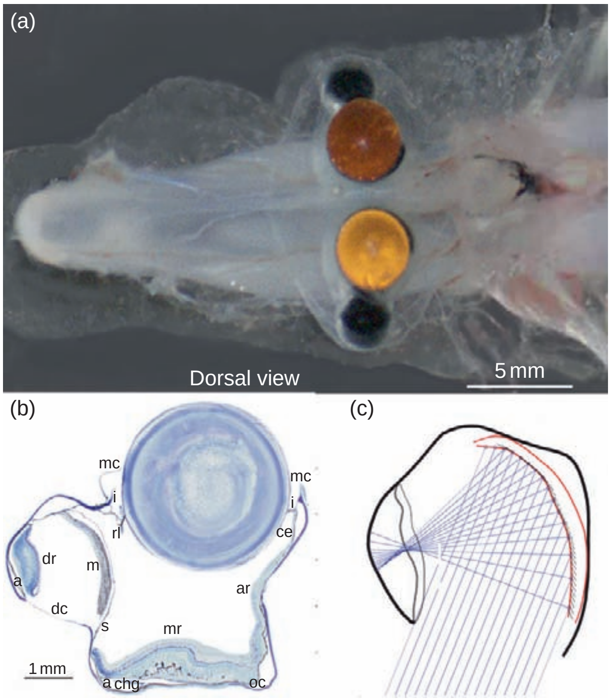


Figure 10 *Dolichopteryx longipes*. (a) Dorsal view of the head. Note the orange eyeshine of the main dorsally directed tubular eye. The black swelling on the lateral face of the tubular eye is the upper surface of the downwardly directed diverticulum.
(b) Transverse section of the right eye showing both the upwardly directed tubular portion and the lateroventrally directed diverticulum. a, argentea; ar, accessory retina; ce, ciliary epithelium; chg, choroid gland; dc, diverticular cornea; dr, diverticular retina; i, iris; m, mirror; mc, main cornea (partially removed); mr, main retina; oc, outer coats of the eye (consisting of sclera, argentea, and choroid); rl, retractor lentis muscle; s, septum between the main tubular eye and the diverticulum.
(c) Schematic diagram of a transverse section of the diverticulum, showing the output of a model that traced rays from a distant point source, located lateroventral to the diverticulum. Light rays are reflected onto the lateral diverticular retina by reflective plates on its medial wall forming a mirror and focusing light at the retinal outer limiting membrane (indicated by the dotted line within the retina). From Wagner HJ, Douglas RH, Frank TM, Roberts NW, and Partridge JC (2009) A novel vertebrate eye using both refractive and reflective optics. *Current Biology* 19(2): 108–114.

known instance of image formation by a mirror in a vertebrate and allows the spookfish to see focused images both dorsally, with the refractive tubular eye, and ventro-laterally, with the reflective diverticulum.

Lenses

In terrestrial animals the major refractive component is the cornea. Underwater, however, as the cornea has a similar refractive index to water, it is optically ineffective and most refraction in fish is performed by an optically powerful, spherical, lens.

Counterintuitively, the lenses of some mesopelagic fish absorb short wavelengths (**Figure 11**) and thus appear bright yellow (**Figures 5(a)**, **5(b)**, **5(d)**, and **6(a)**). Intraocular short-wave-absorbing filters are common in shallow water and terrestrial animals, where light levels are high and they serve to protect the retina from damaging short wavelengths and improve image quality by decreasing the amount of Rayleigh scatter and chromatic aberration. Deep-sea fish, in their light-limited environment, are unlikely to benefit from these functions. In fact, such lenses potentially decrease sensitivity in an already photon-limited environment. The chemical diversity of the pigmentation in yellow lenses, however, suggests that they have evolved on a number of occasions in mesopelagic species and implies that they are important. Perhaps they increase the contrast, and hence visibility, of relatively long-wave-rich bioluminescent signals seen against the short-wave-dominated background radiation.

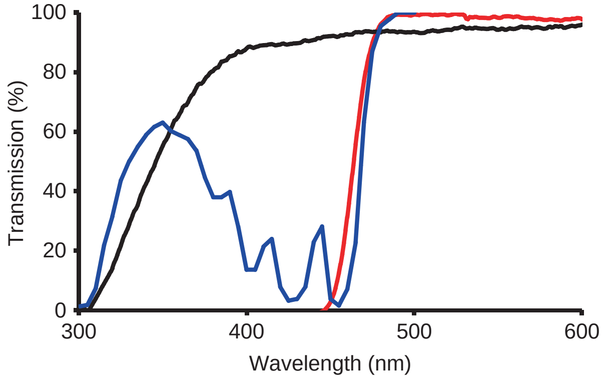


Figure 11 Spectral transmission of the lenses of three mesopelagic teleosts. The unpigmented lenses of *Protomyctophum germanium* (black) are as transparent as a vertebrate lens can be, transmitting all radiation down to around 300 nm, below which the structural proteins and nucleic acids absorb the radiation. The lenses of *Argyropelecus sladeni* (blue) appear yellow to the human eye and contain carotenoids that remove most light between 400 and 460 nm. *Benthalbella macropinna* lenses (red) also appear yellow and absorb most light below 450 nm.

Retinal Structure

The retina of deep-sea fish is similar to that of other vertebrates, consisting of six neural cell types and neuroglia arranged in 10 more or less well-defined layers (**Figure 12**) (**see also Vision**: Inner Retina and Ganglion Cells). Light traverses the retina and is absorbed by visual pigments housed within the membranes of disks located in the outer segments of photoreceptors, which lie adjacent to a simple epithelial layer, the retinal pigment epithelium (RPE). The resulting electrophysiological signal is transmitted vitreally through the remainder of the photoreceptor, whose output is processed by the other cells of the retina before being transferred to the brain by ganglion cells whose axons form the bulk of the optic nerve.

Most vertebrates, including surface dwelling fish, have two types of image-forming photoreceptors: rods and cones (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments). The high packing density of cones, their relatively low degree of postreceptor convergence, and the inhibitory interactions between different spectral cone types provide color perception with high spatial and temporal resolution. However, detailed color vision is a luxury only afforded by high (photopic) light levels. In photon-limited (scotopic) environments a more fundamental requirement is to capture enough light to enable any vision at all. This is provided by the rods, whose extensive temporal and spatial summation provide vision with high sensitivity.

Fish living near the water surface have both rods and cones and switch from one to the other using a variety of physiological, biochemical, and morphological processes at twilight. Although rods far outnumber cones in most species, nocturnal animals have relatively more rods but as they are still occasionally exposed to higher light levels, they retain a significant number of cones. Although a few species of deep-sea fish possess a small population of cones, especially as larvae when they may live in shallower water, and a few species even have a cone-dominated retinae, the majority of deep-sea fishes never experience photopic illumination and thus have pure-rod retinae.

Retinal modifications to increase sensitivity

Photon capture in many deep-sea fish is increased in comparison with other vertebrates by either an increase in length and/or width of the rod outer segments (**Figure 13(C)**) or the possession of several layers of rod outer segments which are of similar size to those of other animals (**Figures 13(B)** and **14**). Thus, their photoreceptor layer is considerably thicker (*c.* 120–180 μm) than that of other vertebrates. The relatively lower density of ganglion cells in deep-sea species, as evidenced by the relatively thinner inner retinal layers, results in the output of many photoreceptors converging onto relatively

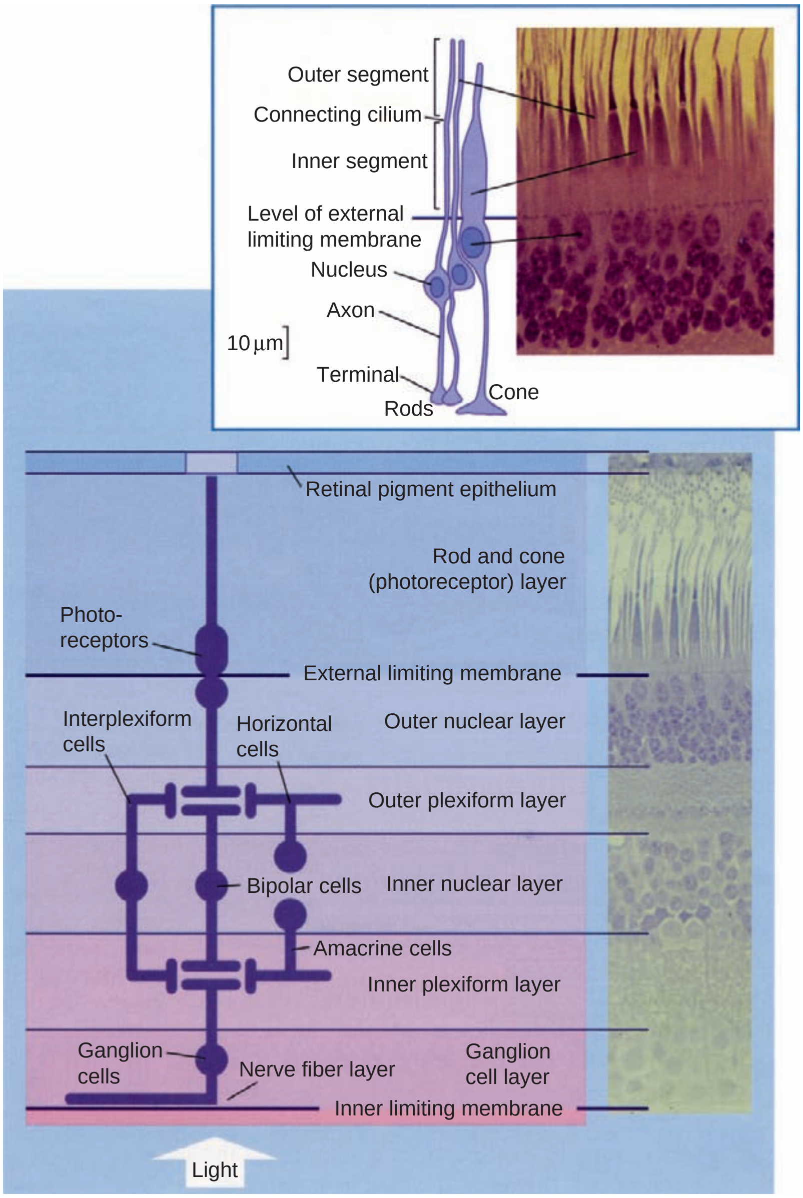


Figure 12 Section of a human retina with a diagram of the six neural cell types within it. Details of rods and cones are shown in the inset. From Douglas RH (2006) Visions of the deep. *Biological Sciences Review* 19(1): 36–41.

few ganglion cells – a further adaptation to enhance sensitivity. The density of visual pigment in deep-sea rods is also higher than in shallower water species, further increasing their sensitivity.

Photoreceptor renewal

In vertebrates, new photoreceptor disks are continually being formed at the base of the outer segment and older ones are pushed toward the RPE eventually to be phagocytosed by it. Furthermore, following its isomerization by light, the rod visual pigment chromophore travels to the RPE to be reisomerized.

However, in comparison to their shallow water relatives, the RPE of deep-sea fish is much reduced (**Figure 13**). This, and the possession in some species of a multibanked retina whose vitreal rods are not in contact with the RPE (**Figures 13**(**B**) and **14**), leads to the question of how, or if, the visual pigments of deep-sea fish regenerate and older outer segment disks are phagocytosed. Perhaps as the number of rod layers in multibanked retinae increases with age, the rods do not undergo renewal and a new layer is simply added once the current one has been exhausted. This idea receives some support because ERG recordings from the multibanked retina of conger eels indicate that only the most vitread layer of

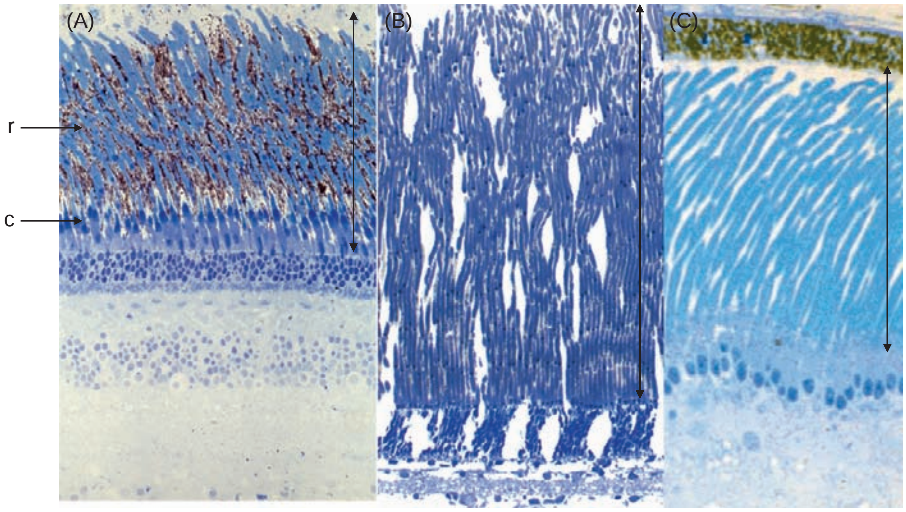


Figure 13 The retinal structure of various teleosts to show how deep-sea fish optimize sensitivity. (A) A shallower water fish, the tench (*Tinca tinca*), has both cones (c) and relatively smaller rods (r). (B) The deep-sea dragonfish (*Aristostomias grimaldi*) increases light capture with rods arranged in several banks. (C) The deep-sea bluntsnout smooth-head (*Xenodermichthys copei*), on the other hand, increases photon capture with very long rod outer segments. The two deep-sea species have a much wider region of rod outer segments (double headed arrow) relative to the rest of the retina. Their retinal pigment epithelium (brown pigmented area) is also much reduced in comparison with the shallow water tench and, unlike the tench, they have no cones. (B, C) Photo courtesy of H.-J. Wagner.

rods is active. Anatomical studies also suggest that, although all outer segment tiers in a multibanked retina show some signs of membrane synthesis, this is greatest in the most vitreal rods, and markers for rod proliferation indicate greater activity in the rods immediately adjacent to the external limiting membrane. However, all deep-sea species studied showed some phagosomes within the RPE, indicating a degree of photoreceptor disk-shedding even in deep-sea fish.

Regional retinal specializations

The distribution of vertebrate photoreceptors is not uniform and most terrestrial animals and shallower water species, for example, have areas of higher cone density providing increased spatial resolving power. Such high acuity areas are associated with high ganglion cell densities, while high-sensitivity regions have relatively fewer ganglion cells due to increased convergence of photoreceptor output.

Superficially, deep-sea fish, with their pure rod retinae, would seem to have no need for high spatial resolution. However, without an ability to appreciate spatial detail they would not be able to estimate the location of point source bioluminescence or image the more extended visual scenes of shallower water. Clearly, deep-sea fish would benefit from retinal regions for maximizing acuity. The retinal location of any such regions indicates the area of visual space of significance to an animal, revealing clues about the behavior of species whose observation *in situ* is very difficult. Furthermore, the spatial separation of photoreceptors and/or ganglion cells in terrestrial and shallow water animals are good predictors of behaviorally determined acuities. Similar measures in deep-sea fish, therefore, provide a reliable indication of spatial acuity in animals not accessible to psychophysical measurements.

Although some deep-sea fish have a uniform mosaic of ganglion cells throughout the retina, many possess one or more regions of increased ganglion cell density specialized for increased resolving power. The benthic tripod fish, *Bathypterois dubius*, for example, has a small centripetal gradient of ganglion cell density and two areae retinae characterized by a higher density of ganglion cells (**Figure 15(a)**). Thus, it views prey both behind and in front with increased precision as it perches above the sea floor on its elongated pelvic fins awaiting passing crustaceans. In some species, the inner retinal layers are pushed aside to minimize light scatter, forming either shallow (concaviclivate) or deeper (convexiclivate) (**Figure 16**) depressions. The fovea in the temporal retina of *Rouleina attrita*, for example (**Figure 15(b)**), gives it a binocular area of high spatial resolution in front of the mouth. Such deep fovea might, as in some birds and reptiles, also function to magnify the image, further increasing resolution.

Some of the most complex mesopelagic eyes are the dorsally directed eyes of the Scopelarchidae (**Figures 5(a)**, **5(b)**, **6(a)**, and **7**). Seven distinct retinal specializations are apparent in *Scopelarchus michaelsarsi* (**Figure 17**), including an area centralis whose ganglion cell density could yield a resolving power of 7.4 cycles per degree, which is surprisingly better than the spatial acuity

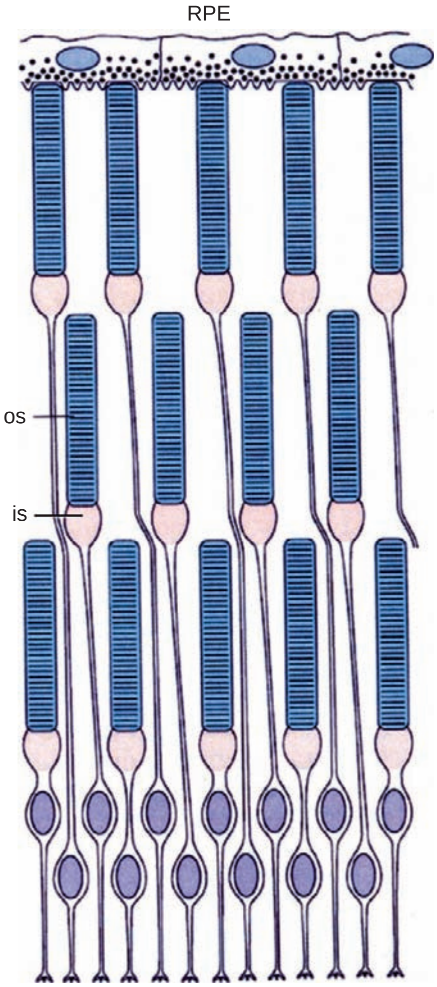


Figure 14 Schematic diagram of the outer part of a retina with three banks of rod outer segments (os) showing the thin retinal pigment epithelium (RPE). is, photoreceptor inner segment. From Warrant E (2004) Vision in the dimmest habitats on Earth. *Journal of Comparative Physiology A* 19: 765–789.

of many shallow-water fish and comparable to that of some predatory reef species. Scopelarchid retinae also contain areas in which *c.* 35 rods are grouped into bundles isolated by reflective tapetal cups within the RPE. Light entering such cups will be trapped and undergo multiple reflections ensuring maximal stimulation of all rods within a bundle, so that each group of rods functions as a highly sensitive, albeit coarse grain, macro-receptor. Similarly grouped receptors are found in other deep-sea species as well as a few shallow-water animals living in turbid environments.

In some deep-sea species, a local increase in thickness of the photoreceptor layer, caused either by an increase in outer segment length or by the formation of multiple banks of rods, causes a bulge in the sclera. In *Bajacalifornia drakei* this fovea externa is composed of 21–28 rows of rods, while the rest of the retina is made up of only two such banks. However, these structures are not associated with an increased ganglion cell density and the resulting high convergence of photoreceptor outputs indicates that they subserve enhanced sensitivity rather than acuity.

The deeper a species lives, the more important bioluminescence is as a source of visual information. Because sensitivity to such point sources is not increased by spatial summation, deeper-dwelling species generally have higher ganglion cell densities than shallower-dwelling mesopelagic fish. The increased acuity afforded by a lack of such convergence will allow accurate localization of bioluminescent signals.

Tapeta

Sensitivity in many deep-sea fish is enhanced by a reflective layer in either the RPE or choroid. Such tapeta, which are responsible for the 'eye shine' seen in many nocturnal animals (**Figures 10**(**a**) and **18**), give photons not absorbed by the visual pigments when first incident on the retina, a second chance of activating the rods. Reflection can be either broadband, resulting in silver/white tapeta, or more spectrally selective, producing eyeshine whose color is related to the animal's photic environment (**Figure 19**).

Visual Pigments

Light incident on the photoreceptors is absorbed by visual pigments which in darkness are composed of a protein, opsin, bound to a light-absorbing vitamin A-derived chromophore, in a 'bent' 11-*cis* configuration (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments). On absorption of a photon the chromophore 'straightens' into an *all-trans* isoform and separates from the opsin which itself changes shape, setting in motion a G-protein-coupled enzyme cascade that ultimately results in the hyperpolarization of the photoreceptor, which is the primary event of vertebrate vision.

In most species, the chromophore is the vitamin A_1-derived retinal, forming a class of pigments known as rhodopsins. In some animals the chromophore is the longer wavelength absorbing vitamin A_2-derived 3,4-di-dehydroretinal, forming porphyropsin visual pigments. If an animal possesses two different visual pigments based on a single opsin combined with either retinal or 3,4-di-dehydroretinal, these form a so-called rhodopsin/porphyropsin pigment pair.

Vertebrate visual pigments have a smooth, bell-shaped absorption spectrum that peaks at a wavelength of maximum absorption ($\lambda_{\max}$) (**Figure 20**).

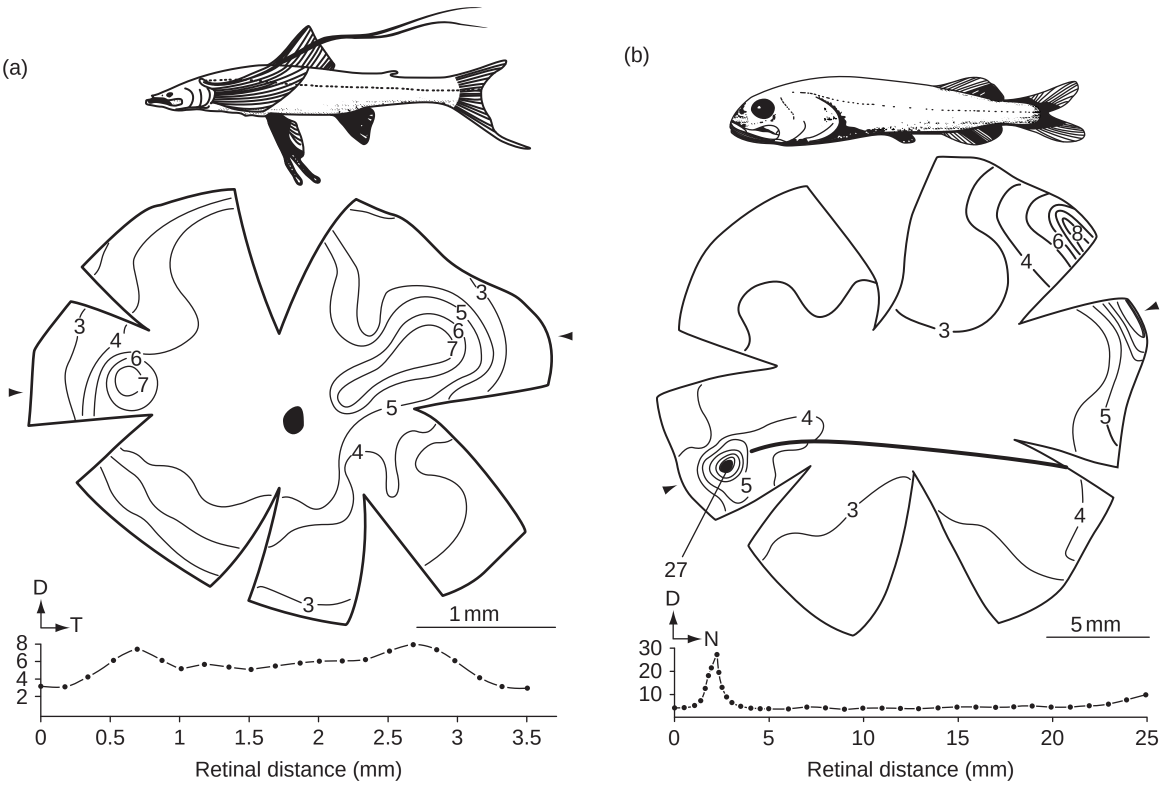


Figure 15 Ganglion cell density in the eyes of (a) *Bathypterois dubius* and (b) *Rouleina attrita*. The isodensity contour maps show the density of neurons in the ganglion cell layer in retinal whole mounts. All densities are $\times 10^3$ cells mm^{-3}. The graph is a density profile along a temporal–nasal transect between the arrows. The optic nerve head and falciform process are depicted in black. In (a) the optic nerve is punctuate while in (b) it is horizontally elongate and coincident with the falciform process. D, dorsal; N, nasal; T, temporal. From Wagner HJ, Fröhlich E, Negishi K, and Collin SP (1998) The eyes of deep-sea fish II. Functional morphology of the retina. *Progress in Retinal and Eye Research* 17(4): 537–685.

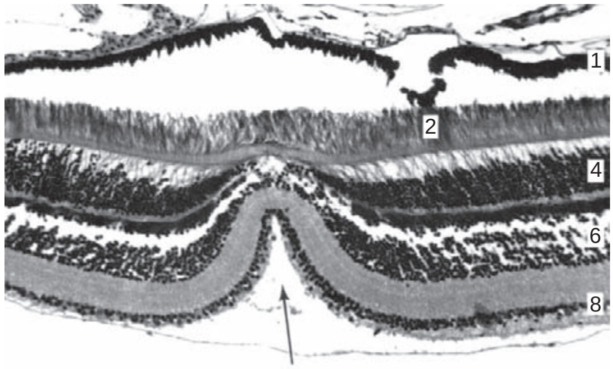


Figure 16 Horizontal section through the center of the convexiclivate temporal fovea of *Bathylaco nigricans*. The gaps between the RPE (1) and the neural retina and within the inner nuclear layer (6) are artifacts. 2, layer of rod outer/inner segments; 4, outer nuclear layer; 8, ganglion cell layer. From Munk O (1968) On the eye and so-called preorbital light organ of the isospondylous deep-sea fish *Bathylaco nigricans* Goode and Bean, 1986. *Galathea Report* 9: 211–218.

The spectral properties of a visual pigment are determined both by the nature of its chromophore and the structure of its opsin, which, in vertebrates, is composed of a single chain of around 350 amino acids that forms seven transmembrane α-helical loops within the outer segment disk membrane. These loops form a bundle surrounding a hollow cavity, the chromophore-binding pocket. On their own the chromophores retinal and 3,4-di-dehydroretinal absorb maximally at 380 and 400 nm, respectively. Interactions between these

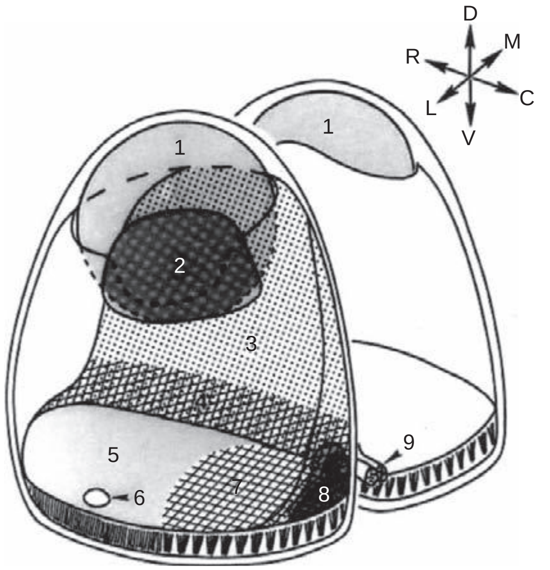


Figure 17 Schematic representation of the tubular eyes of *Scopelarchus michaelsarsi* showing the position and relative size of six retinal specializations (retinal diverticulum not shown) in the left eye: 1, spherical lens; 2, lens pad; 3, grouped rod-like photoreceptors of the accessory retina; 4, grouped rod- and cone-like photoreceptors in the ventral region of the accessory retina; 5, ungrouped rod-like photoreceptors in the main retina; 6, *area centralis* in the centrolateral region of the main retina; 7, grouped rod-like photoreceptors of the main retina; 8, temporal region of large ganglion cells (*area giganto cellularis*) in the main retina; 9, optic nerve. C, caudal; D, dorsal; L, lateral; M, medial; R, rostral; V, ventral. From Collin SP, Hoskins RV, and Partridge JC (1998) Seven retinal specializations in the tubular eye of the deep-sea Pearleye, *Scopelarchus michaelsarsi*: A case study in visual optimization. *Brain Behavior and Evolution* 51: 291–314.

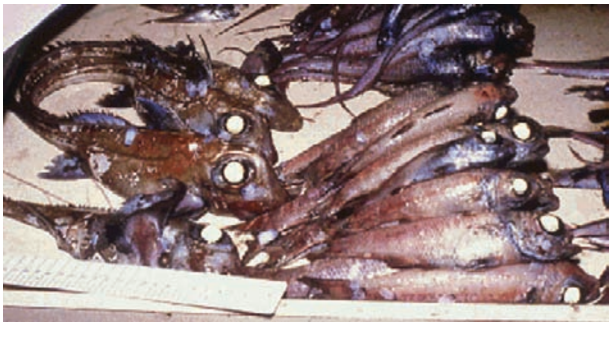


Figure 18 'Eyeshine' caused by a reflective tapetum in freshly caught deep-sea chimaerids (left) and teleosts (right).

chromophores and the opsin amino acid residues in the vicinity of the binding pocket generally results in wavelengths of maximum absorption being displaced to longer wavelengths. As few as nine opsin amino acid sites are responsible for the hypsochromatic shift of most deep-sea fish visual pigments.

Species with a Single Visual Pigment

In 1936, Clarke and Bayliss independently suggested that in order to maximize their sensitivity, deep-sea fish may have their maximum sensitivity shifted toward shorter wavelengths by comparison with shallow-water species, that is, matching the shorter wavelength spectral distribution of the residual sunlight. The available data seem to confirm a hypsochromic shift in deep-sea fish visual pigment λ_{max}. Among 248 species of deep-sea fish examined to date, 219 contain a single visual pigment whose absorption maximum falls, as predicted, between 470 and 495 nm (**Figure 21**). This superficial matching of deep-sea visual pigments to the residual sunlight is often cited as a classic adaptation of a sensory system to a specific environment. However, recent modeling suggests that, in the majority of the World's oceans, maximum sensitivity to residual sunlight requires visual pigments absorbing at even shorter wavelengths and that the observed visual pigments are better suited for the visualization of bioluminescence, that could be visible at distances of up to 30 m.

Multipigment Species

Only 12% of deep-sea species examined have two and rarely three visual pigments whose λ_{max} values are often at longer or, rarely, shorter wavelengths than those of single-pigment species (**Figure 21**). Seven of these multi-pigment species are stomiid dragonfish that are discussed below. Of the remaining 22 species, only *Scopelarchus analis* has three visual pigments (λ_{max} 444, 479, and 505 nm), which is in line with its complex ocular morphology (see above); the rest have two with an average difference in λ_{max} of 26 nm (**Figure 22**).

The possession of more than one spectral type of visual pigment will extend the spectral range of an animal and, if the outputs of the photoreceptors are summed, will improve its overall sensitivity if the spectral environment is sufficiently broad. Color vision is possible following opponent interactions between two photoreceptors with different spectral sensitivities. As some nocturnal terrestrial species have color vision at low light levels, it is conceivable multiple pigments in deep-sea fish could form the basis of a rod-based color vision system. However, such opponency would inevitably decrease absolute sensitivity, which is at a premium in the deep sea. Significantly, the arrangement and complexity of horizontal cells in the retinae of deep-sea fish is very different to that in shallow-water species where antagonistic interaction between chromatic channels is normally associated with color vision. Thus, deep-sea fish lack the traditional neural circuitry associated with

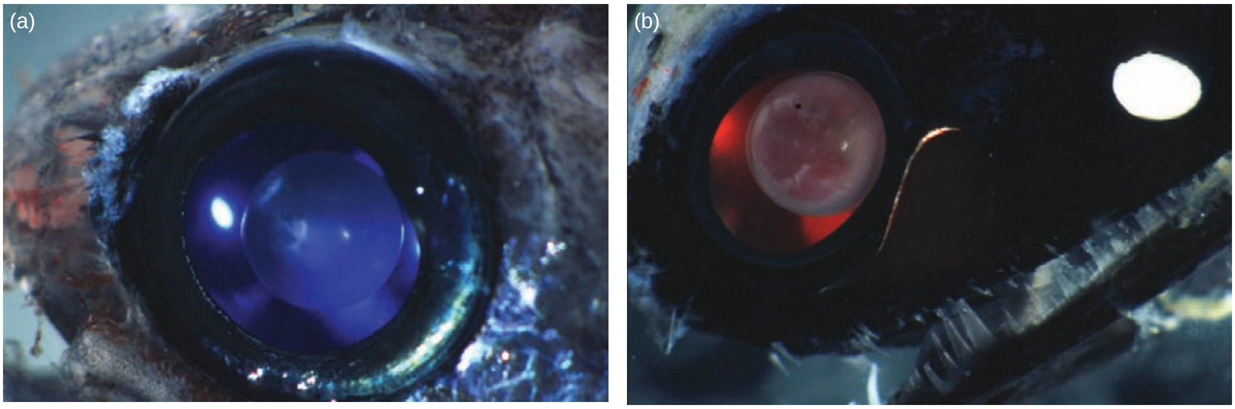


Figure 19 Tapetal reflection in (a) a myctophid and (b) the stomiid *Malacosteus niger*. The coloration is the 'eye shine' produced by reflected light from the tapetum. The short wavelengths reflected by the myctophid match those predominant in both residual sunlight and bioluminescence and are coincident with the maximum sensitivity of most deep-sea fish. The red tapetum of *Malacosteus niger* is one of several adaptations that enable this species to see its own far-red bioluminescence. Photos courtesy of J. Marshall.

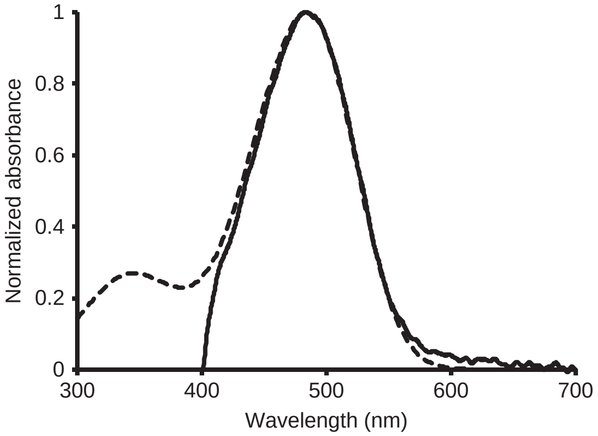


Figure 20 The single rhodopsin visual pigment of the longjaw bigscale (*Scopeloberyx robusts*). This fish can live at depths in excess of 4000 m and has a visual pigment typical of a deep-sea fish. The solid curve is the difference spectrum obtained by subtracting the absorption of a fully bleached detergent retinal extract from a dark adapted spectrum of the same extract before the bleach. The dotted curve represents the best fitting visual pigment template ($\lambda_{\max}$ 484.3 nm). In subsequent figures only the visual pigment templates are shown.

color vision. Unfortunately, the behavioral experiments necessary to show color vision are impossible in captured deep-sea fish.

Long-Wave Sensitivity

Stomiid dragonfish of the genera *Aristostomias*, *Pachystomias*, and *Malacosteus* not only produce blue/green bioluminescence similar to that emitted by other deep-sea species from postorbital photophores, but uniquely also emit long-wave light from photophores located below their eyes (**Figure 23**). Deep-sea species with conventional visual pigments with $\lambda_{\max}$ values around 480 nm (**Figure 20**) will be insensitive to this far-red bioluminescence whose emission spectrum peaks sharply beyond 700 nm (**Figures 24** and **25**). The dragonfish, however, are sensitive in this part of the spectrum and, thus, have what can be regarded as a private waveband they can use for intraspecific signaling or the illumination of prey, immune from detection by other animals.

Retinal extracts show all three genera of dragonfish possess a minimum of two visual pigments, forming a rhodopsin/porphyropsin pigment pair, with absorption maxima shifted toward longer wavelengths in comparison with other deep-sea fish (**Figures 24** and **25**). However, retinal whole mounts and microspectrophotometry of *Aristostomias* reveal an additional third visual pigment maximally sensitive around 581 nm (**Figure 24**), further enhancing photosensitivity to long wavelengths.

Dragonfish of the genus *Malacosteus*, although they posses two of the long-wave shifted visual pigments found in *Aristostomias* lack the third, most long-wave-shifted pigment (**Figure 25**). Uniquely, however, their photoreceptors contain bacteriochlorophyll-derivatives that absorb strongly around 670 nm and act as a photosensitizer that bleaches the visual pigment following the absorption of far-red illumination. Perhaps surprisingly, chlorophyll derivatives, when added to salamander rods and bovine rhodopsin *in vitro,* can enhance long-wave sensitivity considerably despite the fact that such species do not normally contain chlorophylls. This suggests all

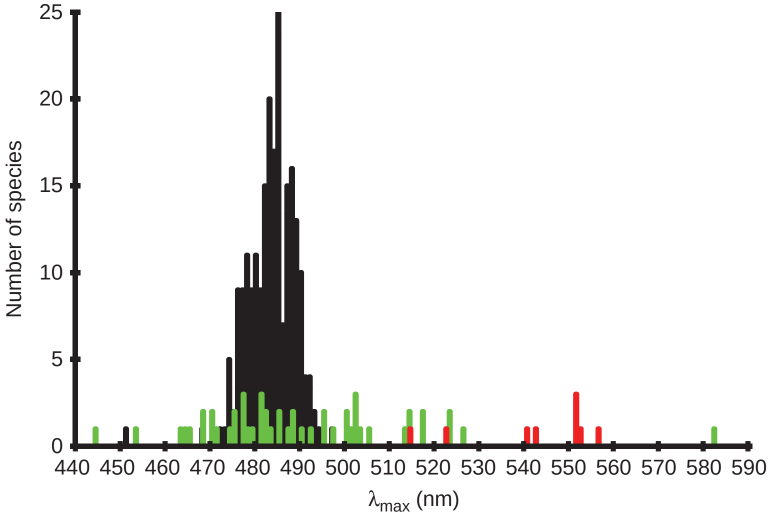


Figure 21 Wavelength of maximum absorption ($\lambda_{\max}$) of 279 visual pigments isolated from the eyes of 248 species of deep-sea fish by either extraction or microspectrophotometry. Where possible we have used data obtained in our laboratory (173 species published and 39 species unpublished). The data for the remaining 36 species come from the literature. Black bars are the rhodopsins from 219 single pigment species, green bars the rhodopsins from species with more than one visual pigment, and red bars the porphyropsins in these species.

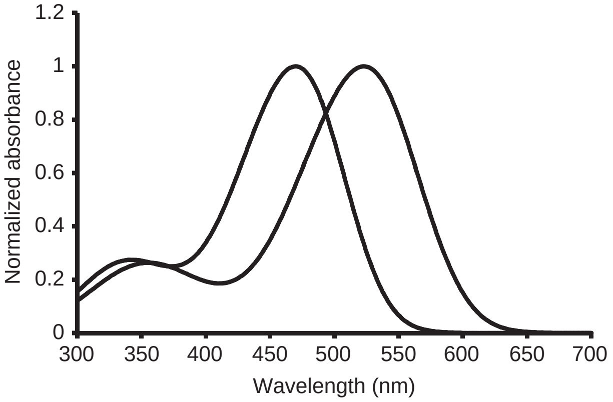


Figure 22 Two rhodopsin visual pigments isolated by partial bleaching of *Benthalbella elongata* retinal extracts ($\lambda_{\max}$ 470 and 523 nm). The retina of the closely related *B. macropinna* also contains two rhodopsins ($\lambda_{\max}$ 453 and 482 nm).

vertebrate photoreceptors might have the potential for such photosensitization.

Given the huge selective advantage such a 'private wavelength' would give stomiids, it is perhaps not surprising that myctophids, which form a significant part of the diet of most dragonfish, may also be more long-wave sensitive than other deep-sea fish, despite not producing long-wave bioluminescence. *Myctophum nitidulum*, for example, posses two visual pigments, the most long-wave sensitive of which ($\lambda_{\max}$ 522 nm) makes these animals significantly more red sensitive than other, non-stomiid deep-sea fish (**Figure 21**) and modeling suggests that *Myctophum nitidulum* could detect stomiid bioluminescence up to a distance of 1 m. Preliminary data suggest a second myctophid, *Bolinichthys longipes*, like *Malacosteus*, posses a bacteriochlorophyll-like substance in its retina (**Figure 26**), which might theoretically allow the detection of stomiid bioluminescence up to a distance of 7 m. It appears as if the dragonfish and myctophids may be involved in a sensory arms race within the deep sea involving sensitivity to far-red light.

Effect of Ambient Pressure on Visual Pigments

The literature relating the absorption spectra of deep-sea visual pigments to their environment is potentially subject to a serious artifact: all visual pigment analyses have been done at atmospheric pressure (0.1 MPa). However, at the average depth of the ocean (4000 m)

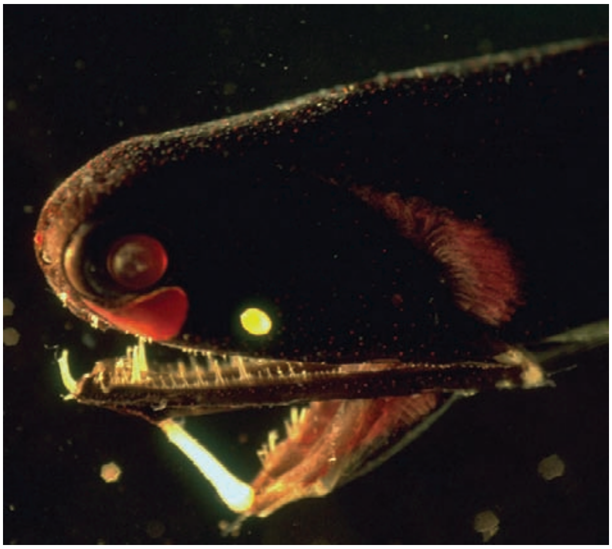


Figure 23 Stomiid losejaw dragonfish *Malacosteus niger* showing the teardrop-shaped suborbital photophore that produces long-wave bioluminescence and the postorbital photophore that emits conventional short-wave bioluminescence. Photo courtesy of T. Frank.

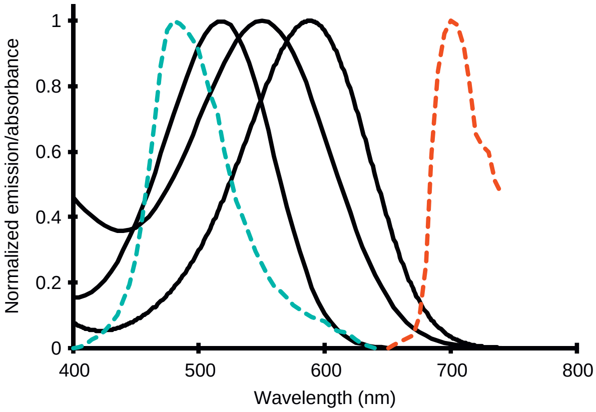


Figure 24 Three visual pigments isolated from the retina of *Aristostomias tittmanni* by both whole-mount spectroscopy of fresh retinae and microspectrophotometry (black lines). The two short-wave-sensitive pigments (λ_{max} 520 and 551 nm) form a rhodopsin/porphyropsin pair. The most long-wave-sensitive pigment is best described by the template for a rhodopsin pigment with λ_{max} 588 m. The dotted lines represent the emission spectra of the postorbital (blue) and suborbital (red) photophores, respectively.

animals will be subjected to 400 times the pressure experienced at the water surface. Because the absorption characteristics of a visual pigment are partially determined by the complex tertiary structure of its opsin protein, it is possible that the absorption characteristics of the visual pigments at depth may be different to those measured at atmospheric pressure. Luckily, however, when visual pigment solutions are subjected to such

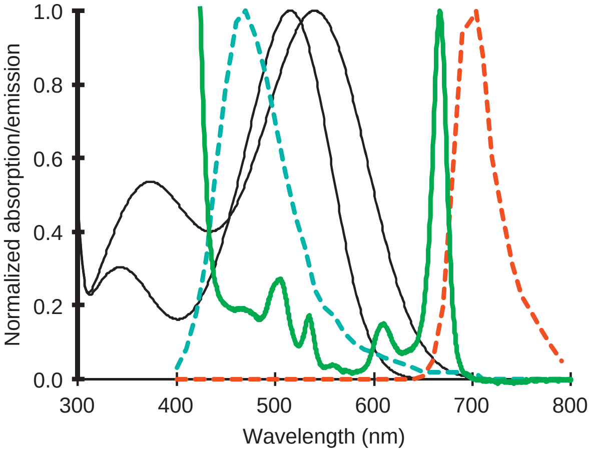


Figure 25 Two visual pigments isolated from the retina of *Malacosteus niger* by detergent extract, microspectrophotometry, and spectroscopy of outer segment suspensions (black lines). These represent a rhodopsin/porphyropsin pigment pair (λ_{max} 515 and 540 nm). The photoreceptor outer segments of this species also contain a form of bacteriochlorophyll (green curve) which acts as a 'photosensitizer', absorbing the red bioluminescence (red dotted curve) emitted by its suborbital photophore and activating the visual pigments. The bioluminescence emitted by the postorbital photophore is shown in blue. Modified from Douglas RH, Partridge JC, Dulai KS, *et al*. (1999) Enhanced retinal longwave sensitivity using a chlorophyll-derived photosensitiser in *Malacosteus niger*, a deep-sea dragon fish with far red bioluminescence. *Vision Research* 39: 2817–2832.

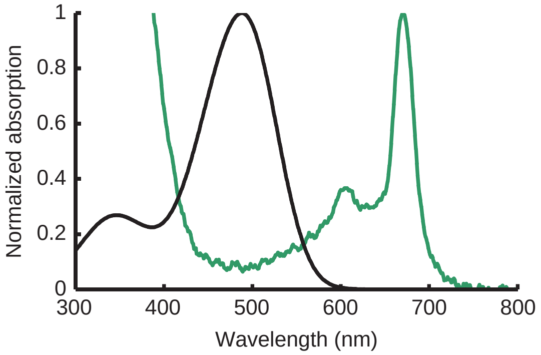


Figure 26 Partial bleaching of retinal extracts reveals only a single visual pigment in *Bollinichthys longipes* (black line). However, these extracts also contain a photostable substance (shown in green and represented by the absorption spectrum of a bleached extract) whose spectrum is very similar to the bacteriochlorophyll-derived photosensitizer of *Malacosteus niger*.

pressures in the laboratory their absorption spectra are little changed. It would be interesting to see if visual pigments in cell membranes, or their rates of regeneration, as well as bioluminescence emission spectra, are similarly unaffected by pressure.

See also: **Design and Physiology of Arteries and Veins**: The Retia. **The Skin**: Bioluminescence in Fishes. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Inner Retina and Ganglion Cells; Photoreceptors and Visual Pigments; Physiological Optics in Fishes.

Further Reading

Collin SP, Hoskins RV, and Partridge JC (1997) Tubular eyes of deep-sea fishes: A comparative study of retinal topography. *Brain Behavior and Evolution* 50: 335–357.

Collin SP, Hoskins RV, and Partridge JC (1998) Seven retinal specializations in the tubular eye of the deep-sea Pearleye, *Scopelarchus michaelsarsi*: A case study in visual optimization. *Brain Behavior and Evolution* 51: 291–314.

Denton EJ (1990) Light and vision at depths greater than 200 metres. In: Herring PJ, Campbell AK, Whitfield M, and Maddock L (eds.) *Light and Life in the Sea*, pp. 127–148. Cambridge: Cambridge University Press.

Douglas RH, Partridge JC, and Marshall NJ (1998) The eyes of deep-sea fish I: Lens pigmentation, tapeta and visual pigments. *Progress in Retinal and Eye Research* 17(4): 597–636.

Douglas RH, Hunt DM, and Bowmaker JK (2003) Spectral sensitivity tuning in the deep-sea. In: Collin SP and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments*, pp. 323–342. New York: Springer.

Fröhlich E and Wagner H-J (1995) Patterns of rod proliferation in deep-sea fish retinae. *Vision Research* 35(13): 1799–1811.

Fröhlich E and Wagner H-J (1996) Rod outer segment renewal in the retinae of deep-sea fish. *Vision Research* 36(19): 3183–3194.

Hunt DM and Bowmaker JK (2006) Visual pigments and visual communication in deepwater environments. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes,* vol. 2, pp. 457–483. Enfield, NH: Science Publishers.

Locket NA (1977) Adaptations to the deep-sea environment. In: Crescitelli F (ed.) *Handbook of Sensory Physiology, Vol. VII/5. The Visual System in Vertebrates*, pp. 67–192. Berlin: Springer.

Locket NA (1985) The multiple bank rod fovea of *Bajacalifornia drakei*, an alepocephalid deep-sea teleost. *Proceedings of the Royal Society of London, Series B* 224: 7–22.

Munk O (1966) Ocular anatomy of some deep-sea teleosts. *Dana Report* 70: 1–62.

Robison BH and Reisenbichler KR (2008) *Macropinna microstoma* and the paradox of its tubular eyes. *Copeia* 2008(4): 780–784.

Turner JR, White EM, Collins MA, Partridge JC, and Douglas RH (2009) Vision in lanternfish (Myctophidae): Adaptations for viewing bioluminescence in the deep-sea. *Deep-Sea Research Part I – Oceanographic Research Papers* 56(6): 1003–1017.

Wagner HJ (2001) Brain areas in abyssal demersal fishes. *Brain, Behavior and Evolution* 57(3): 301–316.

Wagner HJ (2001) Sensory brain areas in mesopelagic fishes. *Brain, Behavior and Evolution* 57(3): 117–133.

Wagner HJ, Douglas RH, Frank TM, Roberts NW, and Partridge JC (2009) A novel vertebrate eye using both refractive and reflective optics. *Current Biology* 19(2): 108–114.

Wagner HJ, Fröhlich E, Negishi K, and Collin SP (1998) The eyes of deep-sea fish II. Functional morphology of the retina. *Progress in Retinal and Eye Research* 17(4): 537–685.

Warrant EJ and Locket NA (2004) Vision in the deep sea. *Biological Reviews* 79(3): 671–712.

Widder EA (1999) Bioluminescence. In: Archer SN, Djamgoz MBA, Loew ER, Partridge JC, and Vallerga S (eds.) *Adaptive Mechanisms in the Ecology of Vision*, pp. 555–582. Dordrecht: Kluwer Academic Publishers.

SENSING THE ENVIRONMENT

Smell, Taste, and Chemical Sensing

Contents

Chemoreception (Smell and Taste): An Introduction
Morphology of the Gustatory (Taste) System in Fishes
Morphology of the Olfactory (Smell) System in Fishes
Neurophysiology of Olfaction
Neurophysiology of Gustation
Chemosensory Behavior

Chemoreception (Smell and Taste): An Introduction

TJ Hara, University of Manitoba, Winnipeg, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Further Reading

Glossary

Electro-olfactogram (EOG) The summed electrical response recorded from a group of receptor cells in response to an odorant stimulus.

Gustation and gustatory Refers to the sense of taste.

Odorants Chemicals in the water that stimulate the olfactory receptors.

Olfaction and olfactory Refers to the sense of smell.

Pheromones Odorants that are secreted by animals that have a specific function to influence the development or behavior of conspecifics.

Receptor A signal transduction molecule that is activated by a hormone.

Tastants Chemicals in the water that stimulate the gustatory receptors.

The last decade saw important advances in our understanding of the functions of the chemosensory systems. The function of chemical senses, or chemoreception, is the most ancient of sensory systems, having evolved 500 million years ago. They mediate the animals' functions most basic to the survival of the individuals and species: feeding and reproduction. Fish live in the aquatic environment, and their sensory systems are in continuous interactions with their physical and chemical perturbations in the surrounding water. The aquatic environment is similar to the terrestrial, in that it contains a multitude of chemical mixtures. However, the aquatic environment differs from the terrestrial one in the ways that chemical substances can be distributed: (1) molecules of chemical substances need to be in solutions rather than in the gaseous phase to be transported, and (2) water is a much slower carrier medium, both in terms of diffusion and convection or currents. Thus, solubility, rather than volatility, of chemical compounds determines their capacity as chemical cues. Consequently, nonvolatile compounds with relatively low molecular weights are superior substances for fish chemoreception and have been implicated in various behavioral roles.

Fish, like all other vertebrates, detect chemical stimuli through olfaction, gustation, and less well-defined senses represented by the general chemical senses and the solitary chemosensory cell. The two major channels of chemoreception – and gustation (or taste) – are discussed elsewhere in this encyclopedia. In terrestrial vertebrates, olfaction is normally distinguished as a distance chemical sense with high sensitivity and specificity, and gustation is primarily a contact or close-range sense with moderate sensitivity. In water, in contrast, these senses both act as distance receptors, detecting stimuli located remote from receptors, similar to hearing and vision, and act as contact receptors, detecting stimuli that are in direct contact with body surface, similar to touch and pain receptors. Thus, the aquatic environment surrounding fishes makes their chemical senses unique, and distinction between these two sensory modalities is not always as clear as in terrestrial organisms. In air or water, however, all chemoreception ultimately is an aquatic phenomenon, because the receptors are covered with fluid materials where the initial sensory transduction process takes place. Although chemo-sensitivities of the solitary chemosensory cell, the trigeminal system, and the lateral line system have been suggested, their biological relevancy has not been demonstrated, and therefore will not be addressed here. All the sensory systems are innately stimulated by chemical substances, whether specific or nonspecific, and all are prone to mechanical stimulation.

Fish, constituting more than half of the total number of *c.* 50 000 recognized living vertebrate species, are a pivotal group that has served as a model for the study of the vertebrate chemosensory system. The fishes often possess specific, sensitive, and readily accessible chemosensory organs. The ability to define specificity and sensitivity has permitted a critical evaluation of the several transduction sequences that follow the initial receptor-binding step. Unfortunately, however, little is known about the chemical senses of the majority of fish species that most likely have diverse behavioral ecology and taxonomy; consequently, elsewhere in this encyclopedia, discussion focuses on the fish species cyprinids (carp, goldfish), ictalurids (catfishes), zebrafish, and salmonids. Historically, the existence of the sense of smell in fish has long been controversial. The earliest description of the olfactory system in fish is probably the one by Monro in 1785: he wrote "... In all fishes, external openings or nostrils for smell are very evident, generally two on each side in the osseous fishes (a), which, on each side of the head (b), lead to a complex organ, the surface of which is of considerable extent; and upon them a pair of large or olfactory nerves terminates, with the addition of some branches from nerves resembling our fifth pair (c). In some fishes, as in the head and nose, passes through a cineritious ball (d), resembling the cineritious matter connected in our body to the olfactory nerve within the cranium. There can, therefore, be no doubt that they enjoy the sense of smelling; nay, there is great reason to believe that, suited to their surrounding element, they are much more sensible of odorous bodies dissolved in water, and applied by its medium, than we should be, if the application of the object was to be made to our organ of smell by the same medium."

What seemed to be the first paper dedicated exclusively to the olfactory sense of fishes was by Duméril in 1807, and by the 1850s the existence of three types of cells: sensory, supporting, and basal cells in the olfactory epithelium were recognized. Also, the connection of nerve fibers to the cells characterized as sensory cells was established for the first time. Nevertheless, Nagel in 1894, for instance, denied the existence of true olfactory sense in aquatic animals, suggesting that the organ of smell can be stimulated only by gaseous substances, implying that chemical stimulation in aquatic animals could only be mediated by taste. Taste buds, constituting the structural basis of the peripheral gustatory organ, were first described in fish as terminal buds (also termed as end buds or cutaneous buds) in the early 1800s before their function as organs of taste became firmly established. The most convincing evidence indicating that distinct olfactory and gustatory functions exist in fish was obtained by Strieck in 1924. Although the peripheral and central organization of the gustatory pathways in fishes was only described at the beginning of the twentieth century, similar studies in mammalian systems came much later.

As in other biological fields, experimental approaches to the functional aspects of chemoreception lagged far behind general morphological, anatomical, and histological studies. In particular, application of electrophysiological work to clarify the function and interrelationships of sensory mechanisms is very recent, and had to await progress in the physical sciences and its instrumentation. With respect to the fish system, it came as recently as 1938, when Adrian and Ludwig recorded an electrical discharge in the olfactory organs of catfish *(Ameiurus melas)*, carp *(Cyprinus carpio)*, and tench *(Tinca tinca)* in response to stimulation of the olfactory epithelium with fluid from decaying animal tissue. In fact, this marked the first study of the vertebrate olfactory system using an electrophysiological technique. The application of olfactory epithelium response (electro-olfactogram) and bulb recording techniques developed by Ottoson to the research on fishes greatly expanded progress in contemporaneous works.

One of the most characteristic features of the chemical senses, olfactory in particular, is that olfactory receptor neurons are the certified neurons belonging to the brain but miles away out in the open water, nosing around the world, whose axons carry external information directly to the brain. Unlike any other neurons of the vertebrate central nervous system, olfactory neurons are replaced every few weeks. Furthermore, chemoreceptive membranes are directly exposed to the aquatic environment and are not protected by external barriers or internal detoxifying system. Therefore, the physiological importance, chemical reactivity, structural complexity, and exposed location of chemosensory membranes combine to make them prime targets for interactions with aquatic contaminants.

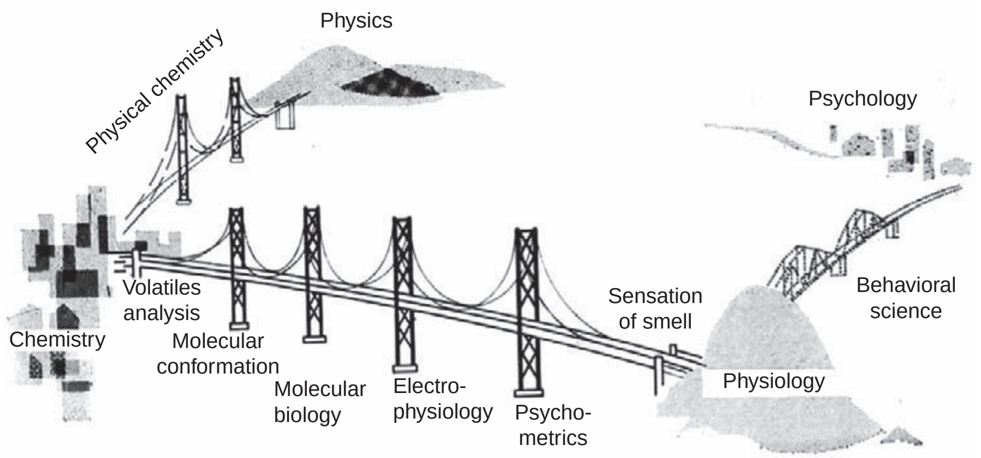


Figure 1 The Bay Bridge analogy for odor research. Modified from Amoore JE (1970) *Molecular Basis of Odor*, 200pp. Springfield, IL: Charles C. Thomas.

The last span between chemistry and physiology, a San Francisco Bay Bridge Analogy used by Amoore (**Figure 1**) some 40 years ago, has now been bridged by the discovery of a large multigene family of G-protein-coupled olfactory receptor genes first in rodents, and has opened a new era in olfaction as well as gustation research. Amoore had proposed that selective deficiencies in the ability to smell certain odors (specific anosmias) derive from genetic variations in genes encoding specific odorant receptor proteins. Direct evidence for the existence of such receptors was subsequently provided by studies in which radioactive odorants (amino acids) specifically bind to membrane fractions collected from olfactory cilia of salmonid fishes. The Nobel prize-winning work by Buck and Axel illuminates the logic of the most enigmatic of sensory systems, and homologous families of olfactory receptor genes have now been identified in a variety of vertebrate species, including fishes. The multigene family is a set of similar but not identical genes that encode the different members of a family of related proteins, and presumed to have arisen by duplication and divergence of an ancestral gene. Phylogenetic analyses of olfactory receptor genes suggest that the most recent common ancestor between fishes and tetrapods had at least nine ancestral olfactory receptor genes, and all olfactory receptor genes identified are classified into nine groups, each of which originated from one ancestral gene. The sizes of the receptor repertoires of vertebrate species are extremely large and are estimated to contain as many as 1000 individual genes. These observations suggest that approximately 1% of all genes are devoted to the detection of odors, making this the largest gene family thus far identified in vertebrates. Such an enormous amount of genetic information devoted to smell perhaps reflects the significance of this sensory system for the survival of most vertebrate species. Although many olfactory, pheromone, and gustatory receptors have been partially cloned or identified through genome sequencings, the identification of the ligands or odorants, or tastants, which activate individual receptors (deorphanization), has proved to be difficult. One of the few examples of deorphanized receptors is the 5.24-amino-acid receptor identified in the goldfish olfactory epithelium by Speca *et al.* in 1999. Here, again, fish have been proved to serve as important leading models for understanding the basic physiology of the chemosensory systems.

The gustatory system in fish, on the other hand, has evolved differently, reflecting their diverse modes of lives in the aquatic environment. Hypertrophy of the system has occurred independently in several groups, notably in the siluroids and the cyprinids. Traditionally, the gustatory system has been thought to be the primary channel for the detection of chemical cues for feeding. However, recent studies demonstrate that in many fish species feeding is triggered primarily through olfaction, complemented by gustation, and there is growing evidence suggesting that the CO_2 sensitivity of gustatory receptors might be involved in the ventilatory/respiratory regulation in fishes (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior and Neurophysiology of Olfaction). Therefore, the statement such as, "Where studied, gustation in fish appears to be exclusively associated with feeding" may no longer be valid. Analyses of genomic sequences encoding proteins homologous to mammalian gustatory receptors identify two families of candidate fish gustatory receptors in pufferfish, medaka, and zebrafish, suggesting that vertebrates commonly possess gustatory receptors of the G-protein-coupled and signaling pathways. Identification of olfactory and gustatory receptors during the last couple of decades has provided extraordinary insight into the strategies for information coding used by the chemosensory systems. Moreover, the integration of molecular, physiological, and morphological approaches has peeled away some mysteries in these sensory systems. The articles such as **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior, Morphology of the Gustatory (Taste) System in Fishes, Morphology of the Olfactory (Smell) System in Fishes, Neurophysiology of Gustation, and Neurophysiology of Olfaction describe fundamental features of organization and function of both the olfactory and gustatory systems of fishes, with occasional comparison with those of terrestrial vertebrates, and provide up-to-date information on their roles in behaviors. The discussion will be centered on the chemosensory systems of limited fish groups, namely salmonids, goldfish, and catfishes, which have served as important models for extensive studies. However, no attempt has been made to be all inclusive; the reader is referred to the review listed in the Further Reading section (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior, Morphology of the Gustatory (Taste) System in Fishes, Morphology of the Olfactory (Smell) System in Fishes, Neurophysiology of Gustation, and Neurophysiology of Olfaction). Of course, two classical works, one by Herrick in 1905 and the other by Sheldon in 1912, are still very alive one hundred years after their publication; the former celebrated its centenary 2 years back, and the latter will do the same in a couple of years.

See also: **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior; Morphology of the Gustatory (Taste) System in Fishes; Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Gustation; Neurophysiology of Olfaction.

Further Reading

Adrian ED and Ludwig C (1938) Nervous discharges from the olfactory organs of fish. *Journal of Physiology* 94: 441–460.

Amoore JE (1970) *Molecular Basis of Odor*, 200pp. Springfield, IL: Charles C. Thomas.

Hara TJ (1975) Olfaction in fish. *Progress in Neurobiology* 5: 271–335.

Hara TJ (ed.) (1982) *Chemoreception in Fishes*. Amsterdam: Elsevier.

Hara TJ (ed.) (1992) *Fish Chemoreception*, pp. 79–102. London: Chapman and Hall.

Hara TJ and Zielinski BS (eds.) (2007) *Sensory Systems Neuroscience, Volume 25: Fish Physiology*, 523pp. New York: Academic Press.

Herrick CJ (1905) The central gustatory paths in the brains of bony fishes. *Journal of Comparative Neurology and Psychology* 15: 375–456.

Kleerkoper H (1969) *Olfaction in Fishes,* 222pp. Bloomington, IN: Indiana University Press.

Laberge F and Hara TJ (2001) Neurobiology of fish olfaction: A review. *Brain Research Reviews* 36: 46–59.

Monro A (1785) *The Structure and Physiology of Fishes. Explained and Compared with Those of Man and Other Animals*. Edinburgh: Charles Elliot.

Nagel W (1894) Vergleihend-physiologische und anatomische Untersuchungen an den Geruchs- und Geschmackssinn und ihre Organe. *Biologie und Zoologie* 18: 1–207.

Niimura Y and Nei M (2005) Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods. *Proceedings of the National Academy of Sciences of the United States of America* 102: 6039–6044.

Ottoson D (1956) Analysis of the electrical activity of the olfactory epithelium. *Acta Physiologica Scandinavica* 35(supplement): 1–83.

Reutter K and Kapoor BG (eds.) (2005) *Fish Chemosenses*. Enfield, NH: Science Publishers.

Sheldon RE (1912) The olfactory tracts and centers in teleosts. *Journal of Comparative Neurology* 22: 177–339.

Speca DJ, Lin DM, Sorensen PW, *et al.* (1999) Functional identification of a goldfish odorant receptor. *Neuron* 23: 487–498.

Strieck F (1924) Untersuchungen uber den Geruchs- und Geschmackssinn der Elritzen. *Zeitschrift für Vergleichende Physiologie* 2: 122–154.

Von der Emde G, Mogdans J, and Kapoor BG (eds.) (2004) *The Senses of Fish Adaptations for the Reception of Natural Stimuli*. New Delhi: Narosa Publishing.

Morphology of the Gustatory (Taste) System in Fishes

TJ Hara, University of Manitoba, Winnipeg, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Peripheral Gustatory System
Central Gustatory Pathways
Palatal Organs and Barbels
Alliance with the Trigeminal Nerve
Concluding Remarks
Further Reading

Glossary

Desmosome A plaque-like structure on the surface of the cell that functions in adhesion with adjacent cells.

Perigemmal Surrounding a budlike or bulblike body; denoting a mode of nerve termination by fibrils surrounding an end bulb.

Peripheral Gustatory System

Taste Buds

Distribution

Taste buds, also termed terminal buds, end buds, or cutaneous buds, constitute the structural basis of the peripheral gustatory organ. The term gustatory system is used to designate these organs, together with their nervous pathways toward and within the brain. In teleosts, taste buds are distributed in five subpopulations, to varying degrees: (1) oral, (2) palatal and laryngeal, (3) branchial (gills), (4) cutaneous, and (5) barbels (**Figure 1**).

The taste bud distribution patterns reflect feeding habits, strategies, and habitats of fishes. Most teleosts lack external taste buds, although some have independently evolved external taste buds on specialized parts of the body, such as barbels, elongated fin rays, and the lips of the mouth. Catfishes and goatfish (*Parupeneus* sp.) are a notable exception to the general condition among teleosts, as they have evolved thousands of external taste buds distributed over the barbels and the entire body surface. Yellow bullhead catfish (*Ictalurus natalis*), for example, have more than 175 000 taste buds on the entire body surface alone. In goatfish, the entire surface of the barbels is distributed with taste buds, with the densities of 150 to 250 mm^{-2}. Carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) have evolved a highly sophisticated food separation system on the roof of the mouth (palatal organ) that is studded with thousands of taste buds. In rainbow trout (*Oncorhynchus mykiss*), a visual feeder, taste buds are restricted to the lips, the palates, and gill rakers with the highest density of 30 per mm^2 totaling 3000–4000. In amago salmon (*Oncorhynchus rhodurus*), the number of taste buds in the oral cavity increases during the first 60 days post-hatching, then sharply increases over the next 300 days, reaching a total number of more than 15 000, at which time smolt transformation takes place. Freshwater species generally have well-developed taste buds in the oropharyngeal cavity and gill arches/rakers, which is thought to be an adaptation to freshwater habitats where a wider range of physical and chemical changes exist than in sea water.

Structure

Most taste buds are bulbiform or flask-shaped, which vary in size (45–75 μm in length and 30–50 μm in width) depending on the thickness of the epithelial layer, and are oriented perpendicular to the skin surface (**Figure 2**). The sensory area, or taste bud pore, is about 10 μm in diameter. A taste bud is buried in the epithelial layers or sits on a dermal papilla without being partitioned by membranes or any other tissues from the surrounding epithelial cells. Generally, three gustatory cell types constitute a fish taste bud: (1) those ending with a rod-shaped apical protrusion or cilia (0.5 μm thick and 1.5–3.0 μm long; rod cell or light cell); (2) those ending with microvilli (0.1–0.2 μm in diameter and 0.5–1.0 μm in length; microvillar cell or dark cell); and (3) basal cell. The total number of gustatory cells in a taste bud varies greatly; for example, 5 cells in *Pomatoschistus*, 67 in *Corydoras*, and as many as 100 in *Ciliata mustela* (60 rod cells and 49 microvillar cells). It is believed that the taste bud evolved while fish took possession of their own distinct ecological niche, thus resulting in considerable variation in their micromorphology. Some believe that taste buds are species specific, and no common type of taste bud exists in fish. Furthermore, in channel catfish (*Ictalurus punctatus*), taste buds innervated by the facial nerve contain significantly more cells than those innervated by the vagal nerve (216.3 ± 34.6 vs. 135.0 ± 19.2), and show heterogeneous distribution patterns of immunoreactivities for metabolites, notably more γ-aminobutyric acid (GABA) positive cells (microvillar cells) exist in the vagal innervated taste buds.

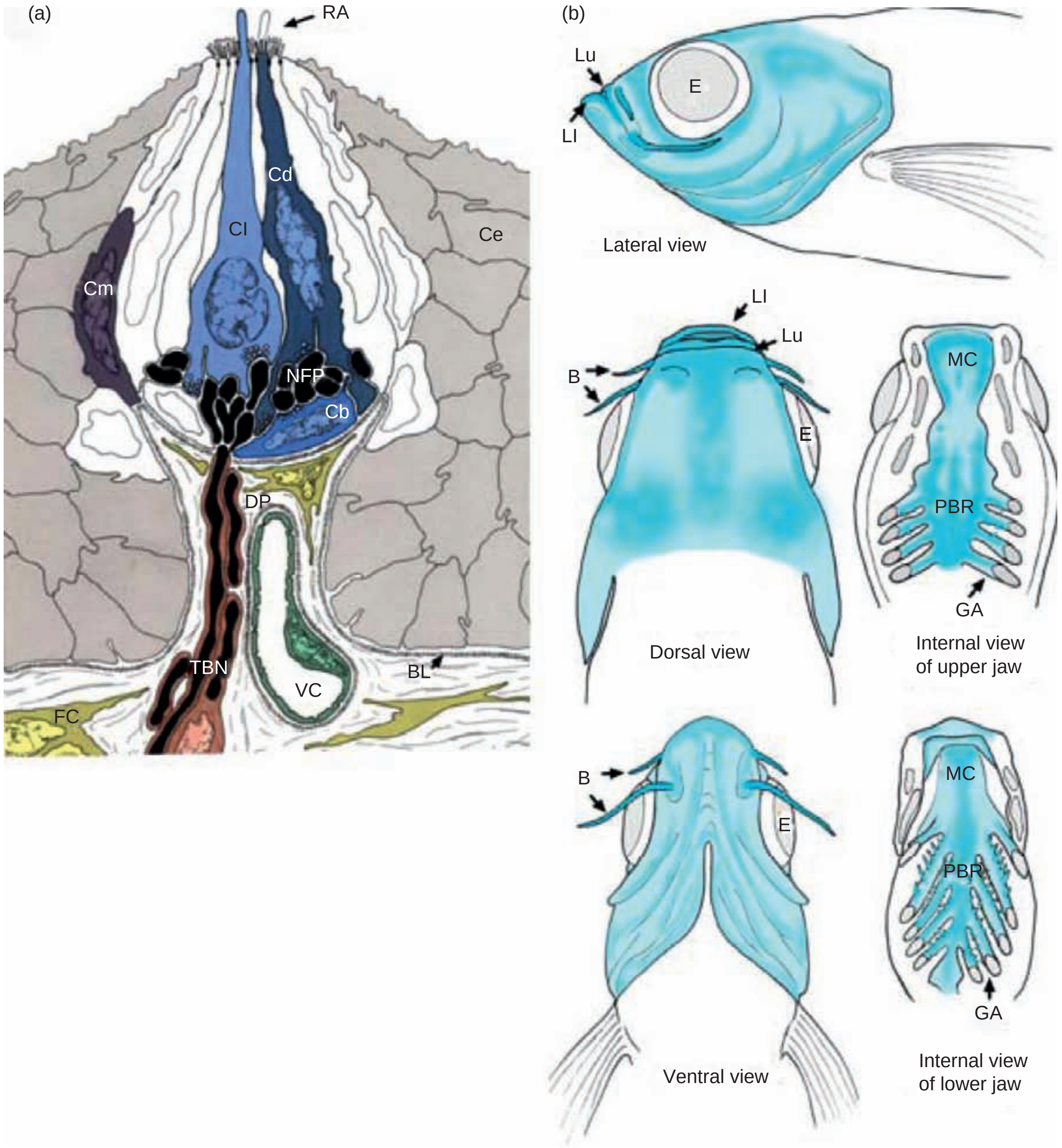


Figure 1 (a) Schematic drawing of a taste bud (TB) typical of teleosts. Dark cells (Cd) with microvilli, light cells (Cl) with a single rod-like protrusion, and basal cells (Cb) constitute a pear- or onion-shaped TB sitting on a dermal papilla (DP). Marginal cells (Cm), not belonging to the TB proper, form the interface between the TB and the epithelial cells (Ce). The TB nerve (TBN) reaches into the TB to form the nerve fiber plexus (NFP). (b) Distribution of TBs in juvenile and adult zebrafish (*Danio rerio*). High-density distribution of TBs is marked by darker shades of color. B, barbels; BL, basal lamina; E, eye; GA, gill arches; Ll, lower lip; Lu, upper lip; MC, mouth cavity; PBR, pharyngo-branchial region; and RA, receptive area. Modified from Hansen A, Reutter K, and Zeiske E (2002) Taste development in Zebrafish, *Danio rerio. Developmental Dynamics* 223: 483–496.

The rod cell, most likely a receptor cell, is cylindrical to spindle shaped. Its supranuclear cytoplasm contains numerous electron-dense tubules (0.4 − 0.6 μm), and the infranuclear portion is characterized by the presence of abundant vesicles (50–70 nm in diameter). Microvillar cells, often believed to be a supporting cell, but, could well be a receptor cell, are characterized by numerous fine filaments (*c.* 50 nm in diameter), running through the entire cytoplasm either diffuse or in bundles. Junctional complexes exist between microvillar cells and between the rod and microvillar cells.

The basal cells, typically one to five per taste bud, are situated at the bottom of the taste bud in depressions of the basal lamina, perpendicular to its longitudinal axis. They attach to both receptor and supporting cells by desmosomes. The basal cells contain many lucent vesicles

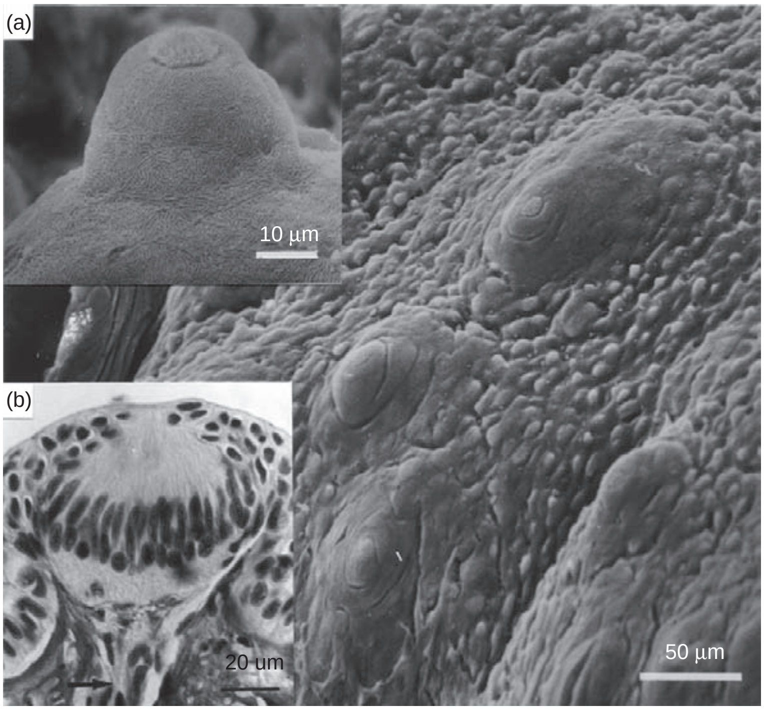


Figure 2 Morphology and distribution of taste buds on the palate of rainbow trout *Oncorhynchus mykiss*. (a) Scanning electron micrograph of a single taste bud located on an elevated papilla. (b) Light micrograph of a longitudinal section through a taste bud on the palate. Adapted from Hara TJ (2005) Olfactory responses to amino acids in rainbow trout: Revisited. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp. 31–64. Enfield: Science Publishers.

(40–50 nm in diameter), rich in serotonin (5-HT), and possess synaptic connections with both cell types as well as with nerve fibers, suggesting that they might act as modulators of gustatory activity. Unlike those of mammalian taste bud, the basal cells of fish are not the precursors of other cell types within the taste buds.

Taste buds are innervated by bundles of unmyelinated nerve fibers that enter from below into their basal portion passing by the side of the basal cells. Generally, one to three nerve bundles supply each taste bud, but the number and pattern of the supply vary among species and locations, even within the same species. Upon entering a taste bud, they further branch out and make an intricate intragemmal nerve plexus, some components of which make synaptic contacts either with the rod cells or with the microvillar cells. However, the synaptic specialization at these nerve-ending receptor junctions is not always clear such as those found in the central nervous system. Even in the mammalian taste buds, synaptic contacts between gustatory cells and nerve endings are not all clear-cut. Synapses are clustered with vesicles (40–70 nm) within the cell cytoplasm adjacent to the presynaptic membrane. In mammals, adenosine-5′-triphosphate (ATP), and not 5-HT, is suggested to serve as a key neurotransmitter linking taste buds to sensory nerve fibers. Mice with genetically knockout ionotropic purinergic receptors (P2Xs) either reduce or eliminate gustatory nerve and behavioral responses to sweeteners, glutamate, and bitter substances, leaving the responsiveness to touch and temperature intact, while 5-HT knockout mice reduce the behavioral responsiveness to pain, leaving the chemical sensitivity intact.

Development

Gustatory cells of vertebrates, unlike all other sensory receptor cells and neurons, are unique in that they originate from local epithelial tissue elements of primary endoderm, and not from neurogenic ectoderm (neural tube, neural crest, or ectodermal placodes). Nevertheless, gustatory cells form synapses and are capable of generating receptor potentials, and like epithelial cells they have a limited life span and regularly replace themselves.

Ontogenetically, taste buds generally develop later than their counterpart olfactory system. For example, in rainbow trout the earliest taste buds are seen only in larvae 8 days post-hatching. Taste bud primordia appear first around the mouth and within the oropharyngial regions a few days prior to hatching, far ahead of those in the head region, in zebrafish (*Danio rerio*) and channel catfish (*Ictalurus punctatus*). The primordial cells accumulated beneath the single layer of epithelial cells begin to elongate from the basal lamina to the epithelial surface by 4 days post-fertilization. By 10 days post-fertilization, the primordia begin to erupt as mature taste buds, at which time basal cells and nerve fibers are clearly seen and the dermal papillae begin to form beneath the basal cells. Although details of the development of the innervation of taste buds are still unknown, taste buds are believed to arise from within the specified epithelium (early specification) rather than being induced (nerve induction) by peripheral nerves. Thus, the taste bud, while maintaining its entity, is a constituent of the epithelium without partition from the surrounding epithelial cells. The average life span of taste bud cells on the channel catfish barbel is 12–42 days. Unlike the olfactory epithelium, in which the basal cells function as progenitors for the receptor neurons, epithelial cells surrounding the taste buds divide and some of their daughter cells migrate into taste buds to form gustatory cells.

Central Gustatory Pathways

Primary Gustatory Centers

The gustatory system is the only vertebrate sensory system in which three cranial nerves carry all peripheral gustatory information. Depending upon the location of the taste buds, either the facial (cranial nerve VII), glossopharyngeal (IX), or vagal (X) gustatory nerves innervate the gustatory cells. Generally, all cutaneous taste buds on the body surface and rostral oral regions are innervated by the facial nerve, while taste buds within the posterior oral cavity and pharyngo-branchial region are innervated by the glossopharyngeal and vagal nerves. Somatotopic organization generally exists in the termination of axons in the primary gustatory nucleus (PGN), the mammalian equivalent of the nucleus of the solitary tract (NST). The gustatory nerves from the different regions of the body thus enter the nucleus in approximately the same order as they are located in the body, that is, axons from more rostral regions of the mouth (or body) enter the more rostral portions of the nucleus (**Figure 3(a)**).

Both the vagal and facial lobes have two main types of secondary neurons: (1) large specialized neurons superficially arranged over the lobes – the chief secondary gustatory neurons and (2) small intrinsic neurons, filling the interior of the lobe. The chief gustatory neurons, directly receiving the peripheral gustatory terminals of the first order, give rise to the long paths of the secondary connection primarily to the superior secondary gustatory nucleus (SGN) in the isthmus, while the intrinsic neurons function as interneurons within the lobe.

Secondary and Tertiary Gustatory Nuclei (SGN and TGN)

The major efferent projections of the primary gustatory center ascend via two pathways (**Figure 4**). Typically, the primary centers project to the superior SGN in the pons (or isthmus), to the tertiary gustatory relay nucleus in the caudal diencephalon, and then to the telencephalic gustatory area. The SGN neurons also project to the hypothalamic inferior lobe (IL) and to a posterior thalamic nucleus. The caudal part of the diencephalon is called the preglomerular nuclear complex. In the rainbow trout, neurons of the preoptic nucleus project to the SGN, suggesting that preoptic SGN and/or preoptic primary gustatory column projections may be important neuroregulatory circuits in fishes. The IL is a large and highly differentiated brain part in teleosts comparable to other major brain parts such as cerebellum, optic tectum, or telencephalon, and is believed to represent a multisensory integration center. Thus, the ascending gustatory connection pattern is shared by all teleosts and seems to represent the ancestral patterns for teleosts. The SGN is the homolog of the parabranchial nucleus, that is, the pontine taste area of mammals.

Telencephalic Gustatory Areas

Telencephalic projections of the secondary and tertiary gustatory neurons have been demonstrated in carp, channel catfish, goldfish, and rainbow trout. Generally, gustatory

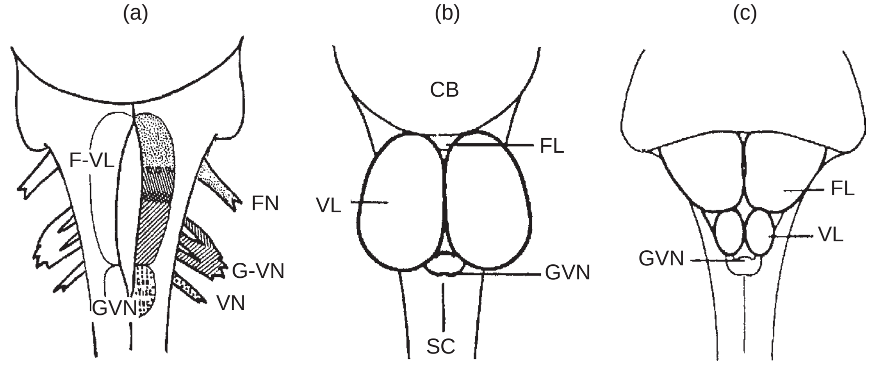


Figure 3 Diagram showing the dorsal view of gustatory and general visceral nuclei in the medulla of unspecialized teleosts (a), goldfish, *Carassius auratus* (b), and catfish, *Ictalurus punctatus* (c). Facial (FN), glossopharyngeal-vagal (G-VN), and vagal nerve (VN) terminate in rostrocaudal sequence in gustatory lobes. CB, cerebellum; FL, facial lobe; F-VL, facial-vagal lobe; GVN, general visceral nucleus; and SC, spinal cord. Adapted from Finger TE (1998) Organization of chemosensory systems within the brain of bony fishes. In: Atema J, Fay RR, Popper AN, and Tavolga WN (eds.) *Sensory Biology of Aquatic Animals*, pp. 339–363. New York: Springer-Verlag, and Kanwal JS and Finger TE (1992) Central representation and projections of gustatory systems. In: Hara TJ (eds.) *Fish Chemoreception*, pp. 79–125. London: Chapman and Hall.

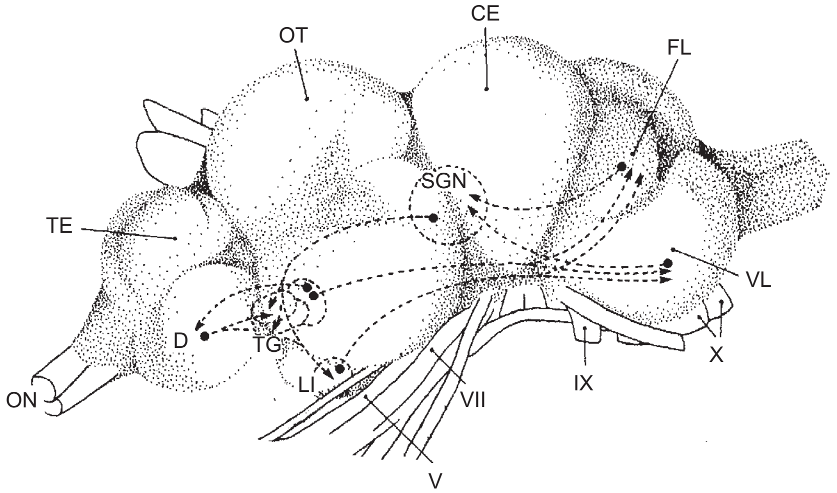


Figure 4 Schematic diagram of the dorsolateral view of the brain of a cyprinid fish showing the entrance of trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) nerves, and the main central gustatory connections. CE, cerebellum; D, dorsal telencephalic area; FL, facial lobe; LI, hypothalamic inferior lobe; ON, olfactory nerve; OT, optic tectum; SGN, secondary gustatory nucleus; TE, telencephalon; and TG, tertiary gustatory nucleus. Modified from Luiten PGM (1975) The central projections of the trigeminal, facial and anterior lateral line nerve in the carp (*Cyprinus carpio L.*). *Journal of Comparative Neurology* 160: 399–417.

nuclei in the telencephalic region include medial and central portions of area dorsalis, which receive projections from the pTGN. In tilapia (*Oreochromis niloticus*), for example, preglomerular TGN (pTGN) projects to the dorsal region of area dorsalis pars medialis (dMm), while in rainbow trout, it projects to the medial region of the dorsal telencephalic area (Dm) and the ventral telencephalon. In addition, in tilapia, the SGN projects to area ventralis pars intermedia (Vi) and area dorsalis pars posterior (Dp). Note that the Vi and the Dp of tilapia and Dm and the ventral telencephalon in salmonids receive direct projections from the olfactory bulb. As Herrick pointed out with regard to the major importance of the relation of gustation and olfaction, the secondary center is the area olfactoria of the forebrain, whose commissure bears the same relation to the secondary tracts as do those of the secondary gustatory nuclei. The main tertiary tract passes to the inferior lobe, which is, in fishes, the central correlation station for all sensory impression. The olfactory and gustatory tertiary tracts end together throughout the inferior lobe and they have a common descending conduction path, the tractus lobobulbaris (**Figure 4**).

The ascending fiber connections of the central gustatory system in teleosts are essentially similar to those of mammals. The mammalian SGN projects directly to the amygdala, which receives direct projections from the olfactory bulb. Phylogenetically, the Vi and Dp are respectively considered as a part of the amygdala and the lateral pallium.

Descending Projections to Facial and Vagal Lobes

The inferior lobe and lobobulbar lobe develop extensive descending fiber projections to the primary gustatory centers in the facial (cyprinids) and vagal (catfishes) lobes as well as in the viscerosensory column (trout). The vagal lobe, as described, projects to motoneurons that innervate the oropharyngeal musculature, which controls swallowing or intraoral food handling in cyprinids (**Figure 5**). By contrast, the facial lobe of catfishes projects to somatosensory nuclei in the caudal medulla, which may provide a means to correlate gustatory and somatosensory inputs from a single locus on the body surface. Furthermore, the return path for all sensory activities of the inferior lobe is the tractus lobobulbaris passing into the oblongata, through which the descending neurons of the inferior lobe communicate with the peripheral motoneurons of the oblongata, and probably also of the spinal cord, thus providing for the most complex reflexes that a fish can achieve.

Palatal Organs and Barbels

Although the gustatory system is generally well developed throughout fishes, two groups of fishes, cyprinids and ictalurids, as well as goatfishes, have become highly specialized for the use of sophisticated food-handling apparatus, the palatal organ and the barbel, and therefore, deserves special attention here. Along with the expansive taste buds, they have developed the most complex neural organization seen in any fish central nervous system. Both groups of fishes are bottom feeders, and have evolved highly sophisticated mechanisms of food detection and separation. In goldfishes and carps, the palatal organ, which is a muscular structure attached to the roof of the mouth and studded with numerous taste buds, combined with the surface of the gill arches, manipulate to separate palatable food materials from the

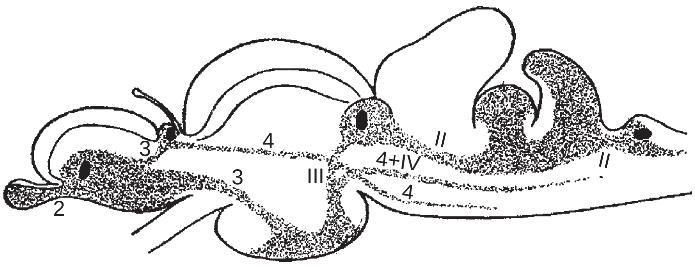


Figure 5 Diagram showing the relations of the gustatory and olfactory centers in teleosts, as represented by the shaded areas. The black spots represent the position of commissures of secondary or tertiary fibers. Numbers 2, 3, and 4 represent the olfactory conduction paths of the second, third, and fourth orders, respectively, and II, III, and IV represent the gustatory conduction paths of the second, third, and fourth orders, respectively. From Herrick CJ (1905) The central gustatory paths in the brains of bony fishes. *Journal of Comparative Neurology and Psychology* 15: 375–456.

unpalatable. The vagal nerve terminates in the gustatory nucleus as in other fishes, but the gustatory nuclei in these fishes have largely bulged out to form a prominent vagal lobe (cf. **Figure 3(b)**). The internal structure of the vagal lobe is topographically organized, that is, the entire structural feature of the oropharyngeal cavity is mapped onto the vagal lobe, so that the anterior end of the palatal organ is represented anteriorly in the lobe and progressing posteriorly. The vagal lobe consists of a series of nine layers of neurons with a complex organization. In addition to a topographical organization of mouth structure in the vagal lobe, gustatory nerves from each of the oropharyngeal structures terminate in specific layers of the lobe, that is, nerves innervating taste buds of the palatal organ project to layer 6, those innervating the gill arches project to layers 2 and 4, and so on. In parallel with the sensory neuron inputs, the outputs of the motor neurons innervating musculatures of the palatal organ and gill arches, occupying the deepest layer of the lobe, are also organized in a topographic fashion (**Figure 6**).

In catfishes, the facial lobe is large, lying rostro-medial to the vagal lobes (cf. **Figure 3(c)**). The facial lobe is organized into six longitudinal columns or lobules extending rostrocaudally and arranged alongside each other, and receives the gustatory axons of the ventrolateral facial nerve. Each lobule receives segregated input from discrete portions of the body surface. The three medial lobes in channel catfish respectively receive input from the medial and lateral mandibular barbels and the maxillary barbels. The two ventral lobules receive inputs from the nasal barbel and pectoral fin and a large dorsolateral lobule from the face and trunk. Thus, the entire extraoral surface of the body is mapped somatotopically onto the facial lobe in a well-defined manner.

In goatfish, taste buds are evenly distributed on a single pair of barbels extending vertically from the lower jaw. Unlike the palatal organs in cyprinids, no muscular structure exists within the barbels. Longitudinally running nerve branch originating from the main nerve trunk of the facial nerve sends a pair of circumferential branches medially and laterally around the margins of a barbel which innervate four taste bud clusters, each containing 14 taste buds, at each proximal–distal level. Thus, each longitudinal nerve, consisting approximately 90 fibers, innervate a total of 56 taste buds, forming a functional unit.

Alliance with the Trigeminal Nerve

The complex gustatory system as has been described makes itself even more complex by the fact that the facial nerve is closely allied with the trigeminal nerve, and strong tactile component exists in the gustatory sensory signal. In vertebrates, the trigeminal nerve generally carries the sensory nerves including pain, temperature, touch, and proprioception from the jaws and head as well as the motor neurons controlling the jaw muscles. Catfish barbells are heavily innervated both by trigeminal and facial nerves, both of which project to the facial lobe by three branches – ophthalmic, maxillary, and mandibular. The trigeminal fibers terminate not only in the principal and spinal trigeminal nuclei but also throughout the facial lobe, with the exception of the lateral-most lobule which contains a representation of taste buds innervated by the recurrent branch of the facial nerve. The trigeminal fibers are coarser than those of the facial which terminate within the same structural loci, and its input to the primary gustatory complex is restricted to those portions of the nucleus receiving sensory inputs from the face and barbels.

Contrary to its central projections, virtually nothing is known about the peripheral termination of the trigeminal nerve, except for the observations that in sea catfish and goatfish some nerve fibers terminate inside taste buds perigemmally without ending on gustatory cells. It thus appears as if the trigeminal fibers have failed to locate their target cells, that is, they become orphaned gustatory nerves. Thus, the relationship between gustatory and tactile sensibility is well recognized but its implications are not fully understood. It seems there may be no pure gustatory area at any level of the gustatory neuraxis.

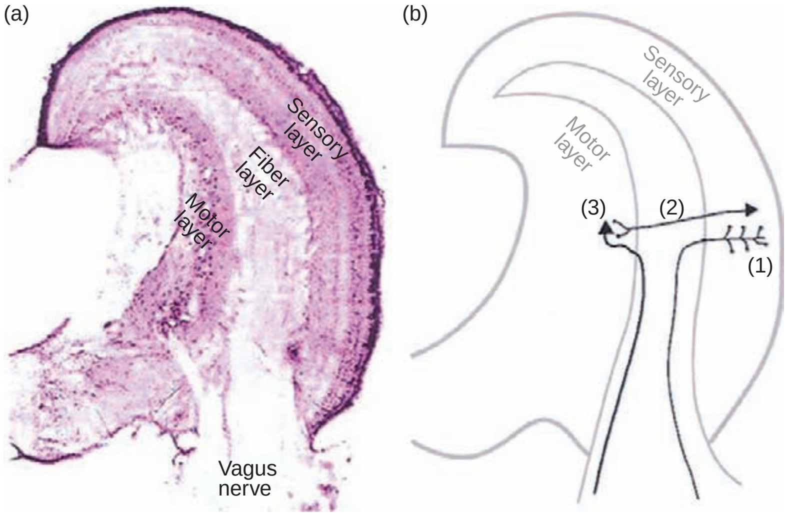


Figure 6 (a) Micrograph of the vagal lobe showing the overall laminar organization. The sensory fibers terminate superficially in the sensory layer; the motor output to the palate originates from motoneurons of the motor layer. (b) Hypothesized reflex circuit utilized in food sorting. Sensory information from a particular point in the oral cavity will terminate in a limited portion of the sensory layer (1) where it activates local interneurons (2). These interneurons have axons that traverse the fiber layer to terminate on the underlying motoneurons (3). The motoneurons that receive the reflex connection innervate muscles in the same local region of the palate from which the sensory input arises. Adapted from Finger TE (2008) Sorting food from stones: The vagal taste system in goldfish, *Carassius auratus*. *Journal of Comparative Physiology A* 194: 135–143.

Concluding Remarks

A great deal of effort has been expended to work out, at the peripheral end, the details of the structure of taste buds including distribution on the body surface, fins, lips, and in the mouth, which provide fishes with a highly detailed gustatory map, chemical or tactile, of the environment as well as the means to detect food and reject noxious objects. Centrally, details of the extremely well-developed vagal/facial lobe system in cyprinids, silurids, and goatfish have enhanced our understanding of the way gustatory information is processed in the central nervous system in vertebrates in general. Despite these exciting advances, two of the major aspects of the peripheral gustatory system still remain unexplored: (1) characterizing homo- or heterogeneity of receptor cells, whether it be the light or dark, or rod or microvillar cells, within a taste bud and synaptic contacts with innervating gustatory nerves and (2) identifying gustatory cells that have the high percentage of the chemical, or tactile, sensitivity.

See also: **Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Gustation; Neurophysiology of Olfaction.

Further Reading

Atema J (1971) Structures and functions of the sense of taste in the catfish (*Ictalurus natalis*). *Brain, Behavior and Evolution* 4: 273–294.

Eram M and Michel WC (2005) Morphological and biochemical heterogeneity in facial and vagal nerve innervated taste buds of the channel catfish, *Ictalurus punctatus*. *Journal of Comparative Physiology A* 486: 132–144.

Finger TE (2008) Sorting food from stones: The vagal taste system in goldfish, *Carassius auratus*. *Journal of Comparative Physiology A* 194: 135–143.

Finger TE and Silver WL (eds.) (1987) *Neurobiology of Taste and Smell*. New York: Wiley.

Hara TJ (ed.) (2002) *Fish Chemoreception*. London: Chapman and Hall.

Hara TJ and Zielinski BS (eds.) (2007) *Sensory System Neuroscience, Fish Physiology*, vol. 25, pp 45–96. New York: Elsevier.

Herrick CJ (1905) The central gustatory paths in the brains of bony fishes. *Journal of Comparative Neurology and Psychology* 15: 375–456.

Hirata Y (1966) Fine structure of the terminal buds on the barbels of some fishes. *Archivum Histologicum Japonicum* 26: 507–523.

Jakubowski M and Whitear M (1990) Comparative morphology and cytology of taste buds. *Zeitschrift für mikroskopisch-anatomische Forschung* 104: 529–560.

Kiyohara S, Yamashita S, Lamb CF, and Finger TE (1999) Distribution of trigeminal fibers in the primary facial gustatory center of channel catfish, *Ictalurus punctatus*. *Brain Research* 841: 93–100.

Northcutt RG (2004) Taste buds: Development and evolution. *Brain, Behavior and Evolution* 64: 198–206.

Northcutt (2005) Taste bud development in the channel catfish. *Journal of Comparative Neurology* 482: 1–16.

Raderman-Little R (1979) The effect of temperature on the turnover of taste bud cells in catfish. *Cell and Tissue Kinetics* 12: 269–280.

Reutter K and Kapoor BG (eds.) (2005) *Fish Chemosenses*. Enfield, NH: Science Publishers.

Reutter K and Witt M (1993) Morphology of vertebrate taste organs and their nerve supply. In: Simon SA and Roper SD (eds.) *Mechanisms of Taste Transduction*, pp 29–82. Boca Raton, FL: CRC Press.

Stone LM, Finger TE, Tam PPL, and Tan S-S (1995) Taste receptor cells arise from local epithelium, not neurogenic ectoderm. *Proceedings of the National Academy of Sciences of the United States of America* 92: 1916–1920.

Toyoshima K, Nada O, and Shimamura A (1984) Fine structure of monoamine containing basal cells in the taste buds of the barbels of three species of teleosts. *Cell and Tissue Research* 235: 479–484.

Wullimann MF (1998) The central nervous system. In: Evans DH (ed.) *The Physiology of Fishes*, pp. 245–282. Boca Raton, FL: CRC Press.

Morphology of the Olfactory (Smell) System in Fishes

TJ Hara, University of Manitoba, Winnipeg, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Peripheral Olfactory Organ
Olfactory Bulb and Central Projections
Concluding Remarks
Further Reading

Glossary

Anlage In embryology, an embryonic area capable of forming a structure: the primordium or bud.

Desmosome A plaque-like structure on the surface of a cell that functions in adhesion with adjacent cells.

Placodes Plate-like thickenings of embryonic ectoderm from which definitive structures develop.

Stroma Refers to the supporting framework of an animal organ typically consisting of connective tissue.

Peripheral Olfactory Organ

Development of the Olfactory Organ

Ontogenetically, the peripheral olfactory organ is the first chemosensory organ to develop in fish, preceding the gustatory. It originates from an anlage formed by the ectoderm and remains ectodermal throughout its development. In rainbow trout (*Oncorhynchus mykiss*), the anlage appears on the ventrolateral part of the head just in front of the rudimentary eyes as early as 11 days postfertilization, and 5–8 mm in length (**Figure 1**). Separate ventrolateral olfactory placodes emerge from the thickened ectoderm, built by cells that separate from the anterior neural plate. The homogeneous population of the olfactory placodal cells then differentiates into the olfactory organ. On 12–16 days postfertilization, the embryos rapidly elongate, primordial organ systems become visible, and the olfactory placodes thicken and begin to form a central depression. At hatching, the olfactory pits, now rectangular-shaped grooves, migrate to a dorsolateral position on the head. In embryos, 15–20 mm in length, the median portion of the external lip of the elongating olfactory pit expands toward the internal lip, resulting in the formation of distinct anterior and posterior nares. The salmonid type of nasal formation is widespread among teleosts, and is phylogenetically considered to be archetypical.

There is considerable variation in size, structure, and degree of development of the olfactory organs. The paired olfactory pits are usually located on the dorsal side of the head. Eels and morays have large and elongated olfactory pits, extending from the tip of the snout to the orbit of the eye. Certain puffers (Tetrodontidae), in contrast, have completely lost the nasal sac. However, in most teleost species, the olfactory organ lies somewhere between these two extremes. In few cases, there are differences between sexes of the same species, for example, bathypelagic anglerfishes.

Each nasal pit generally has two openings, anterior inlet and posterior outlet, which are separated by a nasal flap or bridge. A current of water enters the anterior and leaves through the posterior opening, either passively through the locomotion of the fish in water, or actively by ciliary action within the pits or by the pumping action of the nasal sac. In some teleosts, two accessory nasal sacs, the ethmoid and the lacrimal sac, a single nasal sac, or a fused nasal sac assist in ventilating unidirectional water flow through the nasal cavity. The occurrence of these nasal sacs has long been associated with the need to regulate water flow in sedentary bottom-dwelling fish, but this is questionable in many cases (**Table 1**).

Olfactory Epithelium

The olfactory epithelium lines the olfactory cavity and is generally raised from the floor of the organ into a complicated series of folds or lamellae to make rosette-like arrangements. The arrangement, shape, and degree of development of the lamellae in the olfactory rosette of teleosts vary considerably among species. The largest number of lamellae (over 200) occurs in Anguilliformes, while few or no lamella exists in some Atheriniformes and Tetrodontiformes. Normally, a central ridge is formed rostro-caudally on the bottom of the olfactory chamber from which a varying number of transverse lamellae radiate. In some teleosts, a series of parallel lamellae occur in the absence of the ridge. A new lamella develops caudad and progresses rostrad, so that the oldest and largest lamella is located most posteriorly. The number of the lamellae increases to some extent with growth of

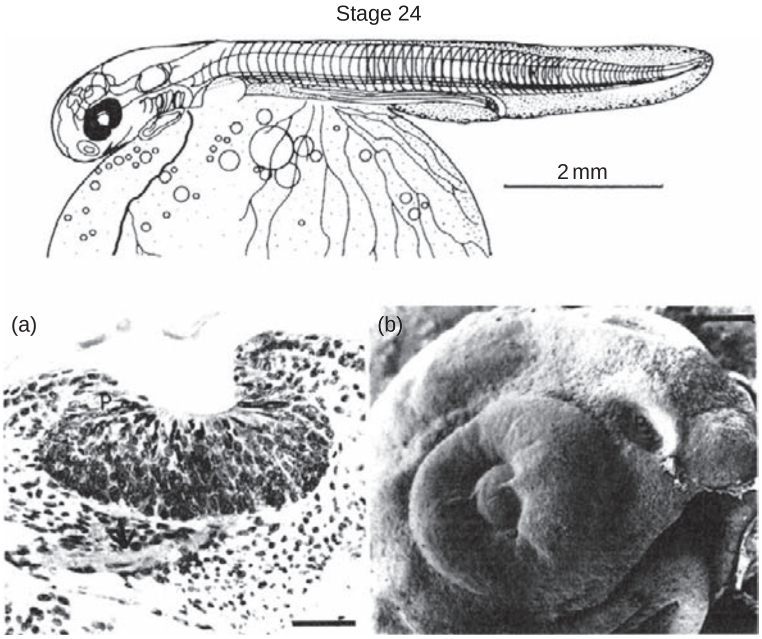


Figure 1 Formation of the olfactory placode and development of the olfactory neuron in rainbow trout embryo. (a) Histologically identifiable olfactory nerve (arrow) extending from the placode (P) to the brain; bar = 200 μm. (b) Scanning electron micrograph of the olfactory placode (P); Scale = 50 μm. Adapted from Hara and Zielinski (1989) Structural and functional development of the olfactory organ in teleosts. *Transactions of the American Fisheries Society* 118: 183–194.

individuals, but remains constant after the fish reaches a certain stage in growth. Secondary folding of the lamella occurs in some species. Most notable are salmonids; there are 5–10 secondary folds per lamella in adults, but none in the parr and younger stages (**Figure 2**).

The olfactory lamella is composed of two layers of epithelium enclosing a thin stromal sheet. The epithelium, columnar pseudo-stratified (65–85 μm in thickness), is separated into two regions: sensory and nonsensory (**Figure 3**). The sensory epithelium shows various distribution patterns: (1) continuous except for the lamella margin (*Anguilla*, *Ictalurus*), (2) separated regularly by the nonsensory epithelium (Salmoniformes), (3) interspersed irregularly with the nonsensory epithelium (*Gasterosteus*, *Thunnus*), and (4) scattered in islets (*Phoxinus*, *Cyprinus*, *and Carassius*). The sensory epithelium consists of three main cell types: (1) receptor cells (olfactory receptor (OR) neurons), (2) supporting cells, and (3) basal cells. The nonsensory epithelium is of a stratified squamous type, usually thinner than the sensory epithelium. It consists of epidermal cells, ciliated nonsensory cells, and basal cells. Mucus cells (goblet cells) usually occur only within the nonsensory epithelium. The stromal sheet which is separated by a basal lamina from the epithelium is filled with loose fibers and cell bodies of the connective tissue. It also contains blood capillaries and bundles of axons arising from the bottom of the receptor neurons. In addition to the olfactory axons, free nerve endings are generally present, some of which are identified as sensory fibers of the trigeminal nerves.

Olfactory Receptor Neurons

Traditionally, two morphologically and ontogenetically distinct receptor neuron types have been recognized in teleosts: ciliated and microvillar (**Figure 4**). This situation contrasts with those of terrestrial vertebrates including humans, where the olfactory neurons are segregated into two separate compartments: (1) ciliated neurons populate the main olfactory epithelium, and (2) microvillar neurons lie in the vomeronasal organ. In rainbow trout embryos, the ciliated form precedes the microvillar form; the former appears when the olfactory placode is folded to form a groove-like structure rostrad to the eye, and predominate until immature microvillar receptor neurons develop at about 28 days postfertilization. A third neuron type, crypt cells have recently been added to the list of receptor neurons. The crypt cell type is widely distributed in teleost fishes, but has not yet been reported in salmonids. The scarcity, however, makes them difficult to find in ordinary electron-microscopic preparations, despite the fact that they line up in a queue in some species. Elasmobranchs and Australian lungfish (*Neoceratodus forsteri*) have only microvillar-type neurons, while river lamprey (*Lampetra fluviatilis*) has only the ciliated. There is currently no evidence to suggest that different neuron types mediate responses to different odorants.

The bipolar olfactory neuron has a cylindrical dendrite that terminates at the free surface of the epithelium. The distal end of the dendrite forms a swelling (olfactory

Table 1 The gross morphology of the peripheral olfactory organ in teleost fishes, showing the occurrence of olfactory epithelial folding, accessory nasal sacs, and habitat

Taxon (some common names)	*Olfactory epithelium. Genus species*	*Number of nasal sacs. Genus species*	*Reference*	*Habitat*
Osteoglossiformes (knifefishes)	ML – *Mormyrus*	0 – *Mormyrus*	Burne (1909)	Demersal
	– *Pantodon buchholizi*		Hanson and Finger (2000)	Pelagic
	– *Pollimyrus castelnavi*			Demersal
Anguilliformes (eels)	ML – *Anguilla anguilla*	0 – *A. anguilla*	Burne (1909), Teichmann (1954)	Demersal
	– *Conger vulgaris*	*C.* – *vulgaris*	Burne (1909)	Demersal
	– *A. japonica, C. myriaster*		Yamamoto (1982)	
Clupeiformes (herrings)	ML – *Clupea harengus*	1 – *C. harengus*	Burne (1909)	Demersal
	– *Clupanodan punctatus*		Yamamoto (1982)	Demersal
Cypriniformes (carps and minnows)	ML – *Carassius auratus*		Liermann (1933)	Demersal
	– *Cyprinus carpio*	0 – *C. carpio*	Yamamoto (1982) and Doving *et al.* (1977)	Demersal/pelagic
	– *Danio rerio*		Hansen and Zeiske (1993)	Pelagic
	– *Phoxinus phoxinus*		Teichmann (1954)	Pelagic
	– *Tinca tinca*	0 – *T. tinca*	Doving *et al.* (1977)	Pelagic
Siluriformes (catfishes)	ML – *Ictalurus punctatus*		Caprio and Raderman-Little (1978)	Demersal
	– *Siluris glanis*	1 – *S. glanis*[*]	Burne (1909), Jakubowski and Kunysz (1979)	Demersal
	– *Plotosus lineatus*		Theisner *et al.* (1991)	Demersal
Esociformes (pikes)	ML – Esox lucius	0 – *E. lucius*	Burne (1909) and Teichmann (1954)	Demersal
Salmoniformes (trouts and salmons)	ML – *Onchorynchus mykiss*	0 – *S. trutta*	Teichmann (1954)	Pelagic
	– *Salmo trutta*	I – *Salmo salar*	Doving *et al.* (1977)	Demersal
			Burne (1909)	
	– *Salvelinus alpinus*		Hanson and Finger (2000)	Demersal
	– *Coregonus clupeaformis*		Hara *et al.* (1973)	Demersal
Gadiformes (cods)	ML – *Melanogrammus aeglefinus*[a]		Burne (1909)	Demersal
	– *Gadus morhua*		"	Demersal
	– *Lota lota*		Teichmann (1954)	Demersal
	– *Muraenolepis microps*	0 – *M. microps*	Eastman and Lanoo (2001)	Demersal

Atheriniformes (silversides)	IL – *Cololabis saira*	1 – *C. sentaniensis*	Yamamoto (1982)	Pelagic
	– *Hemiramphus sajori*[b]		Yamamoto (1982)	
	ML – *Chilatherina sentaniensis*		Yamamoto (1982)	Demersal
	– *Glossolepis incisus*	– *G.incisus*	Zeiske *et al.* (1979)	Demersal
	– *Melanotaenia sentaniensis*	– *Melanotaenia* sp.	Yamamoto (1982)	
	– *Iso hawaiiensis*		Yamamoto (1992)	Reef
Beloniformes (gars)	F – *Oryzias latipes*		Yamamoto (1982)	
	– *Belone vulgaris*	0 – *B. vulgaris*	Burne (1909) and Theisen *et al.* (1980)	Demersal
	– *Cheilopogon agoo*		Yamamoto (1982)	
Cyprinodontiformes (killifishes, swordtails)	F – *Austrofundulus transilis*	1 – *A. transilis*	Zeiske (1974)	Pelagic
	– *Cynolebias whitei*[c]	– *C. whitei.*	Zeiske (1974)	
	– *Pterolebias peruensis*[d]	– *P. peruensis.*	Yamamoto (1982)	
	– *Gambusia affinis*		Zeiske (1974)	Demersal
	F:IL – *Cynolebias nigripinnis*[e]	– *C. nigripinnis*	Zeiske (1974)	
	– *Rivulus cylindraceus*	– *R. cylindraceus.*	Zeiske (1974)	Benthopelagic
	IL-3L – *Aplocheilus lineatus*	– *A. lineatus*	Zeiske (1974)	Pelagic
	– *A.dayi*	– *A. dayi*	Kux *et al.* (1988)	
	ML – *Fundulus chrysotus*		Kux *et al.* (1988)	Demersal
	– *Valencia hispanica*		Kux *et al.* (1988)	Demersal
	– *Procatopus similes*		Kux *et al.* (1988)	Demersal
	– *Aplocheillchthys spliauchen*		Kux *et al.* (1988)	Benthopelagic
	– *Xiphophorus helleri*			Demersal
Gasterosteiformes (seahorses, pipefishes, sticklebacks)	F – *Hippocampus coronatus*[f]		Yamamoto (1982)	Demersal
	– *Sygnathus*		Liermann (1933)	Demersal
	1L – *Spinachia spinachia*	1 – *S. spinachia*	Theisen (1982)	Demersal
	2L – *Gasterosteus aculeatus*		Wunder (1957)	Demersal
Synbranchiformes	F – *Fluta alba*[g]		Yamamoto (1982)	Demersal
Perociformes (perches, clingfishes, gobies, tunas, cichlids)	F – *Conidens laticephalus*[h]		Yamamoto (1982)	
	– *Omobrabchus elegans*		Yamamoto (1982)	Demarsal
	IL – *Acanthogobius flavintanus*		Yamamoto (1982)	Pelagic
	– *Ammodytes personatus*		Yamamoto (1982)	Pelagic
	– *Odonoamblyopus rubicundus*		Yamamoto (1982)	Demarsal
	– *Neogobius melanostomus*	2 – *N. melanostomus*	Belanger *et al.* (2003)	Benthopelagic

(*Continued*)

Table 1 (Continued)

Taxon (some common names)	*Olfactory epithelium. Genus species*	*Number of nasal sacs. Genus species*	*Reference*	*Habitat*
	ML – *Mugil chelo*[i]	1 – *M. chelo*	Burne (1909)	Demersal
	– *Trachinus vipera*[j]	– *T. vipera*	Burne (1909) and Teichmann (1954)	Pelagic
	– *Perca fluvitilis*	2 – *P. fluvitilis*	Yamamoto (1982)	Pelagic
	– *Leiognaths nuchalis*		Yamamoto (1982)	Pelagic
	– *Toxotes jaculator*[k]		Yamamoto (1982)	Pelagic
	– *Sphyraena japonica*		Yamamoto (1982)	Pelagic
	– *Thunnus thunnus*		Yamamoto (1982)	Demersal
	– *Scomber japonicus*		Yamamoto (1982)	Reef
	– *Tilapia zillii*	2 – *T. zillii*	Feshelson (1997)	Reef
	– *Archicentrus nigrofasciatu*		Hanson and Finger (2000)	
Pleuronectiformes (flounders)	ML – *Pleuronectes flesus*[l]	2 – *P. flesus*	Burne (1909)	Demersal
Tetraodontiformes (puffers, boxfishes)	F – *Rudarius ercodes.*		Yamamoto (1982)	Reef
	– *Ostraction ruberculatus*		Yamamoto (1982)	
	IL – *Navodon modestus*[m]		Yamamoto (1982)	
	ML – *Fugu niphoble*[n]		Yamamoto (1982)	Demersal

[a] *Melanogrammus aeglefinus* was originally *Gadus aeglefinus* (Fishbase).

[b] *Hemiramphus sajori* is also known as *Hyporhamphus sajori* (Fishbase).

[c] *Cynolebias whitei* is the original name for this species, also currently called *Nematolebias whitei* (Fishbase).

[d] *Pterolebias peruensis* is the original name for this species, also currently called *Aphyolebias nigripinnis* (Fisgbase).

[e] *Cynolebias nigripinnis* is the original name for this species, also currently called *Austrolebias nigripinnis* (Fishbase).

[f] *Hippocampus coronatus* and *Sygnathus* species are listed under the Sygnathiformes order in Fishbase.

[g] *Fluta alba* is also known as *Muraena alba* and *Monopterus albus* (Fishbase).

[h] *Conidens laticephalus* was formally listed under the *Gobiesociformes* order.

[i] *Mugil chelo* is also known as *Mugil labrasus* and more currently, *Chelon labrosus* (Fishbase).

[j] *Trachinus vipera* is the original name for this species, also currently called *Echiichthys vipera* (Fishbase).

[k] *Toxotes jaculator* was originally known as *Sciaena jaculatrix* and is also currently known as *Toxotes jaculatrix* (Fishbase).

[l] *Pleuronectes flesus* is the original name for this species, also currently called *Platichthys flesus* (Fishbase).

[m] *Navodon modestus* was originally knows as *Monacanthus modestus*, and is also currently known as *Thamnaconus modestus* (Fishbase).

[n] *Fugu niphobles* was originally known as *Spheroides niphobles*, and is also currently known as *Takifugu niphobles* (Fishbase).

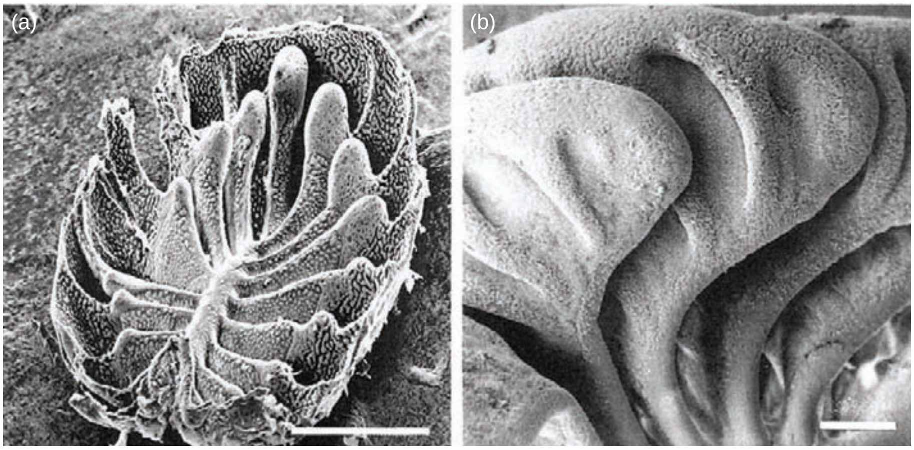


Figure 2 Scanning electron micrographs of an olfactory rosette of goldfish (a) and olfactory lamellae of rainbow trout (b). Scale = 300 μm. Adapted from Hara (2000).

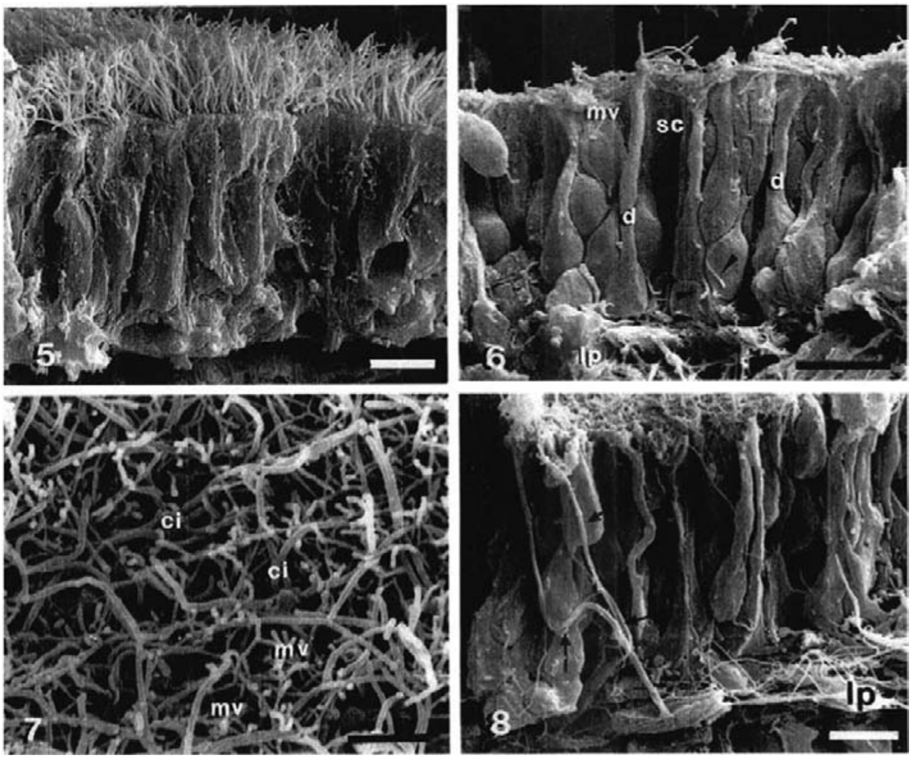


Figure 3 Scanning electron micrographs of fractions of nonsensory (a), sensory (b, d), and the surface of the sensory epithelium of zebrafish. Scale = 5 μm (a, b, d) and 2 μm (c). Adapted from Hansen and Zeiske (1998).

knob or olfactory vesicle), and protrudes slightly (about 1 μm) above the epithelial surface. Each dendrite, 1.5–2.5 μm in diameter, is enclosed entirely by supporting cells with tight junctions near the epithelial surface. The proximal part of the perikaryon (the cell body containing the nucleus) tapers to form an axon. The ciliated neuron has four to eight cilia radiating from an olfactory knob. Each cilium measures 2–7 μm in length and 0.2–0.3 μm in diameter. The cilia show the 9 + 2 arrangement of microtubules, which is identical with that of common kinocilia. The microvillar neuron bears from 30–80 microvilli (2–5 μm long and about 0.1 μm wide) depending upon species. The crypt neurons with ovoid to oval cell bodies (2–3 times the diameters of the other two types) are located in the upper third of the olfactory epithelium. Their axons travel toward the basal lamina and join the axons bundles of other neurons (olfactory nerve; cranial nerve I). The most striking feature is the

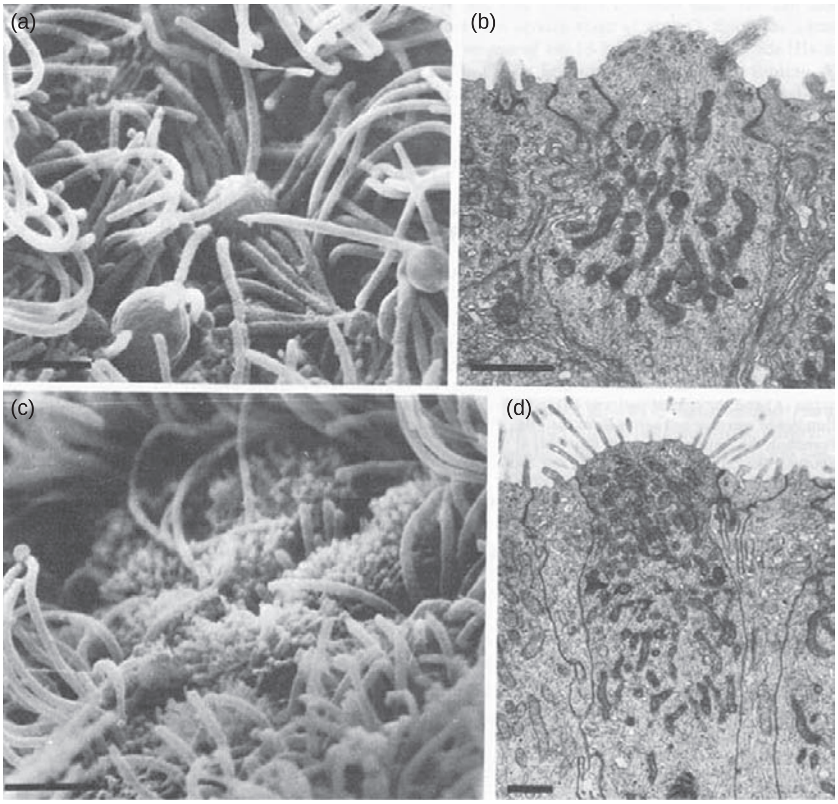


Figure 4 Scanning (a, c) and transmission (b, d) electron micrographs of ciliated and microvillar olfactory neurons of rainbow trout. Scale = 3, 4, 0.3, and 1 μm in (a), (b), (c), and (d), respectively. Adapted from Zielinski and Hara (1989).

apical invagination bordered by microvilli, forming a crypt. They bear cilia as well as microvilli. These cilia do not extend above the surface of the olfactory epithelium, and microvilli encircle the apical rim of the neurons. Thus, the crypt neurons have characteristics of both ciliated and microvillar neurons, assuming an aspect of a compound receptor neuron (**Figure 5**). This limited exposure to the environment could pose a serious problem in accessing to stimulant molecules.

Approximately 5–10 million olfactory neurons comprise the sensory epithelium on each side of the nose in an average teleost. A correlation exists between the receptor neuron morphology, the type of odorant receptor, and G-proteins expressed. An odorant receptor specific G-protein, $G\alpha_{olf}$ is expressed in ciliated olfactory neurons distributed homogeneously across the entire sensory epithelium of several fishes including goldfish. In contrast, microvillar neurons express $G\alpha_o$, $G\alpha_q$, or $G\alpha_{i\text{-}3}$, and, not surprisingly, crypt neurons express both $G\alpha_o$ and $G\alpha_q$ (**Figure 6**). Double-transgenic zebrafish show that the ciliated neurons project axons mostly to the dorsal and medial olfactory bulb regions, whereas the microvillar neurons project to the lateral region, which is established in very early embryonic stages (**Figure 7**).

Olfactory (Odorant) Receptors (ORs)

The initial step in olfaction requires the interaction of odorant molecules with specific receptors on OR neurons. This sensory information is then transmitted to the brain where it is decoded (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Olfaction). A large multigene family that encodes ORs on olfactory neurons was first identified in rodents. Subsequent studies identified homologous gene families in a variety of vertebrate species, including fishes: catfish, zebrafish, goldfish, medaka, loaches, and salmon. These genes belong to the superfamily encoding G-protein-coupled receptors (GPCRs), whose protein products exhibit a seven-transmembrane domain structure. The repertoire of OR genes is extremely large, numbering around 100 in fish to as many as 1000 for some mammals. Whole genome analysis reveals the complete set of 143 OR genes in zebrafish, 44 in fugu, and 42 in *Tetrodon.* Interestingly, the OR gene family identified in fishes is smaller in number but much more diverse than in mammals, and the gene groups found in mammals are nearly absent in fishes. Fish ORs belong to one of three families of GPCRs, the family C (or 3) GPCRs, along with those for glutamate and γ-aminobutyric acid, for Ca^{2+}, for sweet and amino acid taste compounds, and for some mammalian

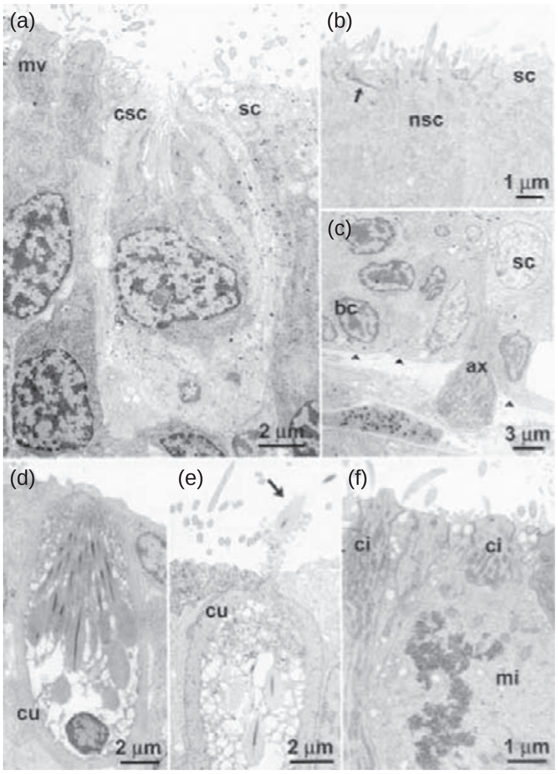


Figure 5 Transmission electron micrographs of the teleosts olfactory epithelia, showing receptor neurons and other cellular components. ax, axon bundles; bc, basal cell; ci, ciliated neuron; csc, specialized supporting cells; cu, thick cuticula; mi, mitosis; mv, microvillar neuron; nsc, nonsensory cell. Adapted from Hansen and Zielinski (2005) Diversity in the olfactory epithelium of bony fishes: Development, lamellar arrangement, sensory neuron cell types and transduction components. *Journal of Neurocytology* 34: 183–2005.

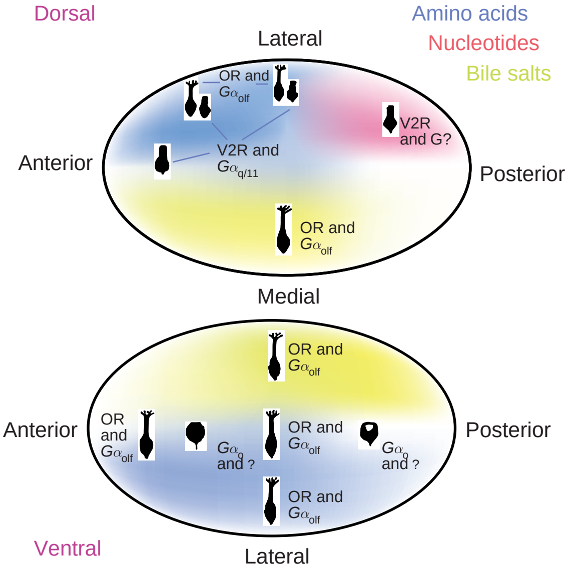


Figure 6 Diagram of the channel catfish olfactory bulb showing the type of olfactory receptor neurons that project to each region of the olfactory bulb along with their G-proteins and receptor types where known. Adapted from Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, and Finger TE (2003) Correlation between olfactory receptor cell type and function in the channel catfish. *Journal of Neuroscience* 23: 9328–9339.

pheromone molecules (**Figure 8**). One of the most important characteristic features of these ORs is the possession of a large extracellular domain that is responsible for ligand recognition, and is structurally similar to the metabotropic glutamate receptor.

In situ hybridization experiments with channel catfish provide strong evidence that individual olfactory neurons express a limited subset of ORs. Assuming that the catfish genome encodes as few as 100 OR genes, each neuron expresses one or a few ORs. Furthermore, neurons expressing specific ORs appear to be randomly distributed within the olfactory epithelium (**Figure 9**). These data are consistent with a model in which randomly dispersed olfactory neurons with common receptor specificities project to common glomeruli in the olfactory bulb.

Nonreceptor Cells

The supporting cells are columnar epithelial cells extending vertically from the epithelial surface to the basal lamina, forming a mosaic interspersed with receptor neurons. The free surface is flat, with a few microvilli. Supporting cells adjoin receptor neurons, ciliated nonreceptor cells, and other supporting cells at the free surface by an apical tight junction. Small vesicles in various sizes accumulate in the apical cytoplasm. The function of the supporting cells in fish olfaction is not defined, but in higher vertebrates they may have a role in the binding of odor molecules as they dissociate from OR sites. Crypt neurons are surrounded by special type of supporting cells that have numerous microvilli at their apical rim, and wrap around a crypt neuron. Crypt supporting and regular supporting cells are connected by numerous desmosomes.

The basal cells are small and oval-shaped, forming a discontinuous layer just above the basal lamina, interspersed by the bases of supporting cells in the sensory epithelium. They have numerous projections enclosing a bundle of receptor neuron axons. The basal cells in the sensory epithelium are assumed to be the progenitors of the receptor neurons and supporting cells. Increased mitotic figures are seen in the basal region in the reconstituting epithelium after degeneration.

Ciliated nonsensory cells are typical columnar cells with a wide flat surface, from which long kinocilia (20–30 μm), showing the 9 + 2 axial pattern of microtubules, extend. These cilia move water over the olfactory lamellae, presumably assisting in the transport of stimulant molecules within the olfactory organ.

Epidermal cells fill the nonciliated part of the nonsensory epithelium and are structurally identical with those of the fish epidermis. They are usually flattened in the

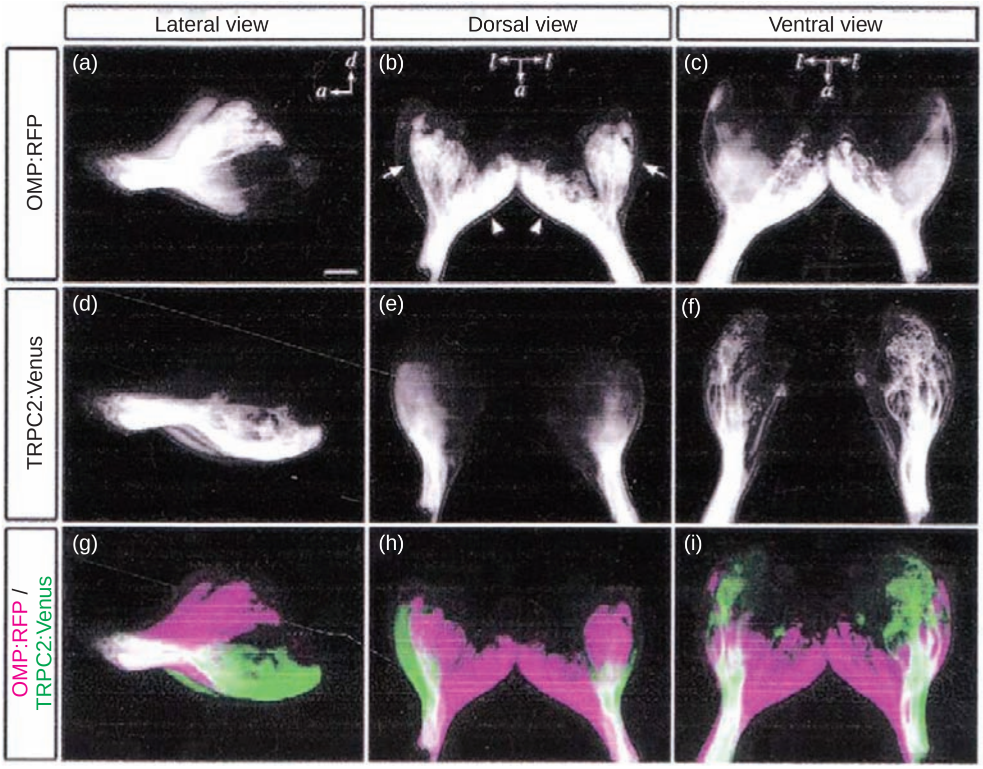


Figure 7 Differential projections of two types of olfactory neuron (ON) in zebrafish, ciliated ONs (magenta) project their axons to almost entire dorsal and the ventromedial regions of the olfactory bulb, whereas microvillar ONs (green) project their axons exclusively to the ventrolateral region. Adapted from Sato *et al*. (2005) Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish. *Journal of Neuroscience* 25: 4889–4897.

surface layer of the epithelium, and shows a prominent microridge arranged in fingerprint-like patterns in the free surface on the cell.

Mucus cells (goblet cells) contain many secretory granules in the cytoplasm with various electron densities, which are secreted between adjacent cells and are the major component of the mucus layer that covers the olfactory epithelium. In salmonids, additional mucus cells are present in the nonsensory epithelia on the ridges of the secondary folds and in the neuroepithelium.

In addition, in rainbow trout, magneto-receptor cells are present in the lamina propria of the olfactory epithelium, which is linked to the brain through the superficial ophthalmic branch of the trigeminal nerve. The cells contain magnetite crystals and respond to changes in the intensity of an imposed magnetic field, which is implicated in the magnetic orientation in salmonids navigation (**see also Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes).

Regeneration of OR Neurons after Degeneration

As mentioned, the OR neurons have a wonderful property described by Thomas as “this population of brain cells, unlike any other neurons of the vertebrate central nervous system, turn over every few weeks; cells wear out, die, and are replaced by brand-new cells rewired to the same deep centers miles back in the brain, sensing and remembering the same wonderful smells.”

Severance of the olfactory axons results in complete degeneration of all existing mature neurons, followed by reconstitution of new receptors from stem cells in the neuroepithelium of adult animals. The time course of degeneration and regeneration varies for different species. In rainbow trout, massive degeneration of receptor neurons takes place between 14 and 28 days following nerve section. The declining populations of neurons observed histologically are supported by the gradual decline in their activities when monitored by electro-olfactogram (EOG) responses and biochemical binding (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Olfaction). The supporting cells remain unaffected during the process. Reconstitution of the neuroepithelium is initiated by an outburst of mitosis in the basal cells. The numbers of newly differentiated receptor neurons returns to normal in 84 days. Following either olfactory nerve transection or the removal of the olfactory bulb in goldfish, the OR neurons show similar degeneration and subsequent neuron death. However, the time course of the renewal of neurons is different, with more rapid and complete renewal in axotomized fish.

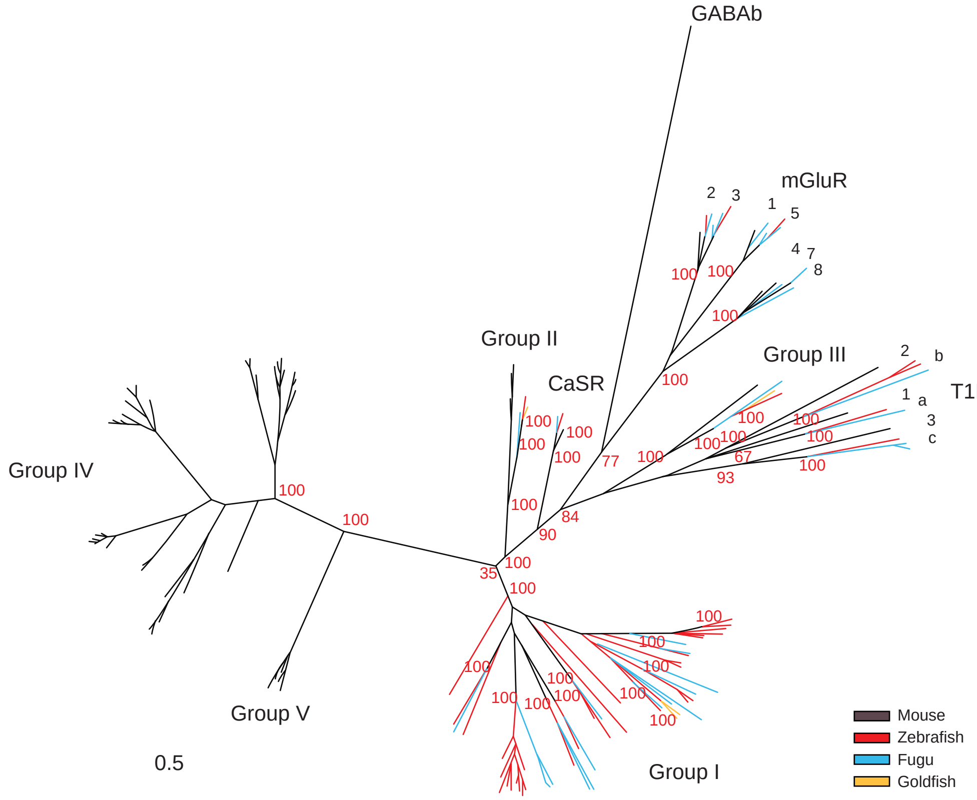


Figure 8 Phylogeny of C family GPCRs from zebrafish, fugu, goldfish, and mouse. Most of the fish receptors form a clade (group I) distinct from the mouse receptors (groups II, IV, and V). Group II contains both fish receptor and mouse sequences. Note that goldfish 5.24 belongs to group III. Adapted from Alioto and Ngai (2006).

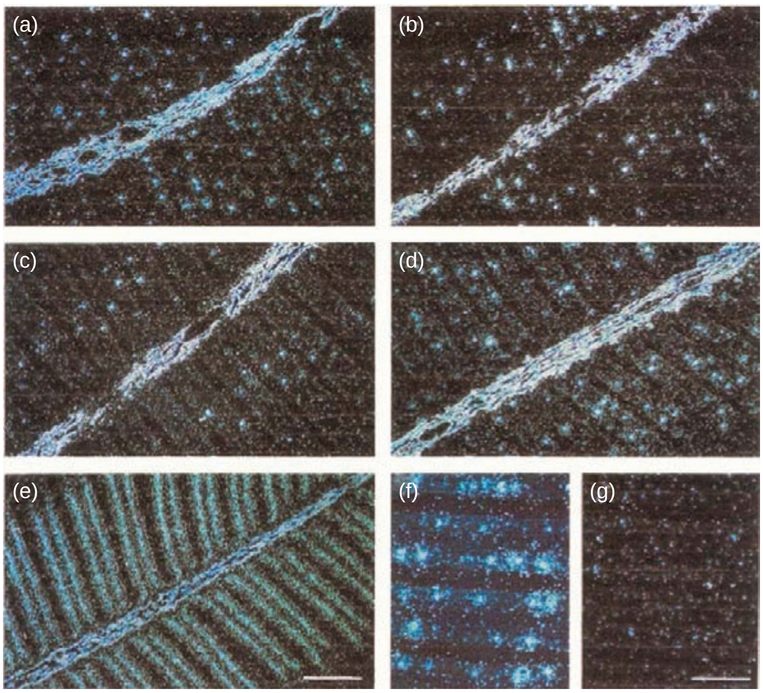


Figure 9 *In situ* hybridization experiments in channel catfish demonstrating that the four receptor subfamilies are expressed in different and largely nonoverlapping subsets of neurons, suggesting that each neuron expresses one or a few receptors. Adapted from Ngai *et al.* (1993).

Olfactory Bulb and Central Projections

The Olfactory Nerve

The axons of the olfactory neuron are unmyelinated, pass through the lamellae, and form the olfactory nerve, fila olfactoria (cranial nerve 1). They are centrally directed axons of the receptor neurons, arise in the nasal mucosa, where they make synaptic contact and terminate in the olfactory bulb. In carp (*Cyprinus carpio*), the olfactory nerve consists of two main bundles, medial and lateral. The medial bundle is derived from the more rostral lamellae, while the lateral from more caudal lamellae. The fibers of the two bundles often cross before they reach the olfactory bulb so that fibers from each reach all parts of the bulb. A number of large scattered ganglion cells lie between the two olfactory nerve bundles, forming the ganglion of the nervus terminalis, or terminal nerve (TN). Dendrites from these cells run mesad to form a bundle of fibers on the mesad aspect of each olfactory bulb. In rainbow trout, subsets of receptor neurons enter the bulb segregated and project to specific regions of the glomerular layer. The olfactory fibers are extremely small (~0.14 μm), and do not branch until they terminate. The length of the nerves varies depending upon the relative positions of the olfactory bulb in different species. The olfactory nerves do not increase in number by mitosis in the post-embryonic stage. However, they do regenerate after degeneration of the olfactory epithelium or nerve. Generally, there are two types of position: (1) the olfactory bulbs are located close to the noses, the olfactory nerves are very short, and long olfactory tracts are present (cyprinids, ictalurids, and silurids) and (2) the bulbs are close to the hemispheres of the forebrain so that long olfactory nerves are present (eels, salmonids, and majority of teleosts).

The Olfactory Bulb

The olfactory bulb is the first relay station receiving the primary olfactory nerve inputs and sending its output signals to target areas in the telencephalon and diencephalon. The organization of the teleost olfactory bulb is basically similar to that of other vertebrates, with variations in differentiation of neuronal elements and development of intra-bulbar local circuitry. Thus, evolutionarily, the olfactory bulb may be one of the most conserved regions of the vertebrate central nervous system. As in all vertebrates, the fish olfactory bulb shows a concentrically laminated structure. Generally, four layers are recognized from superficial to deep: (1) olfactory nerve layer, (2) glomerular layer, (3) mitral cell layer, and (4) internal or granule cell layer. Bundles of olfactory nerve fibers are interwoven in the outermost layer of the bulb. However, the individual nerve fibers do not branch before entering the glomeruli, which are regular in size and shape. The glomeruli, well-defined spherical bodies, may be the most distinctive feature of the olfactory bulb. Important synaptic interactions between the primary and secondary olfactory neurons, mitral cells, take place in the glomeruli, suggesting physiological significance. As mentioned above, the incoming olfactory nerve fibers do not divide before entering the glomeruli, although they branch freely within them. Hence, the axon of a receptor neuron does not terminate in more than one glomerulus, and each glomerulus receives impulses from a segregated and independent collection of ORs, indicating that glomeruli are discrete functional units. This indicates that point-to-point connections like those seen in the visual system (the retinal–tectal projection) do not exist in the epithelium bulb projection.

Fish mitral cells, though comparable in every respect with those of other vertebrates, have several main dendrites reaching out to branch in two or more glomeruli and can also contact diffuse olfactory neuronal terminal fields. Recent study shows that the majority of zebrafish mitral cells innervate a single glomerulus rather than multiple glomeruli, a feature similar to mammalian mitral cell system. Mitral cell dendrites receive numerous synaptic inputs from the olfactory nerve, where they form excitatory synapses, and at the same time they make numerous dendro-dendritic reciprocal synapses (excitatory to and inhibitory from) with the granule cells. Another cell type, ruffed cells, also occurs in some teleosts; they make synaptic contacts with other cell types including the primary olfactory neurons, suggesting a distinct function. Unlike higher vertebrates, the mitral cells of fish can also contact diffuse olfactory neurons terminal fields. These aglomerular plexii seem to be unique to fish.

In higher vertebrates, the accessory olfactory bulb is clearly separable from the main bulbar formation. It forms its own eminence on the lateral surface of the forebrain between the bulb and the hemisphere but of variable size according to the degree of development of the vomeronasal formation. Microscopically, it resembles the main olfactory bulb, except that the mitral cells are more scattered in the accessory bulb and a definite inner plexiform layer is lacking. Like in the fish olfactory bulb, each mitral cell has two, three, or more dendrites ending in the glomeruli. *In situ* hybridization and Northern blot analyses show that the vomeronasal sensory neurons express a transduction cascade distinct from that in the olfactory epithelium. Some consider that the fish pattern of mitral cell glomerular innervation is reminiscent of the mammalian accessory olfactory bulb.

The fact that the architecture of the glomerular population in vertebrates is highly stereotyped is an important aspect of information processing in olfaction. The number of glomeruli per olfactory bulb in teleosts ranges from

80 to several hundreds. The zebrafish olfactory bulb has about 80 glomeruli, of which 22 are identifiable individually by their characteristic position and morphology. Although the goldfish olfactory bulb contains many more glomeruli, their distribution pattern is essentially bilaterally symmetrical. In rainbow trout, subsets of olfactory neurons that are widely distributed and intermingled with the other receptor neurons in the olfactory epithelium and nerve segregate as they enter the olfactory bulb, where most axons terminate in nine discrete terminal fields in the glomerular layer, except a few that project into the ventral medial telencephalon. Recent *in situ* hybridization studies in channel catfish (*Ictalurus punctatus*) also show that a specific OR neuron is distributed evenly all over the epithelium. If this is the general case, a particular olfactory glomerulus would process olfactory information derived from only very few or one receptor types.

Olfactory Tract and Central Projections

The secondary olfactory projections originate in the large mitral cells of the olfactory bulb and generally run in the lateral and medial olfactory tract. Almost 100 years ago, Sheldon (**Figure 10**) described that in carp the lateral olfactory tract is mainly formed by projection fibers from the lateral part of the olfactory bulb, while the medial olfactory tract carries most fibers from the medial olfactory bulb (cf. **Figure 8**). In goldfish, the lateral tract consists of 10–14 fasciculi, in which myelinated fibers (mean diameter 0.7 μm) are prominent, whereas the medial tract contains two large fasciculi containing numerous unmyelinated nerve fibers (mean diameter 0.17 μm) as well as myelinated fibers.

Generally, the lateral olfactory tract projects to the dorsal part of the posterior telencephalon, with a small branch running ventrally in the telencephalon. The medial olfactory tract has two major branches, the dorsolateral and ventromedial bundles. The latter exclusively innervates the ventral telencephalon, preoptic area, and the diencephalon. The dorsolateral bundles terminate in various areas of the dorsal and ventral telencephalon as well as the preoptic area and the diencephalon. A significant portion of the olfactory tract fibers projects contralaterally through different commissures. The contralateral bulb and the anterior commissure also receive an input from the olfactory bulb. Centrifugal connections from the telencephalon to the olfactory bulb also run in the medial part of the medial olfactory tract. In goldfish, electrical stimulation of the opposite olfactory bulb or the anterior

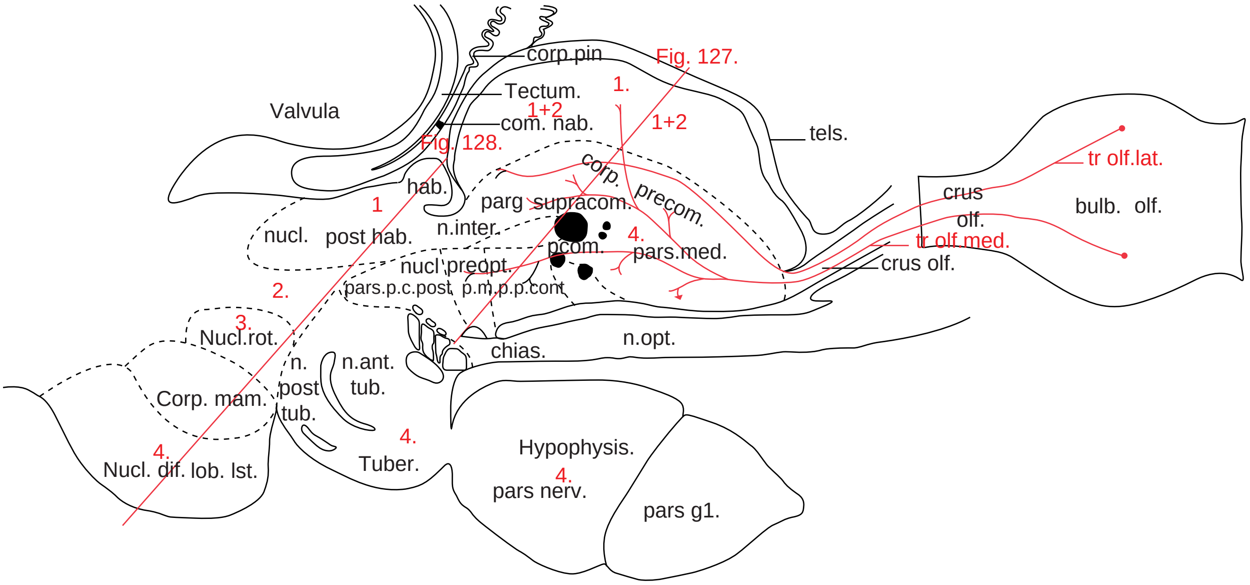


Figure 10 Projections of the olfactory centers on a parasagittal plane, near the mesoncephalon, showing the relation of the different centers to the four primitive columns in carp. 1, pars dorso-medialis hemisphaerii and epithalamus; 2, pars dorso-lateralis hemisphaerii and pars dorsalis thalami; 3, pars ventro-lateralis hemisphaerii and pars ventralis thalami; 4, pars ventro-medialis hemisphaerii and hypothalamus; tr. olf. lat., lateral olfactory tract; tr. olf. med., medial olfactory tract. Abbreviations: bulb, olfactory bulb; chias, optic chiasma; hab, habenula; com. corp. precom, commissural corporium precommissuralium; com. hipp. pars. ant, commussura hippocampi, pars anterior; com. hab., commissural habenularum; com. nucl. preopt., commissura nucleorum preopticorum; corp. mam., corpus mammillare; corp. precom., corpus precommissulare; cru. olf., crus olfactorium ; n. ant. tub., nucleus anterior tuberis; n. opt., nervus opticus; n. post. tub., nucleus posteriro tuberis; nucl. rot., nucleus rotundus nucl. post. hab, nucleus posterior habenularis; pars. gl., pars glandularis of the hypophysis; pars. med., pars medialis of the corpus precommissurale; pars. nerv, pars nervosa of the hypophysis; pars. p. c. post., pars parvocellularis of the nucleus preopticus; p. m., pars magnocellularis of the nucleus preopticus; p. p. c. ant., pars parvocellularis anterior of the nucleus preopticus; pars. supracom., pars suprecommissuralis of the corpus precommissurale; tectum., tectum mescencephali; tela, so-called pallium; tr. olf. Lat., lateral olfactory tract; tr. olf. med., medial olfactory tract; valvula, valvula cerebella. Adapted from Sheldon (1912).

commissure depresses both intrinsic and afferent-evoked olfactory bulbar activities, while sectioning of the olfactory tract results in augmentation of the bulbar activities evoked by chemical stimulation. All of these results suggest that olfactory bulbar responses to the normal afferent stimuli can be modulated by influence from the other bulb and from more posterior parts of the brain.

Little has been established about the central neural pathways of the fish olfactory system. The third-order neurons in the olfactory bulb have not been clearly identified in any fish species. However, one thing is certain, the central olfactory pathways of fishes are believed to be quite different from mammals. This is primarily because the teleostean telencephalon is dominated by eversion of pallial masses, instead of evagination as in other vertebrates, making the identification of major pallial and subpallial division difficult. However, there is growing evidence supporting the hypothesis that the teleostean dorsal telencephalic area, homologous to olfactory cortex, represents the pallium, whereas the ventral telencephalic area represents the subpallium. The latter is connected reciprocally to the hypothalamus, suggesting its involvement in the control of reproductive physiology and behavior. In channel catfish, a simple odotopy, the spatial mapping of different odorants, is maintained above the level of the olfactory bulb, in which feeding cues, amino acid responses are represented in the lateral pallial, whereas bile acid signals are represented in the medial forebrain centers, homologous to portions of amygdala. Recent genetic labeling studies in zebrafish, however, show that the topographic odor map in the olfactory bulb is not maintained intact, but reorganized in higher olfactory centers. Individual mitral cells project axons to multiple target regions in the forebrain, and those innervating the same glomerulus do not necessarily display the same axon trajectory.

Extrabulbar Projections of Olfactory Nerves

The olfactory system is not uniform; not all olfactory neuronal axons terminate in the olfactory bulb. In many fish species, a small subset of these neurons project directly to the forebrain bypassing the bulb and enter the ventromedial telencephalon. An electron microscopic study shows that the medial olfactory tract of carp contains numerous fine unmyelinated nerve fibers. Extrabulbar olfactory fibers of brown trout run in the medial olfactory tract and project to the ventral and commissural nuclei of the area ventralis telencephali, the periventricular preoptic region, the organum vasculosum laminae, and the postoptic hypothalamus. These studies strongly suggest that these extrabulbar fibers, and not the TN, are the primary mediators of neuroendocrine responses to pheromones. In support of this, a population of chemosensory cells responsive to a putative pheromone is located in the ventromedial area that links the olfactory bulb to the telencephalon. The existence of the extrabulbar olfactory fibers is consistent with earlier findings that olfactory stimulation directly activates hypothalamic neurosecretory cells in goldfish (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes). The olfactory–endocrine neuronal pathway seen in fishes may possibly be a ascendant of the olfactory afferent-GnRH (LHRH) neuronal system recently described in mice, in which a small population of olfactory sensory neurons located in a medial zone of the olfactory mucosa is the major conduit of pheromonal cues that regulate GnRH synthesis and release.

Concluding Remarks

Although the basic plan of the olfactory organ of fishes is consistent throughout all fishes, its developmental and molecular anatomical characteristics exhibit great diversity. OR neurons are the only neurons that transmit information from the outside world directly onto the brain, and yet they turn over considerably, unlike any other vertebrate neurons. The fish olfactory system, unlike in mammals, does not possess the vomeronasal system, but instead has three types of receptor neurons, ciliated, microvillar, and crypt. Crypt cells are a common feature of actinopterigian fishes. Cilia and microvilli cells both surround the apical surfaces, which are not directly open to the outside lumen. There is little evidence to suggest that fish microvillar cells are homologous to tetrapod vomeronasal cells, or that the fish ciliated cells are homologous to tetrapod olfactory neurons.

The axons of olfactory neurons project to the ipsilateral olfactory bulb, where they form synapses with mitral cells within glomeruli, forming a discrete functional unit. The olfactory bulb also receives centrifugal fiber inputs from the contralateral bulb as well as from higher brain structures. In addition, a subset of olfactory primary neurons projects directly to the forebrain bypassing the bulb, creating two olfactory subsystems. Thus, olfactory bulb-derived signals may ultimately reach multiple brain regions, including the cortex, the presumed location of conscious perception of odors leading to behaviors such as feeding, whereas extrabulbar signals are targeted to hypothalamic structures that are implicated in reproductive physiology and behavior.

Molecular biological findings suggest that fish olfactory C-family GPCRs have evolved to detect and discriminate a spectrum of amino acids, and have diverged significantly from a common ancestral gene with mammals. Current research is actively pursuing the molecular identities and cellular mechanisms that underlie amino acid sensing. Binding of amino acids with ORs depends on highly conserved residues in the N-terminal domains that bind the α-amino and α-carboxylate functional groups.

The development of conditioned knockout fish GPCRs, for example, the goldfish 5.24 that selectively lacks amino acid sensitivity will allow the systematic analysis of the key physiological issues in fish olfaction.

See also: **Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes. **Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes; Neurophysiology of Olfaction.

Further Reading

Allison AC (1953) The morphology of the olfactory system in the vertebrates. *Biological Research* 28: 195–244.

Hansen A and Zielinski BS (2005) Diversity in the olfactory epithelium of bony fishes: Development, lamellar arrangement, sensory neuron cell types and transduction components. *Journal of Neurocytology* 34: 183–2005.

Hara TJ (ed.) (1992) *Chemoreception in Fishes*. Amsterdam: Elsevier.

Hara TJ and Zielinski B (1989) Structural and functional development of the olfactory organ in teleosts. *Transactions of the American Fisheries Society* 118: 183–194.

Ichikawa M and Ueda K (1977) Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*: A study of retrograde degeneration. *Cell and Tissue Research* 183: 445–455.

Laberge F and Hara TJ (2001) Neurobiology of fish olfaction: A review. *Brain Research Reviews* 36: 46–59.

Miyasaka N, Morimoto K, Tsubokawa T, *et al.* (2009) From the olfactory bulb to higher brain centers: Genetic visualization of secondary olfactory pathways in zebrafish. *Journal of Neuroscience* 29: 4756–4767.

Nikonov A, Finger TE, and Caprio J (2005) Beyond the olfactory bulb: An odotopic map in the forebrain. *Proceedings of the National Academy of Sciences of the United States of America* 102: 18688–18693.

Ostrander GK (ed.) (2000) *The Laboratory Fish*. London: Academic Press.

Pin J-P, Galvez T, and Prézeau L (2003) Evolution, structure, and activation mechanisms of family 3/C G-protein-coupled receptors. *Pharmacology and Therapeutics* 98: 325–354.

Riddle DR and Oakley B (1992) Immunocytochemical identification of primary olfactory afferents in rainbow trout. *Journal of Comparative Neurology* 324: 575–589.

Sato Y, Miyasaka N, and Yoshihara Y (2005) Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish. *Journal of Neuroscience* 25: 4889–4897.

Sheldon RE (1912) The olfactory tracts and centers in teleosts. *Journal of Comparative Neurology* 22: 177–339.

Thomas L (1984) *Late Night Thoughts on Listening to Mahler's Ninth Symphony*. 168pp. Toronto: Bantam Books.

Westerman RA and Wilson JAF (1968) The fine structure of the olfactory tract in the teleosts *Carassius auratus* L. *Zeitschrift für Zellforschung* 91: 186–199.

Whitlock K (2004) A new model for olfactory placode development. *Brain, Behavior, and Evolution* 64: 126–140.

Zielinski BS and Hara TJ (1992) Ciliated and microvillar receptor cells degenerate and then differentiate in the olfactory epithelium of rainbow trout following olfactory nerve section. *Microscopy Research and Technique* 23: 22–27.

Zielinski BS and Hara TJ (2007) Olfaction. In: Hara TJ and Zielinski BS (eds.) *Sensory Systems Neuroscience, Volume 25: Fish Physiology*, pp.1–33. New York: Academic Press.

Neurophysiology of Olfaction

TJ Hara, University of Manitoba, Winnipeg, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Olfactory Signal Transduction
Potency and Specificity of Olfactory Stimuli
Ciliated, Microvillar, or Crypt Receptor Neurons – Functional Specialization?
Signal Processing in the Olfactory Bulb and Telencephalon
Concluding Remarks
Further Reading

Glossary

Amino acids Molecules with an amine group, a carboxylic group, and one of 20 R groups that are the building blocks of proteins.

Bile acids Steroid acids found predominantly in the bile of vertebrates. The two major bile acids are cholic acid and chenodeoxycholic acid.

Epitope Any site on an antigen molecule at which an antibody can bind.

Heterologous expression Expression of a gene in a cell that normally does not express that gene.

Kynurenine An amino acid that is a metabolite of another amino acid, tryptophan.

Odorant A chemical that binds to and stimulates an olfactory receptor.

PGFs Specific types of prostaglandins that act as odorants.

Prostaglandins Any of various oxygenated fatty acids of animals that are formed as cyclooxygenase metabolites, especially from unsaturated fatty acids composed of a chain of 20 carbon atoms, and that perform a variety of hormone-like actions.

Tuned The extent to which a receptor is specialized to respond to a specific chemical stimulus. Broadly tuned receptors respond to a variety of chemical stimuli.

Zwitter ion An ion with both a positive and a negative charge.

Olfactory Signal Transduction

The initial event of olfaction is binding of an odorant molecule with a specific membrane receptor located in the cilia or microvilli of the olfactory receptor neuron. The molecular era of research into olfaction and gustation came of age in 1991 with the discovery of a large multigene family encoding odorant receptors (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction and Morphology of the Olfactory (Smell) System in Fishes). The olfactory receptor gene families are extremely large; in mammals they appear to contain 500–1000 genes, whereas the gene family of fish contains about 100 genes. Each neuron expresses only a single receptor gene. Transgenic and *in situ* hybridization studies in fish have identified mammalian and nonmammalian types of receptor genes in several fish species. A direct demonstration that one member of these gene families could initiate a physiological response to odorants was achieved by expression in heterologous cells (i.e., cells that normally do not express olfactory genes) in goldfish (*Carassius auratus*).

The molecular olfactory receptors thus expressed are G-protein-coupled seven transmembrane proteins, which exhibit seven hydrophobic domains that are likely to serve as membrane-spanning α-helices (**Figure 1**). The transmembrane domains that show exceptional diversity are believed to be involved in ligand binding. The binding of odorant molecules to receptors induces a transduction cascade that ultimately leads to depolarization of the neuron and action potential generation in the olfactory axon. A variety of receptor-mediated transduction events are known. Fish use at least two processes: one that alters the concentration of second messengers, mediated most probably by guanosine 5′-triphosphate-binding proteins (G-proteins), and another that affects intracellular ionic activity via stimulus-gated ion channels. Odorant binding to receptor in the membrane activates G-proteins that stimulate the activity of adenylcyclase (AC) in olfactory cilia. AC generates cyclic AMP (cAMP) from ATP, which directly gates an ion channel in the membrane, allowing influx of Na and Ca ions (**Figure 1**). This leads to change in membrane potential which results in action potential generation in the axons. This transduction scheme is

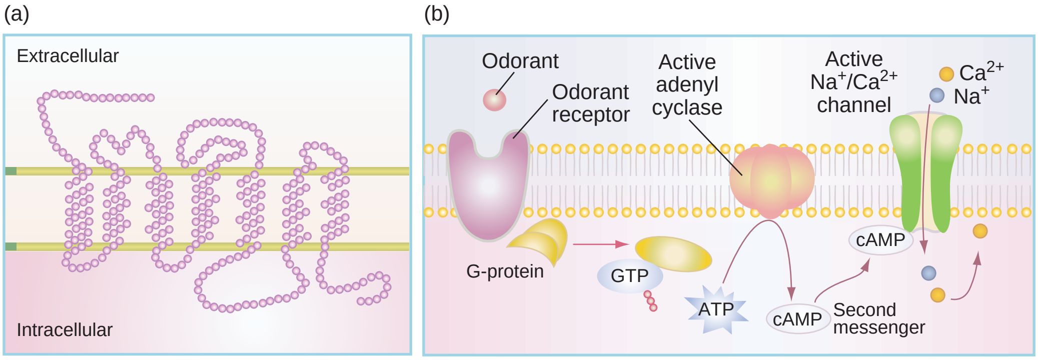


Figure 1 Diagrams representing seven transmembrane domain structure of an olfactory receptor (a), and olfactory signal transduction pathway in vertebrates (b). An odorant interacts with an odorant receptor, resulting in G-protein activation, stimulation of adenyl cyclase activity, opening of a cyclic-nucleotide gated channel by elevated cAMP levels, influx of cations and depolarization. Adapted from Mombaerts (2001) How smell develops. *Nature Neuroscience* 4: 1192–1198.

widely distributed among vertebrates. In teleosts, two other second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG), are also involved in transduction. However, the particular transduction pathways associated with specific odorants are largely unknown.

Potency and Specificity of Olfactory Stimuli

To date, four main classes of chemical compounds have been identified as specific olfactory stimuli (odorants/pheromones) for fish and their stimulatory effectiveness characterized: amino acids, bile acids, sex steroids, and prostaglandins (PGs). These four odorant classes, detected by separate receptor types, are typically nonodorous to humans. Of these, amino acids are by far the most widely studied chemicals in fish olfaction. Their high sensitivity and specificity for the fish olfactory system have been shown in more than 20 fish species. Two less well-characterized chemical groups are nucleotides and polyamines. For the ultimate understanding of the molecular basis of the physiological responses, it is essential to rigorously characterize the receptor–response systems *in vivo*.

Amino Acids

At physiological pH values, amino acids exist naturally in a zwitterion state, with the carboxylic acid moiety ionized and the basic amino group protonated. The entire class of amino acids has a common backbone of an organic carboxylic acid group and an amino group attached to a saturated carbon atom. The simplest is glycine, in which the saturated carbon atom is unsubstituted, rendering it optically inactive. The rest of the 20 most common amino acids are optically active and exist as both D- and L-stereoisomers. Naturally occurring amino acids are, for the most part, the L-isomers. Amino acid side chains are polar, nonpolar, or practically neutral.

Electrophysiological and optical imaging studies in salmonids, channel catfish (*Ictalurus punctatus*), and zebrafish (*Danio rerio*) show that: (1) only α-amino acids are stimulatory at biologically relevant concentrations, (2) the natural L-isomer of an amino acid is significantly more stimulatory than its D-isomer, (3) ionized α-amino and α-carboxyl groups are required, (4) the α-hydrogen of an amino acid must be free, and (5) the size and polar nature of the fourth α-moiety are important factors for stimulatory effectiveness (**Figure 2**). The amino acids effective as odorants are thus characterized by being simple, short, and straight chained, with only certain substituent groups.

The olfactory spectrum of amino acids is generally similar across all fish species examined. An amino acid receptor model involving two charged subsites, one anionic and the other cationic, capable of interacting with ionized amino and carboxylic groups of stimulant amino acid molecule is hypothesized. Biochemical evidence for the existence of such binding sites came from studies on binding experiments using radioactively labeled amino acids on an isolated olfactory preparation from trout. Physical parameters such as saturability, reversibility, affinity, and quantity of binding sites are reasonably consistent with receptor criteria. In addition, good agreement between the rank order of binding and electrophysiological potency further supports the conclusion that binding data reflect the interaction of amino acids with physiologically relevant receptors. Amino acids assert their activities through multiple receptor systems; note, for example, that in **Figure 2** cysteine, glutamate, and arginine evoke different maximal effect, suggesting the involvement of different receptor-mediated physiological functions.

All lines of experimental evidence indicate that in channel catfish, Arctic char (*Salvelinus alpinus*), and rainbow trout (*Onchorynchus mykiss*) at least three receptor types

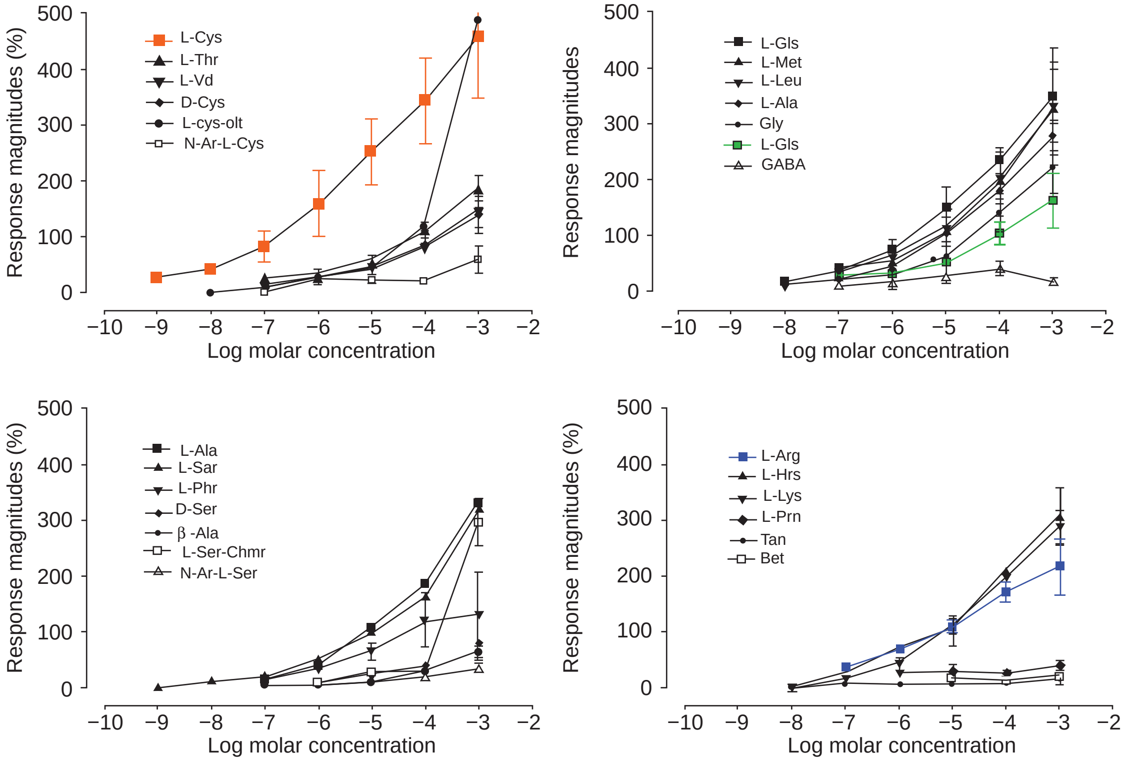


Figure 2 Concentration–response (*C–R*) relationships of EOG responses of rainbow trout to amino acids and related chemicals (arbitrarily grouped). Note that neutral amino acids (represented by cysteine; red), glutamate (green), and arginine (blue) evoke different maximal responses. L-Cys-OMe, L-cysteine methyl ester; N-Ac-L-Cys, *N*-acetyl-L-cysteine; GABA, γ-aminobutyric acid; Tau, taurine; Bet, betaine. Modified from Hara (2005) Olfactory responses to amino acids in rainbow trout: Revisited. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp: 31–64. Enfield, NH: Science Publishers.

(cysteine- (or, neutral amino acid-), arginine- (or, basic guanidine-containing amino acid-), and glutamate- (or, acidic amino acid-) receptor types) exist, which play dominant roles in the discrimination of amino acids (**Figure 3**). Specific binding of alanine of the salmonid olfactory preparation is inhibited by neutral amino acids, but only partially by arginine or glutamate. Each receptor recognizes the characteristic features, or epitope, of an amino acid molecule, including (1) hydrocarbon chain length, (2) difference in functional groups, and (3) position of functional groups. Although the three receptor types are quite specific under the natural condition, because all amino acids have some of these features in common (generic structural plan), they could all function as partial agonists, activating all available receptors depending on their affinities and concentrations. For example, the cysteine receptor is activated not only by its primary ligand cysteine, but also by other neutral amino acids including alanine, glutamine, and methionine, with lesser affinities, and generates receptor potentials accordingly. Also, electrophysiological and binding analyses show that the cloned goldfish olfactory receptor 5.24 is preferentially tuned to recognize basic amino acids; of 20 amino acids tested for competition binding, arginine and lysine have the highest affinities, suggesting that this receptor may in fact represent an Arg-, or more generally guanidino-containing amino acid receptor for this species. This would verify the existence of the Arg-receptor predicted electrophysiologically earlier in salmonids. It should be noted that the 5.24 receptor shares amino acid sequence identity to mammalian pheromone receptors, the Ca-sensing receptors, the T1R taste receptors, and the metabotropic glutamate receptors (mGluRs).

Recently, kynurenine, a metabolite of tryptophan and technically an amino acid, released by ovulating female masu salmon has been found to be a potent olfactory stimulant for spermiating males, with an amazingly low detection threshold of 10^{-14} M. Even immature or sexually regressed males as well as ovulated females detect it at 10^{-12}–10^{-10} M. The urine of the reproductively mature female masu salmon contains high levels of kynurenine that is released into the water at a rate of 0.40×10^{-6} mole/female per hour, where it is thought to act as a male-attracting pheromone (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior). Whether this is a reproductive pheromonal cue specific for this salmonid species and behaves like ordinary amino acid odorants await future study.

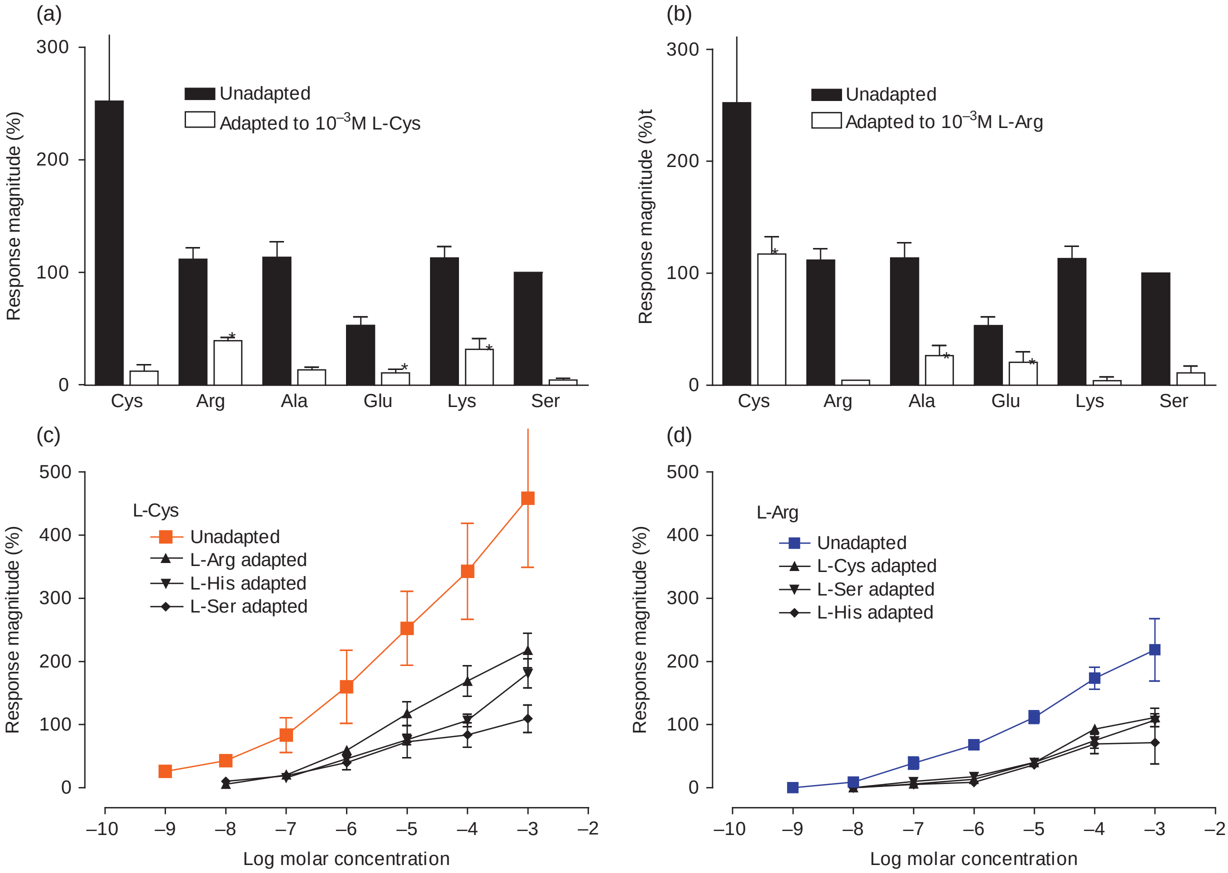


Figure 3 Bar graphs (top) show inhibition of selected amino acids when adapted (pretreated) to cysteine (a) or arginine (b). Effects of adaptation to various amino acids on C–R relations of cysteine (c) and arginine (d) (bottom). Modified from Hara (2005) Olfactory responses to amino acids in rainbow trout: Revisited. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp: 31–64. Enfield, NH: Science Publishers.

Bile Acids

Bile acids, synthesized in the liver from cholesterol, are a family of structurally related sterols. They are essential for food digestion and various aspects of organic and anionic transport in the digestive system, which are then either resorbed or released to the environment. Bile acid composition and conjugation pattern are markedly different in various animals; however, closely related species tend to have similar bile acids. In teleosts, 5α-cyprinol is widely produced in ostariophysians, whereas in salmonids taurocholic acid and taurochenodeoxycholic acid are released. Lake char (*Salvelinus namaycush*), for example, release an average of 4 nmol min^{-1} bile acids per kg of body weight via feces into water.

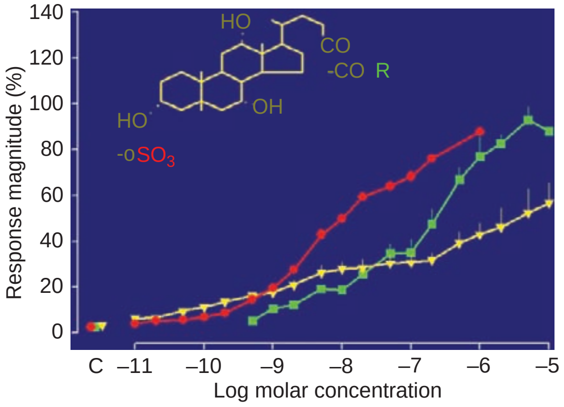


Figure 4 Concentration–response relationships for chenodeoxycholic acid (yellow), taurocholic acid (green) and taurolithocholic acid 3α-sulfate (red), representing respectively free, amidated, and sulfated bile acids. Inset: structural formula of cholic acid, a typical bile acid in vertebrates.

High olfactory sensitivity and specificity to bile acids have been shown in salmonids, zebrafish, and channel catfish. In lake char, 9 out of 38 bile acids tested are detected at thresholds 2×10^{-11}–5×10^{-10} M (**Figure 4**). The most stimulatory include chenodeoxycholic acid, cholic acid, taurochenodeoxycholic acid, and taurolithocholic acid 3α-sulfate. Structure–activity analysis indicates that substituents in the side chain or hydroxyl sulfation are determinant elements for the recognition of individual bile acid receptors, while the position and orientation of hydroxyl or the type of amidation are important for effective stimulation. Lake char also detect petromyzonol sulfate, a sea lamprey (*Petromyzon marinus*) bile acid, at 10^{-9} M. The *C–R* relationship, cross-adaptation, and binary mixture studies suggest that the detection of bile acids is mediated by at least four receptor subtypes: free-, amidated-, 3a-sulfated-, and petromyzonol-like bile acid receptors. These bile acid receptors are specific and independent of olfactory receptors for other known fish odorants, including amino acids, PGs, and gonadal steroids. Petromyzonol sulfate is detected by its own highly specific and independent receptor site in the sea lamprey. Besides petromyzonol sulfate, of 10 bile acids tested allocholic acid is the most potent bile acid in this species.

Gonadal Steroids

Steroid hormone, 17*α*,20*β*-hydroxy-4-pregnen-3-one (17,20P), promotes oocyte final maturation in preovulatory female goldfish and functions as a pheromone that stimulates serum gonadotrophin (GtH) release, enhanced sperm production, and sexual arousal in mature males. Little is known about exactly how these hormonal pheromones are released in any fish species. Of the 24 sex steroids tested for olfactory stimulatory effectiveness in goldfish, 17,20P is the most stimulatory, with a detection threshold of 10^{-13} M, and at a concentration of 10^{-8} M elicits an electro-olfactogram (EOG) response 3 times that elicited by 10^{-5} M L-serine (**Figure 5**). Mature male and female as well as gonadally regressed fish respond to all compounds tested, suggesting that differences in olfactory receptor functions are not responsible for the fact that only mature males exhibit behavioral and endocrine responses to pheromonal 17,20P.

The olfactory responsiveness to steroid hormones seems to be highly species specific; only members of Cyprinidae (11 species), including common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*), detect any of the steroids. Interestingly, crucian carp show high gender specificity; most olfactory bulb neurons in males respond exclusively to one of the steroids, but females show little responsiveness. No olfactory sensitivity to steroid hormones in salmonids has been shown, except that precocious male Atlantic salmon (*Salmo salar*) become acutely sensitive to testosterone, with a threshold concentration of 10^{-14} M, and, amazingly, only during October of the year. In African catfish (*Clarias gariepinus*), some steroid glucuronides found in the male seminal vesicles stimulate the female olfactory system, at a threshold 10^{-11} M. These fish are insensitive to 17,20P.

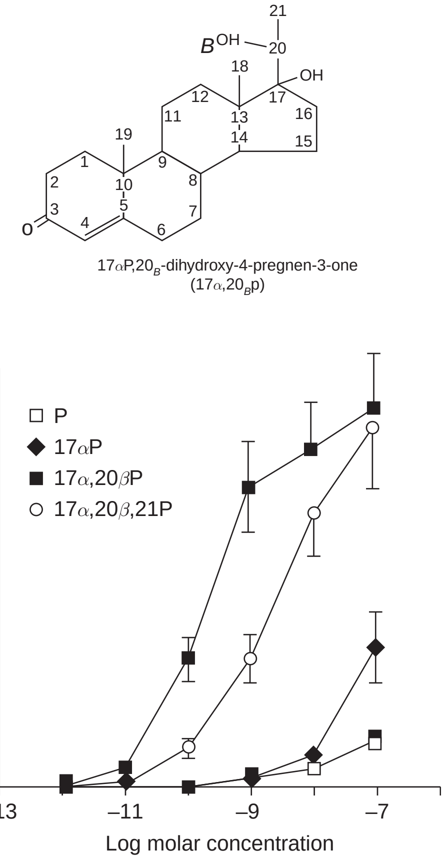


Figure 5 Electro-olfactogram responses to progesterone (P), 17*α*-hydroxyprogesterone (17*α*P), 17*α*,20*β*-dihydroxyprogesterone (17*α*,20*β*P), and 17*α*,20*β*,21-trihydroxyprogesterone (17*α*,20*β*,21P) recorded from mature male goldfish. Inset: structural formula of 17*α*,20*β*P. Adapted from Sorensen PW, Hara TJ, Stacey NE, and Dulka JG (1990) Extreme olfactory specificity of male goldfish to the preovulatory steroidal pheromone 17*α*,20*β*-dihydroxy-4-pregnen-3-one. *Journal of Comparative Physiology* A 166: 373–383.

Prostaglandins

To date, 14 cyprinids, 4 catostomids, 4 salmonids, and 1 cobitid are known to have acute olfactory sensitivity to PGs. F-type $PGF_{2\alpha}$, and its metabolites, for example, are released into the water by ovulating female goldfish, where they function as a pheromone that triggers male courtship. Of the PGs tested, $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ are the most potent, detected at a threshold of 10^{-10} and 10^{-12} M, respectively. Goldfish have separate olfactory receptor sites for $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ that are independent from those that detect other olfactory stimuli (**Figure 6**). In a Japanese cobitid (*Misgurnus anguillicaudatus*), ovarian $PGF_{2\alpha}$ increases during ovulation, and males show sexual behavior toward females as well as unovulated females treated with PGFs. These fish detect PGFs at thresholds between 10^{-13} and 10^{-10} M, and exhibit extremely high EOG response magnitudes; at 10^{-7} M they induce 8–12 times that of 10^{-5} M L-serine.

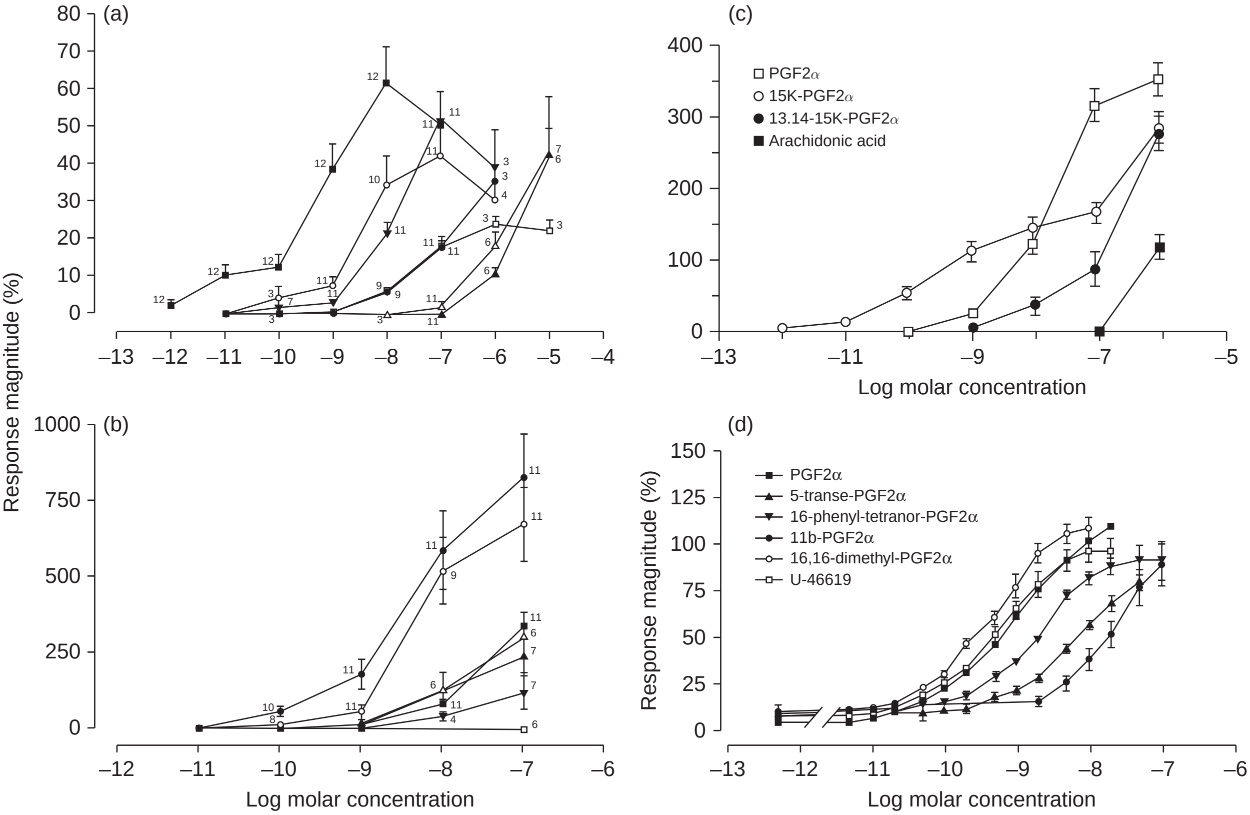


Figure 6 Electro-olfactogram responses to prostaglandins recorded from (a) male goldfish, (b) Arctic char, (c) brown trout, and (d) lake whitefish. Adapted from (c) Sorensen *et al.* (1989) and (d) Sveinsson and Hara (2000), and (a and b) Laberge F and Hara TJ (2003) Behavioural and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonids fishes. *Journal of Fish Biology* 62: 206–221.

In Arctic char (*S. alpinus*), lake whitefish (*Coregonus cluperformis*), and brown trout (*Salmo trutta*), PGs play important behavioral roles. Of 19 PGs and analogs tested in Arctic char, PGF_{2a}, 16,16-dimethyl-PGF_{2a}, and U-46619 are the most stimulatory (thresholds, 10^{-9}–10^{-8} M). No differences exist in the olfactory sensitivity and specificity for PGFs at different stages of sexual maturity. PGFs are released by ripe male Arctic char on the spawning grounds to attract females and stimulate digging behavior. Brown trout are most sensitive to PGF_{2a} with a threshold of 10^{-11} M, while lake whitefish are most sensitive to both 15-keto-PGF_{2a} and 13,14-dihydro-PGF_{2a}. Cross-adaptation and binary mixture experiments show that only one olfactory receptive mechanism is involved. Exposure to PGFs increases locomotor activity in both lake whitefish and brown trout; female brown trout exhibit digging and nest probing behavior. Most interestingly, PGFs are potent stimuli for these salmonids when measured by EOG, but they do not induce electroencephalogram (EEG) responses in the olfactory bulb (**Figure** 7). The synaptic responses due to the hormonal pheromones in the olfactory bulb may be asynchronous, because the recorded EEG response in the bulb reflects a synchronized activity of a number of synapses. Alternatively, olfactory responses to hormonal pheromones are mediated by extrabulbar primary olfactory fibers that bypass the bulb.

Noticeably, all salmonids tested, including rainbow trout, amago salmon (*Oncorhynchus rhodurus*), sockeye salmon (*O. nerka*), and possibly masu salmon (*O. masou*), excluding Japanese ayu (*Pleccoglossus altivelis*), lack the olfactory sensitivity to PGs. It is also striking that in lake whitefish, in contrast to other odorant classes, a distinct neural structure exists in the olfactory bulb-telencephalon area, through which PGF signals are integrated. Also, the olfactory signals due to sex steroids and PGFs are mediated by the medial olfactory tracts in goldfish. These results provide evidence for the existence of the two functional olfactory subsystems in fish, one for odorants and the other for pheromones, as seen in mammals. Olfactory signals from pheromones are processed in a manner distinct from those for odorants such as amino acids and bile acids, and may be mediated by extra-bulbar primary olfactory fibers bypassing the olfactory bulb.

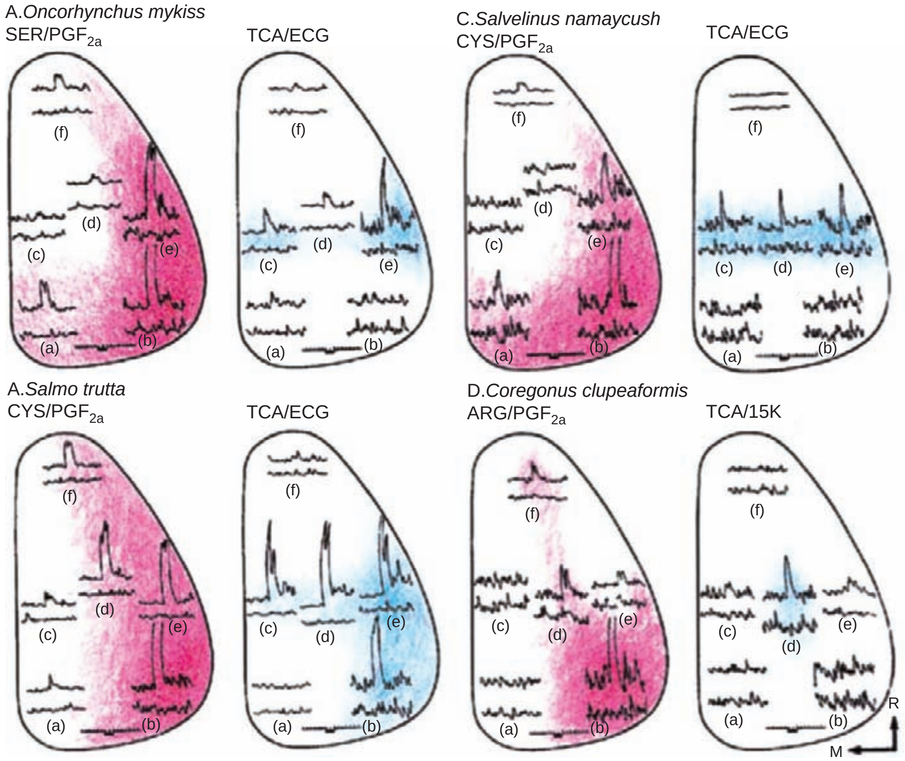


Figure 7 Spatial distribution of electroencephalographic responses to amino acids (Ser, Arg, and Cys), bile acid (TCA), prostaglandin (PGF2, 15K), and sex steroid (ECG) onto the surface of the right olfactory bulb in rainbow trout, brown trout, lake char, and lake whitefish. Recordings are made at six positions: (a) medioposterior, (b) lateroposterior, (c) mid-medial, (d) central, (e) mid-lateral, and (f) rostral. R, rostral; M, medial. Modified from Hara TJ and Zhang C (1998) Topographic bulbar projections and dual neural pathways of the primary olfactory neurons in salmonids fishes. *Neuroscience* 82: 301–313.

Other Chemical Compounds

A new family of chemosensory receptors, called trace-amine-associated receptors (TAARs), that show amino acid sequence identity with receptors for biogenic amines (serotonin, dopamine, etc.) are present in human, mouse, and fish. As many as 57 TAARs receptors exist in zebrafish, while fugu (*Takifugu rubripes*) has only eight TAARs. In mouse, at least three can recognize volatile amines in urine, indicating they may be involved in the detection of social cues. The expansion of these genes in zebrafish is thought to have been shaped by repeated gene duplications, which makes the evolutionary history of this group of receptors more complex than for other GPCR families. In zebrafish, diamine and putrescine (1,4-diaminobutane), largely responsible for the foul odor of putrefying flesh, are nonstimulatory; whereas in goldfish, putrescine, cadaverine, and spermine are stimulatory, with thresholds of 10^{-8}–10^{-7} M, and elicit feeding behavior. Receptors for these polyamines are independent from those of amino acids, bile acids, or single amine-containing compounds. A monoamine, agmatine, the decarboxylation product of arginine, activates the olfactory systems, with thresholds 10^{-9}–10^{-8} M in goldfish, lake whitefish, and walleye (*Stizostedion vitreum*), but not in rainbow trout, brown trout, and lake char.

Morpholine, phenethyl alcohol, and hypoxanthine-3(N)-oxide have been implicated in chemosensory behaviors; however, none has been subjected to electrophysiological scrutiny (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior).

Ciliated, Microvillar, or Crypt Receptor Neurons – Functional Specialization?

Ontogenetically and morphologically different ciliated and microvillar receptor neurons generally exist in the fish olfactory epithelium. Immunocytochemically, the ciliated neuron expresses G-protein G_{olf}, whereas the microvillar expresses $G_{\alpha o}$ (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Gustation). Electrophysiologically, no significant difference exists in membrane characteristics, including Ca^{2+}-activated Cl-conductance between the ciliated and microvillar neuron types in adult rainbow trout. The ciliated are much more abundant than the microvillar by the ratio of 11 to 1 and the latter respond specifically to amino acids (specialist) while the former respond to a wide variety of odorants (generalist). However, young developing embryos contain only ciliated neurons yet respond to both amino acid and bile acids. In goldfish, ciliated neurons regenerate before the microvillar following the olfactory nerve axotomy, and the former respond to amino acids and the latter to pheromones (e.g., PGFs). Consistent with this, *in situ* hybridization studies in goldfish suggest that odorant receptors are expressed in ciliated neurons, while pheromone receptors in the microvillar neurons. It could be confusing that odorant receptors preferentially tuned to recognize amino acid lysine and arginine, with significant similarities with some vertebrate vomeronasal organ receptors (V2Rs), are expressed in microvillar neurons.

In nonteleosts, lemon shark (*Negaprion brevirostris*) with only microvillar neurons and sea lamprey with only ciliated neurons both respond to amino acids. Taken together, there seems to be no clear-cut functional differentiation for the two neuron types; ciliated neurons respond generally to amino acids in some fish species, while the microvillar respond to pheromones in other fish species, and vice versa.

Signal Processing in the Olfactory Bulb and Telencephalon

In discussing signal coding in the olfactory bulb, it is important to recall the following two remarkable features of the axonal projection from the olfactory epithelium to the olfactory bulb: (1) individual glomeruli receive input from olfactory neurons that are distributed throughout the entire regions of the olfactory epithelium and (2) glomeruli are functional units that respond differentially to different odorants. Each odorant could elicit activity in more than one glomerulus, and the number of glomeruli involved varies among odorants and is dependent on stimulus concentration. However, higher concentrations of one same odorant could lead to the activation of more glomeruli, consequently resulting in the loss of specificity.

Odorant stimulation of nares induces large rhythmic oscillations in the olfactory bulb (induced waves; also known as EEG responses); spontaneous electrical activity (intrinsic waves) is interrupted by oscillatory waves that are terminated on cessation of the stimulus. In salmonids, EEG responses to amino acids and bile acids are induced in two segregated regions of the olfactory bulb. When olfactory rosettes are subjected to partial lamellectomy (removal of the anterior, posterior, medial, or lateral halves), both the EOGs and EEGs to these odorants are uniformly reduced and the degree of reduction does not depend on the rosette region removed, suggesting that olfactory neurons responsive to amino acids and bile acids are randomly distributed throughout the olfactory epithelium, and yet project to spatially segregated region. Furthermore, neither PGs nor sex steroids elicit EEG responses, despite strong activation of receptor neurons (**Figure 7**). In lake whitefish, a species characterized by high EOG and behavioral sensitivities to PGFs, a neuron population specifically responsive to PGs exists in the ventro-medial brain tissue strip connecting the olfactory bulb to the telencephalon. Furthermore, unlike responses to other odorants, this neuron population does not induce synchronized oscillatory waves (EEG responses) upon stimulation with PGFs, despite massive discharges. This is the first demonstration that the olfactory bulb-telencephalon area of the brain is a distinct neural structure through which putative reproductive pheromone signals are integrated.

In goldfish, by contrast, stimulation with pheromones activates olfactory neurons in the medial portion of the bulb. More precisely, in channel catfish ciliated neurons project predominantly to region of the olfactory bulb that respond to bile acid (medial) and amino acids (ventral), while microvillar neurons project almost entirely to the dorsal surface of the bulb where responses to amino acids (anterior) and nucleotides (posterior) predominate. Furthermore, gender specific bulbar chemotopy exists in crucian carp (*C. carassius*), the closest relative of goldfish, where neurons specifically sensitive to sex pheromones (17,20P, 17,20PS, androstenedione, and PGFs) are located in a central part of the ventral olfactory bulb in males. The majority of the responded olfactory bulb neurons are activated exclusively by one of the four sex pheromones. In females, of the 96 bulb neurons recorded, only one showed such a specific activation.

How is the EEG response topography established for different odorant classes correlated with olfactory bulbar neurons? Is the relatively independent amino acid receptor mechanisms maintained in the response profiles of the olfactory bulb neurons? Like rainbow trout, amino acids, bile acid, and PGFs activate independent receptor types in brown trout. Within the class of amino acids, different receptor types also are partially independent. Neurons responsive to bile acids and amino acids are segregated to the mid-dorsal and latero-posterior olfactory bulb, respectively (**Figure 8**). A majority of responsive bulb neurons (mitral cells) shows specificity for one odorant class. Neurons responsive to bile acids, though at extremely high concentrations, located in the medial region of the olfactory bulb of channel catfish are also activated by at least one member of each of the three classes of bile acids. Importantly, amino acid-responsive neurons gain responsiveness to new amino acids, or recruit more responsive neurons, with increasing stimulant concentration, resulting in the loss of the receptor specificity. A similar gradual loss of specificity in the activity patterns is also shown in zebrafish and a mammalian heterologous system. Thus, different odorant classes activate specific

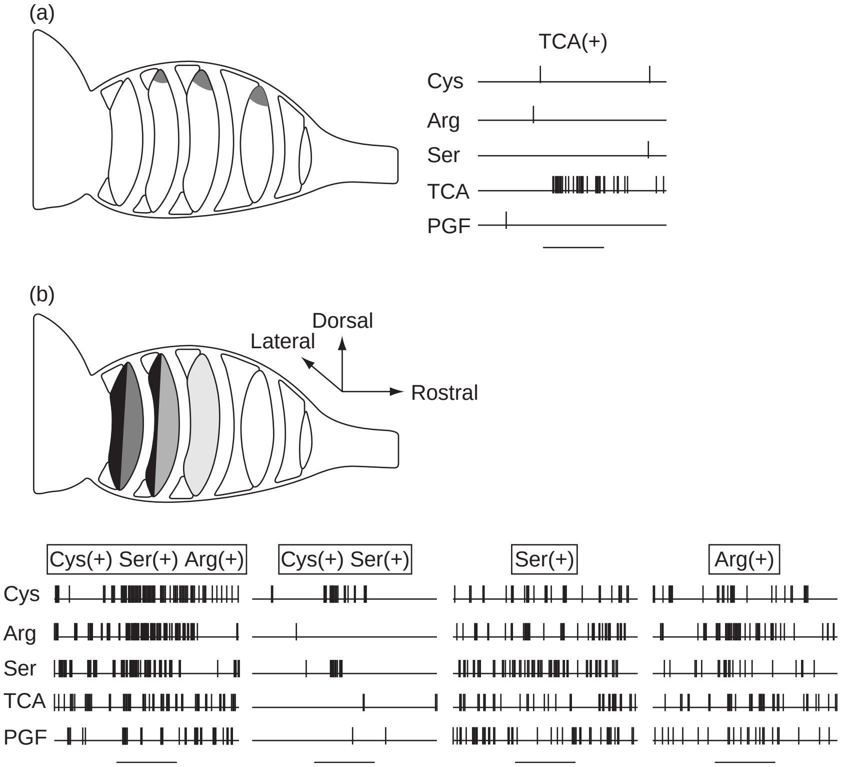


Figure 8 Distribution of bile acid (a) and amino acid (b) responsive units in the brown trout olfactory bulb. The drawings represent sections of the left olfactory bulb, the olfactory nerve to the right and the telencephalon to the left. The neuron response type is specified in boxes above the traces. Adapted from Laberage F and Hara TJ (2004) Electrophysiological demonstration of independent olfactory receptor types and associated neural responses in the trout olfactory bulb. *Comparative Biochemistry and Physiology* 137A: 397–408.

neurons located in different regions of the bulb, and that information distinguishing the related amino acids can be represented in a limited number of bulbar neurons with distinct response profiles, which could potentially encode amino acid quality. This supports, in principle, the idea that slight alterations in an odorant molecule, or a change in its concentration, can change its code, potentially explaining how such changes can alter perceived odor quality.

From the olfactory bulb, olfactory signals are transmitted to the telencephalon via the olfactory tracts. At present, little is known about how sensory information about different odorants might be projected in telencephalon. One study in channel catfish shows that a simple odotopy, the spatial mapping of different odorants, is basically maintained beyond the olfactory bulb. Amino acids are represented in lateral, pallial portion of the telencephalon, equivalent to the olfactory cortex of amniote vertebrates, whereas bile acids are represented in medial telencephalon, possibly homologous to portions of the amygdala. The teleost telencephalon receives inputs from other sensory systems and may function as a higher integrative center as in the terrestrial vertebrates.

Concluding Remarks

Olfaction begins at the olfactory epithelium, where millions of receptor neurons transduce the binding of odorant/pheromone molecules into a neural signal. The genomes of each fish species contain as many as 100 genes for olfactory receptors, with each neuron expressing only one gene. The receptor is a member of a multigene family of G-protein-coupled receptors, sharing sequence similarities with the calcium sensing, metabotropic glutamate, and vomeronasal receptors.

Considerable data on olfactory respond to several odorant classes in different fish species. How is information about an odorant's identity encoded by the olfactory system? A few attempts to identify specific odorant-receptor activation mechanisms have largely failed to identify unifying patterns, probably in part due to fish and protocol variability. To date, amino acid–receptor interactions in rainbow trout have been most studied.

The fish peripheral olfactory code is determined solely by the specific binding affinity between odorant and its specific receptor. Each odorant is recognized by a single receptor, and not combination of receptors, with one receptor to one glomerulus. Morphologically distinct neuron types use different transduction cascades and maintain distinct patterns of projections to the olfactory bulb. As a consequence, each odorant is represented spatially in the olfactory bulb by a unique combination, or ensemble, of glomeruli. Each glomerulus could serve as part of the code for numerous different odorants, thus providing for the discrimination of a vast variety of different odors. This contrasts with the mammalian model in which each odorant is recognized by a variety of olfactory receptors, many of which recognize different structural features of the odorant.

The olfactory system of fish, the only vertebrate group in which the sensitivity and specificity data are available for specific ligands, provides an excellent starting point for the molecular characterization of odorant receptors. For example, the 5.24 odorant receptor is an amino acid-sensing receptor expressed in the olfactory epithelium of goldfish not tuned broadly to detect all 20 naturally occurring amino acids. This is why goldfish 5.24 and its ortholog from zebrafish, receptor ZO6, have very different ligand specificities.

See also: **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction; Chemosensory Behavior; Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Gustation.

Further Reading

Buck LB (1996) Information coding in the vertebrate olfactory system. *Annual Review of Neuroscience* 19: 517–544.

Hara TJ (1975) Olfaction in fish. *Progress in Neurobiology* 5: 271–335.

Hara TJ (ed.) (1992) *Chemoreception in Fishes*. Amsterdam: Elsevier.

Hara TJ (ed.) (1992) *Fish Chemoreception*. London: Chapman and Hall.

Hara TJ (1994) The diversity of chemical stimulation in fish olfaction and gustation. *Reviews in Fish Biology and Fisheries* 4: 1–35.

Hara TJ (2005) Olfactory responses to amino acids in rainbow trout: Revisited. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp. 31–64. Enfield, NH: Science Publishers.

Li W and Sorensen PW (1997) Highly independent olfactory receptor sites for conspecific bile acids in the sea lamprey, *Petromyzon marinus*. *Journal of Comparative Physiology A* 180: 429–438.

Michel WC, Sanderson MJ, Olson JK, and Lipschitz DL (2003) Evidence for a novel transduction pathway mediating detection of polyamine by the zebrafish olfactory system. *Journal of Experimental Biology* 206: 1697–1706.

Mombaerts P (1999) Molecular biology of odorant receptors in vertebrates. *Annual Review of Neuroscience* 22: 487–509.

Mombaerts P (2001) How smell develops. *Nature Neuroscience* 4: 1192–1198.

Sato K and Suzuki N (2001) Whole-cell response characteristics of ciliated and microvillus olfactory receptor neurons to amino acids, pheromone candidates and urine in rainbow trout. *Chemical Senses* 26: 1145–1156.

Satou M (1992) Synaptic organization of the olfactory bulb and its central projection. In: Hara TJ (ed.) *Fish Chemoreception*, pp. 40–59. London: Chapman and Hall.

Sorensen PW and Caprio J (1998) Chemoreception. In: Evans DH (ed.) *The Physiology of Fishes*, 2nd edn., pp. 375–405. Boca Raton, FL: CRC Press.

Stacey NE and Sorensen PW (2005) Hormones, pheromones, and reproductive behavior. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish, Volume 24: Fish Physiology Series*, pp. 357–412. New York: Academic Press.

Zielinski BS and Hara TJ (2007) Olfaction. In: Hara TJH and Zielinski BS (eds.) *Sensory Systems Neuroscience*, *Volume 25: Fish Physiology Series*, pp. 1–43. New York: Academic Press.

Neurophysiology of Gustation

TJ Hara, University of Manitoba, Winnipeg, MB, canada

© 2011 Elsevier Inc. All rights reserved.

Responses to Chemical Stimuli
Molecular Basis of Gustatory Receptors and Transduction
Responses to Mechanical/Tactile Stimuli
Concluding Remarks
Further Reading

Glossary

Amino acids Molecules with an amine group, a carboxylic group, and one of 20 R groups that are the building blocks of proteins.

Bile acids Steroid acids found predominantly in the bile of vertebrates. The two major bile acids are cholic acid and chenodeoxycholic acid.

Channels Proteins located in cell membranes that control the movement of ions across the membrane.

Gustation The sense of taste.

Response magnitude The intensity or size of the response of a receptor to a stimulus, in this case the response to a chemical stimulus.

Tuned Refers to the extent to which a receptor is specialized to respond to a specific chemical stimulus. Broadly tuned receptors respond to a variety of chemical stimuli.

Responses to Chemical Stimuli

General

The pioneering recordings of electrical responses of facial nerve in response to various stimuli in bullhead catfish (*Ameiurus nebulosus*) by Hoagland in 1933 clearly demonstrated that the fish gustatory systems are very sensitive to mechanical stimuli (touch and water current) but generally insensitive to the classical vertebrate taste stimuli (salts, sugar, and acids). Subsequent studies over the last several decades show that fish gustatory receptors are stimulated by dilute aqueous solutions of low-molecular-weight substances, including amino acids, peptides, and steroids. Of these, amino acids are by far the best-studied gustatory chemicals for fish. Even so, response spectra for amino acids vary considerably among fish species, in contrast to those of the olfactory system (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Olfaction). Of the 27 species of teleosts (comprising 14 families and 20 genera) whose facial gustatory amino acid specificities have been examined systematically, two general types have been identified: type 1, 10 species whose gustatory system respond to many different amino acids (i.e., wide response range), for example, channel catfish, and type 2, 17 species that are highly selective and respond to only a few amino acids (i.e., limited response range), for example, salmonids.

Olfaction in vertebrates is recognized as a distance chemical sense with high sensitivity and specificity. In contrast, gustation is primarily a contact or close-range sense with moderate sensitivity. However, the chemical sense of fish is unique in that many studies indicate that gustatory receptors are equally sensitive to the same chemical stimuli or even more sensitive than olfactory making the distinction between these two sensory modalities blurred (**Figure 1**). Furthermore, the gustatory system may not be the sole player in feeding in fish. In fact, recent studies demonstrate that feeding behavior in fish is initiated primarily by olfaction, and complemented by gustation.

Amino Acids

Most electrophysiological responses of the peripheral gustatory receptors are recorded from branches of the facial nerves (whole bundles or single fibers) innervating taste buds inside the upper or lower lips, or maxillary barbells. Electrical responses to amino acids are generally characterized by a fast adapting, rapidly returning to the baseline activity even with continuous stimulation, which contrasts with a slow-adapting olfactory response. Threshold concentrations for the more stimulatory amino acids range from 10^{-8}–10^{-7} M for most fish species tested to 10^{-11}–10^{-10} M for channel catfish. Threshold determinations, however, are highly sensitive to the background water in studies when the receptors are perfused with water. Natural water is required for response activation; replacing natural perfusing water with distilled

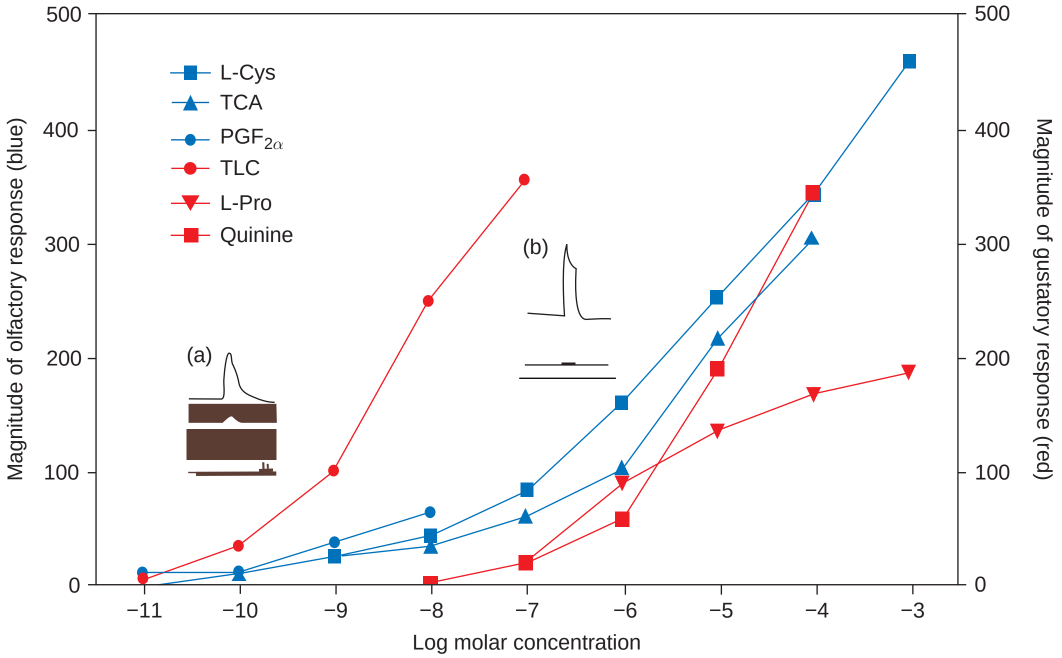


Figure 1 Comparison of the olfactory electro-olfactogram (EOG) and gustatory (palatal nerve response) sensitivities to representative chemical stimuli in rainbow trout. Insets; typical integrated palatine nerve response (a) and EOG (b), respectively. Modified from Hara (1994) The diversity of chemical stimulation in fish olfaction and gustation. *Reviews in Fish Biology and Fisheries* 4: 1–35.

water eliminates responses to stimulant chemicals, and artificial water only partially restores it. Thus, unknown factor beyond cations in the natural water plays an essential role in eliciting maximal gustatory reception.

All fish species in type 2, typically salmonids, respond to L-proline (L-Pro), L-alanine (L-Ala), and several other amino acids and related compounds, to a varying degree (**Figure 2**). Both carp and goldfish can also be placed in type 1. In rainbow trout, only L-Pro, L-Ala, hydroxyl-L-proline (L-Hpr), and L-α-aminoguanidinopropionic acid (L-Agp) are stimulatory, with thresholds 10^{-8}–10^{-5} M, although some strain differences exist. No difference exists in the response patterns to amino acids recorded either from the facial or from glossopharyngeal nerves. A structure–activity study indicates that heterocyclic-imino acids with four-, five-, or six-membered rings, and also of thioproline and hydroxyproline are effective.

The most characteristic feature common to all species of type 1 is that L-Pro is the dominant stimulant amino acid and it is the only naturally occurring amino acid that is not detected by the olfactory system (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Olfaction). Chemically, proline is unique because an amino group is secondary and it cannot act as a hydrogen donor lacking a hydrogen atom on the amide group, which is required for binding of odorant molecules. These data suggest that the gustatory and olfactory systems detect distinct portions of amino acid molecules, thus minimizing redundancy. Cross-adaptation (desensitization) data further suggest that three receptor types are involved in the detection of these amino acids: (1) proline-receptor (L-Pro, L-Hpr, and L-Ala), (2) betaine-receptor (Bet and L-Agp), and (3) leucine-receptor (L-Leu, L-Phe). Of the salmonids, *Salvelinus* spp. are the simplest, detecting only three amino acids L-Pro, L-Hpr, and L-Ala. Salmonids may have developed gustatory receptors for L-Pro and, with phylogenetic advancement, gained greater response capabilities by acquiring new receptor types and by decreasing specificity of existing receptors.

Type 1 fishes detect virtually all natural amino acids by gustation, with the sensitivities higher (lower thresholds) than those of their olfactory counterpart. In channel catfish, for example, responses of the facial nerves innervating the maxillary barbels to L-Ala (the most effective for this species) show thresholds averaging 5×10^{-11} M. Analyses of single fiber responses reveal two clusters: L-Ala and L-Arg. Fibers comprising the latter cluster are divided into two subclusters, Arg and Arg/Pro, and the former, interestingly, is not responsive to Pro but broadly tuned. Responses of the superficial ophthalmic nerves innervating the nasal barbels and the palatine nerves innervating the anterior palate are similar in their amino acid specificities (**Figures 3** and **4**). Fishes in which Ala and Arg are the two major gustatory amino acids are also characterized by high sensitivity to Gly and include most marine species examined.

In summary, although an accurate estimation of the number of effective amino acids and their detection

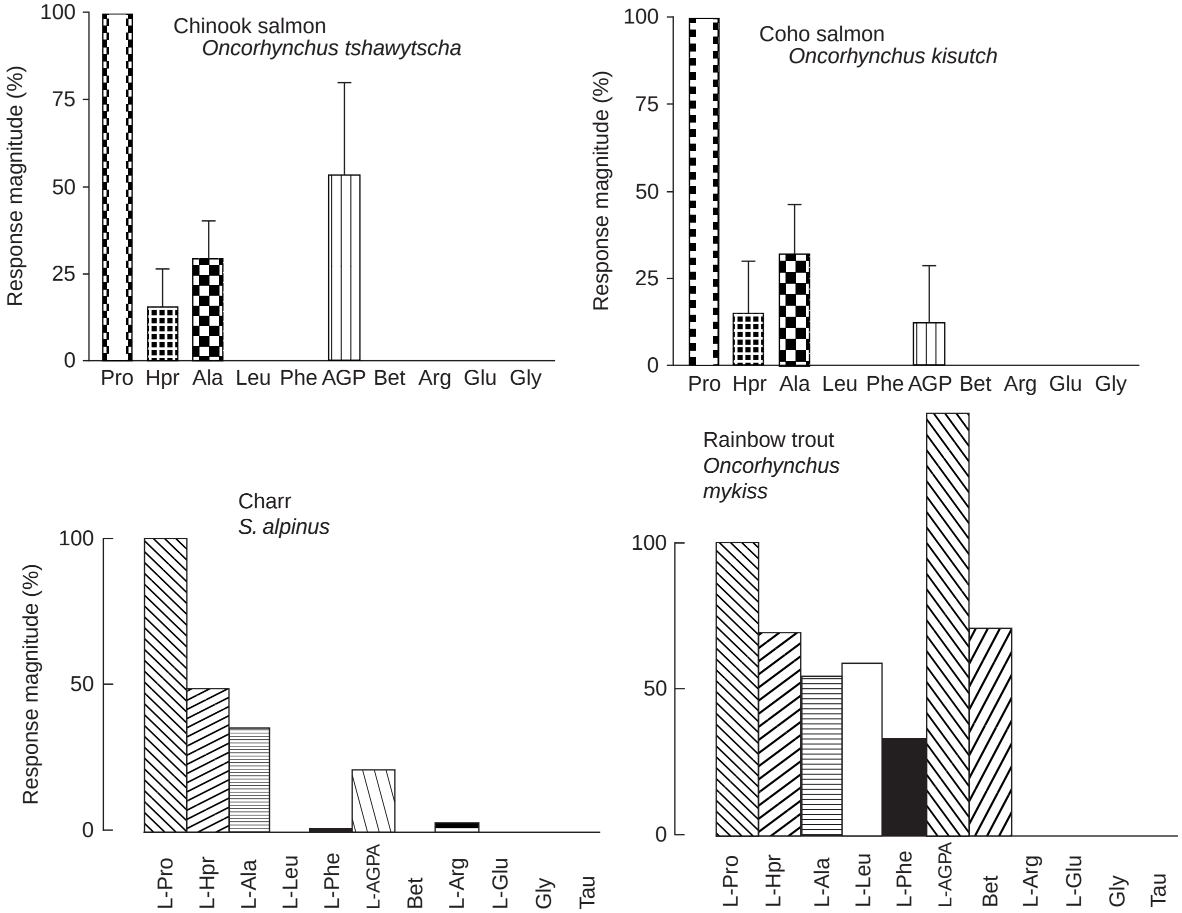


Figure 2 Relative stimulatory effectiveness of amino acids on the palatine nerve responses in four salmonid species. All chemicals are tested at 10^{-3} M. Pro, L-proline; Hpr, hydroxyl-L-proline; Ala, L-alanine; Leu, L-leucine; Phe, L-phenylalanine; AGP, L-α-amino-β-guanidinopropionic acid; Bet, betaine; Arg, L-arginine; Glu, L-glutamic acid; Gly, glycine. Adapted from Hara *et al*. (1999) The variability of the gustatory sensibility in salmonids, with special reference to strain differences in rainbow trout, *Oncorhynchus mikiss*. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 13–24.

thresholds may be difficult to make due to inconsistencies in experimental conditions employed by different researchers, it is not unreasonable to conclude that Pro is the dominant amino acid in the type 2 fishes, minimizing the redundancy between the gustatory and olfactory systems. In all type 1 fishes, Ala and Arg are dominant, and considerable overlaps exist between the two fiber systems.

Quinine, Strychnine, and Tetrodotoxin

Quinine hydrochloride (Q·HCl), strychnine, and the pufferfish toxin tetrodotoxin (TTX) stimulate the gustatory receptors of salmonids. Both Q·HCl and strychnine taste extremely bitter to humans, whereas TTX is a potent neurotoxin, specifically blocking voltage-gated Na-channels on the surface of nerve and skeletal muscle membranes. Rainbow trout and three char species (*Salvelinus* spp.) detect these chemicals at thresholds 10^{-8}–10^{-7} M, with extremely high-response magnitudes (**Figure 5**). Pretreatment with strychnine in rainbow trout inhibits responses to Q·HCl and TTX, but without any effect on amino acid and bile acid responses, suggesting that all three chemicals might share the same receptor and transduction mechanisms, despite vastly different molecular structures (**Figure 4**). Or, as in mammals, a receptor family with a wide response diversity coexists in a single gustatory cell. In channel catfish, Q·HCl suppresses responses of chemo-sensitive fibers but not the tactile. It also suppresses responses to other bitter substances, denatonium benzoate (the most bitter substance known) and caffeine, neither of which suppresses amino acid responses. It is suggested that the Q·HCl inhibition is caused by blocking voltage-gated calcium channels in the gustatory cells.

TTX and a paralytic shellfish toxin, saxitoxin (STX), are potent gustatory stimuli for salmonids. Rainbow trout detect TTX at 2×10^{-7} M, and at 10^{-5} M it evokes a response magnitude 4 times that of 10^{-3} M L-Pro (cf. **Figure 2**). These two chemicals are vastly different structurally, do not cross-adapt, and are therefore unlikely to share the same

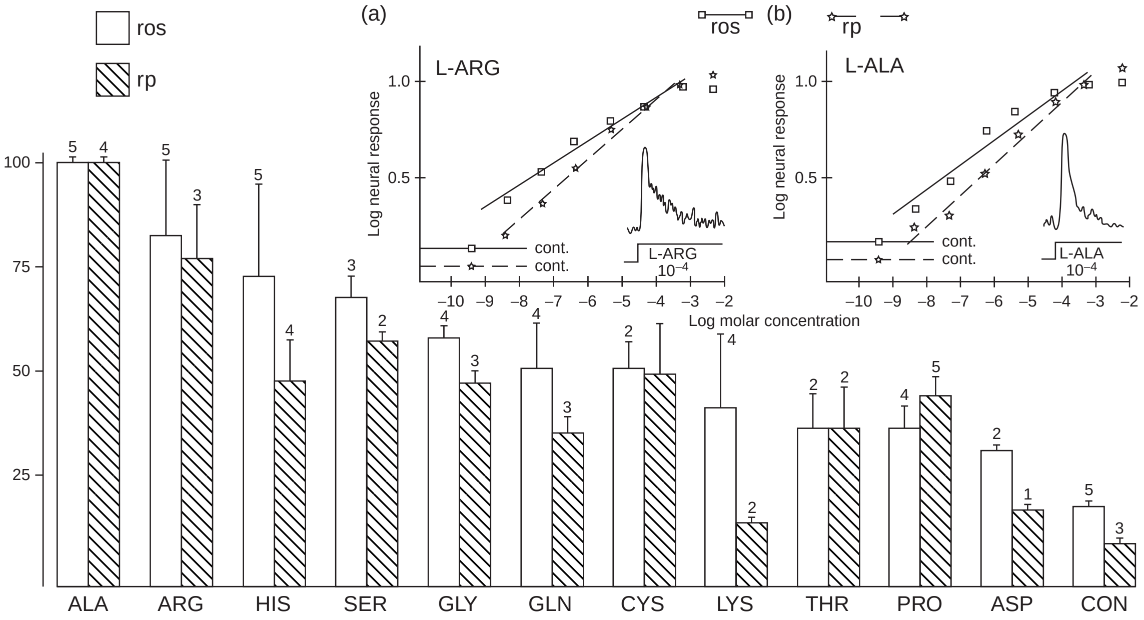


Figure 3 Relative stimulatory effectiveness of amino acids recorded from ophthalmic superficial (ros) and palatine (rp) nerves of channel catfish. All chemicals are tested at 10^{-3} M. Insets: Concentration–response curves for L-arginine (a) and L-alanine (b) recorded from ros and rp. Adapted from Kanwal JS, Hidaka I, and Caprio J (1987) Taste responses to amino acids from facial nerve branches innervating oral and extra-oral taste buds in the channel catfish, *Ictalurus punctatus*. *Brain Research* 406: 105–112.

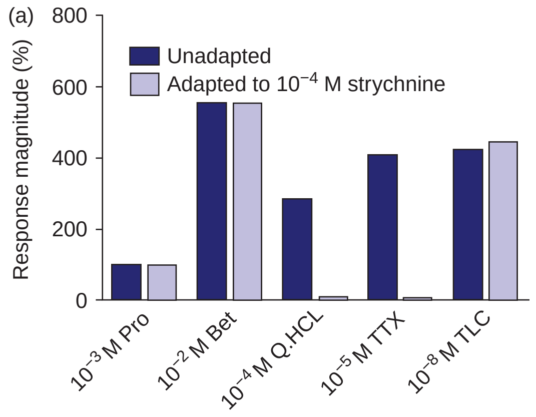


Figure 4 Interactions between strychnine, quinine (Q·HCl), bile acids (taurolithocholic acid, TLC) and tetrodotoxin (TTX) in rainbow trout, demonstrating that Q·HCl and TTX are inhibited by strychnine, but L-Pro, betaine (Bet), and TLC are unaffected. Adapted from Yamashita *et al*. (2006) Gustatory responses to feeding- and non-feeding-stimulant chemicals, with special emphasis on amino acids, in rainbow trout. *Journal of Fish Biology* 68: 783–800.

receptor type. TTX and Q·HCl cross-adapt each other. TTX and STX are known as two of the most potent neurotoxins, with a lethal toxicity to humans 300 times that of cyanide. Both exert essentially the same toxic action by blocking the voltage-gated sodium channels in neural tissues including the gustatory receptors in higher vertebrates. In fish, however, the treatment of the gustatory receptors with TTX does not affect amino acid-induced responses, suggesting that TTX-sensitive sodium channels may not be involved in the gustatory transduction mechanisms in fishes.

Bile Acids

To date, the gustatory systems of only salmonids are known to be extremely sensitive to some bile acids. In rainbow trout, taurocholic acid (TCA), one of the most potent bile acids tested, is detected at a threshold concentration 10^{-12} M, with the maximum response magnitude almost double that of Pro, making TCA as sensitive as its counterpart the olfactory (cf. **Figure 1**). Both taurine- and glycine-conjugated forms are generally more stimulatory than the free form of TCA (**Figure 6**). The response pattern to bile acid is characterized by its slow adapting, tonic response. Contrary to the olfactory, responses to all tested bile acids are inhibited by pretreatment with taurolithocholic acid (TLC), suggesting that all bile acids share the same receptor/transduction system. Or, alternatively, a nonreceptor system may be involved in the bile acid stimulation. The only other fish species whose gustatory systems have been shown similar response sensitivities and specificities to bile acids are three char species (*Salvelinus alpinus*, *S. fontinalis*, and *S. namaycush*). The gustatory systems of carp, sea catfish, and tilapia are not sensitive to bile acids, up to a concentration of 10^{-4} M.

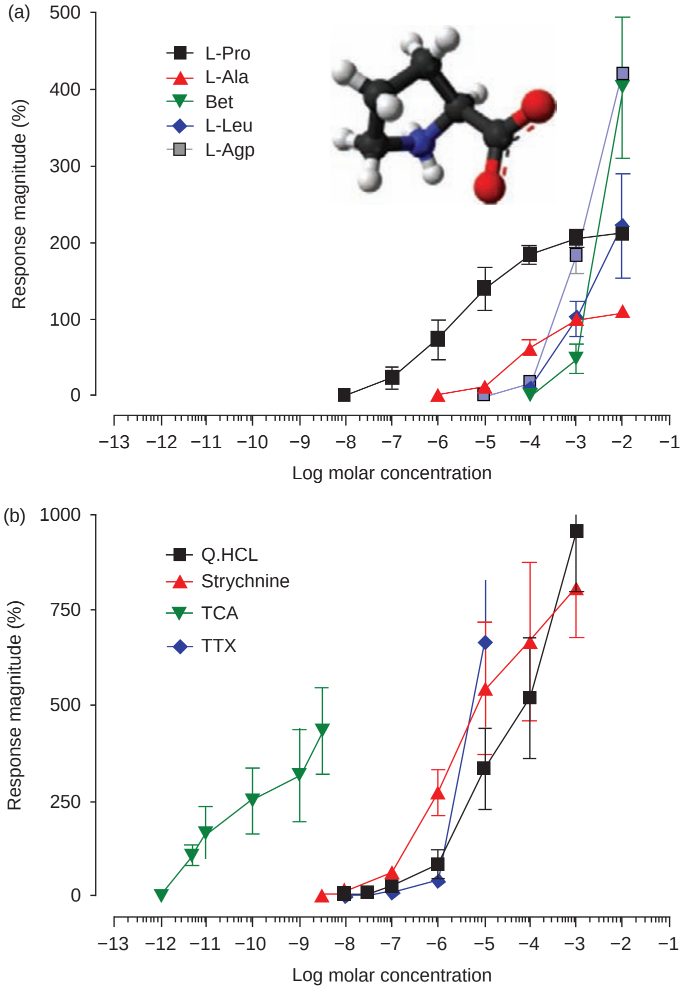


Figure 5 Concentration–response relationships of gustatory responses recorded from the rainbow trout palatine nerve bundles. L-Pro, L-proline; L-Ala, L-alanine; Bet, betaine; L-Leu, L-leucine; L-Agp, L-α-amino-β-guanidinopropionic acid; Q·HCl, quinine·HCl; TCA, taurocholic acid; and TTX, tetrodotoxin. Inset: molecular structure of L-Pro. Adapted from Yamashita *et al*. (2006) Gustatory responses to feeding- and non-feeding-stimulant chemicals, with special emphasis on amino acids, in rainbow trout. *Journal of Fish Biology* 68: 783–800.

CO_2/pH

Fish generally orient negatively to localized increase in hydrogen ions (H^+) concentration, where pH (or a change in pH) is a major directive factor in the distribution of fishes. However, the effects of acid are often confounded with those of CO_2, which is released when acid is added to water containing carbonate salts. In pH-controlled decarbonated natural water, rainbow trout detect CO_2 with a threshold 4×10^{-5} M, which is only slightly higher than the CO_2 level found in natural water (1.8×10^{-5} M) equilibrated with air. The threshold for H^+ is approximately 4×10^{-5} M. Responses to CO_2 are independent of pH, and little affected by pretreatment with amino acid or bile acid (**Figure 7**). None of the single gustatory fibers responsive to CO_2 respond to other stimuli, except Q·HCl. The rainbow trout gustatory receptors are thus capable of distinguishing between CO_2 and H^+, and CO_2 receptors are distinct from those that detect other gustatory stimuli. The CO_2-responsive single fibers are characterized by higher amplitudes of action potentials compared with those responsive to amino acids. The concentration–response curves for both CO_2 and H^+ recorded from a whole palatal nerve bundles show two discrete components, a slow increase from the threshold up to about 2×10^{-3} M and pH 3, respectively, followed by a sharp increment with further increase in concentration. It is assumed that the former responses represent respective receptor-mediated responses transmitted through the palatine nerve, whereas the latter are caused by activation of nonreceptor mechanisms involving trigeminal nerves. In support of this, responses of the palatal gustatory nerves of the Japanese eel to CO_2, when recorded from the facial nerves that lack a trigeminal component, exhibit a single component concentration–response curve. Also in mammals, although

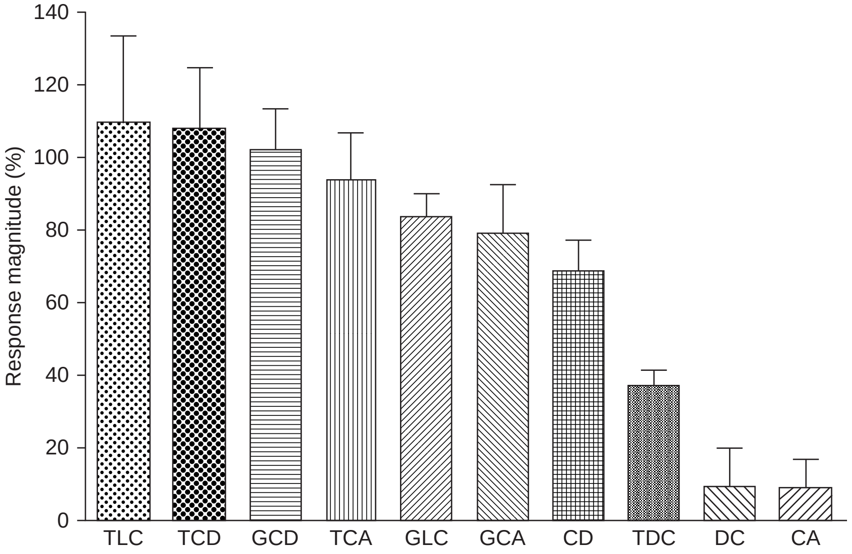


Figure 6 Relative stimulatory effectiveness of bile acids at 10^{-8} M in eliciting gustatory responses in rainbow trout. TLC, taurolithocholic acid; TLD, taurolithodeoxycholic acid; GCD, glycochenodeoxycholic acid; TCA, taurocholic acid; GLC, glycolithocholic acid; GCA, glycocholic acid; CD, chenodeoxycholic acid; TDC, taurodeoxycholic acid; DC, deoxycholic acid; CA, cholic acid. Modified from Yamashita *et al*. (2006) Gustatory responses to feeding- and non-feeding-stimulant chemicals, with special emphasis on amino acids, in rainbow trout. *Journal of Fish Biology* 68: 783–800.

taste buds are predominantly distributed in the oral cavity, especially on the tongue and palate, they are also found on the epithelia of the pharynx and larynx. Both the intraepithelial-free nerve endings and the taste buds are sensitive to CO_2, and suggested that they may play a role in respiratory modulation rather than food appreciation.

Molecular Basis of Gustatory Receptors and Transduction

Analyses of genomic sequences encoding proteins homologous to mammalian T1Rs and T2Rs reveal two families of G-protein-coupled gustatory receptors fT1Rs and fT2Rs in pufferfish (*Fugu rubripes*), medaka (*Oryzias latipes*), and zebrafish. These receptor genes show high (60–70%) similarities to those of mammals: T1R and T2R. The former consist of three subtypes, and are expressed in different gustatory cell populations, whereas the latter, consisting of two to three subtypes, show low degree of similarity to those of mammals, and are expressed in different cell groups from those for T1R genes. This suggests that two types of distinct gustatory receptor and signaling pathways are common to vertebrates. Electrophysiologically, the zebrafish gustatory system is of type 2 fish, responding primarily to L-Pro and L-Ala. A calcium imaging analysis of T1Rs and T2Rs from both zebrafish and medaka, using an HEK293T (human embryonic kidney 293T) heterologous expression system, further reveals that both T1R1/3 and a series of T1R2/3s respond to several amino acids, while zebrafish T2R5 and medaka T2R1 respond to denatonium, one of the most bitter substances. Interestingly, mouse T1R1+3 receptors recognize a broad range of L-amino acids, both in cell-based assays and *in vivo.* T1R1 and T1R3 combine to function as a broadly tuned L-amino acid sensor specifically responding to most of the 20 amino acids. By contrast, the T2R family containing approximately 30 genes represents bitter receptors, detecting diverse organic molecules (caffeine, nicotine, and strychnine) that originate from plants. Unlike T1Rs, most T2Rs are co-expressed in one cell population that does not contain T1Rs, suggesting that each gustatory receptor cell may be capable of recognizing multiple bitter substances. Thus, the discovery and functional characterization of the cell and receptors for amino acids, bitter and sweet tastes in mammals suggest that receptors for these gustatory substances are expressed in distinct populations of cells that operate independently of each other to trigger stereotypic behavioral responses.

Two possible transduction sequences for gustation have been suggested. The binding of gustatory stimulus to receptors activate G-proteins, which, in turn, activate second messenger producing enzyme phospholipase C (PLC). PLC metabolizes phophatydylinositol biphosphate (PIP2) into two second messengers: inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+}, while DAG activates protein kinase C (PKC). An increase in Ca^{2+} activity triggers neurotransmitter release. In contrast, binding of L-Arg and L-Pro to their respective receptors is

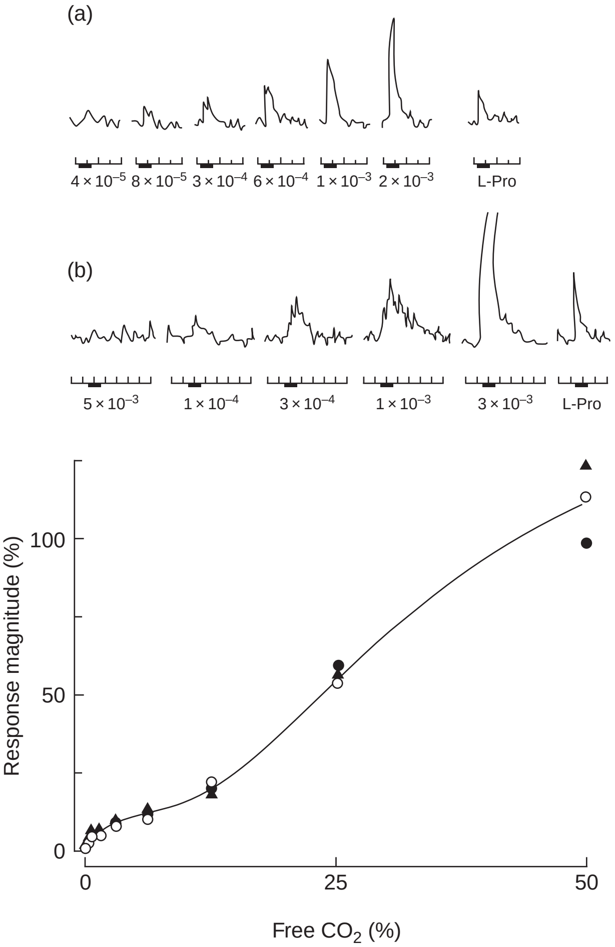


Figure 7 Typical gustatory responses to CO_2 (a) and HCl (b), recorded from the rainbow trout palatine nerve bundles. Concentration–response curves (bottom) show the effects of pretreatment with 10^{-3} M L-proline (open circles) and 10^{-8} M taurolithocholic acid (solid triangles) on CO_2 responses. Control responses to CO_2 before adaptation are shown by solid circles. Adapted from Yamashita *et al*. (1989) Specificity of the gustatory chemoreceptors for CO_2 and H^+ in rainbow trout (*Oncorhynchus mikiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1730–1734.

directly coupled to the activation of nonselective cation channels. Stimulation of gustatory receptor cells release neurotransmitters onto afferent nerves that innervate taste buds. Analogous to neurotransmitter release in other excitable cells, it is expected that neurotransmitter release in gustatory cells depends on an increase in intracellular Ca^{2+} ($[Ca^{2+}]$). In isolated channel catfish gustatory cells, some respond to L-Arg with an increase in $[Ca^{2+}]$, while others elicit decrease in $[Ca^{2+}]$. These heterogeneous responses of the $[Ca^{2+}]$ to L-Arg is consistent with depolarization or hyperpolarization of gustatory cells, suggesting that excitatory and inhibitory responses to a single gustatory stimulus can potentially play a role in increasing the sensitivity of the gustatory system.

Responses to Mechanical/Tactile Stimuli

Behavioral studies show that both chemical and mechanical (tactile) cues are closely involved in feeding in many fish species (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior). For example, catfish find food by touch but not by vision; at the moment food touches a barbel or the fish's body, the fish turns toward it with reflex speed and snaps it up. The need for direct contact with food suggests that relevant receptors are tactile and that the central mechanosensory pathways are involved in activating the feeding behavior. However, as we have seen (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes), the barbels are richly supplied with taste buds exclusively innervated by the facial nerve and paralleled by the free nerve ending plexus via the trigeminal nerve. A question then arises whether the mechanosensitivity originates from the taste bud proper (gustatory nerves) or adjacent nervous components such as trigeminal nerves.

As every fish chemosensory electrophysiologist encounters, it is essential to eliminate electrical noises caused by mechanical disturbances by changing water flow during stimulus application, before any genuine response due to chemical stimuli can be established. Hoagland, in his pioneering study, not only found high sensitivity of the catfish facial nerve complex, but also noticed the striking differences in the impulse amplitudes, that is, low amplitude due to chemical stimuli and high amplitude to the mechanical stimuli (touching or water current). He suspected that the specificity of impulses for the two sense modalities is correlated with cell sizes of origin of the axons: large cells in the Gasserian ganglion supplying tactile (trigeminal) nerve endings and small cells of origin in the geniculate ganglion sending axons to taste buds. However, since the finding that the carp glossopharyngeal nerve, consisting primarily of gustatory nature, responded to both chemical and tactile stimuli, a number of studies ensued identifying the origin of the bimodal nature of the gustatory nerve activities in catfish, carp, puffer, and rainbow trout. These studies show that the majority of fibers are highly sensitive to mechanical stimuli (touching, sliding, brushing, and water current). Although most fibers respond to either chemical or tactile stimuli, small fractions of the facial nerve fibers innervating maxillary barbels and recurrent facial nerve innervating the flank of channel catfish contain bimodal fibers responding to both chemical and tactile stimulation. These chemosensory and tactile fibers terminate in the facial and vagal lobes overlapped and loosely organized in a somatotopic manner in carp and catfish. In rainbow trout, none of single palatine nerve fibers sensitive to touch stimulation respond to amino acids, bile acids,

Table 1 Gustatory responses of single fibers of the palatine nerves (VIIth cranial nerve) to CO_2, and other chemical and tactile stimulation in rainbow trout (*Oncorhynchus mykiss*) + = increased firing frequency; − = no response

Fiber	*CO_2*	L-*Proline*	L-*alanine*	*Betaine*	*TLCA*[a]	*Quinine-HCL*	*Touch*
1	+	−	−	−	−	+	−
2	+	−	−	−	−	−	−
3	+	−	−	−	−	−	−
4	+	−	−	−	−	−	−
5	+	−	−	−	−	+	−
6	−	+	+	−	−	−	−
7	−	−	−	−	+	−	−
8	−	−	−	−	−	−	+
9	−	−	−	+	−	−	
10	−	−	−		+	−	

[a] Taurolithocholic acid.

Q·HCl, or CO_2, while none of the chemosensitives respond to tactile stimulation. Thus, the facial nerve fibers innervating the anterior palate contain two types of afferent fibers, chemosensory and mechanosensory, although it is not clear whether they are derived from the gustatory receptor cell or the synapsing fiber *per se.* (**Table 1**)

Thus, the VII, IX, and X gustatory nerves all comprise chemical as well as mechano-sensitive fibers. The next logical question is whether all the mechanical sensitivity is derived from taste buds, *per se.* One of the morphological features shared by both the gustatory and olfactory, let alone the lateral-line system, is that all have developed ciliated cells of one type or another, directly exposed to the aqueous environment. Ciliated olfactory receptor neurons and lateral-line hair cells are characterized by their cilia having 9 + 2 microtubule configuration.

Concluding Remarks

The gustatory system is generally very well developed in fishes and highly specialized in several groups; the best investigated being the cyprinids, silurids, and mullids. Coupled with expansive peripheral receptors, they all have developed elaborate central structures, probably the most complex neural organization found in any vertebrate central nervous system. Intra- and extrapharyngeal distributions of taste buds provide fishes with detailed chemical and tactile information, as well as the means to detect food items and reject inedible or noxious substances. The extraordinary development of the vagal/facial lobe complex organized topographically receives very specific inputs. Amino acids, particularly proline, alanine, and possibly arginine, comprise major gustatory chemicals in most fish species. Gustatory receptors detect other chemical groups with extreme sensitivity, including bile acids, nucleotides and bitter substances. Gustatory receptors highly sensitive to CO_2 in fish gill epithelium suggest a linkage with ventilatory drive of much greater importance than has previously been acknowledged.

Using heterologous expression systems, functional characterization of the cells and receptors for amino acids and bitter substances provides a view of how gustatory signals are peripherally encoded. These receptors are expressed in distinct populations of gustatory cells that operate independently. With the application of new and powerful molecular techniques, we are hopeful that a more complete understanding of the physiology of the fish gustatory system will arrive soon.

See also: **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior; Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Olfaction.

Further Reading

Caprio J (1978) Olfaction and taste in the channel catfish: An electrophysiological study of the responses to amino acids and derivatives. *Journal of Comparative Physiology* 123: 357–371.

Hara TJ (1994) The diversity of chemical stimulation in fish olfaction and gustation. *Reviews in Fish Biology and Fisheries* 4: 1–35.

Hara TJ, Carolsfeld J, and Kitamura S (1999) The variability of the gustatory sensibility in salmonids, with special reference to strain differences in rainbow trout, *Oncorhynchus mikiss*. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 13–24.

Hara TJ and Zielinski BS (eds.) (2007) *Sensory Systems Neuroscience, Volume 25: Fish Physiology*. New York: Elsevier

Herrick CJ (1905) The central gustatory paths in the brains of bony fishes. *Journal of Comparative Neurology and Psychology* 15: 375–456.

Hoagland H (1933) Specific nerve impulses from gustatory and tactile receptors in catfish. *Journal of General Physiology* 16: 685–693.

Kitada Y and Hara TJ (1994) Effects of diluted natural water and altered ionic environments on gustatory responses in rainbow trout (*Oncorhynchus mikiss*). *Journal of Experimental Biology* 186: 173–186.

Kohbara J, Miche WC, and Caprio J (1992) Responses of single facial taste fibers in the channel catfish, *Ictalurus punctatus*, to amino acids. *Journal of Neurophysiology* 68: 1012–1026.

Konishi J and Zotterman Y (1961) Taste function in the carp. An electrophysiological study on gustatory fibers. *Acta Physiologica Scandinavica* 52: 150–161.

Marui T and Caprio J (1992) Teleost gustation. In: Hara TJ (ed.) *Fish Chemoreception*, pp 171–198. London: Chapman and Hall.
Ogawa K, Marui T, and Caprio J (1997) Quinine suppression of single facial taste fiber responses in the channel catfish. *Brain Research* 769: 263–272.
Oike H, Nagai T, Furuyama A, *et al.* (2007) Characterization of ligands for fish taste receptors. *Journal of Neuroscience* 27: 5584–5592.
Reutter K and Kapoor BG (eds.) (2005) *Fish Chemosenses*. Enfield, NH: Science Publishers.
Sorensen PW and Caprio J (1998) Chemoreception. In: Evans DH (ed.) *The Physiology of Fishes*, 2nd edn., pp 375–405. Boca Raton, FL: CRC Press.
Sveinsson T and Hara TJ (2000) Olfactory sensitivity and specificity of Arctic char, *Salvelinus alpinus*, to a putative male pheromone, prostaglandin $F_{2\alpha}$. *Physiology and Behavior* 69: 301–307.
Yamamori K, Nakamura M, Matsui T, and Hara TJ (1988) Gustatory responses to tetrodotoxin and saxitoxin in fish: A possible mechanism for avoiding marine toxins. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 2182–2186.
Yamashita S, Evans RE, and Hara TJ (1989) Specificity of the gustatory chemoreceptors for CO_2 and H^+ in rainbow trout (*Oncorhynchus mikiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1730–1734.
Yamashita S, Yamada T, and Hara TJ (2006) Gustatory responses to feeding- and non-feeding-stimulant chemicals, with special emphasis on amino acids, in rainbow trout. *Journal of Fish Biology* 68: 783–800.
Zviman MM, Restrepo D, and Teeter JH (1996) Single taste stimuli elicit either increase or decrease in intracellular calcium in isolated catfish taste cells. *Journal of Membrane Biology* 149: 81–88.

Chemosensory Behavior

TJ Hara, University of Manitoba, Winnipeg, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Feeding Behavior
Reproductive Behavior
Alarm Substance and Fright Reaction
Homing of Salmon
Further Reading

Glossary

Anosmic The absence or loss of sense of smell.

Pheromones Odorants secreted by animals that have a specific function to influence the development or behavior of conspecifics.

Feeding Behavior

Gustatory, Olfactory, or Tactile – Historical Development

Feeding behavior of fish is complex and differs between species. Typically, three phases of food search can be differentiated: (1) an initial period of arousal or excitement, when the fish is alerted to the presence of the stimulus, (2) a subsequent search or exploratory phase to locate the source, and (3) a consummation phase, in which the fish seeks to ingest the potential food. In reality, however, these phases are a continuum without distinct transitions.

Various sensory systems contribute to the process of feeding. Their roles and significance vary at different phases and among fish species. Whether feeding behavior in fishes is mediated by gustatory, tactile, or olfactory stimuli has long been a subject of investigation. Bateson, as early as in 1890, listed more than 15 fish species that detect food by olfaction alone. Herrick, on the other hand, reported that barbeled fishes are able to localize food by a combination of tactile and gustatory stimulation, and emphasized that memory plays a part in gustatory and tactile discrimination, while vision and olfaction assumes greater importance during intake. Similarly, working on catfish (*Amiurus nebulosus*), both Parker and Olmsted claimed that responses to food substances are due to stimulation of the olfactory organ, and that blinded or barbel-less fish find food as readily as normal fish. In contrast, Bardach and others found that catfish (*Plotosus* sp.) can find distant chemical clues by gustation alone; olfactory deprivation does not impair their searching ability. By selective ablation of the entire sensory areas of the facial and vagal lobes, Atema further characterized distinct functional roles played by the two systems in catfish (*Ictalurus* sp); the former is involved in localization and pickup of food (in combination with tactile inputs), whereas the latter in the control of swallowing. Goatfish (*Upeneoides bensasi*), a mullet, seem to rely exclusively on barbels for feeding; they are unable to recognize a packet of meat, or to find a food hidden under mud when the barbels are removed, whereas blocking of olfaction has no effect. Barbels play a primary role in detecting food in all barbeled fishes (**see also Smell, Taste, and Chemical Sensing:** Morphology of the Gustatory (Taste) System in Fishes).

Contrary to the general belief, the gustatory system is not the sole player in feeding in fish, and at the same time, not limited to it. In fact, as described below, recent studies demonstrate that feeding behavior is initiated primarily by olfaction, and complemented by gustation. Ontogenetically, taste buds generally develop later than the olfactory system in larvae, by which time their feeding already has been established.

Searching for Feeding Stimulating Substances

Identifying the active ingredients in food has also attracted the interest of many investigators, sometimes from scientific curiosity and sometimes for its potential practical application. Some of the species studied include Japanese eel (*Anguilla japonica*), pigfish (*Orthopristis chrysopterus*), pinfish (*Lagodon rhomboids*), winter flounder (*Pseudopleuronectes americanus*), mummichog (*Fundulus heteroclitus*), Atlantic silverside (*Menidia menidia*), puffer (*Fugu pardalis*), and cod (*Godus morhua*).

All these studies, examining different aspects of feeding behavior elicited by natural food and/or food extract in diverse fish species, have shown conclusively that amino acids act either singly or in combination to play a major role in stimulating feeding behavior. Each species selectively responds to a specific mixture of compounds, but synthetic mixtures of amino acids seldom attain the effectiveness of the original extracts of natural foods. As Herrick emphasized, memory could play a part in olfactory and gustatory discrimination, while behavioral responses to chemical compounds may be learned by experience and probably are stored in temporary memory. Here, an observation on juvenile sockeye salmon (*Oncorhynchus nerka*) is suggestive; they respond only to extracts of those foods constituting their current diet, but they change their extract preference concomitantly after gradual weaning to a new diet.

Feeding Behavior Is Triggered by Single Amino Acids

The mechanisms by which feeding behavior are triggered by amino acids will be examined in two fish groups: (1) fishes in which olfaction is primarily used complemented, or in combination with gustation (salmonids and goldfish, *Carassius auratus*), and (2) fishes in which gustation is solely involved throughout feeding behavior (catfishes).

Rainbow trout, lake char (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), and goldfish respond to 10^{-6} M Cys, the most potent olfactory stimulating amino acid determined electrophysiologically (**see also Smell, Taste, and Chemical Sensing:** Neurophysiology of Olfaction), by increasing locomotor activity, an initial arousal behavior, in exactly the same fashion as in response to food extracts. The enhanced locomotor activity is followed by search behavior patterns distinct for each species: (1) bottom searching in rainbow trout, (2) exploratory behavior against the tank wall in lake whitefish, (3) surfacing/jumping in lake char, and (4) gravel picking in goldfish (**Figures 1** and **2**). Ala and Lys as well as Pro, a gustatory specific amino acid, are all effective at 10^{-6} M, to varying degrees, in most species (**Figure 3**). It is important to note that the behavioral responses are stereotypic, eliciting the same behavioral patterns regardless of the type of stimuli, and concentration-dependent in all species, with thresholds 10^{-9}–10^{-8} M, which approximates the physiological thresholds (**see also Smell, Taste, and Chemical Sensing:** Neurophysiology of Olfaction). Effects of Arg and Glu vary in different species suppressing locomotor activity in rainbow trout, while stimulating feeding behavior in others. For example, in rainbow trout, the elevated locomotor activity by Cys is suppressed, when a mixture of Cys and Arg is introduced (**Figure 4**). Goldfish display typical gravel pecking searching behavior, positioning their bodies almost perpendicular to the bottom, accompanied by increased locomotor activity in response to stimulation with food extract or amino acids. Unlike in rainbow trout, Arg, Glu, and Pro are as effective as food extracts and other amino acids in inducing both locomotor and pecking activities (**Figure 3**). These results clearly indicate that single amino acids initiate appetitive feeding behavior (arousal and searching) primarily by olfaction, and interchangeably and/or complemented by gustation in naive fishes. It is thus highly likely that, in the absence of visual cues, the olfactory system plays a major role in triggering feeding behavior in these species, and possibly others. Agmatine, decarboxylated arginine, also elicits strong feeding behavior in goldfish, most likely through olfaction, because it evokes electro-olfactogram (EOG) responses with a threshold 10^{-9} M in goldfish as well as in lake whitefish and walleye, but not in rainbow trout (**Figure 3**). Agmatine is converted by bacteria from Arg to form polyamines such as putrescine, cadavarine, and spermine that stimulate the goldfish olfactory system and

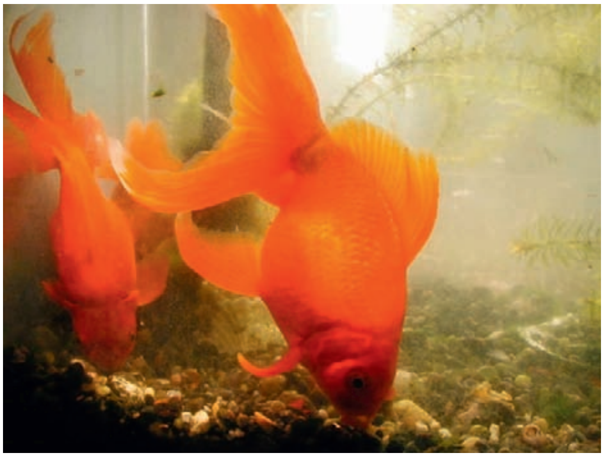


Figure 2 Pecking behavior of goldfish; food is sorted from gravel.

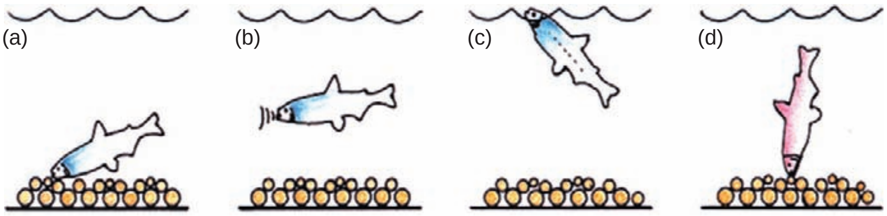


Figure 1 Diagram showing feeding behavior patterns elicited by food extract and chemical stimulation in fishes. On detection of stimuli, fish increase locomotor activity (appetitive behavior) followed by species-specific search behavior patterns: (a) bottom searching in rainbow trout, (b) exploratory/escape behavior against the wall in lake whitefish, (c) surfacing/jumping in lake char, and (d) gravel pecking in goldfish. Modified from Hara (2005).

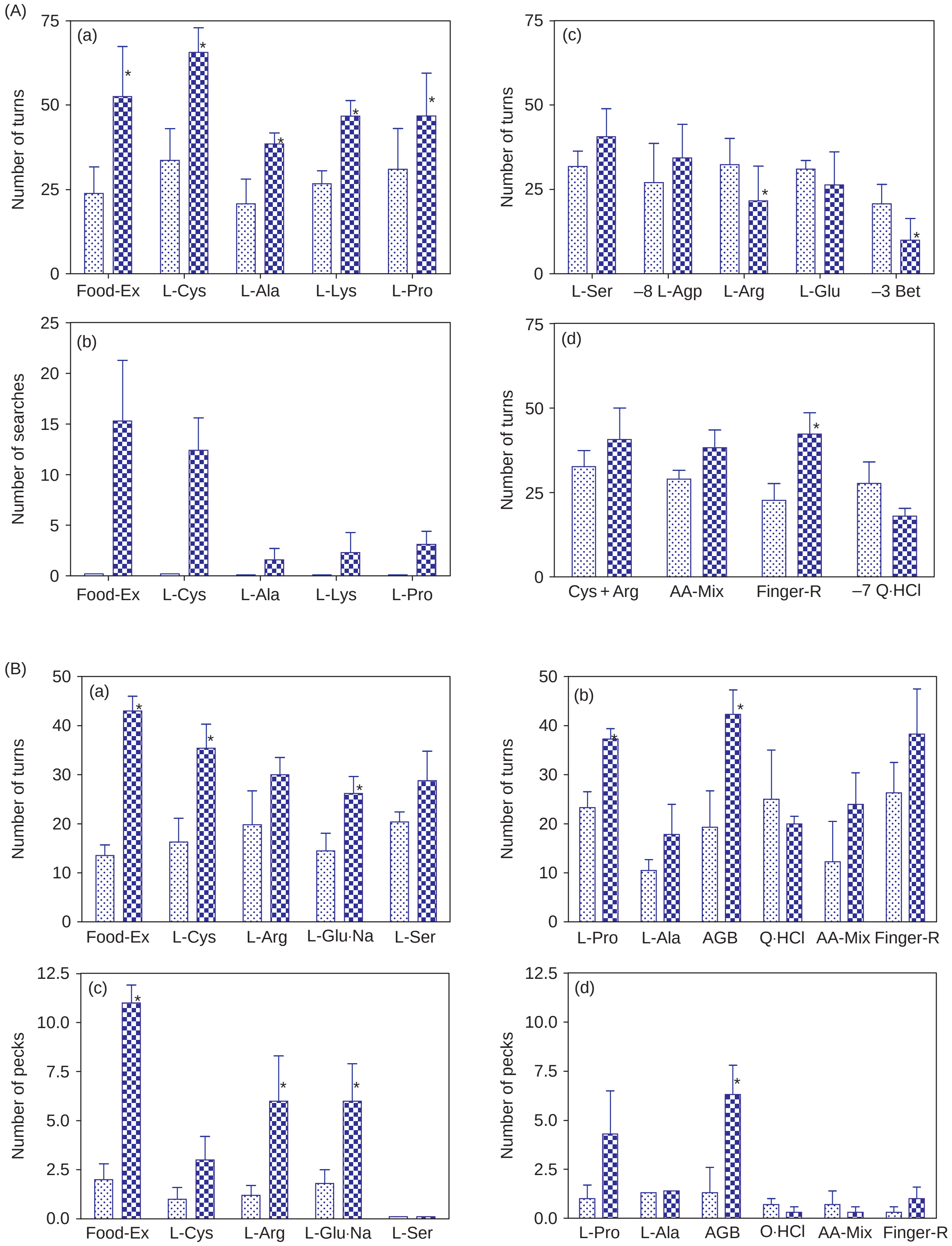


Figure 3 Feeding behavior elicited by food extract (Food-Ex) and chemicals at 10^{-6} M in rainbow trout (A) and goldfish (B). Light bars, control; dark bars, stimulus. L-Agp, L-α-amino-guanidinopropionic acid; AA-Mix, amino acid mixture containing all tested amino acids; Finger-R, finger rinse; Q·HCl, aquinine·HCl; LGlu·Na, monosodium L-glutamate (MSG); AGB, 1-amino-4-guanidinobutane (agmatine). Modified from Hara (2006).

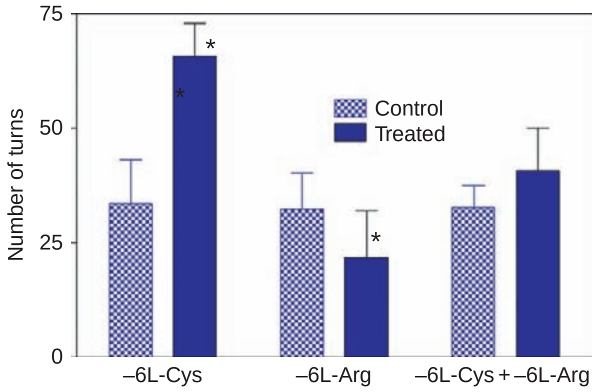


Figure 4 In rainbow trout, L-Cys stimulates locomotor activity (arousal behavior), while L-Arg suppresses it. Thus, when a mixture of both is applied, the Cys-elevated locomotor activity is suppressed.

elicit feeding behavior. Omnivorous cyprinids and catfishes are not only predators of live invertebrates but are also scavengers of dead baits.

Similarly, channel catfish elicit the entire sequence of feeding behavior in response to single amino acids. Arg, Ala, and Pro ($>10^{-7}$–10^{-4} M) induce consummatory behavioral patterns, turning, pumping of water across the gill arches, and biting–snapping. However, feeding behavior in these fish is indistinguishable in both the intact and anosmic, indicating that the entire sequence of feeding behavior is mediated by gustation alone, although which subpopulations of the taste bud systems (oral cavity, barbels, flank, and gills) are involved in which behavioral patterns is not clear. The behavioral pattern elicited by either Ala or Arg is identical, despite the fact that considerable differences exist in the biochemical (receptor binding) and biophysical (transduction) characteristics between the two, though, electrophysiologically, both the olfactory and gustatory systems are equally sensitive to the same amino acid spectrum. Toxicants can directly or indirectly affect the fish's olfactory ability (**see also Toxicology:** The Effects of Toxicants on Olfaction in Fishes).

Avoiding and Spitting the Noxious/Unpalatable

Alkaloids such as quinine, strychnine, and caffeine stimulate the fish gustatory receptors at extremely low concentrations, and induce avoidance behavior and suppress locomotor activities in salmonids and goldfish. When food pellets soaked in quinine solution are sprinkled on the surface of an aquarium, both rainbow trout and goldfish pick off, apparently by vision; the former ingest them immediately, but the latter spit them out after a brief mastication. Salmonids are able to avoid the noxious at a distance, but not once they are taken into the mouth. Goldfish, by contrast, have the specialized palatal organ that enables them to sort food particles from un-palatable particulates. They suck up the mixture, if mixed with gravel, sand, or mud, and manipulate the mix in the mouth, then finally spit out. The well-developed reflex system in the vagal lobe activates the musculature of the palatal organ to effect the sorting operation (**see also Smell, Taste, and Chemical Sensing:** Neurophysiology of Gustation). The protrusion of the upper jaw and the palatal organ is basic to the substrate feeding habits of many cyprinoids (**Figure 5**).

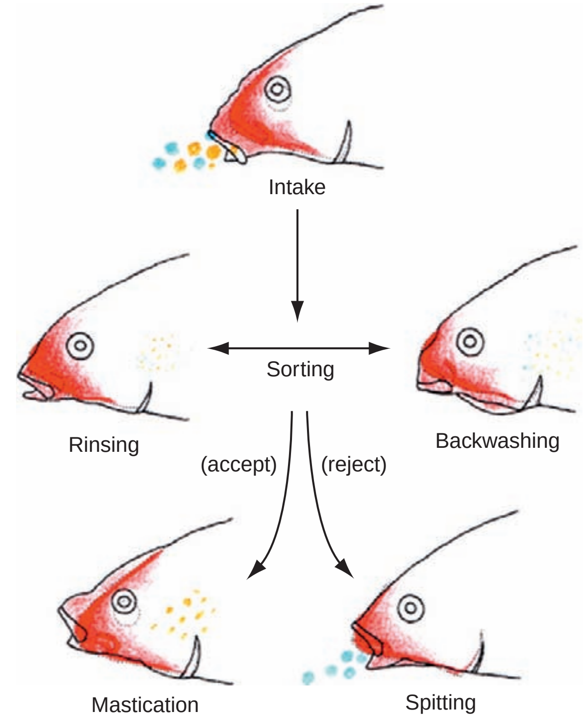


Figure 5 Feeding behavior of goldfish includes: (1) a suctorial intake of food particles, (2) a sorting stage where food is separated from nonfood within the oropharyngeal cavity, and (3) a final stage involving either acceptance or rejection of the particles. Modified from Lamb and Finger (1995).

Reproductive Behavior

In fish, olfaction exerts a functional role in every aspect of the reproductive process, from initial attraction and recognition of sexual status to sexual development of the young. The demonstration that hormones and their metabolites commonly serve as reproductive pheromones in fish has revolutionized our understanding of the importance of chemical signals in influencing vertebrate behavior in general (**see also Hormones in Communication:** Hormonal Pheromones). Pre-ovulatory female goldfish sequentially release two hormonal pheromones, $17\alpha,20\beta$-dihydroxy-4-pregnen-3-one (17 $\alpha,20\beta$P) and prostaglandins (PGFs). The former induces female oocyte maturation and is released into the water to function as a pre-ovulatory pheromone. This pheromone is detected by the male olfactory system and stimulates the synthesis of testicular $17\alpha,20\ \beta$P, which in turn evokes an increase in milt production by the time of ovulation and spawning. At the time of ovulation, females produce PGFs to mediate follicular rupture and to trigger female spawning behavior. Circulating PGFs are subsequently metabolized and released into the water, where they function as a post-ovulatory pheromone that stimulates male sexual arousal, thus resulting in spawning synchrony (**Figure 6**). This goldfish model of hormonal pheromone system, coupled with the development of the EOG method of pheromone assay, has dramatically accelerated the investigation into both the complexity and diversity of their functions in a variety of fishes.

In all salmonids except lake char, the female selects the spawning site and digs a redd by turning on her side and flexing her tail (**Figure 7**). The first convincing evidence that spawning behavior is controlled by hormonal pheromones came from the studies on Arctic char, *Salvelinus alpinus*. Actively spawning male Arctic char release PGF2α into the water, which attracts ovulated females and evokes their digging behavior, and also attracts ripe males. In brown trout (*Salmo trutta)*, exposure to PGF2α and 13,14-dihydro-PGF2α increases swimming activity in individually exposed fish, and further stimulates digging and nest-probing behaviors in females. In addition, in lake whitefish, PGF2α and 15-keto-PGF2α increase locomotor activity. However, rainbow trout that lack the olfactory sensitivity to these chemicals do not show any behavioral response. Thus, the *Oncorhynchus* genus including rainbow trout, may have lost the use of PGFs, and instead employ other chemical compounds. In fact, masu salmon (*Oncorhynchus masou*), which are nonsensitive to PGFs, use an amino acid (L-kynurenine) that is released from ovulated female urine as a powerful male-attracting pheromone. Furthermore, as in the sea lamprey (*Petromyzon marinus*), the high olfactory sensitivity and specificity to bile acids released by conspecifics are suggestive of their functioning as pheromones in salmonid in general. Curiously, a cyprinid rose bitterling (*Rhodeus ocellatus*) exhibits a peculiar sexual behavior in response to general amino acids, known to be feeding. Exposure to amino acids causes male fish to nudge against females under dark condition, while they elicit spermiation under light condition (**see also Hagfishes and Lamprey:** Lampreys: Energetics and Development and Lampreys: Environmental Physiology).

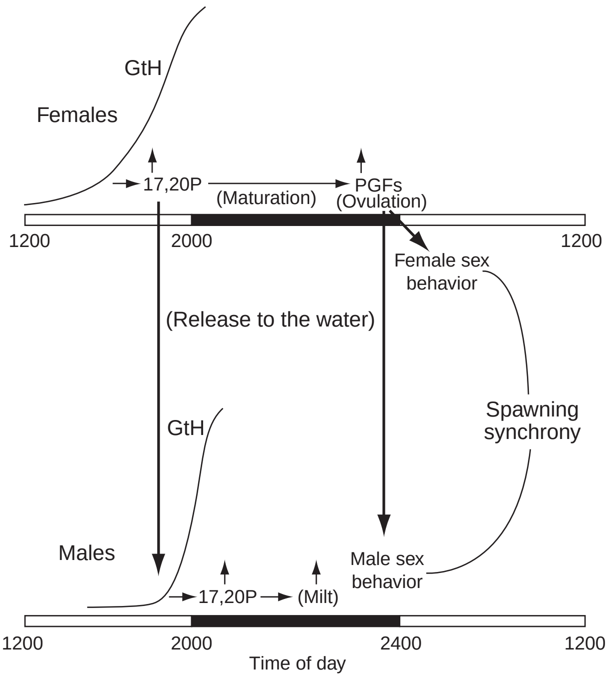


Figure 6 A model of the goldfish sexual pheromone system. Environmental cues trigger an ovulatory surge in gonadotropic hormone (GtH) in vitellogenic females that subsequently stimulates $17\alpha,20\beta$-dihydroxy-4-pregnen-3-one ($17,20\beta$P) synthesis by the ovary. Hormonal $17,20\beta$P induces final oocyte maturation and is released to the water where it functions as a preovulatory pheromone. This evokes a surge in circulating GtH in males, which stimulates the synthesis of testicular $17,20\beta$P. Elevated hormonal $17,20\beta$P in turn evokes an increase in milt production by the time of ovulation and spawning. At the time of ovulation, females produce F-prostaglandins (PGFs) to mediate follicular rupture and trigger female spawning behavior. Circulating PGFs are subsequently metabolized and released to the water where they function as a postovulatory pheromone that stimulates male sexual arousal, effecting spawning synchrony. Adapted from Sorensen *et al*. (1988).

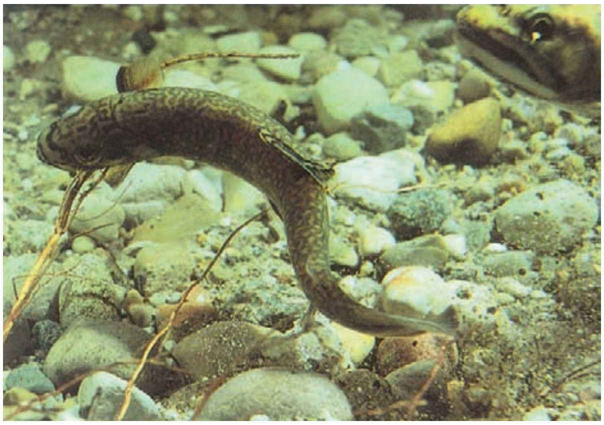


Figure 7 Digging behavior triggered by putative pheromones, prostaglandins in trout.

Alarm Substance and Fright Reaction

Nearly 70 years ago, Von Frisch discovered that a school of minnow (*Phoxinus phoxinus*) become frightened and dispersed, when an injured individual is introduced. An incident that first drew his attention to this problem was his observation of a kingfisher swooping down and attacking a school of minnows. After successfully capturing one, the bird dropped the minnow when flying away.

The other minnows, still schooling, suddenly dispersed. The general characteristics of the phenomenon are that when the skin of a fish is damaged by a predator, alarm substance cells are broken and release alarm substance (Schreckstoff). Nearby conspecifics smell the alarm substance and show a fright reaction (Schreckreaktion). The fright reaction thus produced may then be treated as a visual signal by other conspecifics, leading to rapid transmission of the danger signal through a group of fish. Thus, the alarm-signal system consists of two main components: a chemical component, the alarm substance, and a behavioral component, the fright reaction.

Despite extensive investigations, there remain a number of unanswered questions regarding the fundamental issues of the alarm substance–fright reaction: (1) characterization of the alarm substance, and its olfactory activation, (2) release of the putative alarm substance, hypoxantine-3-N-oxide into the surrounding water, and (3) the nature of the fright reaction. The alarm substance–fright reaction system is generally understood to be restricted primarily in ostariophysan species, though not universal. The fright reaction is not species specific, but the intensity of the response is related to the phylogenetic proximity of the species. Furthermore, the fright reaction is genetically determined, and appears at a certain stage of development, regardless of prior experience. Individuals likely gain protection as a result of their responses to alarm substance released by a conspecific upon attack by a predator. However, whether the release of alarm substance can provide protection or other benefits to the damaged individual is highly questionable.

Putative Alarm Substance

The alarm substance is believed to be contained in a large epidermal cell, the club cell (**Figure 8**), but not without controversy, because they are distributed abundantly in the orobuccal cavity. Unlike mucous cells, club cells lack openings at the skin surface, and release their contents only when the skin is injured. The alarm substance has variously been characterized as being purine or a pterine-like, small-ringed or double-ringed, polypeptide-like or histamine-like. Of these, hypoxanthine-3(N)-oxide (H3NO) is thought to be the most likely candidate alarm substance in the minnows. Both fathead minnows (*Pimephales promels*) and finescale dace (*Chrosomus neogaeus*) exhibit anti-predator responses (increased shoaling and decreased area use) when exposed to conspecific skin extract, synthetic H3NO or purine-N-oxide, but not upon exposure to a molecule lacking a nitrogen oxide functional group. Thus, the nitrogen oxide group is likely the determinant in triggering fright reaction, and H3NO may be one of several possible molecules that function as a chemical alarm cue. In support of this, the fish no longer detect alarm substance under acidic pHs, possibly due to nonreversible covalent changes in the molecules. Despite these efforts in laboratory investigations, skin extracts have been used exclusively as the source of the alarm substance, of which basic chemical characterization is still lacking. It should be noted that the epidermal layer of the fish skin also contains numerous mucous cells, and that the mucus, while primarily serving as a protector of the skin, also plays an important role in individual recognition and schooling. Amino acids, the main ingredients of olfactory stimuli in the mucus, are responsible for these behaviors (**see also The Skin:** Functional Morphology of the Integumentary System in Fishes).

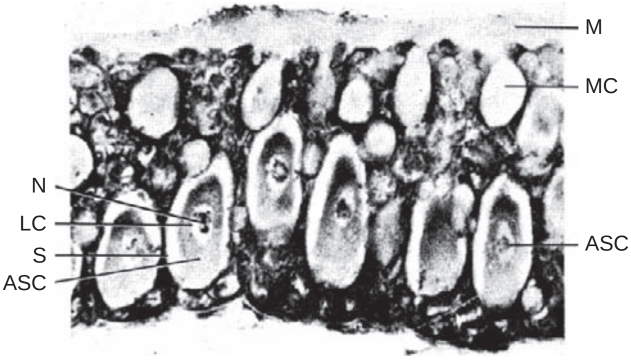


Figure 8 Photomicrograph showing club cells (alarm substance cells) in the epidermis of the minnow (*Phoxinus phoxinus*). ASC, alarm substance cells; LC, light colored region; M, mucus; N, nucleus. Adapted from Pfeiffer (1960).

Olfactory Detection

It is generally accepted that an alarm substance is detected by the olfactory system. This is based on the von Frisch's classical experiment, in which minnows with their olfactory nerves and bulbs removed, lost their responsiveness to alarm substances, even though the possible involvement of gustatory and/or other senses was not totally ruled out. As seen below, anosmic fathead minnows failed to respond behaviorally to predator odor, demonstrating the role of olfaction. Thus, despite a long history of studies on the alarm substance–fright reaction, no direct physiological evidence has been presented, including the studies where the structure–activity relationship of H3NO is investigated, indicating that the substance is indeed detected via the olfactory system.

Fright Reaction

The fright reaction is not stereotyped; von Frisch defines seven behavior patterns, changing from the slight crowding to sudden fright, and to rapid swimming into the hiding place depending on the type of stimuli and its strength. For instance, slow and fast movements, resting and motionlessness, aggressive interactions and area use, time under shelter and time moving, and so on are indiscriminately employed, depending on fish species in different laboratory conditions. In a recent field experiment, wild European minnows failed to modify their behavior or leave the area when exposed to skin extract, which cast a doubt on the hypothesis that the primary function of club cells is to signal alarm to conspecifics. This strongly contrasts with those behavioral patterns described in the feeding and reproductive behaviors, where the response patterns are generally stereotyped, and only the intensity of the specific behavior pattern increases as the stimulus increases. In the case of feeding, fish are generally very cautious, responding only to extracts of those foods constituting their current diet, and establish their extract preference after gradual weaning over to a new one. In some fish species, the elevated locomotor activity, an arousal, by a single amino acid is suppressed, a fright reaction, by other amino acids. This also raises a question whether some of the behavior patterns comprising the fright reaction described might be ones caused by general substances indifferent of alarm substance.

Evolution of Alarm Substance

While the evolution of the signals involving alarm substance–fright reaction has been a topic of great debate and controversy, recent studies now reveal that the alarm substance has evolved as a defense against environmental stressors such as pathogens, parasites, and ultraviolet (UV) radiation, and that it is secondarily co-opted by predators and prey as a chemical signal. This conclusion is based on the recent studies indicating that predation pressure does not influence alarm cell production in fishes; rather, it is stimulated by exposure to skin-penetrating pathogens (water molds, parasites, and UV radiation). Basic chemical characterization of the contents of club cells is again lacking. These authors hypothesize that individual ostariophysan and percid fishes benefit from investing in club cells owing to the protective function the cells provide. When these cells are ruptured by predation, nearby receivers have driven the development of receptors and responses to these alarm cues, thus reducing the probability of predation (**see also Hormones in Communication:** Hormonal Pheromones).

Predator–Prey Interactions

Not to be confused with the alarm substance–fright reaction system, *per se*, is the threat-sensitive predator avoidance hypothesis, which states that prey behave according to the degree of threat posed by their predators. For example, pike-experienced fathead minnows have the ability to differentiate individual pike in a mixture of odors from several pike, and can assess the relative concentration of the odor from each pike. The information carried by predator odors allows preys to accurately assess their level of risk, and respond to predation threats in a threat-sensitive manner. However, if the fathead minnow/pike predator/prey system is to be used as a model system for studying predator odor recognition, researchers need to establish that behavioral responses of minnows to pike water used in these studies is indeed specific for the predator pike.

Homing of Salmon

Evidence of Homing

Salmon are known for their spectacular migration (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle). This impressive feat comprises three migratory phases: (1) downstream journey of the young to the oceans, (2) return of spawning adults to the coastal area near the entrance to their home streams, and (3) the upstream migration. Phases 1 and 2 are thought to rely primarily on non-chemosensory mechanisms, and will not be discussed here. Over 100 years ago, in 1880, Buckland postulated an olfactory basis for salmonid homing, and his statement is quoted even today. The olfactory hypothesis, cast into modern terms by Hasler and his students, proposes that during a developmental period (parr-smolt transformation, PST), salmon imprint to home-stream odors, and adults later use this odorant memory as a migratory cue to guide them back to their natal home stream. The results of olfactory impairment experiments repeated over 20 times using seven different salmonids species are remarkably consistent; in 16 experiments, the olfactory sense appeared to be necessary for correct homing, and, in addition, two studies demonstrated that blind fish homed nearly as well as control fish, indicating that vision is not essential for relocating the original stream during upstream migration.

Olfactory Imprinting

Experimental evidence indicates that olfaction is essential for successful completion of the homing migration. Transfer studies clearly indicate that the salmon do not return to the stream to which their parents returned, but return instead to the stream where they themselves spent the period between the time of transfer and release. Thus, homing is not a genetically determined character, but depends on learning during early critical life of salmon. Salmon must learn the chemical cue without reinforcement during ocean residence, and respond to it as adults.

To test this hypothesis, Hasler and his colleagues exposed juvenile coho salmon (*Oncorhynchus kisutch*) to one of two synthetic chemicals, morpholine or β-phenylethyl alcohol (PEA) at Lake Michigan tributaries, and were able to attract the salmon into unfamiliar streams scented with one of these chemicals during their spawning migration 1.5 years later. Approximately 95% of the morpholine fish were captured in the morpholine-scented stream and 92% of the PEA fish were recovered in the PEA-scented stream. Further studies show that coho salmon learn the odors of their home stream during the PST, a developmental process characterized by physiological and behavioral changes, which prepare freshwater residents (parr) for life at sea. These results are consistent with the general finding that salmon reared at one site but released from a second site prior to, or during PST, return as adult to the release site, not the rearing site. This olfactory imprinting during PST has become an important component of salmon enhancement programs.

Molecular Mechanisms of Imprinting

The most characteristic feature of the olfactory imprinting in salmon is that it is linked to a sensitive period of development. Electrophysiological and biochemical studies provide compelling evidence that populations of olfactory receptor neurons are selectively tuned to respond to odor molecules present during a sensitive period, 'peripheral odor memories'. Olfactory neurons isolated from PEA-imprinted fish are nearly twice as likely to respond to PEA compared with those of nive fish, and neurons from the imprinted showed a sixfold increase in responsiveness to PEA compared with the control. However, neurons isolated from both groups responded similarly to the amino acid L-serine, suggesting that the change in the sensitivity is specific to the imprinted odorant. Generally, in olfaction, receptor activation by odors stimulates adenylyl/guanylyl cyclase activities, causing a rapid increase in intracellular cyclic adenosine monophosphate, which, in turn, activates cyclic nucleotide-gated channels, resulting in membrane depolarization and the generation of action potentials (**see also Smell, Taste, and Chemical Sensing:** Neurophysiology of Olfaction). Stimulation of guanylyl cyclase activity is significantly greater in olfactory cilia isolated from PEA-imprinted salmon compared with PEA-naive fish only at the time of the homing migration, 2 years after PEA exposure. These results suggest that exposing salmon smolts to nanomolar concentration of PEA for as little as 10 days could be correlated with dramatic and measurable changes in their peripheral sensitivity to odors even years later. However, before these data are reconciled into a workable model, the following questions would have been answered: (1) do salmon possess a specific olfactory receptor gene for either morpholine or PEA, and thereby detect them physiologically, and (2) why do salmon become sensitive selectively to exposed morpholine or PEA, when there exist hundreds of other odorants including L-serine? Modern olfactory theory explains that olfactory receptors are encoded by a large multigene family belonging to the superfamily that encodes G-protein-coupled receptors, whose repertoire is around 100 in fishes. Each olfactory neuron expresses one or a few genetically determined olfactory receptors.

What makes the home stream so specific to migrating salmon? A number of attempts have been made to isolate and identify the home stream odor substance, which has been variously characterized as being volatile, water-soluble, neutral, dialyzable, and heat labile. Recent studies show that amino acids in stream water are essential for the homing migration of salmon. Each stream contains a specific amino acid composite that derives from organisms in a tributary. It would not be surprising if amino acids play an essential role in all aspects of behaviors involving feeding, reproduction, kin-recognition, and possibly predator–prey interaction.

See also: **Fish Migrations:** Pacific Salmon Migration: Completing the Cycle. **Hormones in Communication:** Hormonal Pheromones. **Smell, Taste, and Chemical Sensing:** Chemoreception (Smell and Taste): An Introduction; Morphology of the Gustatory (Taste) System in Fishes; Neurophysiology of Gustation; Neurophysiology of Olfaction. **Toxicology:** The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Brown GE, Adrian JC, Jr., NaderiNT, Harvey MC, and Kelly JM (2003) Nitrogen oxides elicit antipredator responses in juvenile channel catfish, but not in convict cichlids or rainbow trout: Conservation of the ostariophysan alarm pheromone. *Journal of Chemical Ecology* 29: 1781–1796.

Buckland J (1880) *Natural History of British Fishes*. London: Unwin.

Chivers DP, Wisenden BD, Hindman CJ, *et al.* (2007) Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: Possible defense against pathogens, parasites and UVB radiation. *Proceedings of the Royal Society B* 274: 2611–2619.

Donaldson LR and Allen GH (1957) Return of silver salmon *Oncorhynchus kisutch* (Walbaum) to point of release. *Transactions of the American Fisheries Society* 87: 13–22.

Døving KB, Hamdan EH, Höglund E, Kasumyan A, and Tuvikene AO (2005) Review of the chemical and physiological basis of alarm reactions in cyprinids. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp. 133–163. Enfield, NH: Science Publishers.

Finger TE (2008) Sorting food from stones: The vagal taste system in goldfish, *Carassius auratus*. *Journal of Comparative Physiology A* 194: 135–143.

Hara TJ (1975) Olfaction in fish. *Progress in Neurobiology* 5: 271–335.

Hara TJ (ed.) (1982) *Chemoreception in Fishes*, pp. 307–326. Amsterdam: Elsevier.

Hara TJ (ed.) (1992) *Fish Chemoreception*, pp. 288–320. London: Chapman and Hall.

Hasler AD and Scholz AT (1983) *Olfactory Imprinting and Homing in Salmon*. Berlin: Springer.

Johnson NS, Yun S-S, Thompson HT, Brant CO, and Li W (2009) A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps. *Proceedings of the National Academy of Sciences of the United States of America* 106: 1021–1026.

Laberge F and Hara TJ (2003) Behavioral and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonids fishes. *Journal of Fish Biology* 62: 206–221.

Magurran AE, Irving PW, and Henderson PA (1996) Is there a fish alarm pheromone? A wild study and critique. *Proceedings of the Royal Society B* 263: 1551–1556.

Nevitt G and Dittman A (1998) A new model for olfactory imprinting in salmon. *Integrative Biology* 1: 215–223.

Satô M (1937) Further studies on the barbels of a Japanese goatfish, *Upeneoides bensasi* (Temminek & Schlegel). *Science Reports of the Research Institutes, Tohoku University, Series* 4(11): 297–302.

Sloman KA, Wilson RW, and Balshine S (eds) Behavior and Physiology of Fish, Vol. 24: Fish Physiology pp. 359–412 New York, NY: Elsevier.

Smith RJF (1992) Alarm signals in fish. *Reviews in Fish Biology and Fisheries* 2: 33–63.

Sveinsson T and Hara TJ (2000) Olfactory sensitivity and specificity of Arctic char, *Salvelinus alpinus*, to a putative male pheromone, prostaglandin $F_{2\alpha}$. *Physiology and Behavior* 69: 301–307.

Von Frisch (1941) Dir Bedeutung des Geruchssinnes in Leben der Fische. *Naturwissenschaften* 29: 321–333.

Yambe H, Kitamura S, Kamio M, *et al.* (2006) L-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. *Proceedings of the National Academy of Sciences of the United States of America* 103: 15370–15374.

SENSING THE ENVIRONMENT

Hearing and Lateral Line

Contents

Fish Bioacoustics: An Introduction
Vestibular System Anatomy and Physiology
Auditory System Morphology
The Ear and Hearing in Sharks, Skates, and Rays
Biomechanics of the Inner Ear in Fishes
Psychoacoustics: What Fish Hear
Auditory/Lateral Line CNS: Anatomy
Physiology of the Ear and Brain: How Fish Hear
Sound Source Localization and Directional Hearing in Fishes
Effects of Human-Generated Sound on Fish
Acoustic Behavior
Vocal Behavior of Fishes: Anatomy and Physiology
Lateral Line Neuroethology
Lateral Line Structure

Fish Bioacoustics: An Introduction

AN Popper and BM Casper, University of Maryland, College Park, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Origin of the Ear and Hearing
Origin of Knowledge of Fish Hearing
Perspectives on Fish Bioacoustics
Conclusions
Further Reading

Glossary

Agnatha Primitive jawless (a = without; gnathos = jaw) fish dominant in the early Paleozoic, and represented today by hagfish and lamprey.

Bony fish The majority of extant fishes – more than 30 000 known species.

Cupula A gelatinous structure found associated with sensory hair cells in the vestibular part of the ear, the macula neglecta, and the lateral line. Motion of the cupula stimulates the sensory cells.

Eighth cranial nerve The nerve that transmits information between the ear and brain.

Elasmobranch The subclass of fishes (Elasmobranchii) found within the class Chondricthyes that includes sharks, skates, and rays, making up the Chondricthyes or cartilaginous fishes. Their bones are made up of cartilage rather than calcium found in all other vertebrates.

Inner ear The part of the ear that is located inside of the head that is responsible for detection of sound (hearing) and movement of the head relative to gravity (vestibular). It consists of three semicircular canals and three otolith end organs, as well as a macula neglecta.

Lateral line Sensory structure consisting of a series of mechanoreceptor cells on the surface of the body of fishes and for detection of water motion relative to the body and low-frequency sound.

Neuromast The functional unit of the lateral line consisting of a patch of hair cells, supporting cells and the overlying cupula.

Otolith A structure found in the inner ear made up of calcium carbonate. Motion of the otolith relative to the sensory cells of the ear results in stimulation of the cells and detection of sounds.

Sensory epithelium The tissue that contains the sensory hair cells in the ear and lateral line.

Sensory hair cell The cell that is the stimulus detector used in the ear and lateral line. The cell has special cilia on its apical (top) surface that are bent by either the cupula or otolith. Once bent, various chemical events start and result in the release of a chemical (neurotransmitter) that then stimulate the eighth nerve that carries the signal to the brain.
Sound localization Determination of the direction of the source of sound around the body of an organism.
Soundscape The acoustic environment around an animal. It may consist of sounds produced by biotic (biological) and abiotic (nonbiological) sources.
Swimbladder A gas-filled structure in the abdominal cavity of many bony fishes. The swimbladder is involved with buoyancy control as well as in sound production and detection.
Teleost The most recently evolved of all the bony fishes (Osteichthyes). Teleosts include everything from goldfish to tuna to salmon.
Vestibular The sense that is found in the ear that detects motion of the head and body relative to gravity.

Origin of the Ear and Hearing

Where and why did hearing evolve among the vertebrates? While not a question that people often ask themselves, the answers are of considerable interest, and may even help humans think about their own sense of hearing. The general view of humans is that hearing has reached its pinnacle with speech, and that we are the foremost users of sound.

The truth, however, is that hearing is highly evolved in most vertebrates, and while no groups other than humans use speech, numerous species use sound to communicate and to learn about their environment. Indeed, it is very likely that the major selective pressure for the evolution of hearing in vertebrates was not for communication, but so that the most primitive animals could glean information about their environments.

Consider that the first vertebrates arose in water. Water is not very conducive to visual communication because the environment is often dark due to murkiness of the water or depth (as well as night), and objects can often block direct visualization of things at any substantial distance from an animal. Moreover, because the eyes are firmly planted in a specific location on the head, the visual field, or area around an animal that is in sight at any one time, is limited. It turns out, however, that the underwater environment is ideal for use of sound. Sound travels very rapidly in water (about five times faster than in air) and it attenuates (loses energy) much more slowly than in air. As a consequence, a sound can travel a considerable distance and it is not interfered with by objects in the environment, darkness, or even the currents that prevent chemical cues from moving quickly or in a straight line.

Thus, one may speculate that the very earliest vertebrates may have evolved a mechanism to detect sound so that they could gather information about objects that were at considerable distances, and all around them, and so they could gather the same information with or without visual cues. Sounds, though detectable to the earliest vertebrates, may not have been communication sounds that are used now, but more likely they were abiotic sounds produced by the surf, rain on the water, movement of currents around rocks, and other things in the environment. Sounds may also have been biotic, or produced by living things, but in the earliest days of vertebrate evolution these sounds may not have been intentionally produced. Such biotic sounds may have been produced by the swimming of predators or prey, chewing of animals on coral reefs, and in any number of other ways, all of which would tell the animal a good deal about its extended environment – its acoustic scene.

It is hard to know what the earliest sound-sensing devices were because there is not a good fossil record of sensory receptors in aquatic organisms. However, in 1967 Willem van Bergeijk speculated that the earliest receptors were located on the skin, and were probably somewhat similar to the surface lateral line system found in modern fishes and sharks, as described by Montgomery and Coombs in **Hearing and Lateral Line**: Lateral Line Neuroethology and by Webb in **Hearing and Lateral Line:** Lateral Line Structure. The most primitive lateral line probably only detected very low frequency water motions, even as it does today.

Van Bergeijk then went on to speculate that the lateral line evolved so that a region became embedded under the skin, as is found in the modern lateral line. He went on to suggest that a portion of this lateral line then developed some kind of associated structure, now known as the otolith, and became the most primitive ear. Since this ear had both a mass (the otolith) and receptor cells (the sensory cells of the lateral line), it would function as primitive accelerometer that could detect low-frequency signals, both from head motion and sound. Once the ear was established, one can hypothesize that, over time, it evolved the ability to detect higher-frequency sounds.

Finally, as the ear evolved, there may have been the co-evolution of better hearing and the production of sounds used in communication.

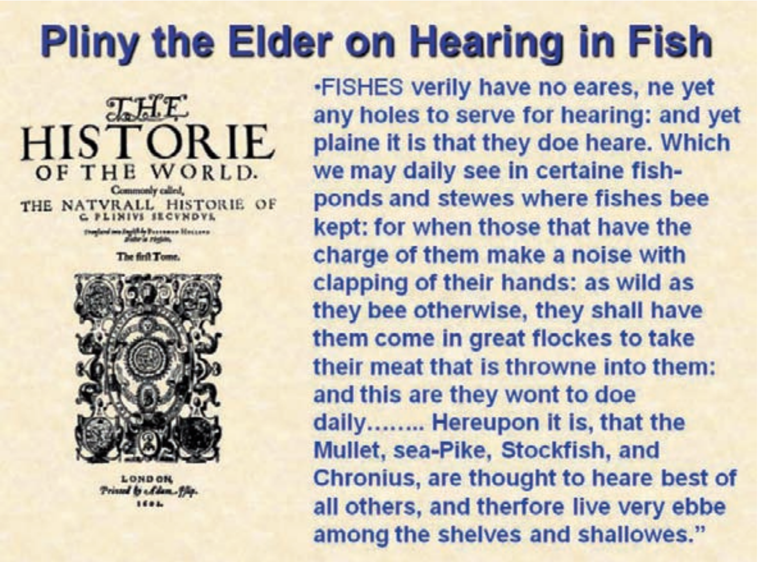
Origin of Knowledge of Fish Hearing

Historically, it appears that Aristotle and other older thinkers understood that fishes make sounds. Indeed, in about 350 BCE Aristotle wrote: "Fishes can produce no voice, for they have no lungs, nor windpipe and pharynx; but they emit certain inarticulate sounds and squeaks, which is what is called their 'voice', as the lyra or gurnard, and the sciaena (for these fishes make a grunting kind of noise) and the caprus or boar-fish in the river Achelous, and the chalcis and the cuckoo-fish; for the chalcis makes a sort piping sound, and the cuckoo-fish makes a sound greatly like the cry of the cuckoo, and is nicknamed from the circumstance. The apparent voice in all these fishes is a sound caused in some cases by a rubbing motion of their gills, which by the way are prickly, or in other cases by internal parts about their bellies; for they all have air or wind inside them, by rubbing and moving which they produce the sounds. Some cartilaginous fish seem to squeak. But in these cases the term 'voice' is inappropriate; the more correct expression would be 'sound'."

It may have been Gaius Plinius Secundus, better known a Pliny the Elder, who first wrote about fish hearing sometime in the earliest part of the first century CE (see **Figure 1** for a 1601 translation). Other more recent people further mentioned fish hearing in their writing, though mechanisms were not discussed. For example, Izaak Walton, in his monumental *The Complete Angler*, in 1653 wrote: "...has made me to believe that *Eeles* unbed themselves, and stir at the noise of the Thunder, and not only as some think, by the motion or the stirring of the earth, which is occasioned by that Thunder" (chapter V). He also noted that "And this reason of Sir *Francis Bacons* Exper. 792 has made me crave pardon of one that I laught at, for affirming that he knew *Carps* come to a certain place in a Pond to be fed at the ringing of a Bel; and it shall be a rule for me to make as little noise as I can when I am a fishing" (chapter V).

However, despite these observations, nothing was really known about fish ears until the first description of a fish ear by Giulio Cesare Casserius, who, in 1601, published *De vocis auditusq[ue] organis historia anatomica singulari*. This volume included the first description of otoliths. This was followed by the brilliant anatomical work of E. H. Weber (1820) who, in his book *De Aure et Auditu Hominis et Animalium. Pars I. De Aure Animalium Aquatilium*, described the Weberian ossicles, a series of bones in the otophysan fishes (goldfish, catfish, and relatives) that are involved with hearing, as well as other structures we now associate with hearing by fishes (**Figures 2(a)**, **2(b)**, and **3(a)**) (**see also Hearing and Lateral Line**: Auditory System Morphology and Psychoacoustics: What Fish Hear). This was followed in 1881 by a two-volume anatomical work by G. Retzius that presented a brilliant set of drawings of the ears of vertebrates from fish to man (see **Figure 3(b)** and figures in **Hearing and Lateral Line**: Auditory System Morphology and The Ear and Hearing in Sharks, Skates, and Rays).

However, it was not until the early 1900s when the great American physiologist G. H. Parker did a series of



Pliny the Elder on Hearing in Fish

THE
HISTORIE
OF THE WORLD.
Commonly called,
THE NATVRALL HISTORIE OF
C. PLINIVS SECVNDVS.
The first Tome.
LONDON,
Printed by Adam Islip.

•FISHES verily have no eares, ne yet any holes to serve for hearing: and yet plaine it is that they doe heare. Which we may daily see in certaine fish-ponds and stewes where fishes bee kept: for when those that have the charge of them make a noise with clapping of their hands: as wild as they bee otherwise, they shall have them come in great flockes to take their meat that is throwne into them: and this are they wont to doe daily........ Hereupon it is, that the Mullet, sea-Pike, Stockfish, and Chronius, are thought to heare best of all others, and therfore live very ebbe among the shelves and shallowes."

Figure 1 Quotation from Pliny the Elder on what may be the first written commentary on fish hearing and sound production. From a translation in 1601.

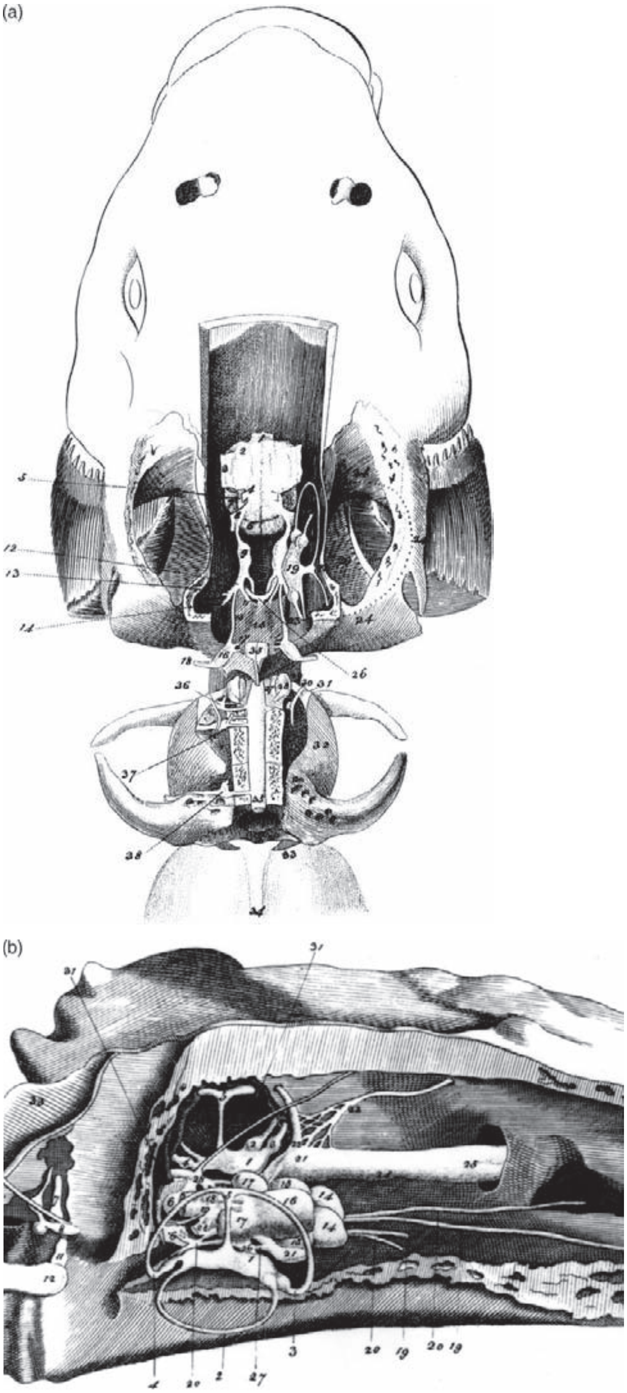


Figure 2 Figures drawn by Weber in 1820; one of the first anatomical descriptions of the ear and the very first description of bones now known as Weberian ossicles (**see also Hearing and Lateral Line:** Auditory System Morphology). (a). Dorsal view of the head of the carp (*Cyprinus carpio*) showing the cranium and auditory apparatus exposed and part of the brain removed. Major structures are: 4 – auditory nerve; 10, 11 saccular nerve; 19 – membranous labyrinth (inner ear, **see also Hearing and Lateral Line:** Auditory System Morphology); 20–23 parts of the vestibular system (**see also Hearing and Lateral Line:** Vestibular System Anatomy and Physiology); 27–33 – bones of what are now know as the Weberian ossicles; 34 – swimbladder. (b) Medial dissection of the head of a catfish (*Silurus glanis*), showing the brain and auditory apparatus. Major structures are: 1 – utricle; 2–5 – parts of the semicircular canals; 6 – saccule; 14 – olfactory lobe of the brain (**see also Hearing and Lateral Line:** Auditory/Lateral Line CNS – Anatomy); 15 – optic lobe; 16 and 17 – parts of the cerebellum. (a) From Plate IV, Fig. 23. (b) From Plate V, Figure 30.

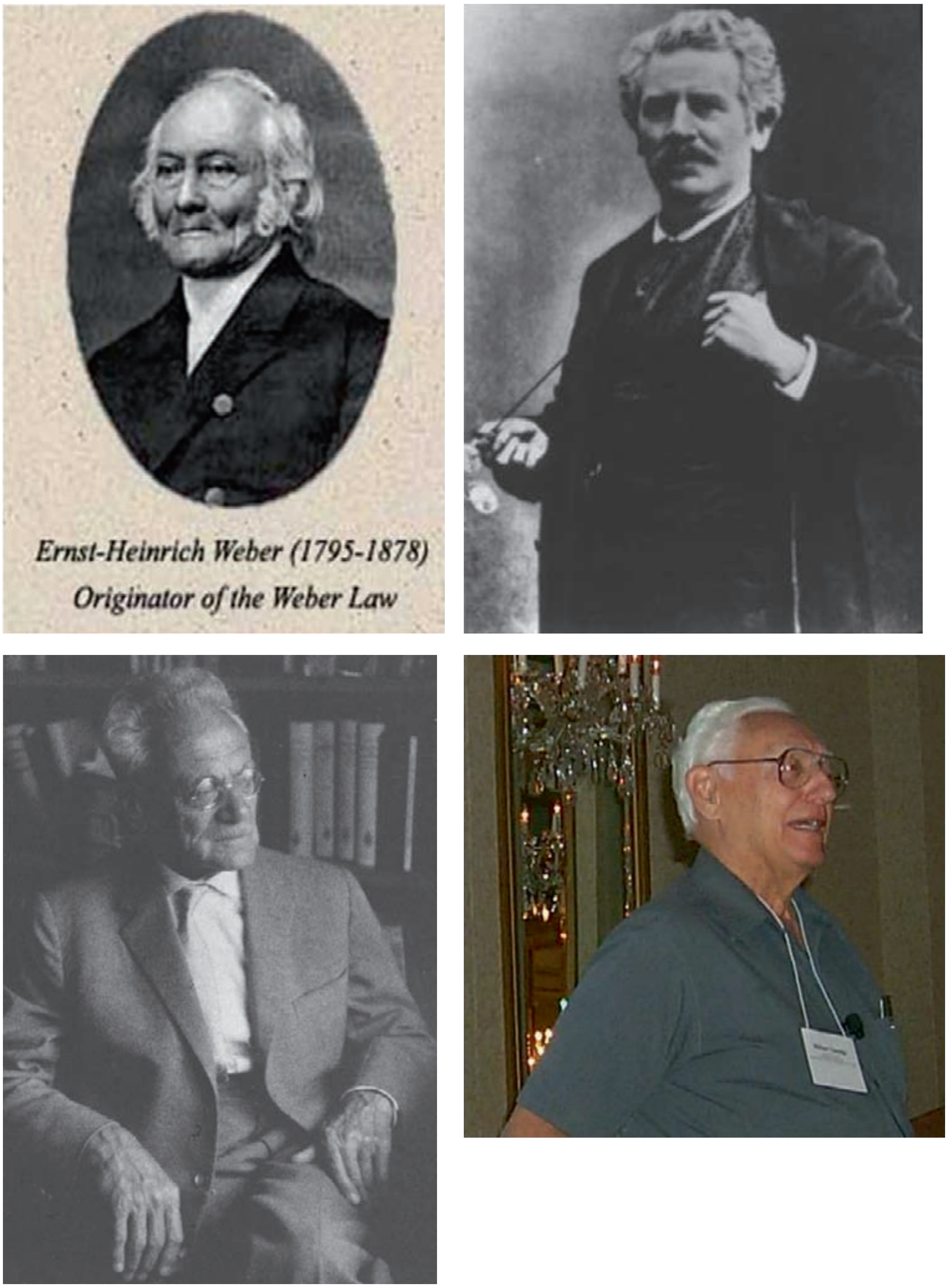


Figure 3 Pictures of some of the leading scholars in fish hearing and sound communication. (a) Ernst-Heinrich Weber (see **Figure 2**); (b) Magnus Gustav Retzius (**see also Hearing and Lateral Line:** Auditory System Morphology and The Ear and Hearing in Sharks, Skates, and Rays); (c) Karl von Frisch (see **Figure 4**); (d) William N. Tavolga, the modern pioneer in the field.

definitive experiments that showed that fishes could hear. This was followed by a series of wonderful studies by Karl von Frisch (who later went on to win the Nobel prize for his brilliant deciphering of the dance language of bees) and his students Stetter and Dijkgraaf, who not only further demonstrated that fishes could hear, but also that they could discriminate between sounds (**Figure 3**(**c**)). However, von Frisch and his students were not able to establish if fishes can tell the direction of a sound source (sound source localization), an area of intense research even today (**see also Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes).

While there was some work on fish hearing in the first half of the twentieth century, studies of fish bioacoustics first really took off with a conference organized in 1963 by William Tavolga (**Figure 3**(**d**)) and a definitive study that he did with Jerome Wodinsky that measured hearing

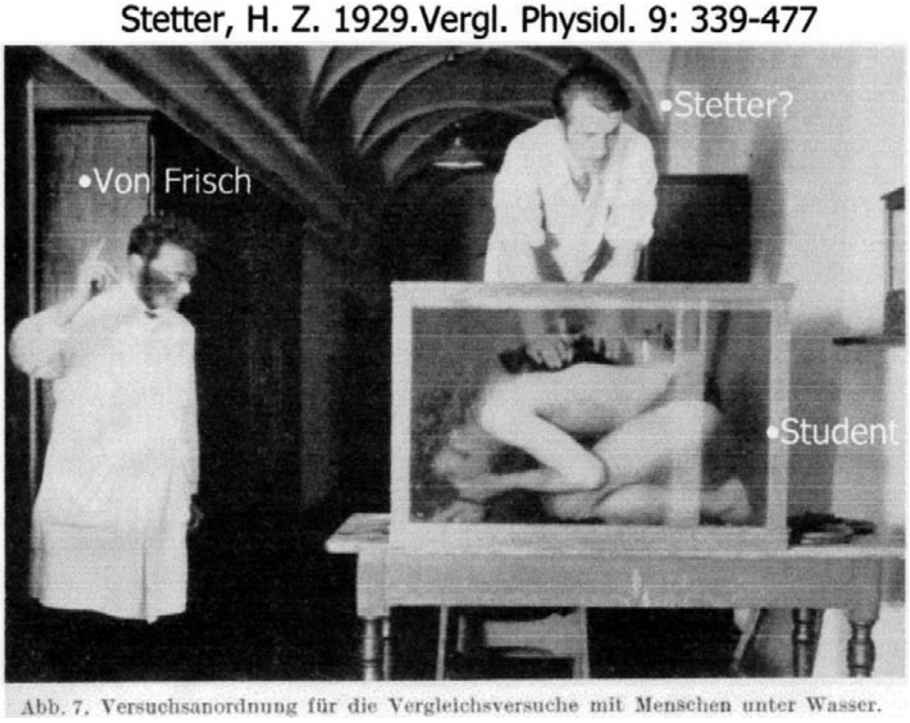

Stetter, H. Z. 1929.Vergl. Physiol. 9: 339-477
•Stetter?
•Von Frisch
•Student
Abb. 7. Versuchsanordnung für die Vergleichsversuche mit Menschen unter Wasser.

Figure 4 A figure from Stetter (1929) showing how experiments were done to measure hearing. A person (presumably a student or technician, but unknown) is underwater listening to the sound. On the left is the teacher Professor Karl von Frisch, and on the right is H. Z. Stetter

capabilities of nine species of marine fishes. Tavolga also provided a definitive history of the field in 1971 and this, combined with another history by James Moulton in 1963, provides a complete historical perspective on how the field grew until the mid-1900s.

Perspectives on Fish Bioacoustics

Bioacoustics is an encompassing term that covers hearing, sound production, and communication using sound. This section attempts to provide an overview of these topics (as well as the lateral line and vestibular systems) as they are associated with fishes. These topics certainly do not cover everything that is known about fish bioacoustics, but they are intended to serve as a primer for someone new to the field, while also offering a review and stepping-off point for the next generation of scientists.

The ear originally served to enable fishes to orient within a three-dimensional environment. This detection of movement is the function of the vestibular part of the inner ear and brain. It is a sense that is vital to all vertebrates as discussed by Baker and Straka in **Hearing and Lateral Line**: Vestibular System Anatomy and Physiology, but one about which most people are unaware until the sense does not function correctly.

The thrust of bioacoustics is involved with hearing and sound production, as well as the detection of water motion. Hearing involves the inner ear and, in some cases, ancillary structures such as the swimbladder. Detection of water motion is the function of the lateral line. Sound production involves a wide range of structures, including, in many species, the swimbladder. Before one can truly comprehend the importance of sound and the auditory system to the behavior and survival of fishes, it is first important to understand how fishes detect a signal and the types of signals to which they might be listening. The fish auditory system is not as well understood as the auditory system of terrestrial vertebrates, but it contains all of the necessary components for the detection of sound in an aquatic environment including anatomical structures that are very similar to those in terrestrial vertebrates (including mammals). Moreover, the auditory structures of fishes are capable of performing all the major functions of hearing that are found in more so-called advanced vertebrates.

The anatomy of the ear is described in detail for fishes by Popper in **Hearing and Lateral Line**: Auditory System Morphology and for sharks and rays (elasmobranchs) by Casper in **Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays, while the mechanisms of the ear that result in sound detection are described by Hastings in **Hearing and Lateral Line**: Biomechanics of the Inner Ear in Fishes. In addition to understanding the ear and its function, one must consider hearing capabilities of fishes because they reflect not only ear function, but also processing of sound by the brain. Thus, Fay, in **Hearing and Lateral Line**: Psychoacoustics: What Fish Hear, discusses hearing capabilities of fishes, whereas how the brain processes sounds is considered from the standpoint of brain anatomy by McCormick in **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy and Physiology by Lu in **Hearing and Lateral Line**: Physiology of the Ear and Brain: How Fish Hear.

It is also important to understand what types of sounds fishes are listening to. The auditory soundscape makes up the sum total of what fishes encounter in their aquatic environments and is discussed by Fay in **Hearing and Lateral Line**: Psychoacoustics: What Fish Hear. It is also important to understand the physical properties of how sound travels within water and the adaptations fishes utilize to accommodate for these factors as discussed by Hastings in **Hearing and Lateral Line**: Biomechanics of the Inner Ear in Fishes. Further associated with these physical properties of sound is the ability, and difficulties, of fishes to localize the source of a sound (estimate the direction of the sound), and this is discussed by Zeddies, Fay, and Sisneros in **Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes.

One of the critical issues today in fish bioacoustics is not just what and how fishes hear, but how the sounds produced by humans in the waters may affect the way fishes hear and use sound. This question is of growing national and international concern, and it is discussed by Hawkins in **Hearing and Lateral Line**: Effects of Human-Generated Sound on Fish.

The other part of bioacoustics is the production of sound. Humans have known for thousands of years that fishes produce sounds (**Figure 1**), but we are only just realizing how many species accomplish this. Luczkovich, Sprague, and Krahforst in **Hearing and Lateral Line**: Acoustic Behavior, as well as Kaatz in **Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling, provide an overview of the sounds fishes produce and the behavioral contexts in which the sounds are used. Sound production by fishes involves mechanisms very different from those in terrestrial animals because there is generally no release of air by fishes, whereas sound production in terrestrial animals generally involves the release of air over some kind of structure, such as the mammalian larynx. The mechanisms used by fishes are discussed by Ladich and Bass in **Hearing and Lateral Line**: Vocal Behavior of Fishes: Anatomy and Physiology.

As mentioned several times in this article, the lateral line is a very early evolved vertebrate system that uses sensory hair cells for the detection of signals. These hair cells are very similar to those of the ear in fishes and terrestrial vertebrates. The function of the lateral line was not really understood until the last half of the twentieth century, and we now know that it shares a place with the auditory system in the overall mechanosensory system in fishes and represents a unique sensory receptor that is only found in aquatic organisms. The lateral line provides for what Dijkgraff called ‘distance touch’, and enables fishes to sense their immediate surroundings as well as other organisms that might be within their vicinity. (The detection of water motion by the lateral line is called Svenning, and was named by Platt, Fay, and Popper in honor of Professor Sven Dijkgaaf.) The role of the lateral line in fish behavior is discussed by Montgomery and Coombs in **Hearing and Lateral Line**: Lateral Line Neuroethology, while Webb, in **Hearing and Lateral Line**: Lateral Line Structure discusses how the basic organization of the lateral line system is similar among most fishes.

Conclusions

The history of our understanding of the role that sound plays in the lives of fishes is a long one, stretching back at least to 350 BCE. However, the first formal scientific recognition of how fishes might hear was not until 1601, and then it was not until around 1902 that it was first demonstrated experimentally that fishes hear and that they use their ears for sound detection. Subsequently, the twentieth century saw a substantial increase in interest in bioacoustics and contributions from a wide range of investigators who asked, and continue to ask, questions about communication, sound production, hearing, the lateral line, and the vestibular sense. The articles referred to here provide a broad overview of our current knowledge as well as insights into the most important scientific questions on fish bioacoustics for the next decades.

See also: **Hearing and Lateral Line**: Acoustic Behavior; Auditory/Lateral Line CNS: Anatomy; Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Effects of Human-Generated Sound on Fish; Lateral Line Neuroethology; Lateral Line Structure; Physiology of the Ear and Brain: How Fish Hear; Psychoacoustics: What Fish Hear; Sound Source Localization and Directional Hearing in Fishes; The Ear and Hearing in Sharks, Skates, and Rays; Vestibular System Anatomy and Physiology; Vocal Behavior of Fishes: Anatomy and Physiology. **Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling.

Further Reading

Moulton JM (1963) Acoustic behaviour of fishes. In: Busnel R-G (ed.) *Acoustic Behaviour of Animals*, pp. 655–693. Amsterdam: Elsevier.

Parker GH (1902) Hearing and allied senses in fishes. *Bulletin of the United States Fish Commission* 22: 45–64.
Popper AN and Dooling RJ (2004) Animal bioacoustics. In: Bass HE and Cavanaugh WJ (eds.) *ASA at 75*, pp. 52–62. Melville, NY: Acoustical Society of America.
Retzius G (1881) *Das Gehörorgan der Wirbelthiere.* Vol I. Stockholm: Samson and Wallin.
Riva A, Orrù B, Pirino A, and Riva FT (2001) Iulius Casserius (1552–1616): The self-made anatomist of padua's golden age. *The Anatomical Record (The New Anatomist)* 265: 168–175.
Stetter H (1929) Untersuchungen über den Gehörsinn der Fische, besonders von *Phoxinus laevis* L. und *Amiurus nebulosus* Raf. *Zeitschrift Fuer Vergleichende Physiologie* 9: 339–477.
Tavolga WN and Wodinsky J (1963) Auditory capacities in fishes. Pure tone thresholds in nine species of marine teleosts. *Bulletin of the American Museum of Natural History* 126: 177–240.
Tavolga WN (1964) *Marine Bio-Acoustics*. Oxford: Pergamon Press.
Tavolga WN (1971) Sound production and detection. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol V. pp. 135–205. New York: Academic Press.
van Bergeijk WA (1967) The evolution of vertebrate hearing. In: Neff WD (ed.) *Contributions to Sensory Physiology*, pp. 1–49. New York: Academic Press.
von Frisch K (1923) Ein Zwergwels der kommt, wenn man ihm pfeift. *Biologisches Zentralblatt (Leipzig)* 43: 439–446.
Walton I (1653. *The Complete Angler.* facsimile edition, http://www.gutenberg.org/files/9198/9198-h/9198-h.htm (accessed June 2010)
Webb JF, Fay RR, and Popper AN (eds.) (2008) *Fish Bioacoustics*. New York: Springer Science + Business Media, LLC.
Weber EH (1820) De Aure et Auditu Hominis et Animalium. Pars I. De Aure Animalium Aquatilium. Gerhard Fleischer, Leipzig. 134 pp. http://books.google.com/books?id=jvc6AAAAcAAJ&printsec=frontcover&dq=De+Aure+et+Auditu+Hominis+et+Animalium&cd=2#v=onepage&q=&f=false (accessed June 2010).

Relevant Websites

http://homepage.univie.ac.at – Acoustic Communication in Fishes: The Ladich Lab.
http://www.dosits.org – Discovery of Sound in the Sea.
http://www.greektexts.com – History of Animals.
http://www.popperlab.umd.edu – Laboratory of Bioacoustics.
http://www.gutenberg.org – Project Gutenberg; The Project Gutenberg EBook of The Compleat Angler, by Izaak Walton.
http://www.bio.miami.edu – Zhongmin (John) Lu Associate Professor.

Vestibular System Anatomy and Physiology

H Straka, Ludwig-Maximilians-Universität, Martinsried-Planegg, Germany

R Baker, New York University Medical Center, New York, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Organization of the Vestibular Sensory Periphery
Central Location of Vestibular-Related Octavolateral Nuclei
Origin of the Octavolateral Vestibular Subgroups
Central Terminations of Semicircular Canal and Utricular Afferents
Hindbrain Vestibuloocular Origins and Projections to Extraocular Motor Nuclei
Horizontal Vestibuloocular Subgroups
Vestibulospinal Projections for Locomotor Control and Posture Stabilization
Auxiliary Vestibular Connections for Gaze and Posture Control
Conservation of Fish Vestibular Organization in Other Vertebrates
Further Reading

Glossary

Egocentric direction Navigation in space with a self-centered reference frame.

Gaze stabilization Maintenance of stable eye and/or head position in respect to the environment.

Gravitoinertial function Detection of static changes in head/body position relative to the Earth's gravitation vector.

Labyrinth A peripheral sensory compartment within the inner ear that includes semicircular canal and otolith end organs.

Lagena An otolith end organ specialized, together with the sacculus, for detecting acoustic stimuli.

Octavolateral nuclei Assembly of functional subgroups in the hindbrain that process sensory signals related to detection of water (lateral line) or object motion (electroreceptive), acoustic stimuli (hearing), or body motion (vestibular).

Otolith Solid calcium carbonate crystal in the saccule, lagena, and utricle, which are involved in the detection of vestibular and auditory stimuli.

Otolith end organs Separate structures that contain sensory epithelia overlain by solid calcium carbonate crystals for detection of vestibular and auditory signals.

Placode Region in the embryonic epithelial layer which gives rise to sensory organs and other structures.

Sacculus An otolith end organ specialized, together with the lagena, for detecting acoustic stimuli.

Semicircular canals Three interconnected membranous ducts within the inner ear that detect angular rotations during turning.

Utricle An otolith end organ specialized for detecting both static and dynamic head motions.

Vestibuloocular reflex Compensatory eye movements in response to vestibular stimulation that minimizes retinal slip to facilitate visual processing.

Introduction

Vestibular neurons and circuits process information related to self-motion (i.e., head/body) in all vertebrates, including fish. The vestibular system is one component of a multisensory brainstem network because other sensory organs also detect self-motion (e.g., eyes and lateral line). Importantly, the most prominent signals triggering the motor behaviors essential for both escape and predation originate from sensory end organs in the inner ear.

All fish use the vestibular system to transduce and then process information about their body position and motion in three-dimensional (3D) space, irrespective of either active or passive body movement arising from underwater currents. For a fish to maintain a particular posture in water, all motion-related sensory signals have to be transformed within the central vestibular networks into motor commands appropriate for controlling the eyes, fins, body, and tail. The coordinated temporal and spatial activation of multiple and widely distributed appendages stabilize whole body position both at rest and during active swimming.

As expected, all vertebrates are confronted with the effects of individual locomotor actions on their ability to perceive the surrounding environment. They must distinguish self from world/object motion. Self-generated as well as passive body motion cause retinal image displacements, with a resultant degradation in the processing of visual information. To maintain visual acuity, retinal image drift must be counteracted by dynamic (rapid) compensatory adjustments in eye position, largely provided by vestibuloocular reflexes (VORs). In essence, a fish must have precise knowledge about its body motion to maintain retinal constancy of objects in the environment by distinguishing whether the objects or their retinal locations are either stationary or moving. Achieving stable gaze with the VOR illustrates perfectly how the vestibular system functions to enable continuous accurate perception of objects moving in the external world with respect to the self. Thus, the fact that the eyes are held still with respect to the environment under all conditions of body motion, especially independent of object motion, evolved many hundreds of millions of years ago by innovations in the hindbrain developing in parallel with cerebellar circuitry.

Evolution also further utilized the vestibular system to compute the perceptual attribute commonly referred to as egocentric direction. Viewed from the context of motor control, a robust egocentric reference frame must exist in the hindbrain and cerebellar circuitry for an animal to choose an appropriate locomotor trajectory during swimming. Thus, vestibular signals that originate from activation of the equilibrium organs in the inner ear during active and passive body motion play a crucial role in the ability to precisely perceive the environment and maintain appropriate body position during locomotion. Perhaps the most significant vestibular attribute, therefore, is distinguishing self-motion from that of the world. Without doubt, vestibular reflexes are essential for survival independent of the ecological niche, life style, and locomotor pattern of a particular species of fish. They allow a fish to distinguish self and self-motion from object motion in the world.

Organization of the Vestibular Sensory Periphery

Sensory signals related to body position and motion are processed and transformed into respective motor commands in an assembly of hindbrain neuronal subgroups, collectively described in the literature as the octavolateral nuclei (**see also Hearing and Lateral Line:** Auditory/Lateral Line CNS: Anatomy). Functionally, these heterogeneous subgroups of neurons process four different sensory modalities: lateral line for detection of either water motion (mechanoreceptive) (**see also Hearing and Lateral Line:** Lateral Line Structure and Lateral Line Neuroethology) or objects (electroreceptive) (**see also Detection and Generation of Electric Signals:** Detection and Generation of Electric Signals in Fishes: An Introduction), auditory for acoustic stimuli (**see also Hearing and Lateral Line:** Auditory System Morphology, The Ear and Hearing in Sharks, Skates, and Rays, and Psychoacoustics: What Fish Hear), and vestibular for body motion. The peripherally located epithelial end organs for these sensory modalities appear during early development from a common embryological region called the 'placode' (**Figure 1**). Individual placodal regions give rise either to lateral line/electroreceptive sensory structures on the body surface (AD, AV, M, P, and ST in **Figure 1**) or to inner ear vestibular/acoustic organs (OT in **Figure 1**). The sensory transduction mechanism in all cases is largely based on the same ancestral hair cell type (**see also Hearing and Lateral Line:** Auditory System Morphology). This ubiquitous mechanoreceptor-type cell is used to detect and convert respective physical forces into neuronal activity, clearly distinguishing between electro- and mechano-sensory stimuli.

Peripheral vestibular end organs of most fish consist of three separate semicircular canals attached to a single sac-like structure, the utriculus (fishes; **Figure 2**) (**see also Hearing and Lateral Line:** Auditory System Morphology). Two additional sac-like structures, the sacculus and the lagena, are largely utilized for hearing in nearly all fish species (**see also Hearing and Lateral Line:** The Ear and Hearing in Sharks, Skates, and Rays, Psychoacoustics: What Fish Hear, Physiology of the Ear and Brain: How Fish Hear, and Sound Source Localization and Directional Hearing in Fishes). Three semicircular canals are oriented at right angles from each other comprising three different planes, which likely offer the optimum physical arrangement for sensing 3D motion in a neutrally buoyant environment. Nevertheless, only one (hagfish; **Figure 2**) or two vertical canals (lampreys; **Figure 2**) were present in the most recent jawless fish ancestors; hence, the addition of a horizontal canal in

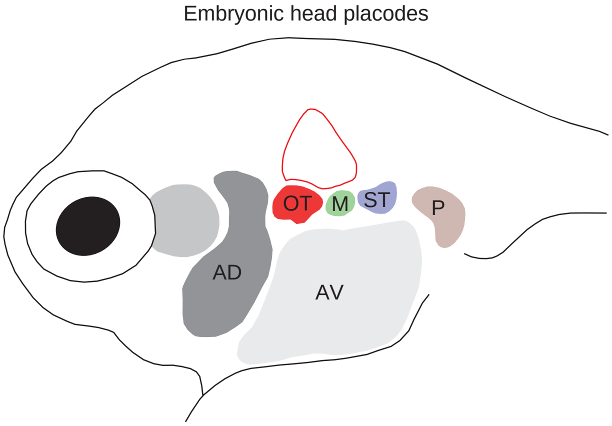


Figure 1 Ontogenetic origin of mechanoreceptive sensory organs (lateral line, electroreception, inner ear vestibular, and auditory sensation). Schematic side view of a fish embryo depicting common placodal origins. AD, anterodorsal placode; AV, anteroventral placode; M, middle placode; OT, otic placode; P, posterior placode; ST, supratemporal placode.

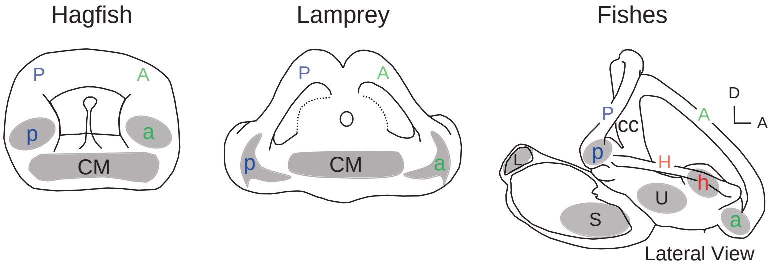


Figure 2 Phylogeny of vestibular labyrinths. A single semicircular canal with two patches (a, p) of sensory epithelia for sensing both anterior (A) and posterior (P) motion (hagfish). Separate anterior (A) and posterior (P) semicircular canal with specialized (a, p) sensory epithelia are found in jawless fish (lamprey). A common central macular organ (CM) is present for graviception in both species. In elasmobranchs and bony fishes, two vertical (A, P) and one horizontal (H) semicircular canal are oriented perpendicular to each other for detection of 3D angular acceleration by cupular deflections (a, p, h). Three separate macula organs (U, L, and S) serve as graviceptive (utricle) and pressure-detecting sensors (lagena, saccule).

elasmobranchs and bony fish arose as a new invention during fish evolution. This newly acquired mirror-image arrangement of the sensory periphery (fishes; **Figure 2**) is a major structural design of the vestibular system that allows the semicircular canals and the utricle to act reciprocally during head motion.

The semicircular canals, as well as the otolith organs (utricle, sacculus, and lagena), are filled with endolymph, a potassium-rich fluid that plays an essential role in the sensory signal transduction process. Each canal has an expansion known as an 'ampulla' containing a crista, covered by a gelatinous cupula, which is deformed by fluid movements during head/body motion. The canals detect turning movements of the head/body about their respective axis (**Figure 2**), thus acting as force transducers of angular acceleration. The utricle contains a single calcium carbonate stone, called an 'otolith', overlying a fixed macula of hair cells and this organization is highly effective for transducing linear acceleration. The presence of single otolithic masses in teleosts is the reason for initially referring to these sensory structures as otolith organs (**see also Hearing and Lateral Line:** Auditory System Morphology). The major role of the utricle in fish relates to sensing inertia through detection of static changes in head/body position relative to the Earth's gravitation vector, thereby creating an internal frame of reference for establishing egocentric direction responsible for orientation and locomotor behaviors. These major utricular roles observed in fish fit well with an earlier evolutionary appearance of this end organ than that of the semicircular canals, which require a critical spatial dimension to allow fluid movements for the detection of head motion. For this reason, it seems inappropriate to call the peripheral vestibular end organs simply the inner ear (**see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes).

Peripheral hair cells in both the lateral line/electrosensory neuromasts on the body surface as well as the specialized vestibular/auditory end organs in the inner ear are chemically linked to first-order afferent nerve fibers that forward the sensory signals to distinct nuclear regions in the dorsal hindbrain. Vestibular afferent fibers that innervate the different semicircular canal organs and utricle are segregated from all other VIIIth nerve components. In addition, vestibular afferents project exclusively to the most lateral octavolateral subgroups in the hindbrain. Based on an evolutionarily conserved neuronal projection to brainstem and spinal regions, largely responsible for the control of balance and locomotion, the lateral portions of the octavolateral nuclei can be distinguished from the more medial and dorsal nuclei that process acoustic and lateral line sensory signals (**Figure 3**).

Central Location of Vestibular-Related Octavolateral Nuclei

The vestibular nuclei occupy a considerable part of the dorso-lateral hindbrain between the Vth and Xth cranial nerves (**Figure 3**) (**see also Hearing and Lateral Line:** Auditory/Lateral Line CNS: Anatomy). Comparison of morphological features in several species of fish generally identifies five major subdivisions of second-order neurons in the vestibular nuclei. Moreover, distinct functional groups, identified according to their neuronal signals, are associated with a particular behavior (e.g., eye or body motion) and correlate reasonably well with the anatomical map. The similarity of structure and function of the major vestibular cell groups that control eye, fin, body, and tail motion across different fish species, therefore, allows vestibuloocular, vestibulospinal, vestibulocommissural, and vestibulocerebellar neurons to be identified as distinct populations. By and large, the current delineation of vestibular subgroups is based on: (1) regionally restricted terminations of first-order afferent fibers from the different peripheral vestibular end organs; (2) anatomical tract tracing from efferent projection areas in the midbrain, hindbrain, and spinal cord; and (3) electrophysiological

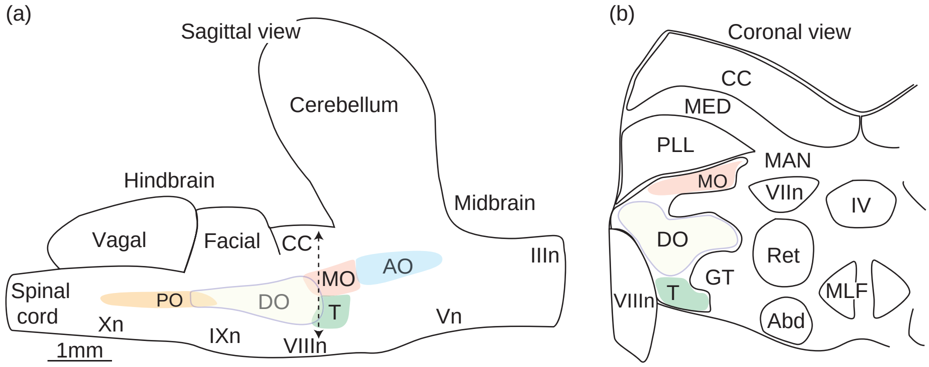


Figure 3 Spatial arrangement of goldfish octavolateral vestibular nuclei. Schematic sagittal (a) and coronal (b) diagrams depicting the location of brainstem nuclei. Arrow with dashed line in the sagittal view indicates the level of the coronal section. Abd, abducens motor nucleus; AO, DO, MO, PO, anterior, descending, medial, posterior octavolateral nuclei; CC, crus cerebelli; GT, gustatory tract; IIIn, VIIn, VIIIn, IXn, Xn, and Vn, oculomotor, facial, vestibular, glossopharyngeal, vagal, trigeminal nerves; MAN, medial auditory nucleus; MLF, medial longitudinal fasciculus; MED, medial nucleus; PLL, posterior lateral line nucleus; Ret, reticular formation; T, tangential nucleus.

correlates of second-order neurons during different behavioral paradigms. The most comprehensive topographical mapping and electrophysiological studies have been carried out in goldfish (*Carassius auratus*), and this species is used here as an example to summarize the overall organization of the vestibular subgroups within the octavolateral nuclei.

Origin of the Octavolateral Vestibular Subgroups

All vestibular subgroups take their origin from a hindbrain segmental framework, which begins in early development as eight well-defined neuroepithelial compartments. The early embryonic hindbrain patterning serves as a mechanistic guide that directs the development of particular vestibular functional phenotypes involved in stabilization of retinal images and body posture. Combinatorial expression of spatially restricted regulatory genes plays a dominant patterning role in the development of any neuron, vestibuloocular, or vestibulospinal, in any of the eight hindbrain compartments. This genetically specified compartmental blueprint is conserved between all species of fish, as well as for subsequent terrestrial vertebrates with only minor modifications.

Vestibular neuronal subgroups evolved long before modern fishes in the earliest and now-extinct groups of jawless vertebrates. Interestingly, in living lampreys, the two vertically oriented semicircular canals, anterior and posterior (**Figure 2**), contribute equally to detection of the horizontal components of angular head acceleration. Hence, there are two well-delineated horizontal vestibular subgroups in the hindbrain. However, a third (horizontal) canal was added to the ontogenetic blueprint in modern sharks and fishes (**Figure 2**) such that the two separate horizontal vestibular subgroups receive a common afferent input from one canal rather than a separate signal from the original anterior and posterior canal design. Notably, this structural design of two separate horizontal vestibular subgroups, unique to central horizontal eye movement circuitry, has been conserved ever since it evolved in jawless fishes to present-day mammals.

Ever since the earliest jawless vertebrates, the vestibular subgroups appear to align with the peripheral location of the anterior and posterior semicircular canals surrounding a central otolith. However, such a stereotyped arrangement is more apparent than real because first-order vestibular afferents exhibit the capability to contact neurons in any of the vestibular subnuclei. Thus, it is likely that the vestibular system in jawless and jawed fishes was quickly converted from a point-to-point, hardwired canal/otolith projection to a highly integrated network in which the appropriate vestibular signal could be utilized independent of the canal of origin but more dependent on the functional role of the targeted motor circuit.

The primitive vestibular system largely relayed purely sensory signals to subsets of neurons that effectively could both generate and relay online signals to motor nuclei. However, in most cases, the vestibular signal can be equally correlated with the sensory stimulus (head motion) or the motor response (eye motion). It turns out that, quite early in evolution, fishes had converted the sensory-related neural activity in many of their vestibular subgroups into motor coordinates (e.g., as seen in goldfish second-order neurons) that exhibit all of the horizontal eye movement signals for visuo-vestibular-induced eye movements. From a systems control point of view, the vestibular subgroups are much more than simple sensory relay neurons; they also represent the motor command for appropriate contractions of eye, fin, body, and tail muscles.

Central Terminations of Semicircular Canal and Utricular Afferents

The distribution of first-order afferent fibers from individual canals and the utricle allows individual vestibular nuclei to be recognized with respect to the direction of head/body motion. Anterior and posterior canal afferents terminate predominantly, though not exclusively, in the anterior octavolateral nucleus (AO) and the tangential nucleus (T) (**Figure 3**). Afferent projections from these two canals are less dense in the descending octavolateral nucleus (DO) and the posterior octavolateral nucleus (PO) (**Figure 3**). In contrast, the horizontal canal afferent termination coincides with one set of smaller neurons in the magnocellular octavolateral nucleus (MO), as well as more uniformly in another subset located throughout DO and T (**Figure 3**). First-order utricular afferents terminate predominantly in MO, T, and DO, thus overlapping to a considerable extent with those from the horizontal canal. Significant for all sensorimotor behaviors in fish, there are virtually no vestibular nuclei that receive exclusive afferent projections from a single peripheral end organ. Rather, first-order afferents overlap more or less completely, even though hot spots of terminations of fibers from particular vestibular organs exist in some nuclei. This widespread vestibular afferent pattern thus differs from topological maps of other sensory systems such as the closely related visual system. The absence of a sensory point-to-point map is consistent with the finding that signal processing in central vestibular nuclei is spatially organized more according to efferent (largely motor) targets rather than topological hindbrain location. Observations on the distribution of afferent inputs are, in turn, quite compatible with the demonstrated roles of the vestibular subgroups in sensorimotor transformations contributing to the control of gaze (eye/head) and posture.

Hindbrain Vestibuloocular Origins and Projections to Extraocular Motor Nuclei

Perhaps the dominant structural attribute of the vestibular system is the spatially precise projection of vestibular subgroups to extraocular motoneurons located in the oculomotor, trochlear, and abducens nuclei. These three-neuronal VOR pathways consisting of first- and second-order vestibular and extraocular motoneurons are responsible for compensatory, 3D eye movements in all species of fish. Continuous, unblurred vision during high frequency and large amplitudes of head motion would not be possible without the vestibular nuclei.

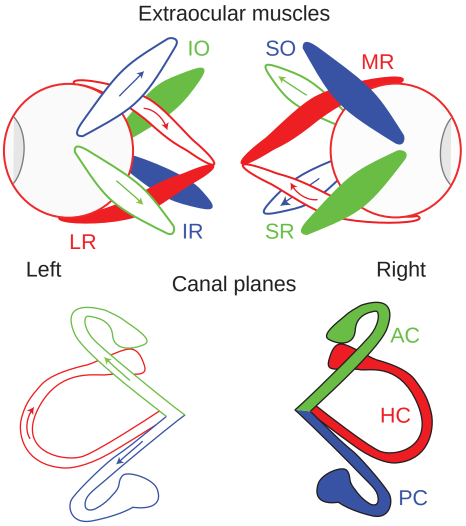


Figure 4 Extraocular muscles and pulling directions (view from dorsal) are illustrated with respect to semicircular canal orientation. Horizontal (HC, red), anterior (AC, green), and posterior vertical canal (PC, blue) are aligned with matching pairs of synergistic ipsi- and contralateral eye muscles (see matching color codes). LR, MR, lateral, medial rectus; IO, SO, inferior, superior rectus; IR, SR, inferior, superior rectus.

Compensatory eye movements are produced in fish, as in all other vertebrates, by a set of six eye muscles that insert on each eye bulb, such that any horizontal, vertical, or torsional movement can be performed (**Figure 4**). A major organizational principle of the angular VOR is the approximate alignment of the axes of a particular semicircular canal and the pulling directions of synergistic sets of two extraocular muscles (**Figure 4**). This arrangement illustrates the intricate link that has evolved between sensory and extraocular reference frames that in many species nearly perfectly stabilizes vision in response to eye/head body motion. The vestibular neuronal subgroups critical for sensorimotor transformation of angular as well as linear acceleration signals are located laterally within the borders of AO and DO/PO (**Figure 3**). In general terms, the fundamental rule underlying any vestibuloocular connection is matching the spatial vector orientations for body movements around the roll and pitch axes with the extraocular motoneurons producing vertical and torsional eye movements. Afferent input from the two vertical canals comprises the major input for vestibular subgroups activating extraocular muscles (SR, IR, SO, and IO) with corresponding spatially aligned pulling directions (**Figure 4**).

Second-order vestibular neurons form behaviorally different and spatially nonoverlapping subgroups within the vestibular portion of the octavolateral nuclei. The functionally distinguishable oculomotor/trochlear-projecting vestibuloocular neurons originate mainly from a rostral (AO) and to a minor extent from a caudal vestibular area (DO/PO). Each respective subgroup of vestibuloocular neurons receives afferent labyrinthine input from either the anterior or posterior vertical semicircular canal compatible with the spatial specificity of their target vertical and oblique extraocular motoneurons (SO, IO, SR, and IR). An additional organizational principle of the vestibulomotor projections is a push–pull arrangement that allows excitation of particular sets of eye muscles and simultaneous inhibition of the respective sets of antagonists. Accordingly, vestibuloocular projections consist of inhibitory neurons, whose axons remain ipsilateral, and excitatory neurons, whose axons cross the midline to match agonist/antagonistic extraocular motor targets. Axons from the excitatory and inhibitory vestibuloocular subgroups reach their respective subdivisions in the oculomotor/trochlear nuclei by pathways in the ipsi- or contralateral medial longitudinal fasciculus (MLF) (**Figure 3**).

Horizontal Vestibuloocular Subgroups

Horizontal eye movements (upper panel in **Figure 5**) are particularly important for scanning the visual horizon and both eyes move conjointly producing conjugate movements that together encompass a much larger oculomotor range. However, conjugacy of eye movements in fish is much more apparent than real because a fovea is absent and the fusion of targets between the two eyes (stereoscopic vision) is

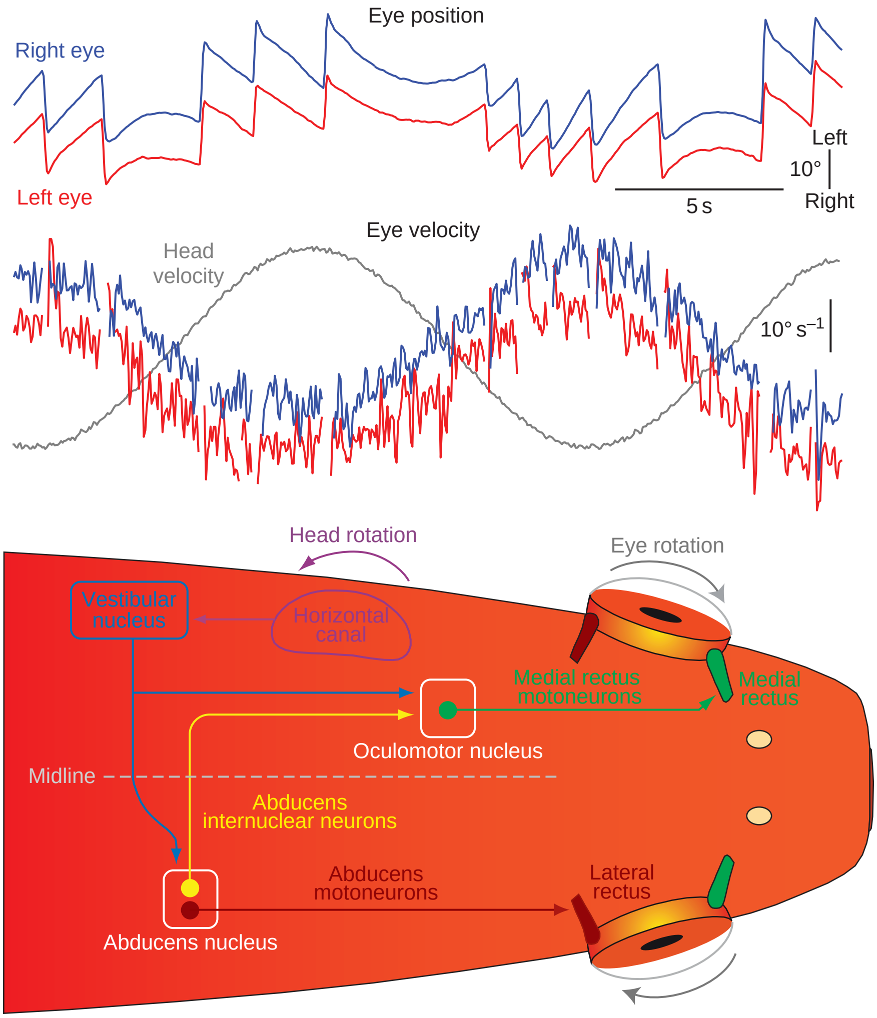


Figure 5 Brainstem circuitry and compensatory conjugate horizontal angular vestibuloocular reflexes. Both right (blue) and left (red) eye motion are equal and opposite to horizontal head rotation as illustrated best by the eye vs. head velocity traces. A simplified view of the brainstem vestibuloocular pathways is shown in the lower schematic.

limited, if present at all. The classical push–pull organization present for vertical eye movements with a nearly mirror-symmetrical, crossed excitation, and an uncrossed inhibition is only established for abducens motoneurons that innervate the ipsilateral lateral rectus (LR) muscle and internuclear neurons that innervate the contralateral medial rectus (MR; **Figure 5**). There is no direct uncrossed vestibular inhibition of MR motoneurons because internuclear neurons receive the same vestibular inhibitory input as LR motoneurons. This intrinsic difference in organization likely facilitates an independent bilateral activation of MR motoneurons for converging eye movements without activation of the LR motoneurons and vice versa for convergence and divergence eye movements.

The principal vestibular subgroups implicated in the horizontal VOR conveying a dynamic head/eye velocity signal are located in the ventral part of DO with projections to four separate, ventrally located, subgroups of abducens (Abd) motoneurons and internuclear (AI) neurons (simplified view with single subgroups in **Figure 5**). A subgroup of horizontal head velocity neurons is located in the rostral MO that project exclusively to ipsilateral MR motoneurons. While the head velocity signal on AI and MO subpopulations might be considered as redundant, it likely is not, because the MO neurons subserve an essential high-frequency vestibular role not provided by AI neurons. In addition, this vestibular subgroup is maintained in mammals, giving rise to a well-delineated pathway (ascending tract of Deiter's) to the oculomotor nucleus. The highly conserved locations of horizontal canal-related, second-order vestibuloocular neurons within the vertebrate hindbrain clearly indicate an organizational principle centered on motor coordinates rather than topography of spatially segregated inputs from the sensory periphery.

Both vertical and horizontal canal-related vestibuloocular neurons are complemented by a densely clustered subgroup that forms the T nucleus just caudal to the VIIIth nerve entry (**Figure 3**). The functional role of this nucleus is clearly demonstrated by the close relationship between torsional angle of the eye and head tilt that appears even in larval fish before semicircular canals become operational. This nucleus serves as a gravitoinertial center with crossed excitatory projections to the midbrain oculomotor/trochlear nuclei and the contralateral spinal cord. Second-order vestibular neurons in the tangential nucleus receive major inputs from the utricle as well as minor inputs from the three canals in a spatially specific pattern. In contrast to vestibular neurons in the semicircular canal-specific nuclei, tangential neurons exhibit a sustained sensitivity after small tonic changes of the head position, clearly indicating an appreciable tonic utricular influence. The likely convergence of spatially specific afferent canal inputs with sensory signals from utricular sectors with the same spatial vector orientation in individual tangential neurons forms a structural substrate that makes this nucleus a gravitoinertial relay center for vestibuloocular as well as postural reflexes.

Vestibulospinal Projections for Locomotor Control and Posture Stabilization

Vestibulospinal projections control gravitoinertial-related postural reflexes through the activation of spinal interneurons and motoneurons. Vestibular signals to spinal targets exhibit either low dynamics that are more correlated with changes in body posture, or high dynamics that are involved in the initiation of fast locomotor actions. Vestibulospinal neurons are generally well localized within a restricted region that coincides with a central position along the rostro-caudal extent of the vestibular areas in the octavolateral nuclei. This central location within the vestibular column is compatible with an early evolutionary appearance of gravitoinertial function utilizing utricular signals for compensating pitch and roll deviations of body position in space. The populations of ipsi- and contralaterally descending vestibulospinal neurons largely located in MO and the dorsal part of DO are morphologically heterogeneous and convey otolith as well as semicircular canal signals to the spinal cord. A number of vestibulospinal neurons are also located in the T nucleus intermingled with those that project to the contralateral oculomotor/trochlear nuclei, suggesting that this nucleus serves as a gravitoinertial relay center coupling eye motion and body posture control.

Auxiliary Vestibular Connections for Gaze and Posture Control

Commissural projections through midline crossing axons interconnect bilateral vestibular nuclei. Functionally, the spatially specific inhibitory brainstem commissural projections that interconnect vestibular neurons with bilateral coplanar canal-related signals (e.g., ipsilateral posterior–contralateral anterior; see **Figure 4**) reinforce the differential detection of angular head acceleration signals due to the mirror-image arrangement of the semicircular canals in the periphery on both sides. This connection increases the sensitivity for detection of angular head acceleration, such that very small signals can be distinguished. A somewhat similar organization exists for utricular commissural connections that link second-order vestibular neurons with signals from spatially aligned bilateral utricular epithelial sectors. However, the specific functional interconnections are more complex given the 360° response sensitivity of the sensory epithelium on each side. Vestibular commissural neurons that interconnect the two sides essentially subdivide into rostral and caudal subgroups in the AO and DO/PO, respectively. Given the dense projection and termination of anterior and posterior semicircular canal afferent fibers in AO, DO, and PO regions where commissural neurons are located, these latter cell groups likely establish a push–pull connectivity to increase the sensitivity for detection of vertical coplanar semicircular canal signals.

Vestibular projections to the vestibulolateral lobe of the cerebellum in fish play an important role in the adaptation and plasticity of gaze-stabilizing reflexes. These connections are evolutionarily very old and are based on an intimate phylogenetic linkage between the octavolateral nuclei and cerebellum. Both, first- and second-order vestibular projections, convey angular head/body acceleration as mossy fiber input to the granular layer of the vestibulocerebellum. In turn, Purkinje cells from the caudal lobe of the vestibulocerebellum provide a direct output to particular vestibuloocular subgroups. The major locations for vestibulocerebellar projection neurons are a rostral subgroup with ipsilateral projection predominance in AO and a second one in DO/PO. The caudal vestibular subpopulation is contiguous with another population of cerebellar-projecting neurons located ventrolaterally in the caudal hindbrain. Together, along with cerebellar-projecting inferior olivary neurons, these populations appear to have a common ontogenetic origin, such that all precerebellar nuclei responsible for eye/head motion derive from a common caudal hindbrain region.

Conservation of Fish Vestibular Organization in Other Vertebrates

Comparison of the spatial distribution of individual vestibular end-organ terminations among different groups of fish, such as damselfish, goby, toadfish, and elasmobranchs, reveals a vestibular pattern similar to that in goldfish. Notably, central projections from comparable populations of vestibular neurons originate from corresponding subdivisions, even though specific morphological details, particularly size, differ between the various species. This conservation also includes connectivity with particular targets, even in the exceptional case of flatfish, where both peripheral and central structural rearrangements occur during development. A comparison of vestibular system anatomy and physiology of fish with tetrapods also reveals a remarkably conserved pattern. In fact, afferent projections from the different vestibular end organs in all tetrapod taxa terminate differentially in areas that correspond to those in fish. Moreover, similar subgroups of major vestibular projection neurons originate from homologous positions in the hindbrain of mammals, birds, and amphibians. These comparative attributes suggest that, from basic wiring through function, the vestibular blueprint was established quite early during vertebrate evolution and has been largely conserved unchanged throughout 400 million years of vertebrate phylogeny.

See also: **Detection and Generation of Electric Signals:** Detection and Generation of Electric Signals in Fishes: An Introduction. **Hearing and Lateral Line:** Auditory/Lateral Line CNS: Anatomy; Auditory System Morphology; Lateral Line Neuroethology; Lateral Line Structure; Psychoacoustics: What Fish Hear; The Ear and Hearing in Sharks, Skates, and Rays.

Further Reading

Gibbs MA and Northcutt RG (2004) Development of the lateral line system in the shovelnose sturgeon. *Brain Behavior and Evolution* 64: 70–84.

Graf W and Baker R (1983) Adaptive changes of the vestibulo-ocular reflex in flatfish are achieved by reorganization of central nervous pathways. *Science* 221: 777–779.

Graf W and Baker R (1985) The vestibuloocular reflex of the adult flatfish. II. Vestibulooculomotor connectivity. *Journal of Neurophysiology* 54: 900–916.

Graf W, Spencer R, Baker H, and Baker R (1997) Excitatory and inhibitory vestibular pathways to the extraocular motor nuclei in goldfish. *Journal of Neurophysiology* 77: 2765–2779.

Highstein SM, Kitch R, Carey J, and Baker R (1992) Anatomical organization of the brainstem octavolateralis area of the oyster toadfish, *Opsanus tau*. *Journal of Comparative Neurology* 319: 501–518.

Maruska KP and Tricas TC (2009) Central projections of octavolateralis nerves in the brain of a soniferous damselfish (*Abudefduf abdominalis*). *Journal of Comparative Neurology* 512: 628–650.

McCormick CA and Braford MR, Jr. (1994) Organization of inner ear endorgan projections in the goldfish, *Carassius auratus. Brain Behavior and Evolution* 43: 189–205.

McCormick CA and Hernandez DV (1996) Connections of octaval and lateral line nuclei of the medulla in the goldfish, including the cytoarchitecture of the secondary octaval population in goldfish and catfish. *Brain Behavior and Evolution* 47: 113–137.

McElligott JG, Beeton P, and Polk J (1998) Effect of cerebellar inactivation by lidocaine microdialysis on the vestibuloocular reflex in goldfish. *Journal of Neurophysiology* 79: 1286–1294.

Pastor AM, De la Cruz RR, and Baker R (1994) Eye position and eye velocity integrators reside in separate brainstem nuclei. *Proceedings of National Academy of Sciences of the United States of America* 91: 807–811.

Pastor AM, De la Cruz RR, and Baker R (1997) Characterization of Purkinje cells in the goldfish cerebellum during eye movement and adaptive modification of the vestibulo-ocular reflex. *Progress in Brain Research* 114: 359–381.

Pastor AM, Torres B, Delgado-Garcia JM, and Baker R (1991) Discharge characteristics of medial rectus and abducens motoneurons in the goldfish. *Journal of Neurophysiology* 66: 2125–2140.

Straka H and Dieringer N (2004) Basic organization principles of the VOR: Lessons from frogs. *Progress in Neurobiology* 73: 259–309.

Suwa H, Gilland E, and Baker R (1996) Segmental organization of vestibular and reticular projections to spinal and oculomotor nuclei in the zebrafish and goldfish. *Biological Bulletin* 191: 257–259.

Suwa H, Gilland E, and Baker R (1999) Otolith ocular reflex function of the tangential nucleus in teleost fish. *Annals of the New York Academy of Sciences* 871: 1–14.

Tomchik SM and Lu Z (2005) Octavolateral projections and organization in the medulla of a teleost fish, the sleeper goby (*Dormitator latifrons*). *Journal of Comparative Neurology* 481: 96–117.

Auditory System Morphology

AN Popper, University of Maryland, College Park, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Vertebrate Ears
Fish Ears
Getting Sound to the Ear
Hair Cell Number and Fish Growth
Summary
Further Reading

Glossary

Auditory system The portion of the ear that is involved in sound detection. In fishes, this generally includes the saccule and lagena, though in many species the utricle may also be involved.

Hair cell The major sensory cell in the ear and lateral line of all vertebrates.

Inner ear The part of the ear that is located inside of the head that is responsible for detection of sound (hearing) and movement of the head relative to gravity (vestibular). It consists of three semicircular canals and three otolith end organs, as well as a macula neglecta.

Otolith A calcium carbonate structure that is located in the otolith organs of fishes. In teleosts, the otoliths take on distinct species-specific shapes.

Otolith organs The saccule, lagena, and utricle of the inner ear. Each has a sensory epithelium (or macula) and an otolith (or otoconia).

Semicircular canals Three mutually perpendicular structures associated with the ear and involved in detection of angular acceleration.

Swimbladder A gas-filled chamber in the abdominal cavity of most bony fishes. The swimbladder is used in buoyancy control in most species; it also subsumes roles in hearing and sound production in some species.

Vestibular system The portion of the ear consisting of the semicircular canals and utricle that are involved with the detection of head motion and balance.

Weberian ossicles A series of bones connecting the swimbladder to the inner ear in the otophysan fishes (goldfish and relatives).

Vertebrate Ears

The inner ears in all vertebrates serve two major functions. Portions of the ear make up the ‘vestibular system’ and which, whereas other parts of the ear make up the ‘auditory system’. Though both the vestibular and auditory portions of the inner ear are critical for the survival of any vertebrate, humans tend to be most aware of the hearing function of the ear because sound is such an integral part of communication. Although humans are not aware of the function of the vestibular system unless it is not functioning properly (e.g., in vertigo), it plays a major role in life because it provides information about the position and movement of the head (**see also Hearing and Lateral Line**: Vestibular System Anatomy and Physiology).

Inner Ear Anatomy

All vertebrates, with the exception of the jawless vertebrates (the lampreys), have very similar inner ear structures (**Figure 1**). The inner ears include three semicircular canals, each with its own sensory region (the ampullae), and several otolith end organs and their sensory regions. The basic ear pattern is the same whether one examines a shark (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays), a bony fish, a frog, or a human. The major difference across the various vertebrate groups is that reptiles, birds, and mammals have an elaboration of one of the otolith end organs, the lagena, that may have evolved into the cochlea in mammals and the basilar papilla in reptiles and birds. Many, but not all, vertebrates also have an additional sensory structure, the macula neglecta, the function of which is not known in any vertebrate group other than the sharks and rays where it appears to play a major role in hearing (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays).

Sensory Cells in the Ear

No matter the species, all vertebrate ears (and the lateral line found in fish and some amphibians; **see also Hearing and Lateral Line**: Lateral Line Structure) use the same cell type to detect and respond to motion. This motion may be in the form of head movement in the vestibular system, or sound in the hearing portion of the ear. The sensory hair

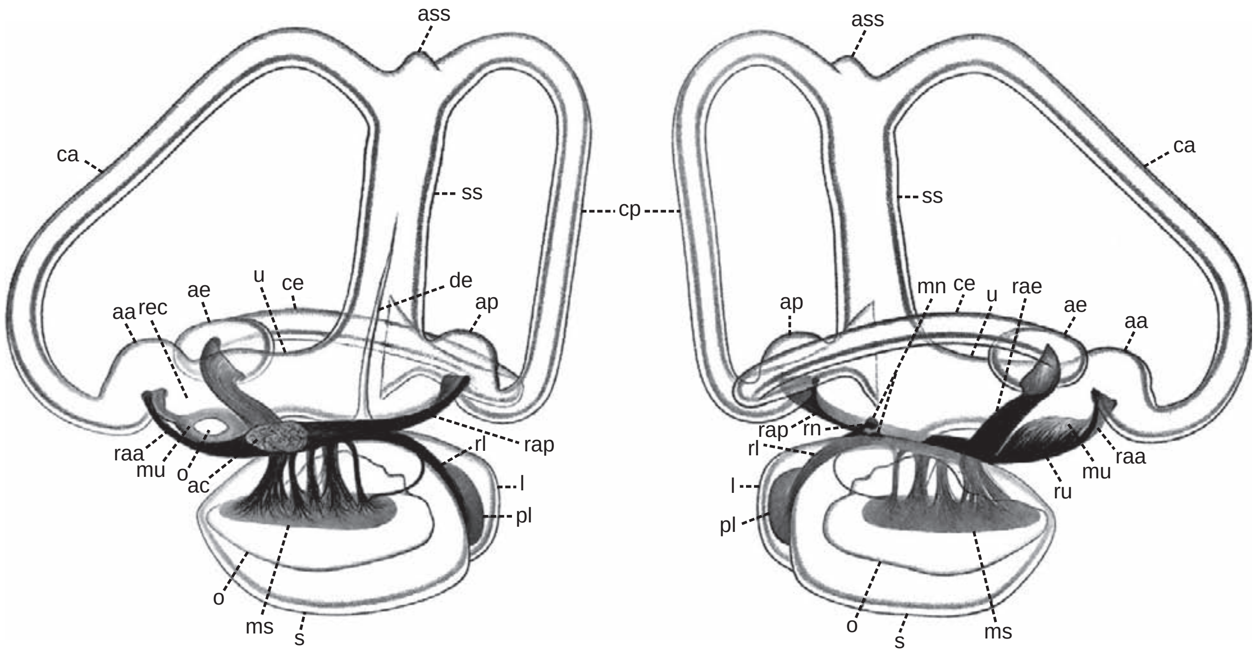


Figure 1 The ear of an Atlantic salmon, *Salmo salar*. The medial side of the ear is on the left and a lateral view is on the right. Legend (major structures): aa, ap – ampullae of semicircular canals; ac – eighth cranial nerve to ear; ca, cp, ce – semicircular canals; de – ductus endolymphaticus; mn – macula neglecta; ms – saccular epithelium (macula sacculus); o – otolith; pl – lagena epithelium (papilla lagena); rac, rap – rami of eighth cranial nerves to semicircular canals; rl – lagena branch of eighth nerve; rs – saccular branch of eighth nerve; ru – utricular branch of eighth nerve; s – saccular chamber; ss – common canal; u – utricular chamber. From Retzius G (1881) *Das Gehörorgan der Wirbelthiere*, vol. I. Stockholm: Samson and Walli.

cell (**Figure 2**) has a cell body that is capped by a number of cilia that project into the lumen of the particular end organ. These cilia are of two types. Most are elaborated microvilli, or stereovilli (also called stereocilia in the older literature) that project from a cuticular plate lying just between the apical (top) membrane of the sensory cell. These stereovilli are often graded in size, in an organ pipe arrangement (**Figure 3**). The longest stereovilli are at one end of the bundle, and this end also has the single kinocilium. The kinocilium, a true cilium, is very similar in structure to the motile cilia of many species, including flagella that propel sperm and bacteria.

The sensory hair cell is a mechanical system that produces a chemical response to the bending of the cilia. When the cilia are bent, a series of chemical events occur that involve channels (tiny pores) opening in the apical ends of the streovilli, thereby letting calcium ions enter the cell. These ions cause a chain of chemical events that result in the release of neurotransmitters from the base of the cells, and these transmitters cause the production of impulses in the neurons of the eighth cranial nerve that innervates the cells (**see also Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy). The signal is then carried to the brain, indicating that some kind of stimulation has taken place.

The sensory hair cell is both morphologically and physiologically polarized. Morphological polarization comes from the fact that the kinocilium is located at one side of the ciliary bundle. The physiological polarization is related to the morphological polarization, and results in a different level of response from the sensory cell depending upon the direction in which it is bent. Thus, in effect, the sensory cell gives a different response to signals from different directions. More specifically, if the ciliary bundle is bent from the shortest stereovilli to the kinocilium, the cell gives maximum response (maximum release of neurotransmitter). However, if the bending is in the opposite direction, the cell releases very little transmitter. Bending in other directions (e.g., perpendicular to the axis of maximum stimulation), the level of neurotransmitter release is somewhat between maximum and minimum release. Thus, in effect, the sensory hair cell gives a different level of response for stimulation in any direction. This graded response is central to the ability of fish to detect the direction of a sound source (**see also Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes).

Ciliary bundles also vary in length in different end organs, or even within a single end organ (**Figure 3**). While the correlation between length and function is not fully understood, there are some data that lead to the suggestion that the longer bundles respond to lower-frequency stimulation, whereas shorter bundles have their best responses to higher-frequency signals.

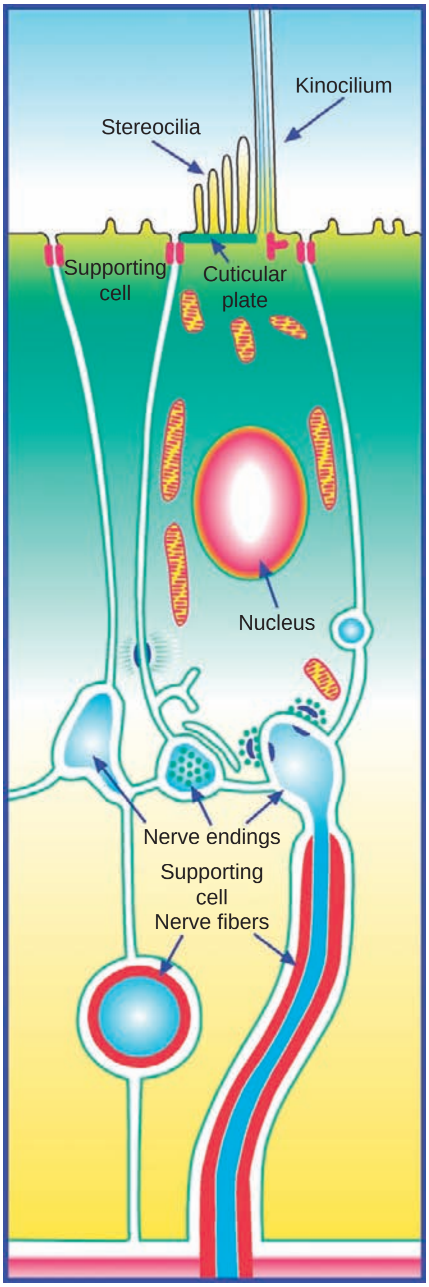


Figure 2 Drawing of a typical fish sensory hair cell. The cell body is innervated by afferent and efferent nerve endings at the basal end. The dorsal end of the cell has a cuticular plate in that are embedded the roots of the stereovilli. The kinocilium is located at one end of the ciliary bundle and terminates at a pair of basal bodies in the cell cytoplasm. Redrawn from Popper AN and Coombs S (1980) Auditory mechanisms in teleost fishes. *American Scientist* 69: 429–440.

Fish Ears

The ear in fishes is located in the cranial cavity just lateral to the brain (**Figure 4**) – there is no outer or middle ear as found in terrestrial animals (although sharks do have a distinct connection to the outside – **see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays). A typical fish ear is shown in **Figure 1**, and other ears, for comparative purposes, are shown in **Figure 5** (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays for illustrations of shark ears). While there are some differences in the shape of the semicircular canals, the greatest variation in shape and size across the fishes is generally associated with the otolithic end organs involved in hearing.

The ear in the Atlantic salmon (*Salmo salar*, **Figure 1**) has three semicircular canals (the anterior, posterior, and lateral (or horizontal)) and they, along with the utricle, make up the major vestibular parts of the ear (though in many species, the utricle also subsumes a role in hearing). A sensory region, or crista, is found at the base of each canal. Each crista has an epithelium of sensory hair cells, and these are overlain with a gelatinous structure, the cupula, that covers the crista. These ear regions have, at times, been referred to as the pars superior.

The ear in fishes also has three otolithic end organs – the saccule, lagena, and utricle (**Figures 1** and **5**). The saccule and lagena, and in many species the utricle, are involved with hearing (the saccule and lagena make up the pars inferior). These otolithic end organs contain very dense calcium carbonate crystals. In Agnathans (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development), sharks and rays (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays), and terrestrial vertebrates, as well as in more primitive bony fishes, the crystals, called otoconia, are embedded in a gelatinous matrix (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays). However in modern bony fishes (teleosts), the crystals are fused into a solid structure called the otolith (**Figure 6**).

The Otoliths

The otoconial mass or the otolith lies in the lumen of the otolith organs and in close contact with the sensory epithelium that contains the sensory hair cells. In teleosts, the otolith and sensory epithelium are connected to one another by a thin otolith membrane. During sound stimulation, the otolith (or the otoconial mass), which is generally about 3 times denser than the fish's body, moves at a different amplitude and phase than does the rest of the body (including the epithelium). This differential movement results in bending of the ciliary bundles and stimulation of the sensory cells, thereby sending a signal to the brain via the innervating eighth nerve (**see also Hearing and Lateral Line**: Biomechanics of the Inner Ear in Fishes).

While the otoconial mass has no particular shape, otoliths take on very specific shapes that are diagnostic of particular species (**Figure 6**). This is particularly true for the otolith of the saccular end organ.

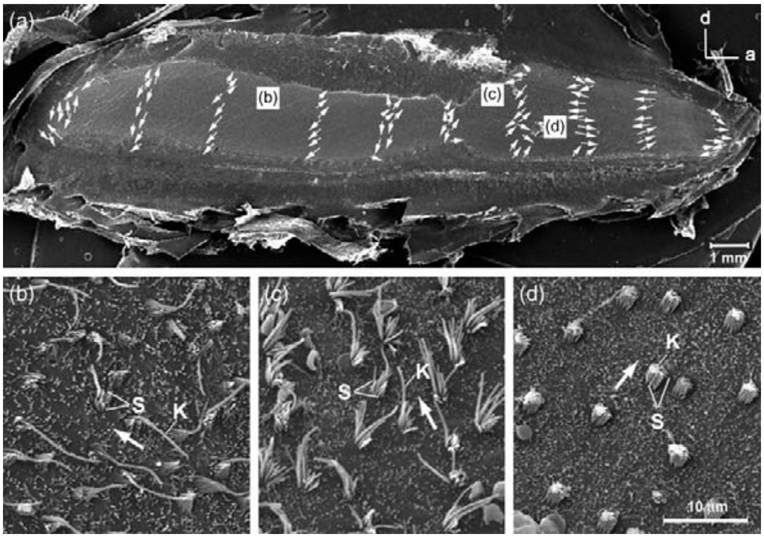


Figure 3 The saccular sensory epithelium of a bluefin tuna, *Thunnus thynnus*. The saccular epithelium (anterior to the right, dorsal to the top). Arrows indicate the direction of orientation of the sensory cells in different regions of the epithelium. The lower images are enlargements of the same lettered regions from the top image. These show the ciliary bundles from the sensory hair cells in the different areas. K – kinocilium; S – stereovilli. From Song J, Matieu A, Soper RF, and Popper AN (2006) Structure of the inner ear of bluefin tuna (*Thunnus thynnus*). *Journal of Fish Biology* 68: 1767–1781.

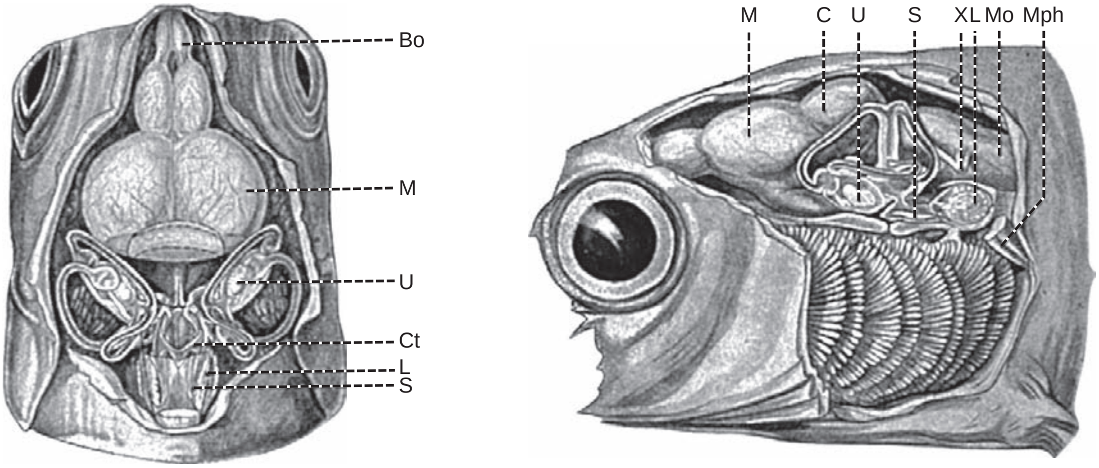


Figure 4 Drawing of the ear and its location in the head from the European minnow, *Phoxinus laevis*. Top shows a dorsal view of the head with the bones removed to reveal the inside of the cranial cavity. The posterior part of the brain is also removed to show the two ears in this otophysan fish. The lower drawing shows a lateral view of the fish, with the brain and ear. Legend: Bo, C, M, Mo, parts of the brain; Ct, transverse canal; L, lagena; Mph, pharyngeal muscles; S, saccule; U, utricle; X, the 10th cranial nerve. From von Frisch K and Stetter H (1932) Untersuchungen über den Sitz des Gohörsinnes bei der Elritze. *Zeitschrift Fuer Vergleichende Physiologie* 17: 686–801.

A characteristic of otoliths is that they increase in size as the fish grows, and this results in the otoliths adding layers of calcium carbonate in a ring-like pattern, much as rings develop in trees. Fisheries biologists use these rings to estimate fish age, and recent studies have shown that there are not only annual rings, but also daily rings that can provide a good deal of information on fish food supply and other aspects of physiology and ecology.

Sensory Epithelia

The sensory surfaces in each of the otolith organs consist of numerous sensory hair cells, each of which is surrounded by supporting cells (**Figure 3**). The sensory cells themselves are organized into groupings, with all of the cells in each group being oriented with the kinocilium on the same side of the cell (**Figure 3**). The size and shape of each orientation group varies among the different otolith organs and among the same otolith organ of

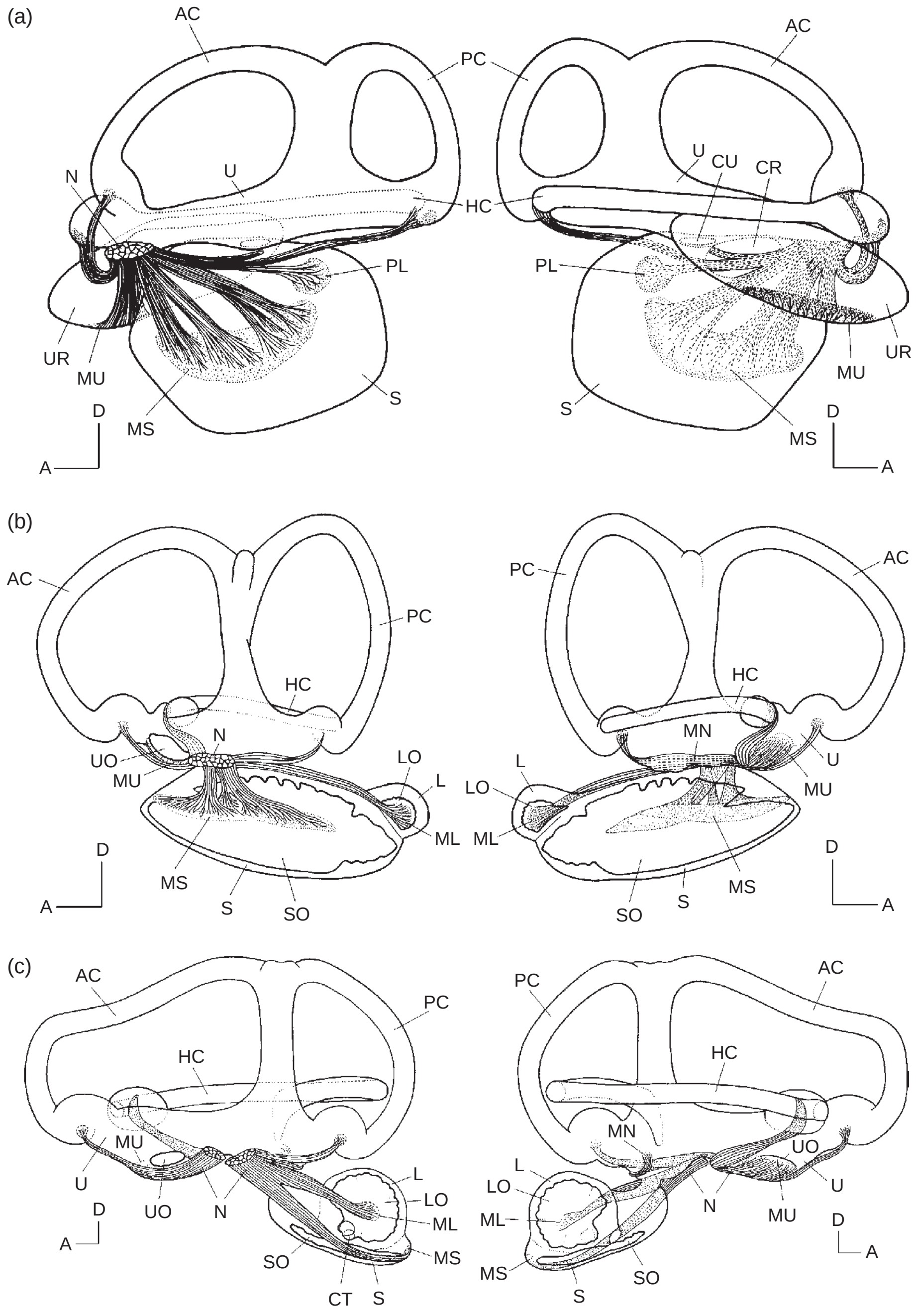


Figure 5 Drawings of the ears from various fish species to show variation. (a) a lungfish (*Ceratodus forsteri*); (b) pike perch (*Stizostedion lucioperca*); (c) European Wels (*Siluris glanis*). Legend: AC, HC, PC – anterior, horizontal, and posterior semicircular canals; L – lagena; LO, SO, UO – lagena, saccular, and utricular otoliths; ML, MS, MU – sensory epithelia of lagena, saccule, and utricle; N – eighth cranial nerve; S – saccule; U – utricle; UR – utricular recess.

different species. For example, **Figure 7** shows the hair cell orientation patterns from a hardhead sea catfish (*Arius felis*), a species with excellent hearing. The saccule has hair cells oriented in two orientation groups, one with the kinocilium oriented dorsally and the other with hair cells oriented ventrally. This bidirectional saccular pattern is similar to that found in primitive fishes, sharks and rays, and most terrestrial vertebrates.

The saccular pattern in hardhead sea catfish can be contrasted to the pattern found in most other modern teleosts where there are at least four hair cell groups on the saccule – two oriented dorsally and ventrally, as in the hardhead sea catfish, and at least two oriented anteriorly and posteriorly (**Figures 8** and **9**). There is considerable variation in this pattern in fishes, and while the functional significance of the differences is unknown, it has generally been suggested that the presence of four groups of cells

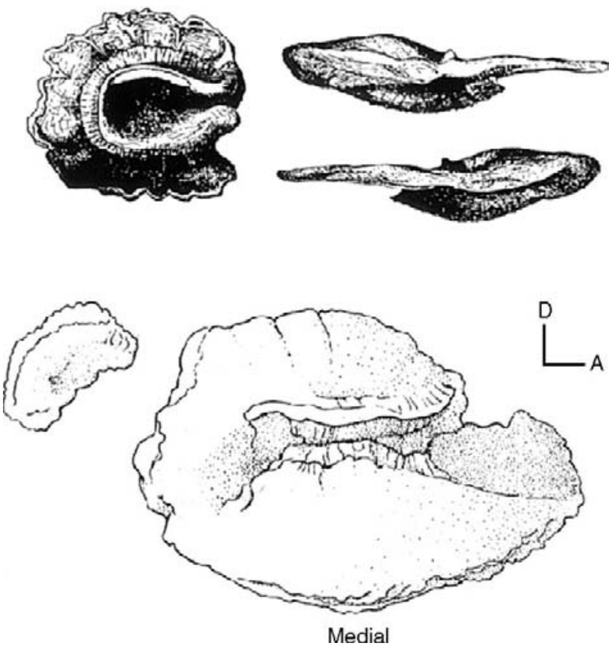


Figure 6 Otoliths from the Eurasian minnow, *Phoxinus laevis*, an otophysan (top) and (bottom) *Zebrasoma verliferum* (sailfin tang). The otolith on the right is from the saccule and left from the lagena. The utricular otolith generally looks like a slightly flattened ball. Note that in the Eurasian minnow the lagena otolith is large as compared with *Zebrasoma*, whereas the saccular otolith is smaller. The shape of the saccular otolith in the Eurasian minnow is rather complex and so two different views are shown along its horizontal axis. Top: From Wohlfahrt TA (1932) Anatomische Untersuchungen über das Labyrinth der Elritze (*Phoxinus laevis* L.). *Zeitschrift Fuer Vergleichende Physiologie* 17: 659–685. Bottom: Popper AN (1983) Organization of the inner ear and processing of acoustic information. In: Northcutt RG and Davis RE (eds.) *Fish Neurobiology and Behavior*, pp. 125–178. Ann Arbor, MI: University of Michigan Press.

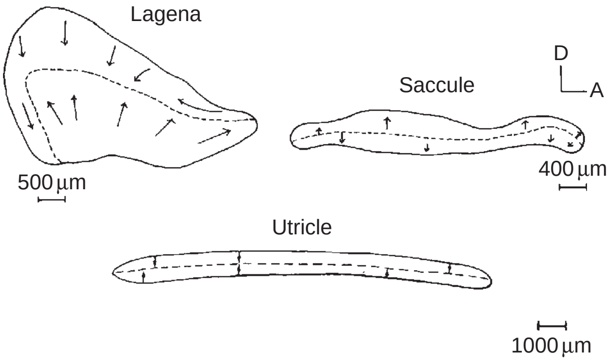


Figure 7 Hair cell orientation patterns from a hardhead sea catfish, *A. felis*. The arrows show the direction of orientation of the hair cells in different epithelial regions (separated by the dashed lines). Each arrow shows the side of the cell on which the kinocilium sits. From Popper AN and Tavolga WN (1981) Structure and function of the ear of the marine catfish, *Arius felis. Journal of Comparative Physiology* 144: 27–34.

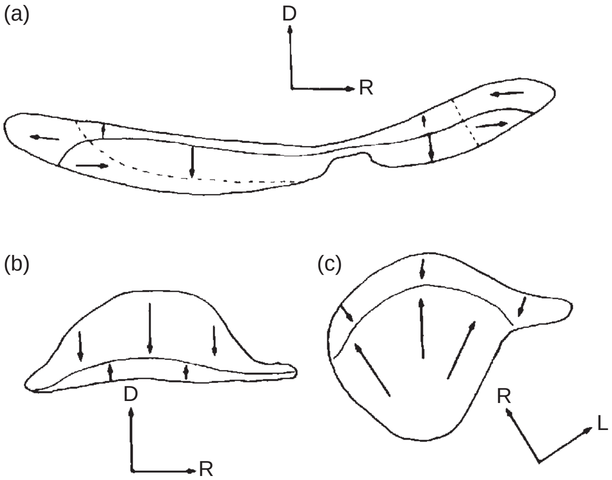


Figure 8 Hair cell orientations for the saccule (a), lagena (b), and utricle (c) from the Mediterranean hake, *M. merluccius*. Note the difference in orientation patterns between the saccule and lagena as compared to the hardhead sea catfish in **Figure 7**. In particular, the saccular epithelium has hair cells orientated both anteriorly and posteriorly as well as dorsally and ventrally. From Lombarte A and Popper AN (1994) Quantitative analyses of postembryonic hair cell addition in the otolithic endorgans of the inner ear of the European hake, *Merluccius merluccius* (Gadiformes, Teleostei). *Journal of Comparative Neurology* 345: 419–428.

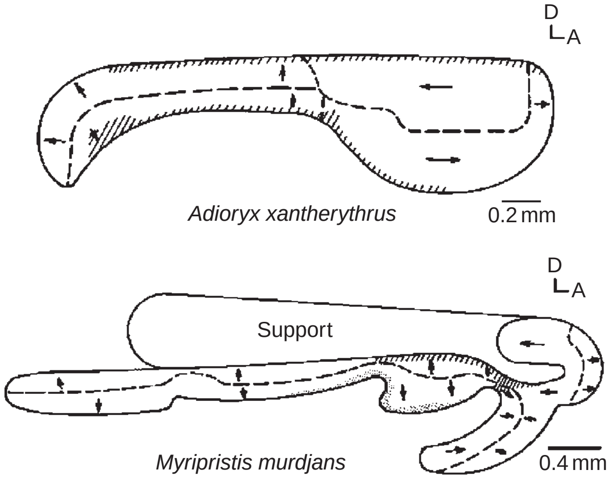


Figure 9 Saccular hair cell orientation patterns from two fishes from the family Holocentridae. The arrows indicate the orientation patterns of the hair cells in different regions. Top is from *Adioryx*, a fish that hears sound to about 1000 Hz, while the more elaborate pattern below is from the related *Myripristis*, a fish that hears to well over 3000 Hz. The anterior end of the swimbladder in *Myripristis* terminates on the chamber of the ear that includes the saccule, while not in *Adioryx*. From Popper AN (1977) A scanning electron microscopic study of the sacculus and lagena in the ears of fifteen species of teleost fishes. *Journal of Morphology* 153: 397–418.

assists fishes in estimating the direction of sounds because the cells in different groups are presumed to have different levels of physiological response to signals from different directions (e.g., sound source localization – **see also**

Hearing and Lateral Line: Sound Source Localization and Directional Hearing in Fishes).

It has also been noted that the more complex patterns of hair cell orientation are often found in fishes that have a wider range of hearing and better hearing sensitivity than fishes with less complex patterns (**Figure 9**). For example, the pinecone soldierfish *Myripristis* saccule has a very complex pattern, whereas the closely related Hawaiian soldierfish *Adioryx* has a simpler pattern. Interestingly, *Myripristis* hears sounds to well over 3 kHz, whereas *Adioryx* does not hear sounds much above 1 kHz.

Getting Sound to the Ear

In contrast to many terrestrial animals that have structures peripheral to the ear that enables the capture of sound and its transmission to the inner ear, there are no specific external sound gathering structures in fishes. Indeed, it is possible for the particle motion component of sound to directly stimulate the ear by producing relative motion between the otolith (or otoconia) and the sensory epithelium (**see also Hearing and Lateral Line**: Biomechanics of the Inner Ear in Fishes).

At the same time, one limitation of direct stimulation is that fish can only detect particle motion with the ear, and there is no direct detection of the pressure component of the sound field. This limits the bandwidth and sensitivity of fish hearing (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear); therefore, fishes that only rely on direct particle motion for sound detection generally do not hear as well as fishes that are able to also detect pressure.

The issue then becomes one of how fish can detect pressure and then convert it into a particle motion signal that can stimulate the ear. This is accomplished by having a discontinuity in the body, most often in the form of a gas bubble that responds to pressure motions. Such a bubble, which is much less dense than the fish body, is most commonly the swimbladder, a gas-filled chamber in the abdominal cavity that is used by most species to control buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes, Swimbladder Function and Buoyancy Control in Fishes) and sound production (**see also Hearing and Lateral Line**: Acoustic Behavior, Vocal Behavior of Fishes: Anatomy and Physiology).

The swimbladder wall then vibrates in response to the pressure component of a sound field and re-radiates the signal, with a substantial particle motion component, to the ear, thereby stimulating the ear. In effect, the fish then becomes a detector of pressure signals. This results in the fish hearing a wide bandwidth of sound. At the same time, the strength of the re-radiated signal will impact hearing capabilities. If the re-radiated signal is weak by the time it gets to the ear, hearing will not be enhanced substantially. However, if the signal is strong, hearing bandwidth and sensitivity may be increased.

In order to increase hearing sensitivity, many species, including those that are taxonomically distinct, have evolved mechanisms whereby the signal from the swimbladder is brought into close proximity to the ear, or even directly to the ear, thereby decreasing any signal losses that might result from attenuation due to the distance between the swimbladder and ear (**Figure 10**). In other words, if the swimbladder is at a considerable distance from the ear, as in salmonids, tunas, and many other commercially important species, the amount of signal from the swimbladder is probably very limited, and this probably correlates with the species generally not detecting sounds to above 800–1000 Hz (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear).

There is a considerable range of mechanisms found in different fish taxa that presumably are present to acoustically couple the motion of the swimbladder to the inner ear. Perhaps best known of these is a series of bones, the Weberian ossicles, found in the goldfish and all other fishes in the taxonomic group Otophysi (this includes carp, catfish, etc.) (**Figure 11**). In these fishes, the posterior-most ossicle (bone) is directly attached to the swimbladder wall, whereas the most anterior bone in the chain makes up a wall of a chamber that contains fluid that is continuous with that of the inner ear. Motion of the swimbladder wall causes the posterior-most bone to move on its connection to the vertebral column, and this motion is transmitted through the ossicles, much as sound is transmitted through the (unrelated) ossicles in the mammalian inner ear, and results in motion of inner ear fluids that directly move the saccular otolith and thereby stimulates the sensory hair cells.

Other fish species have extensions of the swimbladder to a position close to, or actually contacting, the ear (generally the saccule) or additional gas bubbles lying near or in contact with the ear. For example, the aforementioned *Myripristis* has anterior swimbladder extensions that terminate on the bone surrounding the saccular chamber, whereas fishes of the family Mormyridae (elephant-nosed fish) have a separate air bubble that lies within the confines of the semicircular canals and which, in part, makes up the medial wall of the saccular chamber. Similarly, the Anabantidae (bubble nest builders) have an air pocket in the pharynx, which is close to the ear, and this bubble has been shown to enhance hearing in these species compared to related species without such a bubble.

While most specialized structures for bringing sound to the ear are associated with the saccule, in at least one group of fish, the Clupeiformes (herring, shad, and

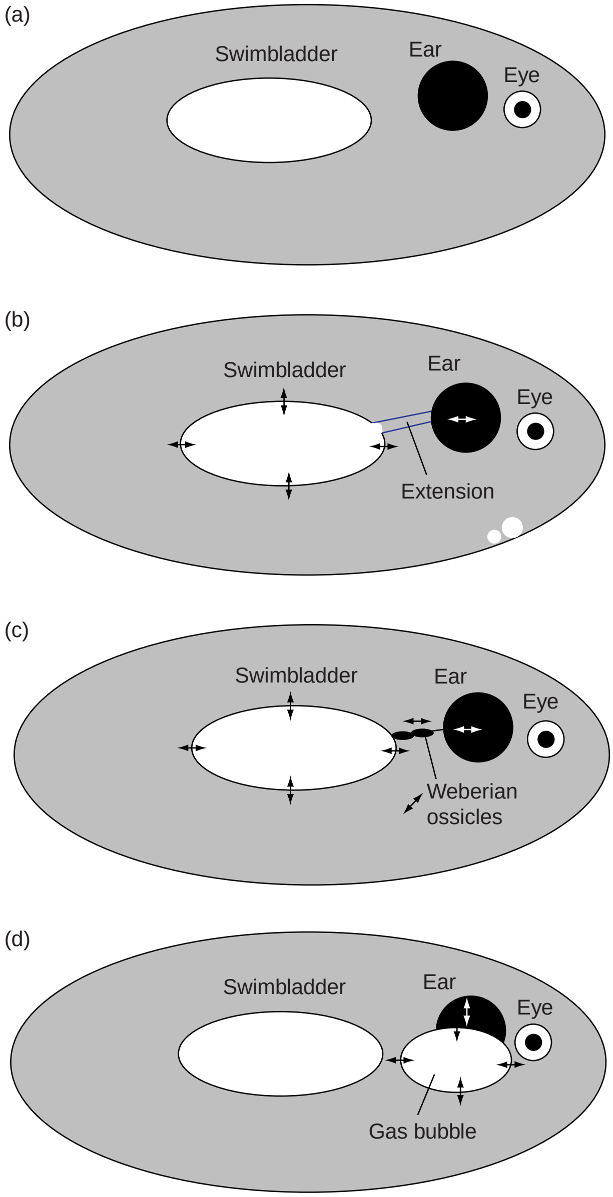


Figure 10 Schematic drawing of the relationship between the ear and the swimbladder in different species. There is no connection in (a), a condition that is typical of species that do not have a wide hearing bandwidth. In (b), the swimbladder projects forward and contacts the inner ear, as in *Myripristis* (**Figure 9**), while (c) shows the condition in the otophysan fish where a series of bones connects the inner ear to the swimbladder (see detailed drawing in **Figure 11**). Finally, panel (d) shows the special condition where there is a secondary bubble of gas near the inner ear, as is found in the mormyrids, anabantids, and clupeids. From Ladich F and Popper AN (2004) Parallel evolution in fish hearing organs. In: Manley GA, Popper AN, and Fay RR (eds.) *Evolution of the Vertebrate Auditory System,* pp. 95–127. New York: Springer.

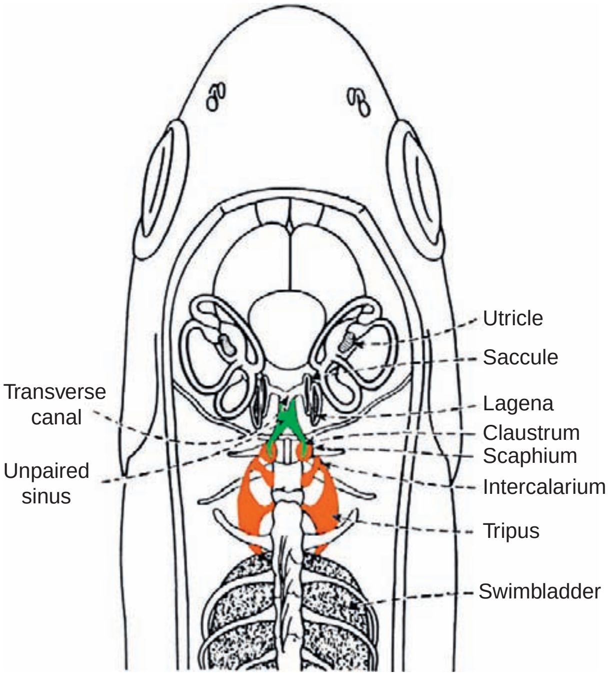


Figure 11 A drawing showing the dorsal view of an otophysan fish with the inner ear, the swimbladder, and the Weberian ossicles. Movement of the wall of the swimbladder results in movement of the Weberian ossicles (orange), and these move the fluids in the sinus (green) and thus of the inner ear and the saccular otolith.

menhaden), there is a thin anterior projection of the swimbladder that terminates in a gas bubble intimate with the utricular chamber of the ear. Moreover, whereas the structure of the utricle is generally the same in almost all aquatic and terrestrial vertebrates, the utricle in Clupeiformes has unique specializations associated with the air bubble. As a consequence, it is thought that this results in the utricle being a major part of the hearing apparatus of these fishes. Moreover, all members of the Clupeiformes can detect sounds to over 4 kHz. In addition, some members of this group, including all members of the genus *Alosa* (e.g., American shad and blueback herring), have even more extreme specializations in the utricle and can detect sounds to over 180 kHz, a range that may be greater than for any other vertebrate other than a few bats and dolphins.

There is considerable inter-specific variation in the way in which the pressure signals are brought to the ear and, quite significantly, it is clear that such specializations evolved over and over again in different fish taxa. Thus, with the exception of the Weberian ossicles in the Otophysi, other specializations are found repeatedly in different fish groups, even when close taxonomic relatives of the same species do not have such specializations. So, for example, anterior elongations of the swimbladder so that it contacts the ear occur in species as diverse as squirrelfish, clown knifefish (*Notopterus chitala*), and in many unrelated deep-sea fish that may have evolved enhanced hearing capabilities to compensate for lack of light.

Hair Cell Number and Fish Growth

One of the unique findings about fishes is that most species grow continuously through the life of the animal. In fishes, the general growth of the body is accompanied by growth of the inner ear, including an increase in the size of the otolith, and an increase in the number of sensory hair cells in each of the sensory epithelia. Thus, for example, a 1-cm-long Mediterranean hake (*Merluccious merluccius*) may have 5000 sensory hair cells in the saccular epithelium, whereas the same species of about 80 cm long (about 9 years old) may have close to 1 million cells per saccular epithelium. This dramatic increase in cell number has been documented in all species in which it has been looked for, including in sharks and rays.

There are many significant questions that arise from this finding. A fundamental question is how fish retain the ability to proliferate sensory hair cells throughout life when species such as mammals, which have the same type of hair cells, have all the hair cells they will ever have at birth, and the only change in number is loss due to cell death with aging.

The other question of interest is what this increase of hair cells means for function of the ear, and, more directly, does hearing change with size of the fish (or age). While data are limited, there is a small amount of evidence that there is a broadening of hearing bandwidth and sensitivity in very young fishes as they add more hair cells. However, a recent study in walleye pollock (*Theragra chalcogramma*) shows that there is no change in hearing ability in fish ranging from 1 to 3 years of age, despite a very substantial growth in the size of the fish and its ear. While still preliminary, these results support a hypothesis that hair cell addition in fishes is probably to maintain a constant level of hearing over time rather than to change hearing capabilities, thereby compensating for other changes associated with growth, such as a change in the relative position of the swimbladder and inner ear.

Related to the vast proliferation of sensory cells in the ear in fishes is the discovery that fishes can regenerate sensory hair cells that are lost due to treatment with ototoxic drugs (those that kill hair cells) or loud sounds (**see also Hearing and Lateral Line**: Effects of Human-Generated Sound on Fish). This ability is not found in mammals, and found only to a limited extent in other tetrapods. In contrast, fishes appear to be able to regenerate large numbers of sensory cells within a few days of damage, and there is good evidence that hearing recovers as the sensory cells are replaced.

Summary

There is very substantial variability in the structure of the ear in fishes, and this variation is particularly associated with the end organs involved with hearing. The functional significance of this variation is not known; however, there are several possible explanations. The first possibility is that the variation represents evolutionary experiments for hearing, each of which provides the brain with similar information about sound. A second possibility is that the differences represent different hearing mechanisms and/or capabilities in different species. What is most interesting, however, is that even when there are differences in ear structure, similar ear patterns have evolved in very different, and taxonomically unrelated, species, suggesting that even with the variation, there are only a limited number of ways that sound detection is most effective.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Swimbladder Function and Buoyancy Control in Fishes. **Hagfishes and Lamprey**: Lampreys: Energetics and Development. **Hearing and Lateral Line**: Acoustic Behavior; Auditory/Lateral Line CNS: Anatomy; Biomechanics of the Inner Ear in Fishes; Effects of Human-Generated Sound on Fish; Lateral Line Structure; Psychoacoustics: What Fish Hear; Sound Source Localization and Directional Hearing in Fishes; The Ear and Hearing in Sharks, Skates, and Rays; Vestibular System Anatomy and Physiology; Vocal Behavior of Fishes: Anatomy and Physiology.

Further Reading

Coffin A, Kelley M, Manley GA, and Popper AN (2004) Evolution of sensory hair cells. In: Manley GA, Popper AN, and Fay RR (eds.) *Evolution of the Vertebrate Auditory System*, pp. 55–94. New York: Springer.

Ladich F and Popper AN (2004) Parallel evolution in fish hearing organs. In: Manley GA, Popper AN, and Fay RR (eds.) *Evolution of the Vertebrate Auditory System*, pp. 95–127. New York: Springer.

Lombarte A and Popper AN (1994) Quantitative analyses of postembryonic hair cell addition in the otolithic endorgans of the inner ear of the European hake, *Merluccius merluccius* (Gadiformes, Teleostei). *Journal of Comparative Neurology* 345: 419–428.

Manley GA, Popper AN, and Fay RR (eds.) (2004) *Evolution of the Vertebrate Auditory System*. New York: Springer.

Popper AN (1977) A scanning electron microscopic study of the sacculus and lagena in the ears of fifteen species of teleost fishes. *Journal of Morphology* 153: 397–418.

Popper AN (1983) Organization of the inner ear and processing of acoustic information. In: Northcutt RG and Davis RE (eds.) *Fish Neurobiology and Behavior*, pp. 125–178. Ann Arbor, MI: University of Michigan Press.

Popper AN and Coombs S (1980) Auditory mechanisms in teleost fishes. *American Scientist* 69: 429–440.

Popper AN, Fay RR, Platt C, and Sand O (2003) Sound detection mechanisms and capabilities of teleost fishes. In: Collin SP and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments*, pp. 3–38. New York: Springer.

Popper AN and Schilt CR (2008) Hearing and acoustic behavior (basic and applied). In: Webb JF, Fay RR, and Popper AN (eds.) *Fish Bioacoustics*, pp. 17–48. New York: Springer.

Popper AN and Tavolga WN (1981) Structure and function of the ear of the marine catfish, *Arius felis*. *Journal of Comparative Physiology* 144: 27–34.

Ramcharitar J, Gannon DP, and Popper AN (2006) Bioacoustics of the family Sciaenidae (croakers and drumfishes). *Transactions of the American Fisheries Society* 135: 1409–1431.

Retzius G (1881) *Das Gehörorgan der Wirbelthiere*, vol. I. Stockholm: Samson and Walli.

Song J, Matieu A, Soper RF, and Popper AN (2006) Structure of the inner ear of bluefin tuna (*Thunnus thynnus*). *Journal of Fish Biology* 68: 1767–1781.

Von Frisch K and Stetter H (1932) Untersuchungen über den Sitz des Gohörsinnes bei der Elritze. *Zeitschrift Fuer Vergleichende Physiologie* 17: 686–801.

Webb JF, Fay RR, and Popper AN (eds.) (2008) *Fish Bioacoustics*. New York: Springer.

Weber EH (1820) *De Aure et Auditu Hominis et Animalium*. *Pars I. De Aure Animalium Aquatilium*. Leipzig: Gerhard Fleischer.

Wohlfahrt TA (1932) Anatomische Untersuchungen über das Labyrinth der Elritze (*Phoxinus laevis* L.). *Zeitschrift Fuer Vergleichende Physiologie* 17: 659–685.

Relevant Websites

http://www.marinebiodiversity.ca – Centre for Marine Biodiversity: Otolith Research Laboratory.

http://www.dosits.org – Discovery of Sound in the Sea.

http://www.popperlab.umd.edu – Laboratory of Aquatic Bioacoustics.

http://www.marine.usf.edu – University of South Florida: College of Marine Science.

http://web2.uwindsor.ca – University of Windsor, Higgs Lab.

The Ear and Hearing in Sharks, Skates, and Rays

BM Casper, University of Maryland, College Park, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Anatomy
Auditory Sensitivity
Summary
Further Reading

Glossary

Elasmobranch The subclass of fishes (Elasmobranchii) found within the class Chondricthyes that includes sharks, skates, and rays, making up the Chondricthyes or cartilaginous fishes. Their bones are made up of cartilage rather than calcium found in all other vertebrates.

Endolymphatic pores and ducts Opening on the dorsal cranium of elasmobranchs known as the endolymphatic pores that lead to a pair of ducts with direct access from the environment to the inner ear.

Fenestra ovalis Located near the saccular chamber, a specialized extension known as the posterior canal duct terminates at its dorsal end as a small membrane known as the fenestra ovalis. On the other side of this membrane is the ventral portion of the parietal fossa making contact with the dorsal cranium. This thin membrane (~7 μm thick) is believed to be part of the acoustic pathway that sound travels from the environment through the parietal fossa to the inner ear.

Otoconia Collections of calcium carbonate granules in a gelatinous matrix that overlie the saccule, utricle, and lagena sensory maculae.

Macula neglecta The nonotoconial end organ of the inner ear. The sensory epithelium of the macula neglecta, found in the posterior canal duct, is covered by a gelatinous cupula, similar to that found in the lateral line organ. Experimental evidence shows that this is one of the acoustically sensitive end organs.

Parietal fossa A depression in the cranium located between the otic capsules. This depression is primarily composed of loose connective tissue, and also contains the endolymphatic ducts. The ventral portion of the parietal fossa maintains contact with the skull, including a small membranous window known as the fenestra ovalis that separates the fossa from the inner ear.

Particle motion The actual movement of water molecules in response to an acoustic disturbance. This is the component of sound that elasmobranchs detect. The bulk of the energy of particle motion is near a sound source and then attenuates quickly with distance.

The elasmobranch fishes, including the sharks, skates, and stingrays, encompass a subclass that contains approximately 1200 extant species. This group of fishes has been around for hundreds of millions of years and, in most respects, has evolved little over this time period. Elasmobranchs represent the ancestral form of many of the teleostean fishes seen today and can provide a window to view many basal forms of anatomical features including the auditory system. However, with very little known about their auditory system across species, generalizations are made from a few species about all features from anatomy through to hearing sensitivities and behavior.

Anatomy

The inner ears of elasmobranchs are found within the otic capsules in the posterior of the chondocranium (skull). Each ear includes four sensory end organs and three semicircular canals, all of which contain groupings of hair cells that respond to unique stimuli and that transmit their information to the brain via the eighth auditory nerve (**Figure 1**(**a**)) (**see also Brain and Nervous System:** Functional Morphology of the Brains of Cartilaginous Fishes). Three of the sensory end organs (the saccule, utricle, and lagena) have their patches of sensory hair cells (maculae) (**see also Hearing and Lateral Line**: Auditory System Morphology) overlain by a calcium carbonate-based structure called the otoconia (**Figures 1**(**a**) and **1**(**b**)). The fourth end organ is the macula neglecta, which has hair cells that are covered only by a gelatinous cupula, unlike the other end organs. The macula neglecta is also uniquely located within the posterior canal duct, near the saccular chamber, rather than the widened ventral portion of the inner ear, where the saccule, utricle, and lagena are found (**Figure 2**).

The semicircular canals are aligned in the *X*, *Y*, and *Z* axes of the fish (**Figure 1**(**b**)) for the detection of

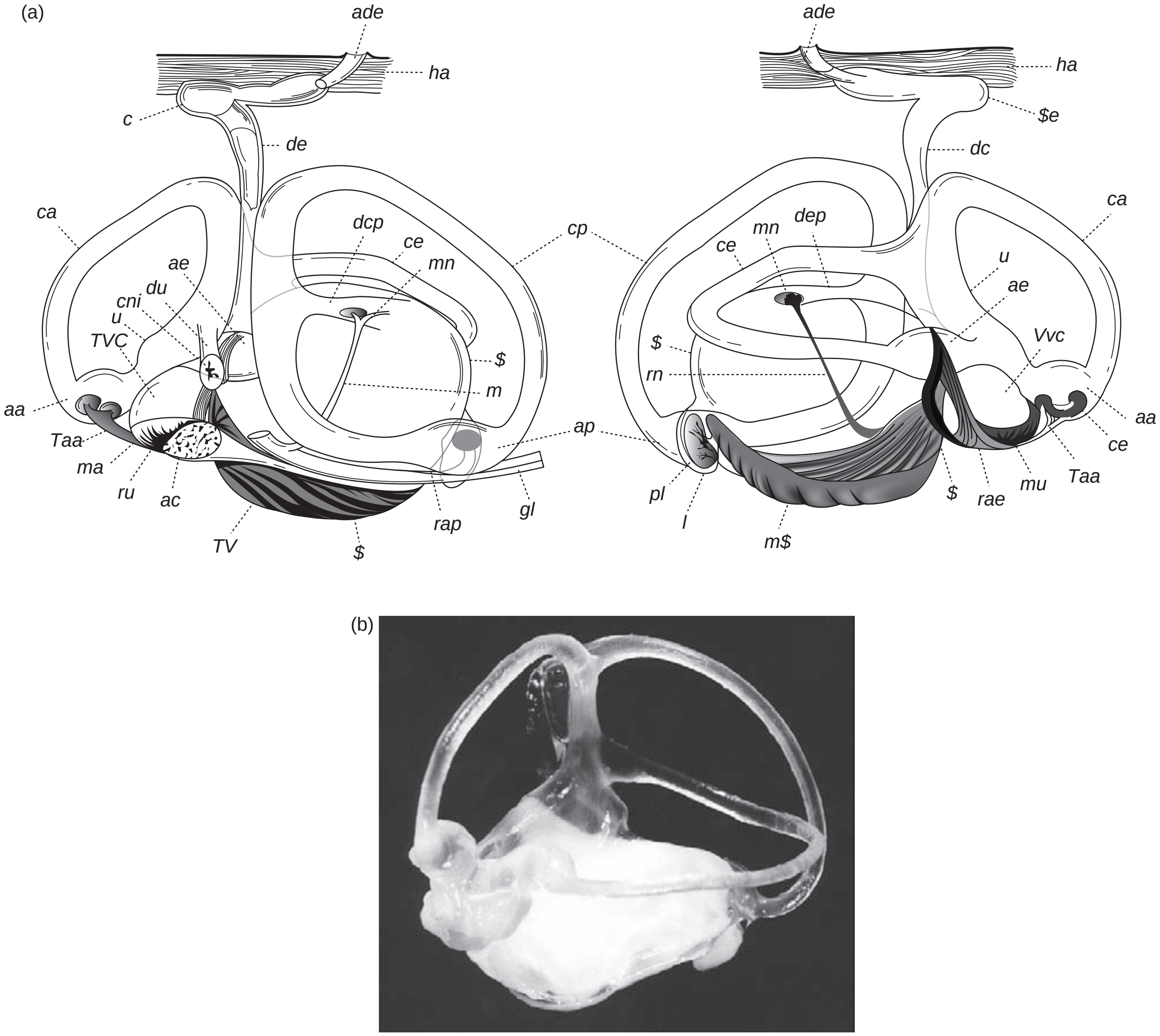


Figure 1 (a) Medial and lateral views of the inner ear of the thornback ray, *R. clavata*. aa – ampulla of anterior canal; ac – acoustic nerve; ade – opening of endolymphatic duct; ae – ampulla of horizontal canal; ap – ampulla of posterior canal; ca – anterior semicircular canal; ce – horizontal semicircular canal; cp – posterior semicircular canal; de – endolymphatic duct; dcp – posterior canal duct; ha – chondrocranium; l – lagena; mn – macula neglecta; mu – utricule macula; pl – lagena macula; raa – ramus anterior ampulla; rap – ramus posterior ampulla; rn – ramus neglectus; rs – ramus sacculus; ru – ramus utriculus; s – saccule; se – endolymphatic sac; u – utricule. (b) Photo of dissected ear of the spotted estuary smooth hound shark, *Mustelus lenticulataus*. Note: Endolymphatic duct was lost during dissecting process. (a) From Retzius G (1881) *Das Gehö rorgan der Wirbelthiere*, vol I. Stockholm: Samson and Wallin. (b) Photo used with permission from John C. Montgomery.

movement in three dimensions (**see also Hearing and Lateral Line:** Vestibular System Anatomy and Physiology). Each semicircular canal has a wider region, called the ampula, which contains a jelly-like cupula overlying a patch of sensory hair cells. As fluid moves through the semicircular canals in response to angular accelerations, the cupula moves causing bending of the hair cells and transmission of information to the brain (**see also Hearing and Lateral Line:** Auditory System Morphology and Vestibular System Anatomy and Physiology).

Elasmobranchs are unique in that they have specialized structures, the endolymphatic ducts, that connect from the saccular chambers to the outside environment (**Figure 2**). The role of these ducts is unclear with regard to hearing, though they may serve a role in otoconial growth and development. The composition of the otoconia in several species of elasmobranchs shows evidence of an exogenous origin for some of the minerals that compose these gelatinous structures. The logical conclusion would be that elasmobranchs are somehow able to facilitate the

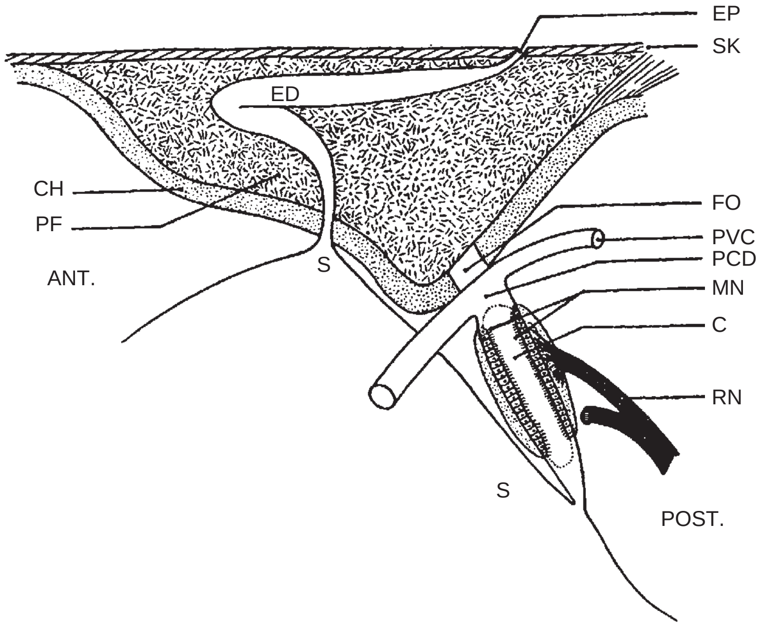


Figure 2 Dorsal portion of elasmobranch ear focusing on the location and relationship of macula neglecta and fenestra ovalis to the parietal fossa. The figure illustrates the nonotolithic pathway of sound travel to the inner ear, in which sound travels through the loose connective tissue of the parietal fossa, impinging on the membranous fenestra ovalis. The movement of this membrane results in the flow of fluid through the posterior canal duct, pushing against the cupula, and stimulating the hair cells of the macula neglecta. C – cupula; CH – chondrocranium; ED – endolymphatic duct; EP – endolymphatic pore; FO – fenestra ovalis; MN – macula neglecta; PCD – posterior canal duct; PVC – posterior vertical canal; PF – parietal fossa; RN – ramus neglectus nerve; S – saccule; SK – skin covering fossa. Modified from Fay RR, Kendall JI, Popper AN, and Tester AL (1974) Vibration detection by the macula neglecta of sharks. *Comparative Biochemistry and Physiology A* 47: 1235–1240, with permission from Elsevier.

movement of particles through the endolymphatic duct, possibly through the contraction of paired muscles connected to the ducts, to increase the mass of otoconial structures. This idea is supported by the presence of sand grains throughout the duct in several species.

In the 1950s, a series of experiments with the thornback ray, *Raja clavata*, were conducted showing that the semicircular canals, along with the lagenar macula and part of the utricular macula, were sensitive to angular accelerations associated with body movements. Conversely, a portion of the utricular macula, all of the saccular macula and the macula neglecta responded to vibrations, and were the likely inner ear end organs responsible for acoustic detection.

The anatomy of the shark inner ear has been examined in great detail, resulting in two hypotheses about the pathways of sound traveling to the inner ear. The otolithic pathway involves the inner ear otoconial end organs (saccule, utricle, and lagena). As the density of a shark's body is approximately equal to that of the surrounding water, sound travels through the shark until it comes in contact with the otoconia, which have a greater density than surrounding tissues in the ear (**see also Hearing and Lateral Line:** Auditory System Morphology). Due to their greater density, the otoconia lag in movement relative to the rest of the body in the sound field. This time lag causes a bending of the cilia on the hair cells and stimulates the ear (**see also Hearing and Lateral Line:** Auditory System Morphology).

The nonotolithic pathway for stimulation of the inner ear involves the macula neglecta. It has been hypothesized that sound travels from above the head, through an area of loose connective tissue above the otic capsule called the parietal fossa, and into the posterior canal duct via the fenestrae ovalis, a membrane on the chondrocranium that separates the parietal fossa region from the macula neglecta in the duct (**Figure 2**). As fluid flows across the macula neglecta, movement of the cupula bends the ciliary bundles. The ciliary bundles of the macula neglecta are directionally polarized in the dorsal/ventral direction (**Figure 3**(**b**); **see also Hearing and Lateral Line:** Auditory System Morphology), further supporting the idea of sound detection from above the head. This pathway has been tested by directly vibrating the parietal fossa while recording from the part of the eighth auditory nerve that receives signals from the macula neglecta. Increased numbers of action potentials were obtained by stimulation of the parietal fossa region compared with stimulation of any other area of the head of the sharks.

Variations in inner ear anatomy exist among elasmobranchs, possibly reflecting differences in feeding behavior and habitat. In general, a more sensitive auditory system would be advantageous for a pelagic, actively swimming

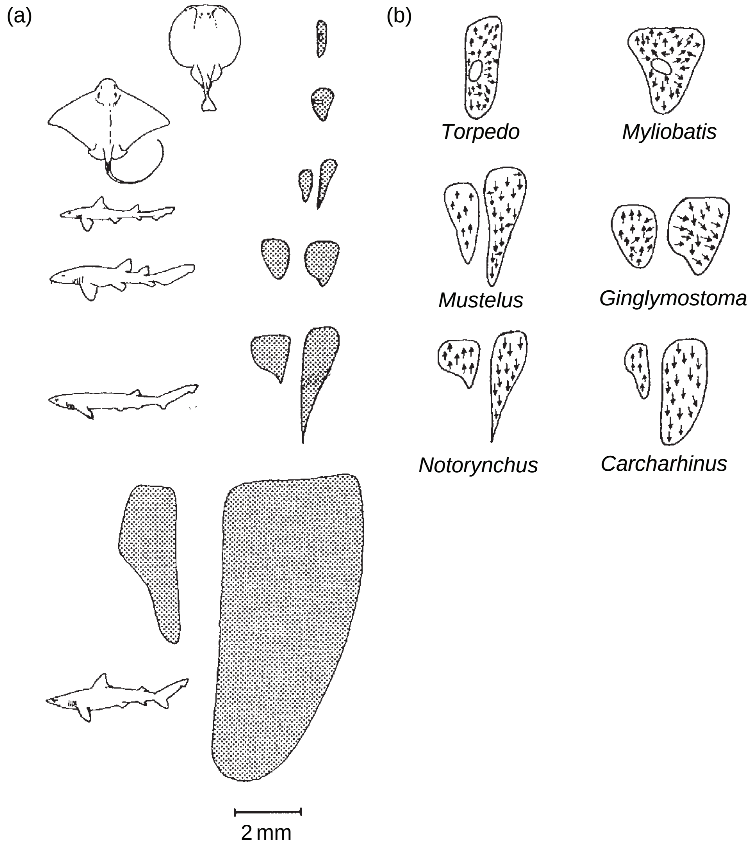


Figure 3 (a) Relative size of sensory epithelium of the macula neglecta for two active, piscivorous elasmobrancs, *Notorynchus* and *Carcharhinus*, and four demersal or nonpiscivorous elasmobranchs*, Myliobatis*, *Torpedo*, *Mustelus*, and *Ginglymostoma*. Note: Body size of all species has been normalized and does not reflect relative size scales. (b) Hair cell orientation patterns (arrows) of the macula neglecta of the same six groups of elasmobranchs from (a). The orientation patterns become more organized as the lifestyle of the species change from benthic to active/piscivorous. Reproduced from Corwin JT (1978) The relation of inner ear structure to the feeding behavior in sharks and rays. In: Becker RP and Johari O (eds.) *Scanning Electron Microscopy II*, pp. 1105–1112. Chicago, IL: Scanning Electron Microscopy, Inc, with permission of Scanning Electron Microscopy, Inc., copyright 1978.

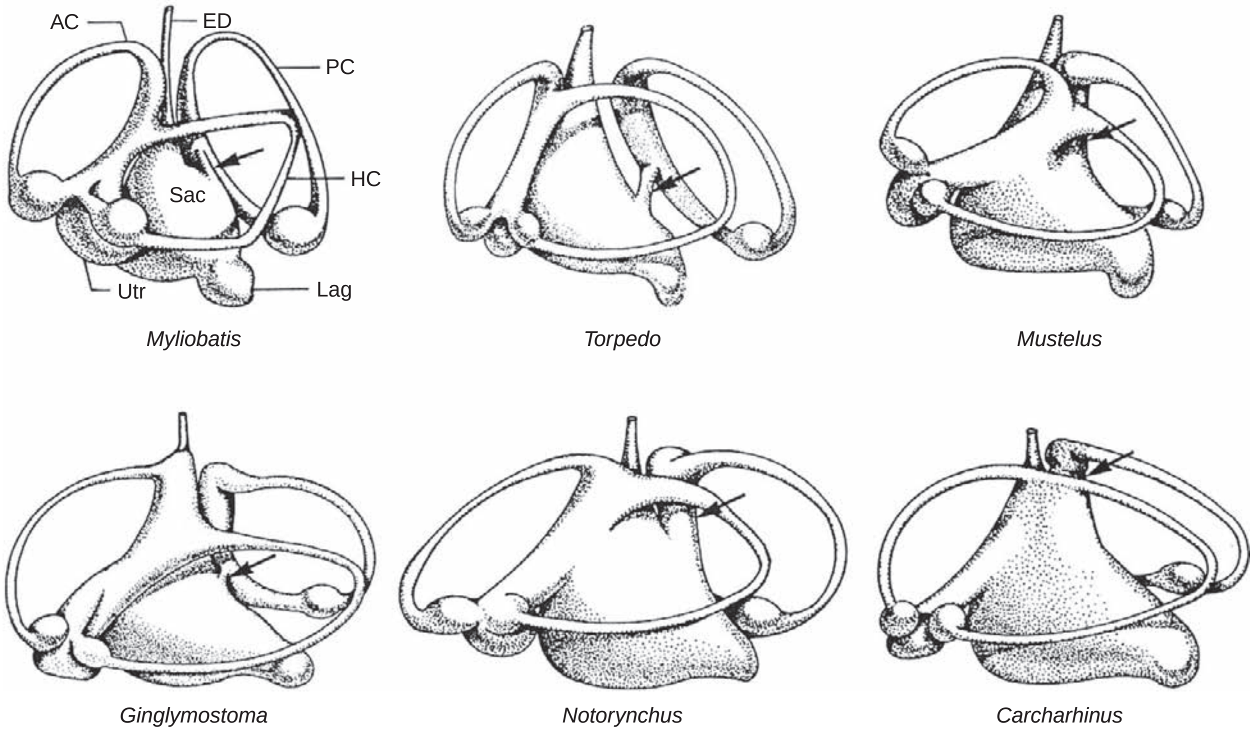
lifestyle, whereas demersal elasmobranchs tend to hunt prey living on or buried in the substrate and likely use other senses (i.e., electroreception, lateral line, and olfaction) to locate their food (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior and **Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs). Correspondingly, pelagic elasmobranchs have a larger macula neglecta than demersal groups, with higher numbers of sensory hair cells spread out over two epithelial surfaces (**Figure** 3(**a**)). These patches of hair cells are organized into two oppositely polarized groups, with one epithelial surface in the dorsal direction and the other in the ventral (**Figure** 3(**b**)). This pelagic group also possesses a larger saccule than demersal elasmobranchs, as well as a larger, more dorsally positioned posterior canal duct and a shorter endolymphatic duct that positions the whole ear closer to the surface of the head (**Figure** 4). The more dorsally located positioning of these ear structures is speculated to lead to an increased ability to detect sounds from the anterior and dorsal directions.

Conversely, demersal elasmobranchs are characterized by a smaller, single epithelial patched macula neglecta (**Figure** 3(**a**)). The hair cells are unorganized and found in fewer numbers than in pelagic species (**Figure** 3(**b**)). Demersal species also have a smaller, more ventrally positioned saccule and posterior canal duct, a longer and narrower endolymphatic duct, and the ear is much farther from the surface of the head due to a deeper or broader parietal fossa (**Figure** 4). These generalizations, however, have been explored in only a few of the extant elasmobranchs. Moreover, cases of intermediate variations between these two categories are found in elasmobranchs that are more euryphagous (feed on fish as well as benthic organisms).

Auditory Sensitivity

Acoustic Attraction Studies

The general methodology to use sound to attract sharks to a location involves powerful underwater speakers to present a wide variety of sounds, varying the frequency



Myliobatis *Torpedo* *Mustelus*

Ginglymostoma *Notorynchus* *Carcharhinus*

Figure 4 Lateral views of elasmobranch labyrinths. Arrows show location and size of posterior canal duct. Note: In the two active, piscivorous elasmobranchs *Notorynchus* and *Carcharhinus*, the larger diameters and more dorsal locations of the ducts compared with the more demersal or nonpiscivorous *Myliobatis*, *Torpedo*, *Mustelus*, and *Ginglymostoma*. AC – anterior semicircular canal; ED – endolymphatic duct with dorsal portion cutoff; HC – horizontal semicircular canal; Lag – lagena; PC – posterior semicircular canal; Sac – saccule; Utr – utricle. Reproduced from Corwin JT (1978) The relation of inner ear structure to the feeding behavior in sharks and rays. In: Becker RP and Johari O (eds.) *Scanning Electron Microscopy II*, pp. 1105–1112. Chicago, IL: Scanning Electron Microscopy, Inc, with permission of Scanning Electron Microscopy, Inc., copyright 1978.

(25–1000 Hz), intensity, duration, tonal (pure tone and broadband noise) versus pulsed and regular versus irregular intervals. In total, 18 different species of sharks (including representatives of family Carcharhinidae, Ginglymostomatidae, Sphyrnidae, Lamnidae, and Alopiidae) were reported to be attracted to auditory signals produced through these speakers. Lower frequencies, with erratically pulsed tones, were the most attractive, with some sharks traveling as far as 400 m to the speaker location. That these erratically pulsed sounds closely mimick the sounds of wounded or distressed fishes, thought to attract sharks, was tested by playing back actual recordings of thrashing fishes as well as the sounds of soniferous fishes. All of these sounds attracted sharks. In cases where sharks were attracted from larger distances, the sound levels were loud enough that similar sounds would likely never be experienced in the sharks' native environments. Sounds played at ambient recorded levels attracted sharks only from <5 m.

While the field is a more ideal location for acoustic behavioral studies, lab studies can answer specific questions. The first behavioral study performed on sharks involved the simple stimulus of knocking on the side of a tank to elicit a startle response in a dogfish, *Mustelus canis*. Severing the eighth auditory nerve completely eliminated this response, providing the first evidence of hearing in elasmobranchs. Decades later, when repeated with a cat shark, *Scyliorhinus cannicula*, severing of the auditory nerve raised the threshold of detection. Lemon sharks, *Negaprion brevirostris*, discriminate between 40 and 60 Hz and orient to speakers placed as close as 9.5° of orientation in a circular pool.

Auditory Threshold Measurements

Most laboratory studies involving elasmobranch hearing have measured auditory thresholds or made more finite measurements of one or more of the individual end organs. These thresholds helped to estimate the range of frequencies (bandwidth) that elasmobranchs can detect as well as the lowest detectable signal by the auditory system.

The macula neglecta always has attracted interest to understand its role. Inspired by the experiment mentioned earlier that involved stimulation of the parietal fossa to elicit a response from the macula neglecta, two similar studies found that stimuli focused on the parietal

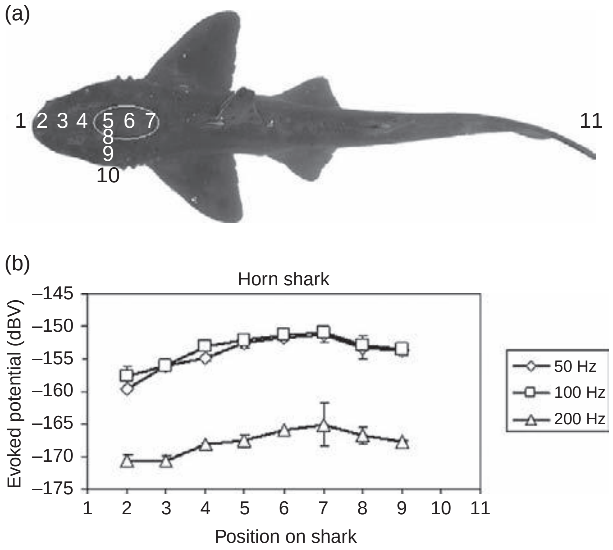


Figure 5 (a, b) Overhead view of the horn shark, *H. francisi*, depicting the different locations stimulated by the dipole stimulus. The oval surrounding locations 5, 6, and 7 depict the parietal fossa region and areas yielding the strongest evoked potential from the dipole stimulus.

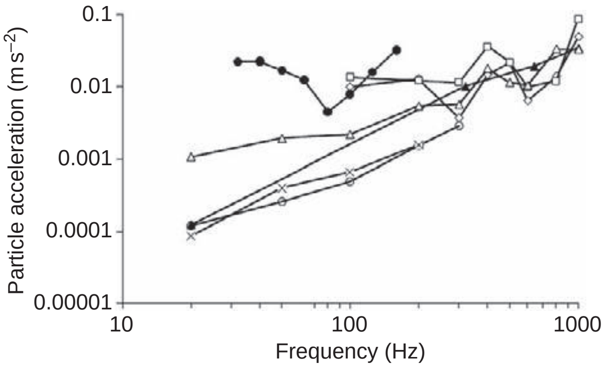


Figure 6 Particle acceleration audiograms of elasmobranchs in response to monopole (underwater speaker) and dipole (vibrating bead) sound stimuli. Open diamond: *G. cirratum*. Open square: *U. jamaicensis*. Open triangle: *R. terraenovae*. ×'s: *Chiloscyllium griseum*-Dipole. Open circle: *Heterodontus francisci*-Dipole. Filled triangle: *N. brevirostris*. Filled circle: *Heterodontus francisci*. The open shapes and ×'s are elasmobranch audiograms obtained using AEPs and the filled shapes are audiograms obtained using classical conditioning methods. Modified from Casper BM and Mann DA (2009) Field hearing measurements of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. *Journal of Fish Biology* 75: 2768–2776, with permission of John Wiley and Sons.

fossa yielded the strongest response from the elasmobranch auditory system. In both cases, without making contact with the skin, the stimuli utilized were an airborne sound source focused on the parietal fossa with the lemon shark, *N. brevirostris*, as well as an underwater vibrating ball to simulate a dipole acoustic stimulus with the horn shark, *Heterodontus francisi* (**Figure 5**).

Hearing thresholds are known from behavioral and electrophysiological studies, but for only a few of the extant elasmobranchs. The earliest audiogram measurements used operant conditioning methods on the bull shark, *Carcharhinus leucas*, and *N. brevirostris*, revealing a range of frequencies of 100–1500 Hz for *C. leucas* and 10–600 Hz for *N. brevirostris*. However, given that the thresholds were obtained with hydrophones and referenced to sound pressure, they do not provide an accurate reflection of auditory thresholds in a group of fishes that detect particle motions and not sound pressure. Lacking any type of air-filled bubble to act as a pressure-to-displacement transducer, elasmobranchs can only detect the particle motion component of sound directly with their inner ears (**see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes, Psychoacoustics: What Fish Hear). Sound pressure thresholds for the little skate, *Raja erinacea*, ranged from 100 to 1000 Hz.

The first particle motion-sensitive audiograms were recorded in the lemon shark using a classical conditioning paradigm and revealed what would be a continuing trend in elasmobranchs, that lowest thresholds were detected in the lowest frequencies measured, with decreasing sensitivities as the frequencies increased (**Figure 6**). Using similar methods, thresholds were obtained in the horn shark but ambient noise levels during experimentation were likely loud enough to result in masking (or artificially raising) of the lower frequencies tested (**see also Hearing and Lateral Line:** Psychoacoustics: What Fish Hear).

Recently, the introduction of the auditory evoked potential (AEP) electrophysiological method has provided the opportunity for measuring hearing in larger numbers of fishes over a shorter period of time than using behavioral methods. Audiograms using AEP have been obtained in the yellow stingray, *Urobatis jamaicensis*, the nurse shark, *Ginglymostoma cirratum*, and the Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (**Figure 6**).

The lemon and sharpnose sharks, which were the two most piscivorous, nondemersal elasmobranchs, have the lowest thresholds, especially at frequencies below 100 Hz. This further supports the anatomical observations that pelagic elasmobranchs could have more sensitive hearing than demersal species. Audiograms are displayed in terms of particle acceleration, which is the likely component of particle motion to stimulate the mass-loaded otoconial end organs.

All of the audiograms described above used an underwater speaker for presenting the acoustic stimuli to the elasmobranchs. An underwater speaker is considered to be a monopole stimulus, in which sound is radiated equally in all directions. A monopole, such as bioacoustical sounds produced by a vibrating fish swimbladder (air-filled cavity within fishes that functions primarily for buoyancy control and, in some fishes, has secondarily evolved vibratory muscles along the bladder that act as a mechanism to produce sounds for communication; **see also Hearing and Lateral Line:** Acoustic Behavior, Vocal Behavior of Fishes: Anatomy and Physiology, **Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling) is often thought to inaccurately portray the majority of acoustic stimuli that an elasmobranch might be detecting in the environment. Dipolar stimuli, such as the low-frequency oscillations created by fishes swimming in the water, are suggested to be more biologically relevant. A study using a dipole stimulus yielded significantly lower thresholds than using a monopole source (**Figure 6**). Although it is unclear if different end organs being stimulated in each study might account for the difference in thresholds, it certainly raises the possibility of dipole stimuli having the potential to be more attractive to sharks.

Discrepancies between Hearing Thresholds and Attraction Experiments

The field attraction studies show that sharks can localize acoustic stimuli from distances up to several hundred meters. In contrast, the hearing thresholds measured in several elasmobranchs suggest that these fishes are capable of hearing sounds, but likely not if the animals are too far away. Particle motion, the component of sound which elasmobranchs can detect, is dominant in the near field (approximately one wavelength from the stimulus source) and then quickly attenuates with distance where the propagating sound pressure wave dominates. For example, the near field of a 100 Hz signal is ~15 m and only ~1.5 m for 1000 Hz, assuming an ideal sound field. The lowest frequency broadcasted in the attraction studies was 25 Hz and would have a near field of ~60 m, certainly less than some of the distances reported. Another member of the genus of *Rhizoprionodon, Rhizoprionodon porosus*, was one of the sharks attracted to sounds in two of the studies. Far-field sound pressure levels estimated from the thresholds of *R. terraenovae* can be compared with the sound levels produced during the field attraction studies involving *R. porosus*. The *R. terraenovae* audiograms were shifted by an estimated critical bandwidth that is assumed to be 10% of the center frequency (i.e., the threshold is lowered by 10 log for direct comparison to the broadband noise stimuli used in the field attractionstudies (**Figure 7**)). Based on the sound pressure thresholds of *R. terraenovae*, it appears that the sound levels from the field attraction experiments were well below the hearing thresholds of the species. These results, however, need to be put into context. *R. terraenovae* was not one of the sharks specifically observed in the attraction studies, and could possibly have different thresholds than *R. porosus*. Moreover, many of the species observed in the attraction studies are pelagic, larger, and more piscivorous than any of the species in which audiograms exist and likely have subtle differences in the ear anatomy that might convey more enhanced hearing abilities than benthic species.

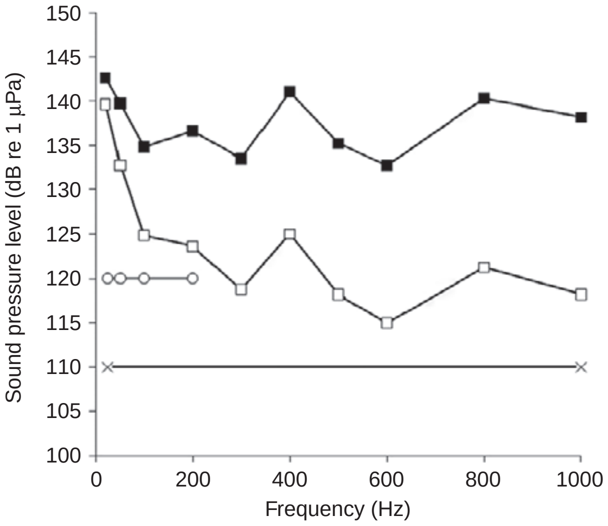


Figure 7 The sound pressure needed to produce particle accelerations equivalent to the Atlantic sharpnose shark, *R. terraenovae*, audiogram in a plane propagating wave (filled square symbols) and this same audiogram shifted by a critical bandwidth to compare with broadband noise signals (open square symbols). The sound pressure levels of broadband noise used in two field attraction experiments in which members of the *Rhizoprionodon* genus were attracted are plotted for comparison, suggesting that *R. terraenovae* would not have been able to detect the sound stimuli being presented. Distances from the sound source to the hydrophone for measurements of sound pressure level were 1 m for Richard (1968) (open circle symbols) and the *R. terraenovae* experiment, while they were made at 18.5 m for Myrberg *et al*. (1969) (× symbols). Reproduced from Casper BM and Mann DA (2009) Field hearing measurements of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. *Journal of Fish Biology* 75: 2768–2776, with permission of John Wiley and Sons.

There are a variety of possible explanations for these observed discrepancies with *R. terraenovae.* It has been suggested that AEP measurements underestimate hearing thresholds in fishes compared to behavioral training methods. Another possible explanation could be that other stimuli may have attracted sharks to the testing locations. For example, in many of the test trials, sharks appeared to be following other fishes into the test area. Several of these fishes, including snapper and grouper, have swimbladders and likely can detect lower level sounds than *R. porosus.* It has also been observed in many of the field attraction experiments that the sharks responded erratically with agitated behaviors close to the speakers, which was likely due to the strong electromagnetic fields these speakers produce. This erratic behavior provides another strong visual stimulus to which other sharks could respond.

Summary

The first experiment to show that sharks can detect sounds was conducted over a century ago, yet, with exception to anatomical features, little is known about elasmobranch audition. Much of what is assumed about elasmobranch hearing is derived from just a few species, and certainly not a reflection of their wide range of body types, behaviors, and ecomorphotypes. Scant information exists on any aspect of audition for any of the larger, oceanic species. Enough observational data exist suggesting that sharks can detect loud, low-frequency sounds. However, beyond a variety of hypotheses, very little experimental data have been compiled to answer how these fishes are able to locate sound sources from any kind of distance or direction. Elasmobranchs provide the opportunity to study an ancestral stage in the evolution of hearing in vertebrates and represent a field of study ripe for further exploration.

See also: **Brain and Nervous System:** Functional Morphology of the Brains of Cartilaginous Fishes. **Detection and Generation of Electric Signals:** Morphology of Electroreceptive Sensory Organs. **Hearing and Lateral Line:** Acoustic Behavior; Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Psychoacoustics: What Fish Hear; Vestibular System Anatomy and Physiology; Vocal Behavior of Fishes: Anatomy and Physiology. **Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling. **Smell, Taste, and Chemical Sensing:** Chemosensory Behavior.

Further Reading

Barry MA (1987) Afferent and efferent connections of the primary octaval nuclei in the clearnose skate, *Raja eglanteria. Journal of Comparative Neurology* 266: 457–477.

Casper BM and Mann DA (2007) Dipole hearing measurements in elasmobranch fishes. *Journal of Experimental Biology* 210: 75–81.

Casper BM and Mann DA (2009) Field hearing measurements of the Atlantic sharpnose shark *Rhizoprionodon terraenovae*. *Journal of Fish Biology* 75: 2768–2776.

Corwin JT (1978) The relation of inner ear structure to the feeding behavior in sharks and rays. In: Becker RP and Johari O (eds.) *Scanning Electron Microscopy II*, pp. 1105–1112. Chicago, IL: Scanning Electron Microscopy, Inc.

Corwin JT (1981) Peripheral auditory physiology in the lemon shark: Evidence of the parallel otolithic and non-otolithic sound detection. *Journal of Comparative Physiology* 142: 379–390.

Corwin JT (1983) Postembryonic growth of the macula neglecta auditory detector in the ray, *Raja clavata*: Continual increases in hair cell number, neural convergence, and physiological sensitivity. *Journal of Comparative Neurology* 217: 345–356.

Corwin JT (1989) Functional anatomy of the auditory system of sharks and rays. *Journal of Experimental Zoology Supplement* 2: 62–74.

Hueter RE, Mann DA, Maruska KP, Sisneros JA, and Demski LS (2004) Sensory biology of elasmobranchs. In: Carrier JC, Musick JA, and Heithaus MR (eds.) *Biology of Sharks and Their Relatives*, pp. 325–368. Boca Raton, FL: CRC Press.

Kalmijn AD (1988) Hydrodynamic and acoustic field detection. In: Atema J, Fay RR, Popper AN, and Tavolga WN (eds.) *Sensory Biology of Aquatic Animals*, pp. 83–130. New York: Springer.

Lowenstein O and Roberts TDM (1951) The localization and analysis of the responses to vibration from the isolated elasmobranch labyrinth. A contribution to the problem of the evolution of hearing in vertebrates. *Journal of Physiology* 114: 471–489.

Myrberg AA, Jr. (1978) Underwater sound – its effect on the behaviour of sharks In: Hodgson ES and Mathewson RF (eds.) *Sensory Biology of Sharks, Skates and Rays*, pp. 391–417. Washington, DC: US Government Printing Office.

Myrberg AA, Jr. (2001) The acoustical biology of elasmobranchs. *Environmental Biology of Fishes* 60: 31–45.

Tester AL, Kendall JI, and Milisen WB (1972) Morphology of the ear of the shark genus *Carcharhinus*, with reference to the macula neglecta. *Pacific Science* 26: 264–274.

Biomechanics of the Inner Ear in Fishes

MC Hastings, Georgia Institute of Technology, Atlanta, GA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Propagation of Sound Underwater
Interaction of Sound with Fishes
Mechanics of the Hearing Process
Summary and Conclusions
Further Reading

Glossary

Acoustic particle velocity Fluctuation in the velocity of fluid particles that is caused by the fluctuating forces exerted on the fluid by acoustic pressure.

Acoustic pressure Fluctuation of pressure (force per unit area) above and below the ambient pressure in the fluid that is caused by sound.

Attenuation Reduction in acoustic power of a propagating sound wave.

Bandwidth Range of frequencies over which a sound is produced or received.

Far field Region far enough away from a sound source that the acoustic pressure behaves in a predictable way and acoustic particle velocity exists only because of the propagating sound wave.

Free field A region that has no reflections in which sound propagates from a source equally in all directions.

Frequency The number of wave cycles in a time interval (measured in Hz = 1 cycle s^{-1} or kHz = 1000 cycles s^{-1}).

Near field A region close to a sound source that has high levels of acoustic pressure and particle velocity which do not propagate into the far field.

Otolith A structure found in the inner ear made up of calcium carbonate and found in most teleost fishes. Motion of the otolith relative to the sensory cells of the ear results in stimulation of the cells and detection of sounds.

Propagation Movement of a sound wave through a fluid.

Swimbladder Gas-filled chamber in the abdominal cavity of many bony fishes that helps to not only control buoyancy, but can also serve as a sound-receiving structure that enhances auditory capability.

Wavelength The distance between repetitions, or cycles, of the spatial fluctuation of acoustic pressure in a sound wave.

Introduction

Sound is an extremely important means of communication for fishes and other aquatic animals. For example, fishes have the ability to detect low levels of sound that can alert them to an oncoming biological or anthropogenic predator, or help locate a mate (**see also Hearing and Lateral Line**: Acoustic Behavior, Vocal Behavior of Fishes: Anatomy and Physiology, Sound Source Localization and Directional Hearing in Fishes, and Fish Bioacoustics: An Introduction). The functional characteristics of auditory systems in fishes are almost identical to those of auditory systems in terrestrial mammals (**see also Hearing and Lateral Line**: Auditory System Morphology and The Ear and Hearing in Sharks, Skates, and Rays), yet fishes do not have an outer ear, middle ear, or cochlea in their inner ear, as do mammals. This article describes the mechanics of sound propagation in water and the interaction of sound with peripheral auditory systems in fishes. Understanding this interaction is critical to understanding reception of sound by fishes and the biomechanics of their ears.

Propagation of Sound Underwater

In air, sound travels at a speed of approximately $340\,\mathrm{m\,s^{-1}}$ and attenuates significantly with distance; however, underwater it travels with little attenuation and at a speed of about $1500\,\mathrm{m\,s^{-1}}$, and thus provides an effective way to transfer information over long distances. Sound is a mechanical disturbance that propagates as a longitudinal wave in air and water. The descriptor, ‘longitudinal’, means that the local motion of the fluid is parallel to the direction of propagation. As the wave propagates, small volumes of fluid particles at successive locations are compressed and then rarefied, creating a fluctuation in pressure above and below the ambient, respectively. This fluctuation is called the acoustic pressure, p. The rate of change in this fluctuation is described by the frequency, f, which has units of cycles per second or hertz (Hz). Tonal sounds, such as whistles, consist of a single frequency while sounds that are more complex are composed of multiple frequencies.

Sound is a function of both time and space, so p also has a repeated variation from one point to the next as the wave propagates. As pressure exerts a force in the fluid, spatial variation in p creates a differential force that causes fluid motion defined as the acoustic particle velocity; its amplitude, v, is proportional to p.

Repetition of spatial variation in p is described by the acoustic wavelength, λ, which has units of length (meters per spatial cycle). Wavelength is equivalent to the sound speed, c, divided by f: $\lambda = cf^{-1}$. So, at the same frequency, wavelength of sound underwater is nearly 4.5 times longer than the wavelength in air. **Figure 1** shows the relationship between λ and f in water.

Sounds range from the quietest whispers to the loudest explosions, so p is expressed on a logarithmic scale, called the decibel (dB) scale, by comparing it to a reference sound with pressure amplitude $p_0 = 2 \times 10^{-5}$ Pa in air and $p_0 = 1 \times 10^{-6}$ Pa in water ($1\,\mathrm{Pa} = 1\,\mathrm{N\,m}^{-2}$). The reference pressures are usually expressed in micropascals (μPa) where 2×10^{-5} Pa $= 20\,\mu$Pa and 1×10^{-6} Pa $=$ 1 μPa. Sound pressure level (L_p) is then defined as $L_\mathrm{p} = 10\ \log\ (p^2/p_0^2)$ dB re p_0, where 're' means 'with reference to'. For example, if $p = 1$ Pa in water then $L_\mathrm{p} = 10\ \log\ ((1\,\mathrm{Pa})^2/(10^{-6}\ \mathrm{Pa})^2) = 120$ dB re 1 μPa, while $p = 1$ Pa in air results in $L_\mathrm{p} = 94$ dB re 20 μPa.

The acoustic pressure and particle velocity combine to produce a flow of acoustic power associated with the passage of a sound wave. Power is the rate of change of energy, and sound power, W, is measured in watts, as is electrical power ($1\,\mathrm{W} = 1\,\mathrm{J\,s}^{-1}$; $1\,\mathrm{J} = 1\,\mathrm{N\,m}$). The amount of sound power is very small, so the reference power is $W_0 = 1 \times 10^{-12}$ W for both air and water. Sound power level (L_W) is then $L_\mathrm{W} = 10\ \log(W/W_0)$ dB re W_0.

Sound Sources

There must be motion of a surface in contact with the fluid to produce sound. Outward motion of this surface moves fluid particles and causes a local compression in the small volume of fluid it touches. This triggers propagation of a wave because pressure increases when the fluid is compressed, and the force due to increased pressure pushes on adjacent fluid causing it to move. Motion of adjacent fluid particles allows the originally compressed particles at the interface with the surface to move into the adjacent volume, which then causes pressure to increase at that location while decreasing in the originally compressed region. This process repeats itself as the wave propagates away from the moving surface, or source.

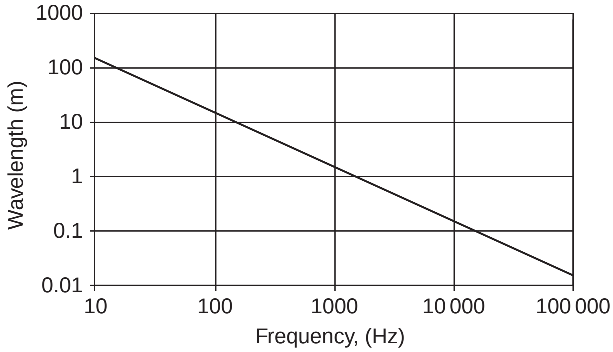


Figure 1 Frequency wavelength chart showing the decrease in wavelength of sound with increasing frequency in water with a sound speed of 1500 m s^{-1}.

The region very close to a sound source is called the 'near field'. In the near field, acoustic particle motion is controlled by a slug-type flow of fluid mass being pushed back and forth by the moving surface. Both p and v are very large near the surface of the source and then spatially decrease until the wave reaches a point far enough away that its propagation is controlled by the characteristics of the fluid and surrounding boundaries. The region at this point and beyond is called the 'far field' of the source. In practical terms, the far field occurs at a distance from the source that is much larger than any physical dimension of the source and is much larger than λ.

The far field should not be confused with an acoustic 'free field'. The term, free field, refers to the surrounding fluid, while far field (or near field) refers to the source. In a free field, the fluid is unbounded and there are no interactions of sound with boundaries, objects, or interfaces, such as the water surface or bottom. A free field is somewhat theoretical; however, it is usually a valid assumption for sound propagation in the middle of the deep ocean, or in situations where λ is very small with respect to the distance between the source and fluid boundaries. A free field is also a good assumption in situations where sound can propagate without interference until the acoustic pressure cannot be distinguished from background noise. In a free field, p and v are related by the characteristic acoustic impedance of the fluid, which is the product of c and fluid density, ρ. This relationship can be expressed as $p = \rho c v$.

When sources are much smaller than the wavelength of sound produced, they can be modeled as simple acoustic point sources. The simplest point sources are an acoustic monopole and dipole. The former is equivalent to a pulsating sphere that produces an outgoing spherically symmetric sound wave at constant frequency in an unbounded fluid. The latter can be realized as a small rigid sphere that oscillates back and forth at constant frequency in an unbounded fluid and produces a sound wave that is not spherically symmetric. The slug flow associated with the near field falls off with the square of the distance from a monopole and with the cube of the distance from a dipole. In the far field of both monopoles and dipoles, the acoustic pressure and particle velocity are in phase and decrease with distance from the source.

There are many moving biological objects, such as the tail of a swimming fish or an insect on the water surface, that create low-frequency waves in water. However, these waves usually do not propagate as sound into the far field because they lack sufficient power. They are local hydrodynamic disturbances detectable at close range by the inner ear and/or lateral line (**see also Hearing and Lateral Line**: Lateral Line Structure, Lateral Line Neuroethology). Hydroacoustic disturbances may appear to be hydrodynamic, very close to the source, and they also propagate into the far field as sound. Examples of hydroacoustic sources include underwater turbines and ship propellers. Whole-body motion of a large swimming animal and a pulsating swimbladder (gas-filled chamber, or chambers, located in the abdomen of most all species of fish) are examples of biological hydroacoustic sources that are often modeled as an acoustic dipole and monopole, respectively.

Transmission Loss

Transmission loss is the ratio of transmitted acoustic power to incident acoustic power. It can be calculated as the difference between the sound energy level at the source and the received (or incident) sound energy level at some distance from the source. In water, sound travels much faster than the strongest ocean currents or mountain streams. These convective motions do not disrupt sound propagation in water as wind does in air, so water is considered a quiescent medium for propagation of sound. Even so, sound energy is lost during propagation because of absorption, spreading, refraction, scattering, and reflection off the surface and bottom. Absorption occurs at the microscopic level when acoustic energy is converted into heat through viscous effects. Although absorption plays a role in propagation of high-frequency signals associated with ship sonar systems and marine mammal echolocation, in the frequency range of hearing in most fishes (**Hearing and Lateral Line**: Psychoacoustics: What Fish Hear), absorption is negligible.

As a wave propagates away from a source, it usually travels radially in all directions as long as it does not encounter a change in fluid characteristics (e.g., temperature), interface with another fluid (such as air), or a solid object. This radial propagation spreads the acoustic energy of the source over larger and larger spherical surfaces that have radii equal to the distance from the source. This is called 'spherical spreading', and p decreases by one-half for every doubling of distance from the source. When the radiated acoustic energy reaches either the surface or the bottom of the water, it is generally reflected back. So, as the wave continues to propagate further from the source, acoustic energy tends to be confined between the surface and bottom and the spreading becomes cylindrical rather than spherical.

Refraction is the bending of a sound wave caused by a change in sound speed. This occurs when sound is transmitted through an interface between two media with differing sound speeds or when a sound-speed gradient is present. Sound speed increases and decreases with temperature, and vertical temperature gradients exist in bodies of freshwater and in the ocean because water layers near the surface are affected by seasonal changes and weather. Sound waves always bend toward the direction of lower sound speed, which causes sound to travel in a curved trajectory rather than a straight path. Refraction by sound-speed gradients in the ocean and large freshwater lakes causes ducting and channeling of sound. These phenomena produce acoustic convergence zones and shadow zones where sound reception is very good and very poor, respectively.

Scattering of sound underwater is analogous to scattering of light in air. One sees objects in air because light that is scattered from them is received by the eye. Likewise, objects underwater can be detected by sound that is scattered from them. Simply put, scattered sound is secondary, or reradiated, sound that would not be present if the scattering object was there. Fish scatter sound, as do most other solid and gaseous objects in the water. Scattering increases transmission loss; however, it also provides acoustic signals related to the environment that fish potentially detect and use to accomplish many necessary life functions, such as navigating in and around objects, avoiding predators, and finding prey or mates (**Hearing and Lateral Line**: Acoustic Behavior).

In many fish habitats (e.g., coral reefs), sound waves interact with the air–water interface at the surface and the sediment and substrate at the bottom. At the surface, the sound wave is nearly perfectly reflected; however, the reflected wave is out of phase with the incident wave. Therefore, p is nearly 0 Pa at the surface, but not for the reflected wave that travels downward through the fluid. At the bottom, sound is partially reflected from hard (e.g., rocky) sediments, absorbed by soft (e.g., muddy) sediments, and scattered by other types (e.g., pebbles and sand). Each surface or bottom interaction attenuates incident sound and increases transmission loss.

Standing Waves and Propagation of Sound in Shallow Water

Most fishes live either in freshwater rivers and lakes, or on the continental shelves in the ocean where maximum water depths are about 200 m. For acoustic frequencies relevant to fishes (**see also Hearing and Lateral Line**: Acoustic Behavior, and Vocal Behavior of Fishes: Anatomy and Physiology), these are shallow-water environments where sound can propagate over distances greater than the water depth only by repeatedly interacting with the surface and bottom. Thus, shallow water is a very noisy and acoustically complex environment that makes understanding the reception of sound by fishes very difficult.

At locations where the ratio of water depth to λ is less than about 100, sound does not propagate freely; instead, it becomes contained in the water column via vertical standing waves called 'modes'. For example, this occurs when the water depth is less than 300 m for sound with $f = 500\,\text{Hz}$. Each mode has a different spatial frequency (cycles per meter), resulting in a large number of vertically oriented pressure minima and maxima. This makes it difficult to predict or monitor sound pressure received by an animal. These variations also create pressure gradients that result in larger acoustic particle motions than would be expected for freely propagating sound.

Each acoustic mode propagates at its own frequency-dependent speed; however, there exists a characteristic cutoff frequency below which a mode cannot propagate. It follows that no sound can propagate at frequencies below the cutoff frequency for the mode having the lowest spatial frequency. This absolute cutoff frequency is a function of water depth and characteristics of the bottom. For example, the absolute cutoff frequency is around 100 Hz for a sandy bottom and 10 m water depth. Sound at frequencies below the absolute cutoff does exist near its source; however, it cannot propagate into the far field.

Similar to the vertically oriented modes described above, standing waves that do not propagate can exist in areas that have many hard and/or soft surfaces that essentially trap sound. These areas include ponds and lakes, inlets and bays, marinas with docks and piers, coral reefs, and underwater caves. Complex sound fields consisting of stationary standing waves are also a problem in small tanks and aquaria often used for fish-acoustics research. Prior to use in experiments, these chambers need to be carefully calibrated by measuring sound pressure at multiple locations for each frequency and temperature of interest.

Interaction of Sound with Fishes

The primary interaction of sound with fishes depends on the length of the fish with respect to λ. Most fish species hear and produce sounds at frequencies typically below 1000 Hz (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear). With $c = 1500\,\text{m}\,\text{s}^{-1}$, λ will be longer than 1.5 m at these low frequencies, so the majority of fishes are fairly small relative to wavelengths of sound at frequencies of importance to them. This means that when a fish encounters a sound wave, its whole body will experience the same acoustic pressure at each point in time as the wave passes. Moreover, because its body is primarily composed of water, the fish will move at approximately the same amplitude and phase as the impinging sound.

Even though the fish tends to move with the sound wave, not all of its internal organs will move together. Different tissues have different densities and characteristic acoustic impedances (ρc). Denser organs, such as bone, will not move as fast as the surrounding tissue due to inertia, while acoustic particle motion will be much larger in tissues with relatively low acoustic impedance, such as the swimbladder, because $v = (p)(\rho c)^{-1}$.

Mechanics of the Hearing Process

Fishes detect and respond to a wide variety of sounds. The exact structure of the inner ear, however, varies among species so they differ in both the range of frequencies (auditory bandwidth) and the lowest sound-pressure level (auditory threshold) that they are able to detect. The inner ear in fishes lies within the cranial cavity just lateral to the brain (**see also Hearing and Lateral Line**: Auditory System Morphology). The semicircular canals detect angular acceleration in fishes just as they do in terrestrial vertebrates (**see also Hearing and Lateral Line**: Vestibular System Anatomy and Physiology). Furthermore, the otolith organs (utricle, saccule, and lagena) serve as vestibular organs and detect the position of the head in the vertical direction relative to gravity and in other directions relative to acceleration of the body, just as the utricle and saccule do in terrestrial vertebrates. In fishes, however, the otolith organs are also involved in detection of sound (**see also Hearing and Lateral Line**: Auditory System Morphology, The Ear and Hearing in Sharks, Skates, and Rays).

Depending on the species, each otolith organ contains either otoconia or a single, dense calcareous stone (otolith) within a chamber filled with endolymph. A sensory epithelium that contains mechanosensory hair cells innervated by the eighth cranial nerve lies on the chamber wall. Each hair cell has an apical group of projections called the ciliary bundle that extends above the surface of the epithelium in which the cell lies. Between the otolith and sensory epithelium is a thin otolithic membrane that connects to both structures and helps keep them in the same position relative to each other. As fishes move with an underwater sound wave and the otoliths or otoconia are much denser than surrounding tissues, these structures lag behind the motion of the rest of the body. This creates relative motion with the sensory epithelium and causes bending of the ciliary bundles and these stimulate the underlying hair cells to send electrical signals to the brain that provide information about sound (**see also Hearing and Lateral Line**: Auditory System Morphology, The Ear and Hearing in Sharks, Skates, and Rays).

Thus, in all fishes, the otolith organs are stimulated directly by the acoustic particle motion associated with the propagating sound wave. This is referred to as the ‘direct path’ of sound to the inner ear. In fishes that have swimbladders, the otolith organs can be stimulated indirectly by particle motion created when sound-pressure fluctuations are transformed into motion by these gas-filled organs. This is referred to as the ‘indirect path’ for sound to excite the inner ear and results in an additional signal proportional to acoustic pressure.

Role of the Swimbladder

The swimbladder has several functions including respiration, provision of buoyancy, and detection of pressure changes. In addition, the swimbladder is stimulated by acoustic pressure causing its wall to move and generate secondary sound, which is again detectable through relative motion of tissues in the inner ear. Additional stimulation of the inner ear via response of the swimbladder increases auditory bandwidth and hearing sensitivity. In some species, the response of the swimbladder to sound as a function of frequency correlates with auditory sensitivity (i.e., lowest hearing thresholds occur at frequencies where the response of the swimbladder to sound is the highest). This effect, however, diminishes with increasing distance between the swimbladder and inner ear because of transmission loss associated with sound propagation through the intervening tissues.

Auxiliary and Unusual Anatomy That Enhance Hearing

Cyprinids and other otophysan species (e.g., goldfishes, catfishes, and carps) possess a series of three or four modified vertebrae, called the Weberian ossicles, that mechanically couples or connects the swimbladder to the fluids of the ear as illustrated in **Figure 2** (**see also Hearing and Lateral Line**: Fish Bioacoustics: An Introduction). The largest ossicle, the tripus, connects the anterior swimbladder chamber to the second ossicle, the intercalarium that connects anteriorly to the scaphium and, in some species, the claustrum. The scaphium and claustrum, when present, are embedded in the walls of the sinus impar, a fluid-filled canal that projects anteriorly and ultimately communicates with the saccule. Expansion of the swimbladder causes forward motion of the ossicles and fluid flow into the saccular chamber, while compression causes fluid flow out of the saccule. This increases relative motion within the saccule, provides additional stimulation of auditory hair cells, and results in lower auditory thresholds and broader auditory bandwidths in these species.

Several species have evolved a number of different mechanisms to couple gas-filled structures other than the swimbladder to the ear, thereby allowing the auditory system to detect signals proportional to acoustic pressure. Anabantids (gouramis) have a suprabranchial air-filled chamber with a membrane-type window located near the saccule. Mormyrids (freshwater elephant-nose fishes) have gas-filled chambers entirely separated from the swimbladder that are located in the semicircular canals and connected to the surface of the saccule. Hearing in several of these species indicates auditory sensitivity at frequencies up to 5000 Hz. Likewise, some sciaenid species (e.g., Atlantic croaker (*Micropogonias undulatus*) and weakfish (*Cynoscion regalis*)) having a swimbladder with a pair of anterior horns that terminate close to the ear have been found to detect higher frequencies than species lacking such structures. Some Myripristinae (soldierfishes) also have a direct connection between the inner ear and anterior projections of the swimbladder that enhances both their hearing sensitivity and bandwidth.

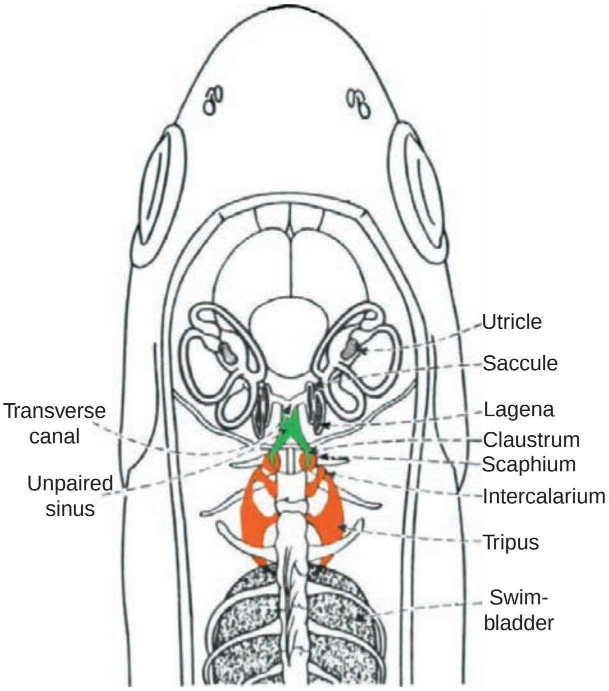


Figure 2 The Weberian ossicles connecting the anterior swimbladder with the inner ear in an otophysan fish.

At least two clupeiform species, American shad (*Alosa sapidissima*) and gulf menhaden (*Brevoortia patronus*), detect ultrasound at frequencies of 100 kHz or higher. In these fishes, the swimbladder, inner ear, and lateral line system are all linked through a pair of bony capsules in the inner ear called auditory bullae. Each bulla is divided into a gas-filled and a liquid-filled part by a membrane. A narrow duct connects the gas-filled part to the swimbladder. Transmission of sound through the membrane in the bulla creates pressure fluctuations in the liquid-filled part, which are transferred directly into the utricle. This provides additional relative motion between the utricular otolith and its sensory epithelia. The utricle is thought to play a role in the detection of ultrasound, but it is not yet understood because not all clupeids with auditory bullae have this capability.

Most auxiliary anatomical structures in fishes improve hearing at higher frequencies. Some species, however, have demonstrated enhanced sensitivity at lower frequencies that may be attributed to oversized otoliths either in the utricle or in the saccule. A larger otolith has more inertia that would increase relative motion with the sensory epithelium when stimulated by low-frequency sound.

Summary and Conclusions

Fishes live in very complex acoustic environments and yet manage to detect and use acoustic signals. There is great diversity in the structure and morphology of their inner ears, as well as other peripheral anatomical structures related to enhanced auditory sensitivity in several species. Biomechanics of the inner ear, swimbladder, and these peripheral structures correlates with the wide range of auditory thresholds and bandwidths found in many fishes.

See also: **Hearing and Lateral Line**: Acoustic Behavior; Auditory System Morphology; Fish Bioacoustics: An Introduction; Lateral Line Neuroethology; Lateral Line Structure; Psychoacoustics: What Fish Hear; Sound Source Localization and Directional Hearing in Fishes; The Ear and Hearing in Sharks, Skates, and Rays; Vestibular System Anatomy and Physiology; Vocal Behavior of Fishes: Anatomy and Physiology.

Further Reading

Au WWL and Hastings MC (2008) *Principles of Marine Bioacoustics*. New York: Springer Science+Business Media.

Fay RR and Popper AN (eds.) (1999) *Comparative Hearing: Fish and Amphibians*. New York: Springer.

Finneran JJ and Hastings MC (2000) A mathematical analysis of the peripheral auditory system mechanics in the goldfish (*Carassius auratus*). *Journal of the Acoustical Society of America* 108: 3035–3043.

Kalmijn AJ (1988) Hydrodynamic and acoustic field detection. In: Atema J, Fay RR, Popper AN, and Tavolga WN (eds.) *Sensory Biology of Aquatic Animals*, pp. 83–130. New York: Springer.

Kuperman WA and Lynch JF (2004) Shallow-water acoustics. *Physics Today* October: 55–61.

Medwin H and Clay CS (1998) *Fundamentals of Acoustical Oceanography*. San Diego, CA: Academic Press.

Rogers PH and Cox M (1988) Underwater sound as a biological stimulus. In: Atema J, Fay RR, Popper AN, and Tavolga WN (eds.) *Sensory Biology of Aquatic Animals*, pp. 131–149. New York: Springer.

Webb JF, Popper AN, and Fay RR (eds.) (2008) *Fish Bioacoustics*. New York: Springer, Science+Business Media.

Relevant Website

http://www.dosits.org – Discovery of Sound in the Sea.

Psychoacoustics: What Fish Hear

RR Fay, Loyola University Chicago, Chicago, IL, USA

© 2011 Elsevier Inc. All rights reserved.

Fish Can Hear!
How Is Hearing Measured?
Classical Conditioning
Instrumental Avoidance Conditioning
Auditory Evoked Potentials
Species Variation in Hearing Ability
The Determinants of Sensitivity
Frequency Discrimination
Auditory Scene Analysis
What Do Fishes Listen to?
Further Reading

Glossary

Audiogram A graphical representation of sound detection thresholds at the range of frequencies that are audible.

Auditory scene analysis A perceptual capacity shared by all vertebrates, including fishes, to segregate and respond independently to the individual sound sources that may be active simultaneously (e.g., the instruments of an orchestra).

Masking The reduction in audibility for one sound (termed 'signal') caused by the simultaneous presence of another sound (termed 'noise'). Masking causes the threshold for the signal to be higher (less detectable) than it normally would without the masking noise.

Otophysic connection An anatomical linkage between a gas bubble (e.g., the swimbladder) and the inner ear that brings the motions of the gas bubble, caused by sound pressure, directly to the ears.

Psychophysics A technology of experimental psychology designed to allow the investigation of the relationships between the physical nature of a stimulus (e.g., sound) and the sensations and perceptions caused by the stimulus.

Soundscape The sum of all the sounds characteristic of an environment.

Weberian ossicles A series of bones that is specially adapted as an otophysic connection between the swimbladder and the inner ear in a group of fishes known as Otophysi.

Fish Can Hear!

About 60 fish species have been evaluated with respect to their hearing abilities, and all have been shown to hear by a behavioral definition of hearing; that is, we observe them responding behaviorally to a sound source following conditioning (see below). Therefore, it has been concluded that all fishes, teleost and nonteleost, can hear. This had been controversial historically (during the early twentieth century), in part because fish have no external ears or any visible suggestion of ears.

The inner ear otolith organs (**see also Hearing and Lateral Line**: Auditory System Morphology) are stimulated directly by sound that passes through the body nearly unimpeded (**see also Hearing and Lateral Line**: Biomechanics of the Inner Ear in Fishes). Otoliths are very dense calcium carbonate stones that respond to sound (move) at a different magnitude and phase of the acoustic particle motion compared with the close-lying sensory epithelium containing sound receptor cells. The saccule is the primary auditory organ in most species.

In some fish species, additional pathways for hearing have evolved that make the animal sensitive to sound pressure as well as acoustic particle motion. These specializations include a swimbladder or other gas cavity in the head or abdomen, and mechanisms that mechanically connect the gas bladder to the ears (an otophysic connection). Vibrations of the gas bladder result from pressure fluctuations (e.g., sound pressures) impinging on the fish. These motions can be transmitted to the ears in some species where they stimulate one or more of the otolithic organs. The best known and understood of these otophysic connections are the Weberian ossicles shared by the group of fishes known as the Otophysi. The species with Weberian ossicles have extreme sensitivity to sound pressure and with hearing extending to higher frequencies (up to 2000–5000 Hz).

How Is Hearing Measured?

There are many physiological and behavioral indications that a given species can hear, but the gold standard in this field is the behavioral audiogram, or the relation between the lowest sound levels that are behaviorally detectable and the frequency of the sound. The term hearing presupposes a behavioral response because it is an ability or capacity of the whole organism to respond to sound. Various methods of behavioral analysis have been applied in hearing studies.

Psychophysics is the science that directly links the physical nature of the stimulus to the perceptions that may arise from sensory stimulation. Combined with psychophysics are the various behavior control technologies that are used to study conditioning and learning in animals. The most common ones are classical, or Pavlovian, conditioning, and instrumental avoidance conditioning. In addition, many recent experiments have used the methods for auditory evoked potentials as a partial substitute for the behavioral methods.

Classical Conditioning

Classical conditioning occurs when a previously neutral stimulus (e.g., a tone) is paired with a stimulus that elicits unconditionally a reflex of some kind (e.g., the suppression of respiration rate or heart rate). With multiple pairings of these stimuli, the previously neutral stimulus (conditioned stimulus) begins to elicit a response very much like the unconditioned reflex response. The occurrence of this response to the conditioned sound stimulus is an indication of hearing. Then the methods of psychophysics are applied to the conditioned stimulus. For example, sounds may be presented with decreasing intensity (e.g., sound pressure level) until the conditioned response fails to occur. The intensity level at which the response fails can be defined as the threshold for hearing that sound. Then, the frequency of the sound is changed and the experiment repeated. A plot of the thresholds as a function of the frequencies tested is the audiogram. It describes the weakest tones that are detectable, and the frequency range of hearing. An audiogram for the goldfish (*Carassius auratus*) is shown in **Figure 1** using these methods.

The audiogram can be interpreted as follows: at all frequencies tested, sound pressures above the audiogram line are detectable, while those levels below the line cannot be heard. The frequency range for the goldfish can be described as from 30 Hz or below to about 2500 Hz. The best frequency of hearing is about 350 Hz. One microPascal ($1\,\mathrm{Pa} = 1\,\mathrm{N\,m^{-2}}$) is the unit of sound pressure against which all sound pressure thresholds are compared. We have confidence in this audiogram because many physiological and behavioral experiments on goldfish confirm that it is specialized for detecting sound pressure (it has Weberian ossicles), and sound pressures were manipulated and measured in this experiment. The greatest sensitivity for goldfish (about 60 dB re: 1 μPa underwater) is comparable to the best sensitivity for humans in air, and many other vertebrate animals. The goldfish is the most studied of fish species in terms of hearing experiments.

Instrumental Avoidance Conditioning

A typical application of avoidance conditioning involves a free-swimming fish in a small aquarium with a partial barrier dividing the aquarium in half. A tone is presented, followed in a few seconds by a noxious stimulus (e.g., very

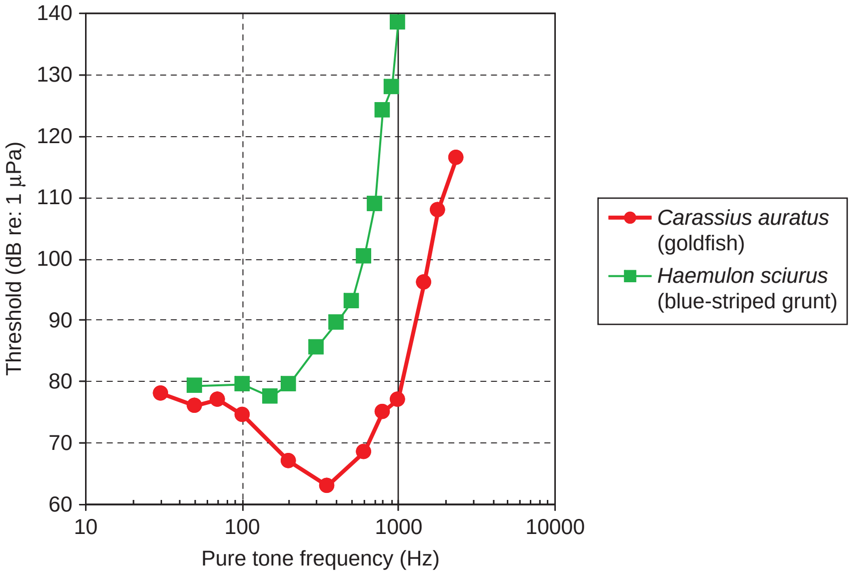


Figure 1 Audiogram for *Carassius auratus* (goldfish) estimated using classical conditioning, and audiogram for *Haemulon sciurus* (blue-striped grunt) using instrumental avoidance conditioning.

mild electric shock). The fish eventually crosses the barrier and the shock is immediately turned off. Almost immediately, the animal learns to swim over the barrier as soon as the shock is started. Then, the animal learns by trial and error that if it swims over the barrier to the other side of the tank during the tone presentation (and before shock onset), it will not get a shock. Swimming over the barrier during the tone is an indication that the fish heard the sound. Once the animal successfully crosses the barrier avoiding a shock in the majority of trials (e.g., 90%), a psychophysical procedure is implemented and thresholds are estimated as in classical conditioning. **Figure 1** shows an audiogram for the blue-striped grunt (*Haemulon sciurus*) measured by avoidance conditioning.

The blue-striped grunt is a species without any known special adaptations for hearing, and therefore we now believe that it may be sensitive to acoustic particle motion, not sound pressure. However, sound pressure was manipulated and measured in this experiment, not particle motion. Thus, this audiogram's interpretation is in doubt, because the relation between sound pressure and particle motion varies in different environments, especially in small aquaria. Nevertheless, we could describe this audiogram as extending from below 50 to 1000 Hz, with a most sensitive frequency of about 150 Hz. This audiogram is different from the goldfish audiogram of **Figure 1**, both in sensitivity and frequency range.

Auditory Evoked Potentials

The synchronous activation of many neurons in the auditory system can be monitored and recorded using surface electrodes on the animal's head. This requires extreme amplification of these minute voltages and the signal averaging of many repetitions of each stimulus. Averaged voltage waves in response to brief sound presentations are used as an indication that the sounds have activated the auditory system. The signal level is then manipulated to determine the lowest level that can still result in repeatable averaged brain waves at all effective frequencies. This results in an evoked potential audiogram, sometimes called an auditory brainstem response (ABR) audiogram. ABR audiograms are now very popular for estimating the frequency range of hearing of various species. The advantage of the ABR method is that it is rapid (usually completed in about 1 h), rather than the several days or weeks required for a behavioral audiogram. The disadvantage of the ABR audiogram is that there is no known relationship between the amplitude of these brain waves at threshold and behavioral responses; thus, the ABR thresholds themselves cannot yet be interpreted as a measure of sensitivity comparable to behavioral sensitivity. In this article, only behavioral audiograms are shown due to the uncertainty of the interpretation of ABR audiograms.

Species Variation in Hearing Ability

Figures 1–4 illustrate some of the variations in hearing abilities of fishes that have been studied. Fishes can be classified into three groups based on their hearing abilities: (1) otophysans and other species that are probably pressure sensitive, (2) species with swimbladders but without any known specializations for the detection of sound pressure, and (3) species without swimbladders without the possibility of sound pressure sensitivity. **Figure 1** illustrates two species representing the first two groups: goldfish – a pressure-sensitive otophysan – and blue-striped grunt – a nonotophysan. **Figure 2** shows the audiograms for three additional pressure-sensitive otophysans (*Cyprinus carpio* (carp), *Arius felis* (marine catfish), and *Astyanax mexicanus* (Mexican tetra)). **Figure 3**

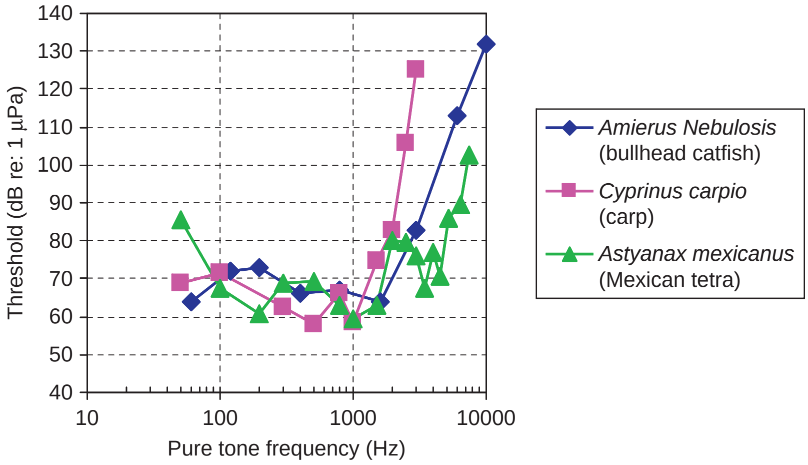


Figure 2 Sound pressure audiograms for selected Otophysi (species having Weberian ossicles).

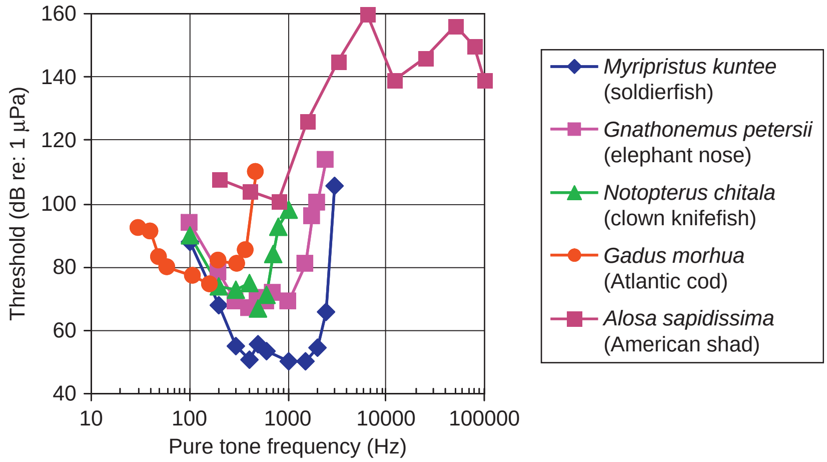


Figure 3 Sound pressure audiograms for selected species that are likely pressure sensitive due to adaptations of the swimbladder or other bubbles of air associated with the ear.

shows selected audiograms for five nonotophysan species (*Myripristus kuntee* (soldierfish), *Ganthonemus petersii* (elephant nose), *Notopterus chitala* (clown knifefish), *Gadus morhua* (Atlantic cod), and *Alosa sapidissima* (American shad)) that are likely to be pressure sensitive, but pressure sensitivity has only been experimentally confirmed for the cod. **Figure 4** shows audiograms for five species (*Pleuronectes platessa* (plaice), *Limanda limanda* (dab), *Negaprion brevirostris* (lemon shark), *Anguilla anguilla* (European eel), and *Salmo salar* (Atlantic salmon)) without swimbladders, or which have been shown to be sensitive only to acoustic particle motion in the frequency range where data are plotted. Note that **Figure 4** shows thresholds in terms of particle acceleration (dB: re: 1 nm s^{-2}). There is a group of species whose audiograms were measured in terms of sound pressure, yet it is probable that they respond only to acoustic particle motion. Thus, these audiograms (not shown) cannot be interpreted until further experiments are done.

Clearly, the otophysans (goldfish of **Figure 1**, and **Figure 2**) hear with the greatest sensitivity at the higher frequencies (up to 5000 Hz in some species). This sensitivity and high-frequency range is due, in part, to the presence of Weberian ossicles, and, in part, to adaptations of the saccular hair cells in these species to respond to the higher-frequency sounds.

Figure 3 shows audiograms for selected nonotophysan species with swimbladder specializations that probably

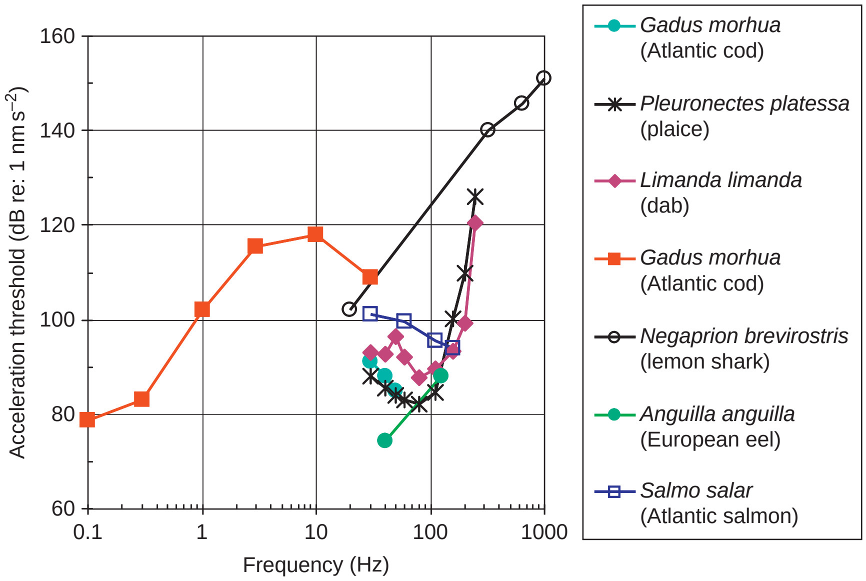


Figure 4 Selected particle acceleration audiograms for species known to be most sensitive to acoustic particle motion in the frequency range where data are displayed.

make them pressure sensitive. Unlike the Otophysi, this group exhibits significant variation in sensitivity as well as in the frequency range of hearing. The Atlantic cod has been shown to be sensitive to acoustic particle motion at low frequencies, but is pressure sensitive at the higher frequencies (above 100–200 Hz), probably due to the forward extensions of the swimbladder, near the ears. The mormyrid *Gnathonemus* sp. has a gas bladder in the inner ear to which the saccule is attached. *Notopterus* sp. and *Myripristus* sp. both have forward extensions of the swimbladder close to the ears. The American shad, *A. sapidissima*, is a herring-like fish with special adaptations of the swimbladder incorporating the utricle that has been shown to have sensitivity at ultrasonic frequencies (>20 000 Hz), but unremarkable sensitivity in the frequency range usual for fishes (<1000 Hz).

The audiograms of **Figure 4** illustrate species either without a swimbladder (*Pleuronectes* sp., *Limanda* sp., and *Negaprion* sp.), or that have been demonstrated to be sensitive to acoustic particle motion (*Salmo* sp., *Anguilla* sp., and *Gadus* sp.) in particular frequency ranges. The ordinate of this figure is in acceleration units rather than sound pressure, and illustrates the nearly constant sensitivity to acceleration at infrasonic frequencies for *Gadus* sp. Sensitivity to particle displacements at 100 Hz is similar for all the species illustrated at about 0.1 nm, or about the diameter of a hydrogen atom. This indeed is very great sensitivity.

The Determinants of Sensitivity

In addition to swimbladder and otophysic adaptations, a fish's hearing sensitivity is determined by the duration of the sound signal and the level of ambient noise present. Sound duration affects sensitivity between <10 and about 400 ms, with greater sensitivity at the longer durations. This growth of sensitivity is common in sensory systems and is known as temporal summation.

Noise is a major determinant of hearing sensitivity under most real-world and uncontrolled situations (**see also Hearing and Lateral Line**: Effects of Human-Generated Sound on Fish) for potential effects on masking of human-generated sounds in the water). For example, the detection and recognition of speech by humans depends on the levels of noise present – as noise level increases, speech becomes harder to hear, or less intelligible, and its threshold for detection becomes higher. A threshold that is determined by noise is termed a masked threshold. Masking refers to the elevation of the detection threshold for one sound (the signal) due to the presence of another sound (e.g., noise). Masking is ubiquitous, and most fishes normally hear under masking conditions in most environments. In general, masking is linear in the sense that an increment of 1 dB in the masking noise causes a 1-dB rise in the threshold for a signal.

Masking has been studied in fishes by using pure tones as signals, and white noise of varying bandwidth as maskers (**see also Hearing and Lateral Line**: Effects of Human-Generated Sound on Fish). The fundamental masking description consists of the level of a just-detectable signal in the presence of wide-band (covering a wide frequency range) noise with a flat spectrum (equal sound pressure at all frequencies). The critical masking ratio (CR) is defined as the sound pressure level in dB of the signal tone that is at threshold (just detectable in the noise) minus the spectrum level (level per Hz) in dB of the masking noise. Large critical ratios occur when the noise is relatively effective as a masker. According to the traditional theories of masking in human hearing, a masker is more or less effective depending on the frequency range of the hypothetical detection filter used to detect the pure tone signal. A filter is a system that is frequency selective, that is, it passes through (responds to) acoustic components in a given frequency range, but rejects (filters out) all other frequency components. Noise is assumed to be integrated or summed over the frequency range of the filter that is assumed to be centered at the signal frequency, so the wider (in frequency) the detection filter, the more noise is admitted, and the masking effect is greater. Therefore, a large CR suggests a wide-band detection filter, and a small CR suggests a narrow-band detection filter. These assumptions have been evaluated in masking studies on fishes and have been shown to be approximately correct.

Figure 5 plots behaviorally measured CRs for goldfish. In general, the CR at the lowest frequency (100 Hz) is about 13 dB and tends to grow linearly with signal frequency through 1000 Hz to about 24 dB. These values are well within the range reported for other vertebrate animals, including humans, and the slope of the masking effect is substantially similar for all vertebrates. The bandwidths of the hypothetical detection filters range from 21 (at 100 Hz) to 257 Hz (at 1200 Hz). All of these bandwidths are significantly narrower than the goldfish's hearing range, and suggest multiple frequency-selective detection channels in the goldfish ear. This has been

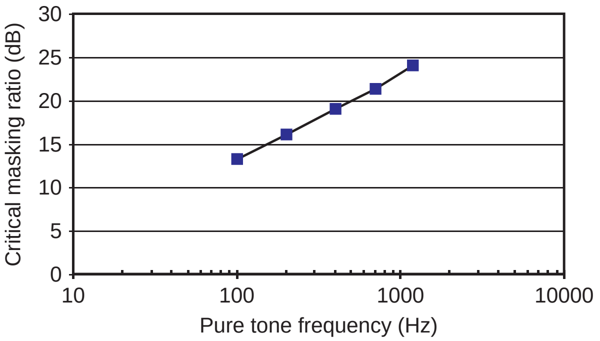


Figure 5 Critical masking ratios (CRs) for the goldfish. The CR is the threshold for the signal tone (in dB) minus the spectrum level of the masking noise (in dB).

confirmed physiologically (**see also Hearing and Lateral Line**: Physiology of the Ear and Brain: How Fish Hear).

CRs have also been measured for the Atlantic cod, haddock (*Melanogrammus aegelfinus*), marine catfish (*Arius felis*), and pinfish (*Lagodon rhomboides*). For the Atlantic cod and haddock, CRs were also measured for different spatial separations of the signal and masker noise sources. It was found that the CR can be up to 8 dB smaller (noise is less effective) when the signal source was straight ahead, and the noise source 90° to the side. Thus, fish demonstrate one feature of the cocktail party effect or the increased detectability of a masked signal when the noise source is displaced in the horizontal plane for human listeners, and suggests detection filters that are selective in the spatial domain as well as in the frequency domain.

Frequency Discrimination

One of the most important aspects of hearing by fishes (and all other vertebrates, including humans) lies in their capacities to discriminate differences between pure tone frequencies (i.e., pitch discrimination). Terrestrial animals accomplish this, in part, due to the organization of their inner ears having a basilar membrane containing sound receptor cells (as in humans and other mammals) and spatial or place mechanism for frequency analysis that appears to be lacking in fishes. So, the extent that fishes can analyze frequency probably depends on the mechanisms other than these familiar place mechanisms.

In a frequency discrimination experiment, the animal must respond one way in the presence of one frequency (F1), and another way in the presence of another frequency (F2). Then the difference (F1 − F2) between these frequencies is made progressively smaller until the animal can no longer differentiate between them (e.g., respond differently). Then this procedure is repeated at a different F1, and the relation between F1 and the smallest discriminable F1 − F2 can be described. **Figure 6** shows this relation for several species. The smallest discriminable change in frequency is plotted as a function of the F1 frequency.

Fishes have been roughly divided into two groups, the otophysans (e.g., European minnow, goldfish, and marine catfish), and the nonotophysans (e.g., *Gobius niger* (goby), *Corvina nigra* (drum), *Sargus annularis* (ringed sea bream), *Cottus scorpius* (bullhead), and *Negaprion brevirostris* (lemon shark)). These data have led to the tentative conclusion that otophysans have more acute frequency discrimination abilities than the nonotophysans. Taking the goldfish as an example, frequency discrimination acuity declines linearly with frequency, being about 3.5 Hz at an F1 of 50 Hz, to about 47 Hz at an F1 of 1000 Hz. The slope of this function and the thresholds themselves place goldfish within the range of variation seen for frequency discrimination among most terrestrial vertebrates. Fish accomplish this without a place mechanism of frequency analysis, and probably rely on a temporal code for frequency, based on the synchrony between the impulses evoked in the auditory nerve and the tone stimulus wave.

Auditory Scene Analysis

When there are multiple, simultaneous sound sources active, the human ability to segregate the sources in perception, and respond to them independently, is

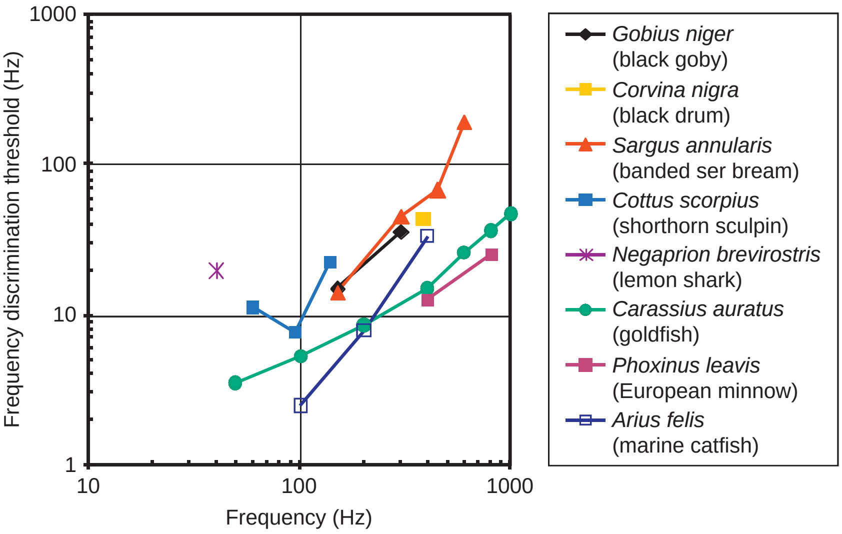


Figure 6 Frequency discrimination thresholds for all species investigated.

known as sound source segregation (**see also Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes). This ability is part of auditory scene analysis (ASA), or the ability to determine the multiple sources that make up a sort of scene in perception. This scene is not necessarily spatial, requiring source localization, but is analogous to the human ability to hear out and recognize individual sound sources (e.g., instruments in an orchestra) even when source localization is not possible (e.g., when listening to a monophonic recording). Scene analysis makes it possible for two (or more) simultaneous sound sources to be heard or determined independently. This phenomenon is well known in human hearing, and has been demonstrated so far only in goldfish (plus the European starling, and some other primates). For example, goldfish can determine that two simultaneous sound sources are present, and respond to the frequency and temporal characteristics of either one, independently. It is presumed that ASA probably operates for all vertebrates and, possibly, some invertebrates. It takes place automatically, without cognition or the focusing of attention. ASA is thought to be a capacity that is necessary for hearing to have survival value, and probably evolved at the same time as hearing itself first evolved among fishes.

What Do Fishes Listen to?

This is a difficult question because we know very little about underwater acoustic ambience. Because many fish species produce sounds (e.g., vocalizations), it was long thought that their hearing was developed for the purposes of sound communication (**see also Hearing and Lateral Line**: Acoustic Behavior). Now, however, it is believed that fish listen to and make use of all the sounds that they can hear (the soundscape or the background noise of each environment), including the swimming, eating, the communication sounds of predators, prey and conspecifics, and the sounds of wind, rain, and moving water, etc. They are thought to use these sounds for soundscape orientation, the same way that humans do, to assess their environment and their place in it. To do this, the capacity for auditory scene analysis is necessary. Sound communication among fishes could only have evolved once the ability to discriminate the sound sources making up the soundscape appeared.

See also: **Hearing and Lateral Line**: Acoustic Behavior; Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Effects of Human-Generated Sound on Fish; Physiology of the Ear and Brain: How Fish Hear; Sound Source Localization and Directional Hearing in Fishes.

Further Reading

Dijkgraaf S (1960) Hearing in bony fishes. *Proceedings of the Royal Society of London B* 152: 51–54.

Fay RR (1970) Auditory frequency discrimination in the goldfish (*Carassius auratus*). *Journal of Comparative and Physiological Psychology* 73: 175–180.

Fay RR (1988) *Hearing in Vertebrates: A Psychophysics Databook*. Winnetka, IL: Hill-Fay Associates.

Fay RR and Popper AN (eds.) (2000) *Springer Handbook of Auditory Research, Vol. 11: Hearing in Fishes and Amphibians*. New York: Springer.

Fay RR and Simmons AM (1999) The sense of hearing in fishes and amphibians. In: Fay RR and Popper AN (eds.) *Springer Handbook of Auditory Research, Vol. 10: Hearing in Fishes and Amphibians*, pp. 269–318. New York: Springer.

Hawkins AD (1981) The hearing abilities of fish. In: Tavolga WN, Popper AN, and Fay RR (eds.) *Hearing and Sound Communication in Fishes*, pp. 109–137. New York: Springer.

Manley G, Fay R, and Popper A (eds.) (2004) *Springer Handbook of Auditory Research, Vol. 22: Evolution of the Vertebrate Auditory System*. New York: Springer.

Popper AN (1970) Auditory capacities of the Mexican blind cave fish (*Astyanax jordani*) and its eyed ancestor (*Astyanax mexicanus*). *Animal Behaviour* 18: 552–562.

Popper AN and Fay RR (1993) Sound detection and processing by fish: Critical review and major research questions. *Brain, Behavior and Evolution* 41: 14–38.

Tavolga WN, Popper AN, and Fay RR (eds.) (1981) *Hearing and Sound Communication in Fishes*. New York: Springer.

Tavolga WN and Wodinsky J (1965) Auditory capacities in fishes: Threshold variability in the blue-striped grunt, *Haemulon sciurus*. *Animal Behaviour* 13: 301–311.

von Frisch K (1938) The sense of hearing in fish. *Nature* 141: 8–11.

Webb J, Popper AN, and Fay RR (eds.) (2008) *Springer Handbook of Auditory Research: Fish Bioacoustics*. New York: Springer.

Auditory/Lateral Line CNS: Anatomy

CA McCormick, Oberlin College, Oberlin, OH, USA

© 2011 Elsevier Inc. All rights reserved.

Evolutionary Overview
Central Anatomy of the Auditory and Mechanosensory Lateral Line Systems
Summary
Further Reading

Glossary

Clupeid Species in the teleost order Clupeiformes, characterized by the presence of a swimbladder-derived gas-filled bulla physically connected to the utricle of the inner ear.

Lateral lemniscus Collection of axons from hindbrain auditory and, when present, lateral line nuclei that terminates mainly in the midbrain roof structure called the torus semicircularis in fish, amphibians, and reptiles, and called the inferior colliculus in mammals.

Neurogenic placodes Embryonic ectodermal thickenings that give rise to neurons and sensory cells such as the ear, lateral line and olfactory organs, and, in some cases, cranial nerves.

Nucleus A discrete population of neurons that can be recognized using histological methods such as a stain for neuronal somata (e.g., cresyl-violet), and that perform a common function (e.g., sound processing).

Otolith organ A collection of sensory hair cells in the inner ear in which the hair cell cilia are covered by a membrane covered by a calcium carbonate mass. The saccule, lagena, and utricle of the inner ear. Each has a sensory epithelium (or macula) and an otolith (or otoconia).

Otophysan A large teleost clade whose members have a series of bones (Weberian ossicles) anatomically connecting the swimbladder to the saccule of the inner ear.

Otophysic connections Anatomical linkage of the inner ear to a gas-filled structure, such as the swimbladder that brings the motions of the gas bubble, caused by sound pressure, directly to the ears.

Primitive A trait that evolved early in evolutionary history.

Primitive vertebrate feature A trait that evolved in the common ancestor of vertebrates.

Teleost The most recently evolved of all the bony fishes (Osteichthyes). Teleosts have rayed fins and a swimbladder, and include everything from goldfish to tuna to salmon.

Evolutionary Overview

Inner Ear Auditory and Lateral Line Sensory Organs

It is likely that the inner ear of the earliest jawed fishes possessed acoustic function because otic hearing organs are present in their descendents: the cartilaginous and bony fishes. Auditory function can be distributed among multiple inner ear sensory organs. Individual otolith end organs – the saccule, utricle, and lagena – may have both acoustic and vestibular roles; such bifunctionality may be the primitive vertebrate condition. In some cartilaginous fish, the macula neglecta is also acoustically responsive.

For further discussion on inner ear end organs, **see also Hearing and Lateral Line:** Auditory System Morphology, The Ear and Hearing in Sharks, Skates, and Rays, Physiology of the Ear and Brain: How Fish Hear, and Vestibular System Anatomy and Physiology.

Mechanosensory lateral line organs are a primitive vertebrate feature. These organs detect pressure gradients resulting from bulk water flow, such as occurs during swimming or the motion of conspecifics and prey. Under certain conditions, sound can also stimulate the mechanosensory lateral line, but the fundamental contribution of lateral line information to auditory processing is unknown. Convergence of lateral line and auditory inputs occurs in a number of brain areas. For further discussion on the lateral line system, **see also Hearing and Lateral Line:** Lateral Line Structure and Lateral Line Neuroethology.

Inner Ear Auditory and Mechanosensory Lateral Line Brain Circuits

The peripheral receptors of the auditory and lateral line systems (as well as in the vestibular inner ear organs) consist of sensory hair cells innervated by bipolar neurons. The anterior and posterior lateral line cranial nerves

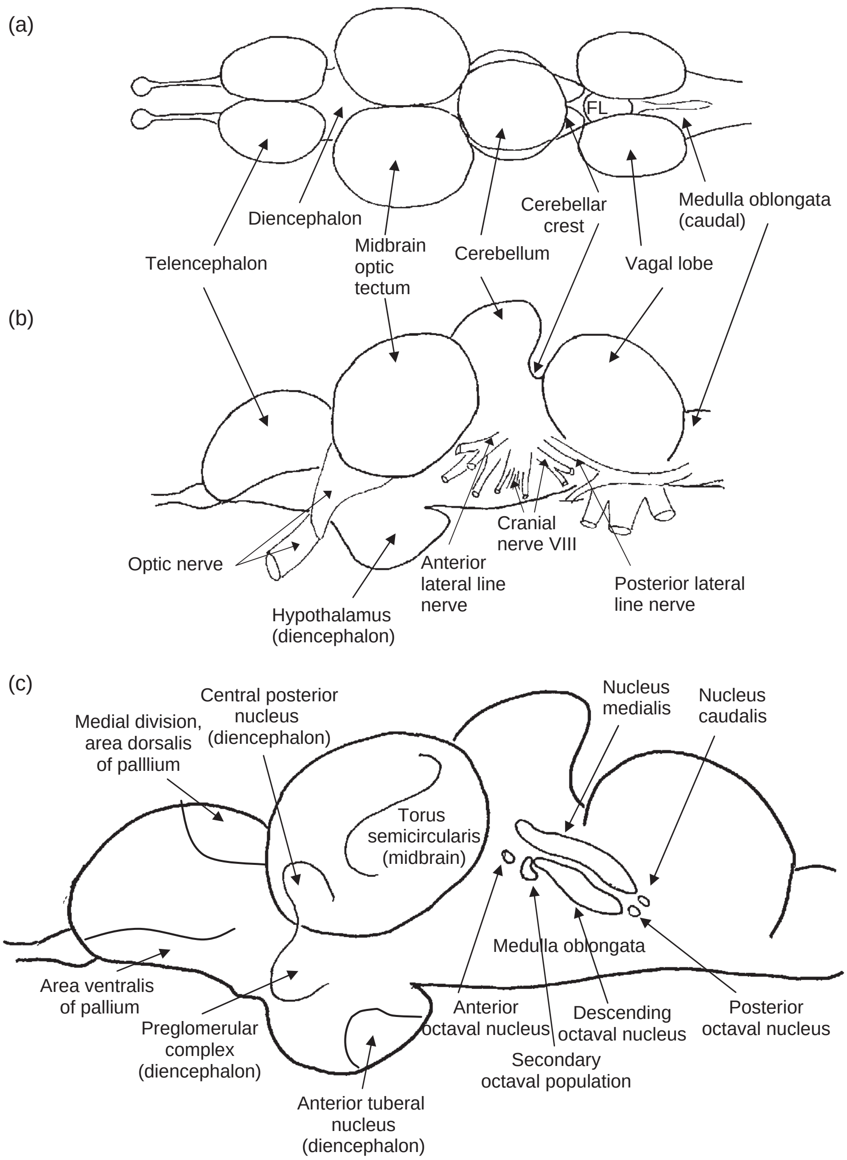

(a)
FL
Diencephalon
Telencephalon
Midbrain optic tectum
Cerebellum
Cerebellar crest
Vagal lobe
Medulla oblongata (caudal)
(b)
Optic nerve
Hypothalamus (diencephalon)
Anterior lateral line nerve
Cranial nerve VIII
Posterior lateral line nerve
(c)
Medial division, area dorsalis of palllium
Central posterior nucleus (diencephalon)
Torus semicircularis (midbrain)
Nucleus medialis
Nucleus caudalis
Medulla oblongata
Area ventralis of pallium
Preglomerular complex (diencephalon)
Anterior tuberal nucleus (diencephalon)
Anterior octaval nucleus
Secondary octaval population
Descending octaval nucleus
Posterior octaval nucleus

Figure 1 Schematic drawings of the brain of the goldfish, *Carassius auratus*: (a) dorsal view of goldfish brain, (b) lateral view of goldfish brain, and (c) lateral view of goldfish brain with cranial nerves removed, showing the locations of some of the major central-processing areas for auditory and lateral line mechanosensory information.

(cranial nerves that are unnumbered) collect sensory input from lateral line mechanoreceptors on the head and trunk, respectively. Cranial nerve VIII (the octaval or stato-acoustic nerve) is an aggregate of nerve branches that collect input from each of the inner ear sensory end organs. Cranial nerve VIII enters the brain at the level of the medulla oblongata (commonly referred to as the medulla) between the entry points of the anterior and posterior lateral line nerves (**Figures 1(a)** and **1(b)**). The lateral line and octaval nerves also contain the axons of central octavolateral efferent neurons that innervate the sensory hair cells. For further discussion on octavolateral efferent neurons, **see also Hearing and Lateral Line:** Physiology of the Ear and Brain: How Fish Hear.

Embryologically, the inner ear and lateral line sensory hair cells and cranial nerves develop from, respectively, the otic and lateral line neurogenic placodes. The lateral line placodes are lost in amniotes and in those amphibians that lack a lateral line at all stages; thus, there is no apparent homolog of this system in most land vertebrates. In contrast, the otic placode is retained in all vertebrates and gives rise to evolutionarily new otic auditory organs that complement (e.g., amphibians) or functionally replace (e.g., mammals) the primitive auditory organs of fish.

The inner ear auditory and the lateral line mechanosensory nerves in jawed fish distribute information to first-order nuclei in the medulla and to areas within the cerebellum. As is the case in other vertebrates, the first-order auditory and lateral line medullary nuclei each form the beginning of a multi-component, ascending central circuit that includes additional processing areas in the medulla, as well as processing areas in the midbrain, diencephalon, and telencephalon (**Figures 1(a)–1(c)**). **Figure 2** summarizes the connections among the main components of the two ascending circuits.

The inner ear auditory and the lateral line mechanosensory systems give rise to largely separate central circuits that are similarly organized (**Figure 2**). Nuclei that receive inputs from both the sensory systems, however, are present at most levels of the brain. Remarkably, the central auditory pathways of jawed fishes and land vertebrates share a common organizational pattern (**Figure 2**) despite the evolution of nonhomologous peripheral auditory organs and, at some or all levels of the brain, auditory nuclei. The auditory pathway of the most primitive jawed fishes may have formed the substrate from which those of later radiations of fish and land vertebrates evolved. An alternative hypothesis is that the auditory pathways of different taxa follow a basal organization plan shared by the auditory and lateral line systems.

In addition to providing input to nuclei in the medulla, other connections of the two sensory systems, which are not described further, include direct inputs to granule cell

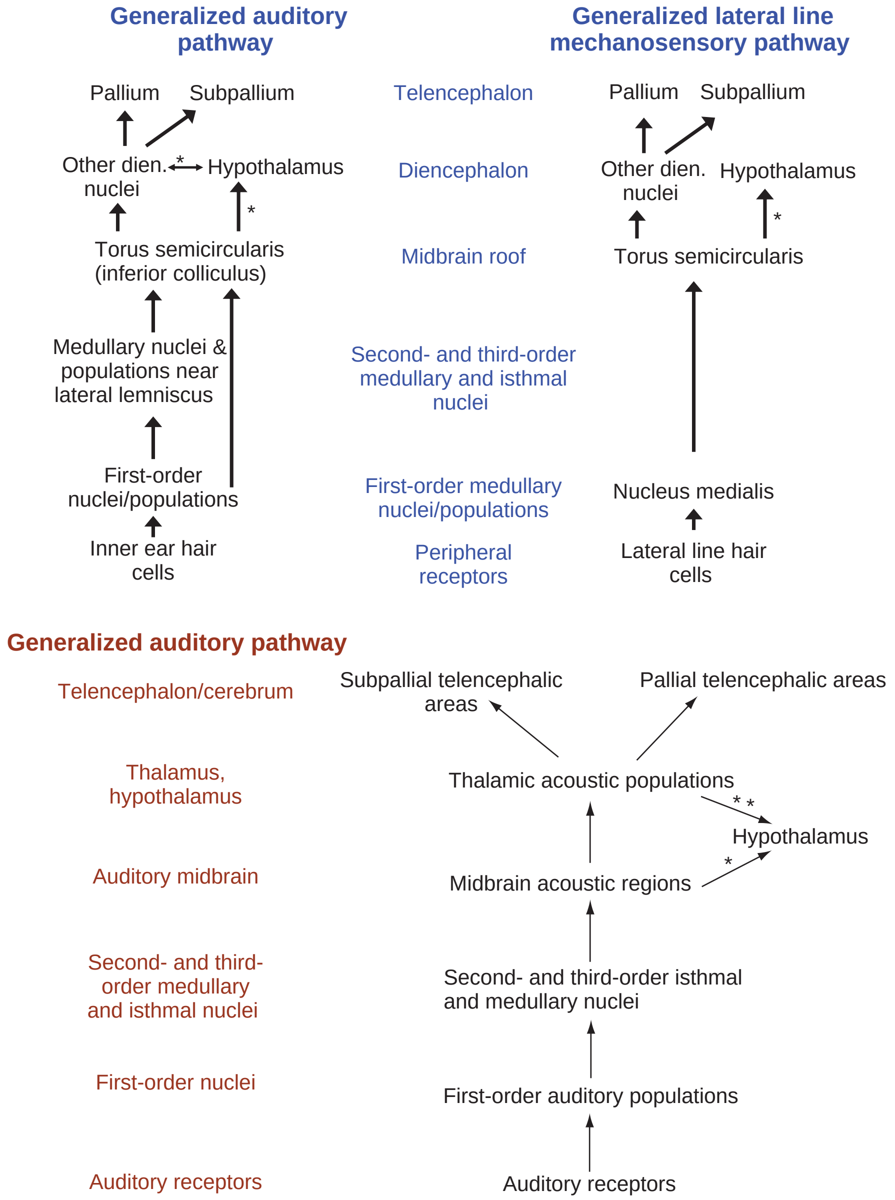

Generalized auditory pathway
Generalized lateral line mechanosensory pathway
Pallium
Subpallium
Telencephalon
Pallium
Subpallium
Other dien. nuclei
*
Hypothalamus
Diencephalon
Other dien. nuclei
Hypothalamus
*
*
Torus semicircularis (inferior colliculus)
Midbrain roof
Torus semicircularis
Medullary nuclei & populations near lateral lemniscus
Second- and third-order medullary and isthmal nuclei
First-order nuclei/populations
First-order medullary nuclei/populations
Nucleus medialis
Inner ear hair cells
Peripheral receptors
Lateral line hair cells
Generalized auditory pathway
Telencephalon/cerebrum
Subpallial telencephalic areas
Pallial telencephalic areas
Thalamus, hypothalamus
Thalamic acoustic populations
* *
Hypothalamus
*
Auditory midbrain
Midbrain acoustic regions
Second- and third-order medullary and isthmal nuclei
Second- and third-order isthmal and medullary nuclei
First-order nuclei
First-order auditory populations
Auditory receptors
Auditory receptors

Figure 2 The pattern of organization of vertebrate central auditory circuits is shown on the left. Lateral line mechanosensory system central pathways, shown on the right, are organized similarly to auditory pathways.

populations in the cerebellum, and, in the case of the inner ear, the Mauthner neuron. For further discussion on the Mauthner neuron, **see also Brain and Nervous System:** Physiology of the Mauthner Cell: Discovery and Properties and Physiology of the Mauthner Cell: Function.

Central Anatomy of the Auditory and Mechanosensory Lateral Line Systems

First- and Higher-Order Nuclei That Project to the Midbrain Torus Semicircularis/Lateral Mesencephalic Complex

In fish, as in other vertebrates, nuclei that receive direct input from auditory fibers of the octaval nerve and from the lateral line nerves are located in the medulla oblongata (**Figures 1(b)**, **1(c)**, **2**, and **3**). Some of the neurons within these nuclei project to acoustic and lateral line regions in the midbrain torus semicircularis (TS) (bony fish)/lateral mesencephalic complex (LMC; cartilaginous fish). This connection is analogous to that from the mammalian cochlear nuclei to the inferior colliculus. In fish, axons from first-order populations course bilaterally within the lateral lemniscus to the midbrain and are joined by axons from second- or higher-order nuclei (**Figure 2**).

Octaval nuclei

Of the four, or five in the case of teleosts, nuclei that receive direct input from the octaval nerve, only the descending octaval (DO) and anterior octaval (AO) nuclei are known to process acoustic information and project to

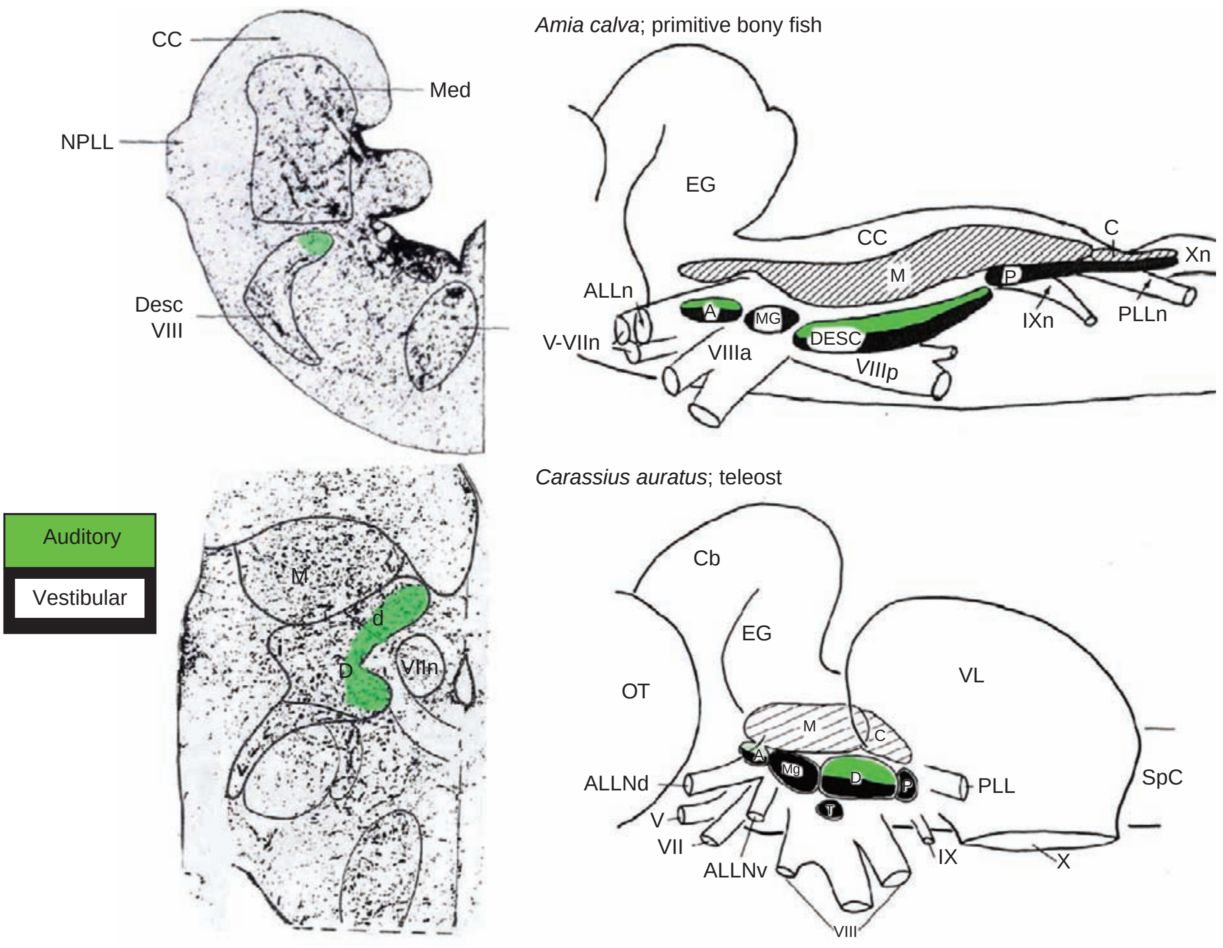


Figure 3 Right: Lateral views of the hindbrain of *Amia calva* (bowfin; top) and *Carassius auratus* (goldfish; bottom) schematically illustrate the locations of lateral line mechanosensory nuclei (diagonal lines), acoustic octaval areas (green), and vestibular octaval areas (black). Left: Transverse, cresyl violet-stained hemisections through the medulla of *Amia calva* (bowfin; top) and *Carassius auratus* (goldfish; bottom). In each species, green delineates the known acoustic region in the descending octaval nucleus. Abbreviations: A, anterior octaval nucleus; ALLn, anterior lateral line nerve; ALLNd, dorsal ramus of anterior lateral line nerve; ALLNv, ventral ramus of anterior lateral line nerve; C, nucleus caudalis; CC, cerebellar crest; D/Desc VIII, descending octaval nucleus; EG, eminentia granularis of cerebellum; IXn, glossopharyngeal nerve; MG, magnocellular octaval nucleus; M/Med, nucleus medialis; NPLL, posterior lateral line nerve; OT, optic tectum; P, posterior octaval nucleus; PLL, posterior lateral line; PLLn, posterior lateral line nerve; SpC, spinal cord; V-VIIn, trigeminal and facial nerves; VIIIa, anterior ramus of octaval nerve; VIIIp, posterior ramus of octaval nerve; and Xn, vagus nerve.

the auditory midbrain (**Figures 1(c)**, **2**, and **3**). However, neither of these nuclei are dedicated to auditory processing as each of them also has areas that receive input from vestibular organs and that have further connections to structures other than the auditory midbrain. Furthermore, it is possible that not all acoustic populations within the octaval nuclei have been discovered. Generally, octaval nerve inputs to DO and AO are ipsilateral, although in some species the octaval nerve distributes to the DO on both sides of the brain (bilaterally). The DO has commissural connections to the contralateral DO. Commissural connections are common throughout the auditory brain, as is the case in other vertebrates. For further discussion on the fish vestibular system, **see also Hearing and Lateral Line:** Vestibular System Anatomy and Physiology.

The largest first-order acoustic population resides in the dorsomedial (dm) zone of the DO (**Figures 4** and **5**). Fibers from the inner ear to the DO are distributed similarly in cartilaginous fish and most bony fish; therefore, this distribution pattern is hypothesized to reflect the primitive condition for jawed vertebrates. Inputs from the saccule, which is believed to be a prominent auditory organ in most fish species, usually provide the majority of input to DO dm zone (**Figure 4**). Fibers from the lagena and utricle overlap saccular inputs at some levels, but are largely laterally or ventrolaterally adjacent to DO dm zone (**Figure 4**). In the goldfish, some of the lagenar and utricular fibers in these adjacent areas contact long lateral dendrites of DO dm neurons, lending additional support to physiological studies that indicate auditory function in all three of the otolith organs. Based on a single cartilaginous and two bony fish species, the macula neglecta terminates largely ventral to dm zone and overlaps inputs from the utricle and posterior semicircular canal crista.

Otolith organ inputs to DO are specialized in clupeid fishes, which include taxa such as shad and herring. The clupeid utricle has a specialized acoustic pressure reception area and it is the utricle, rather than the saccule, that provides the main input to DO dm zone (**Figure 4**). The unrelated sleeper goby potentially illustrates another exception to the typical pattern of otolith inputs; in this species, otolith fibers appear to interdigitate within the dorsal descending nucleus. For additional information on this species, **see also Hearing and Lateral Line:** Physiology of the Ear and Brain: How Fish Hear.

Among fish species, there is a morphological variation in the size of dm area relative to the DO as a whole and in the relationship of dm to the cerebellar crest (CC) (**Figures 1(a)**, **1(b)**, and **5**). Species with the qualitatively largest dm zones have otophysic connections, that is, structural linkage between the inner ear and the swimbladder or other gas-filled structure. Some DO dm cells, especially in those species in which dm zone is particularly large, very likely receive descending input from CC axons. These axons, which originate largely from granule cells in the cerebellum, are known to modulate neuronal activity in the nucleus medialis (M) and may similarly

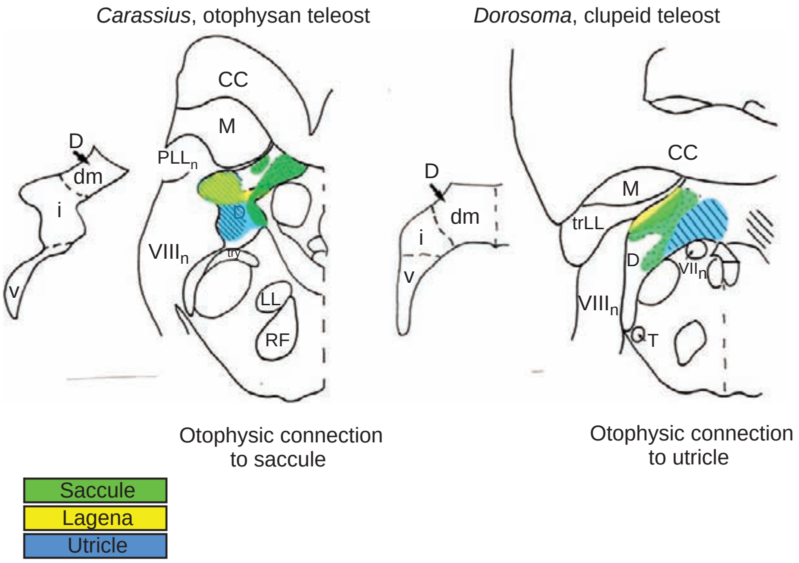
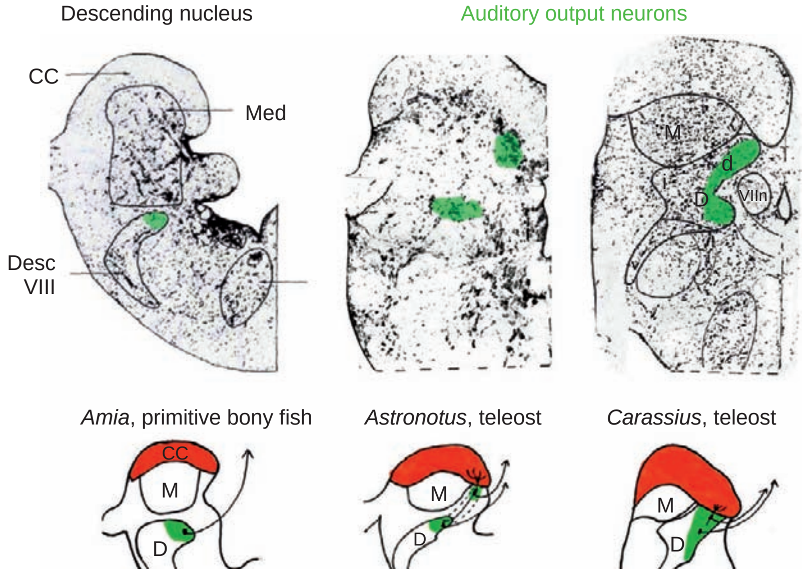

Carassius, otophysan teleost
Dorosoma, clupeid teleost
CC
M
D
dm
i
v
PLLn
D
VIIIn
trV
LL
RF
D
dm
i
v
CC
M
trLL
D
VIIn
VIIIn
T
Otophysic connection to saccule
Otophysic connection to utricle
Saccule
Lagena
Utricle

Figure 4 Transverse hemisections through the medulla of *Carassius auratus* (goldfish; left) and *Dorosoma cepidianum* (gizzard shad, right). Shown are the terminal zones of the saccule (green areas with arrowheads), lagena (yellow area enclosed by the dashed lines), and utricle (blue area with diagonal lines). Abbreviations: CC, cerebellar crest; D, descending octaval nucleus; dm, dorsomedial zone of descending octaval nucleus; i, intermediate zone of descending octaval nucleus; LL, lateral lemniscus; M, nucleus medialis; PLLn, posterior lateral line nerve; RF, reticular formation; trLL, lateral line nerve tracts; T, nucleus tangentialis; trV, descending tract of the trigeminal nerve; v, ventral zone of descending octaval nucleus; VIIn, sensory root of the facial nerve; VIIIn, octaval nerve; and VIIIp, posterior ramus of octaval nerve.



Amia, primitive bony fish *Astronotus*, teleost *Carassius*, teleost

Figure 5 Top: Transverse, cresyl violet-stained hemisections through the medulla of *Amia calva* (bowfin), *Astronotus ocellatus* (oscar), and *Carassius auratus* (goldfish). In each species, green delineates the acoustic region in the descending octaval nucleus. Bottom: Each of the diagrams corresponds to the species above it, showing the locations of neurons in the descending octaval nucleus that receive input from the saccule and project to the acoustic midbrain. The relationship of these auditory output neurons to the cerebellar crest (orange) is also shown. Abbreviations: CC, cerebellar crest; D/Desc VIII, descending octaval nucleus; d, dorsomedial zone of descending octaval nucleus; i, intermediate zone of descending octaval nucleus; M, nucleus medialis; v, ventral zone of descending octaval nucleus; and VIIn, sensory root of the facial nerve.

affect DO dm neurons. CC axons originating from a midbrain acoustic structure (the medial pretoral nucleus, MPT) may also supply DO dm zone in some species. It is possible that the CC provides little or no input to DO dm zone in species in which this zone is small.

Primitively, the dorsal portion of the AO was also acoustic, although this may not be the case in all jawed fish. Fibers from the three otolith organs usually have a distribution pattern similar to that present in the DO. The AO in clupeids has specialized anatomy and inner ear inputs.

In cartilaginous fish, the periventricular nucleus and cell plate C3 receive input from the inner ear and thus potentially receive acoustic input. These structures do not appear to be present in bony fish.

Lateral line nuclei

The lateral line nerves distribute mechanosensory input to a small nucleus caudalis and a much larger nucleus M (**Figures 1–3**). Ascending projections to the midbrain arise only from M and mainly distribute adjacent to the acoustic midbrain area.

M, which is cytoarchitecturally complex, is capped by CC axons that provide a modulatory input to at least the most dorsally located M neurons (**Figure 3**). Terminals of lateral line nerves do not appear to reach these dorsal cells, but may synapse on their ventral dendrites in addition to neurons in other areas of M. Within M, mechanosensory input carried by the posterior lateral line nerve distributes dorsally/dorsolaterally, whereas anterior lateral line fibers from cephalic mechanoreceptors distribute ventrally/ventrolaterally. M gives rise to commissural fibers that supply its contralateral counterpart, and, in addition to its major input to the midbrain lateral line area, other connections that have been reported in individual species include output to the sensory trigeminal nucleus and various octaval nuclei and input from the ipsilateral sensory trigeminal nucleus.

Cross-talk between the auditory and lateral line systems

The vast majority of fibers from inner ear auditory organs and lateral line nerves provide input exclusively to octaval and lateral line nuclei, respectively. However, smaller inputs from the inner ear to M and from the lateral line to the DO dm zone are reported in many species. Each nucleus may therefore contain areas integrating acoustic and lateral line mechanosensory information. Anatomical overlap between the two sensory systems is also present in the isthmus, midbrain, and forebrain. The functional significance of cross-talk between the two systems is not understood and may vary among species. Possibilities include sensory integration supporting sound localization, as well as the specialization of lateral line receptors that are proximate to the inner ear for sound pressure detection.

Higher-order acoustic and/or lateral line nuclei

In bony fish, the secondary octaval (SO) nucleus (**Figure 1(c)**) receives bilateral input from the DO and projects bilaterally to the auditory midbrain (**Figure 2**). The SO has up to three regions with different neuronal morphologies: dorsal, intermediate, and ventral (**Figure 6**). The dorsal and ventral regions may be universally present in teleosts; three regions are usually present in teleosts with otophysic connections. In carp and goldfish, dorsal SO neurons receive descending input from a midbrain auditory area via axons traveling in the CC. An area within SO participates in the vocal circuitry in a teleost, the midshipman *Porichthyes*. For further discussion on this topic, **see also Hearing and Lateral Line:** Vocal Behavior of Fishes: Anatomy and Physiology.

The isthmoreticular (IR) and perilemniscular (PL) nuclei both project to the auditory midbrain (**Figure 2**). The connections of IR and PL are relatively unstudied, but in goldfish PL supplies an auditory diencephalic area – the anterior preglomerular nucleus. Cell groups referred to in various studies as PL, perilemniscal, and paralemniscal may or may not be comparable. In the midshipman paralemniscal neurons project bilaterally to the lateral line area of the midbrain.

Nucleus praeeminentialis (P) receives bilateral input from M and in some species is also known to project to M. Dense input to P from the auditory midbrain was recently demonstrated in the midshipman; this may be a unique connection.

In cartilaginous fish, cell plates C1, C2, and C3 and the nucleus of the lateral lemniscus are mainly higher-order populations (C3 also receives first-order input) that process information from the acoustic or lateral line systems, or from both. It is unknown whether or not any of these populations are homologous to any nuclei in bony fish.

Acoustic and Lateral Line Mechanosensory Areas in the Midbrain TS/LMC

The TS of bony fish (**Figures 1(c)** and **7**) and the LMC of cartilaginous fish are the presumed homologs of the mammalian inferior colliculus. Acoustic-processing areas are adjacent to lateral line mechanosensory areas. The TS of bony fish is known primarily from studies in teleosts and includes an auditory portion – nucleus centralis (NC) – and a lateral line mechanosensory portion – nucleus ventrolateralis (VL) (**Figure 7**). As at lower levels of the brain, anatomical and physiological studies indicate some overlap of the two sensory systems. In certain bony fish species, the boundary between NC and VL is readily apparent only as a result of tract-tracing or via electrophysiological recording. Similarly, the LMC of cartilaginous fish has clearer subdivisions in some species than in others. The LMC includes a lateral line mechanosensory mediodorsal nucleus and a medioventral nucleus that is thought to be acoustic. Relatively, little is known about the connections of the LMC compared with those of the bony fish TS.

In bony fish, NC receives auditory ascending input – much of it bilateral – from the DO dm, AO, SO, IR, and PL (**Figure 2**). The ascending outputs of the NC course

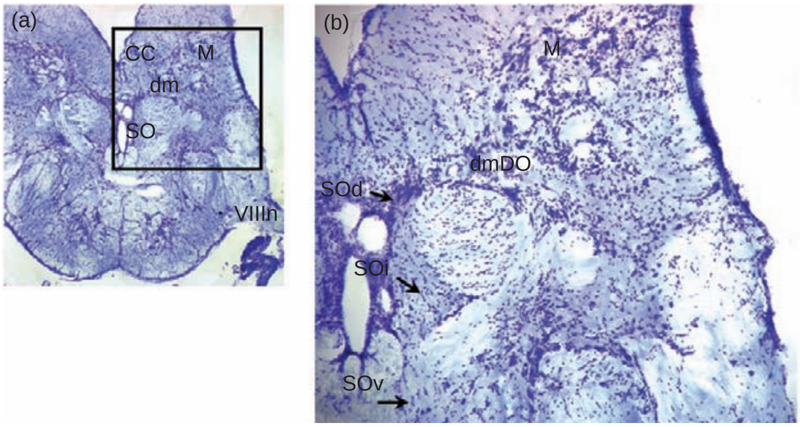


Figure 6 Cresyl violet-stained transverse sections of the goldfish medulla at the level of the anterior root of the VIII (octaval) nerve. The area within the rectangle in (a) is shown at higher power in (b). Abbreviations: CC, cerebellar crest; dm/dmDO, dorsomedial zone of the descending octaval nucleus; M, nucleus medialis; SO, secondary octaval population; SOd, secondary octaval population, dorsal division; SOi, secondary octaval population, intermediate division; SOv, secondary octaval population, ventral division; and VIIIn, octaval nerve.

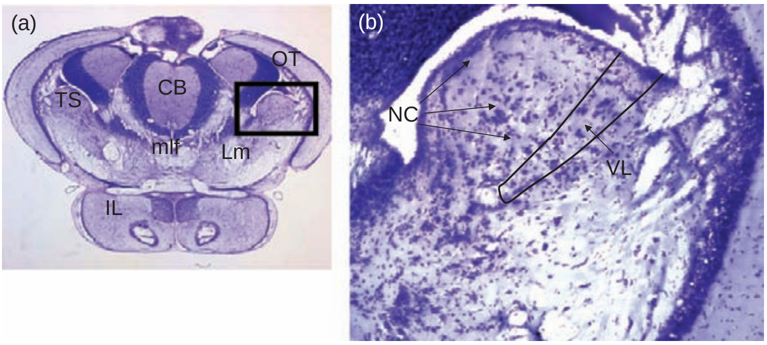


Figure 7 Cresyl violet-stained transverse sections of the goldfish midbrain at the level of the torus semicircularis, encompassed by the rectangle in (a). The torus semicircularis is shown at higher power in (b). Abbreviations: CB, cerebellum; Lm, lateral lemniscus; mlf; medial longitudinal fasciculus; OT, optic tectum; and TS, semicircularis.

to diencephalic nuclei (**Figure 2**) that are generally considered to be auditory; many of these nuclei have descending projections to NC. Some species also have an MPT that receives input from NC. In addition, NC projects to the deep, multisensory layers of the optic tectum and to lower-level acoustic populations in the medulla. Additional connections of NC that may be species specific have been reported.

The VL is the lateral line processing toral area (**Figure 7**). M provides the major ascending input into VL (**Figure 2**); in some species, it may also receive input from other structures at lower levels of the brain, such as the paralemniscal neurons. VL also has reciprocal connections with the optic tectum, primarily its deep layers, and projects ipsilaterally to P.

Acoustic and Lateral Line Mechanosensory Areas of the Forebrain

Forebrain acoustic and lateral line areas have been systematically studied in few species of bony fish. Nonparallel terminology makes species comparisons across the literature daunting. Interestingly, a comparative study of various otophysan species suggests many connectional variations. The following information is drawn primarily from the most recent publications describing forebrain connections in two distantly related teleost groups: the otophysans goldfish (*Carassius*) and carp (*Cyprinus*) and the batrachoidid midshipman. Only major connections are described.

In goldfish (*Carassius*), carp, and midshipman, three areas in the diencephalon are associated with the auditory system, the lateral line mechanosensory system, or both: subdivisions within the preglomerular complex (PG), the central posterior nucleus (CP), and the anterior tuber/tuberal nucleus (AT) of the hypothalamus (**Figure 1(c)**). The posterior thalamic nucleus (PTH) in midshipman may have unique lateral line inputs.

In goldfish and carp, the PG, CP, and AT project to or receive input from specific pallial and subpallial telencephalic areas (**Figures 1(c)** and **2**): the medial division of area dorsalis of the pallium (Dm), the lateral division of area dorsalis of the pallium (Dl), and, within the subpallium, area ventralis dorsal nucleus (Vd), area ventralis ventral nucleus (Vv), and area ventralis supracommissural nucleus (Vs). Based on overall patterns of connectivity, there are two major hypotheses about how Dm and Dl can be compared to regions of the tetrapod pallium. One view holds that Dm and Dl are similar to the tetrapod amygdala and hippocampus, respectively. An alternative view is that Dm and Dl are homologous to isocortical areas.

Connections of preglomerular complex

The PG (**Figure 1(c)**) is composed of several divisions, some of which are further parsed into subnuclei. Two recent studies recognize nucleus preglomerularis anterior (PGa) as an auditory area, but apply slightly different nomenclature to auditory and lateral line regions within nucleus preglomerularis lateralis (PGl).

The strong connections of the NC to the PGa and to the caudal subnucleus of the nucleus preglomerularis lateralis (cPGl; nomenclature of Northcutt), and the connections of PGa and cPGl to the Dm likely comprise the major ascending auditory pathway into the pallium (**Figure 2**). Comparative studies suggest that acoustic processing in Dm may be characteristic of all bony fish. While Dm also receives inputs from the lateral line (including electrosensory input, when present) and gustatory systems, there is evidence suggesting that Dm includes modality-specific subregions.

The PGl-v (ventral part of the rostral subnucleus of nucleus preglomerularis lateralis; nomenclature of Northcutt) receives lateral line input from VL and AT, and presumably visual input from the optic tectum. Terminal zones of rPGl-v axons distribute in Dm and Dl.

Connections of central posterior nucleus

The CP (**Figures 1(c)** and **2**) is reciprocally connected to NC and VL. It is also connected to other forebrain areas that receive auditory and lateral line inputs. CP has a major ascending output to the subpallium (**Figure 1(c)**) – Vd, Vv, and Vs – and a rather minor output to the pallium – Dm.

Connections of anterior tuber of the hypothalamus

The hypothalamus (**Figures 1(b)**, **1(c)**, and **2**) gets input from NC, and in at least some species from VL. In all/most species, auditory and lateral line inputs distribute to a nucleus or region in the AT. In goldfish, AT receives inputs from two other acoustic structures, MPT and PGa, as well as from the suprachiasmatic nucleus. AT projects to a region of the PG – the rPGl-v; the latter nucleus projects to Dm and Dl. Dm gives rise to a strong projection back to AT.

Summary

The similarity of central lateral line and acoustic pathways in fish may be the result of common developmental processes, or rules that organize the location and connectivity of the central components of the two sensory systems. It can be hypothesized that shared developmental processes among fishes and between fish and land vertebrates may underlie the fundamentally similar organization of the acoustic pathways in all vertebrates, despite striking differences in the identity of peripheral auditory receptors and in the anatomical details of central auditory populations.

See also: **Brain and Nervous System:** Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Nervous System: An Introduction; Physiology of the Mauthner Cell: Discovery and Properties; Physiology of the Mauthner Cell: function. **Hearing and Lateral Line:** Auditory System Morphology; Lateral Line Neuroethology; Lateral Line Structure; Physiology of the Ear and Brain: How Fish Hear; The Ear and Hearing in Sharks, Skates, and Rays; Vestibular System Anatomy and Physiology; Vocal Behavior of Fishes: Anatomy and Physiology.

Further Reading

Braford MR, Jr. (2009) Stalking the everted telencephalon: Comparisons of forebrain organization in basal ray-finned fishes and teleosts. *Brain, Behavior and Evolution* 71: 56–76.

Braun CB, Coombs S, and Fay RR (2002) What is the nature of multisensory interaction between the octavolateralis sub-systems? *Brain, Behavior and Evolution* 59: 162–176.

Coombs S and Braun CB (2003) Information processing by the lateral line system. In: Collin S and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments*, pp. 122–138. New York: Springer.

Grothe B, Carr CE, Casseday JH, Fritzsch B, and Koppl C (2004) The evolution of central pathways and their neural processing patterns. In: Manley GA, Popper AN, and Fay RR (eds.) *Evolution of the Vertebrate Auditory System*, pp. 289–359. New York: Springer.

McCormick CA (1999) Anatomy of the central auditory pathways of fish and amphibians. In: Fay RR and Popper AN (eds.) *Comparative Hearing: Fish and Amphibians*, pp. 155–217. New York: Springer.

New JG, Coombs S, McCormick CA, and Oshel PE (1996) Cytoarchitecture of the medial octavolateralis nucleus in the goldfish, *Carassius auratus*. *Journal of Comparative Neurology* 366: 534–546.

Northcutt RG (2006) Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *Journal of Comparative Neurology* 494: 903–943.

Webster DB (1992) An overview of mammalian auditory pathways with an emphasis on humans. In: Webster DB, Popper AN, and Fay RR (eds.) *The Mammalian Auditory Pathway: Neuroanatomy*, pp. 1–22. New York: Springer.

Weeg MS and Bass AH (2000) Central lateral line pathways in a vocalizing fish. *Journal of Comparative Neurology* 418: 41–64.

Yamamoto N and Ito H (2008) Visual, lateral line, and auditory ascending pathways to the dorsal telencephalic area through the rostrolateral region of the lateral preglomerular nucleus in cyprinids. *Journal of Comparative Neurology* 508: 615–647.

Physiology of the Ear and Brain: How Fish Hear

Z Lu, University of Miami, Coral Gables, FL, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Auditory Transduction
Afferent Coding of Sounds
Central Representation of Sounds
Summary
Further Reading

Glossary

Auditory plasticity The ability of the auditory system to make structural and functional changes.

Auditory transduction The transformation of acoustic stimuli into electrical signals by sensory hair cells in the ear.

Coefficient of synchronization A parameter to describe the strength of phase locking of a neuron, ranging from 0 (random firing) to 1 (perfect phase locking).

Inter-spike interval histogram A histogram of the distribution of inter-spike intervals in time, showing the temporal response pattern of a neuron.

Octavolateral efferent The process of a neuron in the brain that projects to octavolateral organs such as the inner ear and lateral line; this output from the brain alters the sensitivity and thus regulates octavolateral inputs to the brain.

Otolithic afferent The process of a ganglion neuron that conveys the sensory input from an otolithic organ (the lagena, saccule, or utricle) to the brain.

Period histogram A histogram of the distribution of spike rates over the stimulus period. A peak in the period histogram indicates phase locking, whereas no peak indicates a random response.

Peri-stimulus time histogram A histogram of the spike rate over the stimulus duration, showing the temporal response pattern of a neuron. It is also called a post-stimulus time histogram.

Phase locking A temporal response feature of a neuron that fires spikes at the same phase per cycle of a pure tone.

$Q_{10\ \mathrm{dB}}$ A ratio of the characteristic frequency over the frequency bandwidth at 10 dB above the lowest threshold; it represents the sharpness of a tuning curve.

Rate-level function A plot of the spike rate vs. the stimulus level of a neuron that is used to reveal the neural coding of stimulus intensity.

Threshold The lowest stimulus level that evokes neural activity.

Torus semicircularis A major octavolateral nucleus in the midbrain of fishes.

Tuning curve A plot of the threshold vs. the stimulus frequency for a single neuron, showing its characteristic frequency (the most sensitive frequency), the best sensitivity (the lowest threshold), and its sharpness of tuning ($Q_{10\ \mathrm{dB}}$).

Introduction

Hearing is an important sense in fishes. The fish ear contains three otolithic organs (the saccule, utricle, and lagena), and these play a key role in the ability of fishes to hear and maintain balance. In the majority of fishes that have been examined, the saccule is considered the major auditory organ, whereas the lagena and utricle may play a minor role in hearing. The otolithic organ of fishes is a fluid-filled sac composed of a sensory epithelium attached to a calcareous otolith via an otolithic membrane (**Figure 1**) (**see also Hearing and Lateral Line**: Auditory System Morphology).

The sensory epithelium has many sensory hair cells, each of which has an eccentrically placed kinocilium and a cluster of stereocilia. The height of stereocilia decreases as the distance they are located from the kinocilium increases. Thus, each hair cell is morphologically polarized. Hair cells in otolithic organs are innervated by otolithic ganglion neurons, and these ganglion neurons convey auditory and vestibular inputs to the brain for central processing (**see also Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy). In addition, the brain has octavolateral efferent neurons that receive sensory inputs and project to octavolateral organs such as the ear and the lateral line. The octavolateral system in fishes includes octavolateral organs as well as octavolateral nuclei in the brain.

The ear functions in hearing and balance; the lateral line system mainly detects net water movement over the fish body (**see also Hearing and Lateral Line**: Lateral Line Neuroethology). Very few studies have

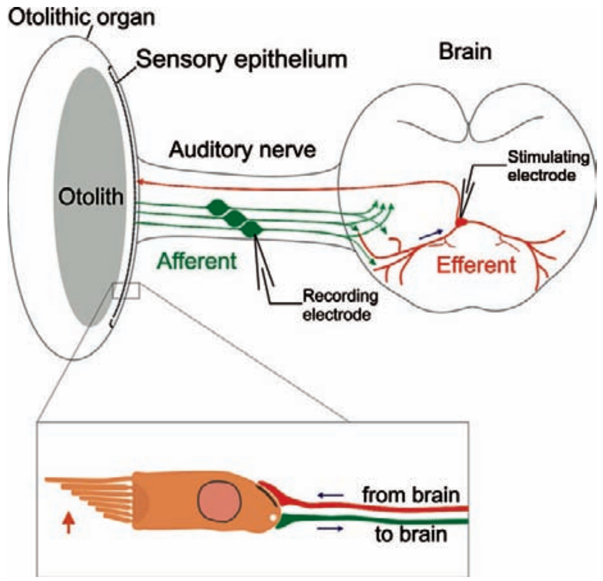


Figure 1 A schematic drawing of the auditory system of fishes, showing an otolithic organ that is innervated by auditory ganglion neurons (green) and an octavolateral efferent neuron (red). Auditory afferents send encoded acoustic information to the brain whereas the auditory efferent projects to hair cells in the otolithic organ. A metal electrode is used to stimulate the octavolateral efferent neuron in the brain while acoustically evoked neural responses from an auditory ganglion neuron are recorded by a microelectrode. The enlargement of the sensory epithelium shows a sensory hair cell with its morphological and functional polarization indicated by the arrow near the ciliary bundle. Adapted from Tomchik SM and Lu Z (2004) Morphology and physiology of otolithic organ efferents. *Society for Neuroscience* Abstract 530.4.

been conducted on auditory physiology of major fish groups, such as lampreys, sharks, and sturgeons, that have a sensory epithelium or otolithic organs and are most likely able to detect the particle motion mode of sound. This article focuses primarily on the physiology of the auditory portion of the octavolateral system in teleost fishes including the sensory hair cells, auditory afferents, major auditory nuclei in the brain, and auditory efferents.

Auditory Transduction

The first step of auditory processing in fishes is auditory transduction, the transformation of acoustic energy into an electrical signal, such as a receptor potential, by sensory hair cells in the ear. When a sound wave reaches an otolithic organ, it sets the otolithic organ in motion. Due to a significant difference in tissue density between the sensory epithelium and otolith, the epithelium tends to move along with the sound wave while the otolith tends to remain at rest (**see also Hearing and Lateral Line**: Auditory System Morphology, Biomechanics of the Inner Ear in Fishes). This relative movement between the sensory epithelium and otolith generates a shearing force that bends the ciliary bundles of hair cells.

When hair cell ciliary bundles are bent from the stereocilia toward the kinocilium, hair cells are activated via the depolarization of their cell membranes. However, when the ciliary bundles are bent in the opposite direction, hair cells are inhibited via hyperpolarization of their cell membranes. Therefore, sensory hair cells in the inner ear of fishes are functionally, as well as morphologically, polarized. When hair cells are activated, they release a chemical called a neurotransmitter. The neurotransmitter binds to its receptor in the membrane of the otolithic ganglion neurons, leading to depolarization of the neuron. If the depolarization reaches a certain membrane potential, the neuron fires action potentials (also called spikes). The spikes travel along afferents of the auditory ganglion neurons to convey encoded acoustic information to the brain for central processing.

Afferent Coding of Sounds

Intensity Coding

Sound intensity (level) is an important physical property of sound. How do fishes tell a loud sound from a weak one? First, look at how individual auditory afferents respond to sounds at different sound levels. The relationship between neural activity and sound level can be explained using a rate-level function. As shown in **Figure 2**, auditory afferents do not fire spikes when the sound level is too low, but do begin to fire spikes as sound intensity reaches a certain level called the threshold, which is defined as the lowest sound level that can evoke a neural response. Above threshold, the firing rate increases with sound level until the maximum firing rate is reached. The sound intensity corresponding to the

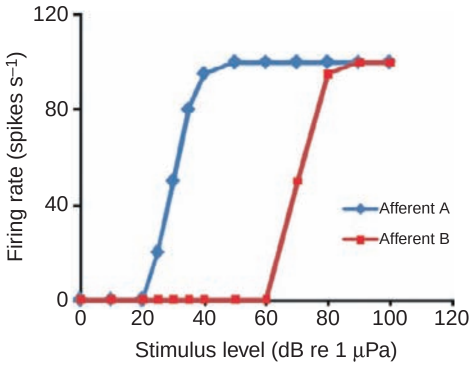


Figure 2 Schematic drawings of rate-level functions of two auditory afferents (A and B) with different thresholds and saturation levels. Firing rates of each auditory afferent increase monotonically with stimulus level in a range between threshold and saturation.

maximum firing rate is called the saturation level. Auditory afferents are unable to increase their firing rate beyond the saturation level. Within the linear portion of a rate-level function between threshold and saturation, neural firing rate is correlated with sound level. Therefore, sound intensity is encoded in the firing rate by auditory afferents. This firing rate is sent to the fish brain for processing to extract the sound intensity information.

Each fish otolithic organ nerve (the saccular nerve) is a collection of afferents and efferents (**Figure 1**). Afferents are sensory fibers that convey inputs from the otolithic organs to the brain, whereas efferents are motor fibers from the brain that project to the otolithic organs. Auditory thresholds vary remarkably among individual auditory afferents. For example, afferent A in **Figure 2** is more sensitive than afferent B because afferent A has a lower threshold (i.e., 20 dB re 1 μPa) than afferent B (60 dB re 1 μPa). Afferent A is useful for encoding sound intensity between 20 and 40 dB re 1 μPa, whereas afferent B is good for encoding sound between 60 and 80 dB re 1 μPa. In other words, individual auditory afferents of fishes often have a hearing dynamic range of about 20 dB. By pooling all auditory afferents together, the auditory nerve is capable of encoding sound levels over a 100-dB range. The sum of the properties of all auditory afferents accounts for the ability of fishes to detect sound intensity (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear).

Frequency Coding

Sound frequency provides animals with information about the nature of a sound source, such as a prey or a predator. Frequency analysis is one of the important functions of the auditory systems in animals. Compared with terrestrial vertebrates, fishes possess a primitive ability to analyze sound frequency.

Any natural sound in the world is a complex sound, which is a sum of many simple sounds (i.e., pure tones). Frequency selectivity of auditory afferents has been studied most thoroughly in teleost fishes such as the goldfish (*Carassius auratus*) and oyster toadfish (*Opsanus tau*). The frequency selectivity of single auditory afferents can be illustrated with a tuning curve. A tuning curve is a plot of thresholds at different sound frequencies (**Figure 3**). Three parameters from a tuning curve are used to describe frequency-response properties of auditory afferents. The best sensitivity (BS) refers to the lowest of thresholds at sound frequencies tested, which indicates how sensitive an auditory afferent is – the lower the BS, the higher the sensitivity of an auditory afferent. The characteristic frequency (CF) of an auditory afferent is the stimulus frequency at BS. $Q_{10\ \mathrm{dB}}$ is defined as CF over the frequency bandwidth at 10 dB above BS and reveals the sharpness of tuning of an auditory afferent.

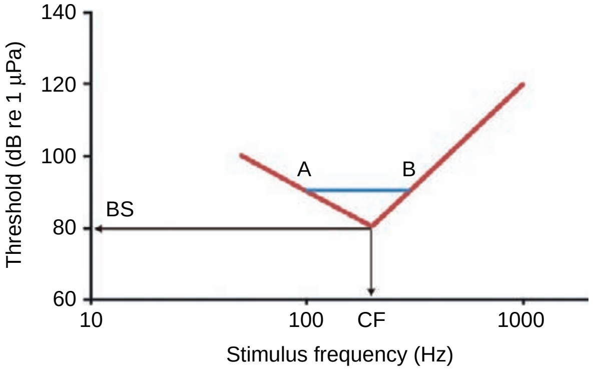


Figure 3 Schematic drawing of a tuning curve of an auditory afferent. BS = 80 dB re 1 μPa. $Q_{10\ \mathrm{dB}}$ = CF/FBW = 1, where CF = 200 Hz and FBW = 200 Hz. FBW is the frequency bandwidth at 10 dB above the BS, which is indicated by the length of AB.

In general, compared with terrestrial vertebrates, auditory afferents of fishes are broadly tuned to relatively low frequencies up to several hundred hertz. It is known that tonotopic organizations (also called tonotopy) are present in most nonfish vertebrates. Tonotopy refers to the spatial representation of sound frequencies in a structure of the auditory system such as the basilar membrane of the cochlea (the hearing organ) in mammals. Auditory afferents innervating hair cells in the basilar membrane are tuned to specific frequencies that correspond to the resonant frequencies of the basilar membrane at the innervating sites. Tonotopy has not been well investigated in fishes. Although some studies provided evidence of a crude tonotopic organization in the goldfish saccule as well as the carp's midbrain, the existence of tonotopy needs to be verified in other fish species.

Temporal Coding

A sound wave is often plotted in time domain (i.e., a function of displacement of medium molecules over time). Temporal coding refers to a neural coding that is based on the timing of spikes, or intervals between spikes. For example, fish auditory afferents are capable of firing spikes that are locked at a certain phase of the cycle of a pure tone; this is called phase locking. Temporal response properties of auditory afferents can be illustrated using the peri-stimulus time histogram (PSTH), inter-spike interval histogram (ISIH), and period histogram (**Figure 4**). PSTH is a histogram of spike times over the duration of a sound presentation. The spike time is the time interval between the beginning of the stimulus and the time at which a spike occurs. Fish auditory afferents often have a sustained temporal response pattern with little adaptation (see the PSTH in **Figure 4**).

Fish auditory afferents tend to phase-lock to tonal stimulation, showing many peaks with a regular inter-peak

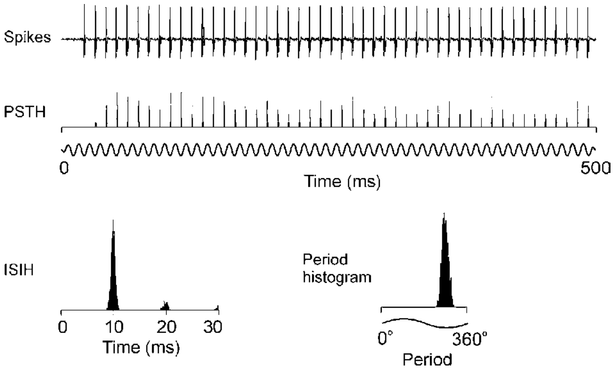


Figure 4 Phase-locking response pattern of an auditory afferent to a 500-ms pure tone at 100 Hz, showing a sweep of spikes, peri-stimulus-time histogram (PSTH), inter-spike interval histogram (ISIH), and period histogram. The histograms were constructed from neural responses of the auditory afferent to 50 presentations of the tone. Modified from Lu Z, Xu Z, and Buchser WJ (2003) Acoustic response properties of lagenar nerve fibers in the sleeper goby, *Dormitator latifrons*. *Journal of Comparative Physiology A* 189: 889–905.

interval over the sound duration in the PSTH. The distribution of time intervals between spikes can be illustrated using the ISIH. For example, in the ISIH shown in **Figure 4**, the major peak occurs at 10 ms, corresponding to the period of the 100-Hz pure tone used. In other words, the information of sound frequency is encoded in the temporal pattern of spikes, the inter-spike interval. The small peak at 20 ms in the figure indicates that the afferent sometimes fired one spike every two stimulus periods. The phase-locking feature of auditory afferents is often displayed with the period histogram, which illustrates the spike time distribution over the stimulus period (see **Figure 4**). A peak in the period histogram indicates that the afferent is locked around a certain phase. The strength of phase locking of auditory afferents can be assessed with the coefficient of synchronization, *R*. *R* ranges from 0 (no phase locking, a flat distribution of spike times in the period histogram) to 1 (perfect phase locking, a vertical line at a phase of the stimulus cycle).

There are several temporal response characteristics of auditory afferents in fishes. Most otolithic afferents are phase locked to tonal stimulation, and the strength of phase locking varies with stimulus level and frequency. Above threshold, phase locking increases with stimulus level until saturation (1 spike every stimulus cycle). At a given stimulus level, phase locking decreases as stimulus frequency moves away from CF. Some auditory afferents of fishes are capable of firing two spikes per stimulus cycle, particularly at stimulus frequencies that are equal or less than 100 Hz. The ISIH of this response pattern shows a major peak at half the stimulus period, and the period histogram has two peaks that are separated by 180°. This double phase locking appears to result from the fact that an auditory ganglion neuron may innervate two hair cell groups that are oriented in opposing directions from one another.

Auditory Plasticity

Auditory plasticity refers to the ability of the auditory system to make structural and functional changes. It is another common feature shared by all vertebrates. Although auditory plasticity has not been thoroughly investigated in fishes, a few studies have demonstrated changes in hearing sensitivity during the development. For example, the auditory sensitivity of juvenile plainfin midshipman fish (*Porichthys notatus*) is about 10–15 dB lower than that of adults. An increase in auditory sensitivity during the development has also been shown in the bicolor damselfish (*Pomacentrus partitus*).

Auditory plasticity can also occur in adulthood. The frequency response of saccular afferents of adult female midshipman varies with seasonal reproductive activity. Specifically, saccular afferents are tuned to higher frequencies in gravid females during the summer than in nonreproductive females during the winter (**see also Hearing and Lateral Line**: Vocal Behavior of Fishes: Anatomy and Physiology). In the summer, midshipman males swim to the shallow water of intertidal zones to build nests and vocalize to attract conspecific females. The advertisement call of males is a sound with multiple frequency components. The fundamental frequency of the midshipman's advertisement call is about 100 Hz, and the second harmonic, 200 Hz, is the most dominant frequency of the mating call. Saccular afferents of summer gravid females are more broadly responsive to the second and third harmonics of the advertisement call, than those of nonreproductive and winter adult midshipman females. This indicates that summer reproductive females are better fit to encode the male's mating call than winter adult females and nonreproductive juveniles. There is strong evidence that this auditory plasticity in the plainfin midshipman fish is mediated by sex hormones.

Central Representation of Sounds

Frequency Tuning

As described above, fish auditory afferents have crude frequency selectivity, that is, they are quite broadly tuned. How is the information of stimulus frequency processed in the brain? Data of frequency selectivity have been collected from the torus semicircularis (a major octavolateral nucleus in the midbrain) and the central posterior nucleus (a major auditory nucleus in the diencephalon) of the goldfish (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes and **Hearing and Lateral Line**:

Auditory/Lateral Line CNS: Anatomy). The $Q_{10\ \mathrm{dB}}$ of auditory neurons in the torus semicircularis is significantly higher than that of saccular afferents, whereas the $Q_{10\ \mathrm{dB}}$ of auditory neurons in the central posterior nucleus is similar to that of saccular afferents. These results indicate that frequency tuning is sharpened at the midbrain, and becomes broad again at the diencephalon. The enhanced tuning at the midbrain is most likely due to a unique inhibitory process in the brain. Evidence has shown that inhibitory side bands appear to sharpen frequency tuning at the level of hindbrain or midbrain. However, the strength of frequency tuning does not continue to increase at higher levels of the brain. It is hypothesized that the auditory midbrain and diencephalon play different roles in frequency analysis. The auditory midbrain contributes to analytic listening (i.e., breakdown of a complex sound into simple components); the auditory diencephalon plays an important role in synthetic listening (i.e., integration of sound components).

Temporal Response Patterns

In contrast to the sustained temporal response pattern with phase locking observed in auditory afferents, various temporal response patterns have been found for auditory neurons in the brain. They include onset, bursting, chopper, pauser, and buildup, which resemble those observed in the mammalian cochlear nucleus. These temporal response patterns have not been observed in auditory afferents, suggesting that they are response patterns unique to the brain. Although phase locking, even strong phase locking, is found in the brain, the percentage of phase-locked auditory neurons tends to decrease along the auditory pathway from the periphery to the brain. It appears that strong phase locking at the auditory periphery is converted into distinctive temporal responses with little phase locking in the brain.

Transformation from the Peripheral Code to a Central Code

Central auditory processing of natural sounds has been reported in several teleosts. For example, the waveform of the advertisement call (or hum) of male plainfin midshipman fish is similar to a 100-Hz pure tone. Like those of other teleosts, saccular afferents of the midshipman are phase locked to fine tonal structures of the mating call. Once this phase-locking information is conveyed to the brain, the brain transforms the peripheral phase-locking code into a new central code. Experimental results indicate that auditory neurons in the torus semicircularis of the midshipman do not show strong phase locking to individual tonal components of the male advertisement call. Instead, they are responsive to the frequency difference of concurrent hums. In the fish natural habitat, males with nests are located not far from one another. Thus, the sound received by a midshipman female is frequently a concurrent signal that is composed of two or more hums. The concurrent signal is often a beat because frequencies of two hums differ by only a few hertz. Auditory midbrain neurons of midshipman females are able to encode the frequency of the beat. Therefore, the peripheral phase-locking code is transformed into a beat frequency code in the fish midbrain.

Another example of central auditory processing of natural sounds involves the mormyrid fish (*Pollimyrus adspersus*), a weakly electric fish that also uses sound in communication. It has been documented that mormyrid males produce sounds such as grunts, moans, and growls to court conspecific females. These courtship sounds consist of click trains with inter-click intervals ranging from 5 to 50 ms. Auditory afferents of the mormyrids fire spikes that are time-locked to individual clicks but show no significant relationship to any inter-click intervals. This time-locked information at the auditory periphery is converted into a new code in the brain that enables the fish to extract temporal information of clicks of courtship sounds. Evidence shows that auditory neurons in the midbrain of this species are selectively responsive to the inter-click intervals that are consistent with those of courtship sounds. Therefore, temporal features of the courtship sounds are extracted in the midbrain of the mormyrid fish.

Role of Octavolateral Efferents

Like other vertebrates, fishes have an auditory system that consists of both afferent and efferent pathways as illustrated in **Figure 1**. In addition to the afferent pathway, octavolateral efferent neurons in the brain innervate auditory, vestibular, and lateral line organs to regulate their responses. Although the octavolateral efferent system has not been well investigated in fishes, results of some recent studies shed new light on the structure and function of the system. There are up to three octavolateral efferent nuclei in the brain of fishes, depending on the species. For example, the sleeper goby (*Dormitator latifrons*) has a single efferent nucleus that is centered on the midline of the medulla and is composed of 13–29 densely packed cell bodies. Each efferent neuron has bilateral processes that are thought to receive multiple sensory inputs such as auditory, vestibular, visual, somatosensory, and lateral line. Efferent fibers form several branches to project to multiple octavolateral organs including otolithic organs, vestibular ampullae, and lateral line neuromasts.

Compared with auditory afferents, octavolateral efferents have distinctive response properties. First, octavolateral efferents have higher spontaneous activity than otolithic afferents. Second, they are not phase locked to tonal stimulation. Third, all octavolateral efferents have low-pass tuning curves with CFs lower than 20 Hz. Fourth, octavolateral efferents have shallow rate-level functions without saturation up to 50 dB above threshold. Finally, they are omnidirectionally sensitive to sounds whereas most otolithic afferents are directionally selective.

How does efferent activation affect afferent responses from octavolateral organs in fishes? Early studies showed that (1) efferents that synapse on hair cells in auditory and lateral line organs tend to inhibit spontaneous and evoked activity of afferents and (2) efferents that go to vestibular organs or synapse on afferent terminals in auditory organs appear to have primarily excitatory or mixed effects on the afferent responses. The results of a recent study on the sleeper goby show that efferent stimulation inhibits the response of auditory afferents to a tone by reducing the signal-to-noise ratio in quiet conditions. However, when masking noise is added, efferent stimulation can increase the signal-to-noise ratio of auditory afferent responses. These results indicate that efferent feedback can help fishes extract a sound from a noisy environment.

Summary

Fishes possess an auditory system that enables them to process fundamental information of sound. The fish auditory system is part of the octavolateral system that plays an important role in hearing, balance, and detection of water movement. For fish hearing, sensory hair cells in the otolithic organs transduce sounds into spikes, and otolithic organ afferents convey spikes to auditory nuclei in the brain where spikes are analyzed to extract the information of sound such as intensity and frequency. At the auditory periphery, sound intensity is encoded in the spike rate whereas sound frequency is encoded primarily in the inter-spike interval. Peripherally encoded frequency and temporal information is analyzed in the brain so that biologically relevant information of sound can be extracted. Some fish auditory responses change with developmental and environmental factors. In addition, auditory efferent neurons in the brain receiving indirect auditory input innervate the inner ear to regulate the auditory input to the brain. These essential auditory processes observed in fishes appear to be shared by higher vertebrates, including humans.

See also: **Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes. **Hearing and Lateral Line**: Auditory System Morphology; Auditory/Lateral Line CNS: Anatomy; Biomechanics of the Inner Ear in Fishes; Lateral Line Neuroethology; Psychoacoustics: What Fish Hear; Vocal Behavior of Fishes: Anatomy and Physiology.

Further Reading

Bodnar DA and Bass AH (1997) Temporal coding of concurrent acoustic signals in auditory midbrain. *Journal of Neuroscience* 17: 7553–7564.

Crawford JD (1993) Central auditory neurophysiology of a sound-producing mormyrid fish: The mesencephalon of *Pollimyrus isidori*. *Journal of Comparative Physiology A* 172: 1–14.

Crawford JD (1997) Feature-detecting auditory neurons in the brain of a sound-producing fish. *Journal of Comparative Physiology A* 180: 439–450.

Fay RR (1978) Phase-locking in goldfish saccular nerve fibers accounts for frequency discrimination capacities. *Nature* 275: 320–322.

Fay RR (1984) The goldfish ear codes the axis of acoustic particle motion in three dimensions. *Science* 225: 951–954.

Fay RR and Edds-Walton PL (1997) Directional response properties of saccular afferents of the toadfish, *Opsanus tau. Hearing Research* 111: 1–21.

Fay RR and Popper AN (2000) Evolution of hearing in vertebrates: The inner ears and processing. *Hearing Research* 149: 1–10.

Furukawa T and Ishii Y (1967) Neurophysiological studies on hearing in goldfish. *Journal of Neurophysiology* 30: 1377–1403.

Highstein SM, Kitch R, Carey J, and Baker R (1992) Anatomical organization of the brainstem octavolateralis area of the oyster toadfish, *Opsanus tau. Journal of Comparative Neurology* 319: 501–518.

Kozloski J and Crawford JD (2000) Transformation of an auditory temporal code in the medulla of a sound-producing fish. *Journal of Neuroscience* 20: 2400–2408.

Lu Z and Fay RR (1993) Acoustic response properties of single units in the torus semicircularis of the goldfish, *Carassius auratus. Journal of Comparative Physiology A* 173: 33–48.

Lu Z and Fay RR (1995) Acoustic response properties of single neurons in the central posterior nucleus of the thalamus of the goldfish, *Carassius auratus. Journal of Comparative Physiology A* 176: 747–760.

Lu Z and Popper AN (2001) Neural response directionality correlates of hair cell orientation in a fish. *Journal of Comparative Physiology A* 187: 453–465.

Lu Z, Xu Z, and Buchser WJ (2003) Acoustic response properties of lagenar nerve fibers in the sleeper goby, *Dormitator latifrons. Journal of Comparative Physiology A* 189: 889–905.

Popper AN and Fay RR (1999) The auditory periphery in fishes. In: Fay RR and Popper AN (eds.) *Comparative Hearing: Fish and Amphibians*, pp. 43–100. New York, NY: Springer.

Sisneros JA, Forlano P, Deitcher D, and Bass AH (2004) Steroid-dependent plasticity of frequency sensitivity in the adult auditory periphery. *Science* 305: 404–407.

Suzuki A, Kozloski J, and Crawford JD (2002) Temporal encoding for auditory computation: Physiology of primary afferent neurons in sound-producing fish. *Journal of Neuroscience* 22: 6290–6301.

Tomchik SM and Lu Z (2005) Central octavolateral projections and convergence in the medulla of a teleost fish, the sleeper goby (*Dormitator latifrons*). *Journal of Comparative Neurology* 481: 96–117.

Tomchik SM and Lu Z (2006) Anatomy and physiology of octavolateral efferent neurons in a teleost fish. *Journal of Comparative Physiology A* 192: 51–67.

Tomchik SM and Lu Z (2006) Modulation of auditory signal-to-noise ratio by efferent stimulation. *Journal of Neurophysiology* 95: 3562–3570.

Relevant Websites

http://www.nbb.cornell.edu – Bass Laboratory, Department of Neurobiology and Behavior, Cornell University.

http://www.life.popperlab.umd.edu – Dr. Arthur N. Popper's Laboratory of Aquatic Bioacoustics, University of Maryland.

Sound Source Localization and Directional Hearing in Fishes

DG Zeddies, Marine Acoustics, Inc., Arlington, VA, USA

RR Fay, Loyola University Chicago, Chicago, IL, USA

JA Sisneros, University of Washington, Seattle, WA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Behavioral Evidence for Directional Hearing and Sound Source Localization
Theories of Directional Hearing and Sound Source Localization
Brain Mechanisms Underlying Sound Source Localization
Summary and Unresolved Issues
Further Reading

Glossary

Acoustic particle motion Motion of the medium associated with an acoustic disturbance.

Auditory scene analysis Process by which humans and other vertebrates are able to hear out and determine the various sources of sound that make up a given mixture of sources impinging on an organism, such as the individual musical instruments making up an orchestra. This process makes use of the perceptual ability of source segregation.

Otophysan fish Having bones (Weberian ossicles) that link the swimbladder to the ear.

Phonotaxis Movement of an organism relative (positive or negative) to a sound source.

Sound pressure The increase (and decrease) in the density of a medium associated with an acoustic disturbance.

Source segregation The perceptual ability to hear and determine individual sound sources from the mixture of multiple sources in the environment.

Introduction

The underwater acoustic environment can be noisy and complex. To make sense of their world, fishes must assign the sounds they hear to appropriate sources and not confuse sounds that originate from independent sources. It is easy to demonstrate that humans have this ability because, with eyes closed, they can identify a number of separate sound-producing objects around them. This is known as source segregation. This ability has been shown in a number of vertebrate groups, including mammals, birds, and fishes.

Source segregation is a fundamental component of auditory scene analysis, which is the parsing of the acoustic ambience into specific, independent sources. By definition, everything in the scene is significant to the extent that the components of noise, for example, must be segregated from biologically relevant sounds for communication signals to be properly interpreted. It is likely that all organisms that hear, from insects to humans, are capable of source segregation.

An important component of source segregation is sound source localization. Sound source localization gives the acoustic scene a spatial dimension. Surprisingly, it is still unclear whether or not fishes can locate sound sources in this sense. It seems logical that they do, but empirical and theoretical work on this topic is contradictory. All experiments to date that are consistent with sound source localization abilities in fishes could be interpreted as a more restricted capacity of discriminating between sources at different locations (and distances), and not necessarily knowing where the sources are located in an absolute sense.

This article provides an overview of underwater sound and the function of fish ears (for more detailed descriptions, **see also Hearing and Lateral Line:** Auditory System Morphology, Biomechanics of the Inner Ear in Fishes, and Psychoacoustics: What Fish Hear).

Underwater Sound

A thorough treatment of acoustics is beyond the scope of this article but can be found in **Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes. However, for this article, it will be necessary to understand some important differences between sound pressure and acoustic particle motion. Sound pressure is a measure of the fluctuations in the density of the medium due to the presence of sound. Sound pressure is what most terrestrial ears sense and it is measured using a microphone or hydrophone. Particle motion is the movement of the particles that make up the media when sound is present. This movement is described in terms of particle displacement, velocity, or acceleration (**see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes). An important distinction between pressure and particle motion is that pressure is scalar while particle motion is a vector. That is, pressure at any one point is a single number that represents magnitude. Particle motion is a vector quantity, meaning that it is a number composed of two parts, a magnitude and a direction. The difference between a scalar and a vector is that of knowing that a car is moving at the speed of 100 km h^{-1} (scalar) versus knowing that this car is moving at the speed of 100 km h^{-1} due north (vector).

When a disturbance or motion occurs, energy is radiated away from the source in the form of pressure and particle motion. As one moves away from the source, pressure and particle motion decay at different rates. In what is known as the near field, or close to the sound source (**see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes for a more complete description), the amplitude of the particle motion decreases more rapidly with distance than the amplitude of the pressure. In air, particle motion decays within short distances of the source and it is rarely experienced by terrestrial animals. In water, the wavelengths are longer because water is denser than air. Consequently, the near field underwater is larger than in air and particle motion remains high farther from the source. Far from a sound source, the particles of the media still move to cause pressure fluctuations. The particle motion in the far field is radial to the source and proportional to the sound pressure amplitude. The range of the near field and far field depends on the type of sound source and the signal wavelength, which is determined by the frequency of the signal and the speed of sound in the medium (for a quantitative description of sounds fields, **see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes).

The Physics of Fish Ears

The ears of all fishes consist of otolith-containing or otoconia-containing end organs (**see also Hearing and Lateral Line:** Auditory System Morphology) that function as inertial accelerometers. As accelerometers, the otoliths are set in motion by the motion of the medium. More precisely, the body of the fish moves with the same displacement, direction, and phase as the water because fish tissue has about the same acoustic properties as water. Otoliths and otoconia are denser than water and, therefore, move with smaller amplitude and lag in phase relative to body of the fish (**see also Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes). The result is relative movement between the otolith and the macula (sensory epithelium) that contains the sensory hair cells (**see also Hearing and Lateral Line:** Auditory System Morphology). Hair cells are inherently directionally sensitive and their axis of best directional sensitivity varies along the macula (**Figure 1**; **see also Hearing and Lateral Line:** Auditory System Morphology and Biomechanics of the Inner Ear in Fishes). This is the basic mechanism by which all fish ears are thought to function, and it is, presumably, how fishes would get directional information about a sound source.

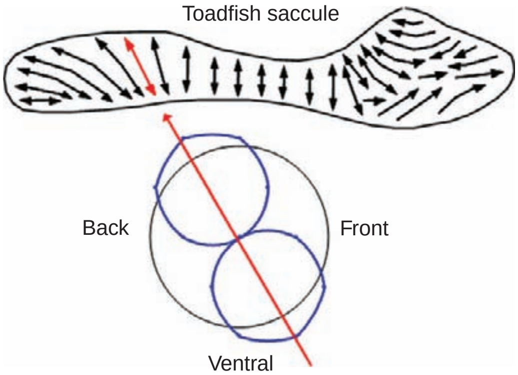


Figure 1 Movement of the otolith in one direction maximally activates only a portion of the hair cells on the macula. Top: Orientation pattern of hair cells on the saccular macula (**see also Hearing and Lateral Line**: Auditory System Morphology). The red arrow shows the group of hair cells maximally activated by the movement of the otolith along the axis indicated by the red arrow in the lower part of the figure. Bottom: The directional response pattern of a nerve cell and hair cell (blue line) with a directional orientation (best axis) equal to the red arrow. This cell will respond best when the direction of particle motion is along the pathway indicated by the red arrow. It will not respond when the motion is 90° to the arrow. Hair cells and nerve cells from different locations on the macula will respond best when the particle and respective otolith motion is along the lines of their respective black arrows.

Some fishes have specialized morphological adaptations that enable them also to detect sound pressure. To effectively sense the pressure component of sound, fishes make use of gas-filled structures such as swimbladders because the gas is compressible and will change volume in a sound field. Such specialized fish species have their gas bladders in close proximity to the ear and/or specializations such as the Weberian ossicles in otophysan fishes (e.g., goldfish (*Carassius auratus*), zebrafish (*Danio rerio*), and catfish (*Ictalurus punctatus*)) that link the swimbladder to the ear (**see also Hearing and Lateral Line:** Auditory System Morphology). The ears of these fishes are stimulated in proportion to the sound pressure level, and sound pressure may be the more relevant component for their hearing.

Behavioral Evidence for Directional Hearing and Sound Source Localization

The first experiments to address sound source localization by fishes were carried out in 1935 on the European minnow (*Phoxinus laevis*). Pioneers Karl Von Frisch and his student Sven Dijkgraaf attempted to train minnows to go to one feeding station randomly selected among four within a lake. The feeding station at which the fish would receive food was marked by a sound emitted at that station prior to feeding. When the fish failed to discriminate between feeding stations, it was concluded that *Phoxinus* could not localize sound sources. Von Frisch convincingly explained these negative results by pointing out that the European minnow was a pressure-sensitive otophysan fish. Otophysans were thought to respond exclusively to sound pressure, via the Weberian ossicles connected to the swimbladder. As previously mentioned, sound pressure at any single point contains no information about the direction of sound propagation. Thus, it was thought that otophysan fishes were unable to perceive direction and were therefore incapable of sound source localization. At that time, no other modes of fish inner ear stimulation, such as the detection of particle motion, were known so it was believed that no fishes were capable of localizing sound sources.

Since then, the behavioral capacity for directional hearing by fishes has been demonstrated in a number of teleost species using psychoacoustic discrimination techniques. Direction-dependent masking was found in several species, including haddock (*Melanogrammus aeglefinus*), pollack (*Pollachius pollachius*), ling (*Molva molva*), and Atlantic cod (*Gadus morhua*). Classic experiments of the 1970s used cardiac conditioning techniques, which measure changes in heart rate in response to an auditory stimulus, to measure the detection thresholds of fishes for a pure tone signal in the presence of a white-noise masker. Masked thresholds were measured as a function of the angular separation of the signal and masking noise sources in the horizontal and vertical planes. It was found that the signal became more detectable or that it was detected at lower signal-to-noise ratio when the angular separation between the signal and noise source was greater than 20° in the horizontal plane and 16° in the vertical plane (i.e., a minimum audible angle (MAA) of 20° horizontal, and 16° vertical). In the early 1980s, it also was found that Atlantic cod could discriminate sound source distance. Experiments showed that cod could discriminate between pure tones emitted alternatively from two aligned underwater speakers at different distances. The cod's ability to discriminate distance was thought to be based on the distance-dependent phase angle between sound pressure and acoustic particle acceleration within the near field of the sound source. Overall, these discrimination experiments provided strong evidence that fishes are well able to discriminate between sound sources in three-dimensional space, but they did not demonstrate that fishes could correctly locate the sound sources.

Some of the best behavioral evidence of sound source localization by fishes comes from a series of conditioning experiments on the Ballan wrasse (*Labrus berggylta*) and Atlantic cod. In these free-field experiments, fishes were conditioned to make a response (turn and orient in the direction of the sound source) whenever the source changed location (**Figure 2**). The sources were separated by angles ranging from 7° to 71° in the horizontal plane, and the results showed that the fish could detect the spatial changes in source location with a MAA of 22°. These experiments also showed that fishes could detect the direction of sound propagation and that this ability required two intact inner ears. Although these experiments clearly demonstrated the ability of fishes to detect spatial changes in the sound source, it could still be argued that the experiments did not demonstrate that fishes could correctly determine the location of sound sources. Nevertheless, these experiments stood as the best evidence for sound source localization by fishes.

Recently, the plainfin midshipman fish (*Porichthys notatus*) was shown to exhibit phonotaxis by approaching

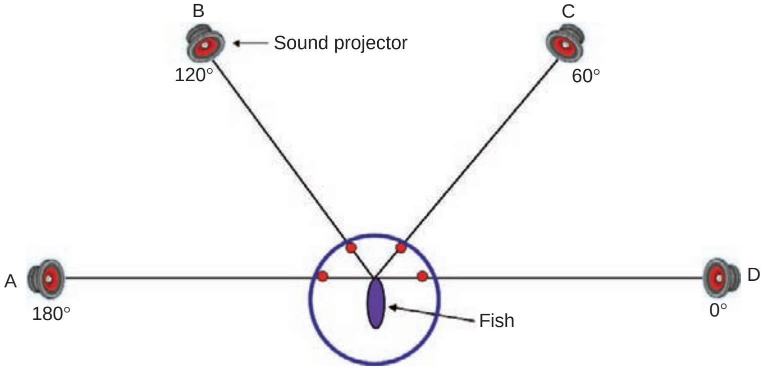


Figure 2 Diagram of four alternative choice experiments that investigated directional hearing in Atlantic cod (*Gadus morhua*). Fish were housed in a netted cage while a stimulus tone of 75 Hz was played from one of four sound projectors (A–D) at different directions. The fish were free to move in the cage and trained to orient toward the food dispenser (red dot) that was in line with the active sound projector.

a sound source that was broadcasting a version of the male's advertisement call (a continuous, multiharmonic signal with a fundamental frequency of ~90 Hz). In these experiments, gravid female midshipman fish were observed to approach and contact the sound source when released about 100 cm away from the source. These fish took (primarily) direct pathways to the source and were evidently capable of sound source localization, but how did they do it?

Theories of Directional Hearing and Sound Source Localization

The work of the 1970s and 1980s was in stark contrast to the thinking of the early 1960s when Willem van Bergeijk suggested that directional hearing in fishes was entirely dependent upon the lateral line system. In the then dominant acoustico-lateralis hypothesis, van Bergeijk posited that the lateral line and auditory system functioned as a single system that could compute sound source location in the near field. Because van Bergeijk assumed that ear-mediated hearing in fishes only processed sound pressure using the swimbladder as a pressure-to-displacement transformer, he asserted that directional hearing by means of a fish ear was not possible in either the near or far fields. The experiments that surgically eliminated the cod's ear, however, showed that both ears are needed for directional detection at a distance from the source, much like that of terrestrial vertebrates.

Terrestrial animals generally use the differences between the sounds arriving at the two ears (acoustic cues) to compute sound source direction, at least in the horizontal plane. These cues consist of time of arrival differences at the two ears (interaural time differences or ITDs), and also the sound level differences at the two ears (interaural level differences or ILDs) caused by the sound shadowing by the head. Fishes cannot use these acoustic differences because: (1) the speed of sound is over 4 times greater in water than in the less dense air, making ITDs very small; (2) the closeness of the ears further reduces ITD cues; and (3) there is a minimal sound shadow caused by the head because the fish's entire body moves with the sound, making ILDs also very small.

So, it is thought that to the extent that fishes can locate sources using sound, the brain must have access to other information. This information arises within the otolithic ears themselves, and in their direct response to acoustic particle motion. Because hair cells are directional receivers and their orientation varies along the macula, the axis of particle motion can, in general, be established by determining which hair cells were stimulated. In the horizontal plane, all the hair cells of the right and left ears respond best when the axis of particle motion is parallel to the orientation of the ear within the head (**Figure 1**). Each ear is maximally activated by particle motion along different axes. The cue is not caused by acoustic level differences arriving at the ears, but by differences in the responses of the ears themselves to particle motion. In other words, the ears themselves are directional and respond best to different axes of particle motion. The brain may calculate the axis of particle motion by calculating the ratios of right and left ear activation. This process has been called vector detection and is the basis for all current models of directional hearing and sound source localization by fishes.

Vector Detection and the Phase Model

Fishes probably can resolve the particle motion vectors using their two ears and the diversity of hair cell directions. However, as Arie Schuijf has pointed out, this does not entirely solve the problem of sound source localization. The axis of particle motion could be resolved by vector detection, but which end of the axis points toward the source? This question has been termed the 180° ambiguity problem. A solution is possible if fishes obtain independent information about the phase of the sound pressure waveform and the particle motion waveform. Using this information, the direction to the sound source could be computed and the 180° ambiguity resolved. Discrimination experiments found that accurate discrimination was no longer possible when the phase relationship between pressure and particle motion was experimentally reversed. This finding supports the idea that localization of sound sources depends on vector detection using particle motion, followed by phase comparison of the pressure and particle motion waveforms.

However, there are still problems that this theory, the so-called phase model, cannot solve. One is that sharks and other elasmobranches seem to be able to locate sound sources in the near and far fields, yet they probably do not detect sound pressure because they lack a swimbladder or other gas bubble associated with the ears (**see also Hearing and Lateral Line:** The Ear and Hearing in Sharks, Skates, and Rays and **Chondrichthyes**: Physiology of Sharks, Skates, and Rays). Another problem for the phase model theory is that particle motion vectors only point to and away from monopole sound sources. A monopole source is like an underwater bubble of gas that expands and contracts with pressure fluctuations. It is thought that most underwater sound sources are not monopoles, but are dipoles or other types. Dipoles are like a vibrating sphere without volume changes. They produce a sound field in which the particle motion vectors do not always point toward and away from the source, and thus would present confusing particle motion vectors to fishes. One possible solution is that fishes do not know where the sound source is in the way that human listeners (or, presumably, other terrestrial animals) do. For example, fish may sample from multiple locations and then swim in the direction that is loudest.

Brain Mechanisms Underlying Sound Source Localization

Peripheral neural encoding of directional information has been investigated in a few species. In these species, Atlantic cod, oyster toadfish (*Opsanus tau*), plainfin midshipman (*P. notatus*), and sleeper goby (*Dormitator latifrons*) responses from the auditory nerve (individual auditory saccular nerve cells) tend to show perfect directionality (**Figure 3**). It is perfect in the sense that response strength is proportional to the axis of stimulation in only one direction. Nerve cells differ considerably in their best-axis vector, likely because individual saccular nerve cells may contact hair cells that are oriented only in one direction. In other words, each hair cell orientation seems to have a private line to the brain. In some fishes (e.g., the sleeper goby), the afferent neurons from all of the otolithic organs to the brain are sufficiently sensitive to respond to particle motion associated with biologically relevant sounds. This suggests that the brain may be able to use information from all the otolithic organs in the computation of sound direction.

Representation of directional acoustic information in the central nervous system has been studied in three species (goldfish, oyster toadfish, and rainbow trout (*Oncorhynchus mykiss*)). Directional information in the oyster toadfish is present in the descending octaval nucleus (DON) of the brainstem (medulla oblongata; **see also Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes and **Hearing and Lateral Line:** Auditory/Lateral Line CNS: Anatomy and Physiology of the Ear and Brain: How Fish Hear). This is the first auditory nucleus that receives input from both ears, and many of the DON cells are more directionally selective than the saccular nerve cells. Directional selectivity in the DON appears to be enhanced by excitatory–inhibitory interactions within the brainstem, but it is not clear if the processing is based on monaural or binaural inputs, or a combination of both. Some recent evidence for binaural computation has been found. In DON cells of the oyster toadfish, some of the best axes of stimulation could not have been obtained from saccular afferents located on the same side of the head as the recorded DON cells.

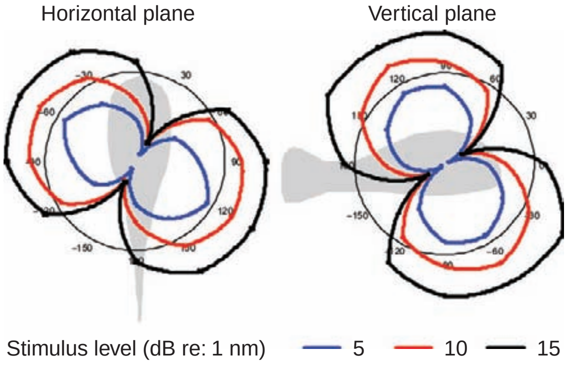


Figure 3 The directional response patterns of one nerve cell of the oyster toadfish (*Opsanus tau*). The left panel shows that the best azimuthal direction is about 60° to the left front, and the right panel shows the best elevation is about 120° (up and to the back). Data are shown for three stimulus levels. Note that the cell responds best to one particular axis of particle motion, but cannot determine as to which end of the axis points toward the sound source.

Directional sensitivity continues to improve at the level of the midbrain in the torus semicircularis (TS). The distribution of best-axis vectors of acoustic particle motion in oyster toadfish TS is much wider than the comparable distribution of best-axis vectors in the saccular nerve cells. The greater range could be due to input from otolithic end organs other than the saccule, or alternatively, due to input from saccular nerve cells from both ears. In either case, all axes of acoustic particle motion are represented in the TS. In contrast to the oyster toadfish, recordings from the TS of the goldfish, a pressure-sensitive otophysan fish, show a narrow distribution of the best axes that is similar to the distribution of best-axis vectors of the saccular nerve cells. Interestingly, the broad distribution of best-axis vectors of the lagenar and utricular afferent nerve cells is not reflected in the properties of the goldfish TS. This lack of diverse best-axis vectors may indicate poor directional hearing in the goldfish and other pressure-sensitive otophysans, just as von Frisch and Dijkgraaf originally reasoned.

Summary and Unresolved Issues

Fishes have available to them a great deal of acoustic information, and fishes that make the best use of that information would, presumably, have the greatest survival and reproductive advantages. The process of making sense of the soundscape is known as auditory scene analysis, and scene analysis requires the ability to segregate information coming from different sound sources. In turn, the fundamental components of sound source segregation are the ability to identify objects emitting sounds, and the ability to estimate where those objects are located. While there is general confidence that at least some species of fishes can accomplish sound source localization, we are ignorant of many aspects of this ability, and this situation presents a whole set of unanswered questions.

Perhaps first among the questions is whether and how fishes like the European minnow and goldfish (and all other species that are primarily pressure sensitive) accomplish sound source localization. Input to the ears is dominated by the swimbladder's response that is proportional to pressure, but a vector detection mechanism based on particle motion from the external source seems to be required for localization. Where does this motional input come from? How can the phase model that requires both pressure and motional inputs be used in species lacking a swimbladder such as elasmobranchs and flatfish? Do fishes perceive the location of sources as human listeners do, or do they merely have a behavioral strategy for approaching sources? In discrimination experiments with multiple sources, fishes are able to discriminate between them, but do they perceive their absolute location or do they merely understand that the sources are somehow different? What is the role of the lateral line system in sound source localization in the near field?

These and other questions remain unanswered. Although we are fairly certain that the axis of particle motion is determined using a vector detection strategy, we are still without a complete account of sound source localization that is generally accepted.

See also: **Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes. **Chondrichthyes:** Physiology of Sharks, Skates, and Rays. **Hearing and Lateral Line:** Auditory/Lateral Line CNS: Anatomy; Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Psychoacoustics: What Fish Hear; Physiology of the Ear and Brain: How Fish Hear; The Ear and Hearing in Sharks, Skates, and Rays.

Further Reading

Bass AH and Clark CW (2003) The physical acoustics of underwater sound communication. In: Megela Simmons A, Popper AN, and Fay RR (eds.) *Acoustic Communication, SHAR,* vol. 16, pp. 15–64. New York, NY: Springer.

Bregman AS (1990) *Auditory Scene Analysis. The Perceptual Organization of Sound*. Cambridge, MA: MIT Press.

Chapman CJ and Johnstone ADF (1974) Some auditory discrimination experiments on marine fish. *Journal of Experimental Biology* 61: 521–528.

Edds-Walton P and Fay RR (2003) Directional selectivity and frequency tuning of midbrain cells in the oyster toadfish, *Opsanus tau*. *Journal of Comparative Physiology A* 189: 527–543.

Edds-Walton P and Fay RR (2008) Directional and frequency response characteristics in the descending octaval nucleus of the toadfish (*Opsanus tau*). *Journal of Comparative Physiology A* 194: 1013–1029.

Fay RR (1984) The goldfish ear codes the axis of acoustic particle motion in three dimensions. *Science* 225: 951–954.

Fay RR (2005) Sound source localization by fishes. In: Popper AN and Fay RR (eds.) *Sound Source Localization, SHAR,* vol. 25, pp. 36–66. New York, NY: Springer.

Fay RR and Edds-Walton PL (1997) Directional response properties of saccular afferents of the toadfish, *Opsanus tau*. *Hearing Research* 111: 1–21.

Fay RR and Edds-Walton PL (2008) Structures and functions of the auditory nervous system of fishes. In: Webb JF, Popper AN, and Fay RR (eds.) *Fish Bioacoustics, SHAR,* vol. 32, pp. 49–98. New York, NY: Springer.

Feng AS and Schellart NAM (1999) Central auditory processing in fish and amphibians. In: Fay RR and Popper AN (eds.) *Comparative Hearing: Fish and Amphibians, SHAR,* vol. 11, pp. 218–268. New York: NY: Springer.

Hawkins AD and Sand O (1977) Directional hearing in the median vertical plane by the cod. *Journal of Comparative Physiology A* 122: 1–8.

Kalmijn AJ (1997) Electric and near-field acoustic detection, a comparative study. *Acta Physiol Scand* 161 (supplement 638): 25–38.

Lu Z, Song J, and Popper AN (1998) Encoding of acoustic directional information by saccular afferents of the sleeper goby, *Dormitator latifrons*. *Journal of Comparative Physiology A* 182: 805–815.

Nelson DR and Gruber SH (1963) Sharks: Attraction by low-frequency sounds. *Science* 142: 975–977.

Popper AN and Fay RR (1999) The auditory periphery in fishes. In: Fay RR and Popper AN (eds.) *Comparative Hearing: Fish and Amphibians, SHAR,* vol. 11, pp. 43–100. New York, NY: Springer.

Rogers PH and Zeddies DG (2008) Multipole mechanisms for directional hearing in fish. In: Webb JF, Popper AN, and Fay RR (eds.) *Fish Bioacoustics, SHAR,* vol. 32, pp. 233–252. New York, NY: Springer.

Sand O and Bleckmann H (2008) Orientation to auditory and lateral line stimuli. In: Webb JF, Popper AN, and Fay RR (eds.) *Fish Bioacoustics, SHAR,* vol. 32, pp. 183–232. New York, NY: Springer.

Schuijf A (1975) Directional hearing of cod (*Gadus morhua)* under approximate free field conditions. *Journal of Comparative Physiology A* 98: 307–332.

Schuijf A and Hawkins AD (1983) Acoustic distance discrimination by the cod. *Nature* 302: 143–144.

Zeddies DG, Fay RR, Alderks PW, Shaub KS, and Sisneros JA (2010) Sound source localization by the plainfin midshipman fish, *Porichthys notatus*. *Journal of the Acoustical Society of America* 127(5): 1304–1313.

Effects of Human-Generated Sound on Fish

AD Hawkins, Loughine Ltd., Aberdeen, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Sound Sources
Impacts on Fish
Regulation and Mitigation
Further Reading

Glossary

Ambient noise The combination of sound from natural sources.

Audiogram A graphical representation of sound detection thresholds at the range of frequencies that are audible.

Auditory threshold The average sound pressure level that is just audible to a subject under quiet conditions.

Background noise The prevailing combination of ambient (natural) noise and man-made noise.

Infrasound Sound that is lower in frequency than 20 Hz, the normal limit of human hearing.

Masking A blocking of the detection of one sound by another.

Mitigation Taking action to minimize the effects of noise.

Noise Unwanted sound; also refers to a sound with a random waveform containing energy across a broad range of frequencies.

Noise-exposure criteria The thresholds set to protect animals from sounds that may harm them.

Particle motion The back-and-forth motion of the component particles of the medium that accompany passage of a sound; it can be expressed as particle displacement (nm), velocity ($\mathrm{nm\,s^{-1}}$), or acceleration ($\mathrm{nm\,s^{-2}}$).

Sound-exposure level (SEL) A measure of the energy of a sound and it depends on both amplitude and duration; the SEL is the time integral of the instantaneous squared sound pressure normalized to a squared reference pressure over a 1-s period (expressed as dB re 1 $\mu\mathrm{Pa}^2\,\mathrm{s}$).

Sound-pressure level (SPL) The fluctuation in pressure above the hydrostatic pressure that characterizes passage of a sound (expressed as dB re 1 μPa).

Soundscape The totality of sounds at a given location.

Ultrasound Very high frequency sound, generally above 20 kHz.

Introduction

Many of the sounds detected in seas, lakes, and rivers are entirely natural. The sounds of falling rain, breaking waves, cracking ice, bubbles, spray, and turbulence provide a continuous but a varying background of noise. Less frequently, earthquakes, volcanic eruptions, and lightning strikes generate intense sounds, traveling over great distances. Sounds from marine mammals, fish, and crustaceans add to the ambient noise. Snapping shrimps and chorusing fish can contribute significantly over a wide band of frequencies, which may mask communication signals from other animals (**see also Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling). Together with the sound of wind-driven waves, these biological sounds may dominate ambient noise levels.

In recent years, humans have added to sea noise. Sounds from shipping, underwater explosions, seismic exploration, offshore construction, and sonars of various types have raised the level of background noise. Typical sound levels at different frequencies from different sources are shown in **Table 1**.

There is concern over the effects of these extraneous sounds upon fish and other aquatic animals. It is known that very intense sounds may kill or injure fish. At lower levels, sound may impair their hearing, affect their ability to orient, or make their vocalizations difficult to detect. It may evoke changes in behavior that may affect spawning migrations or disrupt foraging and feeding, with effects at the population level. Do these sounds have a significant effect upon the fitness of aquatic organisms, affecting their welfare and their survival? If so, how might we reduce them or mitigate their impact?

Sound Sources

Sounds generated by human activities form a significant part of the total underwater background noise. Sound is

Table 1 Characteristics of a number of underwater sound sources

Sound	*Source level (dB re 1 μPa at 1 m)*	*Bandwidth (Hz)*	*Major amplitude (Hz)*	*Duration (ms)*
Explosion (TNT, 1–100 lbs)	272–287 peak	2–1000	6–21	~1–10
Percussive pile driver	185–260 peak	20 to >20 000	40–1000	50
Vibrating pile driver	173–185 rms	10–10 000	100–1500	Continuous
Dredging	168–186 rms	30 to >20 000	<500	Continuous
Drilling	145–190 rms	10–10 000	<100	Continuous
Operating wind turbine	114–130 rms		<200	Continuous
Small ships	160–180 rms	20 to >10 000	>1500	Continuous
Large ships	180–190 rms	20 to >10 000	>1000	Continuous
Low-frequency sonar	215 peak	100–500		600–1000
Medium-frequency sonar	223–235 peak	2800–8200	3500	500–2000
High-frequency sonar	235 peak	Variable		5–10
Seismic air-gun array	260–262	10–1000	10–120	30–60

used for our benefit in underwater communication, navigation, fish finding, research, and sub-sea exploration. However, many sounds are an incidental by-product of other activities, such as commercial fishing, the transportation of goods, drilling for oil and gas, aggregate extraction, the dredging of shipping channels, and the construction of piers and harbors.

Man-made sounds can be continuous or they may be transient or impulsive. Propeller and engine noise from a ship continues for as long as the ship is on the move, although it may change in level and quality as the ship maneuvers. Sounds from some industrial activities, such as drilling or dredging, may be generated for long periods, although they may greatly vary in amplitude with time. Other sounds from activities, such as percussive pile driving, or seismic surveying, are intense but transient. The sounds are intermittent, although they may be repeated many times. Continuous sounds from sources such as ships raise the baseline of ambient underwater noise over wide areas, while transient or impulsive sounds occur less often. It is possible that in many areas, chronic exposure to moderate levels of noise has more significant biological impact than an occasional exposure to extremely loud sounds. Certainly, it is important to distinguish between long-term exposure to noise that affects habitat quality and masks natural sounds, and short-term acute exposure to intense sounds that may evoke a short-term response and which, in some cases, may result in injury or death.

Sound is a back-and-forth disturbance of the component particles of a material medium. As an object vibrates, some of its kinetic energy is transferred to the surrounding gas or liquid. The consequent vibration of particles close to the object is in turn coupled to more distant particles, so that a traveling sound wave is transmitted away from the source at a speed dependent on the density and elasticity of the medium. The passage of the sound wave involves both compression and motion of the medium. The compression wave is observed and measured using an instrument sensitive to pressure variation – a microphone or hydrophone. The particle motion within the medium is more difficult to monitor. It is usual to specify a sound by the sound pressure alone, and to estimate the particle motion, if required, from a knowledge of the acoustic properties of the medium.

The range of sound pressures encountered in water is enormous, and sound levels are therefore specified on a logarithmic scale as decibels (dB) relative to a specified reference level. In water, the reference pressure is 1 μPa. In air, a different reference level is used, so that dB levels in air and water cannot be compared directly. Besides, sound travels much faster in water.

As sounds are variations in pressure with time, they can be described by the different frequency components they contain. Humans can detect or hear sounds over a frequency range extending from 20 Hz (cycles per second) to 20 kHz (20 000 Hz). However, there are many different types of sound. Some are short or transient like a crash or bang, others are long or continuous, like the sound from a boat engine. Some sounds have a tonal or musical quality, while others are rough and unpleasant. The frequency spectrum describes the full range of frequencies within a sound.

Ship noise is very pervasive in some parts of the ocean. It emanates from engines and other machinery, rotating shafts, propellers, and the interaction of water with the hull. The motion of the ship through the water may create low-frequency pressures and water displacements around the ship. Much of the noise from a ship results from cavitation – the collapse of air spaces created by the rotating propellers. Individual ships have their own noise signatures and can be detected over distances of kilometers. Ship noise is greatest close to ports and shipping lanes, and it has become greater as the number of ships on the high seas has increased. In shallow coastal waters, the soundscape may be dominated by sound from small boats fitted with outboard motors.

Construction and industrial activities, both onshore and offshore, may generate significant noise in lakes and rivers, as well as in the sea. These activities include pile driving, dredging, drilling, the use of explosives in construction and decommissioning, the operation of offshore wind farms, and activities at oil- and gas-production facilities. Percussive pile driving may produce especially intense sounds. With this activity, a post, sheet, or tube made of steel or reinforced concrete is driven into the seabed to support a superstructure such as a jetty or a wind turbine. The head of the pile is struck repeatedly, driving it downward until the pile has reached the required depth. The insertion of each pile may take minutes or hours, depending on the size of the pile and the substrate conditions, and there could be many piles. Pile driving generates short pulses of sound with most of the energy concentrated at frequencies below 1000 Hz. Close to the pile, the peak sound pressures associated with each strike may be very high. In addition, compressive, shear, and surface waves are generated within the substrate that may result in large particle accelerations close to the seabed. The use of explosives both in construction and in the removal or decommissioning of structures such as oil platforms also produces very high peak sound levels. The sounds from these activities can travel great distances (tens of kilometers).

Dredging of ports and harbors as well as the extraction of sand and gravel from the seabed create continuous but rather variable levels of sound at low frequencies. Vibrating pile drivers, in contrast to their percussive counterparts, produce continuous sound, so do trawling by commercial fishing vessels, the drilling of oil and gas wells, and tunneling beneath the seabed. Noise is also generated by the operation of offshore wind farms and by oil and gas platforms, although the levels produced during operation are generally lower than those during the construction phase.

Other activities deliberately produce underwater sound. Seismic surveying for oil and gas involves the generation of a low-frequency pulse of sound (with most of the energy in the range 10–120 Hz) that travels through water to enter the seabed. A receiver array detects the returning sound. By analyzing the reflections, it is possible to identify the properties of the seabed, the underlying geology, and the presence of oil and gas deposits. The surveys are conducted from a ship towing an array of air guns. Each air gun produces a short intense pulse of sound that is focused downward by the configuration of the array. Surveys may cover a wide area and last for several weeks.

Sonar is used to measure depth, to map the seabed, to detect fish, to track subsurface vehicles, or to search for military targets. Sonars operate at a range of sound frequencies and are classified as low- (<1 kHz), mid- (1–10 kHz), and high-frequency (>10 kHz) sonars. High-frequency sonars may be detected by only a few fish species, but mid- and low-frequency sonars may operate well within the range of frequencies that can be detected by fish (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays and Psychoacoustics: What Fish Hear). Mid-frequency sonars are perhaps the most common and most relevant to fish, as they are used by many navies to search for submarines. They may have high source levels and generally produce long sound pulses in which the frequency changes.

Other sources of underwater sound include high-frequency acoustic-harassment devices (generally operating above 20 kHz) that are intended to scare marine mammals away from fishing nets, acoustic beacons (used for navigation purposes), and even the underwater components of sonic booms generated by aircraft flying above the sea surface.

Sound travels almost 5 times faster in water than it does in air. Underwater sounds are generally measured using hydrophones sensitive to sound pressure; however, in some cases, particle motion may also be required (because many fish are sensitive to particle motion rather than sound pressure). In a free sound field, particle motion can be estimated, but close to acoustic boundaries the relationship becomes complex and it is necessary to measure particle motion directly. Particle motion can be expressed as particle displacement, particle velocity, or particle acceleration. As motion is a vector, its direction is important. The amplitude of a sound diminishes and its frequency characteristics change with distance from the source. The output from most sources is usually described by the source level – the sound-pressure level (SPL) at 1 m. This is obtained by fitting measurements made at different distances to a model that describes the rate at which sound diminishes with distance. Estimating the sound levels that reach a fish can be difficult, especially if the sound is to be described in terms of particle motion. Yet, it is the sound reaching the fish that is significant. The metrics used to describe and evaluate the impact of sounds include the root mean square (rms) pressure (or particle acceleration), peak sound pressure, received energy (received sound-exposure level (SEL)), rise time, duration, frequency range, roughness, duty cycle, and directionality.

Impacts on Fish

Sound is important to fish. Fish depend on sound to communicate with one another, detect prey and predators, navigate from one place to another, and avoid hazards. Fish larvae use sound to settle in suitable habitats. An important additional function of hearing is auditory-scene analysis – the ability of animals to break down the overall sound field into separate elements to analyze the world around them and assign different components to particular sources (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays, Psychoacoustics: What Fish Hear, and Vocal Behavior of Fishes: Anatomy and Physiology).

Our current knowledge of the impact of underwater sound on fish is fragmentary. In particular, knowledge of the auditory capabilities of fish is limited and relies, with a few notable exceptions, on experiments carried out in the laboratory (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays and Psychoacoustics: What Fish Hear). The presentation of measured sound stimuli to fish under experimental conditions presents major difficulties. Fish are generally most sensitive to low frequencies that are difficult to reproduce with precision, and many fish are sensitive to particle motion that has to be estimated from the measured sound pressures, and in many cases it is not estimated at all. Many of the thresholds and audiograms presented (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear) are unreliable and must be treated with great skepticism. For most fishes, there are no empirical data to describe their hearing characteristics.

Several general conclusions can be drawn from the few audiograms available for fish. Fish are sensitive to a rather restricted range of frequencies compared with birds and mammals. Even the most sensitive fish have relatively poor hearing above 2–3 kHz. There are one or two exceptions: some fishes do appear to be sensitive to very high amplitude, high frequency sounds, including the ultrasonic frequencies generated by some sonars. However, fish are generally most sensitive to relatively low frequencies, including sounds in the infrasonic range (less than 20 Hz).

It is evident that the unaided fish ear is sensitive to particle acceleration and is most responsive to low frequencies. Increased sensitivity and extension of the range to higher frequencies are found in those fish with gas-filled spaces close to or connected with the ear. These fish are sensitive to sound pressure. They include carp (freshwater fish of the order Cypriniformes), which have a connection between the swimbladder and the inner ear (**see also Hearing and Lateral Line**: Auditory System Morphology). At their most sensitive frequencies, fish may not be limited by their absolute sensitivity but by the level of ambient noise, even under relatively quiet sea conditions (**see also Hearing and Lateral Line**: Auditory System Morphology).

There is a hierarchy of ways in which sound affects fish. At one extreme, fish may die when they are exposed to very high energy sounds, for example, close to an explosion or pile driver. In other cases, high-level sounds may injure internal organs. Fish may show temporary hearing loss and damage to the sensory hair cells of the inner ear following exposure to high levels of both continuous and interrupted sound. Lower-level sounds may disrupt behavior patterns, or mask the detection of biologically important sounds. However, in many cases, sounds may simply be detected by fish at a distance without any overt change in behavior. It is possible to think in terms of overlapping zones of impact away from the source, with death and injury occurring at close range, while at extreme distances the animal barely hears the sound (**Figure 1**).

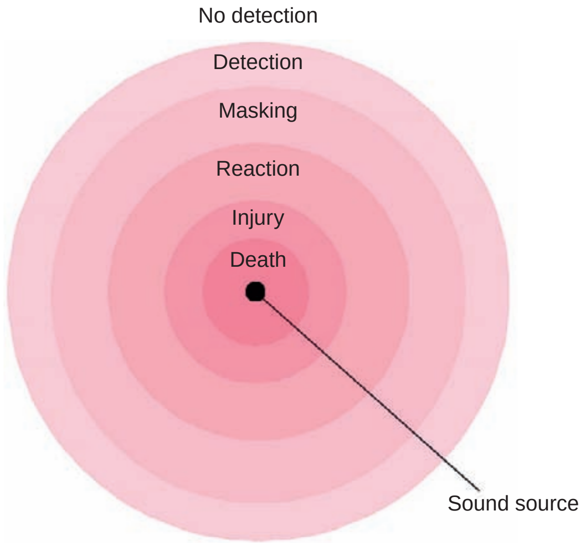


Figure 1 Zones of impact from a sound source. Note that the zones overlap one another.

Studies of fish behavior in response to sounds are difficult to perform. Many factors may influence the results. A careful case-study approach must be adopted, based on well-designed experiments. A great range of impacts has been observed from fish in response to underwater sound. Some fish have shown changes in swimming behavior and orientation, including startle reactions. Schools of pelagic fish dive in response to the firing of seismic air guns, while demersal fish flee from a seismic source across the sea floor. The horizontal and vertical distributions of both pelagic and ground fish change after several days of air-gun operations. Fish cease critically important activities, such as feeding and sound production. Catches in sea fisheries decrease both during and after seismic surveys. Some of the behavioral effects are short term, diminishing after the initial presentation of the sound. Others are longer term and result in fish consistently avoiding a sound source.

So far it has not been possible to grade the magnitude of their response according to the level and characteristics of the sound. Distinctions have been made between involuntary startle reactions, changes in behavior, and outright avoidance, in an attempt to separate inconsequential behavior from significant responses, but the reactions of fish to sounds are often species specific and depend upon the context. We should perhaps not be too concerned about how fish behave in response to sounds. Rather, we should ask what significance this behavior is likely to have for their survival. Will the behavior itself be costly in terms of energy loss or risk to fitness? Will the behavior result in the longer-term impairment of survival through displacement from a preferred area, or will the fish be denied access to a key habitat (**Figure 2**)? From a conservation perspective, the impact of extraneous

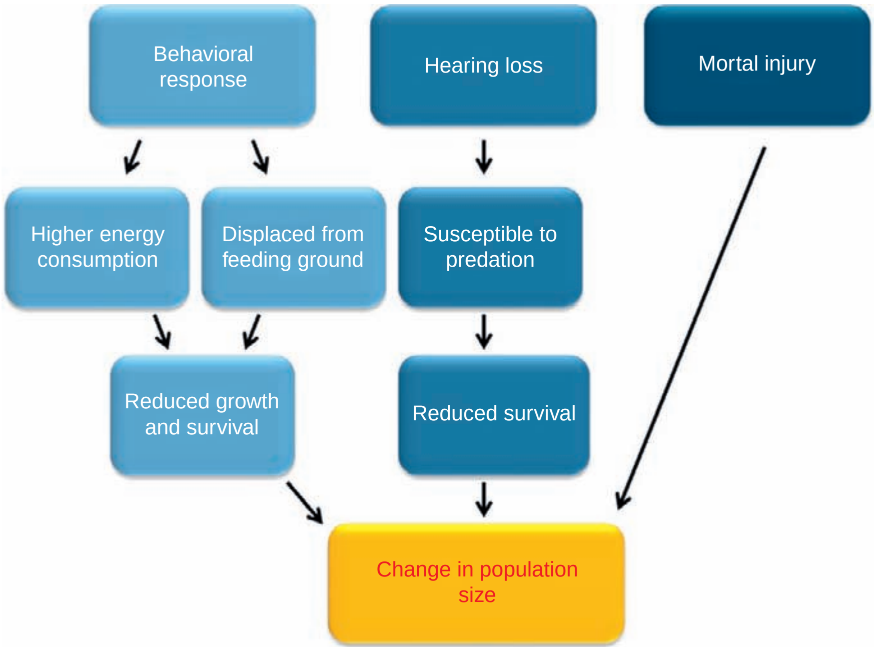

Behavioral response
Hearing loss
Mortal injury
Higher energy consumption
Displaced from feeding ground
Susceptible to predation
Reduced growth and survival
Reduced survival
Change in population size

Figure 2 From a conservation standpoint, it is the impact of sound exposure upon survival which is critical, rather than the immediate response.

sounds on individual fish is less important than their impact on populations and ecosystems, either alone or in combination with other stresses such as commercial fishing. There have been attempts to develop predictive models, based on studies of the disruption to individuals, and examining effects on key life functions such as feeding, growth, reproduction, and migration. However, such models are still in their infancy.

Regulation and Mitigation

Man-made underwater sound becomes significant when it causes harm to living fish. If a man-made sound affects the survival of fish or their ability to reproduce, then it may be important to regulate, eliminate, or reduce noise production. Regulation usually involves the setting of standards or criteria defining the levels of sound that cause a specified degree of response. In setting noise-exposure criteria, it is necessary to have regard to the particular animal or animals being exposed, and their particular sensitivity to sound. It is difficult to extrapolate from one species to another, especially for fish, which are the most diverse of vertebrates. It is also necessary to have some knowledge of the noise levels to which the fish will be exposed, specified by appropriate metrics. A level can then be set which, if exceeded, will constitute a breach in a regulation.

Acute noise-exposure criteria can be used to regulate particular sources, or to place restrictions on the technology that generates the noise. It is perhaps easiest to set these noise-exposure criteria based on the SPL that will result in the death of fish. There has been research into the levels of impulsive sound from explosions that cause lethal injury. It is clear that physical injury from a transient pressure wave is related to the peak-pressure level, the rise time, and the period that the peak pressure acts upon the body. In general, smaller fish are generally more vulnerable than larger fish, and fish with gas inclusions, such as the swimbladder, are more vulnerable than those without.

On the basis that impulsive sounds can harm fish, several regulatory authorities have set standards for the use of pile drivers on the coast and in rivers. One example for pile driving stipulates that SPLs must be maintained below 150 dB rms with reference to 1 μPa for a minimum of half the impacts or strikes; and peak SPLs must be maintained below 180 dB re 1 μPa for all strikes in areas of potential fish presence. The setting of these standards often has been done in an arbitrary way. In particular, a peak value of 180 dB re 1 μPa seems to have gained wide acceptance as an upper limit, without clear justification or critical appraisal. Some researchers have emphasized that several factors, including the total energy, the peak sound pressure (or particle acceleration), and the duration, are important in causing damage rather than the sound pressure *per se.* They have estimated the SEL that results in the death of half the exposed fish based on data for exposure to a single explosive sound and have applied this figure to pile driving for fish of differing size, which gives SEL limits ranging from 195 to 198 dB re 1 μPa^2 s. A dual-criteria approach has also been suggested that includes an interim single-strike criterion for SEL combined with an interim criterion for peak sound pressure. It recommends that criteria for pile driving should be set at an SEL level of 187 dB re 1 μPa^2 s, and a peak SPL of 208 dB re 1 μPa in any single strike. It is important to realize, however, that such criteria are based on very limited data with respect to noise-induced injury. In particular, there are very few data on the cumulative effects of repeated exposure. Measurements of the impact upon fish of large particle accelerations are also lacking.

Some researchers have emphasized that it is important in specifying a level to measure the sound by passing it through a filter that approximates the audiogram of the species. This procedure excludes sound at frequencies that the fish is unable to detect. The approach is not entirely valid because injuries may be inflicted on the fish at frequencies outside its hearing range, and especially by steep rise times and high peak values that are associated with wide bandwidth sounds.

For many fishes that are sensitive to particle motion, the expression of exposure in terms of sound pressure may not be appropriate. The particle acceleration ($m\,s^{-2}$), the acoustic intensity (in $W\,m^{-2}$), or even acoustic intensity over time ($J\,m^{-2}$) is more relevant.

It is much more difficult to decide on noise-exposure criteria for fish based on behavioral responses rather than on temporary hearing loss, injury, or death. Fish are very diverse in their behavior and their responses are often species specific and context dependent. It has been said that sounds that are more than 90 dB above the hearing threshold may cause a strong avoidance response and are therefore especially likely to be harmful. Others have suggested that SPLs of 150 or 160 dB re 1 μPa should be regarded as those at which avoidance behavior is triggered. There are few data to support these assertions and the figures chosen are entirely arbitrary. Much more evidence must be gathered before any simple rule of thumb can be established.

For chronic exposure to moderate-level sounds, where there is no single source but many, quite different criteria must be set. Here, the main problem is the masking of sounds by background noise, as there is considerable overlap between man-made noise and the sounds important to fish. Masking can shorten the range over which sounds can be detected and individuals communicate. There is a dearth of information on long-term trends in ocean noise. Routine monitoring of background noise levels is now underway at a number of experimental sites. It has been suggested that maximum levels should be set for especially sensitive marine sites to protect the natural soundscape.

Besides these regulatory measures, there are a number of options for mitigating the effects of noise. A technique commonly applied with marine mammals is to establish a safety zone for the noise-making activity, ensuring that there are no vulnerable animals within the affected zone. Outside the zone, the sound levels are unlikely to pose harm. With fish, unlike sea mammals, it is not always possible to determine whether there are fish present within the zone of harm, and this procedure is less useful. Other mitigation measures involve attenuation of the sounds themselves. A bubble curtain placed around a pile driver will reduce the sounds transmitted into the water, but not into the substrate. Bubble curtains are certainly successful in reducing the peak amplitude of sounds from pile driver by up to 10 dB, and they also reduce the high-frequency content of the sound. However, they do not reduce the energy transmitted at the lower frequencies to which fish are sensitive. Moreover, fish close to the seabed may respond to the substrate vibrations. Other physical measures that can be effective include placing an absorbent pad between the driven pile and the hammer, and reducing the peak level and the rise time. Raising the level of repeated sounds gradually, to allow fish to escape before the loudest sounds are transmitted, is another measure that is thought to reduce the impact of a sound source and is applicable to both pile driving and seismic surveys.

Perhaps the most effective mitigation measures involve the imposition of spatial and seasonal restrictions to avoid exposure of sensitive species and habitats to noise. Thus, noise-producing activities may be limited to periods and locations when fish are not breeding, migrating, or engaging in other sensitive activities.

See also: **Hearing and Lateral Line**: Auditory System Morphology; Biomechanics of the Inner Ear in Fishes; Psychoacoustics: What Fish Hear; The Ear and Hearing in Sharks, Skates, and Rays; Vocal Behavior of Fishes: Anatomy and Physiology. **Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling;

Further Reading

Engås A, Løkkeborg S, Ona E, and Soldal AV (1993) Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (Melanogrammus aeglefinus). *Fisken og Havet* 3: 111.

McCarthy E (2004) *International Regulation of Underwater Sound: Establishing Rules and Standards to Address Ocean Noise Pollution*. Dordrecht: Kluwer.

OSPAR (2009) Overview of the impacts of anthropogenic underwater sound in the marine environment. Publication Number: 441/2009.

Popper AN, Carlson TJ, Hawkins AD, Southall BL, and Gentry RL (2006) Interim criteria for injury of fish exposed to pile driving operations: A white paper. http://www.wsdot.wa.gov/NR/rdonlyres/84A6313A-9297-42C9-BFA6-750A691E1DB3/0/BA_PileDrivingInterimCriteria.pdf (accessed February 2010).

Popper AN, Halvorsen MB, Kane E, *et al.* (2007) The effects of high-intensity, low-frequency active sonar on rainbow trout. *Journal of the Acoustical Society of America* 122: 623–635.

Popper AN and Hastings MC (2009) The effects of anthropogenic sources of sound on fishes. *Journal of Fish Biology* 75(3): 455–489.

Popper AN and Hastings MC (2009) The effects on fish of human-generated (anthropogenic) sound. *Integrative Zoology* 4: 43–52.
Richardson WJ, Malme CI, Green CR, Jr., and Thomson DH (1995) *Marine Mammals and Noise*. San Diego, CA: Academic Press.
Southall BL, Bowles AE, Ellison WT, *et al.* (2007) Marine mammal noise exposure criteria: Initial scientific recommendations. *Aquatic Mammals* 33: 411–521.
Tavolga WN, Popper AN, and Fay RR (eds.) (1981) *Hearing and Sound Communication in Fishes*. New York, NY: Springer.
Wahlberg M and Westerberg H (2005) Hearing in fish and their reactions to sound from offshore wind farms. *Marine Ecology Progress Series* 288: 295–309.
Webb JF, Fay RR, and Popper AN (eds.) (2008) *Fish Bioacoustics*. New York, NY: Springer.
Wenz GM (1962) Acoustic ambient noise in the ocean: Spectra and sources. *Journal of the Acoustical Society of America* 34: 1936–1956.

Relevant Websites

http://www.dosits.org – Discovery of Sound in the Sea.
http://oceanexplorer.noaa.gov – Ocean Explorer.

Acoustic Behavior

JJ Luczkovich, **MW Sprague**, **and CS Krahforst**, East Carolina University, Greenville, NC, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction – Fish Behavior and Underwater Sound
What Species Make Sound?
Why Do Fishes Make Sound?
Sound Production and Ontogeny, Sex Differences
Sound Production as a Fishery Management Tool
Conclusion
Further Reading

Glossary

Amplitude The maximum pressure deviation from ambient pressure in a sound wave.

Decibel (dB) A logarithmic scale for comparing signal parameters such as sound pressure, particle velocity, voltage, current, etc.

$$\text{Number of decibels} = 20\log_{10}(a_1/a_0),$$

where a_0 and a_1 are the two signal parameters being compared. Decibels are used in acoustics for sound pressure levels and other measurements.

Frequency The number of wave cycles in a time interval (measured in Hz = 1 cycle s^{-1} or kHz = 1000 cycles s^{-1}).

Interpulse interval Time between two pulses in a sound.

Oscillogram A graph produced from an oscilloscope or sound analysis software that records the pressure of a sound wave along a time axis.

Period The time it takes for one cycle of a sound wave to pass by a point.

Power spectral density The total sound power of a 1-Hz-wide band centered at a given frequency in a sound.

Pulse train A set of sound waves, each pulse due to a single set of sonic muscle contractions, or a single contraction for some species.

Sound pressure level A measurement of the sound pressure in decibels comparing the measured sound pressure (a_1 in the decibel formula above) to a reference pressure (a_0 in the decibel formula above). Underwater sound measurements use 1 μPa as the reference pressure.

Spectrogram (also sonogram) A graph representing the sound frequency and intensity variation over time; useful in identifying fish species by their sounds. Most spectrograms are displayed as two-dimensional plots in which the horizontal (x) axis is time (in s), the vertical (y) axis frequency (in Hz), and the color (z) axis is power spectral density (in dB).

Introduction – Fish Behavior and Underwater Sound

Because the underwater world is often dark and turbid, fishes have evolved to take advantage of sounds (**see also Behavior and Physiology**: Linking Fish Behavior and Physiology: An Introduction). Fishes can hear sounds, some better than others, within a frequency range of 10–1000 Hz for most species. Some fishes can hear much higher frequencies, up to 4000 Hz (or ultrasound >20 kHz in some Clupeids) (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays and Psychoacoustics: What Fish Hear). Hearing provides fishes with the ability to analyze their auditory scene – their local (~1 m) and long-distance (~10 m to 1 km) acoustic environment (**see also** Biomechanics of the Inner Ear in Fishes).

Fishes also produce sounds in the same frequencies as they hear for communication (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear). Fishes have evolved specialized sound production mechanisms including stridulation of bones and teeth or contraction of sonic muscles to drive gas-filled swimbladders (**see also Hearing and Lateral Line**: Vocal Behavior of Fishes: Anatomy and Physiology). Recordings of many fish sounds can be played at the 'Relevant websites' listed at the end of this article. It appears that sounds are produced by some of the most primitive fishes (i.e., various species of sturgeons (Acipenseridae) and the reedfishes or bichirs, gray bichir *Polypterus senegalus* and West African bichir *P. retropinnus* Polypteridae)) for the purposes of intraspecific communication. Sound production in fishes has also evolved more than once and has become important for communication in codfish (Gadidae), drumfish (Sciaenidae), toadfishes and midshipmen (Batrachoididae), and many other species. Sound production is used by fishes to communicate along with chemical, tactile, and visual signals (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior and Behavioral Assessment of the Visual Capabilities of Fish).

Fishes produce two different types of sounds: (1) pulsed sounds, in which the sonic muscles contract once to produce each pulse and (2) tonal sounds, in which the sound-producing muscles contract repeatedly to cause a steady-state vibration of the swimbladder. Pulsed sounds, such as the knock produced by silver perch, *Bairdiella chrysoura* (Sciaenidae) (**Figure 1**), have frequencies that depend on the physical properties of the sound-producing mechanism (sonic muscles and swimbladder), and will vary with the size of the individual fish. Tonal sounds, such as the boop produced by oyster toadfish, *Opsanus tau* (Batrachoididae) (**Figure 2**), have frequencies that depend on the vocal pacemaker and muscle contractions, and do not vary with the size of the fish.

The distance that fish sounds travel depends on the loudness of the sound, the loudness of the background noise at the same frequencies as the sound, and the water conditions (e.g., depth, temperature profile, and bottom type). Most sound communication among fishes occurs over short distances, less than 10 m. Although the knock of an individual silver perch can be detected at a maximum distance of 316 m in quiet conditions, the biologically relevant propagation distance is closer to 2 m, especially when background sounds are loud from other fish that are calling nearby. However, the combined sounds of aggregations of fish in the open ocean can be detected by hydrophones at distances of 1–8 km. Conversely, the maximum detection distance can be significantly shorter (<1 m) in shallow streams and ponds where turbulence, wave noise, bottom depth, and the shore limit the sound propagation distances.

Scientists use hydrophones (underwater microphones) to record fishes in the wild and when held captive in aquaria to identify the sound-producing species involved (**Figure 3**). Hydrophones have been attached to remotely operated vehicles (**Figure 4**), submarines, and autonomous sound-recording equipment to capture short- and long-term variations of acoustic fish behavior. Sound recordings are then correlated with biological measurements (size, sex, spawning condition, and gamete production) taken at the same time.

What Species Make Sound?

Many fishes make sounds, including (in addition to the families already noted above) catfishes (multiple families in the order Siluriformes), sculpins (Cottidae), Siamese fighting fishes (Osphronemidae), sea robins and gurnards (Triglidae), pearlfishes (Carapidae), elephant-nose fishes (Mormyridae), damselfish (Pomacentridae), wrasses (Labridae), grunts (Haemulidae), porgies (Sparidae), seabasses and groupers (Serranidae), sunfish (Centrarchidae), cichlids (Cichlidae), and gobies (Gobiidae). This is not an exhaustive list of

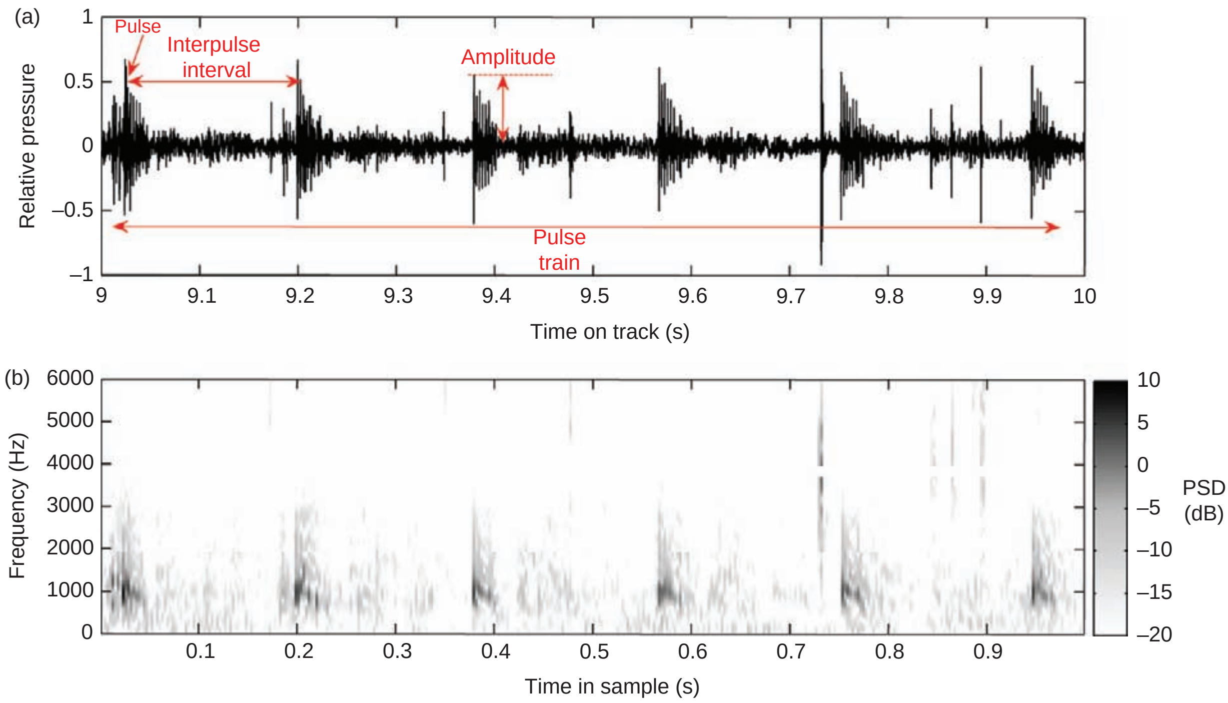


Figure 1 Spectral characteristics illustrated for silver perch, *Bairdiella chrysoura*, pulsed sounds: (a) oscillogram and (b) spectrogram or sonogram.

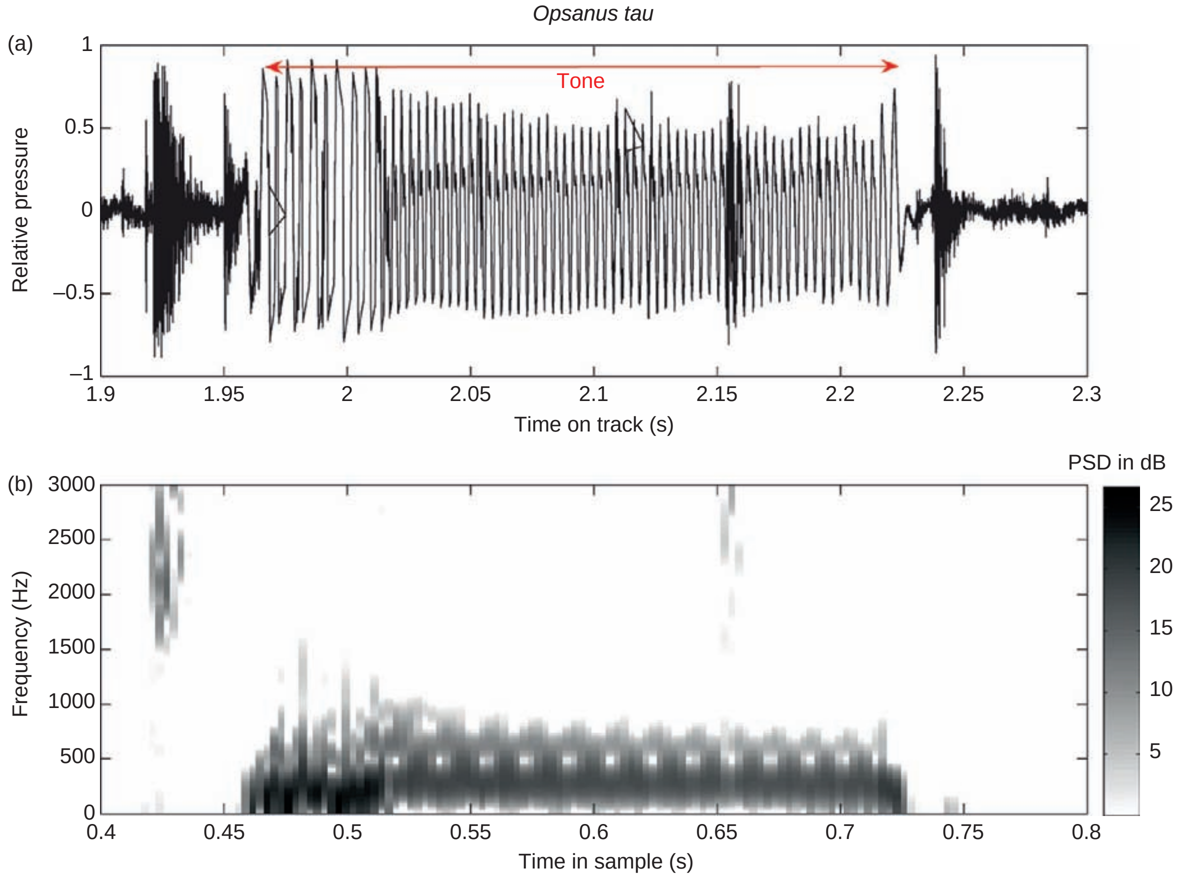


Figure 2 Spectral characteristics of a tonal sound, the oyster toadfish, *Opsanus tau*, boop sound: (a) oscillogram and (b) spectrogram.

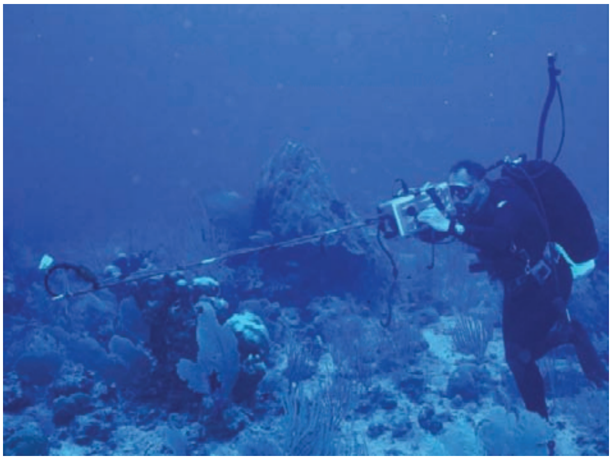


Figure 3 Recording sound and video underwater using a hydrophone mounted on a boom. Photo by Lisa Kerr Lobel. Reproduced from Lobel PS (2001) Sounds produced by spawning fishes. *Marine Technology Society Journal* 35: 19–28.

sound-producing fish families, and at least 700 species are known to make sounds; many species likely to produce sounds have not been studied (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear).

Why Do Fishes Make Sound?

Fishes produce sound for a variety of reasons. These include: (1) incidental sounds produced while swimming and feeding and (2) sounds produced intentionally for communication during aggression, predatory attack (alarm calls), feeding, reproduction, and possibly produced for echolocation. Examples of each type of sound are provided in the subsections below.

Incidental Sounds

Some sounds are incidental and are produced by body movements such as swimming. Anecdotal evidence suggests that these hydrodynamic sounds can be detected by prey fish avoiding predatory fishes. Many fishes are also known to produce sounds incidentally during feeding. These feeding sounds can be simple, like the scraping of teeth on coral as parrotfish, *Sparisoma* sp. and *Scarus* sp. (Scaridae), feed on algae. Other fishes produce sounds when chasing or capturing prey. For example, seahorses, *Hippocampus* sp. (Syngnathidae), make clicking sounds as their skull hits vertebrae when catching prey. Margate, *Haemulon album* (Haemulidae), make both pop and burst

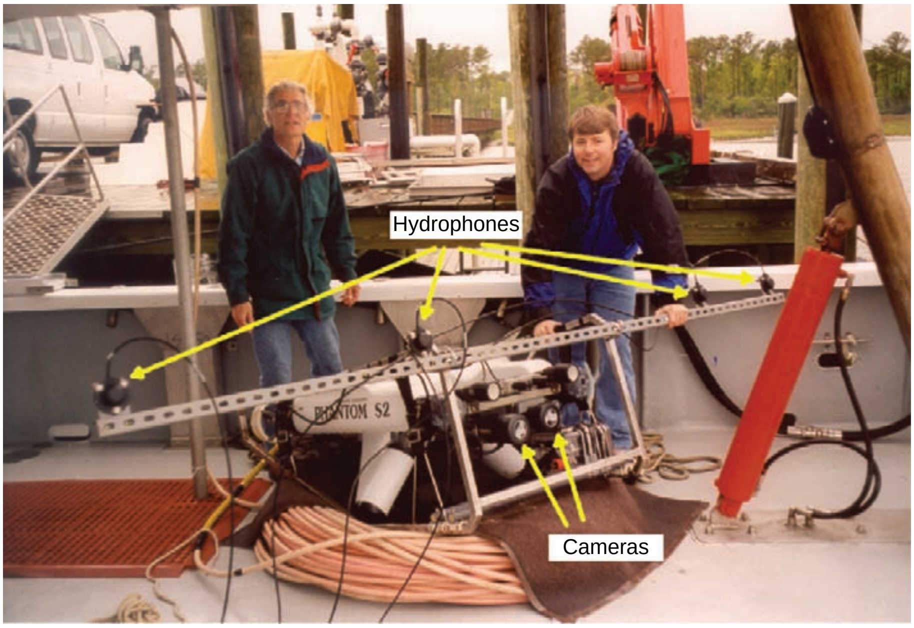


Figure 4 Phantom S2 remote operated vehicle (ROV) with hydrophone boom and integral camera. Photo by Joseph J Luczkovich. Reproduced from Sprague MW and Luczkovich JJ (2004) Measurement of an individual silver perch *Bairdiella chrysoura* sound presure level in a field recording. *Journal of the Acoustical Society of America* 116: 3186–3191.

sounds depending on their feeding mechanism. Popping sounds are made as the fish propels its body forward after plankton, while burst sounds accompany the act of expelling sand from their mouth and opercular openings after a benthic feeding.

Intentional Sounds

Aggressive sounds

There are many examples of fish sounds used in aggressive interactions between individuals of the same species or in territory defense. When several grey gurnard, *Eutrigla gurnardus* (Triglidae), are given food in a laboratory, there is a significant increase in both visual and acoustic displays, with different sound types indicating feeding, fighting, and warning (**Figure 5**). These signals prevent other fish from feeding in the same area. Bicolor damselfish, *Stegastes partitus* (Pomacentridae), are well known for their acoustic and visual displays when approached by an intruder (e.g., SCUBA diver). Bicolor damselfish produce popping sounds with an increasing repetition rate as the intruder approaches their nest. If the intruder is not deterred by the acoustic and visual displays and continues to approach, the damselfish engages in physical battle, biting, and even ramming the intruder.

Alarm calls

Alarm calls denote behaviors alerting conspecifics to predators in the area. For example, longspine squirrelfish, *Holocentrus rufus* (Holocentridae), grunt or make staccato sounds when an intruder, such as another longspine squirrelfish, a bluestriped grunt, *Haemulon scirus*

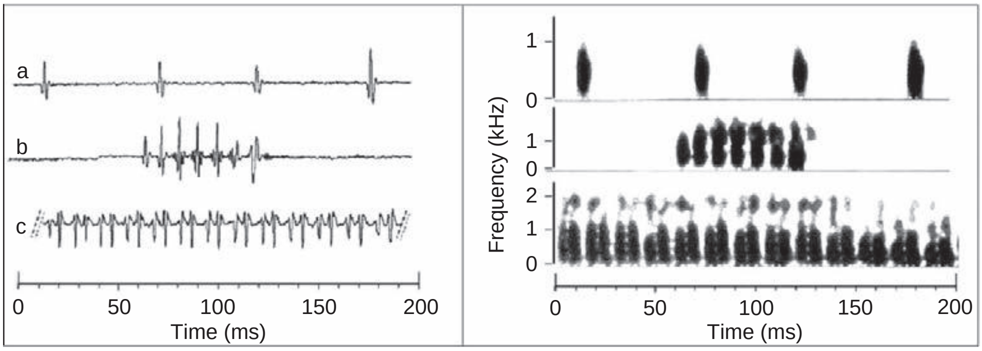


Figure 5 Oscillogram (left) with spectrogram (right) representation of the three types of acoustic signals emitted by grey gurnards *Eutrigla gurnardus* (Triglidae): (a) knocks (feeding); (b) grunts (fighting); and (c) growls (warning). Modified from Amorim MCP, Stratoudakis Y, and Hawkins AD (2004) Sound production during competitive feeding in the grey gurnard. *Journal of Fish Biology* 65: 182–194.

(Haemulidae), a mullet, *Mugil* sp. (Mugilidae), or a spotted moray eel, *Gymnothorax moringa* (Muraenidae), enters their territory. Longspine squirrelfish produced longer staccatos when spotted moray eels were introduced experimentally into their territories compared with the introduction of nonpiscivorous mullet and grunt, suggesting that the longer staccatos may be an alarm call. These squirrelfish alarm calls have been recorded when moray eels and groupers hunt together cooperatively on reefs. These alarm sounds are examples of altruistic behaviors that may facilitate escape behaviors for closely related individuals.

In contrast, longspine squirrelfish have been observed to become silent when the low-frequency (<10 kHz) component of bottlenose dolphin, *Tursiops truncatus*, echolocation sounds was played back. Similarly, a chorus of silver perch (**Figure 6(a)**) was silenced by the sounds of their predator (**Figure 6(b)**). This acoustic avoidance effect was demonstrated by playback experiments using bottlenose dolphin signature whistles, which caused a drop in silver perch sound production of 15 dB (**Figure 6(c)**), but this did not happen when a control sound (700-Hz tone) was played back (**Figure 6(d)**). It is unclear if warning sounds were produced by the silver perch and longspine squirrelfish prior to the silences in these cases.

Spawning/advertisement call

The most well-described fish calls are associated with spawning and reproductive behavior. Sound production, when associated with spawning, is common in turbid water or dark environments, and often occurs during nocturnal spawning events. Generally, only male fishes make spawning sounds as advertisement calls. Acoustic signals may be the mechanisms for female fishes to locate spawning-ready male conspecifics, especially in dark waters. Nocturnal sound production by males is commonly associated with pelagic eggs produced by females in field hydrophone surveys of fishes in the family Sciaenidae: silver perch; weakfish, *Cynoscion regalis*; spotted seatrout, *C. nebulosus*; and red drum, *Sciaenops ocellatus* (**Figure 7**).

In aquarium observations, male red drum used acoustic, visual, and tactile cues during courtship. Males call females with increasingly rapid sounds as spawning approaches, first with two to four short pulse trains, then with rapid drum rolls. When a female responds to the sounds of a male, she begins swimming off the bottom. The male then uses visual (e.g., color change) or tactile (nudging near the female's vent) cues to initiate egg release.

When white seabass, *Atractoscion nobilis* (Sciaenidae), were observed with underwater video and hydrophones in semi-natural oceanic enclosures, a single female spawned with multiple males (up to nine individual males), while the males made sounds variously described as drum rolls, thuds, and a chanting chorus of sounds. During courtship, the males would swim alongside the female with their vents in close proximity to the female's vent (**Figure 8(a)**), making low-frequency (30–200 Hz) pulse trains of sounds (**Figure 9(a)**), some of which were identified as drum rolls and thuds (**Figure 9(b)**). The female exhibited vertical color bars during courtship and spawning, which provided a visual cue to the males

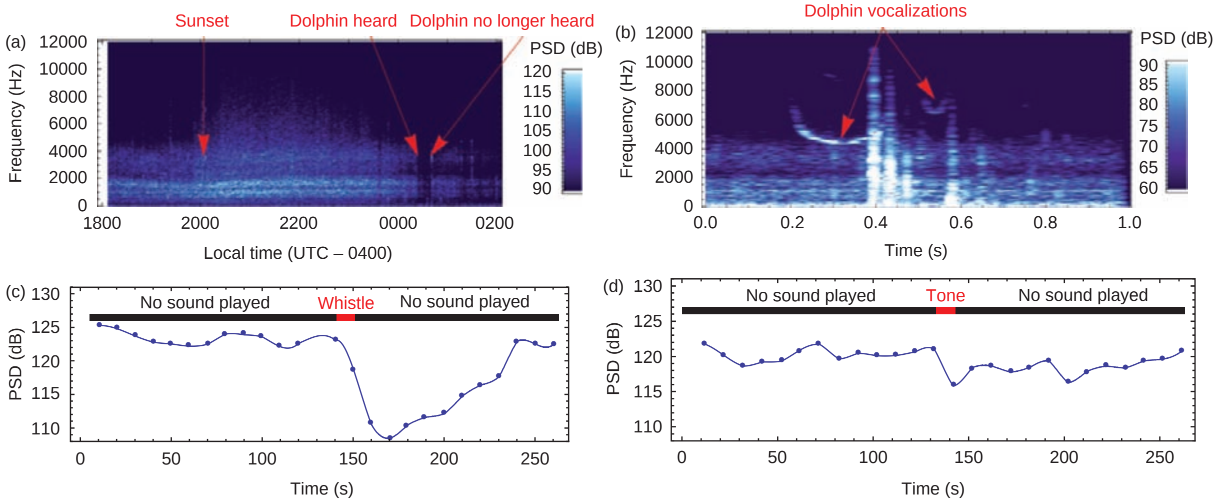


Figure 6 Silver perch, *Bairdiella chrysoura*, acoustic avoidance of bottlenose dolphin, *Tursiops truncatus*, whistles. Power spectral densities (PSDs) given in dB re 1 $\mu Pa^2\ Hz^{-1}$. (a) Composite sonogram showing a silver perch chorus with a sudden quiet period at time 00:24 (24 min after midnight). (b) Sonogram of bottlenose dolphin whistles recorded during the silver perch quiet period in (a). (c) Variation of silver perch loudness, measured by the peak of the power spectrum between frequencies 950 and 1200 Hz, before and after a bottlenose dolphin whistle was played through an underwater speaker. (d) Same measurement as (c) before and after a 700-Hz tone was played through an underwater speaker. Reproduced from Luczkovich JJ, Daniel HJ III, Hutchinson M, et al. (2000) Sounds of sex and death in the sea: Bottlenose dolphin whistles silence mating choruses of silver perch. *Bioacoustics* 11: 323–334.

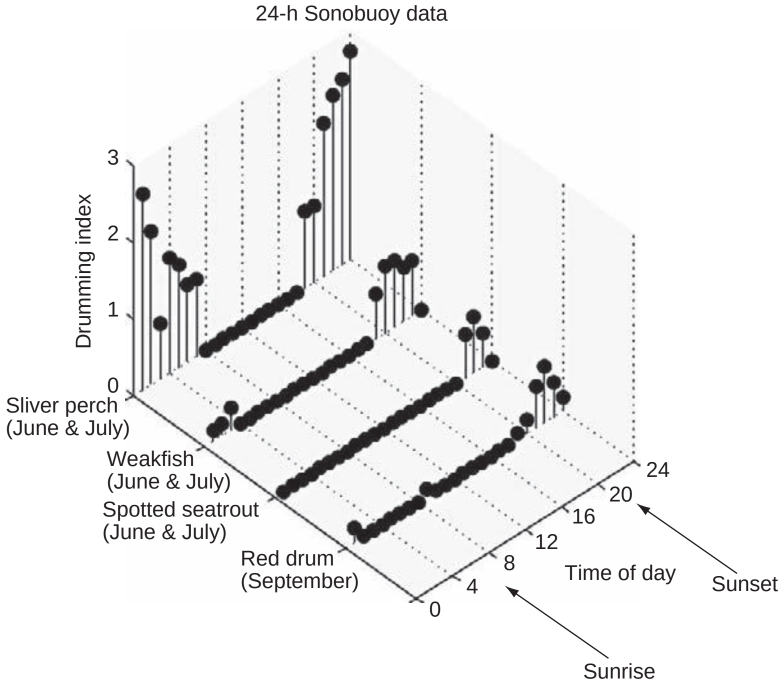


Figure 7 An index of sound production during spawning (drumming index) showing nocturnal and seasonal sound production patterns exhibited by silver perch, *Bairdiella chrysoura*; weakfish, *Cynoscion regalis*; spotted seatrout, *C. nebulosus*; and red drum, *Sciaenops ocellatus*, in Pamlico Sound, North Carolina, USA. An index of 0 corresponds to no fish sound detected, 1 to an individual call detected, 2 to several individual calls detected, and 3 to a chorus of sounds too loud to distinguish individual calls. Reproduced from Luczkovich JJ, Pullinger RC, Johnson SE, and Sprague MW (2008) Identifying sciaenid critical spawning habitats by the use of passive acoutics. *Transactions of the American Fisheries Society* 137: 576–605.

indicating the willingness of the female to spawn. During spawning, a long trail of gametes was observed as the spawning pack swam toward the surface producing a continuous chant (**Figure 9(c)**) during gamete release (**Figure 8(b)**). In this sciaenid species, sound production was made before and during spawning events, and the sound was continuous and loudest during the gamete release.

Female fishes may be able to interpret acoustic signals as a measure of male fitness and use the sounds to choose an appropriate male to fertilize the eggs. The best-studied example of mate choice based on sound production is the plainfin midshipman, *Porichthys notatus* (Batrachoididae). Each summer on the US Pacific coast, plainfin midshipman migrate from deep water to tidepools in the intertidal zone to mate. Males produce a tonal humming sound of 100 Hz with duration lasting from a few minutes to hour-long calls to attract mates. Laboratory experiments have demonstrated that the females are attracted to recorded 100-Hz hums played back alternatively through two underwater speakers. Playback experiments using artificial humming sounds of the same frequency also attract females. When presented with pure tones of various frequencies near the hum frequency, female midshipman showed a preference for 100-Hz tones.

Male damselfishes (Pomacentridae) produce sounds to warn competing males to keep away from their territory and to initiate courtship behavior with a female. In the bicolor damselfish (*Stegastes partitus*), sound playback experiments on a natural reef using males' recorded chirp sounds demonstrated that males use the sound as a warning to other males to keep out. Territory holders can discriminate between neighboring males based on sounds, which carry size-related information (larger damselfish have lower-frequency chirps). Females can also discriminate between males' chirps of different frequencies, choosing the largest males. Male Hawaiian dominos, *Dascyllus albisella*, had significantly longer pulse durations during mating events than when males only were present. During mating, males also complete a signal jump in which they swim upward and then move up and down while producing pulses of sound. Signal jumps differ in interpulse interval, period, pulse duration, and amplitude compared with male aggressive encounters. Signal jumps are related to male vigor and fitness, allowing females to assess a mate using both visual and auditory cues.

Researchers have observed and recorded spawning haddock, *Melanogrammus aeglenus* (Gadidae), in aquaria. Males were observed to participate in several complicated behavioral acts during spawning and courtship, many of which have specific sounds associated with them

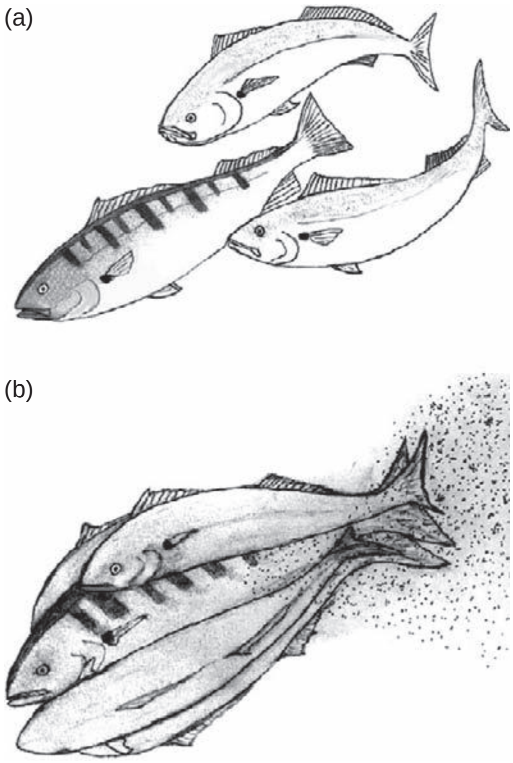


Figure 8 Illustrations of (a) two courting white seabass, *Atractoscion nobilis*, males shadowing and nudging a darkly barred gravid female and (b) a spawning pack of four males and a barred female simultaneously releasing gametes during a broadcast spawning event. Reproduced from Albers SA and Drawbridge MA (2008) White seabass spawning behavior and sound production. *Transactions of the American Fisheries Society* 137: 542–550.

(**Figure 10**). Researchers hypothesized that the associations of distinct sounds with these complicated behaviors are because haddock spawn at depths of over 100 m, where there is little light. Thus, the sounds enhance the visual displays.

Other examples of fishes that produce sound during courtship and mating include the parrotfish, groupers, hamlets, and gobies. In the butter hamlet, *Hypoplectrus unicolor* (Serranidae), both members of a spawning pair are simultaneous hermaphrodites, and both make sounds. The butter hamlet individual acting in the male spawning role (producing spermatozoa) produces an initial courtship sound, a series of 500-Hz pulses. The other individual, acting in the female role (producing eggs), also produces a sound during egg release, which is a frequency-modulated downward sweep (between 600 and 200 Hz), followed by a longer broadband sound (350–1350 Hz). Individuals will then immediately reverse in their mating roles, making the appropriate sounds for their sex role. In striped parrotfish, *Scarus iseri* (Scaridae), group spawning occurs in association with sound production. A spawning rush or upward swimming group of 5–30 individuals of both the sexes produces a low-frequency (30–1200 Hz) broadband sound during the entire spawning event. Grass gobies, *Zosterisessor ophiocephalus* (Gobiidae) are sound producers, having grunting and staccato sounds associated with territorial defense, courtship, and reproduction. Males produce these sounds prior to mating. The frequencies (~330 Hz) of these transient sounds are inversely related to fish size.

Navigation and echolocation

Fishes may use sounds, produced by themselves or other species, as a way of finding their way around. For example, there is evidence that more larval damselfish and cardinalfish (Apogonidae) are attracted to artificial reefs that have sounds of reef animals (fishes, snapping shrimp, and urchins) played through underwater speakers than are attracted to control sites that do not have reef sounds. Larval reef fishes also swim toward the sounds of a coral reef played back through a speaker. Because coral reef sounds can be heard by larvae over a long distance (~100 m), planktonic larval fishes can possibly use sounds as a navigation cue to locate suitable settlement habitats.

It has been suggested that fishes make sounds as a way of learning about the environment around them from the echoes that reflect from the bottom or objects in the sea (**see also Hearing and Lateral Line**: Sound Source Localization and Directional Hearing in Fishes). For example, the sea catfish, *Arius felis* (Ariidae), has been shown experimentally to find its way through a maze with transparent walls, never hitting the walls and all the while making popping sounds; individuals that had been muted by surgically cutting their sonic muscles did not make sounds and did not easily navigate the maze, bumping into the transparent plastic walls (**Figure 11**). Blinded sea catfish were also able to navigate the maze, but did so while making sounds. Deep-sea fishes (families Macrouridae and Moridae) have sonic muscles, unusual inner ear structures with large otoliths, and can produce sounds at great depths (3900 m), loud enough to be detected near the surface by a hydrophone. Sound production by these deep-sea fishes may be used to echolocate in total darkness. Whether fishes actually use sound in this way remains speculative, but is an area of considerable interest.

Sound Production and Ontogeny, Sex Differences

Although fishes produce sounds, this does not occur equally at all life stages or in both sexes. Most studies have identified mature males producing sound, but not females or juveniles. For example, in the drumfish family, weakfish, and spotted seatrout, males produce advertisement calls associated with spawning, but females and immature males do not have enlarged drumming muscles

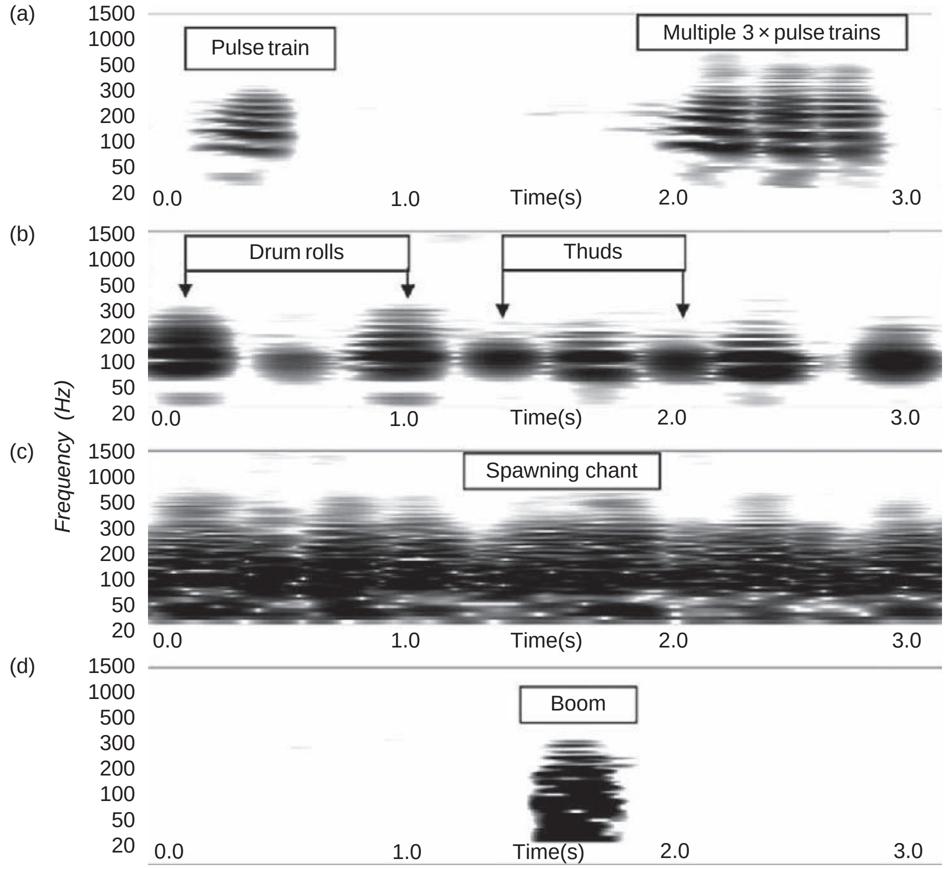


Figure 9 Sonograms displaying: (a) white seabass, *Atractoscion nobilis*, courtship sounds, including single and multiple (33) pulse trains; (b) drumroll and thud sounds produced in rapid succession at the beginning of a spawning event; (c) spawning chant of continuous, overlapping drumroll and thud sounds; and (d) hydrodynamic boom. Sounds recorded from fish held in a net pen in Catalina Harbor, California. Reproduced from Albers SA and Drawbridge MA (2008) *Transactions of the American Fisheries Society* 137: 542–550.

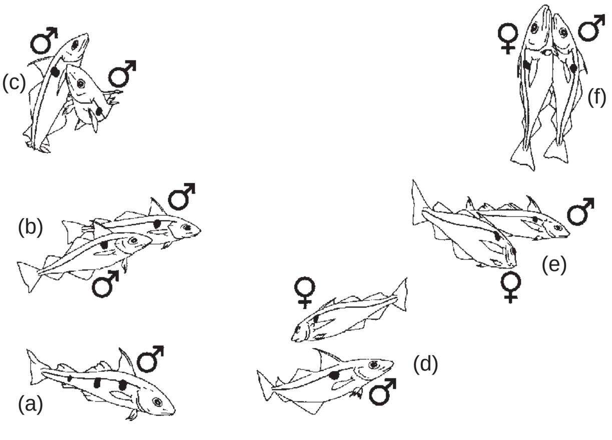


Figure 10 Behavior shown by haddock during courtship: (a) solitary display by a male; (b) lateral display by one male to another; (c) circling of two males in lateral display; (d) lateral display by a male to a female; (e) flicking by a male to a female; (f) mating embrace between male and female, with synchronous release of eggs and milt. From Hawkins AD and Amorim MC (2000) Spawning sounds of the male haddock *Melanogrammus aeglefinus*. *Environmental Biology of Fishes* 59: 29–41.

that are necessary for sound production. This is an example of sexual dimorphism.

In other species (including striped cusk-eel, *Ophidion marginatum* (Ophidiidae), Atlantic croaker, *Micropogonias undulatus* (Sciaenidae), grey gurnards, multiple species of toadfish, midshipmen, and haddock), both sexes produce sound, although differences in anatomy and sound characteristics are apparent. For example, both male and female croaking gouramis, *Trichopsis vittata* (Osphronemidae), produce sounds, but the sounds differ in loudness and spectral characteristics when mating and in agonistic encounters. Female croaking gouramis initiate courtship by producing low-intensity purring sounds, the only species documented to do this.

Sound production in juvenile fishes has not been well studied. Juvenile Lusitanian toadfish, *Halobatrachus didactylus* (Batrachoididae), can produce territorial sounds when they have reached 38 mm in standard length. Atlantic croakers have been shown to have sonic muscles at a standard length of 45 mm and the ability to produce sounds prior to maturity. It has been shown that sounds become louder and lower in frequency as these fishes

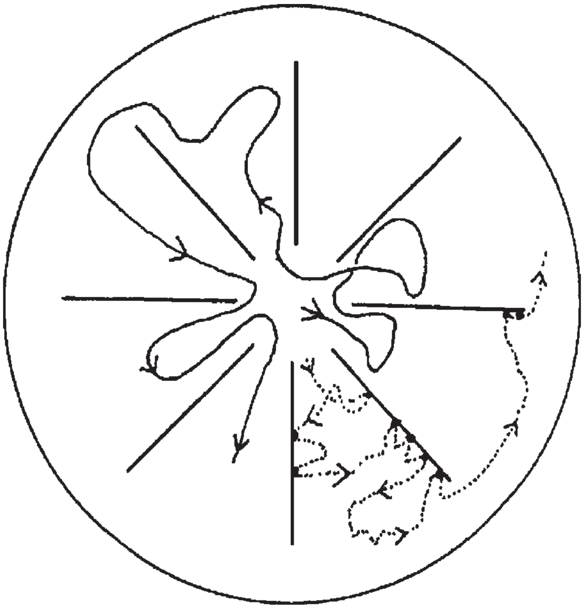


Figure 11 The maze-swimming behavior of sea catfish, *Arius felis*, a normal, sound-producing individual (solid line), and one of a group of eight muted individuals (dashed line). Reproduced from Fig. 6 in Tavolga WN (1976) Acoustic obstacle detection in the sea catfish (*Arius felis*). In: Schuijf A and Hawkins AD (eds.) *Sound Reception in Fishes*, pp. 185–204. Amsterdam: Elsevier, with permission from Elsevier.

grow. There are also differences in the rates of pulsing between juveniles and adults of the same species. These acoustic differences by different life stages may be indicative in changes of behavior (e.g., fighting vs. mating) for these species.

Sound production can vary within a species and sex. Two types of males occur in plainfin midshipman (types I and II), each having different vocal patterns and reproductive strategies. Type I males make the 100-Hz humming sound as an advertisement call to attract females to a spawning nest. Type II males do not make these humming advertisement sounds. Both types of males produce grunts, which are used in agonistic encounters. Instead of initiating courtship behaviors through the hums, type II males will instead employ a sneaker strategy in that they enter the nest of a type I male and spawn with the female, while the type I male is engaged in territorial defense with another type I male. Thus, sound production in midshipman depends upon genetic factors and the physiological state of the males (types I and II, spawning or fighting). Grass gobies also exhibit differences in sound production associated with the alternative reproductive strategies of males: small sneaker males had small dorsal fins, higher frequency (415 Hz), and quieter sounds, while large territory defenders had elongated second dorsal fins, lower-frequency sounds (299 Hz), and louder (by 10 dB) sounds.

Sound Production as a Fishery Management Tool

Scientists have used hydrophones and sound recorders to locate spawning grounds by documenting fishes making sound in the natural environment. Many such studies have been done in association with routine biological sampling, such as egg and larval fish collections using plankton nets, or adult fishes captured by trawl nets and angling. The results of such studies suggest that economically important fishes in the families Sciaenidae, Serranidae, and Gadidae make sound primarily in spawning periods and that sounds can be used as a proxy for spawning behavior. Pelagic egg abundance and sound production of fishes are correlated, both increasing during the mating period. For example, weakfish along the US Atlantic Coast has been shown to increase its sound production during mating periods in May through July each year, when their gonadal development stages are greatest. In addition, the drumming muscles in the male weakfish were also highly developed at that time, due to increased blood levels of testosterone. These results suggest that spawning activities are associated with sound production by male weakfish.

Conclusion

Sound production plays an important role in the survival, social organization, mating success, and navigation capabilities of fishes in dark and turbid aquatic environments. There is a need for more studies linking sound production and specific behaviors in species known to make sound and many more undescribed sound-producing species. Various types of sounds (pulsed and tonal) are produced in association with specific behavior by over 700 species of fishes. Different sounds are produced at different maturity and life stages, with pulsed sound frequencies declining as an individual gets larger. Although most sound producers are males, in some species both sexes make sounds that differ in characteristics.

New technologies based on digital sampling hydrophones connected to automated sound recorders are being used to assess variation in sound production during the day and seasonally, allowing spawning times to be determined remotely. Use of passive acoustic monitoring, combined with active sonar and traditional methods of sampling, can help assess habitat use, size of individuals, and the spawning stock size for economically important sound-producing species and improve fisheries management practices. Scientists now regularly listen to fishes and can identify the species making the sound, their size, and something about their natural behaviors, even when the conditions prevent visual observations. The use of sound by fishes is essential for their survival; humans now appreciate this importance and use it as an aid in conservation of fishes.

See also: **Behavior and Physiology:** Linking Fish Behavior and Physiology: An Introduction. **Hearing and Lateral Line:** Biomechanics of the Inner Ear in Fishes; Psychoacoustics: What Fish Hear; Sound Source Localization and Directional Hearing in Fishes; The Ear and Hearing in Sharks, Skates, and Rays; Vocal Behavior of Fishes: Anatomy and Physiology. **Smell, Taste, and Chemical Sensing:** Chemosensory Behavior. **Vision:** Behavioral Assessment of the Visual Capabilities of Fish.

Further Reading

Albers SA and Drawbridge MA (2008) White seabass spawning behavior and sound production. *Transactions of the American Fisheries Society* 137: 542–550.

Amorim MCP, Stratoudakis Y, and Hawkins AD (2004) Sound production during competitive feeding in the grey gurnard. *Journal of Fish Biology* 65: 182–194.

Bass AH (1990) Sounds from the intertidal zone: Vocalizing fish. *BioScience* 40: 249–258.

Brantley R and Bass A (1994) Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish. *Porichthys notatus girard* (Teleostei, Batrachoididae). *Ethology* 96: 213–232.

Fish MP and Mowbray WH (1970) *Sounds of Western North Atlantic Fishes*. Baltimore, MD: The Johns Hopkins University Press.

Hawkins AD (1986) Underwater sound and fish behavior. In: Pitcher TJ (ed.) *The Behavior of Teleost Fishes*, pp.114–151. Baltimore, MD: The Johns Hopkins University Press.

Hawkins AD and Amorim MC (2000) Spawning sounds of the male haddock *Melanogrammus aeglefinus. Environmental Biology of Fishes* 59: 29–41.

Ladich F and Myrberg AA, Jr. (2006) Agonistic behavior and acoustic communication. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes,* vol. 1, pp. 121–148. Enfield, NH: Science Publishers.

Lobel PS (1992) Sounds produced by spawning fishes. *Environmental Biology of Fishes* 33: 351–358.

Luczkovich JJ, Daniel HJ, III, Hutchinson M, *et al. (2000)* Sounds of sex and death in the sea: Bottlenose dolphin whistles silence mating choruses of silver perch. *Bioacoustics* 11: 323–334.

Luczkovich JJ and Keusenkoethen M (2007) Behavior and sound production by longspine squirrelfish *Holocentrus rufus* during playback of predator and conspecific sounds. In: Pollock NW and Godfrey JM (eds.) *Diving for Science Proceedings of the American Academy of Underwater Sciences 26th Symposium*. Dauphin Island, AL, USA: Rubican Research Repository http://archive.rubicon-foundation.org (accessed on June 2010).

Luczkovich JJ, Mann DA, and Rountree RA (2008) Passive acoustics as a tool in fisheries: An introduction to the American Fisheries Society Symposium. *Transactions of the American Fisheries Society* 137: 533–541.

Luczkovich JJ, Pullinger RC, Johnson SE, and Sprague MW (2008) Identifying the critical spawning habitats of sciaenids using passive acoustics. *Transactions of the American Fisheries Society* 137: 576–605.

Malavasi S, Torricelli P, Lugli M, Pranovi F, and Mainardi D (2003) Male courtship sounds in a teleost with alternative reproductive tactics, the grass goby, *Zosterisessor ophiocephalus. Environmental Biology of Fishes* 66: 231–236.

Mann DA and Lobel PS (1997) Propagation of damselfish (Pomacentridae) courtship sounds. *Journal of the Acoustical Society of America* 101: 3783–3791.

McKibben J and Bass A (2001) Effects of temporal envelope modulation on acoustic signal recognition in a vocal fish, the plainfin midshipman. *The Journal of the Acoustical Society of America* 109: 2934.

Myrberg AA (1997) Sound production by a coral reef fish (*Pomacentrus partitus*): Evidence for a vocal, territorial "keep-out" signal. *Bulletin of Marine Science* 60: 1017–1025.

Tavolga WN (ed.) (1964) *Marine Bio-Acoustics.* Oxford: Pergamon Press.

Tavolga WN (1976) Acoustic obstacle detection in the sea catfish (*Arius felis*). In: Schuijf A and Hawkins AD (eds.) *Sound Reception in Fishes*, pp. 185–204. Amsterdam: Elsevier.

Webb J, Fay R, and Popper A (eds.) (2008) *Fish Bioacoustics*. New York: Springer Verlag.

Relevant Websites

http://macaulaylibrary.org – Cornell University MaCauley Library of Natural Sounds, including a large collection of fish sounds.

http://www.cosmosmagazine.com – Cosmos: The Science of Everything; Deep sea fish may use sound to navigate.

http://www.fishecology.org – Fish and Other Underwater Sounds; Rodney Roundtree Sound Archive.

http://core.ecu.edu – ECU PiratePanel; Fish Sound Archive of the Sciaenid Acoustics Research Team (SART) at East Carolina University, Greenville, NC 27858. http://www.nytimes.com Fish Sounds – Ocean Life – New York Times Science Times Article, 8 April 2008.

http://ocr.org – Ocean Conservation Research: Science and Technology Serving the Sea; Fish Sounds.

http://www.newscientist.com – NewScientist; Reefs with sound attract more fish. New Scientist Article, 16 April 2005.

http://www.gso.uri.edu – URI Graduate School of Oceanography; University of Rhode Island Sounds of the Fishes of the North Atlantic. The collection of recordings of 153 species of fish from Fish MP and Mowbray W (1970) *Sounds of Western North Atlantic Fishes* is available on CD.

http://www.dosits.org – University of Rhode Island. Animals and Sound in the Sea.

Vocal Behavior of Fishes: Anatomy and Physiology

F Ladich, University of Vienna, Vienna, Austria

AH Bass, Cornell University, Ithaca, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Vocalizations
Vocal Organs
Sexual Dimorphisms in Sound-Producing Muscles
Vocal Neuron Control
Concluding Comments
Further Reading

Glossary

Extrinsic drumming muscles A sound-generating muscle that is not entirely attached to the swimbladder, but has its origin on other skeletal structures such as ribs, vertebral processes, or tendons.

Intrinsic drumming muscles A sound-generating muscle that is entirely attached to the swimbladder.

Pectoral girdle/pectoral fin tendons The pectoral girdle connects the anterior appendage (fin or forelimb) to the axial skeleton. Some fishes vibrate a pair of tendons attached to pectoral fin to generate sounds.

Pectoral spine First-enhanced fin ray in catfishes which is used for defense when locked in a perpendicular position and for sound production when rubbed in a groove of the shoulder girdle.

Sonic/vocal motor nuclei Clusters of motor neurons whose cell bodies (somata) form a motor nucleus (not to be confused with the nucleus inside of a cell) that is located in the hindbrain and/or spinal cord of the central nervous system.

Introduction

The diversity of vocalizations and vocal organs among fishes is discussed first, followed by a focus on how sound-producing muscles and the central nervous system (CNS) determine the physical properties of vocal behaviors. Historically, the sound-producing systems of fishes were only referred to as sonic. These systems are also referred to here as vocal, and the associated behaviors as vocalizations, because of the development of the vocal control systems in similar regions of the CNS in both fishes and other vertebrates (see section Vocal Neuron Control).

Vocalizations

Fishes vocalize in various behavioral contexts such as when they are caught, defending territories such as nests, when advertising nest sites, and during courtship and mating (**see also Hearing and Lateral Line**: Acoustic Behavior and **Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling). We estimate that approximately a third of all living fish species vocalize. Given the approximately 30 000 or more species of living fishes, there are more vocal species among fishes than among either mammals or birds. The vocalizing species identified so far belong to the bony fishes that include two major clades known as Actinopterygii and Sarcopterygii (also includes tetrapods). The majority of vocal fish are actinopterygians and include the basal groups of African bichirs (Polypteridae) and sturgeons (Acipenseridae) and the more recently evolved teleosts that comprise the vast majority of actiniopterygians. Jawless (Cyclostomata – lampreys and hagfish) and cartilaginous (Chondrichthyes – sharks, skates, rays, and chimaeras) fishes that are more primitive than bony fishes are not known to vocalize.

The vocalizations of fishes, like those of other vertebrates, vary in their spectral (frequency content) and temporal (e.g., duration and call rate) properties. As discussed in more detail in the next section, most fishes produce sound either by vibrating fast-contracting muscles attached to gas-filled swimbladders or by vibrating some part of the pectoral girdle or fins. Swimbladder or drumming sounds are usually brief (on the order of milliseconds), with the primary energy only a few hundred hertz (**Figures 1(a) and 1(b); Figures 2(a)–2(c)**). Each sound pulse results from a single contraction of the swimbladder muscles. Multiple sound pulses make up a single call that can vary widely in duration and energy content at one or more frequencies. Multi-harmonic signals have a tonal quality with energy peaks at a fundamental

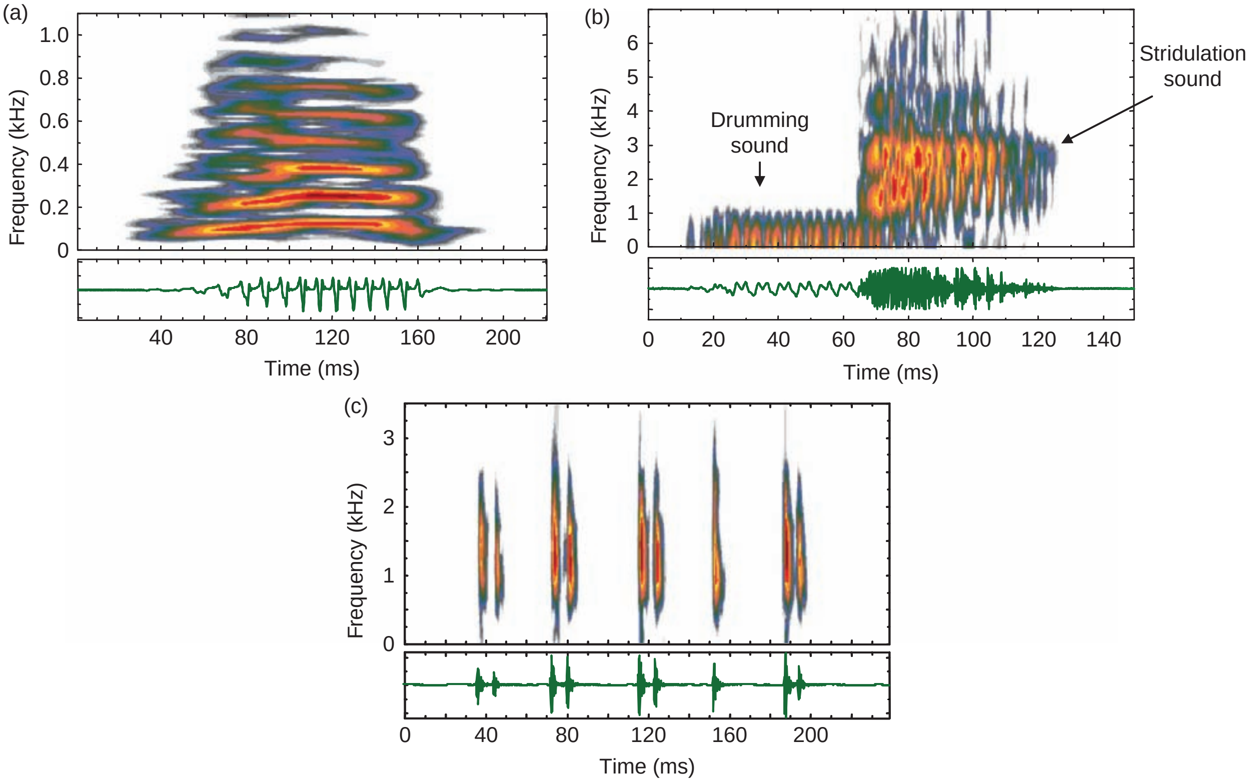


Figure 1 Sonagrams (or spectrograms, above) and waveforms (or oscillograms, below) of vocalizations emitted by various fish species and various sound-generating mechanisms. The sonogram or spectrogram displays the amount of sound energy at each of the frequencies that comprise the call (top) and an oscillogram trace shows the time-varying waveform. (a) A low-frequency multi-harmonic sound produced by drumming muscles in a piranha (*Pygocentrus nattereri*). The main sound energy is concentrated at approximately 200–300 Hz (red color; sound sample, **Sound Clip 1**). (b) A sound produced by a long-whiskered catfish (*Pimelodus blochii*). In the second part the sound, energies are found up to several kilohertz (sound sample, **Sound Clip 2**). (c) A croaking sound emitted during agonistic behavior in a croaking gourami (*Trichopsis vittata*) (sound sample, **Sound Clip 3**).

frequency and at harmonics that are integer multiples of the fundamental (**Figure 1**(**a**); (**Sound Clip 1**); **Figures 2**(**a**) and **2**(**b**)). Some swimbladder drumming sounds are referred to as nonharmonic signals because energy is spread over a broad range of frequencies rather than being concentrated in discrete energy bands; hence, these signals are often referred to as broadband (**Figure 2**(**c**)).

The vocal repertoire of the plainfin midshipman (*Porichthys notatus*), a species that belongs to a larger group of vocal fish known as toadfishes (Batrachoididae), exemplifies the range of call types produced by fishes (**Figure 2**; **Sound 4–6**). During the breeding season, nesting males emit long-duration, multi-harmonic hums that can last for more than 1 h and are used to attract females to their nest. Males also produce very brief, broadband grunts that last for about 100 ms during agonistic encounters. Grunts are often produced in succession to form grunt trains. Males also produce agonistic growls that are essentially a mix of hums and grunts.

The calls of species that vibrate the pectoral fins (stridulation) are generally pulsatile, but typically have a higher frequency content than swimbladder-associated sounds. Pectoral sounds are broadband, with their primary energy close to or greater than 1 kHz. Swimbladder/drumming and pectoral/stridulation sounds may be produced in series or simultaneously as by some catfish (**Figure 1**(**b**); **Sound Clip 2**); Croaking gouramis (*Trichopsis vittata*, Osphronemidae) generate a series of high-frequency double pulses called croaks (**Figure 1**(**c**); **Sound Clip 3**) Long (150–200 ms) and loud croaks are produced by both sexes when defending territories, while brief, lower-intensity sounds are emitted by females prior to mating.

Vocal Organs

The main group of sound-generating mechanisms among fishes involves the swimbladder. The swimbladder (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes) can be vibrated in numerous ways by a pair of muscles variously called drumming, sonic, or vocal muscles.

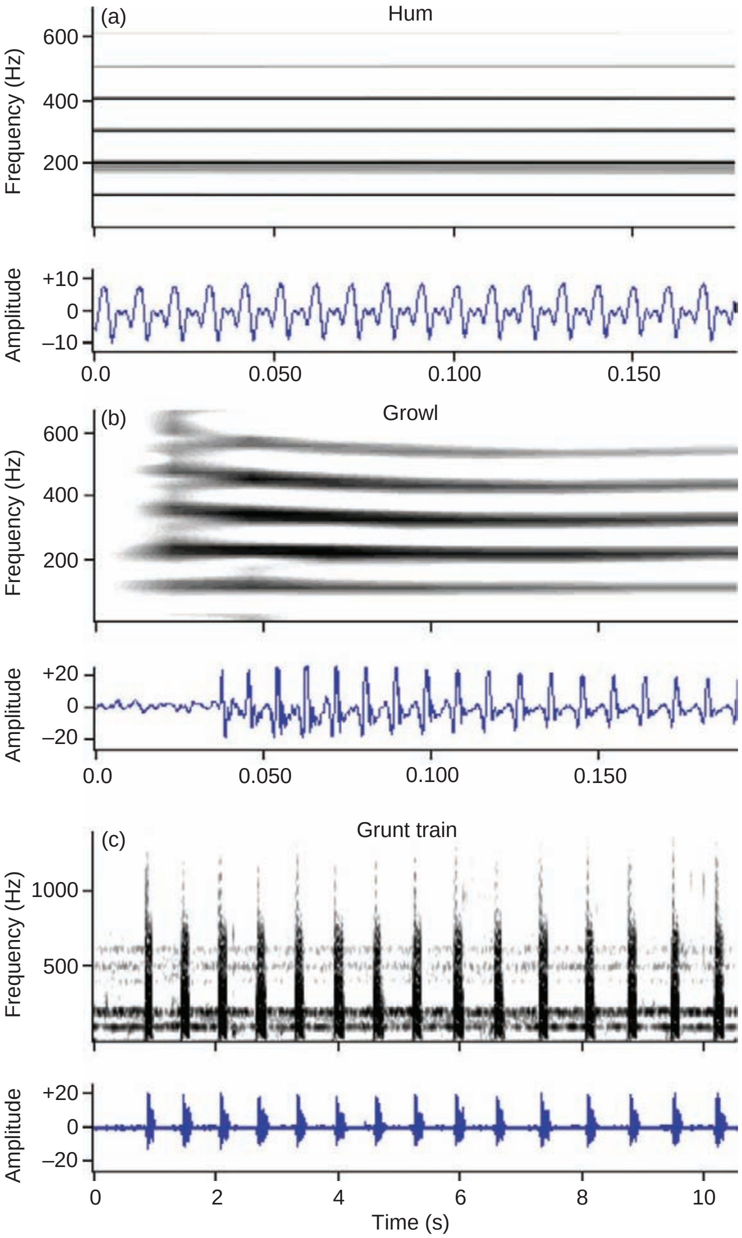


Figure 2 Male plainfin midshipman fish (*Porichthys notatus*) produce three major classes of vocalizations – hum, growl, and grunt train. (a) This hum has a fundamental frequency of 102 Hz with several harmonics (sound sample, **Sound Clip 4**). (b) The growl begins with a grunt region followed by a hum-like region (sound sample, **Sound Clip 5**). (c) The grunt train shows a series of grunts (sound sample, **Sound Clip 6**). Recordings by Marchaterre, MA, Cornell University. From Bass AH and Clark CW (2003) The physical acoustics of underwater sound communication. In: Simmons AM, Fay RR, and Popper AN (eds.) *Acoustic Communication*, pp. 15–64. New York: Springer, with kind permission of Springer Science+Business Media.

Drumming muscles, which are among the fastest contracting striated muscles known among vertebrates, vibrate swimbladders at high rates (50–250 Hz). Based on the origin and insertion of these muscles on parts of the musculoskeletal system, several types of swimbladder mechanisms may be distinguished.

Drumming muscles often have a direct connection to the swimbladder. Intrinsic drumming muscles are entirely attached to the walls of the swimbladder, as found in toadfishes (**Figure 3(a)–3(c)**). Extrinsic muscles originate on a part of the musculoskeletal system other than the swimbladder, but insert either directly or indirectly via tendons or bones on the bladder. For example, the tigerfish (*Terapon jarbua*, Terapontidae) has muscles originating on the medial surfaces of the dorsal part of the shoulder girdle (supracleithra) connected to the skull, and inserting dorsally on the anterior half of the swimbladder (**Figure 4**). The pimelodid catfish (*Pimelodus* sp., Pimelodidae) has muscles

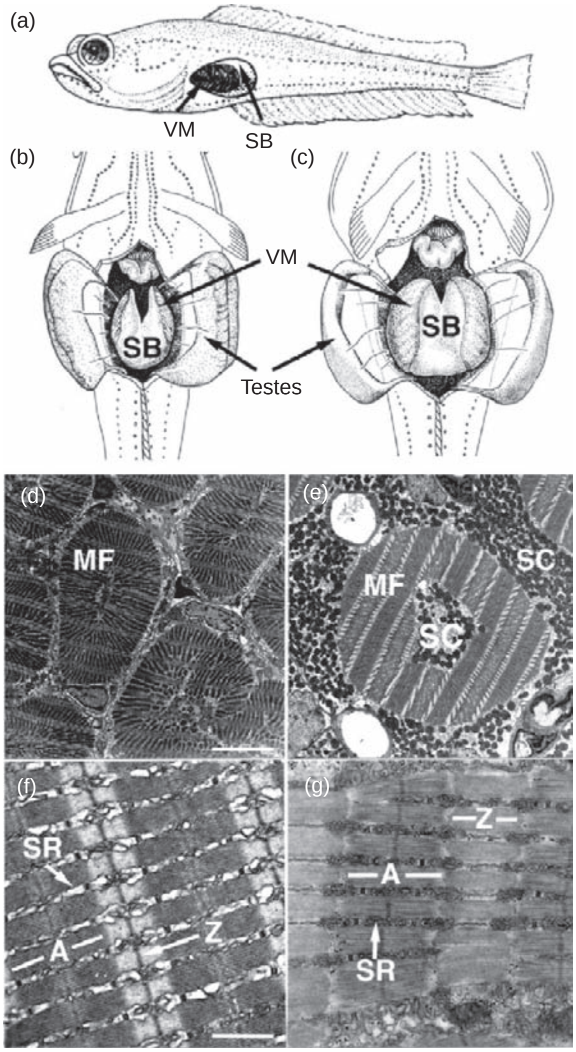


Figure 3 Intrinsic, swimbladder drumming muscles differ between male morphs in the midshipman fish, *Porichthys notatus*. (a) Drawing of a side view shows the location of the swimbladder (SB) with attached vocal muscles (VM). (b, c) Drawings of ventral views with the VMs attached to the SB in a type II, sneaker male (b) and a type I, humming male (c). (d–g) The fine structure of the vocal muscle in type II (d, f) and type I (e, g) males as viewed with an electron microscope. (d, e) As shown in cross section, the muscle fibers of type II (d) and type I (e) males are tubular in shape and include a large region of myofibrils (MF). The myofibrils of type II males are surrounded by a thin band of sarcoplasm (SC) that contains nuclei and mitochondria. The fibers of type I males have a ring of myofibrils with central and lateral zones of SC filled with mitochondria. (f, g) As viewed along the length of a single muscle fiber of a type II (f) and a type I (g) male, the width of Z-lines and the degree of branching of the sarcoplasmic reticulum (SR) are greater in type I males. Scale = 6 μm in (d) and (e), and 0.8 μm (f) and (g). (a–c) Modified from Bass AH and Grober MS (2009) Reproductive plasticity in fish: Evolutionary lability in the patterning of neuroendocrine and behavioral traits underlying divergent sexual phenotypes In: Pfaff D, Arnold A, Etgen A, *et al.* (eds.) *Hormones, Brain, and Behavior*, ch. 16, pp. 580–609. New York: Academic Press. (d–g) Modified from Bass AH and Marchaterre MA (1989) Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): Sexual polymorphism in the ultrastructure of myofibrils. *Journal of Comparative Neurology* 286: 141–153.

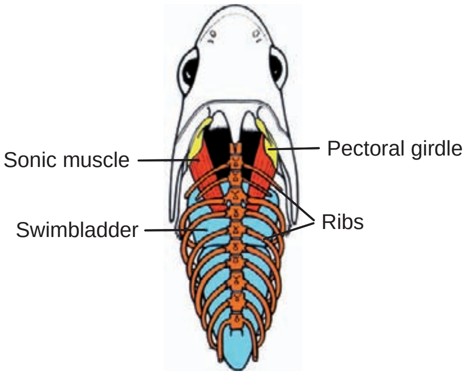


Figure 4 Line drawing of dorsal view showing sound generating mechanisms in the tiperperch (*Terapon jarbua*). The sonic/vocal muscle originates at the pectoral (shoulder) girdle and inserts on the dorsal surface of the swimbladder immediately beneath the anterior ribs. Contractions of the muscle results in sound production. Modified from Zupanc GKH (1982). *Fische und ihr Verhalten*. Melle: Tetra Verlag

originating on a lateral process of the vertebral column and inserting on the swimbladder ventrally (see **Figure 5**(**d**)).

In other cases, sound-producing muscles lack any direct connections to the swimbladder. There is a wide range of such indirect mechanisms. The muscles in black and red piranha (*Serrasalmus rhombeus and Pygocentrus nattereri*; Characidae) originate on the second rib and insert on a broad tendon that surrounds the swimbladder ventrally, similar to the band that wraps around a horse to hold a saddle in place (**Figure 6**). In the unrelated drums and croakers (Sciaenidae), drumming muscles originate on the body wall lateral to the swimbladder and insert on a broad tendon surrounding the dorsal part of the swimbladder. In the jaraqui (*Semaprochilodus insignis*, Prochilodontidae), a close relative of piranhas, the swimbladder is vibrated via large muscles extending between the ribs, allowing these fish to produce loud evening choruses in the Amazonian river system where they live. Several catfish families have bony plates called elastic springs that vibrate the swimbladder. In thorny (Doradidae) and upside-down (Mochokidae) catfishes, drumming muscles pull a disk-shaped elastic spring forward (**Figures 5**(**a**) **and 5**(**b**)), extending the bladder. In sea catfishes (Ariidae), the elastic spring is concave-shaped and pointed (**Figure 5**(**c**)).

The second major group of vocal mechanisms in fishes depends on vibration of either the pectoral/shoulder girdle or the modified pectoral fins. In sculpins (Cottidae), a group that lacks a swimbladder, the entire shoulder girdle is vibrated by muscles extending from the skull to the dorsal edge of the shoulder girdle. Several catfishes possess an enhanced first pectoral fin ray known as the pectoral spine. The friction process of the base of the pectoral spine is rubbed in a groove of the shoulder girdle (**Figure 7**(**a**)) that results in a series of pulsed, high-frequency, stridulatory-like sounds (**Figure 1**(**b**)). Croaking gouramis generate a pair of

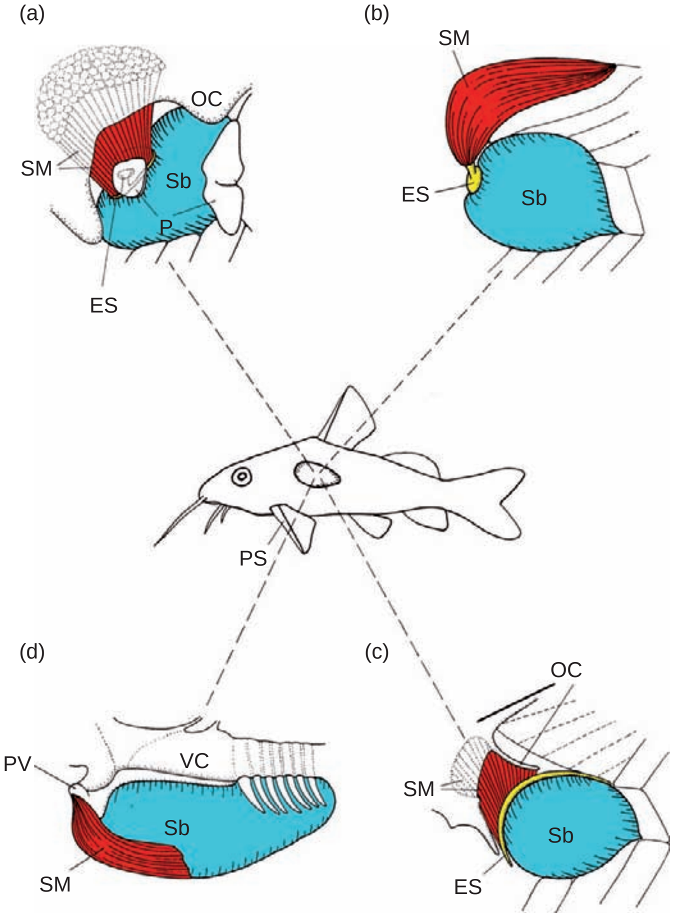


Figure 5 Diversity of sonic/vocal muscles associated with the swimbladder in catfishes. (a) In thorny catfishes (Doradidae), the sonic muscles (SM) originate at the occipital bone (OC) and insert on a bony plate (elastic spring ES) that touches the swimbladder (Sb). The ES is connected via a tendon to a dermal plate (P). (b) In upside-down catfish, the sonic muscle originates in the dorsal musculature and inserts on a small, round ES. (c) In sea catfish, the ES is a broad and pointed bony plate which surrounds the swimbladder dorsally and anteriorly. (d) In pimelodid catfishes, the sonic muscles originate on a process (PV) of the vertebral column (VC) and insert on the swimbladder ventrally. PS, pectoral spine. Modified from Ladich F and Fine ML (2006) Sound generating mechanisms in fishes: A unique diversity in vertebrates. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes*, vol. 1, pp. 3–43. Enfield, NH: Science Publishers.

sound pulses using two enhanced pectoral fin tendons that are stretched during the forward movement (abduction) of the fin and plucked over enhanced bases of fin rays (**Figure 7(b)**). When pectoral fins are beaten alternately, a series of double pulses called croaking sounds are emitted.

Though less well studied, sound also can be produced by other musculoskeletal adaptations. For example, the clownfish (*Amphiprion*, Pomacentridae) makes sounds when the mouth is rapidly shut by a sonic ligament, followed by the collision of teeth. Seahorses (Syngnathidae) generate clicking sounds by rubbing posterior head and neck bones against each other.

There is no clear evolutionary relationship between fish systematics and the diversity of sound-producing mechanisms. Sonic/vocal organs are either a characteristic of an entire order such as the intrinsic drumming muscles in toadfishes, or can occur in only three species in one genus within a suborder, such as tendon plucking in croaking gouramis.

Sexual Dimorphisms in Sound-Producing Muscles

Sound-producing muscles vary in development between sexes. They may be similarly developed in both sexes such as in the croaking gourami (*Trichopsis vittata*), can be much smaller in females such as in the pygmy gourami *T. pumila*, or can be completely absent in one sex such as in the females of several species of drumfishes. The descriptions of sex differences in pectoral mechanisms are few.

Sexual dimorphisms in drumming muscles are best known for the intrinsic muscles among toadfishes (**Figures 3(a)–3(c)**), as exemplified by midshipman fish that have two types of males. Territorial males, known as type I males, build nests under rocky shelters in the intertidal zone from where they produce long-duration, hum advertisement calls to attract females (**Figure 2(a)**). A second group of males (type II) either sneak or satellite spawn. Although neither

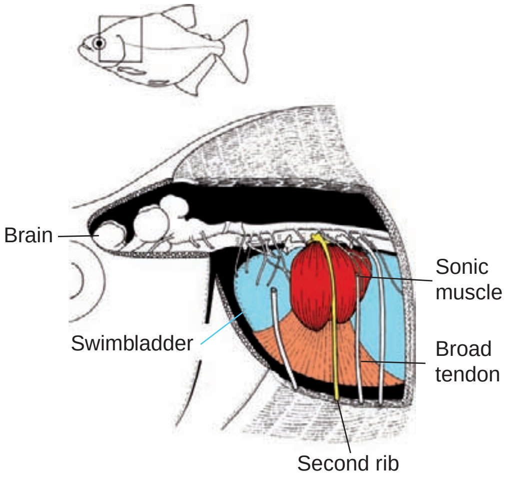


Figure 6 Line drawing of lateral view showing swimbladder region of a black piranha (*Serrasalmus rhombeus*) (insert) and the sonic/vocal muscle in more detail (lower drawing). The sonic muscles originate dorsally at the second rib and insert ventrally at a broad tendon surrounding the swimbladder ventrally. Adapted from Fig. 1 in Ladich F and Bass AH (2005) Sonic motor pathways in piranhas: With a reassessment of phylogenetic patterns of sonic mechanisms among teleosts. *Brain, Behavior and Evolution* 66: 167–176.

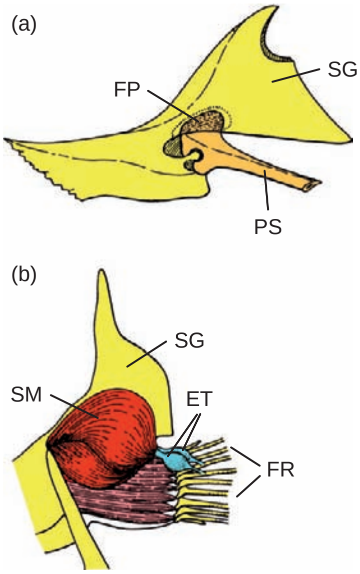


Figure 7 Line drawings of pectoral sound-generating mechanisms. (a) Stridulatory mechanism in catfishes. This lateral view of the left pectoral girdle in a pimelodid catfish shows the shoulder girdle (SG), the enhanced first fine ray (PS, pectoral spine) and the dorsal friction process (FP) with a series of ridges. (b) Pectoral tendon plucking mechanism in the croaking gourami. Contraction of the sonic muscle (SM) results in stretching of both enhanced tendons (ET) and abduction of fin rays (FR). Adapted from Ladich F (2004) Sound production and acoustic communication. In: van der Emde G, Mogdans J, and Kapoor BG (eds.) *The Senses of Fishes: Adaptations for the Reception of Natural Stimuli*, pp. 210–230. New Delhi: Narosa Publ. House.

type II males nor females generate hums, they generate grunts (which type I males also produce, **Figure 2(c)**). The sound-producing muscles of the humming, type I male are sixfold larger than those of females and type II males (e.g., **Figures 3(b)** and **3(c)**). The tubule-shaped muscle fibers have alternating bands of overlap and nonoverlap between thick and thin filaments that gives a striated appearance like other skeletal muscles (**Figures 3(d)–3(g)**). Only type I males have enlarged central and peripheral zones of sarcoplasm (muscle fiber cytoplasm) that are densely filled with mitochondria, clearly adapted to providing energy during their long bouts of humming (**Figures 3(d) and 3(e)**). Type I male muscle fibers differ in other anatomical traits that likely reflect biomechanical adaptations related to humming (see **Figures 3(f)** and **3(g)**).

Vocal Neuron Control

Fishes, like other vertebrate groups, have a vocal control system in the CNS (**Figure 9(a)**). Muscle contraction is evoked by excitation from clusters of nerve cells (motor neurons) that form a sonic or vocal motor nucleus (not to be confused with the nucleus inside of a cell). The sonic/vocal motor nuclei (VMNs) are bilaterally symmetrical; each nucleus elicits the contraction of the sonic muscle on the same side of the body.

There are three major patterns of organization for the VMN of fishes. The first pattern, like that found among all toadfishes (**Figure 9(b)**), has the VMN positioned in the posterior hindbrain and/or anterior spinal cord. A second pattern shows the VMN forming a column along the ventral border of the hindbrain and spinal cord. In contrast,

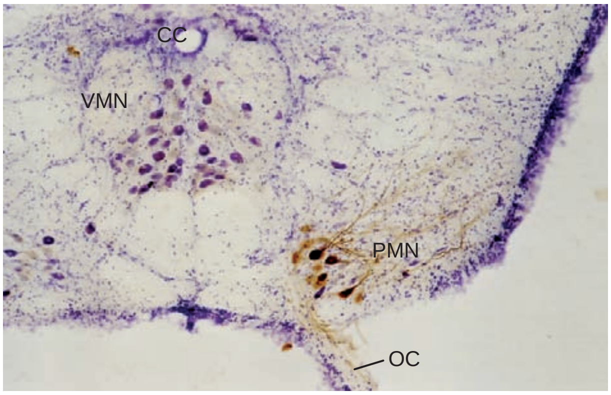


Figure 8 Photomicrograph of neurons (brown) and fibers in brainstem of the mochokid catfish, *Synodontis nigromaculatus*, showing the location of vocal motor neurons. Nonlabeled, swimbladder vocal motor neurons (VMN) are on the midline below the central canal (CC). Labeled pectoral spine motor neurons lie within the ventrolateral motor column (PMN). Axons of the PMN exit the brain via the occipital nerve (OC). From Fine ML and Ladich F (2003) Sound production, spine locking, and related adaptations. In: Arratia G, Kapoor BG, Chardon M, and Diogo R (eds) *Catfishes*, vol. 1, pp. 249–290. Enfield, NH: Science Publishers.

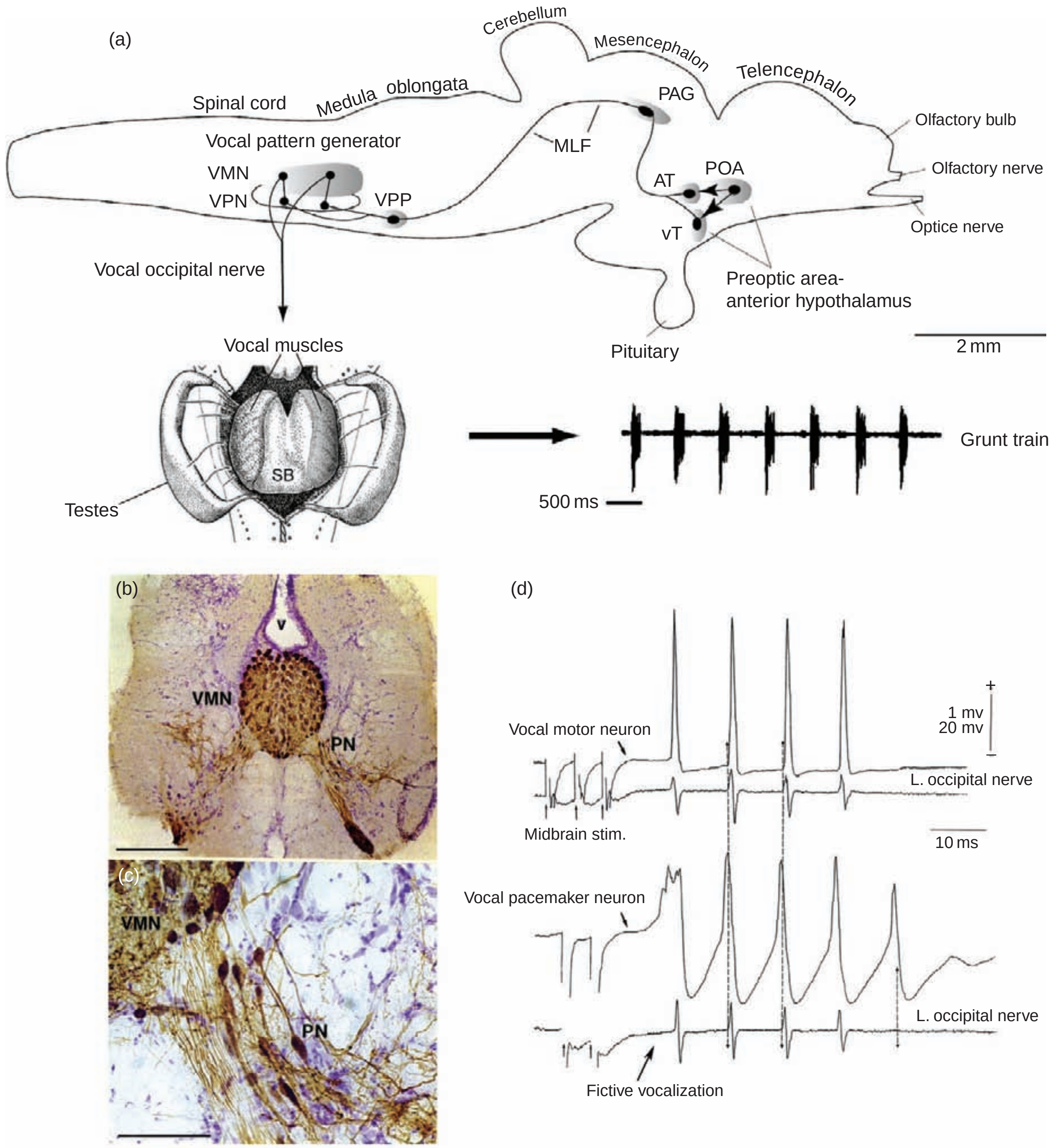


Figure 9 Vocal pattern generator in the midshipman fish, *Porichthys notatus*. (a) Side view of the brain showing vocal network. Solid dots represent the somata of neurons, and lines represent connections between neuron populations (nuclei). Two connected dots indicate reciprocal connections. (a) Nuclei in the preoptic area (POA) and anterior hypothalamus (ventral tuberal nucleus (vT); anterior tuberal nucleus (AT)) send axons to the periaqueductal gray (PAG) in the midbrain. The PAG connects to the vocal pattern generator that extends between the posterior hindbrain and anterior spinal cord, and consists of vocal prepacemaker (VPP), pacemaker (VPN), and motor (VMN) nuclei. VMN axons innervate, via occipital nerve roots, the vocal swimbladder muscles. The arrow points to a grunt train that is produced by a type I male when defending his nest (see **Figure 2**). (b, c) Morphology of the vocal pattern generator. Following application of a compound known as biocytin to the cut end of the nerve that innervates a single vocal muscle, both VMNs and the adjacent pacemaker neurons (PN) are labeled (v, fourth ventricle). Scale = 300 μm in (b)' and 75 μm in (c). (d) Vocal neuron physiology. Shown here are intracellular recordings from single vocal motor and pacemaker neurons following electrical microstimulation in the PAG (see (a)'). Each trace is the average of four. Both intracellular records from neurons (top) and intracranial records (bottom) from the left occipital nerve are shown. The nerve records are referred to as fictive vocalizations because they directly determine the fundamental frequency and duration of natural calls. The fictive call evoked here equals a single grunt. Small arrows at the beginning of the lower traces indicate the beginning of electrical microstimulation that evokes a fictive call. The highly synchronous discharges of the occipital nerve are aligned (vertical lines) with the intracellular recordings to show the similar timing in the firing of pacemaker and motor neurons. Timescale and direction of polarity for all electrical records are indicated. (a) From Forlano PM, Marchaterre MA, Deitcher DL, and Bass AH (2010) Distribution of androgen receptor mRNA expression in vocal, auditory and neuroendocrine circuits in a teleost fish. *Journal of Comparative Neurology* 518: 493–512. (b, c) Adapted from Bass AH, Horvath BJ, and Brothers, E (1996) Nonsequential developmental trajectories lead to dimorphic vocal circuitry for males with alternative reproductive tactics. *Journal of Neurobiology* 30: 493–504. (d) Adapted from Bass AH and Baker R (1990) Sexual dimorphisms in the vocal control system of a teleost fish: Morphology of physiologically identified cells. *Journal of Neurobiology* 21: 1155–1168.

catfishes, such as mochokids and pimelodids with two sound-generating mechanisms, have both a midline nucleus associated with a swimbladder mechanism and a far ventral nucleus associated with a pectoral spine mechanism (PMN, **Figure 8**). A third pattern, found in piranhas and a few other taxa, shows swimbladder muscles innervated solely by spinal motor neurons (not shown).

The vocal motor system has been best studied in toadfishes that have an expansive VMN (**Figures 9(a)** and **9(b)**). The VMN is controlled by other CNS nuclei that together form a vocal motor network. Premotor neurons, called pacemaker neurons (PNs) because they determine the firing rate of motor neurons, are adjacent to the VMN (**Figures 9(b)** and **9(c)**). PNs receive input from a prepacemaker nucleus (VPP, **Figure 9(a)**) that is turned on by other vocal nuclei (**Figure 9(a)**, see legend for abbreviations). Together, VPP and PN directly determine the duration and fundamental frequency of vocalizations.

Studies of larval midshipman and toadfish show that the vocal premotor–motor circuit develops in a segment-like region across the hindbrain–spinal transition. A phylogenetic analysis shows that this pattern is highly conserved between fish, as well as in all the other major lineages of sonic vertebrates – amphibians, reptiles, birds, and mammals (including primates). Thus, the vocal basis for acoustic communication appears to have evolved from an ancestrally shared, developmental compartment originating prior to the evolutionary origin of the group of fishes that gave rise to tetrapods.

Concluding Comments

Fish, like all other major groups of sound-producing animals, vocalize in a wide range of social contexts including territory defense and mate attraction. Fish make sound mainly by vibrating muscles attached to either the swimbladder or parts of the pectoral skeleton. Unlike terrestrial vertebrates, they do not produce sounds with a vocal organ that involves the vibration of membranes in an airflow as in the larynx of amphibians, reptiles, and mammals or the syrinx of birds. However, the muscles used for these functions, like the neural systems used to control sound frequency and duration, have similar evolutionary developmental origins among fishes and terrestrial vertebrates. Given that sound production is widespread among the more than 30 000 species of fish, the work reviewed here represents studies of a very small sample size. There remains much to learn about how and why this remarkably successful group of vertebrates makes and uses sound to reproduce and survive.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. **Hearing and Lateral Line**: Acoustic Behavior. **Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling.

Further Reading

Bass AH and Baker R (1990) Sexual dimorphisms in the vocal control system of a teleost fish: Morphology of physiologically identified cells. *Journal of Neurobiology* 21: 1155–1168.

Bass AH and Clark CW (2003) The physical acoustics of underwater sound communication. In: Simmons AM, Fay RR, and Popper AN (eds.) *Acoustic Communication*, pp. 15–64. New York: Springer.

Bass AH, Gilland E, and Baker R (2008) Evolutionary origins for social vocalization in a vertebrate hindbrain–spinal compartment. *Science* 321: 417–421.

Bass AH, Horvath BJ, and Brothers, E (1996) Nonsequential developmental trajectories lead to dimorphic vocal circuitry for males with alternative reproductive tactics. *Journal of Neurobiology* 30: 493–504.

Bass AH and Ladich F (2008) Vocal-acoustic communication: From neurons to behavior. In: Webb, JF, Fay RR, and Popper AN (eds.) *Fish Bioacoustics: Springer Handbook on Auditory Research,* vol. 32, pp. 253–278. New York: Springer.

Bass AH and Marchaterre MA (1989) Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): Sexual polymorphism in the ultrastructure of myofibrils. *Journal of Comparative Neurology* 286: 141–153.

Bass AH, Marchaterre MA, and Baker R (1994) Vocal–acoustic pathways in a teleost fish. *Journal of Neuroscience* 14: 4025–4039.

Bass AH and Remage-Healey LH (2008) Central pattern generators for social vocalization: Androgen-dependent neurophysiological mechanisms. *Hormones and Behavior* 53: 659–672.

Brantley RK and Bass AH (1994) Alternative male spawning tactics and acoustic signaling in the plainfin midshipman fish. *Porichthys notatus. Ethology* 96: 213–232.

Fine ML and Ladich F (2003) Sound production, spine locking, and related adaptations. In: Arratia G, Kapoor BG, Chardon M, and Diogo R (eds) *Catfishes,* vol. 1, pp. 249–290. Enfield, NH: Science Publishers.

Forlano PM, Marchaterre MA, Deitcher DL, and Bass AH (2010) Distribution of androgen receptor mRNA expression in vocal, auditory and neuroendocrine circuits in a teleost fish. *Journal of Comparative Neurology* 518: 493–512.

Ladich F (2001) The sound-generating (and -detecting) motor system in catfishes: Design of swimbladder muscles in doradids and pimelodids. *Anatomical Record* 263: 297–306.

Ladich F (2004) Sound production and acoustic communication. In: van der Emde G, Mogdans J, and Kapoor BG (eds.) *The Senses of Fishes: Adaptations for the Reception of Natural Stimuli*, pp. 210–230. New Delhi: Narosa Publ. House.

Ladich F and Bass AH (2005) Sonic motor pathways in piranhas: With a reassessment of phylogenetic patterns of sonic mechanisms among teleosts. *Brain, Behavior and Evolution* 66: 167–176.

Ladich F, Collin S, Moller P, and Kapoor BG (2006) *Communication in Fishes,* vol. 1, Enfield, NH: Science Publishers.

Ladich F and Fine ML (2006) Sound generating mechanisms in fishes: A unique diversity in vertebrates. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes,* vol. 1, pp. 3–43. Enfield, NH: Science Publishers.

Rice A and Bass AH (2009) Novel vocal repertoire and paired swimbladders of the three-spined toadfish (*Batrachomoeus trispinosus*): Insights into the diversity of the Batrachoididae. *Journal of Experimental Biology* 212: 1377–1391.

Zupanc GKH (1982) *Fische und ihr Verhalten*. Melle: Tetra Verlag.

Relevant Websites

http://homepage.univie.ac.at – Acoustic Communication in Fishes: The Ladich Lab.

http://www.nbb.cornell.edu – Cornell University: Department of Neurobiology and behavior.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Lateral Line Neuroethology

JC Montgomery, University of Auckland, Auckland, New Zealand

SL Coombs, Bowling Green State University, Bowling Green, OH, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Movement and the Response Characteristics of Lateral Line Submodalities
Feeding
Conclusion
Further Reading

Glossary

Cupula A gelatinous structure covering the hair cells of a neuromast.

Innervation The nerves that carry information to or from a sense organ or muscle.

Mechanosense A sense that encodes a mechanical stimulus.

Neuroethology The combined study of behavior (ethology) and its neural basis.

Neuromast The functional unit of the lateral line consisting of a patch of hair cells, supporting cells and the overlying cupula. Motion of the cupula relative to the sensory cells results in stimulation of the lateral line.

Papillate Describing neuromasts that are located on small skin protuberances.

Rheotaxis Orientation to water currents.

Introduction

In his history of lateral line studies, the great Dutch twentieth-century physiologist Sven Dijkgraaf distinguishes two senses of the phrase 'the function of a sense organ'. The first is the function of the sense organ in the life of the animal as a whole. How does it serve in the quest for food or mates? The second relates to the functional properties of the sense organ itself and its ability to detect and code specific aspects of the stimulus. Although the structural features of a sense organ provide useful insight into function, full characterization requires direct recording of the electrical responses of the sense cells and their afferent nerve fibers. These fibers transmit sensory information from the sense organs to the brain in the form of action potentials that propagate along the nerve. The frequency and temporal pattern of these events represent the output of the sense organs, the input to the brain, and the sensory code for whatever has been sensed. Although electro- or neurophysiology is essential for our understanding of how a sensory system works, Dijkgraaf rightly points out that, neurophysiology does not give conclusive evidence that any particular applied stimulus and observed response has biological relevance. A biologically relevant understanding of a novel sensory system requires a combined approach of neurophysiology and behavior (ethology), which has become the field of neuroethology (**see also Hearing and Lateral Line:** Psychoacoustics: What Fish Hear).

To illustrate these issues, consider being faced with an alien animal (**Figure 1**). The ventral surface of the body has a recognizable mouth and nostrils, but otherwise is covered with numerous pin-sized holes from which canals disappear into the body, and a series of complex subepidermal canals that can be revealed by injection with blue ink. What is the function of these structures? How could one find out?

When these structures were first discovered, they were considered to be the mucus-producing organs. It was not until their innervation was described and examined electrophysiologically, that the sensory nature of these structures was revealed. Neurophysiological characterizations alone, however, proved inconclusive, as these structures responded to many different things, including tactile, thermal, chemical, and electrical stimuli. It took detailed behavioral studies to effectively delineate the function of these structures, which turned out to be a part of not one, but two separate sensory systems: an electrosensory system that senses weak electrical currents (**see also Detection and Generation of Electric Signals:** Morphology of Electroreceptive Sensory Organs) and a mechanosensory lateral line that senses water movement. The discovery process itself, more thoroughly documented on the electrosensory side, is a beautiful illustration of how powerful combined neurophysiological and behavioral approaches can be in elucidating function.

A neuroethology approach thus provides the best insight into how an unfamiliar sensory system such as

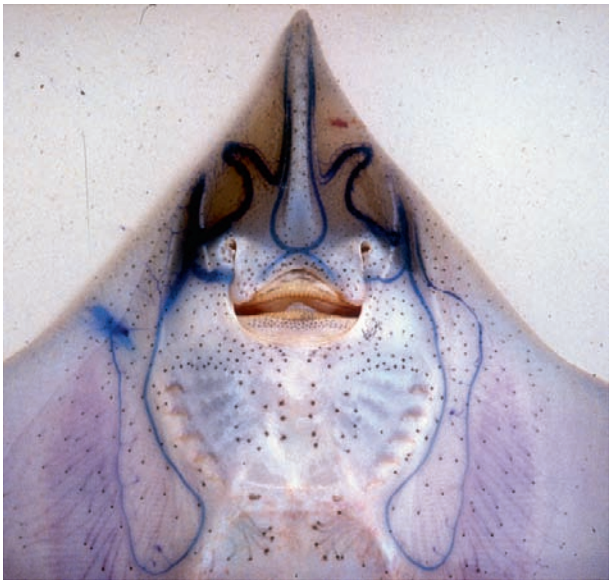


Figure 1 Ventral surface of a skate (*Raja nasuta*). Lateral line canals are injected with blue ink (injection site visible on left side of the figure). Electrosensory pores are pigmented in this species. Note the distribution of both systems around the mouth.

the lateral line provides biologically useful information, and how this information is processed by the brain to guide behavior. Neuroethologists also recognize that function is often best revealed in animals with peculiar (to humans) behavioral and sensory adaptations. For example, a lot has been learned about the neuroethology of the lateral line by studying nocturnal fish with visual limitations and other surface-dwelling species that feed on surface insects. At the same time, it is important to recognize that all fish possess a lateral line, so this sense is likely to provide important (if yet unrevealed) contributions to many aspects of fish behavior. At the most basic level, biological behavior is all about mating, feeding, and moving about safely (and cheaply) in between times. Hydrodynamic information encoded by the lateral line has been shown to play a critical role in each of these behaviors. However, we will concentrate on movement and feeding, as covered in separate sections below.

Movement and the Response Characteristics of Lateral Line Submodalities

If a fish sits facing into a slow uniform flow, or glides in still water, there is a uniform and predictable flow of water over the body. As described in **Hearing and Lateral Line:** Lateral Line Structure, there is a submodality of the lateral line (superficial neuromasts (SNs)) that is anatomically suited to the detection of these flows (**Figure 2**).

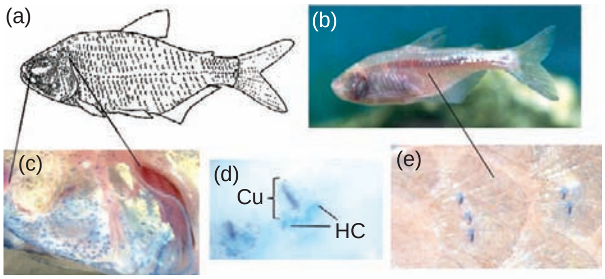


Figure 2 Distribution of SNs (small dots) and canal pores (open circles) (a) on a blind cavefish (*Astyanax fasciatus*) (b). Methylene blue-stained cupulae (Cu) and underlying hair cells (HC) in SNs on the head (c),(d) and trunk scales (e) of a blind cavefish. Line drawing in (a) from Schemmel C (1977) Zur Morphologie Und Funktion Der Sinnesorgan Von Typhilasina Pearsi (Hubbs) (Ophidioidea, Teleostei). Zoo*morphology* 87: 191–202.

From a behavioral standpoint, SNs have been shown to play a part in the orientation to slow flows. Turning and facing the current is called positive rheotaxis and is a common response in fish. It allows fish to hold station in a stream, to intercept food and olfactory information flowing downstream, and perhaps get free gill ventilation (**see also Ventilation and Animal Respiration:** Efficiency of Gas Exchange Organs). For a fish to know that it is moving in a current, it needs a stationary reference frame against which to sense the movement. This can be accomplished with either visual or tactile contact with the bottom or sides of the stream. The need for an established stationary reference frame, and the strength of visually mediated rheotaxis led to the early conclusion that in rheotaxis, the lateral line plays little part. However, later behavioral studies using selective ablation of the SNs showed that this specific submodality of the lateral line does play a useful role in rheotaxis. For a fish sitting in a slow flow, the threshold current speed at which it turns and faces upstream is much lower with an intact SN system than after ablation of this submodality. Interestingly, adding a food odor to the water even further lowers the rheotactic threshold, indicating that fish are sensing flow velocities well below that at which they turn to face upstream (**Figure 3**).

Several studies have examined the electrophysiological response of SNs to flow. In these experiments, the fish is stationary in a flume that is used to manipulate the flow around the fish. **Figure 4** shows the afferent neural response from a presumed SN of water flowing in a head-to-tail direction. The activity of the nerve increases for the duration of the increased flow providing an apparent direct measure of the flow over that part of the fish's body. The simple interpretation would be that the flow past the cupula of the neuromast (**see also Hearing and Lateral Line:** Lateral Line Structure) activates the sense (hair cells and the afferent fiber) in a nonadapting mode, giving a straightforward encoding of flow rate and direction by the firing rate of the afferent nerve. However, this interpretation is complicated by the observation that in

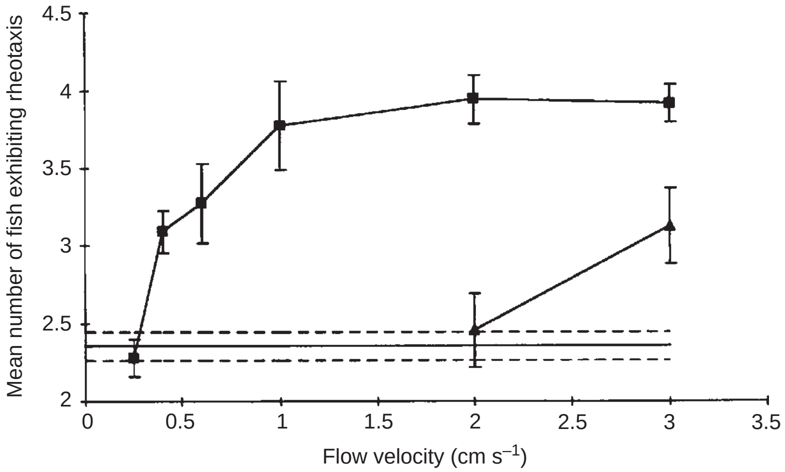


Figure 3 Mean number of cavefish exhibiting rheotaxis in the absence of an odor stimulus (triangles) and in the presence of a dilute odor stimulus (squares). The presence of the diffuse odor lowers the threshold from 2–3 to less than 0.4 cm s^{-1}. Physical ablation of the SNs raises the threshold to >8 cm s^{-1}.

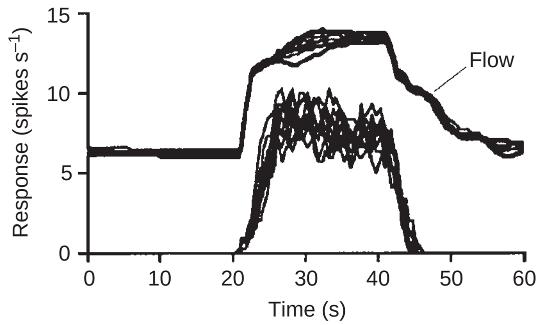


Figure 4 Response of a single anterior lateral line afferent fiber to repeated stimulation. Ten flow profiles (upper traces) are superimposed. The corresponding spike activity (lower traces) was binned (500 ms bins) and smoothed (three-point running average). The flow was increased (from 0.15 to 4 cm s^{-1}) between t = 20 s and t = 40 s. From, Voigt R, Carton AG, and Montgomery JC (2000) Responses of lateral line afferent neurones to water flow. *Journal of Experimental Biology* 203: 2495–2502.

some experiments afferent fibers can increase their firing rate during either head-to-tail, or tail-to-head flows – a finding that is inconsistent with the directional properties of hair cells. In theory, hair cells within each lateral line neuromast should respond best to one direction of flow and be inhibited to the opposite flow direction. One interpretation of this observation is that afferents are not encoding flow direction but only flow amplitude, and that the increased firing rate is not due to the direct flow *per se*, but due to the microturbulance induced over the surface of the fish. These two encoding mechanisms are not mutually exclusive and may operate at different flow rates, or differently in fish with different surface characteristics. The complexities of surface boundary layer flow in nature and in experimental situations, the precise response characteristics of cupula and hair cells, and the connectivity of afferents to hair cell populations are beyond the scope of this article. However, the important point is that the precise nature of sensory encoding by the lateral line can be problematic – even for very simple stimuli – in this case, a stationary fish in a controlled, unidirectional flow.

Obviously, the situation increases in complexity for a fish holding station in a turbulent flow, or a fish gliding or swimming actively. There is behavioral evidence that both SNs and canal neuromasts (CNs) provide information in turbulent flows that allow fish to modify their swimming to maintain station behind objects in a flow, and to enhance swimming efficiency in the presence of turbulence. There is also good evidence that gliding blind cavefish (*Astyanax fasciatus*) use their own self-generated flow to detect stationary features in their environment – an ability called active hydrodynamic imaging. However, electrophysiology in free moving animals becomes a difficult endeavor and there is little direct physiological evidence for how information from SNs and CNs is combined and used in these behavioral contexts. Functional understanding of the role of SNs and CNs is still largely based on the structural and physiological differences described below.

As shown in **Figure 2**, SNs are located on the surface of the skin of the fish, whereas CNs are located within subdermal canals (**see also Hearing and Lateral Line:** Lateral Line Structure). The effect of this is to remove the CNs from the direct influence of surface currents. Instead, they respond to the pressure gradient between the canal pores, responding to accelerating flows rather than constant-velocity flows. This protects the CNs from mechanical damage and also from being over-stimulated by ambient water currents, or by the fishes own motion. This effect is shown in **Figure 5**, where the electrophysiological responses of SNs and CNs are shown as a

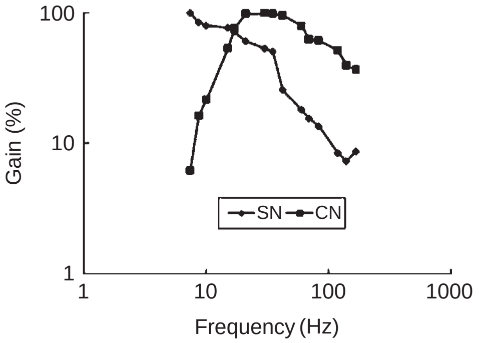


Figure 5 Frequency response characteristics of the superficial neuromasts (SNs) and canal neuromasts (CNs) in the eel (*Anguilla dieffenbachii*). Gain (%) is a relative measure of the response strength. These responses are for a vibrating bead stimulus where the amplitude of the vibration is adjusted at different frequencies to maintain a constant maximal stimulus velocity.

function of frequency. These stimulus-response curves are one of the most important ways of representing sensory function. The representation is borrowed from the field of engineering and is based on the notion that for a linear system, knowledge of how it responds to a range of frequencies allows effective characterization. In other words, you can then predict how it will respond to any arbitrary stimulus wave form. These curves are also called filter functions, showing which frequencies are being passed by the filter and which are being rejected. Using this later description, we can say that with respect to stimulus velocity, the SNs act as low-pass filters, letting low but not high frequencies through, by not responding to higher frequencies. By comparison, canals' neuromasts are band-pass filters, only responding to specific band of frequencies within the 10–80-Hz range.

The above allows further general points to be made about lateral line function, division of labor between submodalities, issues of signal and noise, and comparisons with other sensory systems. First, the filter curves effectively show the frequencies of interest where the gain is highest and sensitivity is best. SNs are interested in low frequencies associated with slow movements, whereas CNs are interested in rapidly changing movements associated with higher frequencies in the tens of hertz range. Filters are typically used to remove unwanted noise that differs in frequency from the signals of interest. Hence, CNs filter out low-frequency noise which leaves them sensitive to higher frequencies of interest; as described in the next section these higher-frequency sources are typically other animals. The ability to filter out steady flow and lower frequencies allows the CNs to operate even when the fish is exposed to ambient currents or is itself moving. The best direct physiological demonstration of this effect is the work showing lateral line afferent fibers responding to a vibrating bead. In stationary water, all fibers show a similar response. But when a background flow is added, one can quickly recognize two classes of fibers: those that become saturated, or over stimulated by the flow and cease to respond to the vibrating stimulus, and those that are unaffected by the flow and continue to encode the vibration. It is reasonable to equate these two classes of response to SNs and CNs, respectively.

Another general point is that frequency-response curves have been obtained for lateral line afferents from a wide variety of fish. It would be reasonable to hypothesize that the details of the curves for CNs would vary, according to the nature of the noise fish typically encountered, and according to the biologically relevant signals of interest. Although detailed characteristics of filter curves do vary across species, data on environmental flow noise and relevant signal characteristics are unavailable for comparison with filter functions to adequately test this hypothesis. What is clear is that within a species, the canal afferents are relatively similar in their response characteristics and not sharply tuned to particular frequencies. In this respect, the lateral line is quite different from the auditory systems that have a range of sharply tuned afferents whose best frequencies vary systematically across the range of frequencies encoded.

There is another important way in which signal and noise can be distinguished in lateral line systems. The swimming and breathing movements that fish make, and associated water flows, provide a strong source of stimulation to the lateral line, which, in most contexts, will be unwanted noise. Given that this is a self-generated noise, there are a number of behavioral and physiological strategies that can be used in mitigation.

The simplest is to cease active movement (as we do when we are listening intently). Some fish hunt using a pause/move behavior known as a salutatory search where prey is detected during the pause phase of the search. As a slightly different example, blind cavefish swim using a kick/glide motion. They sense obstacles using hydrodynamic imaging during the passive glide phase, when the flow field is more uniformly stable and less disturbed by the fish's own irregular, kicking (tail beat) motions.

A second strategy is to turn the lateral line off during active movement. The lateral line end organs have efferent innervation from the brain which can reduce the sensitivity of the lateral line during swimming. Efferent inhibition of lateral line sensitivity may also, in part, explain why blind cavefish typically collide with walls during the active kick (tail beat), but not during the passive glide phase of their swimming motion. Finally, the hindbrain processing center that receives the lateral line input and passes it on up to higher brain centers acts as a sophisticated adaptive filter which can cancel lateral line input that is generated by the animal's own movement.

Feeding

Having considered the physiological characteristics of the lateral line in the functional context of movement, it is now appropriate to add the insights into lateral line neuroethology that arise within the context of feeding. There is evidence that the lateral line contributes to the ability of fish to find the source of an olfactory stimulus being dispersed in a current, but the main consideration here is detection and localization of small moving prey. Lateral-line-mediated prey detection and capture has been studied in a wide range of species. The relevant stimulus and the way in which lateral line information guides behavior depends on whether prey is submerged or on the water surface and whether or not fish detect the current-generating movements of the prey as they occur or whether they detect the history of their movements in a trailing wake. In the case of fish feeding on relatively small, submerged prey, such as crustaceans, the stimulus is the fluctuating current driven by ventilation or swimming movements of appendages. Following prey detection, the approach is typically made in a number of steps until the fish is in the position to take a well-aimed strike at the target. The most likely contribution of the lateral line to this behavior is information about the precise location of nearby targets, both with respect to direction and distance to inform the approach and strike. In some fish the lateral line is essential for the initial detection and final strike behavior, both of which occur at fairly close range (less than 1 body length of the predator). In other, more diurnally active species vision plays a primary role in the initial detection and approach, whereas the lateral line plays a more important role in the final strike at close range.

Information about target location (azimuth, elevation, and distance) is contained in the instantaneous spatial pattern of activity across arrayed lateral line sensors. This spatial pattern is, in turn, converted into a population code or pattern of neural activity across the afferent fibers that innervate the sensors (**Figure 6**). Exactly how this population code is processed by the brain to guide behavior is not well understood, but it is likely integrated with information from other senses in a highly conserved brain region that controls orienting movements in vertebrates. For prey that move very slowly and/or remain relatively fixed in position for sufficient periods of time, snap shots of spatial patterns of activation along the lateral line may be sufficient for target localization by the lateral line. However, for prey that rapidly changes its location, the combined spatio-temporal patterns of activity across the arrayed sensors will be important. For example, some fish are able to intercept the fast-moving crustacean prey provided they are moving along, rather than across, the lateral line receptor array. Benthic stargazers (*Leptoscopus macropygus* and *Genyagnus monopterygius*) effectively catch their fish prey in darkness, but only after the prey crosses the lateral line receptor array around the mouth to trigger a strike. In both of these cases, prey-generated currents propagate at the speed of sound; thus, determinations about prey location from spatial patterns of activation can, in theory, be made instantaneously from spatio-temporal patterns as the prey rapidly changes location.

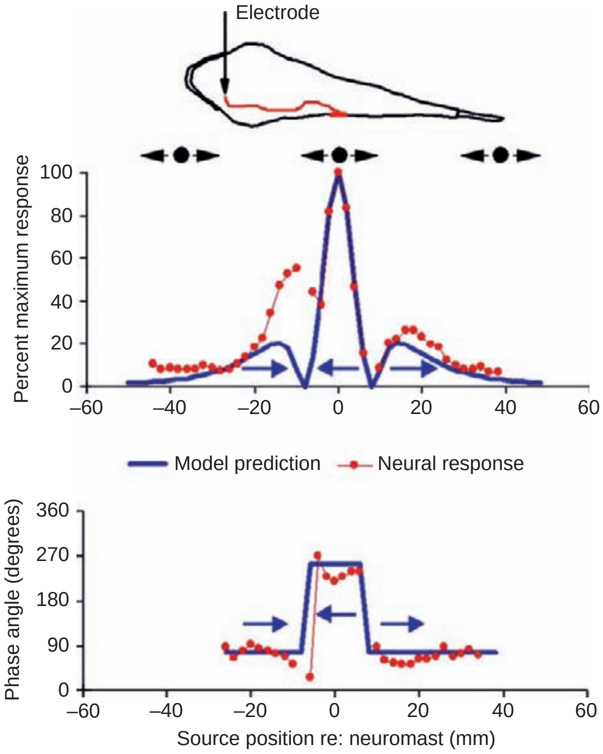


Figure 6 Neural responses to a dipole source compared with modeled predictions. The spike activity of a lateral line nerve fiber innervating a single CN on the trunk canal of a mottled sculpin (*Cottus bairdi*), was measured in response to the slowly changing locations of a 50-Hz vibrating sphere. As the source changed location, the spike activity (thin line with symbols) follows the predicted changes in pressure gradient (thick line). The phase angle, or firing time of the fiber, also follows the modeled predictions, as it shifts by a 180° for every change in pressure-gradient (flow) direction (see arrows). While this figure depicts the response of a single fiber from a single sensor midway along the trunk canal, it can also be used to illustrate the instantaneous spatial pattern of activity that would exist across multiple fibers for a single source position centered on the array.

Nocturnal catfish (*Silurus glanis*), on the other hand, track down their submerged prey in a substantially different way – by intercepting, and then following, the hydrodynamic trail of a swimming prey some time after (up to several minutes) the swimming movements have occurred. In this case, the water disturbances in the trailing wake travel at the speed of bulk water flow, which varies with the swimming speed of the prey. Recent physiological and modeling experiments have shown that the lateral line is capable of encoding information about both the spinning direction and rate at which vortices are shed in the trailing wake of a moving prey or around a submerged obstacle in a stream (**Figure 7**). In principle, fish could use this information to tell them something about the general direction, distance, and perhaps even the identity of the wake source.

Finally, a number of fish and amphibians specialize in the detection of prey that have fallen onto, or are struggling on, the water surface. In this case, the relevant stimulus is a surface wave. The spatial array of receptors that these specialists deploy adjacent to the water surface allows them to estimate direction to the target based on the orientation of the wave front passing over the receptors. Target distance is estimated by the lateral line afferents being able to encode the features of the surface wave that change as the wave propagates from the source. These include the frequencies that are present and how they disburse in time. This is because with surface waves, high frequencies travel faster, but attenuate more quickly with distance. Similar cues (e.g., changes in the spectrum) in slowly propagating hydrodynamic wakes may also inform nocturnal catfish about the distance to submerged wake sources.

Conclusion

It is not possible here to do justice to the wide literature on function and physiology of the lateral line. The neuroethological approach has been emphasized where physiological studies can be rather directly related to biologically relevant behavior in the contexts of movement and feeding. From what was a relatively unknown sensory system in the middle of last century, there is now a good sense of what the lateral line does and how. Dijkgraaf's original characterization of the lateral line as touch at distance remains remarkably apt. The lateral line is a distributed sensory system like touch and through intervening water movement it can locate objects in space, whether they are stationary and inanimate, or moving and alive. Biomechanical filter properties of lateral line structures, central control through the efferent system, and adaptive gain control in the brain enable fish to respond to the biologically important hydrodynamic signals and to distinguish them from self-generated and environmental noise. In addition, disturbances caused by underwater landscapes in self-generated and ambient flow noise may provide fish with lateral line information on the hydrodynamic scene, similar to what has been proposed for acoustic scene analysis. Significant issues remain, both within sensory encoding and within central processing of lateral line information. The practical

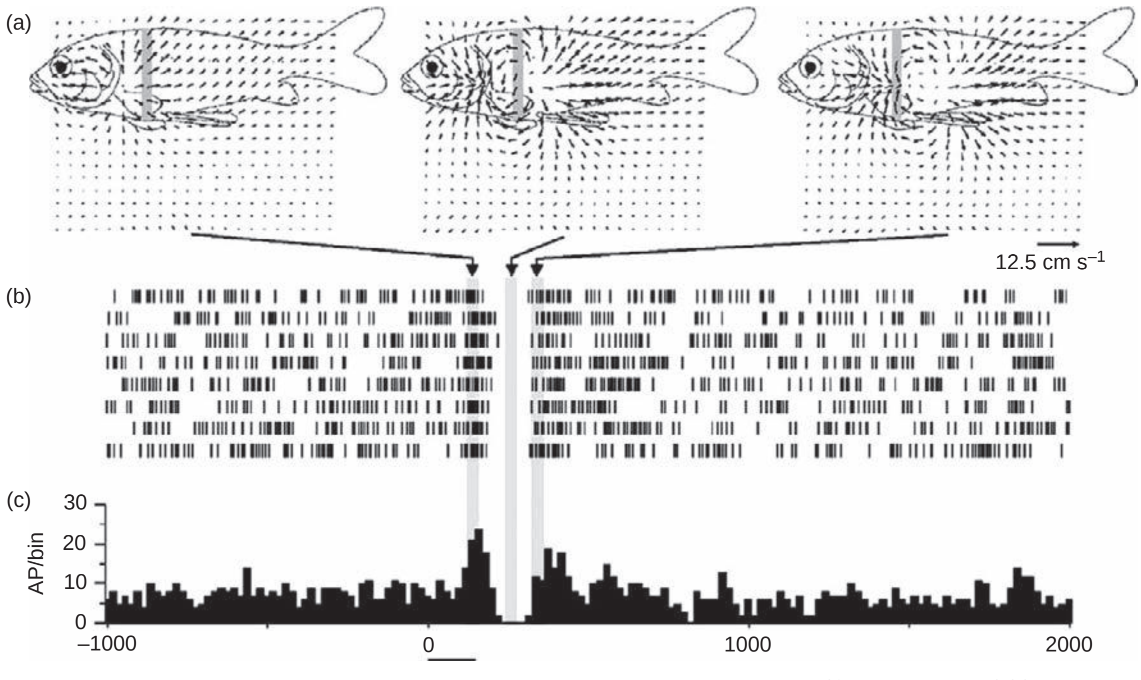


Figure 7 Particle motions associated with a vortex traveling along the surface of a goldfish (*Carassius auratus*) (a) and the corresponding responses of a lateral line nerve fiber (b, c) innervating a CN just behind the operculum of the fish. In (b), each mark represents a single action potential and each row of marks, the time series of responses to a single sweep of the vortex ring down the flank of the fish. In (c), the responses to repeated stimulus sweeps are combined into a peri-stimulus time histogram that shows the accumulated number of action potentials (AP) per each 20 ms bin during the repeated sweeps. Reproduced from Chagnaud BP, Bleckmann H, and Engelmann J (2006) Neural responses of goldfish lateral line afferents to vortex motion. *Journal of Experimental Biology* 209: 327–342, with permission from The Company of Biologists.

difficulties of keeping electrophysiology tightly aligned with an appropriate ethological context are not peculiar to lateral line studies. There is also still much to learn from a comparative perspective. Some 30 000 different species of fish, living in different freshwater and marine habitats, encounter abiotic and biotic flows that range from very orderly and predictable to highly turbulent and chaotic. Thus, the basic superficial/canal dichotomy described here is an incomplete picture of the myriad of other lateral line adaptations that have evolved in different environments – for example, the elaborate membranous canal systems and papillate SNs seen in deep-sea fishes.

Studies of lateral line function and physiology have enjoyed a resurgence in recent years – in part, because new technologies in flow visualization and computational fluid dynamics have found their way into biological studies. In addition, engineers involved in flow and flow sensing have started to recognize the quite remarkable abilities of the fish lateral line, and the potential that these have for advancing our own technologies in this area.

See also: **Blood:** Cellular Composition of the Blood. **Detection and Generation of Electric Signals:** Morphology of Electroreceptive Sensory Organs. **Hearing and Lateral Line:** Lateral Line Structure; Psychoacoustics: What Fish Hear.

Further Reading

Bassett D, Carton AG, and Montgomery JC (2007) Saltatory search in a lateral line predator. *Journal of Fish Biology* 70: 1148–1160.

Bleckmann H (1997) Reception of hydrodynamic stimuli in aquatic and semiaquatic animals. In: Rathmayer W (ed.) *Progress in Zoology,* vol. 6, pp. 1–115. Stuttgart, Jena, New York: Gustav Fischer Verlag.

Bleckmann H (2008) Peripheral and central processing of lateral line information. *Journal of Comparative Physiology A* 194: 145–158.

Chagnaud BP, Bleckmann H, and Engelmann J (2006) Neural responses of goldfish lateral line afferents to vortex motion. *Journal of Experimental Biology* 209: 327–342.

Chagnaud BP, Bleckmann H, and Hofmann MH (2007) Karman vortex street detection by the lateral line. *Journal of Comparative Physiology A* 193: 753–763.

Coombs S, Braun CB, and Donovan B (2001) The orienting response of lake Michigan mottled sculpin is mediated by canal neuromasts. *Journal of Experimental Biology* 204: 337–348.

Coombs S, Hastings M, and Finneran J (1996) Modeling and measuring lateral line excitation patterns to changing dipole source locations. *Journal of Comparative Physiology A* 178: 359–371.

Coombs S and Montgomery JC (1999) The enigmatic lateral line system. In: Popper AN and Fay RR (eds.) *Comparative Hearing: Fishes and Amphibians.* Springer Handbook of Auditory Research vol. 11, pp. 319–362. New York: Springer.

Dijkgraaf S (1963) The functioning and significance of the lateral-line organs. *Biological Reviews* 38: 51–105.

Dijkgraaf S (1989) A short personal review of the history of lateral line research. In: Coombs S, Gorner G, and Münz H (eds.) *The Mechanosensory Lateral Line*: *Neurobiology and Evolution*, pp. 7–16. Berlin: Springer.

Engelmann J, Hanke W, Mogdans J, and Bleckmann H (2000) Hydrodynamic stimuli and the fish lateral line. *Nature* 408: 51–52.

Liao JC (2007) A review of fish swimming mechanics and behaviour in altered flows. *Proceedings of the Royal Society of London, Series B Biological Sciences* 362: 1973–1993.

Marshall NJ (1996) Vision and sensory physiology – the lateral line systems of three deep-sea fish. *Journal of Fish Biology* 49(supplement A): 239–258.

Montgomery JC, Baker CF, and Carton AG (1997) The lateral line can mediate rheotaxis in fish. *Nature* 389: 960–963.

Montgomery JC and Bodznick D (1994) An adaptive filter cancels self-induced noise in the electrosensory and lateral line mechanosensory systems of fish. *Neuroscience Letters* 174: 145–148.

Montgomery JC, Coomb S, and Halstead M (1995) Biology of the mechanosensory lateral line in fishes. *Reviews in Fish Biology and Fisheries* 5: 399–416.

Montgomery JC, Mcdonald F, Baker CF, Carton AG, and Ling N (2003) Sensory integration in the hydrodynamic world of rainbow trout. *Proceedings of the Royal Society of London, Series B Biological Sciences* 270(supplement 2): 195–197.

Pohlmann K, Atema J, and Breithaupt T (2004) The importance of the lateral line in nocturnal predation of piscivorous catfish. *Journal of Experimental Biology* 207: 2971–2978.

Windsor SP, Tan D, and Montgomery JC (2008) Swimming kinematics and hydrodynamic imaging in the blind Mexican cave fish (*Astyanax fasciatus*). *Journal of Experimental Biology* 211: 2950–2959.

Yang Y, Chen J, Engel J, *et al.* (2006) Distant touch hydrodynamic imaging with an artificial lateral line *Proceedings of the National Academy of Sciences of the United States of America* 103: 18891–18895.

Relevant Websites

http://mugwump.pitzer.edu – Fixing Content and Function in Neurobiological Systems: The Neuroethology of Electroreception.

http://www.neuroethology.org – International Society for Neuroethology.

Lateral Line Structure

JF Webb, University of Rhode Island, Kingston, RI, USA

© 2011 Elsevier Inc. All rights reserved.

What Is the Mechanosensory Lateral Line System?
Structure and Function of Neuromast Receptor Organs in Bony Fishes
Structural Variation in the Lateral Line System among Bony Fishes
Development of the Lateral Line System in Bony Fishes
The Lateral Line System of Chondrichthyan Fishes
The Lateral Line System of Jawless Fishes
Further Reading

Glossary

Dermal bone Bone that ossifies in contact with the dermis.

Embryo The developing fish from fertilization through hatch.

Hair cell The major sensory cell in the ear and lateral line of all vertebrates.

Kinocilium Cilium composed of 9 + 2 arrangement of microtubules, which is found in sensory hair cells.

Larva The developing fish from hatch through transformation to the juvenile stage.

Mechanosensory The ability of cells and organisms to recognize and measure mechanical forces.

Neuromast The functional unit of the lateral line consisting of a patch of hair cells, supporting cells and the overlying cupula. Motion of the cupula relative to the sensory cells results in stimulation of the lateral line.

Placode A plate-like thickened area of embryonic ectoderm which gives rise to sensory organs.

Sensory ganglion A collection of the cell bodies of the bipolar sensory neurons that compose the cranial nerves; they sit outside the brain.

Standard length (SL) From the tip of the snout to the base of the fin rays of the caudal fin.

Stereocilia (stereovilli) Microscopic, finger-like extensions of the cell membrane composed of actin and found in sensory hair cells.

What Is the Mechanosensory Lateral Line System?

The mechanosensory lateral line system of fishes detects unidirectional and oscillatory water flows. It is found in all 30 000 + species of living fishes: the ‘Agnatha’ (the jawless fishes, the hagfishes and lampreys), the Chondrichthyes (sharks, skates, rays, and chimaeras), and the Osteichthyes (the bony fishes). It is composed of a spatial array of water flow detectors (neuromasts receptor organs) located on the head, trunk, and tail, and plays critical roles in a variety of behavioral contexts, including prey detection, predator avoidance, communication, and navigation. The lateral line systems of the Chondrichthyes and Osteichthyes are particularly well developed, with superficial neuromasts on the skin and canal neuromasts in a series of lateral line canals on the head and trunk. The lateral line system of the lampreys is composed exclusively of superficial neuromasts located in grooves on the skin of the head and trunk. The lateral line system is only present in some hagfishes, where it is represented by the presence of ciliated sensory cells located in a small number of grooves on the head.

Structure and Function of Neuromast Receptor Organs in Bony Fishes

Neuromasts are small epithelial receptor organs (~10–500 μm in length) that are composed of hundreds or, in some case, thousands of sensory hair cells, in addition to nonsensory support cells (the stem cells from which hair cells differentiate), and mantle cells (**Figure 1**). Each sensory hair cell has a ciliary bundle extending from the cell surface that is composed of a single long kinocilium and many shorter stereocilia that sit to one side of the kinocilium. The length of the kinocilium and stereocilia varies among neuromasts and may have functional implications. The location of the stereocilia relative to the kinocilium establishes the physiological polarity of a hair cell (**Figures 1–3**). All of the hair cells within a neuromast are oriented 180° to one another, thus defining the neuromast’s axis of best physiological sensitivity to water flows (**Figure 3**).

The ciliary bundles of all hair cells in a neuromast are covered by a gelatinous cupula that serves as the biomechanical interface between the hair cells and water in the

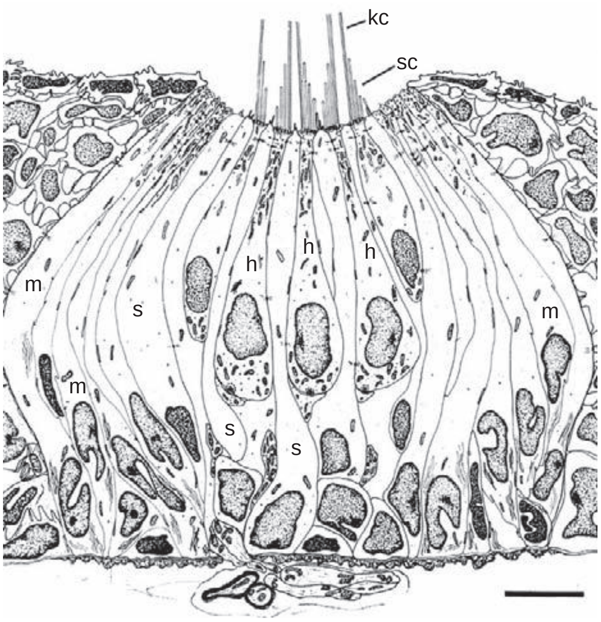


Figure 1 Schematic drawing of a superficial neuromast in Nile tilapia, *Sarotherodon niloticus* (m = mantle cells; s = supporting cells, h = hair cells). Scale = 10 μm. Reproduced from Munz H (1979) Morphology and innervation of the lateral line system in *Sarotherodon niloticus* (L.) (Cichlidae, Teleostei). *Zoomorphology* 93: 73–86, with kind permission of Springer Science+Business Media.

surrounding environment (**Figure 3**) . When water flows past a neuromast, it bends the cupula, and the ciliary bundles contained within it (**Figure 4**). This generates a stimulus (**see also Hearing and Lateral Line**: Lateral Line Neuroethology) that is transmitted to the brain via a lateral line nerve composed of the sensory neurons that innervate the hair cells.

There are two classes of neuromasts in bony fishes (**Figures 4** and **5**) – canal neuromasts in pored, fluid-filled canals, and superficial neuromasts on the skin (sometimes referred to as pit organs). Canal and superficial neuromasts demonstrate a considerable amount of interspecific variation in size and shape (**Figure 6**). Hair cells may be distributed over the entire surface of the neuromast (e.g., canal neuromasts of the zebrafish, *Danio rerio*, **Figure 6**(**b**)), but are more commonly found in a circular or oval area in the center of the neuromast, called the 'sensory strip' (**Figures 3** and **6**). Neuromast morphology is correlated with the morphology of the lateral line canals in which they are found (see below). The hair cell orientation in canal neuromasts is parallel with the long axis of the canal so that water flows in the canal have the potential to stimulate the neuromasts. Superficial neuromasts, which sit on the skin, are generally smaller than canal neuromasts and are often oriented with their axis of best physiological sensitivity parallel or perpendicular to the body axis. Some superficial neuromasts are located in lines or groups in the vicinity of canals (accessory neuromasts, **Figure 5**) and are generally oriented parallel or perpendicular to that of the neuromasts within the canals.

The hair cells that compose the neuromasts are innervated by both sensory afferent neurons (which compose the sensory lateral line nerves) and efferent neurons that form a synapse with each of the hair cells. In the Chondrichthyes and Osteichthyes, several lateral line

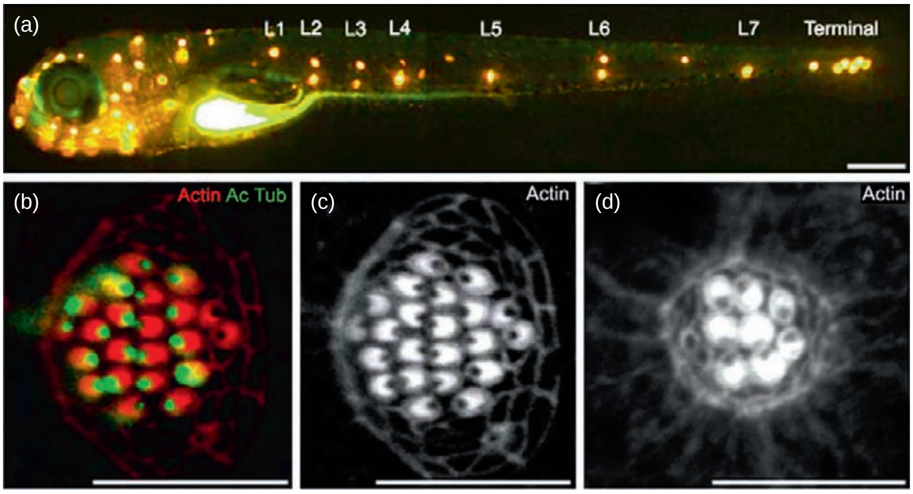


Figure 2 Neuromast distribution and hair cell orientation in zebrafish (*Danio rerio*) larva (6 days postfertilization) as revealed by fluorescent staining. (a) Lateral view showing seven trunk neuromasts (L 1–7) of both sides of fish, and terminal neuromasts. Scale = 100 μm, (b) rostro-caudal axis of hair cell ciliary bundle polarity (neuromast L1) shown with double labeling by rhodamine–phalloidin (stereocilia), and anti-acetylated tubulin (kinocilia). (c) Labeling of rostro-caudally oriented neuromast with rhodamine–phalloidin only, dark spots are locations of kinocilia. (d) Similar labeling of dorso-ventrally oriented neuromast L2. Scale (b)–(d) = 10 μm. Reproduced from Lopez-Shier H, Starr CJ, Kappler FA, Kollmar R, and Hudspeth AJ (2004) Directional cell migration establishes the axes of planar polarity in the posterior lateral-line organ of the zebrafish. *Developmental Cell* 7: 401–412, with permission from Elsevier.

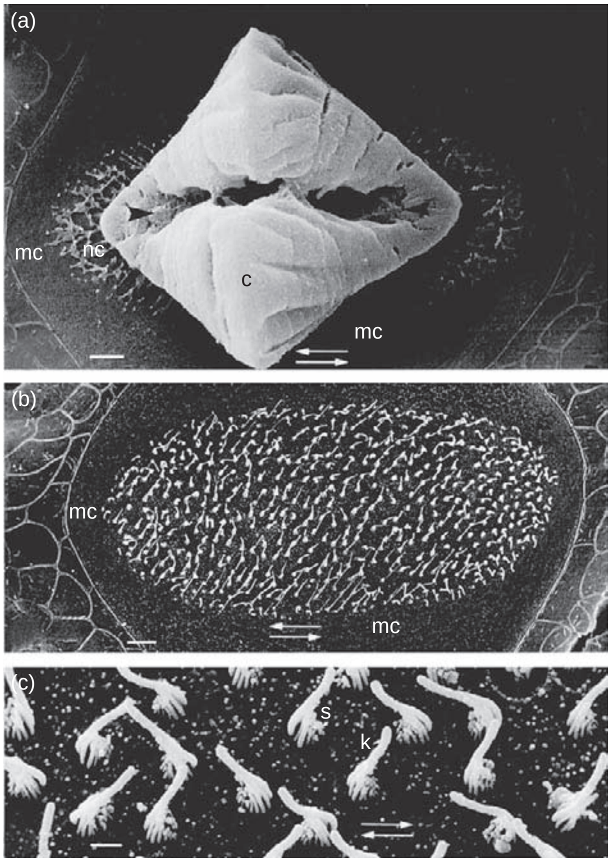


Figure 3 Neuromast of the yellow striped cardinalfish, *Apogon cyanosoma*. (a) Cupula (cu) partially covers the neuromast; mantle cells (mc) and hair cells (hc) are evident; (b) close-up of the surface of the neuromast showing the hair cell population and mantle cells (mc) that define the outer perimeter of the neuromast; and (c) close-up of hair cells showing the kinocilium (k) and stereocilia (s) and their axis of polarization (arrows). Reproduced from Rouse GW and Pickles JO (1991) Ultrastructure of free neuromasts of *Bathygobius fuscus* (Gobiidae) and canal neuromasts of *Apogon cyanosoma* (Apogonidae). *Journal of Morphology* 209: 111–120, with permission of J. Wiley and Sons.

nerves, each defined by a discrete sensory ganglion, innervate neuromasts on the head and trunk. These are distinct from the ganglia of the cranial nerves of vertebrates that are traditionally denoted by roman numerals (e.g., V – trigeminal, VII – facial, IX – glossopharygeal, and X – vagus (**see also Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern and **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy). The anterior lateral line nerve innervates canal and superficial neuromasts above and below the eye. The middle lateral line nerve is composed of a small number of fibers and innervates a small number of neuromasts at the posterior margin of the head. The posterior lateral line nerve innervates lines of canal and/or superficial neuromasts, including the trunk canal, which may extend the length of the trunk. The lateral line nerves project to one or more lateral line centers in the medulla oblongata, which are distinct from the auditory and electroreceptive centers of the hindbrain (**see also Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern and **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy).

Structural Variation in the Lateral Line System among Bony Fishes

In bony fishes, the lateral line canals are contained in a remarkably consistent subset of dermal cranial bones (the lateral line bones, **Figure 7**; **see also The Skeleton**: Bony Fish Skeleton), regardless of the shape of the head, or the size or shape of the individual bones. Neuromasts are found on the floor of the canals, in which one neuromast is found between the adjacent canal pores (with the exception of one group of lungfishes).

Variation in the morphology of the cranial lateral line canals among bony fishes is defined by four canal patterns (**Figure 8**). The narrow canal pattern (**Figure 8(a)**) is the most common and is characterized by well-ossified canals with small pores, which may be extended through soft tissues to the skin surface as tubules. The narrow-branched pattern (**Figure 8(d)**) is similar to the narrow pattern, with the exception that the canal pores extend into the soft tissue overlying the bones as highly branched tubules and end in a large number of terminal pores. The reduced canal pattern (**Figure 8(b)**) occurs when some or all canals do not develop and enclose neuromasts leaving superficial neuromasts on the skin. These replacement neuromasts are often proliferated, occurring in clusters or dense lines. The widened canal pattern (**Figure 8(c)**) evolved convergently in only a dozen or so families of teleost fishes, including those that live on or near the benthos and those that live in the water column at great depths (e.g., mesopelagic and bathypelagic fishes). Widened canals are greater in diameter than narrow canals, and, as a result, may cover much of the head, but they have incompletely ossified canals that leave large openings in the canal roof. Their very large canal neuromasts (often an order of magnitude larger than those in narrow canals in fish of the same size) are often diamond shaped (**Figure 6**) and may sit in constrictions in the canals under a bony bridge, which is the remnant of the ossified canal roof. The epithelium that covers the canals is generally pierced by small pores and is thought to be deformed by water flows that cause fluid to move in the canal, thus stimulating the canal neuromasts.

The lateral line canals on the trunk demonstrate considerable diversity among species (**Figure 9**). One lateral line canal is generally found on the trunk of bony

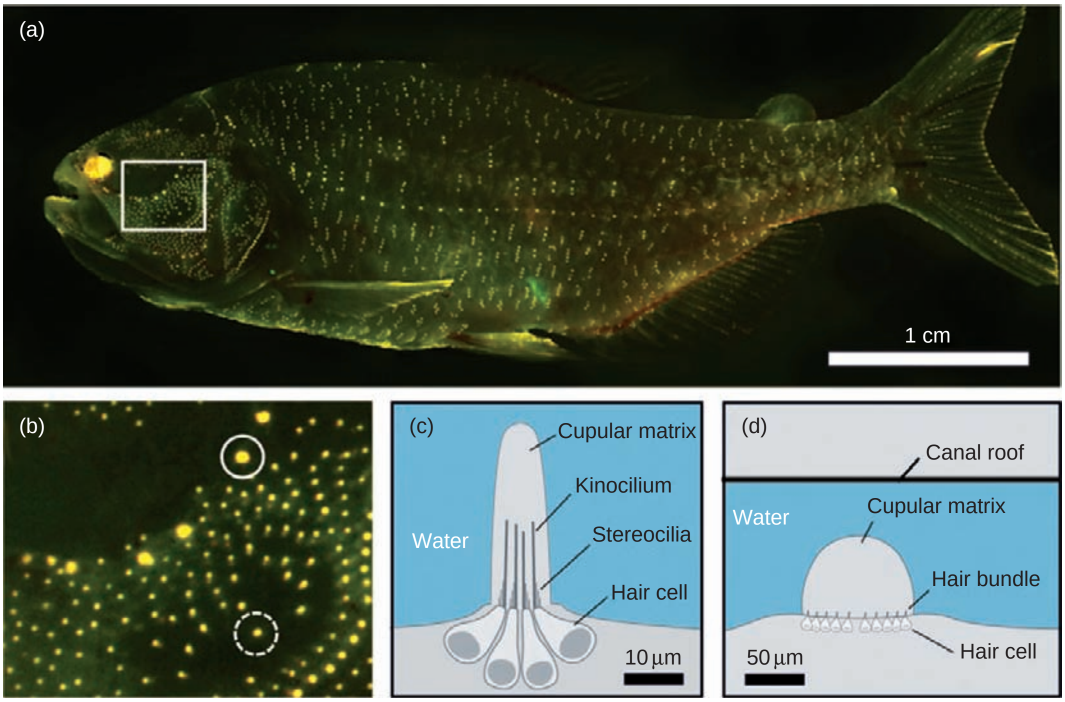


Figure 4 Distribution and structure of canal and superficial neuromasts. (a) Neuromasts marked with fluorescent stain (DASPEI, 2-(4-(dimethylamino)styryl)-*N*-ethylpyridinium iodide) in the Mexican blind cavefish (*Asynanax mexicanus*). (b) Magnified view of white box in (a), showing different sizes of canal (solid circle) and superficial (dashed circle) neuromasts; (c) schematic of superficial neuromast; and (d) schematic of canal neuromast, with each ciliary bundle appearing as a single line. Reproduced from Windsor SP and McHenry MJ (2009) The influence of viscous hydrodynamics on the fish lateral-line system. *Integrative and Comparative Biology* 49: 691–701, with permission of Oxford University Press.

fishes, but multiple canals do occur (e.g., in greenlings, pricklebacks, and some cichlids). The trunk canal is composed of a series of overlapping lateral line scales, each of which contains a canal segment in which one canal neuromast is found (**Figure 10**). The single lateral line canal may run straight down the body to the base of or onto the caudal fin, or in more evolutionarily derived fishes, it may arch above the pectoral fin before returning to the mid-flank (over the horizontal septum) and proceeding to the caudal fin. In some benthic fishes, the canal is placed high on the body (e.g., stargazers), and in fishes that live just under the water surface, it is placed low on the body (e.g., flying fishes). In other fishes, the canal may be incomplete (e.g., damselfishes), or divided into two portions (a disjunct pattern, e.g., cichlids), or a trunk canal may be absent (e.g., herrings and relatives), but superficial neuromasts are widely distributed on the body.

The spectrum of variation in lateral line morphology among bony fishes is fairly well understood, but the functional significance of the variation in lateral line morphology has been well established in only a few cases (e.g., functional differences between canal and superficial neuromasts, and between narrow and widened canals on the head; **see also Hearing and Lateral Line**: Lateral Line Neuroethology). The lateral line system is linked to the swimbladder and/or the inner ear in a limited number of fish groups (e.g., herrings and relatives, some catfishes, and some butterfly fishes), which is likely to broaden the functional roles of the lateral line system.

Development of the Lateral Line System in Bony Fishes

Both the neuromasts and the sensory neurons that innervate the hair cells are derived from a series of specialized patches of ectoderm on the head (the lateral line placodes). The development of the lateral line system commences during the embryonic period (prior to hatch), and extends through the larval (posthatch) and juvenile periods of a fish's life history. Lateral line development on the head occurs in three stages: (1) migration of placode-derived primordia and differentiation of hair cells in embryonic neuromasts, (2) growth of neuromasts (increases in size, changes in shape), and (3) formation of the pored lateral line canals (via intramembranous ossification of dermal bone – without a cartilage model) around a subset of neuromasts (the presumptive canal

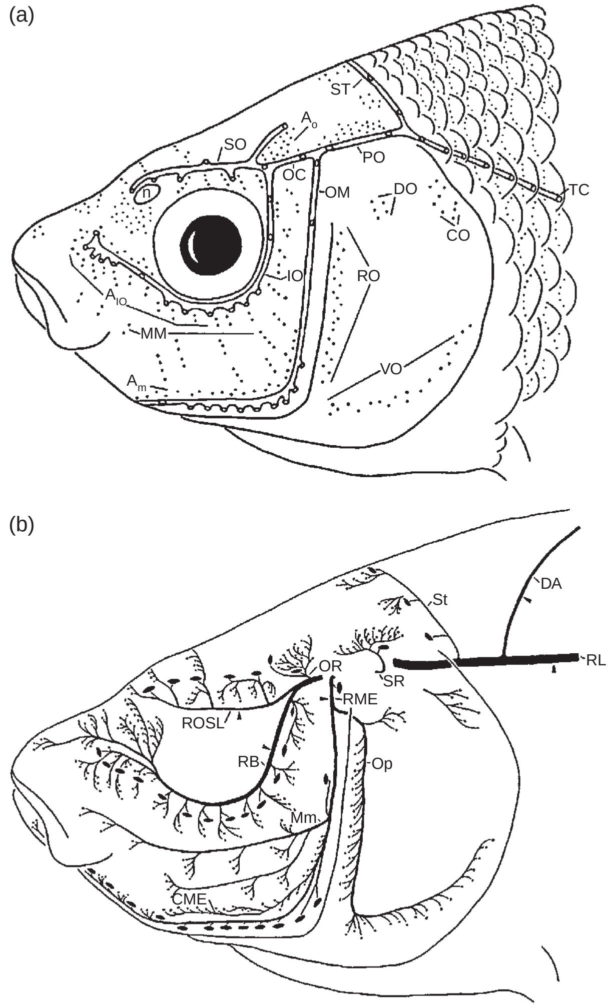


Figure 5 Distribution and innervation of lateral line canals and superficial neuromasts in the goldfish, *Carassius auratus*. (a) Pored lateral line canals and linear series of superficial neuromasts, (b) innervation of canal and superficial neuromasts. Reproduced from Puzdrowski RL (1989) Peripheral distribution and central projections of the lateral-line nerves in goldfish, *Carassius auratus. Brain Behavior and Evolution* 34: 110–120, with permission of Karger AG, Basel.

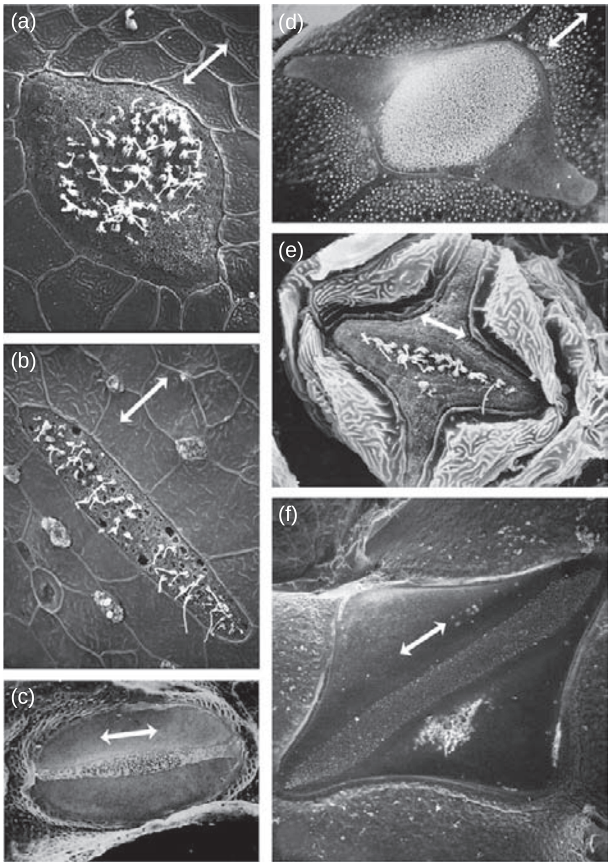


Figure 6 Morphological variation in canal and superficial neuromasts. (a) Superficial neuromast on trunk of windowpane flounder, *Scophthalmus aquosus*; (b) narrow canal neuromast in zebrafish, *Danio rerio*; (c) narrow canal neuromast in mottled sculpin, *Cottus bairdi*; (d) widened canal neuromast in clown knifefish, and *Notopterus chitala*; (e) superficial neuromast from the blind side of the head of California tongue sole, *Symphurus atricauda*; and (f) widened canal neuromast in rex sole, *Glyptocephalus* sp. Arrows indicate the axis of orientation of hair cells, which is parallel to long axis of the canal in which the canal neuromasts are found. (c, d) Courtesy of S Coombs.

neuromasts), as the canals become integrated into a conserved subset of dermal bones.

The genetic mechanisms underlying the first stage of lateral line development, the patterning and differentiation, have become the subject of recent detailed experimental studies using the zebrafish. Following neuromast patterning and differentiation, their size and shape change and two subsets of superficial neuromasts become apparent: presumptive canal neuromasts (those that will become enclosed in bony canals) and superficial neuromasts (that remain on the skin throughout life).

The development of the lateral line canals (the third stage of development) generally begins toward the end of the larval period, which may occur many weeks after fertilization (e.g., 28 days postfertilization in zebrafish). The timing of development generally varies among species, canals, and canal segments within a canal. Formation of individual canal segments is initiated in the vicinity of individual presumptive canal neuromasts, and the pattern of canal development, which has been described in detail in zebrafish and a cichlid, appears to be conserved among bony fishes.

The development of a lateral line canal segment around a single neuromast occurs in several stages (**Figure 11**). After canal segments form around individual canal neuromasts, adjacent canal segments may grow toward one another, and fuse leaving a common pore between them, thus forming a pored canal. The occurrence of one canal neuromast between the adjacent canal pores in most bony fishes is a result of this developmental pattern. Some variation in the morphology of the lateral line system among

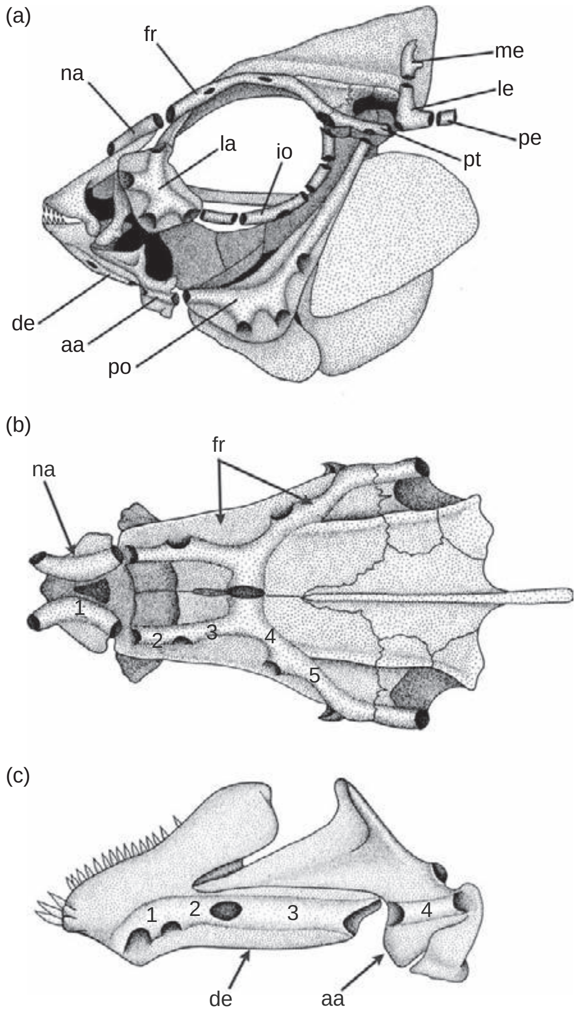


Figure 7 Skull of the convict cichlid, *Archocentrus nigrofasciatus* illustrating the lateral line canals in the dermal bones. (a) Lateral view showing the supraorbital canal in the nasal (na) and frontal (fr) bones, infraorbital canals in the lacrimal (la) and infraorbital series (e.g., io), preopercular canal in the preoperculum (po), mandibular canal in the dentary (de) and angulo-articular (aa), otic canal in the pterotic (pt), supratemporal commissure in the lateral and medial extrascapular bones (le, me) and postotic canal in the posttemporal bone (pe). (b) Dorsal view showing the supraorbital canal (so) in the nasal and frontal bones, (c) ventro-lateral view showing the mandibular canal (md) in the dentary and anguloarticular bones. Numbers indicate the location of neuromasts within the canals. From Tarby ML and Webb JF (2003) Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. *Journal of Morphology* 254: 44–57.

fishes can be attributed to the degree to which this developmental process is completed (e.g., the reduced canal pattern). Throughout the juvenile and adult phases of a fish's life history, the ossified lateral line canals continue to increase in diameter in concert with the increase in the size of the bones in which they are contained.

The development of the lateral line canal on the trunk starts with the establishment of a series of superficial neuromasts along the body axis as a result of the rostro-caudal migration of one or more lateral line placode-derived primordia on the head. At the end of the larval stage, scales begin to develop in a caudal-to-rostral direction, and then the lateral line canal segments develop in the lateral line scales in rostro-caudal sequence (**Figure 10**), resulting in one neuromast within the canal segment of each scale. The distribution of superficial neuromasts on the trunk is not particularly well known in many fishes, but it is common to observe an increase in the number of superficial neuromasts, including the formation of linear stitches on the scales, as in the goldfish (**Figure 5**) and zebrafish.

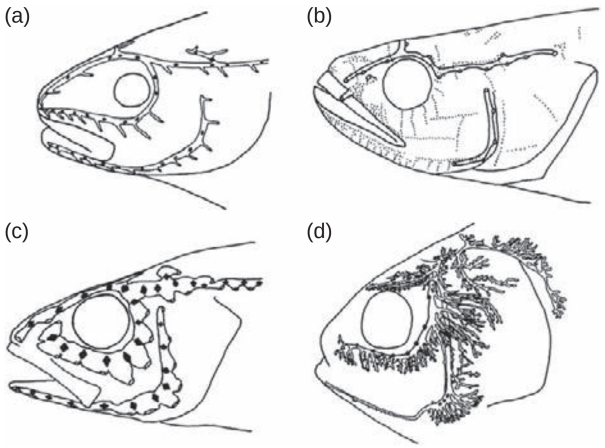


Figure 8 Four types of head canal systems among teleost fishes: (a) narrow-simple canal system, (b) reduced canal system, (c) widened canal system, and (d) narrow-branched canal system. (a, c, and d) Black dots and diamonds are canal neuromasts, (b) lines of dots are superficial neuromasts on skin. Reproduced from Webb JF (1989) Gross morphology and evolution of the mechanoreceptive lateral line system in teleost fishes. *Brain*, *Behavior and Evolution* 33: 34-53, with permission of Karger AG, Basel.

The Lateral Line System of Chondrichthyan Fishes

The mechanosensory lateral line system in the Chondrichthyes (sharks, skates, rays, and chimaeras) consists of lateral line canals containing neuromasts, superficial neuromasts located on the skin, and the vesicles of Savi, which are found only in some rays (**Figures 12** and **13**).

The lateral line canals of sharks, skates, and rays are narrow and cylindrical. They are composed of soft tissue (unlike those of bony fishes), are found in the dermis, and have tubules that extend to the skin surface ending in terminal pores. The number and course of the lateral line canals, which may be quite complex, are related to head shape and body form. On the head of most sharks, pored lateral line canals are distributed between the eyes, below the eyes, and on the upper and lower jaws, and a single

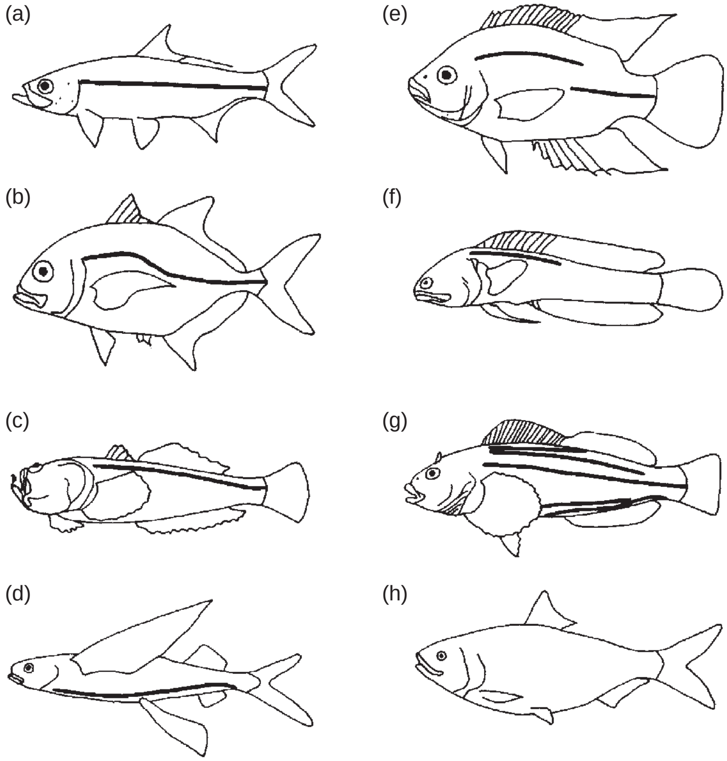


Figure 9 Eight trunk canal patterns among teleost fishes: (a) complete – straight, (b) complete – arched, (c) displaced dorsally, (d) displaced ventrally, (e) disjunct, (f) incomplete, (g) multiple, and (h) absent. Reproduced from Webb JF (1989) Gross morphology and evolution of the mechanoreceptive lateral line system in teleost fishes. *Brain, Behavior and Evolution* 33: 34–53, with permission of Karger AG, Basel.

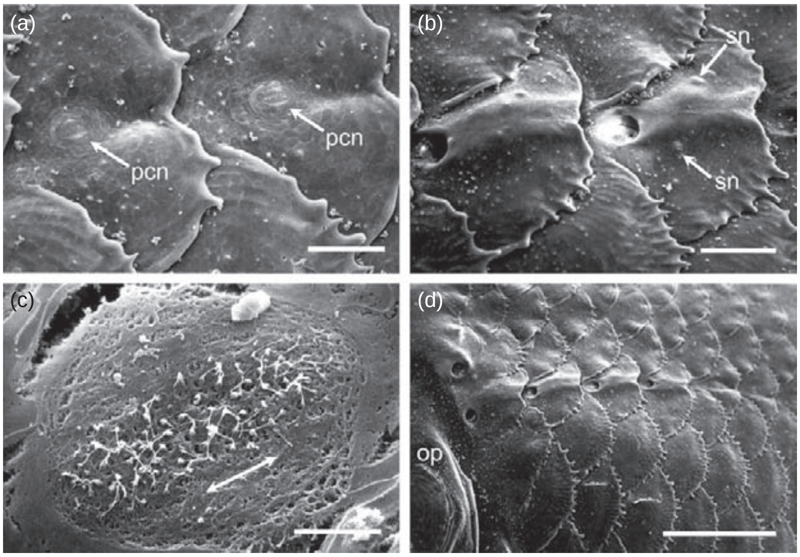


Figure 10 Scanning electron micrograph of the trunk canal in the convict cichlid, *Archocentrus nigrofasciatus* (=*Cichlasoma nigrofasciatum*). (a) Two lateral line scales before canal development, showing presumptive canal neuromasts (pcn); (b) two lateral line scales after canal forms showing additional superficial neuromasts (sn); (c) close-up of a presumptive canal neuromast from (a); arrow indicates axis of best physiological sensitivity as determined by hair cell orientation; and (d) anterior end of the trunk canal (just caudal to operculum, op) showing overlapping scales. From Webb JF (1989) Gross morphology and evolution of the mechanosensory lateral line system in teleost fishes. *Brain Behavior and Evolution* 33: 34–53.

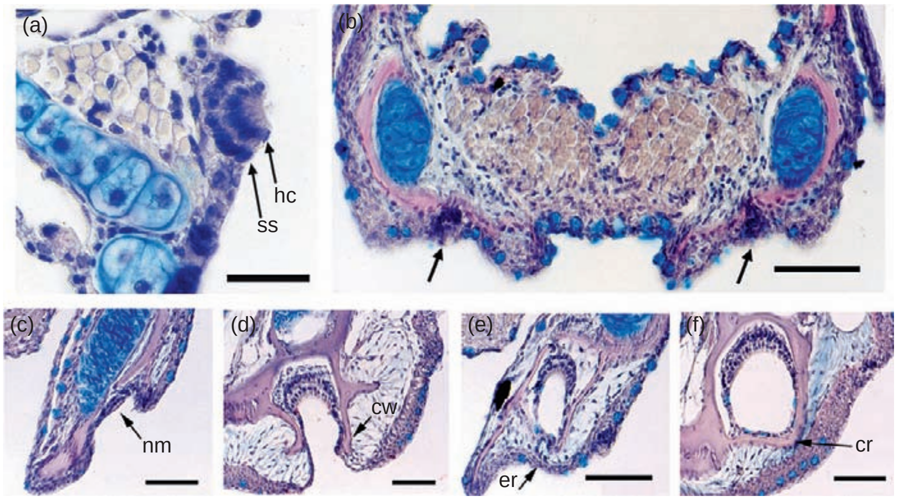


Figure 11 Development of canal segments and canal neuromasts in the zebrafish, *Danio rerio* (4.5–22 mm standard length; SL). (a) Presumptive canal neuromast in the infraorbital series in a 4.5-mm larva, showing hair cells (hc) and support cells (ss). Scale bar = 2 μm. (b)–(f) Development of a segment of the mandibular canal (Scale = 50 mm). (b) Bilateral presumptive canal neuromasts (nm) in mandibular epithelium (stage I), overlying dermal bone (pink) surrounding Meckel's cartilage (blue), 11.5 mm SL. (c) Neuromast (nm) at bottom of epithelial depression (stage IIa), 11 mm SL. (d) Neuromast in canal groove (stage IIb) with ossified canal walls (pink, cw) extending toward surface, 20 mm SL. (e) Neuromast in canal enclosed by epithelial roof (er) with ossified canal walls (pink, stage III), 12 mm SL. (f) Neuromast enclosed in canal with ossified canal roof (pink, cr), 22 mm SL. (c)–(f) From Webb JF and Shirey JE (2003) Post-embryonic development of the lateral line canals and neuromasts in the zebrafish. *Developmental Dynamics* 228: 370–385.

canal moves onto the trunk along the mid-flank to the end of the vertebral column in the upper lobe of the heterocercal tail (**Figure 12**). In the hammerhead sharks (genus *Sphyrna*), some of the canals extend onto the cephalofoil, the lateral extensions of the head that give these fishes their name (**Figure 12**). The dorso-ventrally flattened skates and rays have well-developed canals on both the dorsal and ventral surfaces of the head, including canals that extend onto the dorsal and ventral surfaces of the greatly expanded pectoral fins, which are fused to the head (**Figure 12**).

In addition to pored canals, some species (e.g., Atlantic stingray, *Dasyatis sabina*) may have nonpored canals on the ventral surface of the head or body that likely function differently than pored canals. It is thought that deformation of the skin overlying these canals stimulates the canal neuromasts. Torpedo rays, electric rays, and stingrays also have small structures, the vesicles of Savi, pouches that occur in linear series or clusters on the ventral side of the snout, which contain either one neuromast (e.g., stingrays) or one large and two smaller neuromasts (e.g., torpedo rays, **Figure 12**). Like the nonpored canals, the vesicles of Savi are not open to the external environment and are thought to provide a tactile sense.

The canal neuromasts are distinct in morphology and distribution from those in bony fishes. The neuromasts of bony fishes are well-defined, focal populations of hair cells that are clearly separated by the nonsensory epithelial cells in the canal lining, but the neuromasts of sharks, skates, and rays have been described as being composed of either a continuous or a nearly continuous sensory epithelium. Unlike bony fishes (with the exception of some lungfishes), several neuromasts are located between adjacent canal tubules. As in bony fishes, the hair cells in the sensory epithelia within the canals have well-defined ciliary bundles, and the hair cells are oriented 180° to one another – parallel with the canal axis. Superficial neuromasts sit on the skin, between modified placoid scales in sharks, or on the smooth skin of skates and rays, and are generally found on the dorsal surface of the body (above the trunk canal in sharks, or on the dorsal surface of the body in skates and rays).

The chimaeras (or ratfishes), an enigmatic group of chondrichthyans, have either narrow tubular canals (family Callorhynchidae) or open grooves (families Rhinochimaeridae and Chimaeridae) on the head, and the overall distribution is similar to that in the other chondrichthyan fishes. However, unlike the canals of other Chondrichthyes, the canals and grooves of chimaeras are supported by C-shaped rings (variously described as cartilaginous or calcified). As in sharks, skates, and rays, the canal neuromasts are described as being elongate and almost continuous. However, in some members of the Chimaeridae (e.g., *Hydrolagus*), the open grooves on the underside of the snout have periodic dilations, and discrete neuromasts (not elongated sensory epithelia) are found in the canals between dilations (**Figure 13**). Superficial neuromasts have not been reported in these fishes.

The neuromasts and sensory neurons that innervate the hair cells in chondrichthyan fishes are thought to be derived from cranial placodes as they are in bony fishes, but the way in which the neuromasts differentiate and grow, and the way in which the lateral line canals develop, are not well understood.

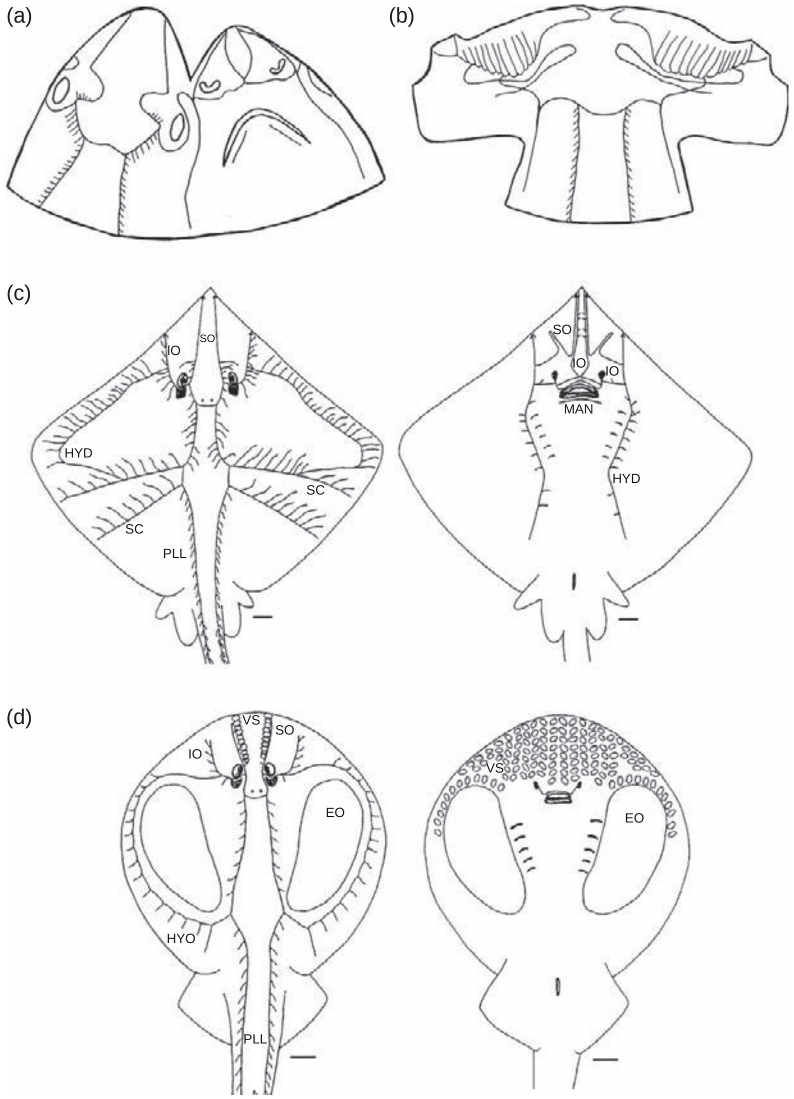


Figure 12 Distribution of lateral line canals in representative chondrichthyan fishes. (a) Grey reef shark, *Carcharhinus menisorrah*, dorsal and ventral views; (b) scalloped hammerhead, *Sphyrna lewini*, dorsal view; (c) clearnose skate, *Raja eglanteria*, dorsal (left) and ventral (right) views; and (d) lesser electric ray, *Narcine brasiliensis*, dorsal (left) and ventral (right) views, showing the vesicles of Savi (vs) and electric organ (eo). (a)–(b) Modified from Chu YT and Wen MC (1979) *Monograph of Fishes of China (No. 2): A Study of the Lateral-Line Canals System and that of Lorenzini Ampulla and Tubules of Elasmobranchiate Fishes of China*. Shanghai: Science and Technology Press; (c)–(d) from Maruska DP (2001) Morphology of the mechanosensory lateral line system in elasmobranch fishes: Ecological and behavioral considerations. *Environmental Biology of Fishes* 60: 47–75. Reprinted with permission of Springer Science + Business Media.

The Lateral Line System of Jawless Fishes

Paleontological investigations have revealed that the lateral line system evolved with the first vertebrates and is present as canals in the bony head shields of the extinct ostracoderms from more than 400 million years ago. In contrast, the lateral line system of the living jawless fishes, the hagfishes, and lampreys, which only have an internal cartilaginous skeleton, consists solely of superficial neuromasts, if at all it is present (**Figure 14**).

The lateral line system of lampreys (order Petromyzontiformes) is similar to that of jawed fishes with respect to the general morphology and distribution of neuromasts on the head, and their innervation by a series of lateral line nerves that project to the hindbrain. However, the neuromasts are reported to lack cupulae. The ammocoetes larvae of lampreys have already developed a functional lateral line system, with neuromasts that are similar to those of adults.

Among hagfishes, only members of the family Eptatretidae (e.g., *Eptatretus*) are known to have a lateral line system; the system is apparently absent in hagfishes of the family Myxinidae. In *Eptatretus stoutii*, the lateral line system consists of a series of about 11 short depressions or

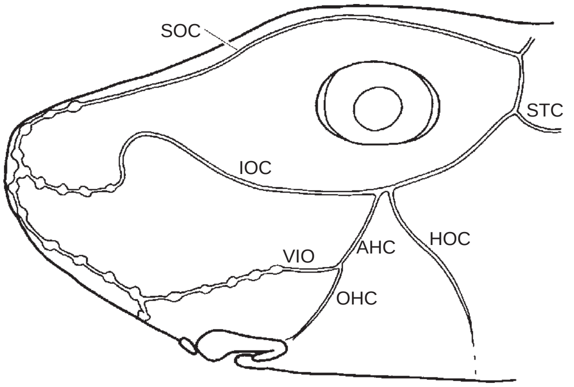


Figure 13 Distribution and morphology of lateral line canals in a representative chimaera, *Hydrolagus collei*. Lateral view showing open grooves laterally, and grooves with periodic dilations rostrally and ventrally. Dots indicate location of pores of the ampullae of Lorenzini of the electrosensory system. Modified from Field RD, Bullock TH, and Lange GD. (1993) Ampullary sense organs, peripheral, central and behavioral electroreception in chimeras) (*Hydrolagus*, Holocephali, Chondrichthyes). *Brain Behavior and Evolution* 41: 269–289.

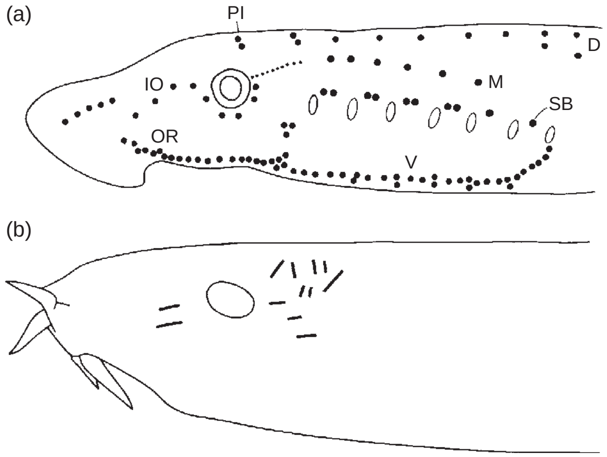


Figure 14 Lateral line system of living jawless fishes. (a) Superficial neuromasts of the European river lamprey, *Lampetra fluviatilis*. D, dorsal trunk line; IO, infraorbital line; M, main trunk line; OR, oral line: PI, pineal line; SB, suprabranchial line; V, ventral head line. (b) Lateral line grooves in a Pacific hagfish, *Eptatretus stouti*. Reproduced from Northcutt RG (1989) The phylogenetic distribution and innervation of craniate mechanoreceptive lateral lines. In: Coombs S, Görner P, and Münz H (eds.) *The Mechanosensory Lateral Line*, pp. 17–78. New York: Springer, with kind permission of Springer Science+Business Media.

grooves rostral and caudal to the eye spot, which appear unpigmented and vary in size, number, and position among individuals. This general morphology is similar to that represented by the early developmental stages of the lateral line system in jawed vertebrates. The depressions contain flask-shaped sensory receptor cells that may have a single cilium and a corolla of shorter microvilli, are reported to lack cupulae, and are unlike the directionally polarized hair cell bundles of the hair cells in the well-developed neuromasts of chondrichthyan and osteichthyan fishes. Sensory cells in the grooves are innervated by bundles of cranial nerve fibers that form two nerves that project to the hindbrain. The function of the sensory cells is not clear, but they are thought to transduce hydrodynamic disturbances. The system is thought to be derived from a small number of cranial placodes, but the development of hagfishes is currently known only from a small number of embryos.

See also: **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern. **Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy; Lateral Line Neuroethology. **The Skeleton**: Bony Fish Skeleton.

Further Reading

Braun CB (1996) The sensory biology of the living jawless fishes: A phylogenetic assessment. *Brain, Behavior and Evolution* 48: 262–276.

Braun CB and Northcutt RG (1997) The lateral line system of hagfishes (Craniata: Myxinoidea). *Acta Zoologica* 78: 247–268.

Chu YT and Wen MC (1979) *Monograph of Fishes of China (No. 2): A Study of the Lateral-Line Canals System and that of Lorenzini Ampulla and Tubules of Elasmobranchiate Fishes of China*. Shanghai: Science and Technology Press.

Coombs S, Görner P, and Münz H (eds.) (1989) *The Mechanosensory Lateral Line: Neurobiology and Evolution*. New York: Springer.

Coombs S, Janssen J, and Webb JF (1988) Diversity of lateral line systems: Phylogenetic, and functional considerations. In: Atema J, Fay RR, Popper AN, and Tavolga WN (eds.) *Sensory Biology of Aquatic Animals*, pp. 553–593. New York: Springer.

Coombs S and Montgomery JC (1999) The enigmatic lateral line system. In: Popper AN and Fay RR (eds.) *Comparative Hearing: Fishes and Amphibians*, pp. 319–362. New York: Springer.

Field RD, Bullock TH, and Lange GD (1993) Ampullary sense organs, peripheral, central and behavioral electroreception in chimeras (*Hydrolagus*, Holocephali, Chondrichthyes). *Brain Behavior and Evolution* 41: 269–289.

Ghysen A and Dambly-Chaudiere C (2007) The lateral line microcosmos. *Genes and Development* 21: 2118–2130.

Kasumyan AO (2003) The lateral line in fish: Structure, function and role in behavior. *Journal of Ichthyology* 43: S175–S213.

Lopez-Schier H, Starr CJ, Kappler FA, Kollmar R, and Hudspeth AJ (2004) Directional cell migration establishes the axes of planar polarity in the posterior lateral-line organ of the zebrafish. *Developmental Cell* 7: 401–412.

Maruska DP (2001) Morphology of the mechanosensory lateral line system in elasmobranch fishes: Ecological and behavioral considerations. *Environmental Biology of Fishes* 60: 47–75.

Northcutt RG, Holmes PH, and Albert JS (2000) Distribution and innervation of lateral line organs in the channel catfish. *Journal of Comparative Neurology* 421: 570–592.

Song J and Northcutt RG (1991) Morphology, distribution and innervation of the lateral-line receptors of the Florida gar, *Lepisosteus platyrhincus*. *Brain Behavior and Evolution* 37: 10–37.

Tarby ML and Webb JF (2003) Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. *Journal of Morphology* 254: 44–57.

Webb JF (1989) Gross morphology and evolution of the mechanosensory lateral line system in teleost fishes. *Brain Behavior and Evolution* 33: 34–53.

Webb JF (2000) Mechanosensory lateral line: Functional morphology and neuroanatomy. In: Ostrander G (ed.) *Handbook of Experimental Animals – The Laboratory Fish*, pp. 236–244. London: Academic Press.

Webb JF (2000) Mechanosensory lateral line: Microscopic anatomy and development. In: Ostrander G (ed.) *Handbook of Experimental Animals – The Laboratory Fish*, pp. 463–470. London: Academic Press.

Webb JF and Northcutt RG (1997) Morphology and distribution of pit organs and canal neuromasts in non-teleost bony fishes. *Brain Behavior and Evolution* 50: 139–151.

Webb JF and Shirey JE (2003) Post-embryonic development of the lateral line canals and neuromasts in the zebrafish. *Developmental Dynamics* 228: 370–385.

Webb JF, Smith WL, and Ketten DR (2006) The laterophysic connection and swim bladder in butterflyfishes in the genus *Chaetodon* (Perciformes: Chaetodontidae). *Journal of Morphology* 267: 1338–1355.

SENSING THE ENVIRONMENT

Detection and Generation of Electric Signals

Contents

Detection and Generation of Electric Signals in Fishes: An Introduction
Morphology of Electroreceptive Sensory Organs
Physiology of Ampullary Electrosensory Systems
Physiology of Tuberous Electrosensory Systems
Active Electrolocation
Electric Organs
Generation of Electric Signals
Development of Electroreceptors and Electric Organs

Detection and Generation of Electric Signals in Fishes: An Introduction

BA Carlson, Washington University in St. Louis, St. Louis, MO, USA

© 2011 Elsevier Inc. All rights reserved.

Further Reading

Glossary

Active electrolocation The ability of weakly electric fish to orient and detect objects based on their electric sense.

Electric field A spatial gradient in electrical potential. In animals, these can be actively generated by an electric organ or passively generated due to the uneven distribution of ions between the inside of the animal and the surrounding water. Direct current (DC) fields are steady, whereas alternating current (AC) fields are changing.

Electric organ An organ specialized for generating external electric fields. Myogenic electric organs are derived from skeletal muscle and are composed of individual cells called 'electrocytes'. Neurogenic electric organs are derived from neuronal axons.

Electric organ discharge (EOD) Electrical discharges resulting from the summed excitation of the cells (electrocytes) of the electric organ of electric fishes that function both in communication and in locating nearby objects. Short (100 ms to several tens of milliseconds) electrical pulses of various voltages (a few millivolts to several hundred volts) are generated at various rates (a few to over 1000 times per second.

Electrocommunication Communication based on electric signals.

Electroreceptor A sensory cell specialized for the detection of external electric fields. Ampullary electroreceptors are sensitive to low-frequency fields up to ~50 Hz, whereas tuberous electroreceptors are sensitive to high-frequency fields up to tens of kilohertz such as those produced by an EOD.

Electrosense The ability to detect electric fields. A passive electrosense is one in which external electric fields are detected, whereas an active electrosense is one in which self-generated electric fields are detected.

Humans have been familiar with the special abilities of strongly electric fish since antiquity. Egyptian tombstones depict images of the electric catfish *Malapterurus electricus*. Ancient Arabic texts refer to both *Malapterurus electricus* and the torpedo rays *Torpedo* spp. as 'thunderer' or 'trembler'. The ancient Romans used the shocks of these fish as therapeutic treatments for a wide range of medical ailments. However, it was not until the eighteenth century that the painful, numbing sensations caused by these fish were convincingly demonstrated to be caused by electricity. This discovery of animal electricity set the stage for the famous experiments of Luigi Galvani, in which he demonstrated that frog leg muscles could be made to contract by connecting nerves of the muscle in series with two different metals and the spinal cord. Galvani incorrectly attributed this effect to a unique form of electricity – an innate vital force housed within animal tissue that was released by the metal. His contemporary, Alessandro Volta, disagreed. Volta maintained that the contractions were not due to intrinsic electricity, but extrinsically generated current provided by contact between the two different metals. Volta ultimately proved correct, but the experiments of Galvani provided the first direct evidence of the electrical basis for nervous and muscular activity in all animals. The controversy engendered by these experiments led to the invention of the first battery to produce a reliable, steady electrical current – the Voltaic pile – which Volta created as an artificial model of an electric organ by stacking alternating disks of two different metals separated by cardboard soaked in brine.

When Darwin published *On the Origin of Species* in 1859, he devoted an entire chapter to problems with his theory of natural selection, which he entitled 'Difficulties on theory'. One area of concern was the evolution of electric organs, of which Darwin remarked "The electric organs of fishes offer another case of special difficulty; it is impossible to conceive by what steps these wondrous organs have been produced…" By that time, anatomists had discovered several groups of fish with relatively small organs similar in structure to the electric organs of *Malapterurus electricus*, *Torpedo* spp. and the electric eel *Electrophorus electricus*. Some of these were shown to generate electric fields, but they were far too weak to serve as any kind of a weapon, leading Du Bois-Reymond to dub them pseudo-electric. Because of morphological similarities between skeletal muscle and electric organ, Darwin correctly concluded that electric organs likely evolved from muscle, with strong electric organs evolving from weaker versions. Due to the absence of any apparent common ancestor, he further concluded (again, correctly) that the electric organs found in different groups of extant fish must have evolved independently. The problem, as Darwin saw it, was explaining how a weak electric organ could serve any adaptive function. Not for another century did Lissman and Möhres provide convincing experimental evidence that weak electric organs are used for communication (electrocommunication) as well as for navigation and orientation (active electrolocation). Such behaviors require a specialized sensory structure – an electroreceptor – for detecting electric fields.

In the late seventeenth century, Stefano Lorenzini described in detail a series of pores and associated canals distributed throughout the body of *Torpedo* spp. that were later found in many species of aquatic vertebrates, the so-called ampullae of Lorenzini. In 1773, John Walsh, the first person to detect actual sparks from an electric fish (an electric eel), provided the first direct evidence for an electric sense. Walsh found that an electric eel would approach a pair of recording electrodes and discharge its organ whenever the electrodes were short-circuited, leading him to conclude that eels could detect electrical conductors. Du Bois-Reymond, consistent with his opinions on weak electric organs, believed that the ampullae of Lorenzini served no useful function. Physiological experiments from the 1930s through the 1960s led various researchers to conclude that the ampullae were used for thermoreception, mechanoreception, chemoreception, and eventually electroreception. The latter was ultimately confirmed through clever behavioral experiments by Kalmijn, which conclusively demonstrated the existence of a passive electrosense in sharks and rays that was used to locate prey. Concomitant to these developments, the discovery of electrocommunication and active electrolocation by Lissman and Möhres had inspired an intensive effort to identify and characterize the necessary receptor cells. This culminated in the discovery of electroreceptive units in the lateral line nerve by Bullock and colleagues that were sensitive to much higher frequencies than the low-frequency ampullary receptors, making them well suited to detecting the specialized discharges of weak electric organs.

Today, research in the field is as varied as the diversity of organisms possessing an electrosense. Molecular biologists, electrophysiologists, neuroanatomists, mathematical modelers, and evolutionary biologists continue to study this distinctly foreign sensory system to ask broadly relevant questions ranging from information processing by sensory systems and the neural organization of motor output to the evolution of animal communication and the process of speciation. The section begins with an overview of electroreceptors (**see also Detection and Generation of Electric Signals:** Morphology of Electroreceptive Sensory Organs) by Jørgen Mørup Jørgensen, who describes the morphology of ampullary electroreceptors used for passive electrolocation and tuberous electroreceptors used for detecting actively generated electric fields for the purposes of active electrolocation and social communication. Jørgensen also summarizes the basic patterns of electroreceptor evolution across vertebrates, highlighting their multiple, independent origins. Michael Hofmann goes on to describe the physiology of ampullary electrosensory systems (**see also Detection and Generation of Electric Signals:** Physiology of Ampullary Electrosensory Systems), while Maurice J Chacron and Eric Fortune describe the physiology of tuberous electrosensory systems (**see also Detection and Generation of Electric Signals:**

Physiology of Tuberous Electrosensory Systems; in both cases, the authors focus on the peripheral encoding of sensory information and mechanisms for the detection of behaviorally relevant stimuli within the central nervous system. Gerhard von der Emde and Jacob Engelmann describe how tuberous electrosensory systems are used in active electrolocation (**see also Detection and Generation of Electric Signals:** Active Electrolocation) for the detection and analysis of objects in the environment. This behavior relies on the active generation of electric fields, and Angel Caputi describes in detail the morphology of the electric organs (**see also Detection and Generation of Electric Signals:** Electric Organs) that generate these fields, paying special attention to the relationship between structure and function. Masashi Kawasaki, discussing the generation of electric signals (**see also Detection and Generation of Electric Signals:** Generation of Electric Signals), describes how the central nervous system controls the timing of electric organ output. Kawasaki considers both the general principles of electromotor circuits as well as the evolutionary variation in these pathways as it relates to differences in electric signaling behavior. The section ends with a description of the development of electroreceptors and electric organs (**see also Detection and Generation of Electric Signals:** Development of Electroreceptors and Electric Organs) by Frank Kirschbaum and Jean-Pierre Denizot. Despite the diversity of approaches and interests, readers will recognize throughout this section an appreciation for comparative approaches to biological problems, as well as a common bind that holds the field together: a deep interest in the amazing capabilities of these animals and the fundamental insights they provide into the origins and operations of our own biology.

See also: **Detection and Generation of Electric Signals:** Active Electrolocation; Development of Electroreceptors and Electric Organs; Electric Organs; Generation of Electric Signals; Morphology of Electroreceptive Sensory Organs; Physiology of Ampullary Electrosensory Systems; Physiology of Tuberous Electrosensory Systems. **Sensory Systems, Perception, and Learning:** Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Bullock TH (1974) An essay on the discovery of sensory receptors and the assignment of their functions together with an introduction to electroreceptors. In: Fessard A (ed.) *Handbook of Sensory Physiology, Vol. III/3: Electroreceptors and Other Specialized Receptors in Lower Vertebrates*, pp. 1–12. Berlin: Springer.

Bullock TH, Hagiwara S, Kusano K, and Negishi K (1961) Evidence for a category of electroreceptors in the lateral line of gymnotid fishes. *Science* 134: 1426–1427.

Bullock TH and Heiligenberg W (eds.) (1986) *Electroreception,* Wiley Series in Neurobiology (Northcutt RG (ed.)). New York: Wiley.

Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) (2005) *Electroreception,* Springer Handbook of Auditory Research, vol. 21. New York: Springer.

Darwin C (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Du Bois-Reymond E (1884) *Untersuchungen über thierische Elektricität,* vol. 3. Berlin: Reimer.

Finger S and Piccolino M (in press) *A History of Electric Fishes: From Ancient Ideas to Modern Physiology.* New York: Oxford University Press.

Galvani L (1792) *De Viribus Electricitatis in Motu Musculari Commentarius cum Joannis Aldini Dissertatione et Notis* (Commentary on the effect of electricity on muscular motion together with Aldani's dissertation and notes), Green RM (trans.), 1953, Cambridge: Elizabeth Licht.

Heiligenberg W (1991) *Neural Nets in Electric Fish,* Computational Neuroscience Series. Cambridge: MIT Press.

Kalmijn AJ (1966) Electro-perception in sharks and rays. *Nature* 212: 1232–1233.

Lissman HW (1951) Continuous electrical signals from the tail of a fish, *Gymnarchus niloticus*. *Nature* 167: 201–202.

Lissman HW (1958) On the function and evolution of electric organs in fish. *Journal of Experimental Biology* 35: 156–191.

Lorenzini S (1678) *Osservazioni intorno alle Torpedini fatte da Stefano Lorenzini Fiorentino e dedicate al Serenissimo Fernando III, Principe di Toscana*. Florence: Onofri.

Möhres FP (1957) Elektrische Entaldungen im Dienste der Revierabgrenzung bei Fischen. *Naturwissenschaften* 44: 431–432.

Moller P (1995) *Electric Fishes: History and Behavior*. New York: Chapman and Hall.

Volta A (1800) On the electricity excited by the mere contact of conducting substances of different kinds. *Philosophical Transactions of the Royal Society* 90: 403–431.

Walsh J (1773) Of the electric property of the Torpedo. *Philosophical Transactions of the Royal Society* 63: 461–480.

Relevant Websites

http://www.bbc.co.uk – BBC page on The 'Sixth Sense' of Weakly Electric Fish.

http://biology4.wustl.edu – Homepage of Dr. Bruce A. Carlson in the Department of Biology at Washington University in St. Louis.

http://www.nbb.cornell.edu – Homepage of Dr. Carl D. Hopkins in the Department of Neurobiology and Behavior at Cornell University.

http://www.psy.jhu.edu – Homepage of Dr. Eric S. Fortune in the Department of Psychological and Brain Sciences at Johns Hopkins University.

http://people.virginia.edu – Homepage of Dr. Masashi Kawasaki in the Department of Biology at the University of Virginia.

http://www.medicine.mcgill.ca – Homepage of Dr. Maurice J. Chacron in the Department of Physiology at Mcill University.

http://alumnus.caltech.edu – Movies of electromotor behavior and computer simulations of weakly electric fish.

http://www.scholarpedia.org – Scholarpedia article on electrolocation.

http://video.google.com – Video of electric eel.

http://en.wikipedia.org – Wikipedia article on electric fish.

Morphology of Electroreceptive Sensory Organs

JM Jørgensen, University of Aarhus, Aarhus, Denmark

© 2011 Elsevier Inc. All rights reserved.

Introduction
Lampreys: Electroreceptive End Buds
Ampullary Organs
Tuberous Electroreceptive Organs
Monotreme Electroreceptive Organs
Further Reading

Glossary

Ampullae of Lorenzini Ampullary organ in cartilaginous and some bony fishes.

Ampullary organs Mucous-filled tubular structures in many fishes with open pores in the skin connecting to the outside and electroreceptors at the blind end.

End buds Cone-shaped sensory organs in the skin of lampreys.

Gymnarchomast Tuberous electrosensory organ in the skin of the bony fish *Gymnarchus* with a single sensory cell that has an apical invagination with numerous microvilli.

Gymnomast Tuberous electrosensory organ in fishes belonging to the family Gymnotidae. Typically, there are a few sensory cells with many microvilli in a closed chamber.

Knollenorgan Tuberous electrosensory organ used for electrocommunication among mormyrid fishes. The sensory cells are equipped with numerous microvilli and are specialized for detecting the electric organ discharge of another conspecific fish.

Mormyromast Tuberous sensory organ used for active electrolocation in the skin of mormyrids. Two types of electrosensory cells are exposed to different parts of a closed chamber.

Siluromast Tuberous organ with a single electroreceptor in the skin of the silurid *Pseudocetopsis*.

Tuberous organ Electrosensory organs in the skin of some teleosts that lack an external opening to a pore.

Introduction

The history of electroreception is a fascinating story involving mysterious, tubular, jelly-filled structures, for a long time believed to be slime-secreting organs (**see also Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction). These organs were first found in elasmobranchs, and, after their discoverer, termed 'ampullae of Lorenzini'. They remained a puzzle for several hundred years after their first description. As anatomical techniques improved, it became clear that these organs were, in fact, sense organs.

It is now clear that these special sensory organs, which are sensitive to many different types of stimuli, including weak electric fields, arose very early in the evolution of vertebrates. With the exception of hagfishes, all other anamniotic craniate classes, including lampreys, cartilaginous fishes, and a number of bony fishes, as well as urodele and caecilian amphibians, have electroreceptors. Organs that are sensitive to weak electric fields have also been identified in monotreme mammals.

It is known that weak electric fields, important for many aquatic vertebrates, are used for prey detection, identification of objects in the environment, and social communication. It is therefore a real mystery why many fishes apparently have lost this valuable sense during evolution.

The structure, or morphology, of electroreceptive sense organs provides a platform for the understanding of their function. This article briefly describes the different types of electroreceptive organs found in various vertebrates.

Lampreys: Electroreceptive End Buds

Adult lampreys have widespread superficial epidermal sensory organs called end buds on the head and trunk. The sensory cells are directly exposed to the surrounding water and thus have no duct. Each end bud consists of 3–30 slender sensory cells that are separated from one another by supporting cells. The apical surface of each sensory cell is equipped with a hair bundle consisting of 80–90 microvilli. Presynaptic synaptic bodies lie opposite to afferent nerve boutons.

The ammocoete larvae of lampreys and newly metamorphosized lampreys do not possess end buds, although they appear to have a sense for weak electric fields.

Instead of end buds, they have a number of scattered multivillous sensory cells. This cell type shares many characteristics with the sensory cells belonging to the octavolateralis system. Such multivillous cells are found in small groups in the gill vent papillae and on the tail.

Solid indication of an electroreceptive sensory system in adult lampreys is the presence of a dorsal octavolateral nucleus in the medulla oblongata and responses to electrosensory stimuli recorded from single afferent nerve fibers. The sensory cells are excited by cathodal stimuli and both end buds and multivillous cells may be categorized as nonteleost ampullary organs lacking a duct.

Ampullary Organs

General Structure

Electroreceptive ampullary organs are characterized by a tube-like duct, with a pore in the skin, and a sensory epithelium at the end of the tube (**see also Detection and Generation of Electric Signals**: Physiology of Ampullary Electrosensory Systems). The canal leading from the sensory epithelium to the environment varies considerably in length. It is filled with a mucous jelly that has a very low electrical resistance. The wall of the ampullary duct is composed of flattened cells that are connected to each other by tight junctions and desmosomes, creating a boundary with high electrical resistance.

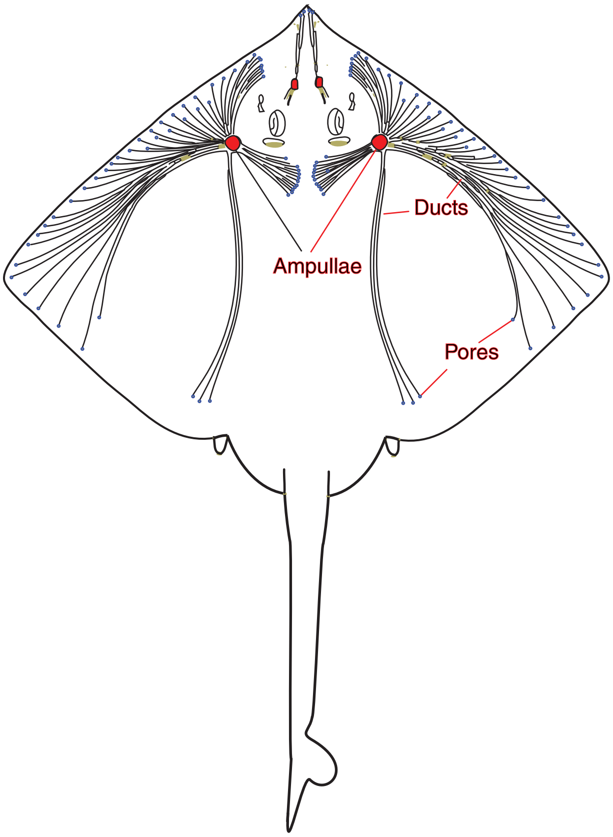


Figure 1 Dorsal view of a ray *Raja* with the position of ampullae of Lorenzini. The tubes spread out from clusters of ampullary ends to cover a large part of the wings and body.

Nonteleost Fishes: The Ampullae of Lorenzini

The ampullae of Lorenzini are defined here as ampullary sense organs that project to a dorsal octavolateral nucleus in the medulla oblongata and are excited by cathodal stimuli. With this definition, the organs of Lorenzini include the electroreceptive organs in nonteleost fishes and the ampullary organs in amphibians.

The morphology of the sensory cells in the ampullae of Lorenzini is characterized by a narrow apical area exposed to the ampullary lumen. Typically, a short cilium extends from a basal body. The cilium may be surrounded by a few microvilli. Basally, synaptic sheets or round bodies form contact with a single or a few afferent nerve endings.

Ampullary Organs in Cartilaginous Fishes (Chondrichthyes)

Cartilaginous fishes include sharks, skates, and rays, as well as chimaeras.

The ampullae in the marine species generally have long canals that lead to sensory cells situated deep in the dermis. However, the canal length as well as shape may vary considerably within the same individual (**Figure 1**). The pores are found in the head region of all species and in the pectoral fins of rays and skates. The sensory epithelium covers the interior of the ampullary end, which often forms several blind ending sacs called diverticulae (**Figure 2**).

The sensory ampullae are located in clusters and the total number of ampullary organs in an individual varies considerably among species. Some species have only 148 ampullae, as found in *Heterodontus francisci*, or 162 ampullae, as found in *Torpedo marmorata*, while other taxa have more than 2000. The maximum number of 2824 has been found in the hammerhead shark, *Sphyrna lewini.*

The ampullae vary in shape, as shown in **Figure 2**. The ampulla may be simple with one enlarged chamber and, in some cases, a few diverticulae. This type is found in electric rays belonging to the genus *Torpedo.* A number of simple ampullae may together form assembled ampullae, as in the shark *Hexanchus.* More common is a lobular type, with 2–80 diverticulae arranged side by side, all of which open into a central ampulla. This type is found in most sharks, rays, and skates. A section of such an ampulla shows the chambers (**Figure 3**). In some chimaeras, the chambers appear as slender pockets forming a finger-shaped ampulla.

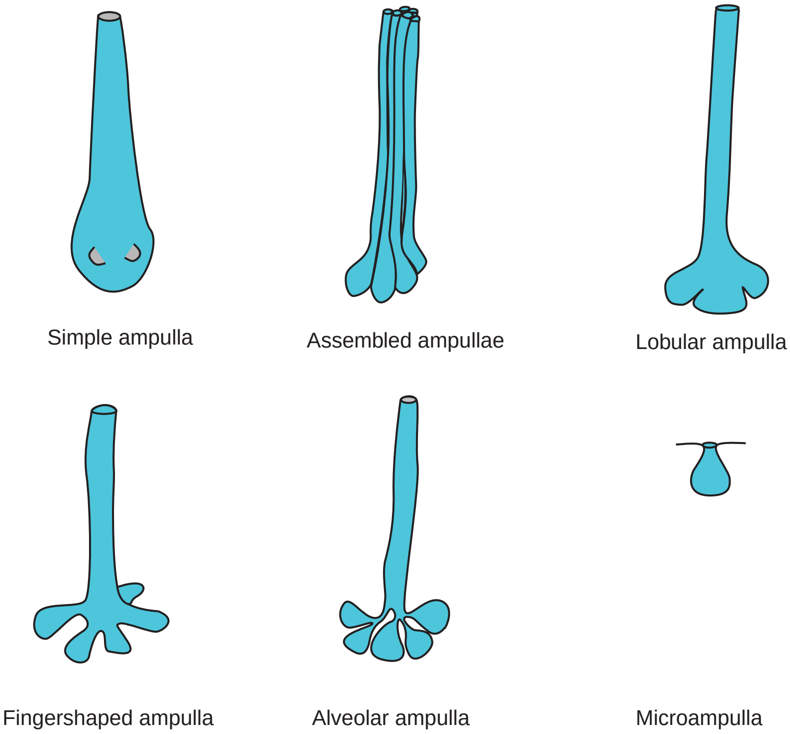


Figure 2 Different types of ampullae of Lorenzini known from various cartilaginous fishes. Not drawn to scale.

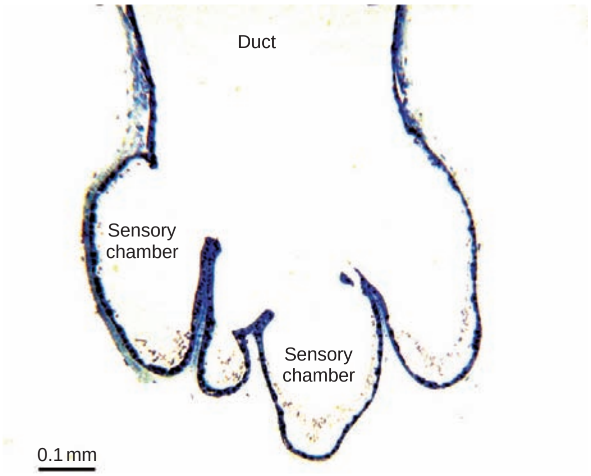


Figure 3 Light micrograph of a section from an ampulla with several sensory chambers from the spotted dogfish (*Scylliorhinus canicula*).

Some sharks, such as the piked dogfish (*Squalus acanthias*), have chambers that are connected to the central canal by slender ducts; this type may be termed alveolar. In permanently freshwater elasmobranchs, such as *Potamotrygon*, short ducts end in simple microampullae.

In a detailed study of 40 species of rays belonging to the genus *Raja*, it was found that the majority of ampullary organs are located on the ventral side of the animal. It also appears that fish-eating rays have their ampullary pores distributed over a larger part of the body than those species that feed primarily on invertebrates. In addition, deep-dwelling species have larger ampullae with more chambers than shallow-water species.

Ampullary Organs in Bichirs and Reedfish (Polypteriniformes)

Extant fishes belonging to this superorder are a small group of African fishes of the genera *Polypterus* and *Erpetoichthys* (synonym *Calamoichthys*). The ampullary organs in these species are distributed on the head, with the highest density on the snout region in front of the eyes. The ampullary organs occur as simple tubes in the skin, except where an ampullary organ seems to divide, forming two organs. The sensory epithelium consists of electroreceptor cells separated by supporting cells. The apical part of the sensory cell is characterized by having a single cilium surrounded by 8–10 microvilli. Basally in the receptor cell, synaptic sheets or rounded synaptic bodies are seen opposite to the afferent nerve endings.

Ampullary Organs in Sturgeons and Paddlefishes (Acipenseriformes)

The ampullary organs of sturgeons have been examined in the genera *Scaphirhynchus*, *Acipenser*, and the paddlefish

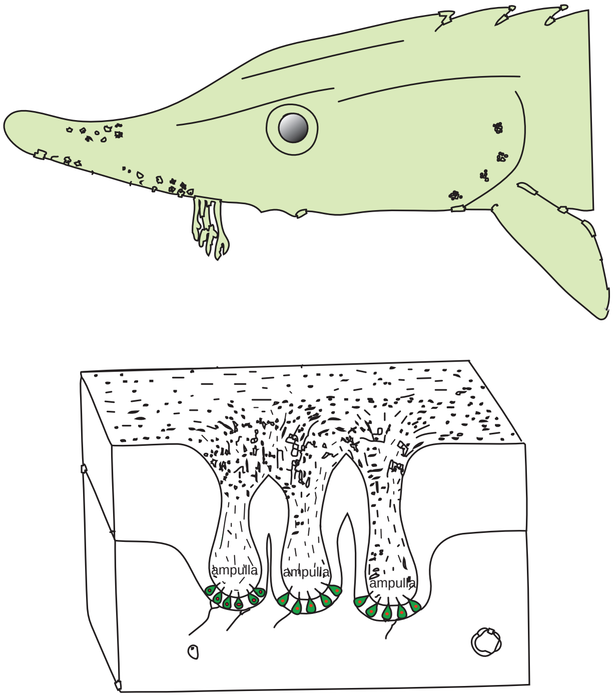


Figure 4 The head of a sturgeon (*Acipenser ruthenus*) with ampullary organs on the snout and gill cover.

Polyodon spathula. In all species examined, the ampullary organs occur on the head, including the rostrum, and, in some species, the opercula. The ampullary organs occur in clusters of 5–30 single organs (**Figure 4**). Occasionally, two to five ampullae may share a common pore. The pear-shaped sensory cells are apically equipped with a cilium, and a single nerve fiber ramifies below the sensory epithelium of each ampulla to form several afferent boutons in contact with the sensory cells. These boutons have synaptic sheets opposite to the afferent boutons.

Recent studies of electroreception in the paddlefish have shown convincingly that this species uses its sensory ampullae for passive detection and feeding on plankton.

Ampullary Organs in Lungfishes (Dipnoi)

The extant lungfishes have ampullary organs embedded in a thick epidermis. Their distribution is unusual among other nonteleost bony fishes, because they are found not only on the head, but also on the trunk and tail fin. The large, pear-shaped sensory cells are equipped with an apical cilium, which may be surrounded by a microvilli (**Figure 5**). Basally in the cells, synaptic sheets surrounded by synaptic vesicles are found opposite to the afferent nerve endings.

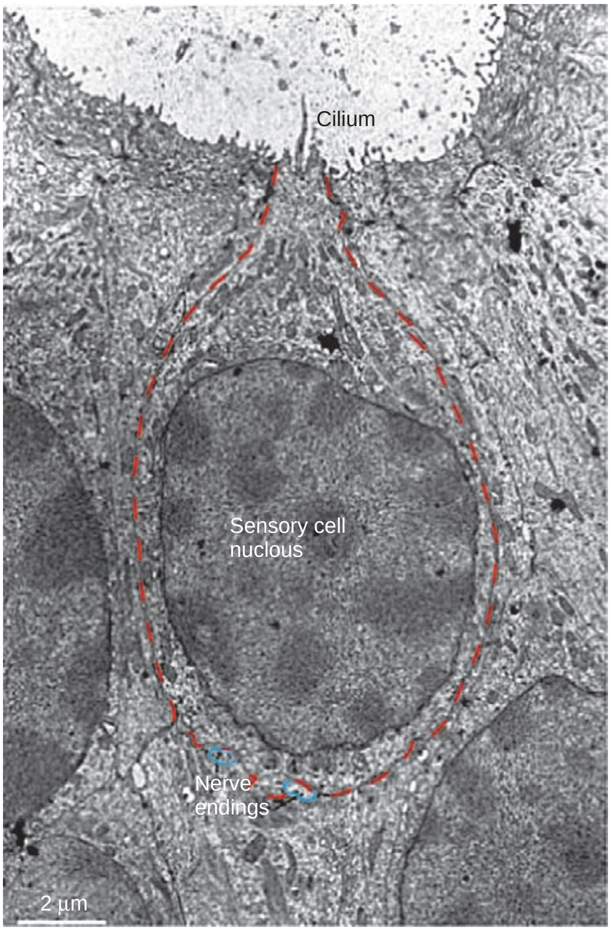


Figure 5 An ampullary sensory cell from an African lungfish (*Protopterus annectens*) as seen using a transmission electron microscope. The sensory cell membrane is marked with a dashed red line. Apically is seen a cilium surrounded by few microvilli. Small nerve endings are seen at the basal end of the sensory cell.

Ampullary Organ in Coelacanths (Coelacanthiformes)

We have information only from *Latimeria chalumnae*, one of the two species in this group. The sensory cells are situated in a rostral organ consisting of an irregular chamber in the ethmoid region. It has three pairs of openings to the surrounding water. As in all other ampullary organs, the tubes leading to the pores are filled with a jelly-like substance. Deep crypts that are lined by a sensory epithelium consisting of sensory and supporting cells are found in a part of the chamber. Although no well-fixed tissue has been examined, it seems that the sensory cells are very much like the ampullary sensory cells found in other nonteleost fishes.

Additional evidence for electroreception is that the rostral organs are innervated by branches of the superficial ophthalmic ramus of the anterodorsal lateral line nerve that projects to a distinct dorsal nucleus in the medulla oblongata.

Ampullary Organs in Caecilian and Urodele Amphibians

A number of species belonging to caecilian and urodele amphibians have ampullary organs that are located on the head. Each ampulla contains 10–30 pear-shaped sensory cells, all of which are innervated by a single afferent nerve fiber.

In larval *Ichthyophis* (Caecilian), each sensory cell has a single apical cilium surrounded by a few microvilli, while the urodeles show a more varied pattern. In the axolotl (*Ambystoma mexicanum*), the sensory cells have approximately 200 microvilli around an eccentric cilium. In the European cave salamander (*Proteus anguinus*), some sensory cells have an apical cilium, while others only possess a basal body. Electroreceptive sensory cells of other urodeles belonging to the genera *Triturus* and *Salamandra* have apparently no cilia or basal bodies, but possess numerous long microvilli.

Basally in the electroreceptive cells, very large synaptic spheres up to 3 μm in diameter are found opposite to the afferent nerve endings. Electrophysiological evidence of electroreception and the role of electroreception during feeding have been demonstrated in the axolotl (*Ambystoma mexicanum*). The nerve fibers from the amphibian ampullary organs project to a dorsal nucleus in the medulla.

Ampullary Organs in Teleosts

Ampullary organs are not found widely in teleosts. However, they have been demonstrated in species belonging to the orders Osteoglossiformes (family Mormyridae with many electroreceptive species, family Gymnarchidae with one species, family Notopteridae with two species with electroreceptive organs: *Xenomystus* and *Papyrocranus*), Siluriformes (many species in all studied families), and Gymnotiformes (many species belonging to all families).

With few exceptions, these electroreceptive teleosts are freshwater teleosts, and a characteristic is a rather short ampullary duct (**Figure 6**). By contrast, in the few electroreceptive saltwater teleosts, for example, *Plotosus* which belongs to the order Siluriformes, the ampullary ducts are long, as found in cartilaginous fishes.

Nerve fibers from the teleost electroreceptors project to a lateral lobe in the medullary octavolateralis area, which is distinct from the dorsal nucleus of nontelostean fishes. Another difference between teleosts and nonteleosts is that the teleost electroreceptors are primarily excited by anodal fields, while nonteleost electroreceptors are excited by cathodal fields. This naturally leads to the hypothesis that the electroreceptive organs of these two main groups have an independent evolutionary history.

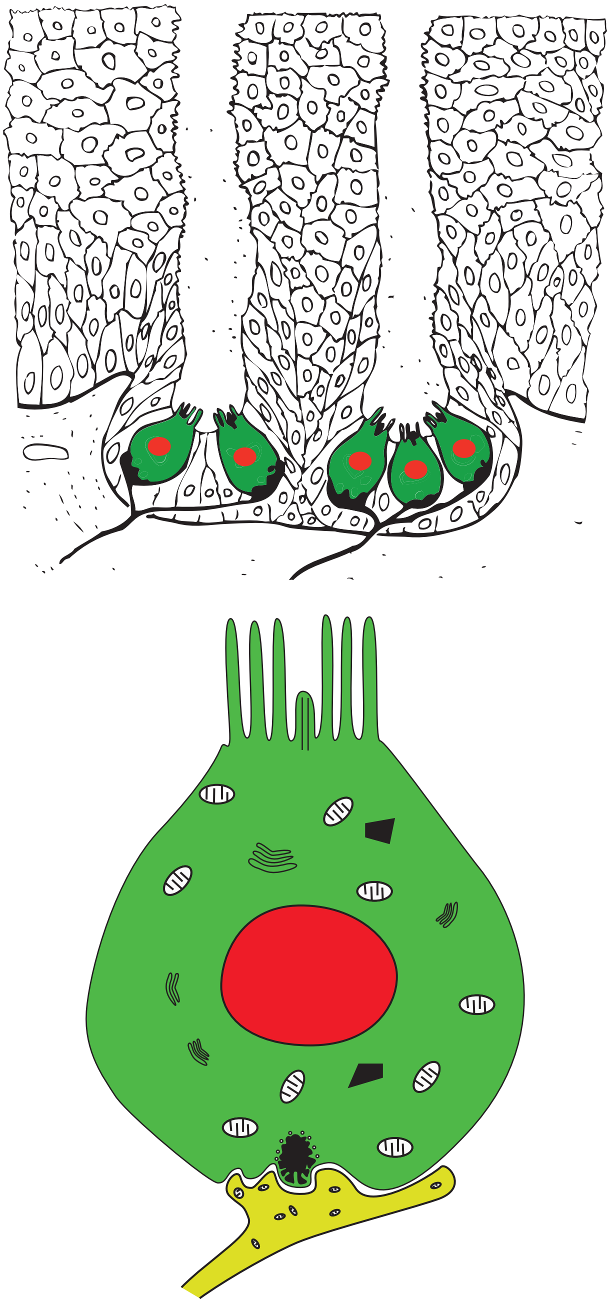


Figure 6 Above are two ampullae from the African knifefish *Xenomystus nigri* (Nothopteridae, Teleostei) and below is an isolated sensory cell with an apical cilium and microvillae.

The evolutionary line leading to osteoglossiform fishes is so far removed from the gymnotiform and siluriform line that it has been suggested that these teleost lines evolved ampullary organs independently. Furthermore, it is commonly believed that the origins of the different electroreceptive cells are the hair cells of the mechanosensory lateral line and inner ear. **Figure 7** shows two

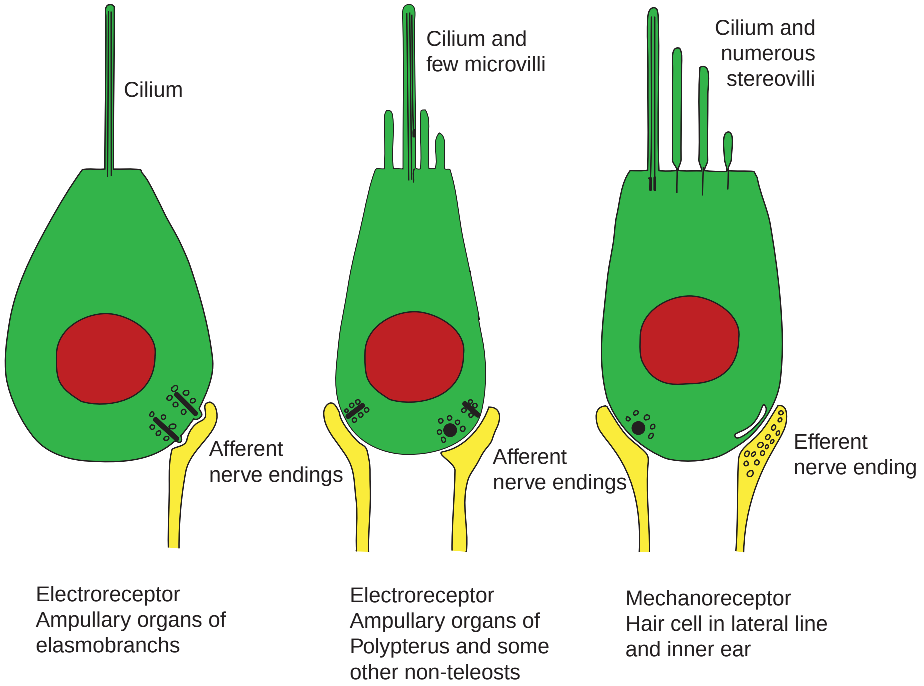

Cilium
Cilium and few microvilli
Cilium and numerous stereovilli
Afferent nerve endings
Afferent nerve endings
Efferent nerve ending
Electroreceptor Ampullary organs of elasmobranchs
Electroreceptor Ampullary organs of Polypterus and some other non-teleosts
Mechanoreceptor Hair cell in lateral line and inner ear

Figure 7 Different sensory cells from the octavolateralis organs. To the left is a pear-shaped sensory cell from the ampullae of Lorenzini of elasmobranchs. In the middle is a sensory cell from the ampullary organ in the bichir *Polypterus.* To the right is a mechanosensory hair cell from the lateral line.

different electroreceptive sensory cells and a lateral line hair cell.

Ampullary Organs in Notopterid Fishes

Fish belonging to the order Osteosteglossiformes contain the family Notopteridae, with many species in Asia and a few in Africa. Only the African species are known to possess electroreceptive organs. The ampullary organs in the African knife fish *Xenomystys nigri* (**Figure 6**) are not only distributed all over the head and along the body, but are particularly numerous slightly dorsal to the horizontal septum. Each organ has four to eight pear-shaped sensory cells, which apically have 40–60 microvilli and a short cilium, up to 1 μm. So far, this species is the only known teleost with an apical cilium on its electroreceptive cells. The cilium arises from a basal body and is found between the microvilli. These microvilli are about 2.5 μm long and have a diameter of 0.2–0.3 μm. Mitochondria are primarily seen in the apical part of the cell and below the centrally located nucleus. One or a few round synaptic bodies surrounded by clear vesicles are found opposite to a single nerve bouton.

Amullary Organs in Mormyrid Fish

Mormyrid ampullary organs are distributed all over the head as well as dorsally and ventrally along the body. The ampullary ducts in two species of *Gnathonemus* have a length of about 150 μm or less and a diameter of 20 μm. Each organ contains three to eight egg- or pear-shaped sensory cells (**Figure 8**), separated by supporting cells. Each sensory cell has a number of apical microvilli, which extend for a short distance into the ampullary canal. The nucleus is found in the center of the sensory cell. In addition to mitochondria and vesicles in all parts of the cytoplasm, the sensory cell has about 20–30 synaptic sheets opposite to the afferent nerve boutons, which contact the basal or lateral parts of the cell. A single nerve fiber divides to contact all of the sensory cells in a single ampullary organ.

Ampullary Organs in the Teleost *Gymnarchus*

Gymnarchid ampullary organs are distributed all over the head and body of the fish. The ampullary lumen is 10–20 μm in diameter and only one sensory cell is generally found in the ampulla (**Figure 8**). The shape of the sensory cell is unique in that the ampullary lumen extends almost half way down into the apical part of the cell. The surface of the cell has a sparse number of short microvilli that are about 1 μm in length and 0.1 μm in diameter. Synaptic sheets surrounded by numerous vesicles are found opposite to several synaptic boutons, all of which are connected to a single nerve fiber.

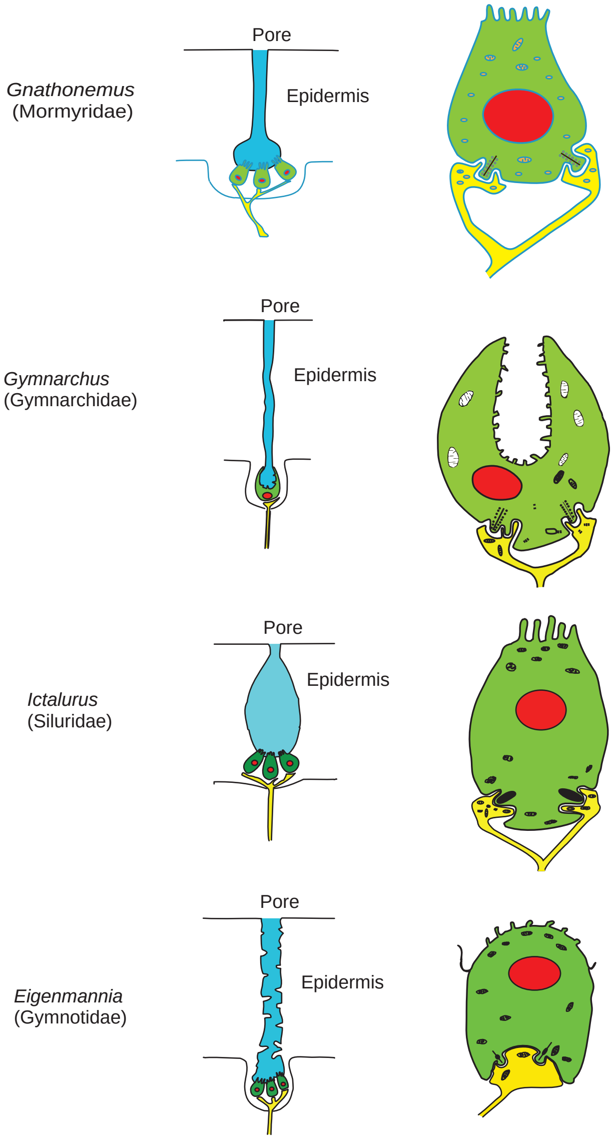


Figure 8 Teleost ampullary organs and individual sensory cells from representatives of four different families. Not drawn to scale.

Ampullary Organs in Silurid Fishes

Silurid ampullary organs are most numerous in the head, but are also found along the side of the body. In *Plotosus,* they are primarily found at the base of the fin rays. The ampullae of the transparent catfish (*Kryptopterus bicirrhus*) are distributed all over the body of the fish.

In the freshwater silurids, the ampullae are situated in the epidermis (**Figure 9**); only a few reach deep into the dermis. In *Pseudocetopsis,* usually only one or two sensory cells are found in the bottom of the ampullae, while the electroreceptive ampullae of *Ictalurus* (*Amiurus*) contain 5–15 sensory cells and *Kryptopterus* has 10–30 sensory cells. In all of the species mentioned, these cells are equipped with a sparse distribution of short microvilli (**Figure 10**). The marine species (e.g., *Plotosus*) have long ducts, reaching about 3 cm deep into the dermis, and the sensory cells have numerous long microvilli.

Ampullary Organs in Gymnotid Fishes

Generally, the Gymnotid ampullary organs are found scattered on the head and in bands along the side of the body. Between two and eight pear-shaped sensory cells protrude slightly into the ampullary lumen (**Figure 8**), exposing about 15–20% of the sensory cell surface to the lumen. Irregularly distributed microvilli that are less than 1 μm in length and with a core of microfilaments are seen on the surface. Numerous mitochondria are found around the central or luminally placed nucleus. Basally, 10–15 synaptic bodies are found opposite to a single large nerve bouton, which measure about 5 μm in diameter. These dark staining presynaptic bodies or rods have a peculiar structure consisting of a roundish body with a rod-like structure pointing centrally into the cell and a foot process that reaches into the synaptic lobe of the sensory cell that projects into the bouton. These synaptic bodies are surrounded by numerous clear vesicles with a diameter of 20–30 nm.

Tuberous Electroreceptive Organs

Tuberous organs have so far only been demonstrated in teleost fishes (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems). They differ from ampullary organs in that they do not have a duct from the surface of the skin to the

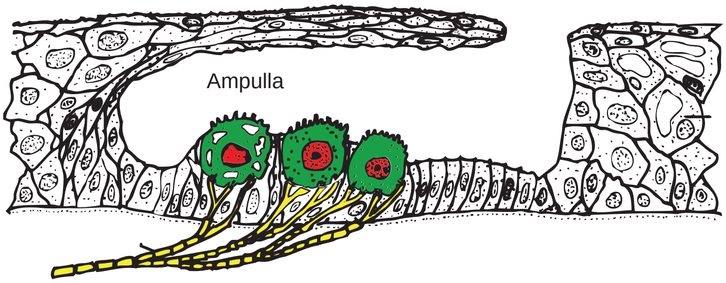


Figure 9 A section of an ampullary organ from the transparent catfish (*Kryptopterus bicirrhus*).

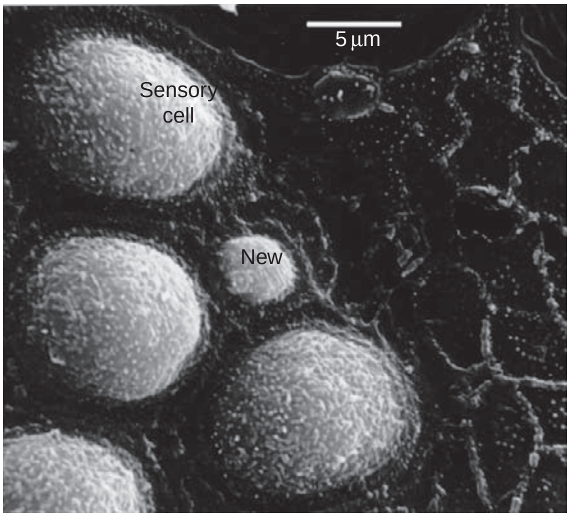


Figure 10 Scanning electron micrograph of an opened ampulla of the transparent catfish (*Kryptopterus bicirrhus*) showing the surface of a few sensory cells with a few small microvilli. The size of the surface of the cells differs considerably, probably due to the cells in different phases of sensory cell turnover.

sensory organ. Instead, the sense organ is covered by a plug of loosely packed epidermal cells. The sensory cells open into an intraepidermal cavity. The sensory cells are different from the ampullary sensory cells, both in structure (see **Figure 11**) and function. It is believed that tuberous organs also have evolved from the mechanosensory hair cell. The primary function of the tuberous sensory cells is believed to be communication by detecting signals from the weak electric organs in other fishes.

Tuberous Organs in Mormyrids

Two distinct types of tuberous organs are found in mormyrids, the so-called knollenorgan and mormyromast (**Figure 11**). Each knollenorgan is composed of three to four sensory cells and a number of supporting cells. These sensory cells lie in a narrow cavity, or sensory chamber, which is limited externally by flattened epidermal cells. Below the sensory cells, a number of supporting cells are found. Some pear-shaped supporting cells, situated below the sensory cells, are filled with rough endoplasmic reticulum, indicating that they are involved in production of the jelly that fills the sensory chamber. The sensory cells are large, up to 40 µm in diameter with an apical or central nucleus. Most of the surface is covered by a large number of microvilli up to 3 µm in length and 0.1–0.15 µm in diameter. A large number of mitochondria are found below the microvilli. Basally, the sensory cell sits on a platform in which the synapses are found. All sensory cells in an organ are innervated by a single nerve fiber that divides to form several boutons on the individual sensory cells.

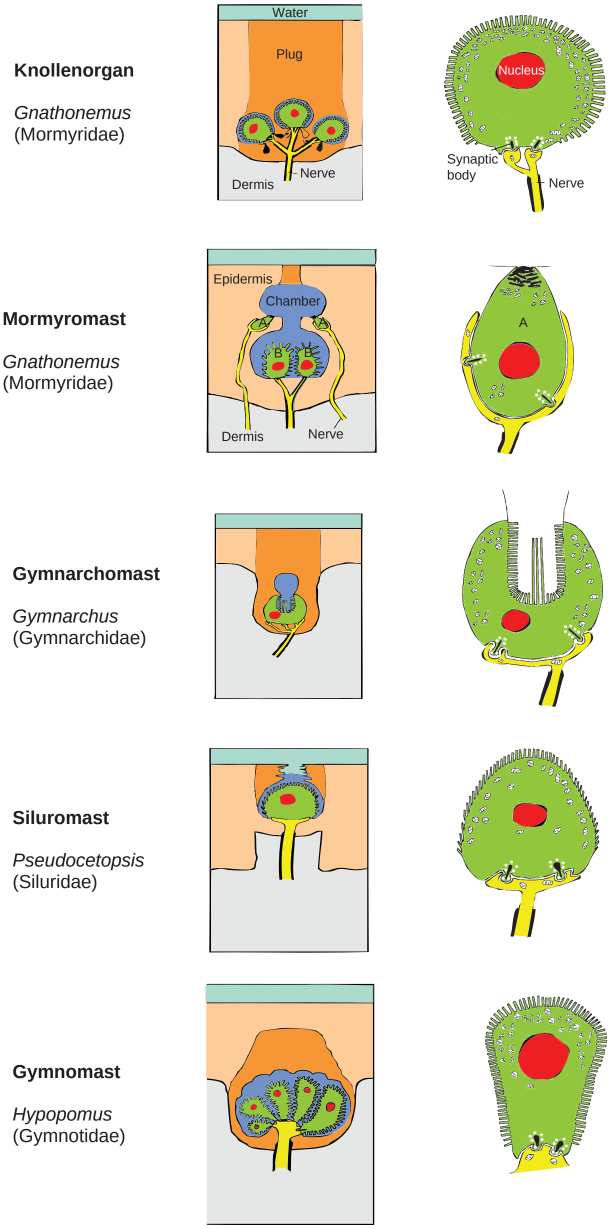


Figure 11 Five different tuberous organs and their sensory cells found in teleost fishes.

The mormyromast is a complicated structure involving at least two different sensory cells with separate innervation. The mormyromast has two chambers that are connected by a narrow canal. The shallower chamber contains type-A sensory cells, while the deeper chamber has type-B sensory cells, which look like the sensory cells of the knollenorgan. Six to thirteen type-A cells lie in a circle in the outer (superficial) chamber and these are separated by supporting cells. The apical part of this pear-shaped type-A cell is characterized by numerous membrane foldings. The type-A cell is embraced by finger-like projections of the afferent nerve that stretches up along the lateral part of the cell. The synaptic sheets indicate the position of synapses. The type-B sensory cell sits on a platform, with the majority of the cell surface exposed to the deep chamber. Numerous long and slender microvilli stretch out into the chamber jelly. The type-B cells form basal contacts with nerve endings, which originate from a single fiber.

Tuberous Organ in *Gymnarchus*

Gymnarchus has a peculiar sensory cell in the tuberous organ, which has been called a gymnarchomast. A single sensory cell opens into a jelly-filled chamber. The cell has an apical depression filled with numerous microvilli. The microvilli originating from the bottom of the depression form a long projection into the apical cavity. The cell nucleus is located basally. A single nerve fiber splits to form several nerve endings at the basolateral part of the sensory cell. In some gymnarchomasts more sensory cells are seen located together, and organs with more than one sensory cell have been termed gymnarchomast type II. However, it is uncertain whether this type of organ is a further developmental stage of the gymnarchomast type I.

Silurid Tuberous Organ

In silurids, tuberous organs have only been described from one species, *Pseudocetopsis*. This organ, the siluromast, is located rather superficially in the epidermis (**Figure 11**). It contains a jelly-filled cavity with a single spherical sensory cell having a diameter of about 25 μm and numerous apical microvilli that increase in height toward the cell apex. The nucleus has a central position. A single afferent nerve flattens out at the base of the sensory cell.

Gymnotid Tuberous Organs

In gymnotids the tuberous organ, the gymnomast, is described in a number of species (**Figure 11**). A cavity contains 25–35 elongate sensory cells. The exposed lateral and apical surfaces of these cells are enlarged by microvilli that stretch out into the gymnomast chamber. In some species, a single nerve fiber splits to innervate all sensory cells with several nerve boutons. In other species, such as *Hypopomus*, the nerve ending is a single large knob that forms synapses with all the sensory cells in a single gymnomast.

Monotreme Electroreceptive Organs

The monotreme mammals include the platypus (*Ornithorhynchus anatinus*) and two species of spiny anteaters (*Tachyglossus aculeata* and *Zaglossus bruijnii*). In all species, the bill or snout contains specialized mucous glands with electroreceptive nerve endings. The platypus has about 40 000 electroreceptive mucous glands, while *Zaglossus* has about 3000 and *Tachyglossus* about 100.

In platypus the electroreceptive organs are distributed in narrow stripes on the entire bill, while the spiny anteaters have their organs on the outermost part of the snout. The sense organs always consist of a large coiled mucous gland, where naked nerve endings open into a capsule of epidermal cells, which forms around the middle portion of the mucous gland.

See also: **Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction; Physiology of Ampullary Electrosensory Systems; Physiology of Tuberous Electrosensory Systems.

Further Reading

Andres KH and von Düring M (1988) Comparative anatomy of vertebrate electroreceptors. *Progress in Brain Research* 74: 113–131.

Bullock TH (1982) Electroreception. *Annual Review in Neurosciences* 5: 121–170.

Bullock TH and Heiligenberg W (eds.) (1986) *Electroreception*. New York: Wiley.

Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) (2005) *Electroreception*. New York: Springer.

Bullock TH, Northcutt RG, and Bodznick DA (1982) Evolution of electroreception. *Trends in Neurosciences* 5: 50–53.

Jørgensen JM (2005) Morphology of electroreceptive sensory organs. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, *Springer Handbook of Auditory Research*, pp. 47–67. New York: Springer.

Jørgensen JM and Bullock TH (1987) Organization of the ampullary organs of the African knife fish *Xenomystus nigri* (Teleostei: Notopteridae). *Journal of Neurocytology* 16: 311–315.

Lissmann HW and Mullinger AM (1968) Organization of ampullary electric receptors in Gymnotidae (Pisces). *Proceedings of the Royal Society Series B* 169: 345–37.

Northcutt RG (1986) Electroreception in nonteleost bony fishes. In: Bullock TH and Heiligenberg W (eds.) *Electroreception*, pp. 257–258. New York: Wiley.

Northcutt RG and Bemis WE (1993) Cranial nerves of the coelacanth, *Latimeria chalumnae* [Osteichthyes: Sarcopterygii: Actinistia], and comparisons with other craniata. *Brain, Behavior and Evolution* 42(supplement 1): 1–76.

Szabo T (1974) Anatomy of the specialized lateral line organs of electroreception. In: Fessard A (ed.) *Electroreceptors and Other Specialized Receptors in Lower Vertebrates. Handbook of Sensory Physiology* III/3, pp.13–58. Berlin: Springer.

Physiology of Ampullary Electrosensory Systems

MH Hofmann, University of Bonn, Bonn, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Sources of Electric Fields
Morphology of the Ampullary Organs
Physiology of the Receptors
Information Processing in the Hindbrain
Higher Electrosensory Areas
Electrosensory-Guided Behavior
Further Reading

Glossary

Alternating current (AC) fields Electrical fields that alternate constantly, e.g., electrical power lines typically carry sinusoidally changing voltages with a frequency of 50–60 Hz.

Direct current (DC) fields Steady electric fields, e.g., batteries supply a more or less constant voltage over their lifetime.

Dorsal octavolateral nucleus (DON) The target of primary afferent electrosensory fibers in the hindbrain of all nonteleost species.

Introduction

The ampullae of Lorenzini were described in 1678 by the Italian physician Stefano Lorenzini in elasmobranchs (**see also Detection and Generation of Electric Signals**: Electric Organs). Their function remained elusive for a long time, until behavioral and physiological studies showed that the receptor cells at the base of the ampullae respond to mechanical stimulation, temperature changes, salinity changes, and electric fields. About 50 years ago studies established that the detection of weak electric fields is the natural function of the ampullae.

Initially thought to be a specialization in elasmobranchs, electrosensory ampullae have been found in all basal fish groups (**Figure 1**). Electroreception was lost, however, in neopterygian fish (teleosts, including gars and bowfin), but re-evolved in some groups of teleosts (catfish, gymnotids, and mormyrids). Electroreception was also lost with the transition to a terrestrial life. Larval salamanders and gymnophians still have electroreceptors, but anurans and all other land vertebrates are not known to have an electric sense. Notable exceptions are *Echidna* and the platypus. These primitive mammals have modified trigeminal nerve fibers sensitive enough to pick up weak electric fields from prey items.

The widespread occurrence of electroreception in basal fish groups suggests that this sensory system played an important role in early vertebrate evolution. Besides some behavioral evidence of electroreception in lobster, there are no invertebrates known to have an electric sense.

Some fish have electric organs and can produce much larger electric fields (**see also Detection and Generation of Electric Signals**: Electric Organs). They probe the environment with this field and can detect any object that distorts this field (**see also Detection and Generation of Electric Signals**: Active Electrolocation). These fields are created actively by fish, which are called active electrosensory fish in contrast to the passive electrosensory fish that only sense external fields. The other difference is that the electric organ discharges consist of higher frequencies that are above the upper frequency limit for detection by the ampullae of Lorenzini. For this reason, the active and passive electrosense are sometimes also called high- and low-frequency electroreception, respectively. Natural stimuli of the ampullae of Lorenzini, therefore, are weak electric fields from animate and inanimate sources that have a low frequency.

Sources of Electric Fields

There are many sources of electric fields in the aquatic environment. Most important are probably those produced by other animals. Every animal has an electric field around its body, which is strongest in the head and gill region. Measured directly at the skin, it can be as high as a few millivolts in freshwater teleosts, but is usually 10 times weaker in seawater elasmobranchs. Invertebrates, a major prey of many fish, also show electric fields comparable to those of fish.

These ever-present, steady, direct current (DC) fields are modulated by the animal's own movement. Respiration is an important source of these modulations, but any movement will change the field to some degree.

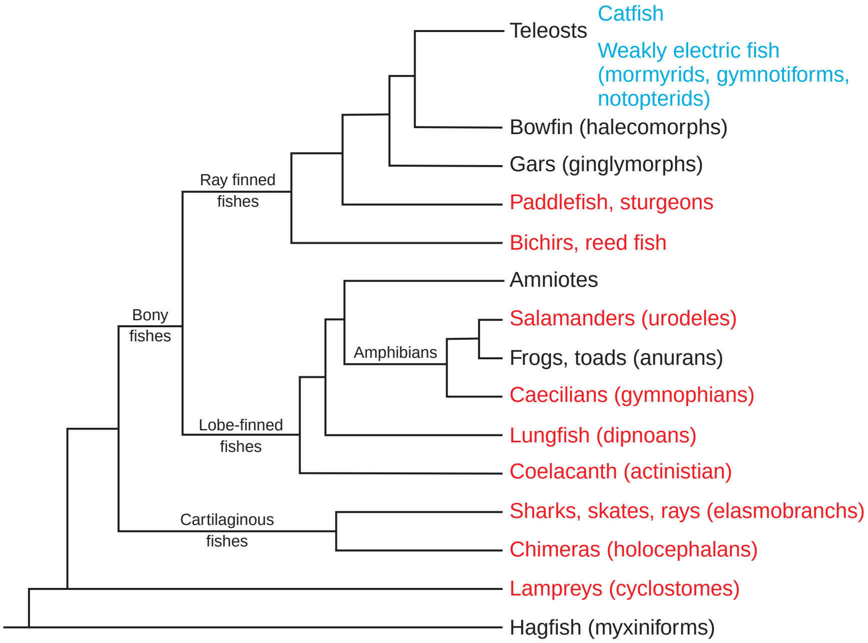


Figure 1 Cladogram of the major fish and amphibian groups, with the presence of electroreception indicated in color. All group names in color represent groups with ampullary organs. The red color represents fish groups with primitive, homologous ampullary organs and the blue-coded groups represent teleosts with derived (re-evolved) ampullary electroreceptors. Note that most teleosts do not have electroreception. Only the few groups shown in the figure re-evolved electroreception.

In addition, the electrical activity of muscles can directly add higher-frequency components. **Figure 2** shows an example of an electric field from a small water flea (*Daphnia*). Both steady DC and higher-frequency, alternating current (AC) fields are present. Because ampullary electroreceptors adapt to steady fields, they do not respond to pure DC electric fields. However, there is always a relative movement between the source and the receiver, so the electric potential at a given receptor changes over time when a source passes by. Consequently, the DC field is transformed into an AC event when the source passes by a receptor, as shown in **Figure 2(a)**.

There are also larger-scale electric fields produced by geophysical processes. Any physical or chemical boundary will produce electric fields that could be used by electrosensory animals for orientation and navigation. Examples are filtration potentials when water is flowing through the substrate or potentials arising between waters with different temperature or salinity. There are also streaming potentials that are proportional to water currents in a river. There is some evidence that catfish and sharks use this information, but little is known about how widespread the detection of large-scale electric fields is.

Some fish can produce larger electric fields with specialized organs (**see also Detection and Generation of Electric Signals**: Electric Organs). Well known are the strong discharges of electric eels and some rays (*Torpedo*) used as a defense or to stun prey. Other groups of fish

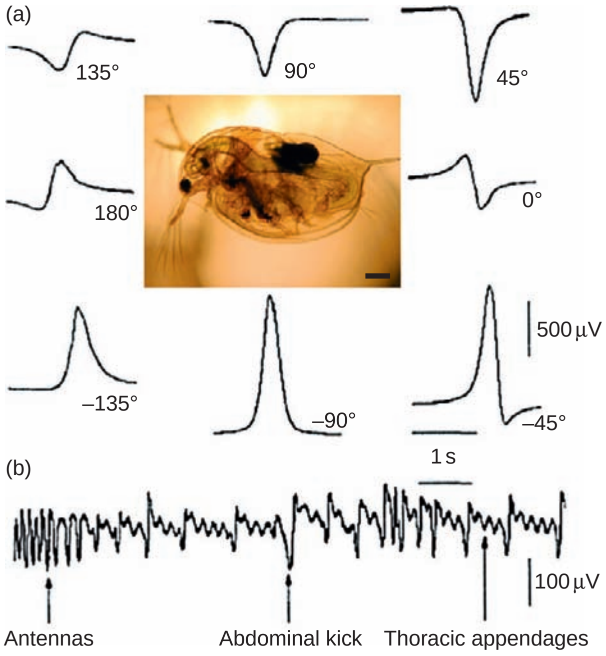


Figure 2 Electrical potential of a water flea (*Daphnia,* scale = 200 μm). (a) Electrical signal of a live *Daphnia* that was fixed on the tip of a stick and swept close to a recording electrode with different body orientations. (b) Higher amplification of higher-frequency components caused by various movements of the appendages. Modified from Wilkens LA (2004) *The Senses of Fishes.* New Delhi: Narosa Publishing House.

have evolved weakly electric organs and use them for electrolocation and navigation (**see also Detection and Generation of Electric Signals**: Active Electrolocation). Although these organs produce higher-frequency electric fields well above the frequency range of the ampullary electroreceptors, they can be detected by very sensitive ampullary receptors. Examples include some catfish that specialize in locating and preying on weakly electric fish. Some rays also produce weak discharges in the low-frequency range and use them to communicate with conspecifics during courtship.

Morphology of the Ampullary Organs

The ampullae of Lorenzini are located in the head and sometimes also on the trunk. They consist of either short (freshwater fish) or long (seawater fish) canals that are connected to the outside with a pore. **Figure 3** gives an example of a fish with a very high number of pores, the paddlefish. Individual ampullae are constantly multiplying by division (**Figure 3**(**b**)). For more details on ampullary morphology, the reader is referred to **Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs. Here, we focus on aspects that are important for the encoding of electric fields.

To measure an electric field, a two-point measurement is necessary. For ampullary organs, one point is the pore in the skin. Because the canal wall has high resistance and the gel inside the canal has low resistance, the canal acts as a cable, carrying the electric potential to the apical surface of the electroreceptor. The receptor cell then measures the voltage between this apical potential and the potential below its basal membrane.

Figure 4(**a**) shows a schematic drawing representing a fish with long canals in an electric field. Electrosensory fish that live in seawater have long canals and the skin has relatively low resistance, that is, the fish is transparent to electric fields. The electrodes to the left and right of the fish produce a quasi-homogenous field. If the voltage between the electrodes is 10 μV and the distance between the electrodes is 10 cm, the field strength in between can be expressed as 1 $\mu V\ cm^{-1}$. Because the length of the canal 1 in **Figure 1**(**a**) is 2 cm, the voltage drop across the receptor is 2 μV. The voltage drop at canal 2 is zero because the canal is perpendicular to the field lines and parallel to the isopotential lines. In summary, the length

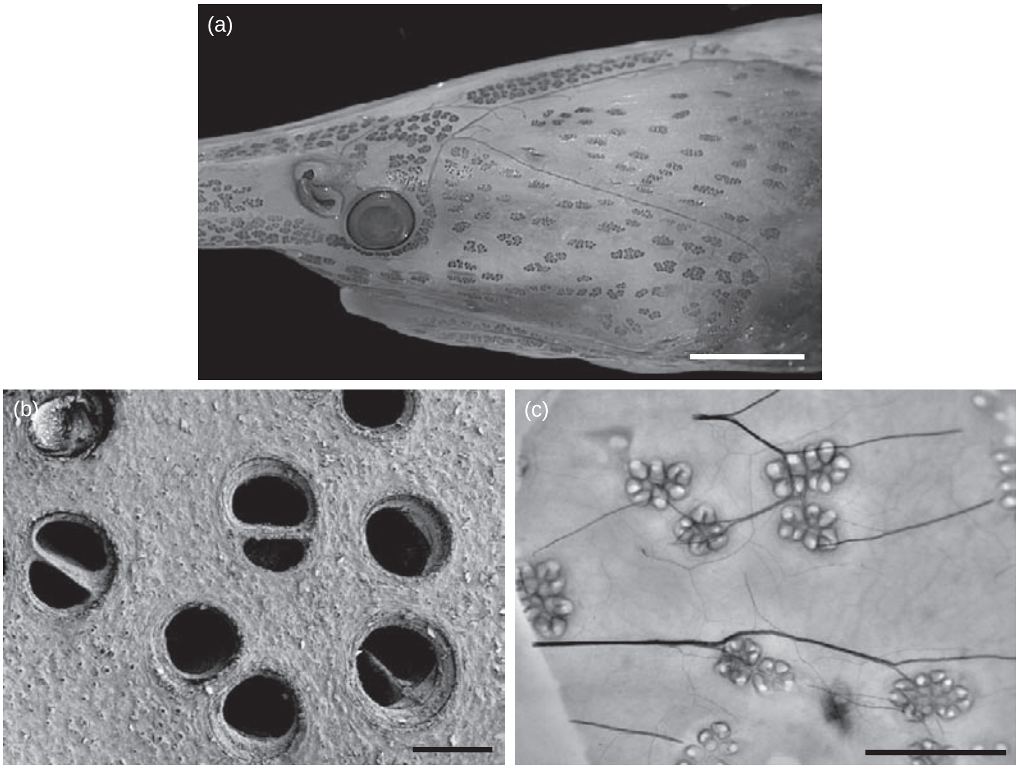


Figure 3 (a) Head of a paddlefish with ampullae of Lorenzini and lateral line canals stained. The ampullae are organized in clusters. Up to 75 000 individual pores have been counted in a larger specimen (scale = 2 cm). (b) Scanning electron microscope of individual pores in the paddlefish. Ampullae are constantly added during growth by division. Several stages of divisions are clearly visible (scale = 0.5 mm). (c) Sudan stain of a skin sample after bleaching with peroxide. The pore clusters are heavily innervated by large, myelinated fibers (scale = 1 cm).

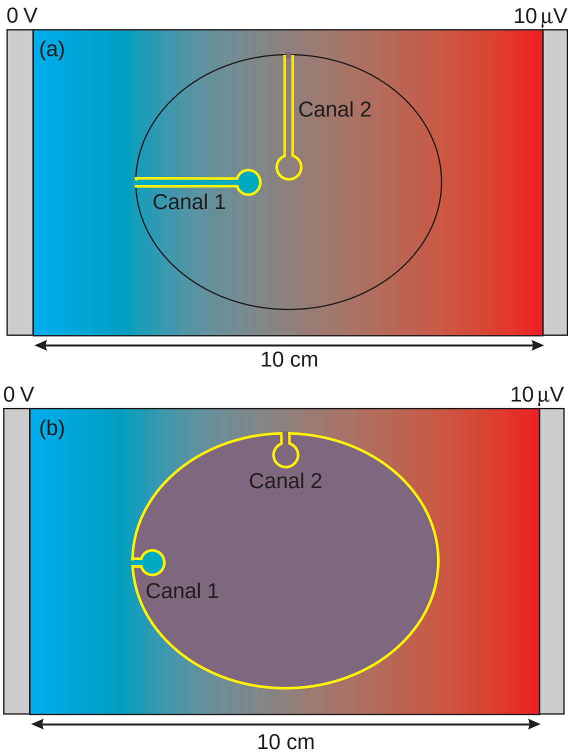


Figure 4 The principle of measuring electric fields with ampullary organs. (a) A saltwater fish with long canals and a low-resistance skin. (b) A freshwater fish with short canals and a high-resistance skin (indicated in yellow). See text for further details.

of the canal determines sensitivity and the orientation of the canal provides information about the orientation of the electric field.

There is one more important aspect to reiterate. Electroreceptors are insensitive to DC fields because they rapidly adapt. Only changes in field strength can be detected, by varying either the source potential or its orientation. If the fish depicted in **Figure 4**(**a**) rests on the bottom, neither receptor would be able to measure anything. Only if the fish starts moving and changing direction would the receptors detect changes in electric field strength.

In freshwater, fish have a skin with relatively high resistance and electric fields cannot penetrate well. However, since the resistance of the body fluids inside the fish is low, the entire fish would assume a potential that is the average of the potentials on the skin (**Figure 4**(**b**)). As a consequence, the canals can be short and their length does not change the sensitivity. Otherwise, the situation is similar to the seawater fish described above.

Although there are always large-scale electric fields in the natural environment, it is currently not well investigated to what extent fish use this information for orientation. It is well established that small electric fields from other fish or invertebrates are detected. **Figure 5** shows a scenario where a small source dipole representing a prey fish is close to a larger electrosensory fish.

If we consider a freshwater fish with a short canal and high skin resistance, the receptor would measure the difference between the potential at the pore and the mean body potential inside. A small dipole source would not have any measurable effect on the potential inside the fish. However, if the dipole is close to one pore, the corresponding receptors at the base of the ampulla would certainly respond to that. The situation is similar in seawater fish with long canals. Because of the remote position of the end of the long canal, the potential there would not be affected by a small dipole close to the pore. As a consequence, the length of the canal is not important, in contrast to the situation in a large field.

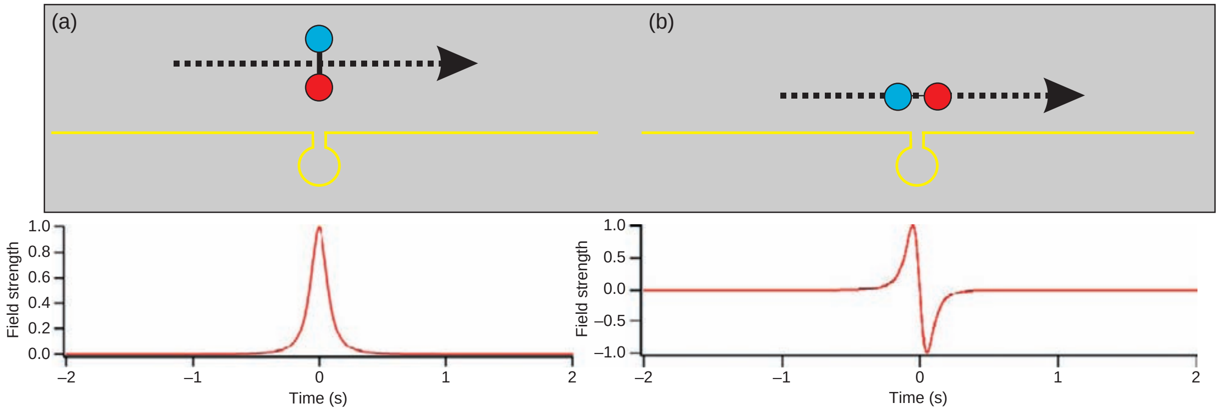


Figure 5 An electrical dipole with a DC field is moved over a receptor with the dipole oriented either perpendicular to the movement direction (a) or parallel to it (b). In the perpendicular mode, the positive pole (red) is always closer to the fish and the signal never becomes negative (graph of (a)). In the parallel mode, the receptor is first closer to the positive (red) pole, but after passing the center of the dipole, the receptor is closer to the negative (blue) pole and the signal at the receptor becomes negative. The field strength is normalized.

The voltage at the pore also depends on the orientation of the dipole. If the dipole is oriented perpendicular to the skin, the closer pole always dominates. If the dipole is oriented parallel to the skin and centered over the pore, both poles cancel each other out and the voltage at the pore is zero. In both cases, however, a static source would not be detected due to the rapid habituation of the receptors. If the source is moving parallel to the skin surface, however, the signal at the pore changes in time in a characteristic way as shown in **Figure 5**. The frequency range of this signal can be determined by calculating the Fourier transformation and it depends on the distance to the dipole and the relative velocity. Frequencies up to 10–30 Hz can be present in realistic situations. This correlates with the upper frequency limit for detection as measured by applying sinusoidal fields with different frequencies.

Physiology of the Receptors

The receptor cells at the base of the ampullae are innervated by afferent nerve fibers that carry information to the hindbrain. The voltage across the receptor cell is greatly amplified and regulates neurotransmitter release at the synapse to the afferent nerve fiber. The nerve fiber produces spontaneous action potentials and the rate of these action potentials is modulated by the neurotransmitter. Apart from the high amplification, the response to electric fields is also filtered. The system is insensitive to DC fields and responds best in the range between 1 and 10 Hz. There is hardly any response above 50 Hz. Filter properties are well investigated in the paddlefish, in which the receptors resemble a derivative filter in combination with a low-pass filter. A derivative filter is a filter where the gain is proportional to the frequency of the signal. This filter is not designed to cut off frequencies, but gradually attenuates lower frequencies.

In all nonteleost species, afferent fibers respond with increased spike rate if the pore becomes more negative and with a decreased spike rate if the pore becomes positive. In teleosts, the response is reversed; spike rate increases if the pore becomes more positive.

Without stimulation, the electroreceptors produce a constant spontaneous rate of action potentials. Detailed analysis of the spike train, however, reveals the presence of oscillations. Apparently, the membrane potential of the receptor cell itself is subject to oscillations that modulate the spike rate of the afferent fiber. Such oscillations are also known to be present in the high-frequency electroreceptors of active electrosensory fish and in mechanosensory vestibular and auditory hair cells, where they contribute to frequency tuning. In the passive electrosensory system, they are thought to increase sensitivity.

Information Processing in the Hindbrain

Electrosensory afferent fibers enter the hindbrain and terminate in the dorsal octavolateral nucleus (DON). Here, they make synaptic contact with large, multipolar secondary neurons that in turn project to the midbrain. These multipolar DON cells also receive extensive input from the cerebellum through the crista cerebellaris. Response properties of these secondary neurons are well investigated in a number of species. In catfish, two populations of neurons are present, which increase their spike rate in response to either positive or negative stimuli. In skates, secondary neurons have focal receptive fields with an inhibitory surround or sometimes two focal points, one inhibitory and the other excitatory. In the paddlefish, response properties of secondary cells are very similar to primary afferents.

One major function of the DON seems to be to filter out unwanted noise. The fish's own movement modulates its own electric field and these movements are detected by their electroreceptors. There are two known mechanisms for canceling out such unwanted noise. Modulations of the fish's own electric field affect all electroreceptors and such noise common to all of them can be filtered out by subtracting the average signal of all receptors from the signals at individual receptors. Only signals different from this common mode would then be transmitted to higher brain areas.

The other mechanism is more complex because it involves an adaptive filter. The modulation of the electric field due to respiration cannot easily be predicted. What is predictable is its timing. Any signals that are reliably correlated with the timing of inspiration and expiration are probably not due to external sources, and through plasticity, the circuit filters out these signals. If an electrical stimulus is given in phase with the fish's own respiration, hindbrain neurons initially respond to it, but slowly adapt with repeated presentation. Parallel fibers from the cerebellum are a major source of input to the secondary hindbrain neurons, and they provide the signal that marks the timing of respiration. The signal coming from the cerebellum is thought to provide a mirror image of the actual electrosensory input caused by respiration and this is used to cancel the sensory input. Indeed, if the artificial stimulus that was paired with the respiration is suddenly stopped, a mirror image of the initial response becomes visible and this also eventually disappears with time.

Higher Electrosensory Areas

Secondary neurons in the hindbrain DON send information to the midbrain. In teleosts, the major target is the torus semicircularis. In elasmobranchs, most fibers terminate more laterally, in an area called the lateral mesencephalic nucleus, but some fibers also project directly to the mesencephalic tectum. In the paddlefish, all three targets receive direct projections from the DON (**Figure 6**). It is not well known how electrosensory information is processed and integrated with information from other modalities. Electrophysiological recordings in the fore- and midbrain have mostly been made to localize electrosensory areas, but responses to different stimuli have rarely been investigated.

In the catfish, there are different populations of neurons with different best frequencies and different latencies in the torus semicircularis. In the rostro-caudal axis, there is evidence for a somatotopic arrangement, that is, neurons with receptive fields on the head are found more rostrally in the torus semicircularis and neurons with receptive field on the trunk more caudally. A similar somatotopic organization is found in the lateral mesencephalic nucleus of an elasmobranch.

One of the biggest problems for the electrosensory system, as opposed to the visual system, is the lack of directional sensitivity of the receptors. In the eye, the lens focuses the image onto the retina and individual photoreceptors only respond to a tiny fraction of the image. In the electrosensory system, the most important effect on response magnitude is the distance from the source to the receptor, but not its location in space. For a large fish capturing tiny water fleas, such as the paddlefish, the location of the prey is important and therefore we expect central mechanisms to enhance spatial resolution. In paddlefish, an edge detection filter in the tectum may be doing just that. Similar to algorithms used widely in digital image processing, tectal neurons seem to calculate the third derivative of electrosensory signals and thus are similar to an algorithm developed for computer vision, the Laplacian of Gaussian filter, that is used to detect edges in images.

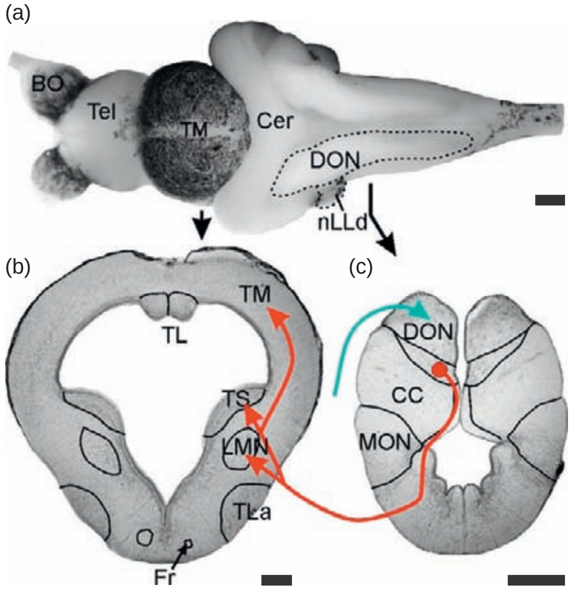


Figure 6 Central projections of the electrosensory system in a paddlefish. (a) Dorsal view of the brain with the large DON in the hindbrain. Arrows indicate the level of the frontal cross sections shown in (b) and (c). Primary afferent fibers first enter the DON in the hindbrain (blue arrow in (c)). Secondary neurons in the DON project to several areas of the midbrain (red arrows from (c) to (b)). BO, bulbus olfactorius; CC, crista cerebellaris; Cer, cerebellum; DON, dorsal octavolateral nucleus; Fr, fasciculus retroflexus; LMN, lateral mesencephalic nucleus; MON, medial octavolateral nucleus; nLLd, dorsal branch of the anterior lateral line nerve; Tel, telencephalon; TL, torus longitudinals; TLa, torus lateralis; TM, tectum mesencephali; TS, torus semicircularis. Scale = 1 mm. Modified from Wilkens LA and Hofmann MH (2007) The paddlefish rostrum as an electrosensory organ: A novel adaptation for plankton feeding. *BioScience* 57: 399–407.

Electrosensory-Guided Behavior

The first evidence that some fish can detect electric fields came from behavioral observations. Catfish avoid larger metal objects and sharks can be attracted to small dipole fields resembling prey. Avoidance and prey capture are probably the most important and best-investigated behaviors. In many situations, the electrosense is the best for sensing prey hidden in the substrate. Like a metal detector, skates and rays scan the substrate for hidden prey, and the visual system is certainly not capable of locating objects underneath the fish's disk. Olfaction and the mechanosensory lateral line are certainly used as well, but for precise localization, the electrosense is preferred. Sharks will strike at an electrical dipole hidden in the sand even if an odor source is located a small distance from the dipole.

Paddlefish are related to sturgeons, but in contrast to sturgeon, which scan the substrate for hidden invertebrates, the paddlefish feeds on tiny water fleas, the major component of freshwater zooplankton. These water fleas can be located in three-dimensional (3D) space using electroreceptors distributed throughout the dorsal and ventral surface of the head. More importantly, the electric field of the water flea is extremely small, and the paddlefish therefore has the highest sensitivity and highest number of receptors of any freshwater electrosensory fish. Their peculiar rostrum carries a large number of receptors to detect the prey well in front of the mouth. Smaller paddlefish feed on individual *Daphnia* and it is critical not to spend too much energy catching each one.

Paddlefish can catch *Daphnia* with their electrosense alone. Even in complete darkness, the number of *Daphnia* caught does not decline. There is one advantage of using the electrosense over the visual system. Murky water contains many particles, but only live prey produce an electric field. This means that small dipole sources always mean prey, but the visual system would suffer from false alarms. Paddlefish therefore can feed in murky waters where other fish cannot.

Another important function of the electrosense is to detect predators. A large approaching dipole field almost always means danger. There are few studies on avoidance responses in electrosensory fish, but large metal objects create corrosion potentials and can scare fish away. For fish conservation it is important to assess the impact of man-made submerged structures on electrosensory fish. Catfish and sturgeons are an important part of the freshwater ecosystem and sturgeons make extensive migrations to their spawning grounds. Man-made structures, especially metallic ones, may have great impact on spawning migrations, but there are virtually no studies on this topic.

There is little information on whether the electrosense is used for orientation and navigation. Large-scale electric fields are present in every aquatic environment and could be used as landmarks. Catfish align to these fields and artificial fields could be used to locate hiding places. However, it is not known to what extent catfish use large-scale fields in a natural environment.

An interesting hypothesis is that fish sense the weak electric field that is induced by swimming through the Earth's magnetic field. Elasmobranchs have the capacity to detect these induced fields. In fact, there are observations that sharks follow magnetic field lines and magnetic anomalies in the seafloor. There are also more controlled experiments on rays that show orientation to magnetic fields. Whether they detect these fields by sensing the electric field induced during swimming or whether they have other magnetosensors is unknown.

In some elasmobranchs electroreceptors are also used for communication. Skates (Rajidae) possess electric organs along the sides of their tail. In contrast to the strong electric discharges of the torpedo ray, skates produce only weak electric fields used mainly during courtship. There is also evidence that electroreception is used for communication in the absence of electric organs. Stingrays (*Urolophus halleri*) do not have electric organs, but strong modulations of their normal DC electric field caused by respiratory movements are detected by conspecifics buried in the sand.

See also: **Detection and Generation of Electric Signals**: Active Electrolocation; Electric Organs; Morphology of Electroreceptive Sensory Organs.

Further Reading

Bodznick D and Montgomery JC (1992) Suppression of ventilatory reafference in the elasmobranch electrosensory system: Medullary neuron receptive fields support a common mode rejection mechanism. *Journal of Experimental Biology* 171: 127–137.

Bodznick D and Montgomery JC (2005) The physiology of low-frequency electrosensory systems. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 132–153. New York: Springer.

Bodznick D, Montgomery JC, and Carey M (1999) Adaptive mechanisms in the elasmobranch hindbrain. *Journal of Experimental Biology* 202: 1357–1364.

Bodznick DA and Boord RL (1986) Electroreception in chondrichthyes: Central anatomy and physiology. In: Heiligenberg W and Bullock TH (eds.) *Electroreception*, pp. 225–256. New York: Wiley.

Bullock TH (1974) An essay on the discovery of sensory receptors and the assignment of their functions together with an introduction to electroreceptors. In: Fessard A (ed.) *Electroreceptors and Other Specialized Receptors in Lower Vertebrates*, pp. 1–12. Berlin: Springer.

Bullock TH and Hopkins CD (2005) Explaining electroreception. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 1–4. New York: Springer.

Finger TE (1986) Electroreception in catfish: Behavior, anatomy and electrophysiology. In: Heiligenberg W and Bullock TH (eds.) *Electroreception*, pp. 287–314. New York: Wiley.

Hofmann MH, Chagnaud BP, and Wilkens LA (2009) An edge detection filter improves spatial resolution in the electrosensory system of the paddlefish. *Journal of Neurophysiology* 102: 797–804.

Hofmann MH and Wilkens LA (2005) Temporal analysis of moving d.c. electric fields in aquatic media. *Physical Biology* 2: 23–28.

Hopkins CD (2005) Passive electrolocation and the sensory guidance of oriented behavior. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 264–289. New York: Springer.

Kalmijn AJ (1974) The detection of electric fields from inanimate and animate sources other than electric organs. In: Fessard A (ed.) *Handbook of Sensory Physiology*, vol. 3, pp. 147–200. New York: Springer.

Northcutt RG (1986) Electroreception in nonteleost bony fishes. In: Heiligenberg W and Bullock TH (eds.) *Electroreception*, pp. 257–285. New York: Wiley.

Tricas TC, Michael SW, and Sisneros JA (1995) Electrosensory optimization to conspecific phasic signals for mating. *Neuroscience Letters* 202: 129–132.

Wilkens LA (2004) *The Senses of Fishes*. New Delhi: Narosa Publishing House.

Wilkens LA and Hofmann MH (2005) Behavior of animals with passive, low frequency electrosensory systems. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 229–263. New York: Springer.

Wilkens LA and Hofmann MH (2007) The paddlefish rostrum as an electrosensory organ: A novel adaptation for plankton feeding. *BioScience* 57: 399–407.

Physiology of Tuberous Electrosensory Systems

ES Fortune, Johns Hopkins University, Baltimore, MD, USA

MJ Chacron, McGill University, Montreal, QC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Amplitude Coding
Time Coding
Physiology of ELL Neurons Receiving Input from Amplitude-Sensitive Electroreceptors
Physiology of Midbrain Neurons: Integration of Sensory Information
Conclusion
Further Reading

Glossary

Amplitude modulation Refers to a signal in which changes in amplitude carry sensory information.

Corollary discharge Refers to a copy of a motor command that is sent from motor areas to sensory areas in the brain. It is often used to predict and eliminate sensory responses to self-generated stimuli.

Electrosense Ability to detect electric fields. A passive electrosense is one in which external electric fields are detected, whereas an active electrosense is one in which self-generated electric fields are detected.

Feedback Refers to projections from central brain areas back to more peripheral sensory areas.

Frequency modulation Refers to a signal in which changes in instantaneous frequency carry sensory information.

Information theory Refers to the mathematical theory of communication developed by Claude Shannon that is used in a variety of applications today.

Neural code Refers to the patterns of neural activity and transformations by which sensory input to motor outputs to give rise to behavioral responses by the organism.

Phase locking Refers to the tendency of certain neurons to fire at a preferred phase of a periodic signal.

Rate code Refers to a neural code in which information is carried solely by the firing rate (i.e., the number of action potentials per unit time) of a neuron.

Temporal code Refers to a neural code in which information is carried by the specific timings of action potentials.

Introduction

Weakly electric fish produce weak electric fields using specialized electrogenic organs. The electrical output of the organ is known as the electric organ discharge, or EOD (**see also Detection and Generation of Electric Signals:** Electric Organs and Generation of Electric Signals). There are two phylogenetically independent orders of weakly electric fishes: the Gymnotiformes of South America and the Mormyriformes of Africa. These clades evolved within two distantly related groups, the ostariophysans and osteoglossomorphs, respectively.

Gymnotiform and mormyriform fishes can both be classified as either pulse type or wave type, depending on the temporal structure of their EOD. Wave-type weakly electric fish generate continuous, highly periodic EOD waveforms (**Figure 1(a)**), whereas pulse-type weakly electric fish emit short, typically less than 2 ms, stereotyped pulses with longer periods of silence, typically greater than 10 ms, between pulses (**Figure 1(b)**). In some species the inter-pulse intervals are nearly constant, whereas in other species the inter-pulse intervals can vary over orders of magnitude.

Both wave- and pulse-type weakly electric fish can detect perturbations of the autogenous EOD using tuberous electroreceptors that are distributed on their skin surface: this is referred to as active electrolocation since the animal actively generates the sensory signal (**see also Detection and Generation of Electric Signals**: Active Electrolocation). Tuberous electroreceptors also detect the EODs of other individuals in the context of communication (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish). There are several classes of tuberous electroreceptors that encode different features of the animal's electric field. All species have at least two classes of tuberous electroreceptors, one type (amplitude coding) that encodes EOD amplitude modulations (AMs), and another type (time

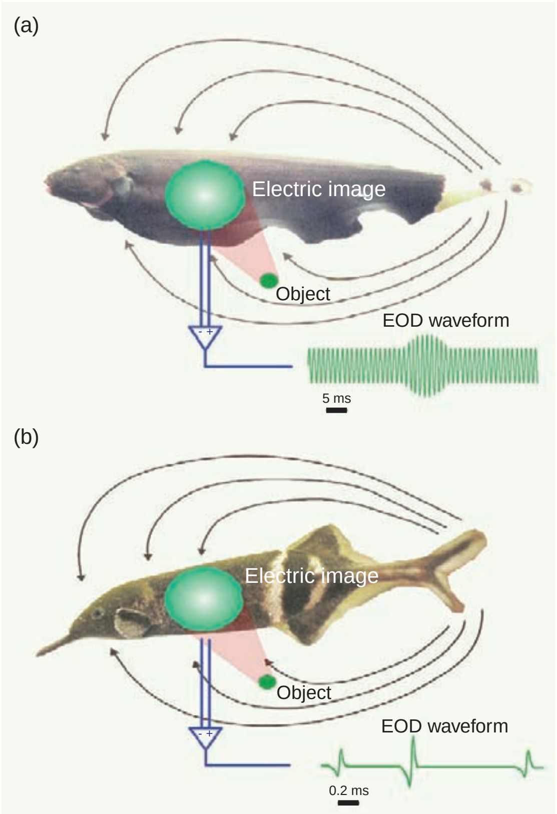


Figure 1 The principle of active electrolocation in weakly electric fish. (a) The wave-type gymnotiform species *Apteronotus albifrons* emits a highly periodic quasi-sinusoidal EOD. (b) The pulse-type mormyriform species *Gnathonemus petersii* emits pulses of electricity with strong variability in the interpulse interval. In both cases, one can easily record the EOD by placing a pair of metal wires close to the animal and connecting them to an amplifier. Objects with a conductivity different than that of the surrounding water (e.g., rocks, plants, other fish, and plankton) will distort the EOD; for example, a conductive object will locally increase the EOD amplitude. The projection of this distortion on the animal's skin surface is referred to as the electric image.

coding) that encodes EOD timing and frequency modulations (FMs). Information from these electroreceptors is transmitted to the electrosensory lateral line lobe (ELL) in the hindbrain. ELL neurons in turn project to the electrosensory midbrain and higher-order areas.

We first review the characteristics of amplitude- and timing-sensitive peripheral electroreceptive neurons in wave- and pulse-type gymnotiform and mormyriform weakly electric fishes. We then review the characteristics of ELL neurons with a particular emphasis on the role of feedback pathways in wave-type gymnotiforms. We conclude by highlighting some of the characteristics of higher-order neurons and their roles in behaviorally relevant computations.

Amplitude Coding

Both mormyriform and gymnotiform weakly electric fish have tuberous electroreceptors embedded in the skin that can encode AMs of the EOD. In gymnotiform fish, these receptors are known as P-units since it was originally found that the probability of firing increased linearly with EOD amplitude. In mormyriform pulse-type fish, these receptors are known as mormyromasts. **Figure 2** illustrates the coding strategy used by wave-type as well as pulse-type gymnotiform and mormyriform weakly electric fishes.

Amplitude Coding in Pulse-Type Fish

Mormyromasts in pulse-type mormyriform fishes usually respond with one or more action potentials to each EOD pulse (**Figure 2(a)**). In mormyromasts, the latency between the EOD pulse in the water and the first action potential decreases for increasing EOD amplitudes. There is very low trial-to-trial variability in the relationship between latency and EOD amplitude – these afferents use a timing code to transmit information about EOD amplitude (**see also Detection and Generation of Electric Signals:** Active Electrolocation).

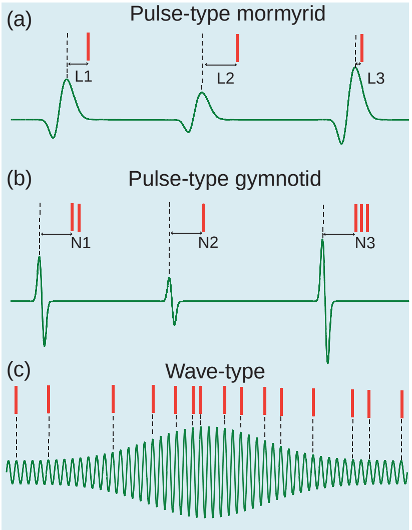


Figure 2 Amplitude coding in wave-type, pulse-type mormyriform, and pulse-type gymnotiform weakly electric fishes. (a) Mormyromasts in mormyriform pulse-type fish typically fire a single action potential (red bars) in response to each EOD pulse. However, the latency L between the EOD pulse and the action potential decreases monotonically with increasing EOD amplitude. (b) By contrast, burst duration coders in gymnotiform pulse-type fish fire a burst of action potentials in response to each EOD pulse. While the latency to first spike does not vary much with increasing EOD amplitude, the number of spikes in the burst (N) increases monotonically with increasing EOD amplitude. (c) P-units in gymnotiform wave-type fish and O-units in mormyriform wave-type fish fire action potentials that are phase-locked to the EOD but skip a random number of EOD cycles between firings. Their probability of firing an action potential increases with EOD amplitude.

Tuberous encoding in pulse-type gymnotiform fishes involves a different strategy. Tuberous neurons in gymnotiform fish exhibit little variation in the latency to the first spike, but instead modulate the number of spikes per burst as a function of EOD amplitude (**Figure 2**(**b**)). These are known as burst duration coders.

Amplitude Coding in Wave-Type Fish

In wave-type gymnotiform fishes, P-units exhibit some phase locking to the EOD with preferred phase near a local maximum. As the firing rates of P-units are usually lower than the EOD frequency, these neurons skip a more-or-less random number of EOD cycles between action potentials (**Figure 2**(**c**)). Increases and decreases in EOD amplitude will increase or decrease the firing rates of these units: the firing rate varies roughly linearly with EOD amplitude. These neurons encode AM information in a rate code. As one might expect, these units exhibit rectification for very low EOD amplitudes, because the firing rate cannot by definition be negative, and these units also saturate for very high EOD amplitudes. Recent studies have shown that these afferents have broad, flat tuning curves over the range of salient frequencies, from near 0 Hz to around 100 Hz.

In wave-type mormyriform fish, O-units have properties similar to those of P-units in wave-type gymnotiform weakly electric fish. Because both gymnotiform and mormyriform clades evolved independently, it is interesting to note that they evolved similar coding strategies for AM information carried by wave-type EODs.

Time Coding

As mentioned previously, weakly electric fish can also detect the timing of their EODs and therefore FMs (i.e., transient changes in the EOD frequency). Contrary to amplitude-coding strategies, time-coding strategies follow the same general principles in both pulse- and wave-type gymnotiform and mormyriform fishes.

Time Coding in Pulse-Type Fish

Pulse-marker units in gymnotiform and knollenorgans in mormyriform pulse-type weakly electric fish fire one phase-locked action potential per EOD pulse. These units therefore track changes in the EOD frequency (**Figure 3**(**a**)). In mormyrids, individual knollenorgans respond at various phases of an EOD pulse, so that relative differences in the timing of action potentials among knollenorgans additionally encode the EOD waveform (**see also Sensory Systems, Perception, and Learning:** Shocking Comments: Electrocommunication in Teleost Fish).

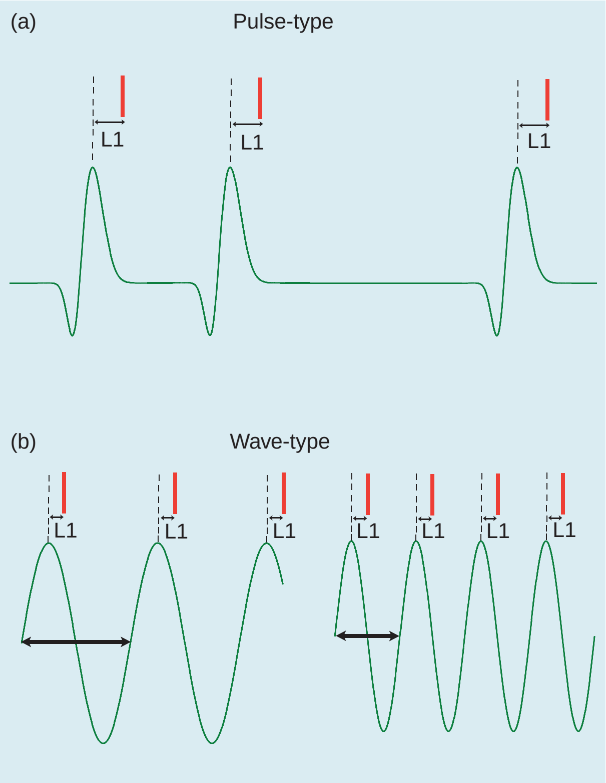


Figure 3 Time coding in wave-type and pulse-type weakly electric fishes. (a) Pulse-marker units in pulse-type gymnotiforms and knollenorgans in mormyriforms fire one action potential per EOD pulse and this tracks changes in EOD timing and frequency. (b) T-units in wave-type gymnotiforms and S-units in wave-type mormyriforms fire one action potential per EOD cycle (i.e., 1:1 phase locking). Their instantaneous firing rates thus provide a measure of the instantaneous EOD frequency.

Time Coding in Wave-Type Fish

T-units in gymnotiform wave-type weakly electric fish and S-units in wave-type mormyriform fish encode the timing of each EOD cycle and therefore the instantaneous EOD frequency by firing one action potential per EOD cycle at a precise phase (**Figure 3**(**b**)).

Projections to Higher-Order Neurons

The various types of electroreceptor units all project into a hindbrain structure known as the ELL. Anatomical and physiological studies have shown that the time-coding pathways preserve fine temporal information carried by time-coding afferents. In all classes of weakly electric fish, neurons receiving input from time-coding afferents have similar physiology: they fire one action potential per EOD cycle or EOD pulse. These neurons receive no descending feedback from central nervous system (CNS) circuits and thus appear to be a feedforward-only system. By contrast, information from amplitude-coding afferents is subject to complex processing that includes

Table 1 Summary of coding strategies used by tuberous electroreceptors

	Amplitude coding	*Frequency coding*
Wave type	*P-unit or O-unit.* Skips a random number of EOD cycles between firings. Probability of firing on any given EOD cycle increases with EOD amplitude.	*T-unit or S-unit*. Fires one action potential per EOD cycle. The phase of firing advances with increasing EOD frequency or amplitude.
Pulse-type gymnotiform	*Burst duration coder.* Fires a burst of spikes for each EOD pulse. The number of spikes in the burst increases with increasing EOD amplitude.	*Pulse marker unit.* Fires one action potential per EOD pulse at a precise phase.
Pulse-type mormyriform	*Mormyromast.* Fires one or more action potentials in response to each EOD pulse. The latency to the first action potential decreases with increasing EOD amplitude.	*Knollenorgan.* Fires a single phase-locked action potential in response to each EOD pulse.

descending feedback in ELL circuits. Tuberous electroreceptors fire not only in response to the animal's own EOD pulse, but also in response to EOD pulses emitted by conspecifics. As described below and reviewed in detail (**see also Detection and Generation of Electric Signals:** Active Electrolocation), pulse-type mormyrid fish generate a corollary discharge of each EOD pulse, allowing them to distinguish between self-generated and externally generated EODs within the ELL. Mormyromasts thereby give rise to a sensory pathway specialized for encoding self-generated EODs, whereas knollenorgans give rise to a sensory pathway specialized for encoding externally generated EODs. The basic properties of the electroreceptor types reviewed here are summarized in **Table 1**.

Physiology of ELL Neurons Receiving Input from Amplitude-Sensitive Electroreceptors

Amplitude Coding in Wave-Type Gymnotiform Fish

The ELL contains multiple segments devoted to processing tuberous input with the number of segments varying from species to species. The anatomy of an ELL segment in the gymnotiform weakly electric fish *Apteronotus leptorhynchus* is shown in **Figure 4(a)**. P-type electroreceptors project to pyramidal cells within the ELL. There are two types of pyramidal cells: basilar pyramidal cells receive direct electroreceptor input on their basilar dendrites, whereas nonbasilar pyramidal cells receive indirect electroreceptor input through local inhibitory interneurons. More recent studies have shown large morphological and molecular heterogeneities within the pyramidal cell population. Pyramidal cells are organized in both basilar and nonbasilar columns each consisting of superficial, intermediate, and deep cells. Pyramidal cells are the sole output neurons of the ELL. While all pyramidal cells project to the midbrain, only deep pyramidal cells give rise to the feedback input that is received mostly by superficial and intermediate pyramidal cells. These feedback projections can account for up to 95% of synaptic input to ELL pyramidal cells.

Pyramidal cell physiology is well characterized in general *in vivo* and there are important differences between superficial, intermediate, and deep pyramidal cells. Deep pyramidal cells have broad tuning curves and the highest spontaneous firing rates (>35 Hz) while superficial pyramidal cells have narrower tuning curves and the lowest firing rates (<15 Hz) with intermediate pyramidal cells having characteristics between these limits. Studies have shown that pyramidal cell tuning is both segment and context specific: pyramidal cells can change their tuning to match the temporal frequency content of both prey-related and communication-related stimuli.

Feedback pathways play important roles in regulating pyramidal cell responses to electroreceptor afferent input and many studies have elucidated their functions, which include gain control, cancellation of reafferent input, and regulation of burst firing, as described below.

Cancellation of Self-Generated stimuli

Sensory processing of electrosensory information is context dependent. For example, a given fish might be more interested in detecting a suitable mate or a prey item at different times of day and periods of its life. Indeed, tuberous electrosensory systems are modulated to vary the relative salience of information from conspecifics or prey items. Another example of context-dependent processing of electrosensory stimuli is the perception of movement. The fish's locomotion through the environment generates broad stimulation of the electrosensory system. This reafferent sensory stimulation must in turn be distinguished from external sensory stimuli such as a nearby predator or prey item. For weakly electric fish, tail movements that bring the tail closer/farther from a given point on the skin will increase/decrease the local EOD amplitude. Such signals can interfere with the detection of signals caused by external sources. In many species, the movement-related information is cancelled in the ELL. Different

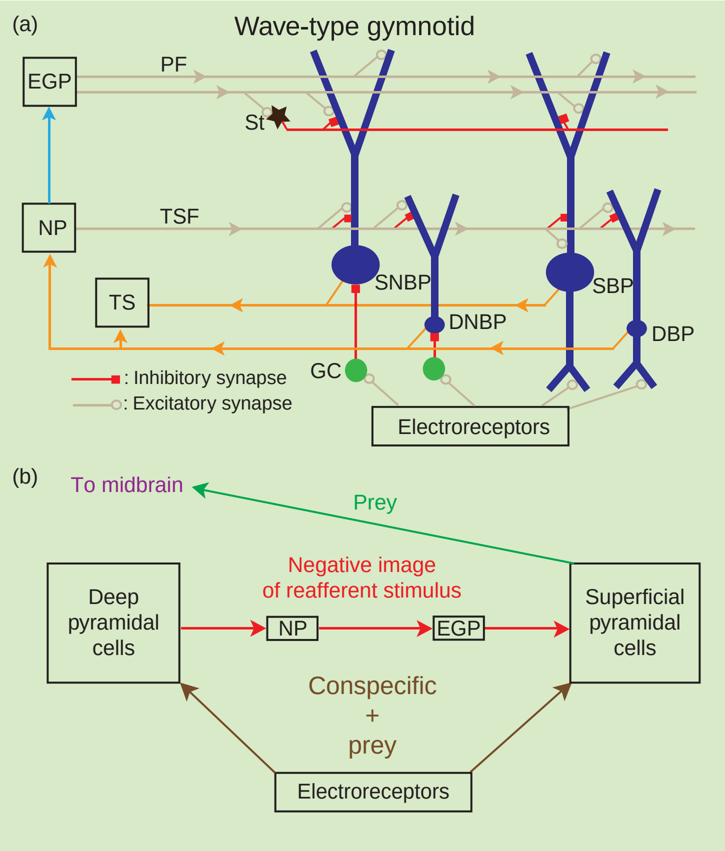


Figure 4 (a) Simplified anatomy of the ELL in *A. leptorhynchus*. Electroreceptors project to pyramidal cells: basilar pyramidal cells receive direct excitatory input while superficial nonbasilar pyramidal cells receive indirect inhibitory input via interneurons called granule cells (GC) There are large heterogeneities within the pyramidal cell population. Superficial basilar and nonbasilar pyramidal cells (SBP, SNBP) have large apical dendrites while deep basilar and nonbasilar pyramidal cells (DBP, DNBP) have small apical dendrites. Only deep pyramidal cells project to the nucleus praeminentialis (NP) while all pyramidal cell types project to the midbrain TS. Most interestingly, it is superficial pyramidal cells that receive the most feedback directly from NP via the tractus stratum fibrosum (TSF) and indirectly from granule cells in the eminentia granularis posterior (EGP) via parallel fibers (PFs). The direct projection from NP to ELL is called the direct feedback pathway while the indirect projection via EGP is called the indirect feedback pathway. (b) Functional circuit for cancellation of spatially diffuse electrosensory stimuli. Both superficial and deep pyramidal cells receive input from electroreceptors that respond to both conspecific and prey-related stimuli. Conspecific-related stimuli are spatially diffuse while stimuli caused by prey are spatially localized and only the former activate the negative image that is received mostly by superficial pyramidal cells, thereby allowing these cells to respond exclusively to prey stimuli.

species use different neural architectures to achieve this cancellation. Pulse-type mormyrid fish use a corollary discharge of the EOD command and an efference copy in order to achieve this function (**see also Detection and Generation of Electric Signals:** Active Electrolocation). Wave-type fish, however, have no such efference copy and must rely on other sensory signals and feedback pathways.

While electroreceptors in wave-type fish are sensitive to EOD AMs caused by tail movement, superficial ELL pyramidal cells are mostly insensitive to these same AMs. A series of elegant experiments performed by Bastian revealed that these neurons receive a negative image of the expected reafferent stimulus: the two inputs will cancel if the negative image has the right amplitude. Tail movements activate proprioceptors that project to the EGP and participate in generating the negative image. However, later studies revealed that tail bending is not required for cancellation; it can occur with tuberous electrosensory input only.

The necessary circuitry to achieve this was recently uncovered: deep pyramidal cells are strongly modulated by self-generated stimuli. In fact, these cells provide the necessary cancellation input to superficial pyramidal cells through feedback pathways. This mechanism relies on the fact that the electric images caused by different behaviorally relevant signals have different spatial extents: electric images caused by a fish's own EOD will cover the entire body surface, electric images caused by conspecifics will tend to cover most, if not all, of the animal's skin surface (these are often referred to as global stimuli), and the electric images caused by prey will only cover a small fraction of the animal's skin (local stimuli). Studies have shown that prey stimuli will not activate the negative image but that spatially diffuse stimuli will. As such, the cancellation circuit segregates spatially localized prey stimuli from spatially diffuse stimuli (**Figure 4**(**b**)).

Regulation of Burst Firing

Another major role for feedback pathways is to regulate the strength of pyramidal cell responses to sensory inputs. ELL pyramidal cells in *A. leptorhynchus* have a burst mechanism that is heavily regulated by feedback pathways. Pyramidal cells have much lower firing rates than receptor afferents (5–50 Hz) and they instead respond to changes in EOD amplitude with bursts of spikes (**Figure 5**(**a**)). Most interestingly, while receptor afferents use rate codes to transmit information about sensory stimuli, pyramidal cells use a timing code: spike bursts carry different information from isolated spikes. Bursts tend to code for low temporal frequencies of AM, while isolated spikes code for higher frequencies (**Figure 5**(**b**)).

Recent studies have focused on understanding the contributions of both ion channels and regulation of ion channel conductances by neuromodulators toward altering the responses of ELL pyramidal cells to sensory input.

Comparison between Wave-Type Gymnotiform and Mormyriform Weakly Electric Fish

Electrophysiological studies of ELL neurons in *Gymnarchus niloticus*, the only known species of mormyriform wave-type fish, show remarkable parallels with gymnotiform weakly electric fish. Separate neuron classes in the ELL respond to either AM or FM. Most surprisingly, these fish also lack a corollary discharge in stark contrast with their pulse-type cousins. Thus, tuberous electrosensory systems that have evolved independently apparently use similar strategies to code for behaviorally relevant stimuli, providing a nice example of convergent evolution. Further studies are needed, however, to understand the cancellation of self-generated stimuli in *G. niloticus*.

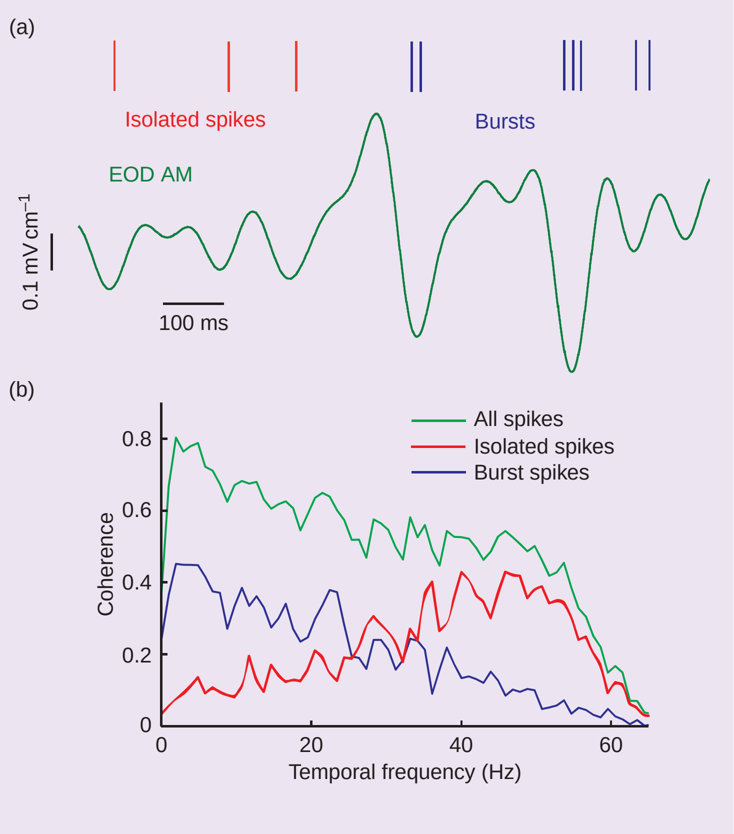


Figure 5 Physiology of ELL pyramidal cells in *A. leptorhynchus*. (a) Time-varying EOD amplitude (green) and spiking response of an example ELL pyramidal cell. This cell fired action potentials that were either isolated (red bars) or part of bursts (blue bars).
(b) Coherence between the spike train of an example pyramidal cell and time-varying EOD amplitude. The coherence computed from the spike train (green) shows that this cell responds to all frequency components contained within the stimulus. However, the coherence computed from burst spikes alone (blue) was greater for low frequencies while the coherence computed from isolated spikes alone (red) was greater for higher frequencies. Hence, action potentials can code for different aspects of the stimulus (here temporal frequency), depending on whether they are isolated or belong to a burst.

Physiology of Midbrain Neurons: Integration of Sensory Information

The torus semicircularis (TS) is a midbrain structure that is used to process electrosensory as well as auditory and mechanosensory information. In gymnotiform weakly electric fish, the TS is somatotopically organized and receives information from all of the maps in the ELL. It is hypertrophied and has distinct layers. Some of the layers in the dorsal torus receive direct tuberous inputs from ELL pyramidal cells, while others receive ampullary inputs (**see also Detection and Generation of Electric Signals**: Physiology of Ampullary Electrosensory Systems). These neurons send information to the ventral layers of TS. Information from P-type and T-type tuberous electrosensory systems is first sent to the ventral layers of the TS. Thus, the TS is a site of convergence, first from the various maps of the ELL in the dorsal TS, and second from P-type and T-type neurons in the deep layers of the TS. The TS is also where selectivity for specific behaviorally relevant stimulus features emerges in the ascending electrosensory pathway.

By contrast, the TS of mormyrid pulse-type fish is divided into separate nuclei that receive input from different groups of afferent neurons: neurons within the nucleus lateralis (NL) receive ampullary/mormyromast input while neurons in the nucleus exterolateralis (EL) and nucleus medialis ventralis (NMV) receive input from knollenorgans. We review some of the properties of midbrain neurons in both gymnotiform and mormyriform fishes below.

Emergence of Selectivity for Salient Stimuli

TS neurons exhibit more selective responses to electrosensory stimuli than do receptor afferents or ELL neurons. Specifically, these neurons often respond to a much narrower range of temporal frequencies or spatiotemporal features than ELL pyramidal cells. The mechanisms underlying the increase in selectivity in the TS have been intensively studied in relation to two behaviorally relevant stimuli – social stimuli related to the ‘jamming avoidance response’ (JAR) and moving electrosensory images.

Tuberous Mechanisms Underlying the JAR

The JAR behavior occurs in some wave-type species when two or more conspecifics are near each other, within ~1 m, and the fish have similar EOD frequencies, within about 10 Hz of each other. This tends to occur more in gregarious species such as *Eigenmannia virescens* (**Figure 6(a)**). When fish are close to each other, the electric fields mix and produce interference patterns. Interference patterns of 3–8 Hz are detrimental and impair each fish’s ability to electrolocate nearby objects. To avoid these detrimental interference patterns, each fish may change its EOD frequency. The JAR behavior differs between species and is best studied in the gymnotiform wave-type weakly electric fish *E. virescens* (**Figure 6(b)**). In this species, the animal with the lower EOD frequency decreases its EOD frequency while the animal with the higher EOD frequency increases its EOD frequency. This behavior thereby increases the frequency of the interference patterns produced by the interactions of the EODs to 20 Hz or more. Interference patterns greater than 20 Hz do not have detrimental effects on electrosensory perception. The JAR behavior is most strongly elicited by interference patterns at rates of 3–8 Hz, but only weakly by electrosensory stimuli at rates above 20 Hz.

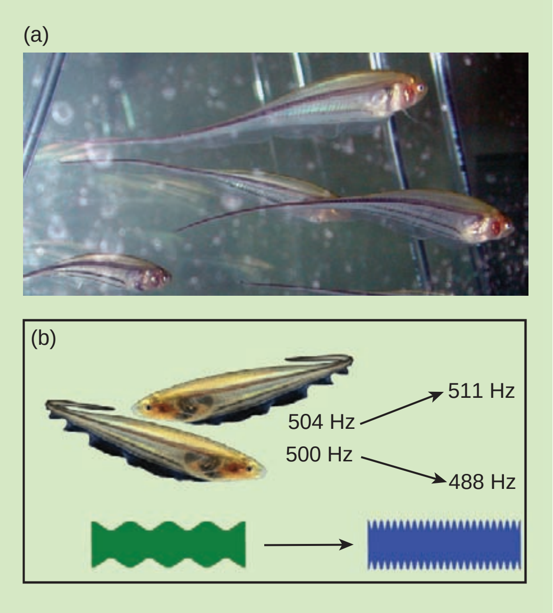


Figure 6 (a) *Eigenmannia viriscens* are gregarious (i.e., they tend to stay in groups). (b) The JAR of *Eigenmannia*. When two conspecifics are in close proximity and the EOD frequencies are within about 10 Hz (504 and 500 Hz shown here), the electric fields combine to produce spatially widespread AMs at the difference in frequencies in the EOD, which here is 4 Hz. The trace below shows a 1-s sample with a 4-Hz AM. This combined electric signal elicits the JAR, in which the higher EOD frequency fish raises its frequency, and vice versa for the lower EOD frequency fish. The result is a higher AM rate; 23 Hz is shown here.

Most surprisingly, the JAR behavior is similar in both wave-type gymnotiforms and *G. niloticus*. Overall, both groups compute the sign of the frequency difference similarly although some computations occur in different brain areas. These computations require simultaneous knowledge of the AMs as well as the phase modulations occurring on different parts of the body.

In wave-type gymnotiform fish, these AMs are encoded by P-units and subsequently encoded by ELL pyramidal cells. Many neurons within the layers 1–5 of TS respond strongly to low-frequency stimuli below

10 Hz and weakly to higher-frequency stimuli above 20 Hz. These low-pass responses are generated by a suite of mechanisms in TS, including passive membrane properties, short-term synaptic depression, and subthreshold membrane conductances. Similar mechanisms are found in the TS of *G. niloticus*.

Phase differences across the body give rise to timing differences between inputs from T-type (in wave-type gymnotiform fishes) or S-type (in *G. niloticus*) afferents across the animal's body. In *Gymnarchus*, these timing differences are detected by ELL neurons, whose sensitivity matches that of the animal's behavior. In wave-type gymnotiform fish, these differences are detected by neurons in lamina 6 of the TS. Specifically, giant adendritic cells within this layer receive input from multiple ELL spherical cells and are thought to average this input. Giant cells then project broadly across lamina 6 onto the somata of small cells that also receive local input from spherical cells on their dendrites. These small cells possibly perform a comparison of the local EOD phase with respect to an EOD phase that is averaged across the animal's body by responding specifically when input from the dendrites is coincident with input from the soma. The sensitivities of small cells to timing differences are still significantly less than that of the animal's and, in contrast to *Gymnarchus*, further integration appears to take place: one must look in the diencephalon to find neurons whose sensitivity matches the behavior.

As mentioned above, some neurons in the deep layers of the TS receive input from both the amplitude-coding and time-coding pathways. These neurons likely play a critical role in the JAR as they compute the sign of the frequency difference between fish, which is required to determine the proper direction of the change in EOD frequency. Some neurons are active when the frequency difference is positive, while others respond when the frequency difference is negative. Similar neurons are found within the TS of *G. niloticus* where nonlinear voltage-dependent conductances enhance their selectivity to particular combinations of AM and PM information.

Processing of EOD Waveform

Some neurons within the TS of pulse-type mormyrids are sensitive to small differences in EOD waveform. This circuit potentially functions in an analogous manner to the circuit found within lamina 6 in gymnotiform wave-type fish. Specifically, small cells within the EL pars anterior receive delayed excitatory input from nELL neurons and inhibitory input from giant cells within the same nucleus. This circuit would allow the small cells to respond selectively to EOD pulses of certain duration in a 'delay line blanking' mechanism. A major difference between the duration coding circuit in pulse-type mormyrids and similar circuits found in wave-type gymnotiform fishes is that the former uses both excitation and inhibition while the latter uses only excitation.

Tuberous Mechanisms for Processing Moving Objects

The electrosensory perception of moving objects is an important function in weakly electric fish. Prey capture can be achieved in complete darkness: under these conditions, it is likely that the tuberous electrosensory system is the primary source of salient sensory information for the control of the behavior. A critical feature for the behavior is the determination of the direction of movement of the prey stimulus. Selectivity for the direction of object motion first emerges at the level of the TS. P-type tuberous neurons in the dorsal layers can have strongly directionally selective responses to moving objects, whereas neurons in the ELL do not. Such neurons respond vigorously when an object moves in a particular direction (head-to-tail or tail-to-head) but weakly or not at all when the object moves in the opposite direction. Direction selectivity in these neurons is thought to derive from short-term synaptic plasticity and further studies are needed to understand the mechanisms giving rise to this computation.

Conclusion

Tuberous electrosensory systems use a variety of strategies to code for natural sensory input. On the one hand, the combination of neural mechanisms and strategies used to achieve the same computation in some distantly related species shows a remarkable level of convergence; on the other, a remarkable level of diversity exists in brain mechanisms and computations among more closely related species. Indeed, biological systems can often implement multiple solutions for the same functions, and the comparative approach is required in order to identify and understand the fundamental principles underlying neuron computations and behavioral control.

See also: **Detection and Generation of Electric Signals:** Active Electrolocation; Electric Organs; Generation of Electric Signals; Physiology of Ampullary Electrosensory Systems. **Sensory Systems, Perception, and Learning:** Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Bastian J (1999) Plasticity of feedback inputs in the apteronotid electrosensory system. *Journal of Experimental Biology* 202: 1327–1337.

Bastian J, Chacron MJ, and Maler L (2004) Plastic and non-plastic cells perform unique roles in a network capable of adaptive redundancy reduction. *Neuron* 41: 767–779.

Bell CC, Han V, and Sawtell NB (2008) Cerebellum-like structures and their implications for cerebellar function. *Annual Review of Neuroscience* 31: 1–24.

Bullock TH (1982) Electroreception. *Annual Review of Neuroscience* 5: 121–170.

Carlson BA and Kawasaki M (2008) From stimulus estimation to combination sensitivity: Encoding and processing of amplitude and timing information in parallel, convergent sensory pathways. *Journal of Computational Neuroscience* 25: 1–24.

Chacron M (2007) Electrolocation. *Scholarpedia* 2(1): 1411, http://www.scholarpedia.org/article/Electrolocation (accessed January 2010).

Chacron MJ, Doiron B, Maler L, Longtin A, and Bastian J (2003) Non-classical receptive field mediates switch in a sensory neuron's frequency tuning. *Nature* 423: 77–81.

Fortune ES (2006) The decoding of electrosensory systems. *Current Opinion in Neurobiology* 16: 474–480.

Hagiwara S, Kusano K, and Negishi K (1962) Physiological properties of electroreceptors of some gymnotids. *Journal of Neurophysiology* 25: 430–449.

Heiligenberg W (1991) *Neural Nets in Electric Fish*. Cambridge, MA: MIT Press.

Kawasaki M and Guo YX (1998) Parallel projection of amplitude and phase information from the hindbrain to the midbrain of the African electric fish *Gymnarchus niloticus*. *Journal of Neuroscience* 18: 7599–7611.

Krahe R and Gabbiani F (2004) Burst firing in sensory systems. *Nature Reviews Neuroscience* 5: 13–23.

Maler L (2009) Receptive field organization across multiple electrosensory maps- I. Columnar organization and estimation of receptive field size. *Journal of Comparative Neurology* 516: 376–393.

Maler L (2009) Receptive field organization across multiple electrosensory maps- II. Computational analysis of the effects of receptive field size on prey localization. *Journal of Comparative Neurology* 516: 394–422.

Mehaffey WH, Ellis LD, Krahe R, Dunn RJ, and Chacron MJ (2008) Ionic and neuromodulatory regulation of burst discharge controls frequency tuning. *Journal of Physiology (Paris)* 102: 195–208.

Oswald AMM, Chacron MJ, Doiron B, Bastian J, and Maler L (2004) Parallel processing of sensory input by bursts and isolated spikes. *Journal of Neuroscience* 24: 4351–4362.

Sawtell NB, Williams A, and Bell CC (2005) From sparks to spikes: Information processing in the electrosensory systems of fish. *Current Opinion in Neurobiology* 15: 437–443.

Scheich H, Bullock, TH, and Hamstra RH (1973) Coding properties of two classes of afferent nerve fibers: High frequency electroreceptors in the electric fish, eigenmania. *Journal of Neurophysiology* 36: 39–60.

Szabo T and Hagiwara M (1967) A latency-change mechanism involved in sensory coding of electric fish. *Physiology and Behavior* 2: 331–335.

Xu-Friedman MA and Hopkins CD (1999) Central mechanisms of temporal analysis in the knollenorgan pathway of mormyrid electric fish. *Journal of Experimental Biology* 202: 1311–1318.

Relevant Websites

http://alumnus.caltech.edu – Caltech Alumni Web Server, Weakly Electric Fish.

http://www.fiu.edu – FIU, Florida International University; Neuroethology and Behavioral Ecology of Communication in Electric Fish.

http://www.bio.indiana.edu – Indiana University, Department of Biology, G. Troy Smith.

http://www.zoologie.uni-bonn.de – Institut für Zoologie der Universität Bonn, Neuroethologie Bonn.

http://www.medicine.mcgill.ca – McGill, Computational Systems, Neuroscience Laboratory.

http://www.neuromech.northwestern.edu – Northwestern University, McCormick School, Biomedical & Mechanical Engineering.

http://people.virginia.edu – University of Virginia, What Is an Electric Fish?

Active Electrolocation

G von der Emde, University of Bonn, Bonn, Germany

J Engelmann, University of Bielefeld, Bielefeld, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
EOD Carrier Modulation as a Source of Environmental Information
Prereceptor Mechanisms
Behavioral Performance during Object Detection and Analysis
Foveation in Electrolocation
Electroreceptors for Active Electrolocation
Central Processes
Further Reading

Glossary

Active electrolocation The ability of weakly electric fish to orient and detect objects based on their electric sense.

Electric image A two-dimensional modulation of the self-produced electric field at the sensory surface (electroreceptive skin) that is caused by the presence of an object in the environment.

Electric organ corollary discharge (EOCD) Neuronal copy of the electromotor command that is sent from motor areas involved in electric organ discharge (EOD) generation to sensory areas involved in processing electrosensory information. Among other functions, it is used to predict and eliminate responses to self-generated stimuli.

Electric organ discharge (EOD) Electrical discharges resulting from the summed excitation of the cells (electrocytes) of the electric organ of electric fishes that function both in communication and in locating nearby objects. Short (100 ms to several tens of milliseconds) electrical pulses of various voltages (a few millivolts to several hundred volts) are generated at various rates (a few to over 1000 times per second).

Fovea Classically, it is the location of the highest density of cone photoreceptors and thus the highest spatial acuity in the eye. More recently, the term is also attributed to regions of high receptor densities within the sensory surface in several other senses.

Reafference The sensory consequence of motor activity.

Somatotopy A topographic map-like representation of a sensory surface in the animal's brain.

Introduction

For acquiring information about their surroundings, weakly electric fish actively emit electric signals with a specialized electric organ (**see also Detection and Generation of Electric Signals**: Electric Organs). Electric organ discharges (EODs) are species specific, either continuous sinusoidal signals or short pulses of 100 μs to a few milliseconds in duration. In African mormyridae, pulse-EODs are constant all-or-nothing events, which are emitted in an irregular train of pulses depending on behavioral context (**Figure 1(b)**; **see also Detection and Generation of Electric Signals**: Generation of Electric Signals). Nearby objects distort the field generated by the EOD (**Figure 1(c)**), and the animal senses these modulations of the EOD to analyze its surroundings.

We focus on the African mormyrid *Gnathonemus petersii* (**Figure 1(a)**), or elephantnose fish. This fish is probably the best-studied mormyrid with respect to its active electrolocation system. It can link and assemble local features of sensory patterns to construct representations of objects in complex scenes and thus solve complex object-recognition tasks.

EOD Carrier Modulation as a Source of Environmental Information

Modulations of the EOD are caused by objects close to the animals' skin that differ in impedance from the surrounding water. Depending on the impedance difference between object and water, the current flowing locally through electroreceptors next to the object is either decreased or increased, resulting in a decrease or increase in voltage at the receptor (**Figure 1(d)**, top). Capacitive properties of nearby objects can additionally alter the waveform of the local EOD (**Figure 1(d)**, bottom).

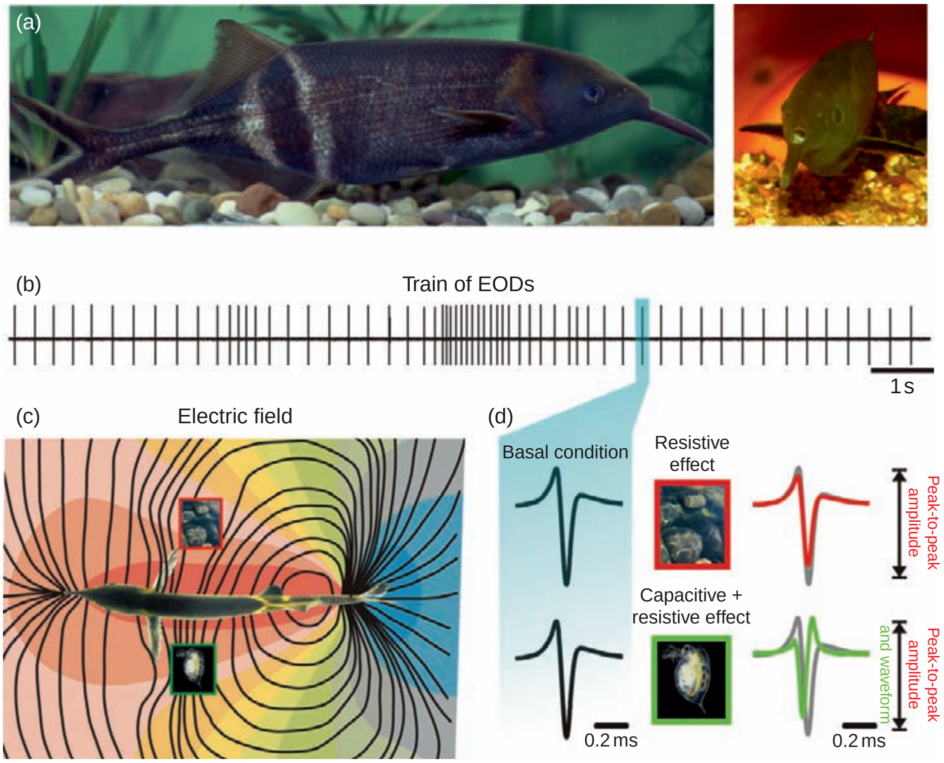


Figure 1 (a) Photograph of a *Gnathonemus petersii*, a member of the African weakly electric Mormyridae family. (b) A sequence of pulse-type electric organ discharges (EODs) emitted by the weakly electric fish *G. petersii*. While the interval between EODs can vary, EOD amplitude is constant. (c) Schematic of the electric field that surrounds the animal when an EOD is emitted. The solid lines indicate the current flow, while the electric field potential intensity is shown in a color code. Insulating objects (stones) locally decrease the amplitude of the field, which means that the current flow is quenched to the sides of the object (above). Conductive and or capacitive objects such as the little prey copepod shown below increases the field intensity locally; thus, they cumulate a higher current density (d) Comparison of the potential modulations of the signal carrier by inanimate, resistive objects (stones) as opposed to a living prey item (copepod) with a complex impedance consisting of resistive and capacitive components. While objects that differ in their resistance from that of water only modulate the peak-to-peak amplitude of the EOD (red signal, top row), complex impedances alter both the EOD amplitude and its waveform (green signal, bottom row). The basal EOD is shown in gray and the modulated EOD in color.

The electric image of the objects is thus projected onto the electroreceptive skin, which covers almost the entire body. In mormyrids, a typical electric image has a centre-surround (or Mexican hat) spatial profile (**Figure 2**). A conductor projects an image with a large center region where the local EOD amplitude is increased, surrounded by a small rim area where the amplitude is decreased. Images of nonconductors are of opposite arrangement: local EOD amplitude decreases centrally and slightly increases in the surrounding rim area.

Electric images are always blurred, or out of focus, because no focusing mechanisms comparable to the lens of an eye exist. Hence, when projecting three-dimensional (3D) objects on the 2D sensory surface, there is no one-to-one relationship between spatial object properties and image shape. In addition to shape, size, and geometrical properties of the object, electric images depend on object distance and location along the fish's body, the fish's body proportions, bending movements of the fish's body, the presence of additional objects, background conductance, and more. For example, electric images of two objects fuse in a nonlinear way depending on the distance between them (**Figure 2**).

Prereceptor Mechanisms

Before a sensory stimulus hits the receptor cells, it is modified by structures external to the receptors. In active electroreception, such prereceptor mechanisms were initially described in South American weakly electric fish, where they enhance the perception of electric images. In *G. petersii*, the low resistive internal electric properties and the elongated shape of the fish's body constitute part of these prereceptor mechanisms. While the EOD waveform is reasonably constant, the EOD amplitude changes from caudal to rostral along the fish's body. The overall effect of the prereceptor mechanisms detailed below is that EOD amplitudes are fairly constant in the head region where highest electroreceptor densities occur.

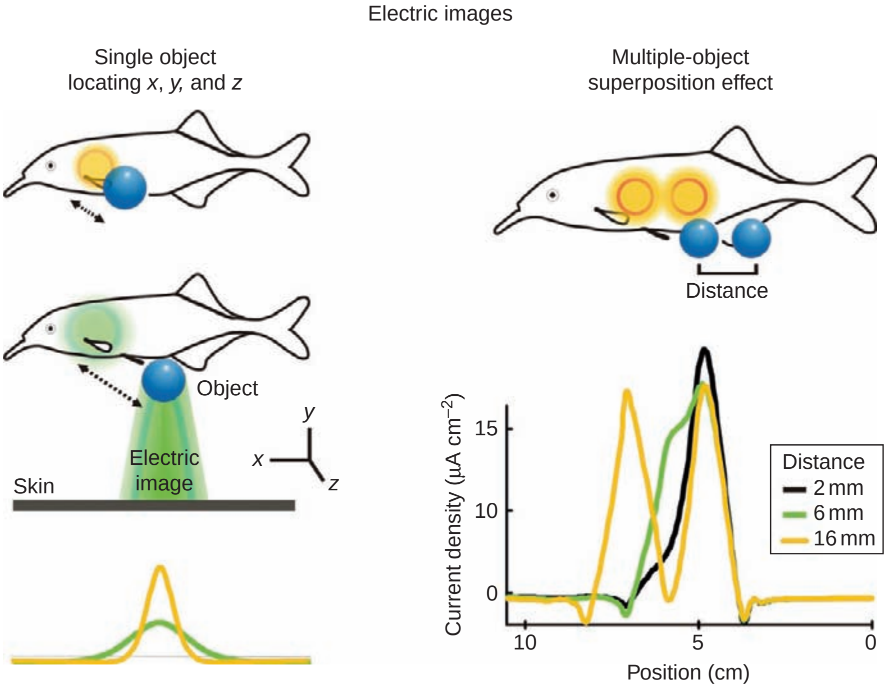

Electric images
Single object locating x, y, and z
Multiple-object superposition effect
Distance
Object
Electric image
Skin
y
x
z
Current density (µA cm^{-2})
15
10
0
10
5
0
Position (cm)
Distance
2 mm
6 mm
16 mm

Figure 2 Schematic drawings explaining the concept and properties of electric images. The left-hand side shows how the two-dimensional (2D) image (the change in current flowing through the animals' skin in the presence of an object with respect to the current pattern without any object present) depends on the distance (*z* coordinate) of an object. With increasing distance, the location of the peak in the image remains constant (*x*, *y*); while the overall amplitude decreases, the image increases in width and the slope of the images becomes more shallow (blur). The right-hand side shows that electric images interact in a nonlinear fashion if two objects are in close proximity. Thus, electric images are apposition images. The bottom data show modeled transects through electric images for two small spheres that are progressively moved closer to each other. Note that for 6-mm interobject distance, the peaks of the electric image begin to fuse to one single peak. Modified from Engelmann J, Bacelo J, Metzen M, *et al.* (2008) Electric imaging through active electrolocation: Implication for the analysis of complex scenes. *Biological Cybernetics* 98(6): 519–539.

The elongated chin appendix (Schnauzenorgan) of *G. petersii* is mainly stimulated by current impinging from the front of the fish. This can be attributed to the presence of two effects: the constantly open mouth funnels current between the mouth and the nares, and the internal tissue of the Schnauzenorgan funnels current stimulating the Schnauzenorgan receptors. The thin and elongated shape of the Schnauzenorgan constitutes an additional mechanism termed 'tip effect', to locally increase EOD amplitude. Thus, several passive prereceptor mechanisms enhance the electrical field properties in a manner ideal for active electrolocation at the region of the animal's body that has the highest fovea-like densities of electroreceptors (see below).

When *G. petersii* forages, they move their Schnauzenorgan rapidly, which might cause changes of the electric-field distribution around the body and hinder active electrolocation with the Schnauzenorgan. However, relying on the tip effect, the electrical field moves together with the Schnauzenorgan. This results in constant EODs at the tip of the Schnauzenorgan, analogous to a spot light that moves along the ground and dynamically scans the environment.

Behavioral Performance during Object Detection and Analysis

During active electrolocation, weakly electric fish detect and analyze several object properties based on the electric images. Among these is the electrical resistance of objects determined by measuring the change in amplitude. Mormyrids and gymnotiforms can also perceive capacitive object properties. This requires that the animals measure either EOD waveform distortions (mormyrids) or phase shifts (gymnotiforms). The range of capacitive values that an animal can detect depends on the duration of its EOD: shorter EODs enable the detection of lower capacitances. The detectable range of capacitances corresponds to the range of capacitive values of animated objects found in the natural habitat of the fish. Therefore, capacitance detection could be used to find living objects such as food or other fish.

Mormyrids also localize objects in 3D space. When presented with two objects at different distances, *G. petersii* can learn to choose the farther of the two objects. This capability constitutes a true sense of

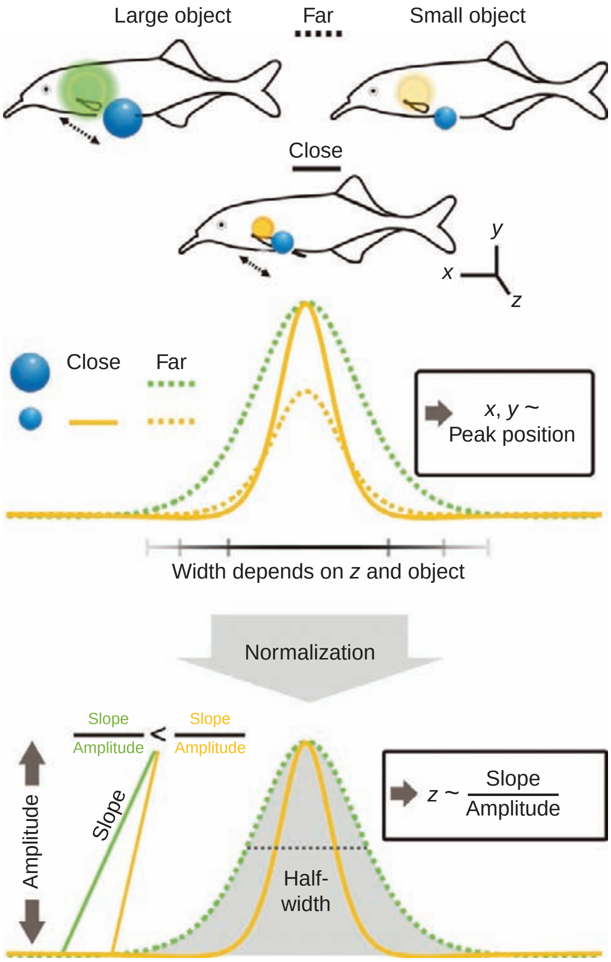


Figure 3 (a) Schematic comparison between the electric images of a small and a larger sphere when both are at the same distance (top row), and for the smaller object at a closer distance (middle). Transects below show the spatial amplitude distributions of the electric images. Note that the positions of the peaks *x* and *y* are similar in all three cases, whereas the widths and slopes of the images depend on size and distance of the objects. (b) The electric images of the closer small sphere (yellow) and the further removed large sphere (green) differ in width and slope but not in amplitude. Normalizing images by their amplitudes results in a size-invariant measure for distance – the slope/amplitude ratio.

depth perception, based on measuring the normalized maximal slope of the electric image (**Figure 3**). Fish can measure the slope of the electric image by determining the intensity change at the image's edges, or the fuzziness of the edges. For the normalized slope, they set this slope value in relation to the maximum intensity in the image's center.

Even though the electric sense lacks focusing mechanisms, weakly electric fish also perceive an object's 3D shape. In a two-alternative forced-choice procedure, *G. petersii* quickly learns to recognize objects of various shapes and discriminate them from different shapes. Shape recognition persists even when the objects are rotated in space, indicating a viewpoint-independent recognition of objects. *G. petersii* also demonstrates size constancy during object recognition, that is, they recognize an object of a certain shape even if its electric image appears larger or smaller because of variation in object distance. Fish can also identify the size of an object independently of its distance (**Figure 4**). For analyzing the shape or size of a new object, fish perform the so-called probing motor acts, that is, they swim around the object, scanning it with their sensory surface from several viewpoints. This is in contrast to distance measurements, which can be achieved instantly and do not require scanning movements.

The electric images of objects located close to each other fuse in a nonlinear way, leading to complex electric images (**Figure 2**). In spite of this effect, *G. petersii* is able to perceive the shape of an object even when it is positioned right in front of a large background (**Figure 4**). This ability persists even if the object and the background are made from the same material, for example, a metal object in front of a metal background. When trained to discriminate between two objects placed next to each other and a solid object of the same length, *G. petersii* is able to detect gaps as small as 2–3 mm between the two objects. It is currently not known how the fish perform these tasks.

These findings show that weakly electric fish have a remarkable ability to analyze complex 3D scenes and can identify object properties even in a natural setting containing many objects of various sizes, shapes, and materials.

Foveation in Electrolocation

In visual systems, foveation is an important mechanism for detailed object analysis. Behavioral, anatomical, and physiological studies reveal several similarities between certain electroreceptive skin regions and the eyes of pigeons. Two separate foveae have been found in *G. petersii*, one located at the Schnauzenorgan and the other between the mouth and the nares, the so-called nasal region. Both match the requirements of a fovea: (1) a higher density of electroreceptors compared to the rest of the sensory epithelium; (2) an overrepresentation of these areas in the brain; (3) structural/morphological and physiological specializations of the receptors and accompanying structures within the foveae; and, finally, (4) the animals show behavioral adaptations for focusing a stimulus onto the foveae for detailed analysis (**Figure 5**).

Similar to the pigeon's eye, the two foveae of *Gnathonemus* serve separate functions: the nasal region is a long-range guidance system that is used to detect obstacles or other objects in front of and to the side of the animal, for example, during foraging. The

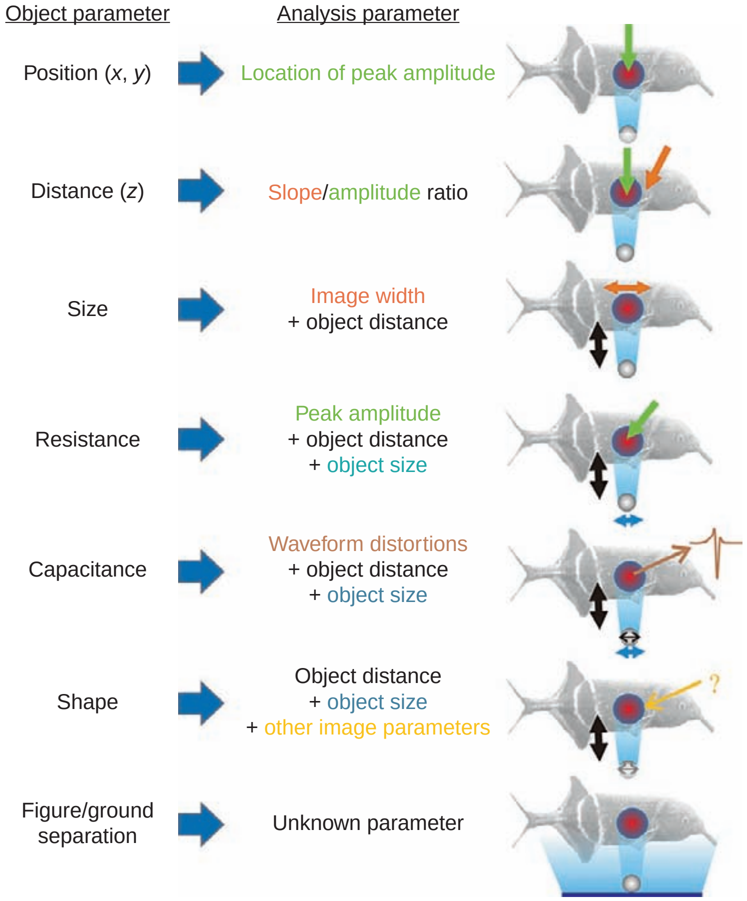


Figure 4 Object properties (object diameter >5 mm) (left) and the parameters of the electric image (right) that *G. petersii* employ for their determination during active electrolocation. In order to recognize most object parameters, combinations of several image parameters are necessary.

Schnauzenorgan, on the other hand, is a short-range movable (prey-) detection system that is used to find and identify prey on the ground or inspect object details. Both systems work simultaneously to ensure an optimal sensory inspection of the nocturnal environment of the fish.

Electroreceptors for Active Electrolocation

Weakly electric fishes have two classes of electroreceptor organs (**see also Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs). Ampullary organs respond to low-frequency electric signals of animate and inanimate origin (**see also Detection and Generation of Electric Signals**: Physiology of Ampullary Electrosensory Systems). These receptor organs are used for passive electrolocation.

The second class of these organs, tuberous electroreceptor organs, are found only in clades that also have electric organs, that is, in the South American Gymnotiformes and the African Mormyriformes. Tuberous receptors are tuned to electric signals with high carrier frequencies, and are generally most sensitive to the dominant frequencies of the fish's own EOD. In both groups, tuberous receptors encode the amplitude and the phase of the fish's own EOD (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems).

Mormyromasts and Knollenorgans are the two types of tuberous electroreceptors found in mormyrid fish. Knollenorgans are exclusively used to detect the EODs of other mormyrids for communication (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish), whereas mormyromasts are responsive to the animal's own EOD and modulations of the EOD caused by the external environment. Each mormyromast houses two types of receptor cells that are tuned to different aspects of the signal carrier, that is, one channel for amplitude (A-cells) and one for waveform encoding (B-cells). Afferents of both types of receptor cells terminate somatotopically in two zones of the electrosensory lateral line lobe (ELL) of the brain.

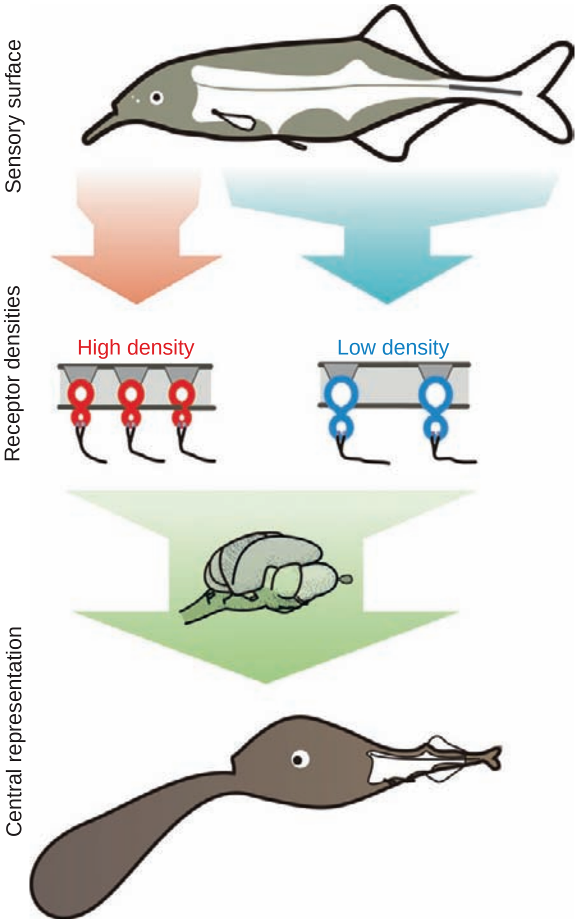


Figure 5 Schematic of the peripheral and central foveation mechanisms in *G. petersii*. The top drawing shows the sensory surface of the fish (light gray filling). The middle part indicates that the Schnauzenorgan and the head region contain the highest densities of mormyromast electroreceptors. This asymmetry of the receptor distribution is further enhanced in the ELL, where a central magnification of the Schnauzenorgan region and the head region takes place. Thus, about 2 times as much brain area is devoted to the processing of information from the Schnauzenorgan than would be expected based on receptor density. In the sketch below, the sizes of the electroreceptive skin areas are shown in proportion to their representation in the brain. Note that in this drawing, the Schnauzenorgan and the nasal region appear overproportionally large relative to the rest of the body.

Peripheral Encoding

Latency coding of EOD amplitude and electrical imaging

In response to an EOD, mormyromast receptor cells generate graded receptor potentials that give rise to action potentials in primary afferents. To an undistorted EOD, most afferents of A- and B-cells respond with a burst of 2–6 action potentials. Both types of afferents respond with an increase in the number of spikes and a decrease in spike latencies when the stimulus amplitude increases. The coding of EOD intensity by the latency of the first spike is continuous, whereas the transition in spike number is discrete. Thus, mormyrid fish are good examples of spike-latency encoding.

As modulations of the EOD amplitude by natural objects are small, animals must extract latency changes in a sub-millisecond range. This is reflected in the precision with which afferents respond to an object moving through the receptive field (see **Figures 6(a)** and **6(b)**). The resulting electric image is a typical Mexican-hat-like modulation of the basal EOD amplitude. This is linearly translated into the spike latencies of the afferents. Only very intense modulations induce additional changes in the number of spikes.

Encoding of waveform

Only the B-cells are extremely sensitive to minute waveform distortions of the local EOD as they occur in the presence of capacitive objects. For capacitance detection, *G. petersii* measures these EOD waveform distortions based on the differential input of the A- and B-type electroreceptor cells (**Figure 7**). However, central mechanisms are needed to extract impedance unambiguously, because both receptor types are affected by amplitude. It should be noted that in nature, capacitive objects will always induce both amplitude and phase modulations of the EOD.

Central Processes

Information Processing in ELL

ELL circuitry

Sensory input of mormyromast afferents is relayed to a layer of granule cells in the ELL. Here, the temporal code of the afferents is transformed into a rate code. Granule cells are either gamma-aminobuytric acid-ergic (GABAergic) or glutamatergic and make synapses on the basal dendrites of both inhibitory interneurons and two types of efferent neurons. Efferent neurons that are inhibited by sensory input are the large ganglion (LG) cells, while large fusiform (LF) cells are excited by sensory input. The most prominent interneurons, the Purkinje-like medium ganglion (MG) cells, provide inhibitory input to these efferent cells (**Figure 8**).

In addition to the peripheral sensory input, several central afferents provide projections to the ELL (**Figure 8**). These central afferents are related to specific neuronal tasks such as noise cancellation, the differentiation of re- and exafferent input, and the decoding of the latency code of the sensory afferents (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems).

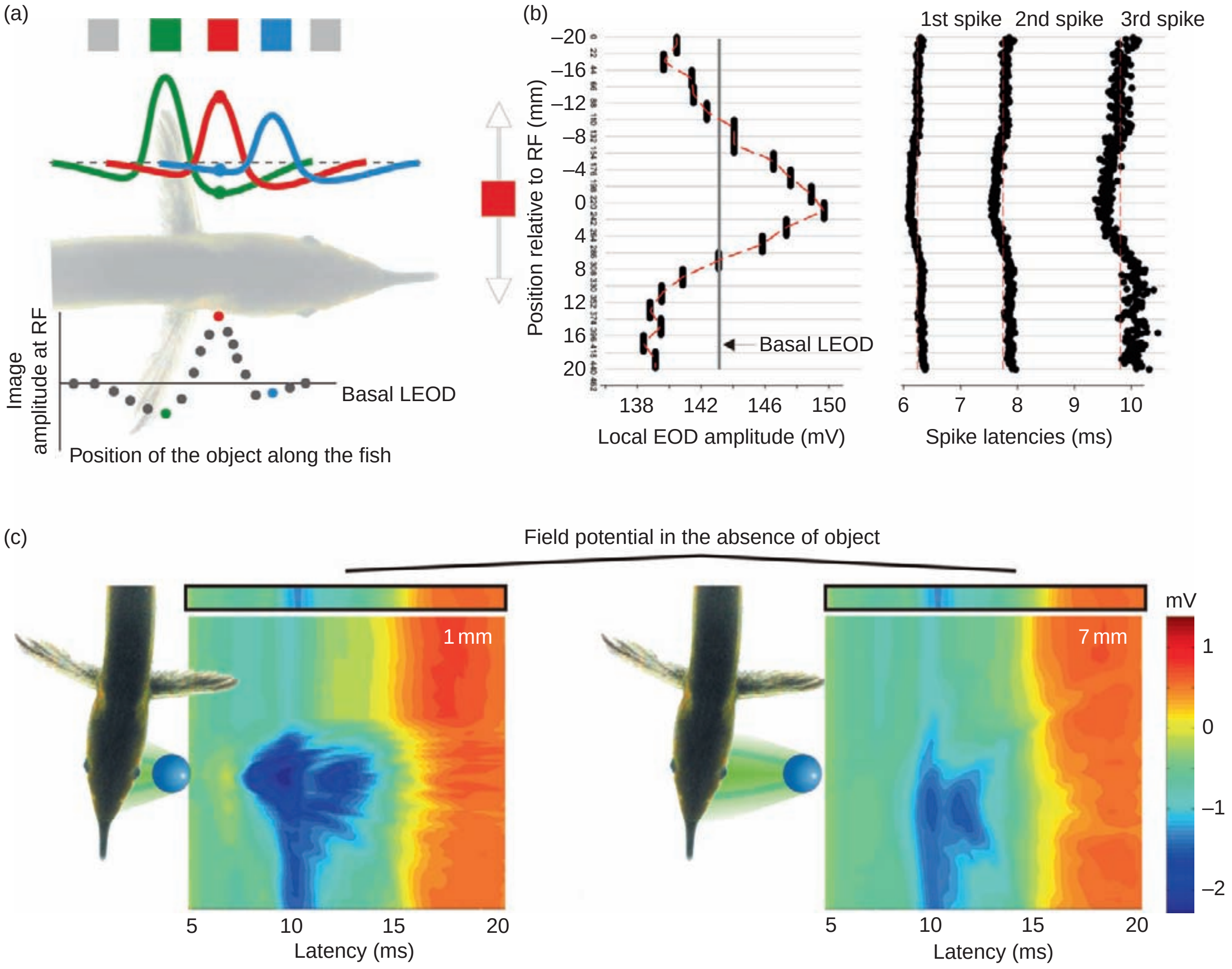


Figure 6 Response patterns of primary afferents and field potentials in ELL. (a) Schematic of the recording and stimulation procedure. An object is moved through the receptive field of a mormyromast (located at the red dot). At each position, the object casts a slightly different image on the skin. The receptor therefore responds to the local modulation of each image as it occurs at the location of the receptor pore on the skin. (b) Raster diagram of the changes in the basal EOD amplitude (left) and the spike latency of a mormyromast A-cell afferent (right) recorded simultaneously. The horizontal lines separate recordings obtained from spatially neighboring positions. Note that the electric image is faithfully encoded in the latency of the first spike. Note that high stimulus amplitudes (peak of the image) lead to short latencies of the spike response and vice versa. (c) Color maps showing the field potential recorded in the ELL as a function of time and object position. The horizontal axis of each graph shows time and the vertical axis represents the location of the object along the longitudinal axis of the fish. The two graphs show results for an object distance of 1 mm (left) and 7 mm (right). The top parts indicate the field potentials in the absence of an object, and any difference to the graph below is due to the presence of the object and the corresponding change in sensory input. As the object is moved further away from the animal (*z*-axis), the width of the neuronal representation contained in the field potential increases but the response amplitudes steeply decline. Data in (c) are modified from Gomez L, Budelli R, Grant K, and Caputi AA (2004) Pre-receptor profile of sensory images and primary afferent neuronal representation in the mormyrid electrosensory system. *Journal of Experimental Biology* 207(14): 2443–2453.

Latency Code to Rate Code Transformation in Granule Cells

The mormyromast receptors provide an example of temporal coding, because the latency of the first spike conveys all the information about amplitude and phase of the external EOD. Afferent fibers form mixed chemical and electrical synapses onto granule cells in the ELL. A second excitatory input from the juxtalobar nucleus, which conveys EOD-time-locked input (electric organ corollary discharge (EOCD)), also synapses on the granule cells. This corollary discharge input gates the reafferent input from the mormyromast electroreceptors, thus preventing granule cells from responding to the EODs of other fish. Granule cells have the potential to transform the latency-encoded stimulus amplitude conveyed by the afferents to a rate code, because they respond gradually to excitatory postsynaptic potentials (EPSPs). In addition, the efficiency of synaptic input

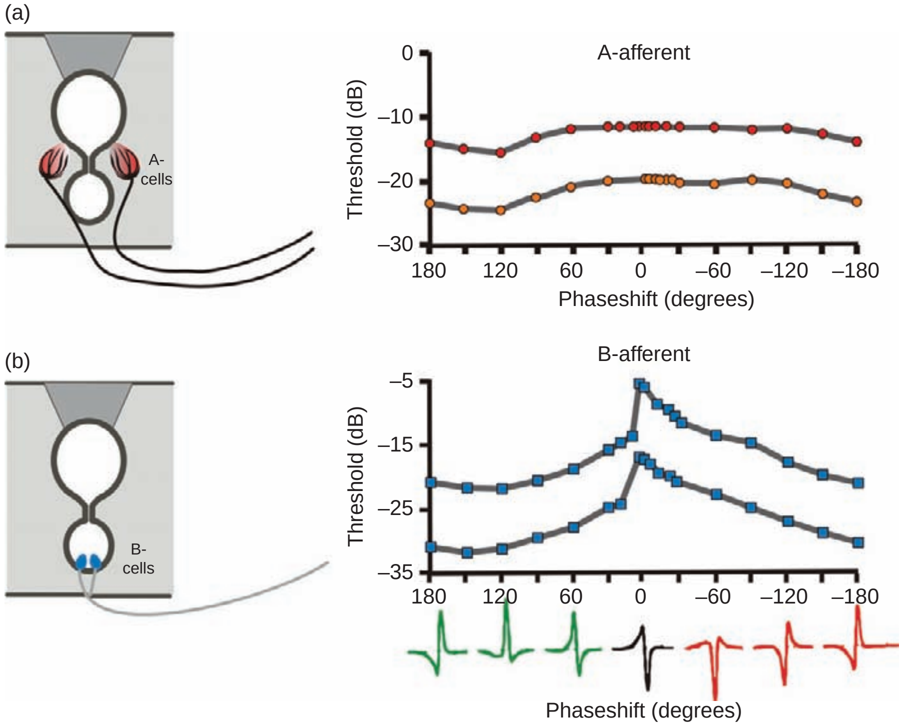


Figure 7 Response of two A- (a) and two B-cell (b) afferents to phase-distorted EODs. Afferents were stimulated with phase-shifted versions of their natural EOD (shown in black). This manipulation only affects the waveform but maintains a constant peak-to-peak amplitude and power spectrum of the EOD. While A-afferents have relatively high thresholds and respond to waveform-distorted EOD similar as they do to their natural EOD (a), B-cell afferents are least sensitive to the undistorted EOD and respond highly sensitive to weak waveform distortions characteristic for living prey items, which correspond to phase shifts in the range between 0° and –45°. Modified from von der Emde G and Bleckmann H (1997) Waveform tuning of electroreceptor cells in the weakly electric fish, *Gnathonemus petersii*. *Journal of Comparative Physiology A* 181(5): 511–524.

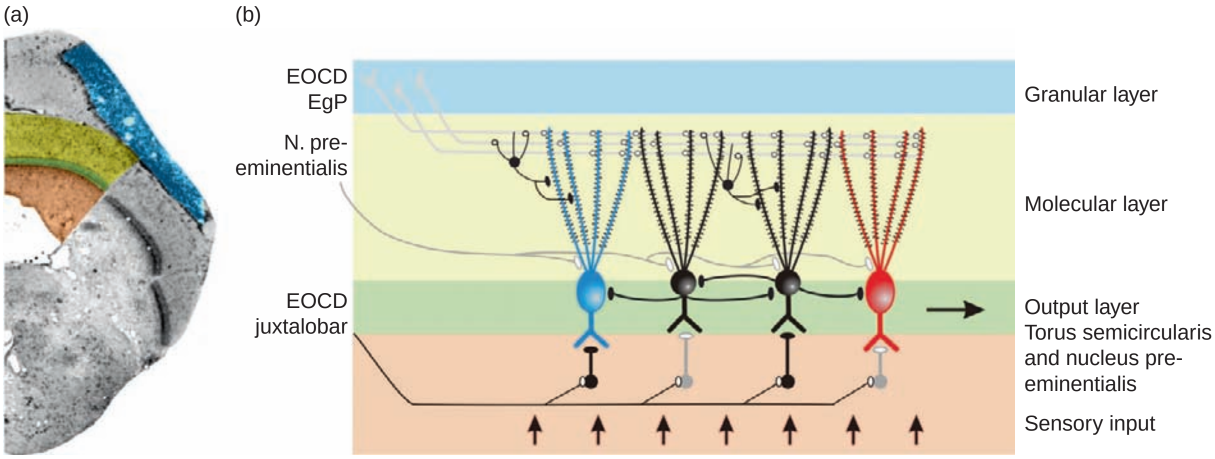


Figure 8 (a) Photomicrograph of a section through the ELL of *Gnathonemus*. Laminar regions of the medial zone of the ELL are highlighted in the same colors as used in the schematic of the ELL (b). (b) Schematic of the layered organization of the ELL. On the left and below, sources of input to the ELL are shown. Primary sensory input from electroreceptors converges on granular cells in the granular layer. These cells also receive strong corollary discharge-related input from the juxtalobar nucleus (electric organ corollary discharge (EOCD)). Local interneurons (black) and ELL efferent cells of two varieties (blue and red) are found in the ganglionic layer (output layer). While large ganglionic cells (I-cells, blue) are inhibited by sensory input, large fusiform cells (E-cells, red) are excited. The local interneurons (medium ganglionic neurons, black) are either inhibited or excited by the sensory input and provide local inhibitory feedback to the efferent cells. Afferents of nucleus preeminentialis supply direct electrosensory feedback input to the basal apical dendrites of these cells of the ganglionic layer. A third central source of afferents comes from the eminentia granularis (EgP), which sends massive bundles of parallel fibers to the molecular layer of the ELL. This input conveys corollary discharge input (EOCD), sensory input of other modalities, as well as predictive signals that drive plasticity in this system. The photo was kindly provided by K. Grant.

in driving granule cells depends on the timing between sensory and juxtalobar input. Thus, the synaptology of granule cells makes them ideal candidates for a coincidence-based transformation of latency-encoded afferent input to a rate code.

Spatial coding

The coding accuracy of finite spatial coordinates depends on the tuning width of the neurons. Narrow receptive fields are best suited to encode 1D (e.g., EOD amplitude) and 2D parameters (localization of a stimulus), while for encoding three dimensions, optimal tuning widths exist. Electric images are 2D by nature, and sensory information of these images is relayed to the topographically ordered array of neurons in ELL (**Figure 9**). This 2D representation enables fish to determine the 3D coordinates of objects (*x*, *y*, *z*). Object location (*x*, *y*) is directly accessible as the location of the maximal amplitude from the map within ELL (**Figure 3(a)**). Object distance (*z*), however, must be extracted using measures related to the amplitude and width of the electric images (**Figure 3(b)**).

With regard to orientation (*x*, *y* coordinates), the spatial resolution ultimately set by the mormyromast density is maintained at the early processing stages. Mormyromast afferents and efferent cells of ELL show characteristically small receptive fields of only a few millimeters in diameter (**Figure 9**). The fine sampling array, especially at the foveal regions, contributes to the acuity in discriminating between two objects (see below).

Specific circuits within ELL enhance the 2D representation by mechanisms of lateral inhibition based on a rapid and topographically specific inhibition of the granular cells (large multipolar intermediate layer (LMI) neurons **Figure 10**). Similar to contrast-enhancing lateral inhibition in the visual system, this might enhance contrast of the otherwise blurred sensory input.

Determining the distance to an object depends on determining the blurriness of the image. Blur itself depends on both object size and distance; thus, a normalization that effectively measures the blurriness of the image is required. Behavioral data suggest that animals detect the so-called slope/amplitude ratio for distance detection. In a simplified approach based on small objects only, this would be equivalent to determining the half-width of a Gaussian electric image.

For simple images, a neuronal algorithm has been proposed (**Figure 10**). In a first step, the peak amplitude of the image can be obtained from the mean activity of the population of all ELL neurons. Normalizing the population response by this mean rate, the number of neurons active above a certain threshold could then indicate half-width or slope as a measure of distance. Optimally, this two-step process is carried out in two parallel somatotopic maps, of which the map used to

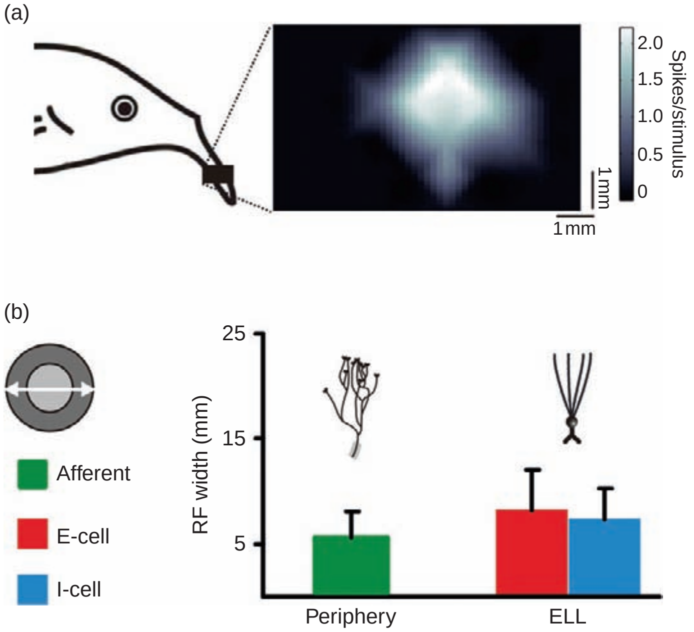


Figure 9 (a) Two-dimensional receptive field of a primary afferent fiber innervating a mormyromast on the Schnauzenorgan. The receptor organ was stimulated by an artificial local dipole, but the dimensions documented here are comparable to those that can be seen during stimulation with small objects in the presence of the EOD. (b) Mean width of receptive fields of primary afferents (green) and of efferent cells (red and blue) of the ELL (large fusiform cells, E-cells, and large ganglionic cells, I-cells). Note that the efferent neurons of the ELL have receptive fields of similar widths than the peripheral afferents, which might enhance the spatial acuity of the system (see text).

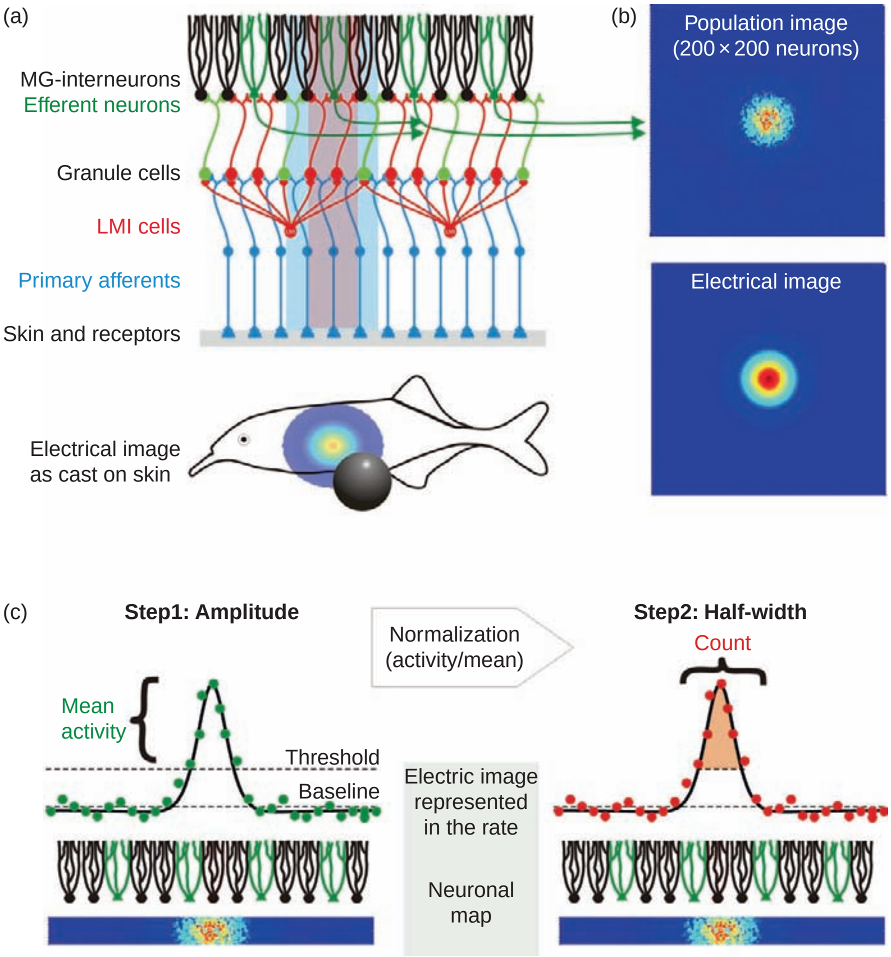


Figure 10 (a) Schematic of the conversion of an object's peripheral electric image on the skin to its neuronal excitation pattern within the ELL network. The spatial spread and blurriness of the neuronal representation is likely to be reduced by the local inhibitory LMI cells (see text). (b) Comparison of a theoretical 2D image cast onto the skin and its neuronal representation in the ELL. The topographical arrangement of the ELL maintains the peripheral image but adds noise. (c) Possible two-step procedure for the extraction of the distance of a small object. In an initial step, electric image amplitude as represented by the ongoing activity of the ELL output could be determined as the mean rate count above a threshold. Second, the response profile of the population would be normalized by this mean activity. It follows that the number of neurons (= the spatial extent of the image) active above a threshold would represent the normalized width of the electric image. In the simplified case of Gaussian-shaped electric images of smaller objects, this is equivalent to determining the slope/amplitude ratio, which was found to explain animal-detection performance in behavioral experiments.

determine the mean amplitude should have wider spatial tuning than that used to determine image width. As two parallel maps of the electrosensory environment exist (maps of the medial and dorsolateral zones of the ELL, medial zone (MZ), and dorsolateral zone (DLZ)), and these converge topographically in the midbrain nucleus lateralis, the MZ and DLZ might be a way to establish parallel processing of the amplitude and width parameters discussed above.

Plasticity

Functionally, plasticity in electrosensory fishes enhances the detection of weak signals embedded in noise by the subtraction of predictions based on the recent sensory past from the current sensory input (**Figure 11**). This is equivalent to forward models where future states of the sensory input are predicted based on associations between recent input and motor commands.

Predictive input is conveyed by the parallel fibers that target the apical dendrites of efferent cells and Purkinje-like MG-cells within the ELL, while sensory information from afferents impinges on the basal dendrites in ELL. Temporal association of sensory and predictive inputs (EOCD) can lead to associative spike-timing-dependent plasticity in MG-cells. Key to this is the occurrence of broad spikes generated in the proximal apical dendrites shortly after (within ~50 ms) the parallel-fiber input (**Figure 12**).

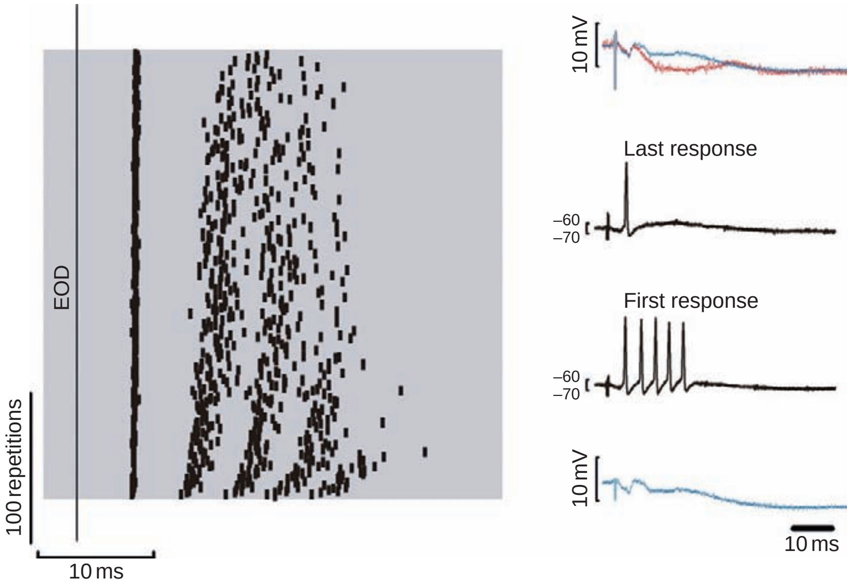


Figure 11 Response of an efferent (LF-cell) neuron of the ELL to the sudden appearance of a small conductive cube. Neuronal spikes are shown in the form of a raster display where the occurrence of each spike is marked by a dot. Experimental time progresses from bottom to top. In the absence of the object, this cell was silent but started spiking heavily on the first presentation of the object (left bottom and first response). After the object was present for a longer time period, the intensity of the sensory response was greatly diminished (left top and last response). This change is due to a change of the corollary discharge input to this cell as is shown on the right. After a certain duration of presenting the object, the corollary discharge input to the cell changes from an initial epsp (blue traces) to an inhibitory postsynaptic potential (ipsp, red trace). Thus, a predictive signal develops which counterbalances the afferent input.

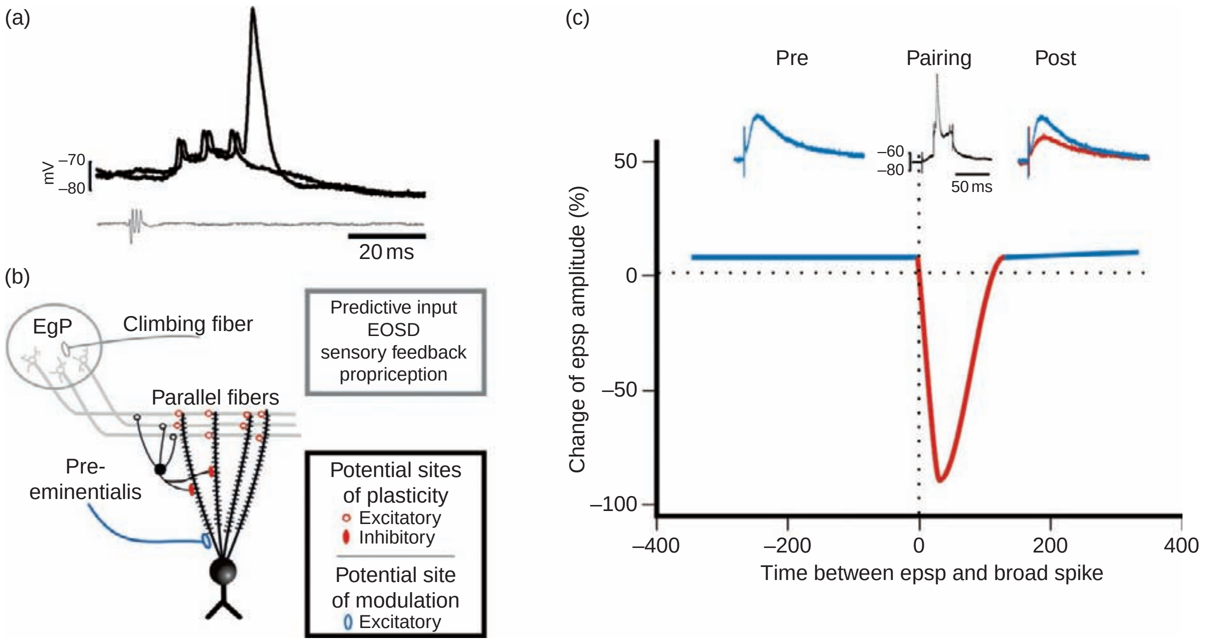


Figure 12 Response properties of medium ganglionic cells and the anti-Hebbian learning rule. (a) Two responses of an MG-cell to the corollary discharge input (in the absence of sensory input). Note that these cells generate axonal spikelets and occasionally complex dendritic broad spikes. (b) Schematic of the connectivity between the climbing fiber/parallel fiber input to the MG-cells and the possible regulatory sites for broad spike generation. (c) Temporal learning rule of MG-cells. Pairing between corollary discharge epsp and broad spikes leads to anti-Hebbian depression of the parallel fiber efficacy (red curve), when the epsp precedes the broad spikes. *In vitro*, additional nonassociative weak potentiation occurs at other times (blue line), but this may be different *in vivo*.

Similar to the complex spikes of the cerebellum, broad spikes can be controlled independently from axonal spikes, most likely by feedback from the preeminential nucleus. This nucleus integrates ELL output with feedback from the midbrain torus semicircularis and thus could provide a source of instructive signals modulating plasticity.

See also: **Detection and Generation of Electric Signals**: Electric Organs; Generation of Electric Signals; Physiology of Ampullary Electrosensory Systems; Physiology of Tuberous Electrosensory Systems. **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Babineau, DJE, Lewis JE, and Longtin A (2007). Spatial acuity and prey detection in weakly electric fish. *PLOS Computational Biology* 3(3): e38.

Bacelo J, Engelmann J, Hollmann M, von der Emde G, and Grant K. (2008) Functional foveae in an electrosensory system. *Journal of Comparative Neurology* 51(3): 342–359.

Bell CC (1990). Mormyromast electroreceptor organs and their afferent fibers in mormyrid fish. II. Intra-axonal recordings show initial stages of central processing. *Journal of Neurophysiology* 63: 303–318.

Bell CC, Han C, and Sawtell NB (2008) Cerebellum-like structures and their implications for cerebellar function. *Annual Review of Neuroscience* 31: 1–24.

Brown WM and Backer A (2006). Optimal neuronal tuning for finite stimulus spaces. *Neural Computation* 18(7): 1511–1526.

Bullock TH, Hopkins CD, Popper AN, and Fay RR. (2005) *Electroreception*. New York: Springer.

Caputi AA and Budelli R (2006) Peripheral electrosensory imaging by weakly electric fish. *Journal of Comparative Physiology A* 192(6): 587–600.

Engelmann J, Bacelo J, Metzen M, *et al.* (2008) Electric imaging through active electrolocation: Implication for the analysis of complex scenes. *Biological Cybernetics* 98(6): 519–539.

Gomez L, Budelli R, Grant K, and Caputi AA (2004) Pre-receptor profile of sensory images and primary afferent neuronal representation in the mormyrid electrosensory system. *Journal of Experimental Biology* 207(14): 2443–2453.

Han VZ, Grant K, and Bell CC (2000) Reversible associative depression and nonassociative potentiation at a parallel fiber synapse. *Neuron* 27(3): 611–622.

Hollmann M, Engelmann J, and von der Emde G (2008) Distribution, density and morphology of electroreceptor organs in mormyrid weakly electric fish: Anatomical investigations of a receptor mosaic. *Journal of Zoology* 276: 149–158.

Lewis JE and Maler L (2001) Neuronal population codes and the perception of object distance in weakly electric fish. *Journal of Neuroscience* 21(8): 2842–2850.

Maler L (2009a) Receptive field organization across multiple electrosensory maps. I. Columnar organization and estimation of receptive field size. *Journal of Comparative Neurology* 516(5): 376–393.

Maler L (2009b) Receptive field organization across multiple electrosensory maps. II. Computational analysis of the effects of receptive field size on prey localization. *Journal of Comparative Neurology* 516(5): 394–422.

Pusch R, von der Emde G, Hollmann M, *et al.* (2008). Active sensing in a mormyrid fish – electric images and peripheral modifications of the signal carrier give evidence of dual foveation. *Journal of Experimental Biology* 211(6): 921–934.

Sawtell NB, Williams A, Roberts PD, von der Emde G, and Bell CC (2006) Effects of sensing behavior on a latency code. *Journal of Neuroscience* 26(32): 8221–8234.

Shuai J, Kashimori Y, Hoshino O, Kambara T, and von der Emde G. (1999) Electroreceptor model of weakly electric fish *Gnathonemus petersii*: II. Cellular origin of inverse waveform tuning. *Biophysical Journal* 76: 3012–3025.

von der Emde, G. (2006) Non-visual environmental imaging and object detection through active electrolocation in weakly electric fish. *Journal of Comparative Physiology A:* 192(6): 601–612.

von der Emde G, Amey M, Engelmann J, *et al.* (2008) Active electrolocation in *Gnathonemus petersii*: Behaviour, sensory performance, and receptor systems. *Journal of Physiology Paris* 102(4–6): 279–290.

von der Emde G, Behr K, Bouton B, *et al.* (2010) 3-dimensional scene perception during active electrolocation in a weakly electric pulse fish. *Frontiers In Behavioral Neuroscience* 4:26. doi:10.3389/fnbeh.2010.00026.

von der Emde G. and Fetz S (2007) Distance, shape and more: Recognition of object features during active electrolocation in a weakly electric fish. *Journal of Experimental Biology* 210(17): 3082–3095.

Relevant Websites

http://biology4.wustl.edu – Carlson Lab: Behaviour and Communication.

http://www.medicine.mcgill.ca – Chacron Lab: Neurocomputational aspects.

http://www.unic.cnrs-gif.fr – Grant Lab: Physiology of electrolocation.

http://www.nbb.cornell.edu – Hopkins Lab: Communication and evolution.

http://biology.mcgill.ca – Krahe Lab: Electrolocation and communication.

http://www.neuromech.northwestern.edu – MacIver Lab with focus on robotics.

http://www.med.uottawa.ca – Maler Lab: Theory of electrolocation.

http://www.nelson.beckman.illinois.edu – Nelson Lab: Extensive bibliography and movies.

http://alumnus.caltech.edu – Rasnow Lab: Computational aspects.

http://www.theangelsproject.org – Robotics.

http://www.fiu.edu – Stoddard Lab.

http:// www.zoologisches-institut.uni-bonn.de – von der Emde Lab: Active electrolocation and bionics.

Electric Organs

AA Caputi, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

© 2011 Elsevier Inc. All rights reserved.

Diversity of Electric Organs and Bioelectrogenesis
Morphological Organization of Electrogenic Systems and Its Role in Electrogenesis
Further Reading

Glossary

Active electrolocation The ability of weakly electric fish to orient and detect objects based on their electric sense.

Carangiform Type of locomotion exhibited by some fish characterized by oscillatory movements of the rear of the body and tail.

Electrocommunication Communication based on electric signals.

Electrocytes Effector cells or syncytium specialized for generating electric fields and located primarily in the tail of electric fish. They generate and control the waveform of the electric organ discharge.

Electromotor neuron Spinal neuron that drives electrocytes to generate an electric field.

Electroreception Sensory modality for the reception and analysis of electric fields.

Fixed action pattern Behavioral sequence that is indivisible and runs to completion. These patterns are invariant and are produced by a commanding neural network in response to external or internal stimuli.

Neural network Circuit of neurons sharing a common control function.

Nodes of Ranvier Gaps in the myelin sheath of axons that create an uninsulated region of the axon where voltage-sensitive ion channels for generating electrical current are located.

Nonspherical topology Any shape of an object that, if inflated, would never achieve a spherical form.

Polyphyletic character A phenotypic character present in multiple taxa that is not present in a common ancestor.

Post-effector mechanisms The passive mechanisms that link effector cells with an external action. The clearest example is the skeleton and connective pulleys that transform muscle contractions into body movements. By analogy, the passive tissues of electric fish transform the fish's body into a distributed electric source.

Size principle States that the threshold of motor neurons grows with the size of the cell body; thus, motor units are recruited according to their size.

Synchronism A relationship among events such that they occur at the same time.

Syncytia (plural of syncytium) A large cell-like structure containing many nuclei derived from the fusion of many individual cells.

Diversity of Electric Organs and Bioelectrogenesis

Electric organs are effectors of the nervous system specialized for emitting electric fields. Bioelectrogenesis has multiple functions. Some electric organs generate strong discharges for use as a weapon. More commonly, electric organs transmit information. In the latter case, the emitted energy carries two types of electrosensory signals. The emitter fish obtains information about surrounding objects by sensing changes in the self-generated electric field (**see also Detection and Generation of Electric Signals**: Active Electrolocation and **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish).

Electrogenesis is found in two orders of Chondrichthyes and four orders of Actinopterygii. The lack of known common electrogenic ancestors suggests that electric organs evolved several times (**Figure 1**). A few basic solutions for electrogenesis have been combined in different ways, yielding a large diversity of electric organs and electric organ discharges (EODs).

One common feature of electric organs is the electrogenic tissue, which is formed by cellular units (called electrocytes) derived from excitable tissue depending on the taxon. Electric organs are classified as neurogenic or myogenic, depending on the cell lineage of the electrocytes. Electrocytes retain a complex repertoire of cell membrane ion channel as the basis of their effector function. They also develop a characteristic geometry and adapt their channel distribution to the organization of the electric organ as a whole (**see also Detection and Generation of Electric Signals**: Development of Electroreceptors and Electric Organs).

Neurogenic organs generate electric fields by the coordinated activation of hundreds of enlargements of electromotor axons running within a connective sheath extended along the fish body. These axons lack a

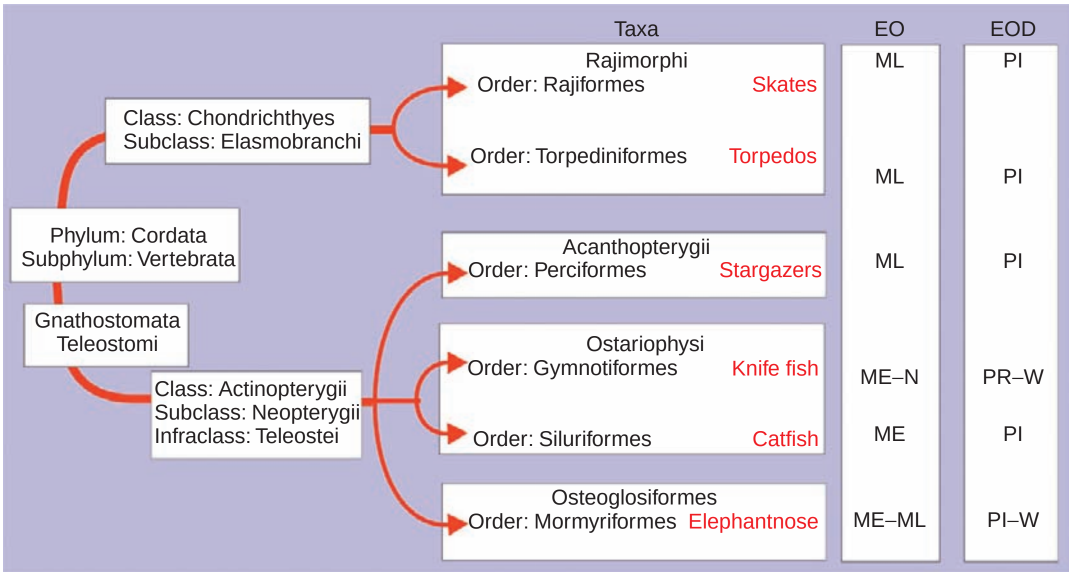


Figure 1 Electrogenesis has been reinvented several times during evolution. Electric organs can be composed of electrocytes of neural (N) or myogenic (M) lineage. Neural organs are extended. Myogenic organs may be localized (ML) or extended (ME) along the fish body and may be driven as a high-frequency continuous wave (W) or in a regular (PR) or irregular (PI) pulsatile manner.

peripheral target and have unique adaptations in their nodes of Ranvier and geometry to maximize current summation.

Myogenic organs are composed of a serial array of electrogenic syncitia derived from the fusion of muscular precursor cells. During development, myogenic electrocytes retain excitability but lose contractile machinery. Depending on the taxon, myogenic electric organs are derived from different muscle groups. Some are localized, either cephalic (stargazers and torpedo rays) or caudal (skates and mormyrids). Other electric organs extend all along the fish body, following the adaptation of body geometry and motor plan for active electroreception (knife fishes).

All these systems conform to a key concept of electrogenesis: relatively large electric fields resulting from the sum of weak currents generated by each electrocyte. Efficient summation of these currents requires the following:

1. A geometric organization allowing a parallel flow of the currents generated by equally oriented patches of membrane.
2. A precise coordination of electrocyte activation by the nervous system. This implies two processes, synchronous activation of identically oriented electrogenic membranes to develop significant amplitude and asynchronous but precisely sequenced activation of opposite-oriented electrogenic membranes to maintain a constant waveform.
3. A post-effector path for electric currents that ultimately integrates the electric organ output and transforms the whole animal body into an electric source.

The activation of the electric organ is repetitive, generally all or none, and follows species-specific spatiotemporal patterns. The timing of the discharge is determined by a command signal generated in the medulla (**see also Detection and Generation of Electric Signals**: Generation of Electric Signals). In pulse fish, the interval between each command is much larger than the duration of the EOD and the electrogenic output consists of a series of discrete pulses of stereotyped waveform characteristic of the species. In wave fish, the discharge interval is regular and similar to the duration of the EOD; the electrogenic output has a continuous sine-wave-like shape with a relatively high frequency (100–1700 Hz). Thus, the rhythms of the neural commands are also characteristic of the species, and, in the case of wave fish, they are also a major determinant of the EOD waveform.

To sum up, electric fish can be schematically grouped as pulse fish having either localized or distributed electric organs and wave fish having electric organs of either neural or muscular lineage (**Figure 1**): (1) Mormyridae, Rajidae, Torpedinidae, and Perciforms have localized organs emitting pulses at irregular intervals; (2) Sternopygydae and Gymnarchidae have myogenic organs emitting continuous waves; (3) Apteronotidae is the only group exhibiting neurogenic organs emitting continuous waves; and (4) Rhamphychthydae, Hypopomidae, and Gymnotidae have more complex electrogenic systems with extended organs emitting complex pulses at regular intervals. Each of these groups exhibits a different combination of mechanisms for waveform generation.

Morphological Organization of Electrogenic Systems and Its Role in Electrogenesis

The individual contribution of electrocytes to the EOD can be either the same or different in amplitude, waveform, and timing. The process of integrating the activity of multiple electrocytes requires a physical path for current summation, as well as a control system. Electrocyte properties (geometry and channel repertoire), neural coordination mechanisms (peripheral wiring, spinal tracts, and motor neuron recruitment), and post-effector mechanisms (connective structures of the electric organ and the fish tissue considered as a current path) have different but complementary roles in determining the waveform of the EOD.

The Electrocytes

The basic rule for understanding electrocyte contribution to the EOD waveform is that active electrocyte membrane patches guide inward currents; the outside of the patch becomes negative and the inside positive. Electrocytes (either from neural or myogenic lineages) show adaptations of their geometry for orienting the external flow of currents and express a channel repertoire whose quality and distribution set the dynamics of their response. As electrocytes are closed surfaces of excitable membrane, only part of the electrocyte must be active at one time to avoid the cancellation of current flow by patches of membrane oriented in opposite directions. Therefore, effective electrogenesis is a subcellular process. In addition, different regions of the electrocyte are activated in a precise sequence to generate multiphasic waveforms. Functionally equivalent regions of different electrocytes have similar ion channels and are oriented in the same direction. Therefore, their synchronous activity generates current flowing in the same direction and each distinct component of the species-specific waveform is generated by a set of functionally equivalent electrocyte regions. As a corollary, membrane patches with opposite orientation are activated in sequence, thereby generating successive wave components with opposite polarity.

Neural electrocytes derive from nerve axons. Unlike most axons, the axons of spinal electromotor neurons of adult Apteronotidae do not have a synaptic target but end as a hairpin-shaped specialization (yellow, **Figure 2(a)**). Neural electrocytes form a plexus running along the body just ventral to the spine. In the electric organ, axons run in a rostral direction for several segments and then turn back in a caudal direction, ending near the point of origin. The thickness of the straight portions of this structure (up to 100 μm in diameter) contrasts with a smaller diameter at the origin, end and turning points. In the regions where the diameter of fiber is small, the nodes of Ranvier are similar to those in typical peripheral nerves. At the thicker portions, the nodes of Ranvier are wide and unexcitable, showing a large number of processes over the entire unmyelinated surface. These nodes have the morphological substrate of a series capacitance that allows a large flow of outward current without firing action potentials. Thus, the action potential running rostrally at the narrow origin of the dorsal branch of the hairpin generates forward currents inside the axon causing a head-positive phase (orange, **Figure 2(b)**). A part of these action currents invades the narrow portion at the turning point, generating a second sink. Action potentials generated there propagate caudally on the ventral arms of the electrocyte, generating the head-negative phase (blue, **Figure 2(b)**).

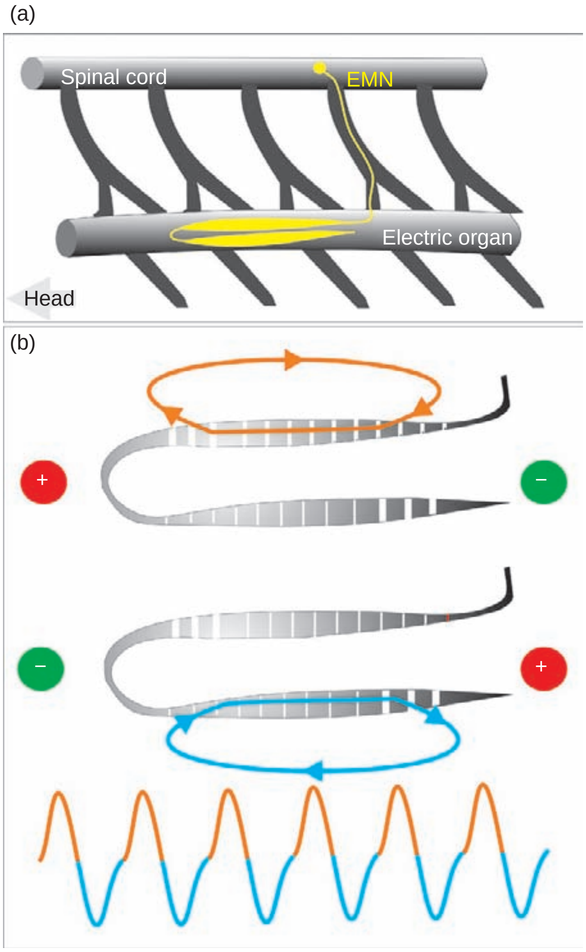


Figure 2 Neural electrocytes. (a) Neural electrocytes consist of two of enlargements of peripheral electromotor axons arranged as a hairpin structure with thick arms and a narrow turning point. (b) Bennett's hypothesis for local wave generation. The action potential running rostrally at the narrow origin of the dorsal branch of the hairpin generates forward currents inside the dilated axon, causing a head-positive phase (orange). A part of these action currents invades the narrow portion, eliciting an action potential at the turning point. This acts as a second sink for action currents that propagate caudally on the ventral arm, generating the head-negative phase (blue).

Myogenic electrocytes are cells under neural control but have different specializations. They respond with monophasic or multiphasic discharges depending on their shape, pattern of innervation, and ion-channel repertoire and distribution. At rest they are a closed, charged, and nonelectrogenic surface (**Figure 3(a)**). This equilibrium is broken by the action of cholinergic synapses on a localized region of each electrocyte that causes an initial external negativity associated with the flow of inward synaptic

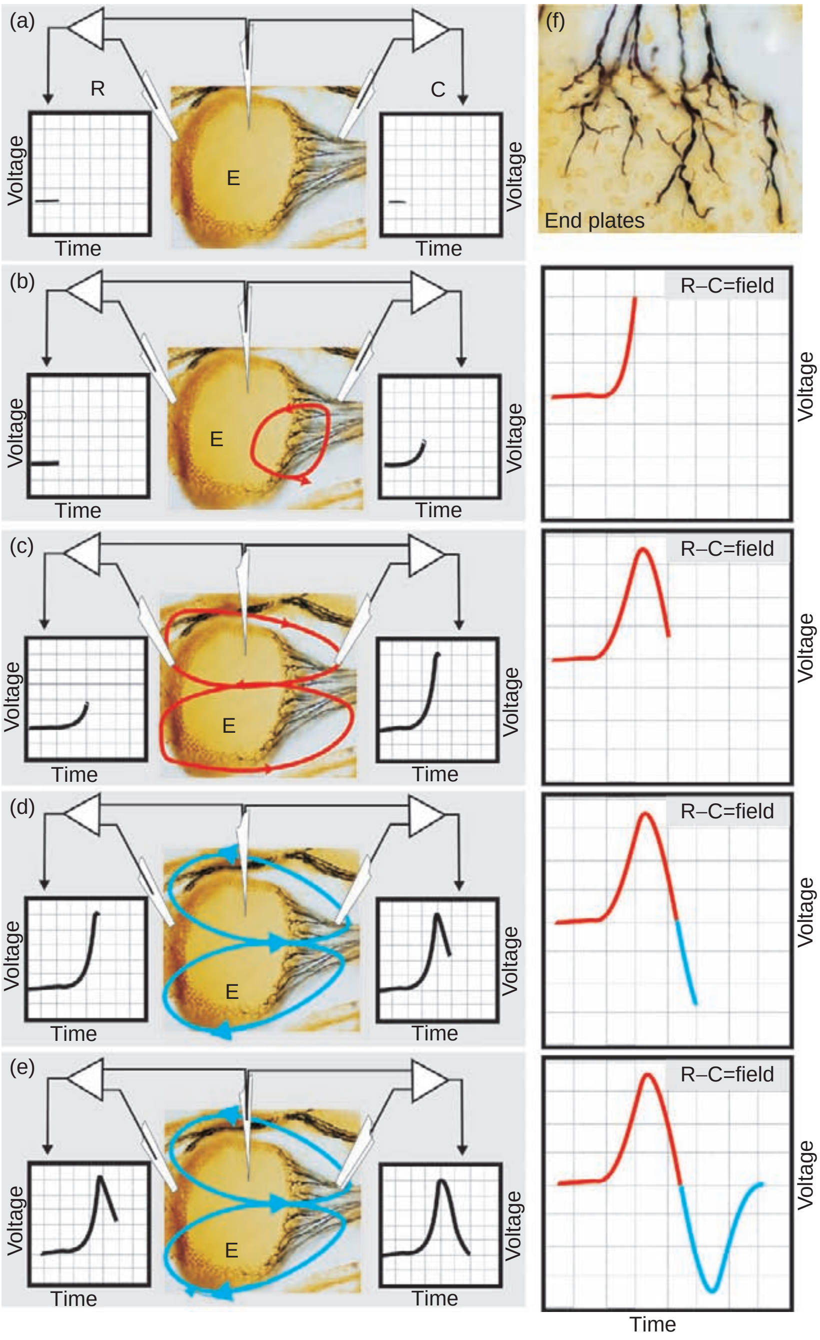


Figure 3 The process of activation of a singly innervated electrocyte. The series of composed figures (a–e) includes a photomicrograph of a singly innervated electrocyte with superimposed schematics representing the flow of currents at five selected times of the EOD as red and blue lines. These currents are associated with changes in the electric field recorded using three microelectrodes (represented as white needles) with their tips placed either inside the electrocyte (E) or outside the electrocyte facing the rostral (R) or the caudal (C) faces, respectively. These electrodes are connected to the differential amplifiers (represented as white triangles) that subtract the potential inside the electrocyte from the potential outside. The time courses of these potential differences (E-R and E-C) are represented in the screens at the rostral and caudal sides of the electrocyte. The time course represented in the right column corresponds to the head-to-tail field (resulting from subtracting R–C) and is the contribution of a single electrocyte to the whole EOD. Color-coding in the traces matches with lines indicating the current flow. (a) At rest, the electrocyte shows a homogeneous internal negativity, but does not generate external fields. (b) Synaptic activity alters the membrane potential locally at the caudal face, generating an inward current (red) that elicits action potentials and consequently caudal sink. (c) Action currents (red) flow outward through the rostral face, generating the head-positive component and exciting the rostral face. (d, e) The consequent secondary sink causes a field of opposite direction that progressively overcomes the head-positive component (red), giving rise to the head-negative component (blue). (f) Synaptic terminals on the surface of the same electrocyte (photomicrograph obtained from another tissue section).

currents (**Figure 3(b)** and inset). These inward currents may also trigger action potentials by exciting adjacent membrane patches, causing an equally oriented and sharper negativity (**Figure 3(c)**). Subsequent activation of the electrocyte membrane may generate additional wave components according to a fixed pattern.

The example in **Figure 3(d)** shows how the opposite face is excited by the action currents generated at the innervated face, resulting in a biphasic discharge. However, electrocyte responses depend on geometry and channel distribution, yielding a large variety of waveforms. In skates, torpedoes, and electric eels, the contribution of electrocytes to the EOD is the simplest. Electrocytes are disk shaped with only one innervated and excitable face. Thus, the EOD consists of a single wave component whose negativity indicates the innervated face. The electrocytes of the African wave fish *Gymnarchus niloticus* combine the activation of the innervated face with a passive response of the opposite face. The activation of caudal faces results in the head-positive crests of a sine-wave-like waveform. Interestingly, in these fish, the time constant of the nonexcitable face matches the frequency of the neural command. Most gymnotiforms show mono-innervated electrocytes on their caudal face, but the rostral face may be excitable or not, depending on the taxon and electrocyte location along the electric organ. *Brachyhypopomus* is a good example of where the innervation is concentrated on a small protrusion of the caudal face (**Figure 4(a)**). Depending on their size and channel repertoire, rostral faces may or may not respond to the depolarizing flow of current generated by the caudal faces. The decrease in size and the inter-electrocyte distance from head-to-tail facilitate the invasion of currents from the caudal to the rostral faces of neighboring electrocytes and, consequently, increase the probability of rostral face firing. As a result, the negative/positive ratio of the locally generated EOD increases from rostral to caudal regions of the fish body (**Figure 5(a)**).

Evolution has led to several solutions for increasing the number of wave components beyond two. In the case of the drum-shaped electrocytes of the tail region of some *Gymnotus* (*G. coropinae*, *G. javari*, *G. carapo*, and other species still not formally described), the channel repertoire of innervated faces allows their re-excitation by the action currents occurring at the opposite face, generating in this way a triphasic waveform (**Figure 4(b)**). Another solution is the sequenced synaptic activation of opposite faces of doubly innervated electrocytes as occurs in the dorsolateral tube of the electric organ of *Gymnotus* and *Rhamphychthys* (**Figure 4(c)**). In common with other gymnotids, their electric organs show several columns of caudally innervated electrocytes generating the main complex of the discharge (**Figures 5(b)** and **5(c)**, red portions of the electric organ and waveform traces). Electrocytes decrease in size and increase in density from head to tail. The regional waveform is monophasic (large rostral electrocytes), biphasic (some *Gymnotus* at the central or caudal regions), or triphasic (*Rhamphychthys* and *Gymnotus* at the tail region). The electric organs of these taxa are characterized by the presence of multiple tubes, each housing electrocytes with different properties (**Figures 5(b)** and **5(c)**, blue and green portions of the electric organ and waveform traces) showing rostrally innervated or doubly innervated electrocytes at some regions of the electric organ. In these electrocytes, synaptic activation in rostral faces occurs earlier than in caudal ones, which in turn occurs synchronously with the activation of caudal faces of singly innervated electrocytes. As a result, a negative component precedes the main complex at the abdominal and central regions of the fish body. Moreover, rostrally innervated faces of abdominal electrocytes are often unable to fire action potentials; this is reflected in fairly smooth negative components generated at the abdominal region (**Figures 5(b)** and **5(c)**, blue portions of the electric organ and waveform traces).

The electrocytes of pulse mormyrids show the largest geometrical refinement. During development, cell fusion occurs according to a precise pattern, creating complex geometries with nonspherical topologies; this means that if an electrocyte were inflated, it would never achieve a spherical form. These electrocytes are fenestrated disks that give rise to finger-like evaginations, which fuse into larger trunks or stalks where the electromotor neurons make synaptic contact. In some species, the stalks traverse electrocyte fenestrations and fuse again with other evaginations on the membrane of the opposite side, acting not only as an electrogenic membrane contributing to the EOD but also as a connecting route between opposite faces (**Figure 4(d)**). Depending on taxa, stalks may go across the electrocyte more than once. These differences in geometry establish the species specificity of the EOD.

Electrocyte shape is not only the expression of genetic traits during early development, but it is also under endocrine control. Sexual differentiation causes changes in the relative areas of excitable and nonexcitable membranes of the electrocytes by adding invaginations of the membrane, and it controls the expression of the ion-channel repertoire. Endocrine signals may also control the expression of channel repertoire at specific regions of the electrocytes, altering the responses of the noninnervated faces in both gymnotids and mormyrids. Interestingly, in the wave fish *Sternopygus*, sex hormones act at the electrocyte level and at the central pacemaker nucleus in such a way that the resulting waveform is a timescaled version of the waveform of nonsexually differentiated fish.

The Bulbospinal and Peripheral Networks

The electric organ of pulse species is commanded either by an irregularly discharging nucleus or by a pacemaker (**see also Detection and Generation of Electric Signals**: Generation of Electric Signals). Command nuclei operate as time integrators, firing irregularly depending on the

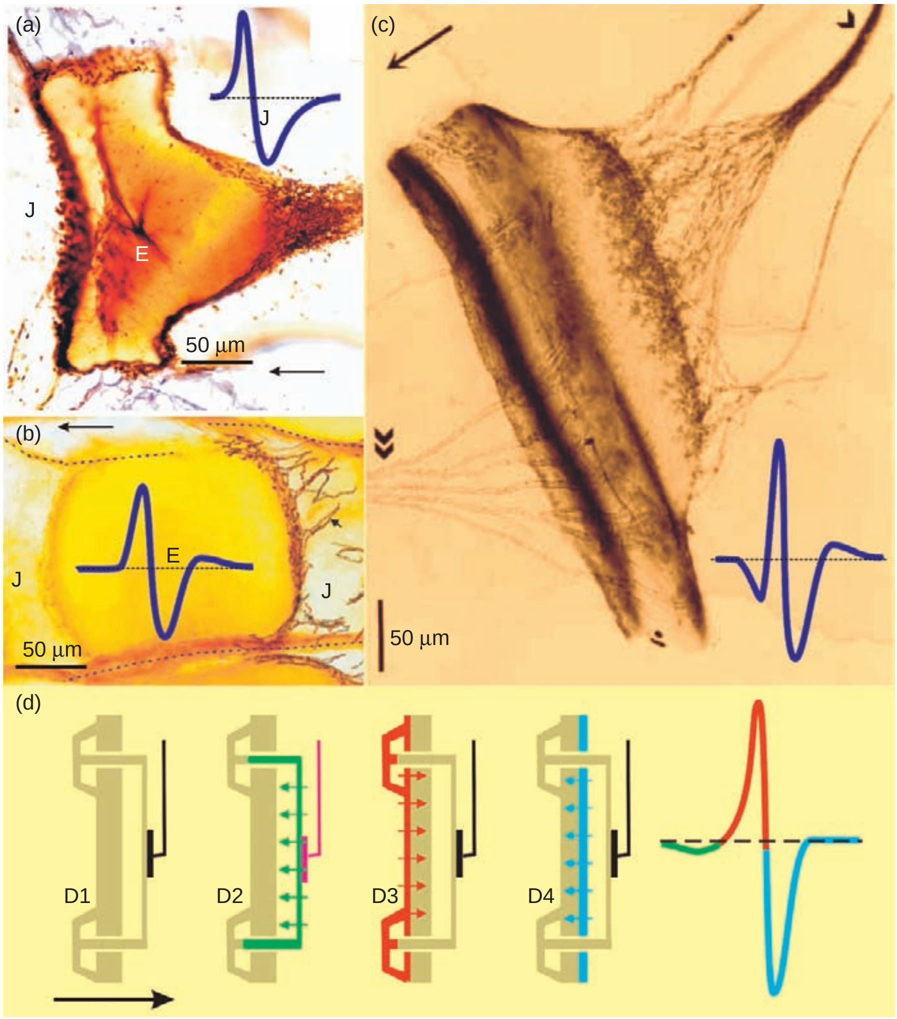


Figure 4 Different types of electrocytes contribute to waveform generation in different ways. (a) Singly innervated electrocyte from *Brachyhypopomus pinnicaudatus.* Nerve terminals contact a caudal protrusion, eliciting the head-positive phase. The negative phase results from current invasion of the rostral face. (b) Caudally innervated electrocytes from the tail region of *Gymnotus carapo* generate a triphasic waveform. Neural activation of caudal faces generates the large initial positive component. Longitudinal currents excite the noninnervated electrocyte face, generating the negative component. The process repeats in the opposite direction, depolarizing again the innervated face to generate the late positive component. (c) Doubly innervated electrocyte from *Rhamphychthys rostratus* shows innervation on the caudal ridge (single thick arrowhead) and also on the marginal zone (double arrowhead). The marginal zone is synaptically activated first, causing the negative phase; next, the synaptic activation of the caudal phase elicits a triphasic complex following a similar sequence as in (c). (d) Schematic of the activation process of a perforated stalked electrocyte from a pulse mormyrid. (D1) Electrocytes have perforant stalks that go through the electrocyte. (D2) Synapses activate the rostral stalks generating a small negative phase at the beginning (green). (D3) Perforant stalks conduct the excitation to the caudal face, generating a head-positive component (red). (D4) Finally, the rostral face is invaded, generating the head-negative component (blue). In all images, the arrow points rostrally.

richness and degree of synchrony of descending motor commands. Pacemaker nuclei generate a regular, frequency-modulated up or down descending motor commands. In all wave species, the electric organs are regularly driven by a pacemaker.

Different control strategies can be described for localized or extended, myogenic or neurogenic types of electric organs:

1. In fish with localized electric organs, the species-specific waveform of the field is based on the synchronous recruitment of similar electrocytes, all generating the same contribution to the external electric field. Thus, in fish with localized myogenic organs, the neural network mainly works as a transmission line to synchronize electrocyte excitation.

In a representative species of Mormyridae, *Gnathonemus petersii*, the electric organ is restricted to the base of the tail and consists of two columns on each side of the midline. In each column, about a 100 electrocytes are enclosed in a tube of connective tissue and are separated by gelatinous tissue. Electrocytes are disk shaped and serially stacked with

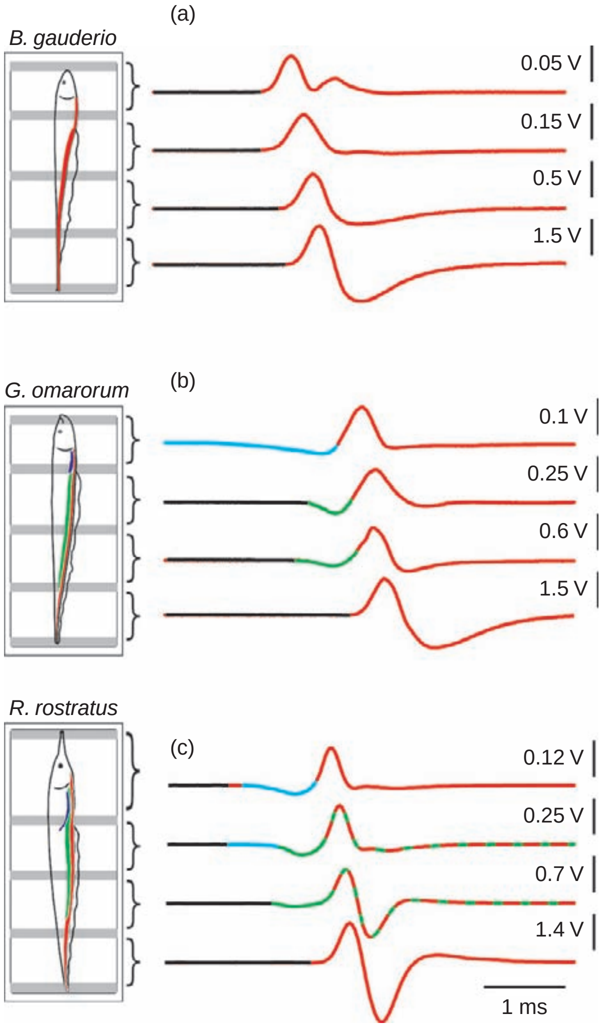


Figure 5 Heterogeneity of electric organs and regional EODs in pulse gymnotids. (a) *Brachyhypopomus pinnicaudatus* shows similar electrocytes all along the electric organ. Due to the decay in size and inter-electrocyte distance, the probability of firing of the rostral faces increases from head to tail, leading to an increase in amplitude of the negative wave (red traces). (b) In addition to the caudally innervated electrocytes generating the main positive–negative complex (red), *G. omarorum* shows two types of doubly innervated electrocytes in one of the tubes composing the electric organ: (1) less-excitable electrocytes at the abdominal region responding to the neural drive with graded potentials reflected in the smoothness of the initial negative wave component (blue) and (2) smaller electrocytes firing action potentials in response to an early command volley on their rostral faces, generating a sharp negative component at the center of the fish body. (c) *Rhamphychthys rostratus* shows three types of electrocytes at the abdominal region: (1) caudally innervated, generating the head-positive component (red), (2) rostrally innervated, generating a smooth negative component (blue) and doubly innervated at the marginal and caudal ridges, generating a triphasic component (green). At the central regions, only caudally innervated and doubly innervated electrocytes are present; and, at the tail region, the entire electrocyte population is caudally innervated. As the electrocytes diminish in size and increase in density toward the tail they become more excitable, leading to tetraphasic contributions of doubly innervated electrocytes and triphasic contributions of caudally innervated electrocytes.

anterior and posterior faces oriented perpendicular to the body axis. They measure about half a millimeter in diameter and are separated by only 50 μm. The current generated by all electrocytes flows along the tube. Thus, the number of electrocytes adds power, but not waveform diversity, and the efficiency of the EOD results from the synchronous activation of the population. To secure this, a synchronized doublet of spikes descends from the command nucleus along the bulbospinal electromotor tract, provoking a synchronized triplet of spikes at the electromotor neuron pool located in the caudal spinal cord. This volley activates the electrocyte stalks provoking in turn the synchronous response of the electrocyte population. The variety of electrocyte shapes, in particular, the number of times that stalks cross the electrocyte body, determines a large variety of EOD waveforms in pulse mormyrids.

2. Extended myogenic electric organs are exhibited by a polyphyletic group of fish formed by species belonging to different evolutionary lines, indicating that this strategy is also a robust solution for active electrolocation and electrocommunication. These types of electric organs generate two types of electric fields: brief pulses of a stereotyped spatiotemporal shape, or continuous quasi-sinusoidal waves shifted in phase along the electric organ. These complex outputs contrast with the input to the electromotor system, which consists of a localized impulse-like pacemaker discharge. Therefore, the nervous system must combine temporal and spatial processing of such simple inputs to achieve a complex electrogenic output.

First, the nervous system synchronizes the activity of electrocytes separated by hundreds of millimeters with 300-μs accuracy. Second, *Rhamphychthidae* and *Gymnotidae* show doubly innervated electrocytes requiring a sequenced recruitment of electromotor neuron pools innervating oppositely oriented membrane patches of the same electrocyte (**Figures 4** and **5**). Thus, in these cases, the neural network works also as a central pattern generator that coordinates the synchronous activation of functionally equivalent membranes and the sequential activation of opposite membranes.

Synchrony and sequence are supported by the synergistic action of neural delay lines implemented in a variety of forms including: (1) different conduction velocities along the spinal cord, (2) different conduction distances in the periphery, and (3) an appropriate distribution of coupling delays (included within this term are the conduction time through fine terminals, synaptic delay, and synaptic efficacy). *Gymnotus omarorum* is a paradigmatic species for illustrating some of these mechanisms.

In the spinal cord of pulse gymnotids, relay axons of different diameters descend through the dorsolateral funiculi (**Figure 6(a)**). Thin and slowly conducting relay fibers end proximally, but most fast conducting fibers end in the caudal portion of the spinal cord. These fibers project on an electromotor neuron layer with somata scattered in a row above the central canal (**Figure 6(b)**). Since electromotor neuron

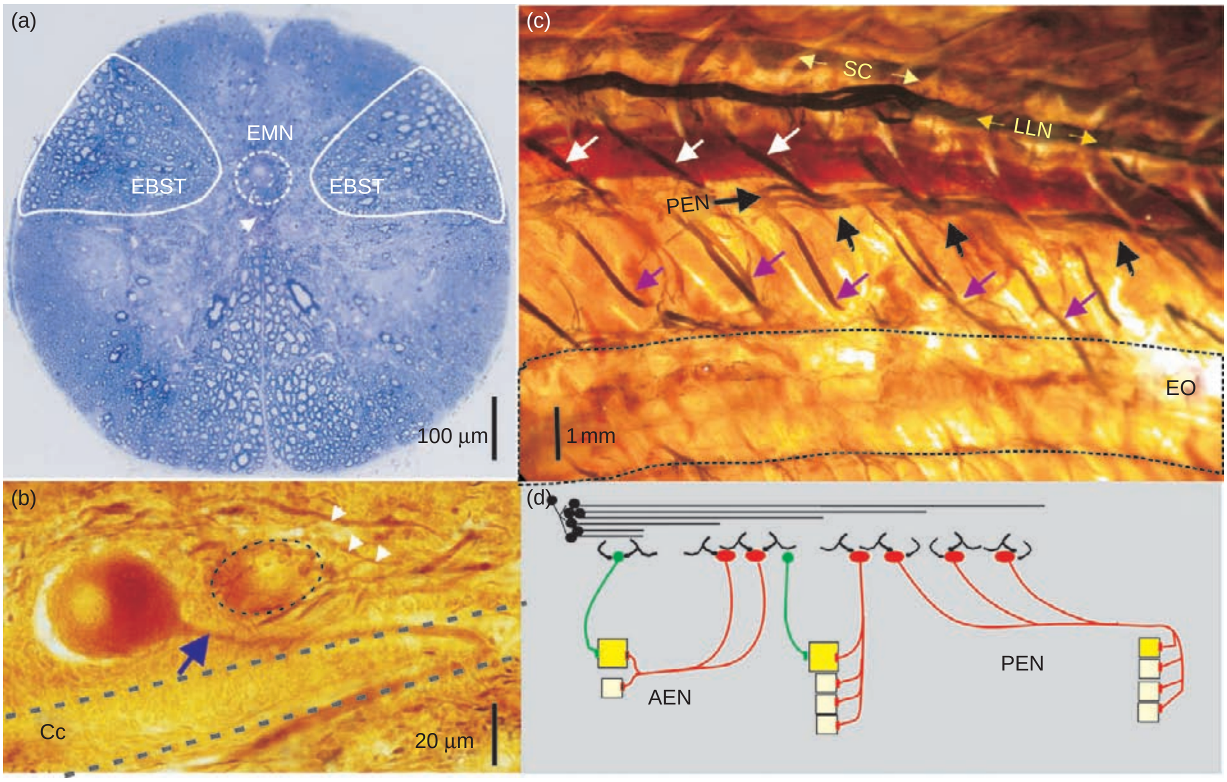


Figure 6 The neural network controlling the electric organ in *G. omarorum*, this network consists of two layers of neurons schematically represented in (d). The relay cell somata form part of the electromotor nucleus at the medulla. Their axons (represented in black in (d)) run along the spinal cord through the dorsolateral funiculi (electromotor bulbar-spinal tract (EBST) enclosed by white contours superimposed on a methylene-blue-stained semi-thin transverse section of the spinal cord in (a)). The terminals of these axons project to electromotor neurons distributed in a row above the central canal (Cc and dotted lines in the silver impregnated section are shown in (b)). Their axons (arrow in (b)) leave the spinal cord through the ventral roots (white arrows in the Osmium-stained material are shown in (c)) and form a neural plexus (PEN black arrows) at the caudal half of the fish's body. From this plexus, segmental nerves project to the electric organ (violet arrows). At the abdominal region, axons innervating the caudal faces of both electrocyte types (doubly and singly innervated) form a recurrent anterior electromotor nerve adding the necessary conduction time to cause their synchronous activation with the rest of the body. (d) At the very caudal regions, the posterior electromotor nerve composed of thick axons projecting to the caudal regions of the electrocytes runs dorsal to the electric organ (d).

activation results from pooled activity of slow and fast fibers, which converge on electromotoneurons in different proportions along the spinal cord, the more caudal the electromotoneuron is located, the more synchronized and faster the activation volley that it receives. In addition, the electric organ has a shortened representation on the spinal cord: in *Gymnotus*, small electromotoneurons projecting to the rostral faces are located on the rostral half of the spinal cord, and large electromotoneurons projecting to caudal faces occupy the central two quarters of the spinal cord (**Figure 6(c)**). Because of the size principle and their proximal location, small neurons projecting to the rostral faces are activated earlier than large neurons projecting to the caudal faces of the same electrocytes. Axons projecting to the rostral faces of the abdominal doubly innervated electrocytes run directly. Conversely, axons projecting to the caudal faces of both doubly and singly innervated electrocytes at the abdominal region are packed as a pair of recurrent nerves (anterior electromotor nerve (AEN), **Figure 6(d)**). This adds a significant conduction distance that delays the caudal activation of caudal faces, separating it from the activation of rostral faces but bringing it close to the activation of caudal faces of electrocytes in the rest of the fish's body. The rest of the axons, projecting to the caudal faces, form a longitudinal plexus referred to as the 'posterior electromotor nerve' (PEN, **Figure 6(d)**). Again, larger fibers projecting to more electrocytes are observed at the tail, shortening the activation time of this region. The activation process of the electric organ of *G. omarorum* is summarized in **Figure 7**.

3. The family Apteronotidae has neural organs. Electromotor neurons respond as forced oscillators driven by a descending tract coming from a pacemaker command nucleus firing at high frequency (about 1000 Hz). Peripheral axons run along a hairpin path showing important changes in diameter (**Figure 2**). At the longitudinal portions they enlarge several times in diameter, increasing the electrogenic area, but their diameter is small at the entry and at the turning

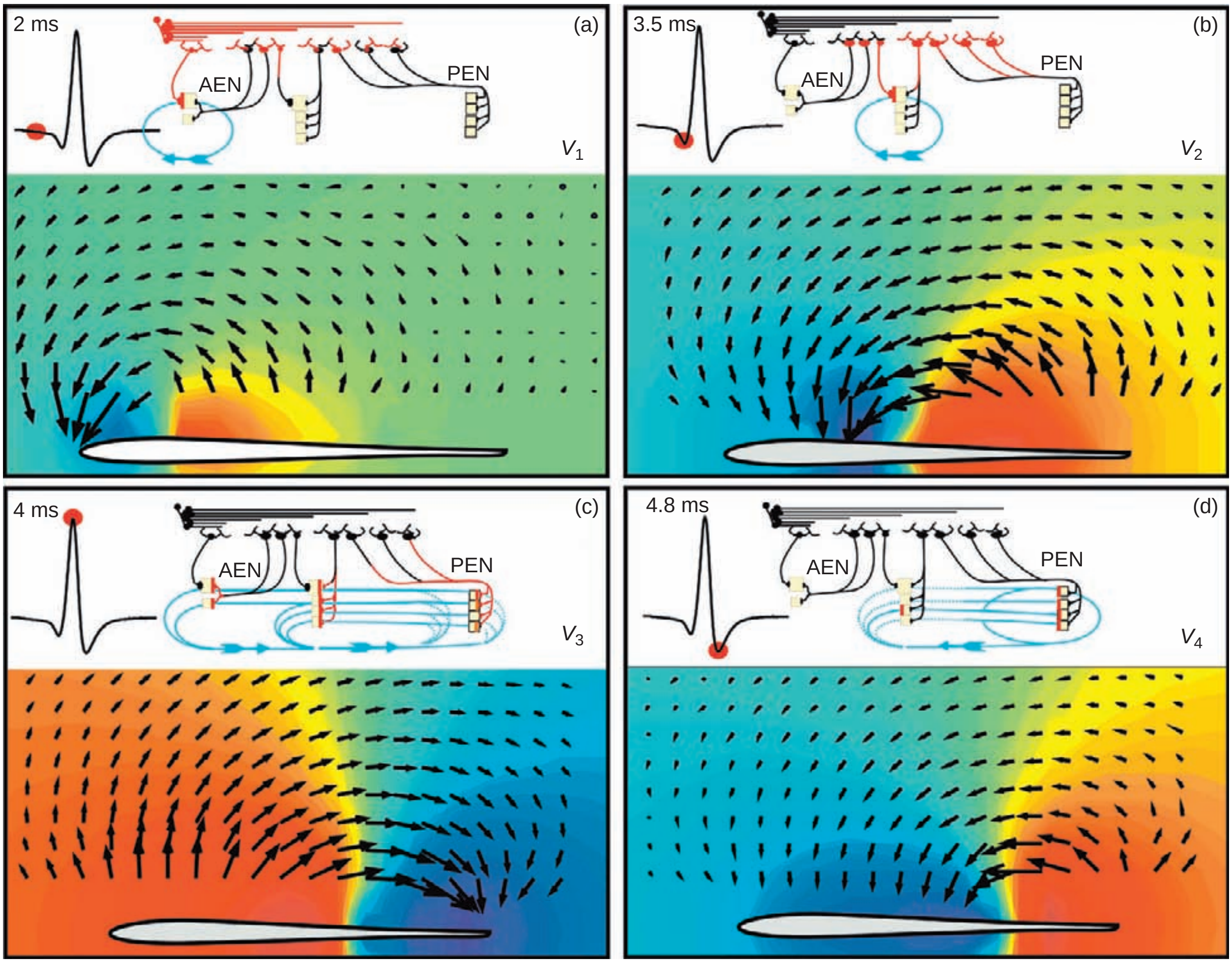


Figure 7 The process of activation of electric organs in *Gymnotus*. Excitation begins with synchronous activation of the central relay nucleus. The descending volley first reaches the small, round electromotor neurons lying in the rostral segments of the cord and projecting to the rostral face of the abdominal electrocytes. (a) After 2 ms, most descending fibers are active at the central portion of the cord. At the same time, the postsynaptic potentials occurring at the rostral face of abdominal electrocytes are generating V_1. (b) Approximately 3.5 ms after the command, small electromotor neurons activate electrocyte rostral faces in the central region, generating V_2. Less-excitable, large, oval electromotor neurons fire later. This, and possibly differential efficacy at peripheral synapses, causes V_3 to occur 0.7 ms after V_2. Axons of large electromotor neurons projecting to the caudal face of abdominal electrocytes follow a recurrent course through the anterior electromotor nerve (AEN), adding an extra delay and compensating for some of the differences in spinal conduction times. (c) Large electromotor neurons occupying the most caudal spinal segments project onto the caudal electric organ through the posterior electromotor nerve (PEN), eliciting V_3 approximately 4.1 ms after the RN discharge. (d) V_4 results when the action potentials generating V_3 propagate to the rostral face of caudally innervated electrocytes about 4.8 ms after the command.

point of the hairpin. Interestingly, the summated duration of these two phases matches the interval between pacemaker discharges. According to the fiber lengths and conduction speeds of the fibers, one can deduce that more than one descending volley has occurred at the descending track and the electromotor axons at the same time. Synchrony is very accurate, but not better than in other gymnotids. Rostral regions of the fish body discharge about 200 μs before the caudal regions (**Figure 8**). However, in high-frequency fish, this phase shift represents up to 20% of the cycle and this phase lag is an important cue for self identification.

Post-Effector Mechanisms

Unitary activities are integrated as an EOD by the passive but no less important action of the electric organ structure and post-effector mechanisms. Electrocytes are packed in series inside connective tubes running parallel along the electric organ, as in the batteries of a hand-held flashlight. This organization maximizes the longitudinal current flow and current summation on the tissue surrounding the electric organ. This flow of current through body tissues of low conductance relative to water transforms the whole fish body into a distributed electrogenic source.

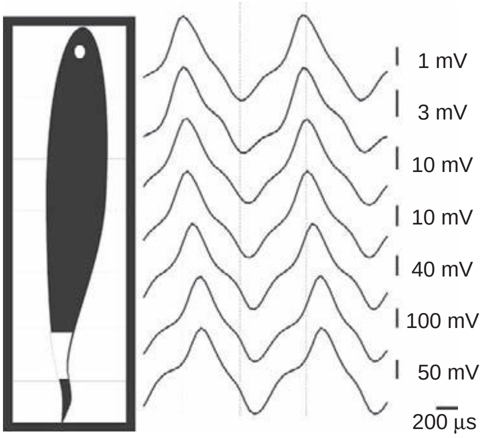


Figure 8 Spatiotemporal pattern of electromotive force generated by *Apteronotus albifroms* recorded with the fish in air. Note that the local waveform is similar but differs in amplitude and phase along the fish's body.

The electrocytes of localized organs often respond to their neural command with a characteristic activation pattern, which determines the waveform of the electric field everywhere in the fish's surroundings. In fish with carangiform locomotion, such as mormyrids, the electric organ is located at the base of the tail but the specialized sensory mosaic is at the head region. The fish's body is a low-conductivity path that connects the cephalic region with the electric organ and also, because of lateral leakage, distributes current for probing the environment. This causes, as an additional effect, an enlargement of the equivalent dipole arm with a consequent increase in 'electrical visibility' of the fish by conspecifics.

Knife fishes optimize these rostral funneling effects with the body geometry by having a long anal fin and a relatively stiff body posture. Furthermore, in pulse knife fishes, the heterogeneous structure of the electric organ determines a different waveform contribution of each region of the electric organ to the integrated output. Thus, for a given time and a given point in the fish surroundings, the local field is a weighted sum of the fields generated at that point by the different portions of the electric organ. The amplitude and polarity as well as the time waveform of the local field strongly depend on the relative position and distance from the electric organ where the field is measured. As a consequence, the field waveform stimulating different regions of the sensory mosaic shows substantial differences from region to region of the skin. In addition, the relatively low conductivity and the knife shape of the fish body cause a rostral funneling of the caudally generated currents, thereby increasing the relative weight of the caudally generated far

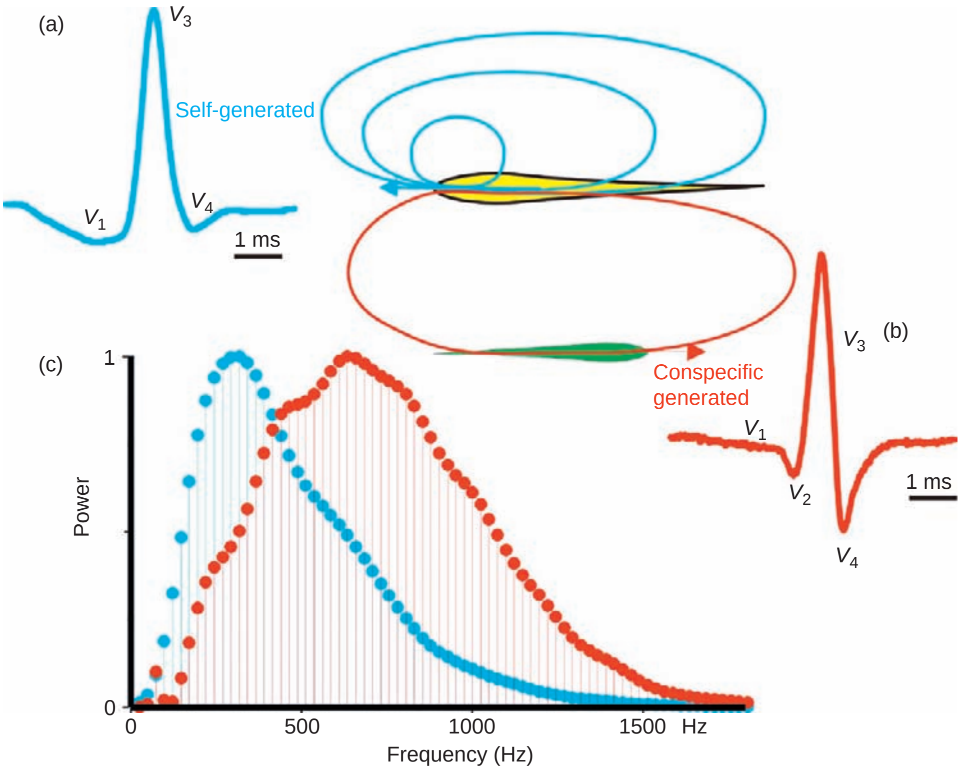


Figure 9 Differences between the self-generated local electric-organ-discharge-associated field (blue) and the conspecific-generated local electric-organ-discharge-associated field at the foveal region of *Gymnotus omarorum* (red). Normalized time waveforms generated by a 22-cm emitting fish (yellow silhouette). Self-generated waveforms show a prominent V_1 (a, blue); the communication signal (b, red) was recorded at the fovea of a parallel 14-cm-long conspecific (green silhouette), 10 cm away. (c) Normalized power spectral density histograms corresponding to the waveforms in (a) (same color-coding). Note the shift in the power spectral density in the self-generated EOD.

field. Here, it is important to recall that these fish, as well as *G. petersii*, have an electrosensory fovea near the oral region. Because of passive filtering, the waveform of the self-generated transcutaneous fields at the rostral region that carry active electrolocation signals is rather different from the fields observed from a distance that carry electrocommunication signals emitted by other fish (**Figure 9**).

See also: **Detection and Generation of Electric Signals**: Active Electrolocation; Development of Electroreceptors and Electric Organs; Generation of Electric Signals. **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Bass AH (1986) Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In: Bullock TH and Heiligenberg W (eds.) *Electroreception*, pp. 13–70. New York: Wiley.

Bennett MVL (1971) Electric organs. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. V, pp. 347–491. London: Academic Press.

Bullock TH and Heiligenberg W (1986) *Electroreception*. New York: Wiley.

Bullock TH, Hopkins CD, Popper AN, and Fay RR (2005) *Electroreception (Springer Handbook of Auditory Research)*. New York: Springer.

Caputi AA (1999) The electric organ discharge of pulse gymnotiformes: The transformation of a single impulse into a complex spatiotemporal electromotor pattern. *Journal of Experimental Biology* 202: 1229–1241.

Caputi AA, Carlson BA, and Macadar O (2005) Electric organs and their control. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception (Springer Handbook of Auditory Research)*, ch. 14, pp. 410–453. New York: Springer.

Caputi AA, Castelló ME, Aguilera P, and Trujillo-Cenóz O (2002) Peripheral aspects of electrolocation and electrocommunication in *G. carapo*. *Journal of Physiology – Paris* 96: 493–505.

Chagas C and Paes de Carvalho A (1961) *Bioelectrogenesis: A Comparative Survey of Its Mechanisms with Particular Emphasis on Electric Fishes*. Amsterdam: Elsevier.

Cox RT and Coates CW (1938) Electrical characteristics of the electric tissue of the electric eel *Electrophorus electricus* (Linnaeus). *Zoologica* 23: 203–212.

Fessard A (1958) Les organes électriques. In: Grassé PP (ed.) *Traité de Zoologie*, *Vol. 13: Agnathes et Poissons: Anatomie, Éthologie, Systé*matique, pp. 143–238. Paris: Masson.

Hopkins CD (1995) Convergent design for electrogenesis and electroreception. *Current Opinion in Neurobiology* 5: 769–777.

Hopkins CD (2009) Electrical perception and communication. In: Squire L (ed.) *Encyclopedia of Neuroscience*, vol. 3, pp. 813–831. Oxford: Academic Press.

Lorenzo D, Silva A, Caputi AA, Borde M, and Macadar O (2001) Electrogeneration in South American weakly electric fish. In: Kapoor BG, Hara TJ, and Hara TJ (eds.) *Sensory Biology of Jawed Fishes: New Insights*, pp. 121–159. Enfield, NH: Science Publishers.

Macadar O (1993) Motor control of waveform generation in *Gymnotus carapo*. *Journal of Comparative Physiology A* 173: 728–729.

Zakon HH and Unguez GA (1999) Development and regeneration of the electric organ. *Journal of Experimental Biology* 202: 1427–1434.

Zakon HH, Zwickl DJ, Ying Lu, and Hillis DM (2008) Molecular evolution of communication signals in electric fish. *Journal of Experimental Biology* 211: 1814–1818.

Generation of Electric Signals

M Kawasaki, University of Virginia, Charlottesville, VA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Physiological Mechanisms of Electric Organ Discharges
Central Control of EOD Time Patterns
Further Reading

Glossary

Action potential A small and transient change of voltage across the cell membrane when the negative resting potential momentarily becomes nearly zero volts and quickly returns to the original level in a few milliseconds. The action potential is possible because of the activity of ion channels, proteins of the cell membrane, selectively allowing certain inorganic ions to pass through their pore.

Central pattern generator Neuronal circuits that generate rhythmic motor output for coordination without the aid of sensory feedback.

Electric organ discharge (EOD) Electrical discharges resulting from the summed excitation of the cells (electrocytes) of the electric organ of electric fishes that function both in communication and in locating nearby objects. Short (100 μs to several tens of milliseconds) electrical pulses of various voltages (a few millivolts to several hundred volts) are generated at various rates (a few to over 1000 times per second).

Gamma-aminobutyric acid (GABA) The most abundant inhibitory neurotransmitter in the vertebrate brain and which usually has an inhibitory influence on the target neuron.

Postsynaptic potential Membrane potential produced by the activities of synapses on an excitable cell. Its amplitude varies depending on the strength of the input signal.

Resting potential The electrical potential inside relative to outside in the resting condition of excitable cells. A few tens of millivolts in amplitude and of negative polarity.

Introduction

Feeble electricity occurs as the by-product of physiological activities of many biological tissues and organs. In contrast, the electric organ of electric fishes produces electricity strong enough to carry out behavioral functions. Electric organs are known only in six taxonomic groups (orders) of fishes: Torpediformes, Rajiformes, Siluriformes, Perciformes, Gymnotiformes, and Mormyriformes (**Figure 1**). The electric organ occurs at various locations in the fishes' body and varies in size and shape depending on the species, but all produce an electric field outside the body (**see also Detection and Generation of Electric Signals**: Electric Organs).

The electrical discharges from the electric organ are called the electric organ discharges (EODs). Each EOD lasts only for a short period of time (100 μs to a few hundred milliseconds depending on species). EODs may be generated in sequence at various inter-EOD intervals. EODs that are separated by relatively long inter-EOD intervals are called pulse-type EODs. Pulse-type fishes may discharge at relatively irregular intervals or rather constant intervals depending on species. Continuous EODs that are separated only by short inter-EOD intervals are called wave-type EODs. Wave-type electric fishes generate EODs uninterrupted day and night throughout their lives for electrolocation.

The strength of EODs differs considerably among electric fishes. The electric eel (*Electrophorus* sp.), electric catfish (*Malapterurus* sp.), electric ray (*Torpedo* sp.), and the electric stargazer (*Astroscopus* sp.) are categorized as strongly electric fishes that generate strong EODs with amplitudes between ~5 and ~800 V. In some *Torpedo* spp., a 50 V peak of an EOD can produce as much as 1 kW of power (20 A of current). These fishes use the strong EODs primarily for defense and stunning prey.

The remaining electric fishes are categorized as weakly electric fishes that generate EODs with amplitudes ranging from a fraction of a volt to several volts. Weakly electric fishes use electricity for electrical behaviors – electrocommunication (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish) and electrolocation (**see also Detection and Generation of Electric Signals**: Active Electrolocation). Though the strongly electric fish were known to ancient humans and demonstrated as electric with the Leyden jar in the

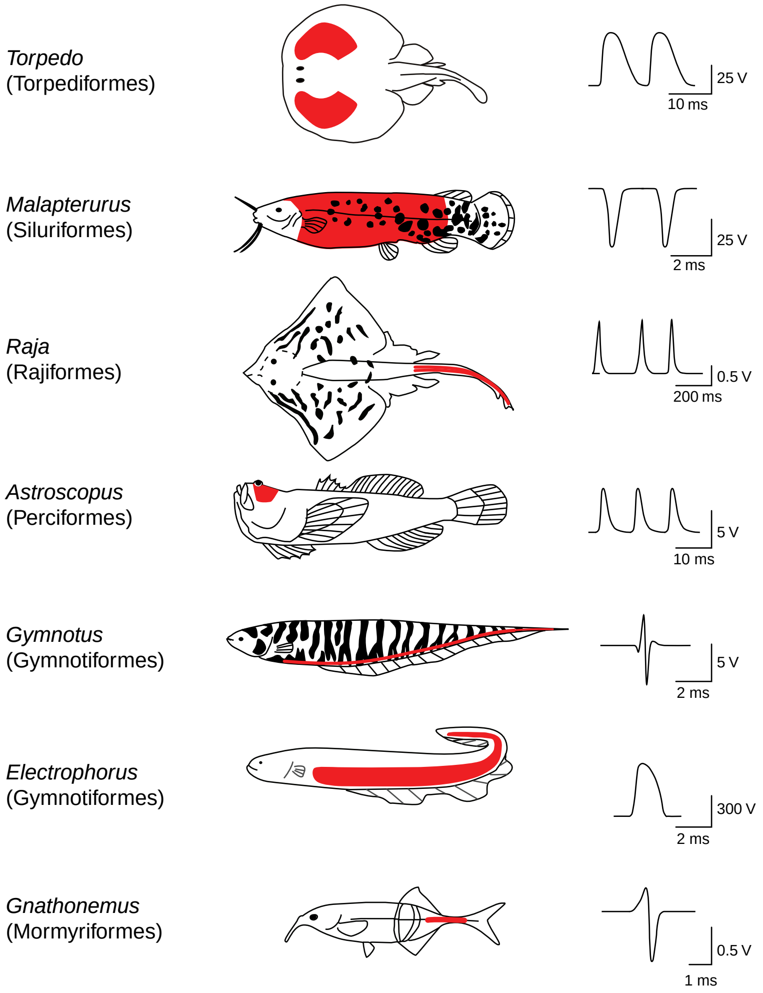


Figure 1 Location of the electric organ (red) and waveform (right) of EODs of representative fish from five major groups of electric fishes. Top view of fish for *Torpedo* and *Raja*, lateral view for other fishes.

sixteenth century, the behavioral functions of low-voltage electricity in weakly electric fishes were recognized only in the 1950s. The phylogeny and ethology of electric fishes, however, indicate that the strongly electric fishes evolved from weakly electric fishes.

The principle of electrical generation is identical for strongly and weakly electric fishes and is based on ionic flow across cell membrane as occurs in ordinary nerve and muscle cells (**see also Detection and Generation of Electric Signals**: Electric Organs). Generating cells in the electric organ are called electrocytes. The electrocytes maintain a charge difference between the inside and outside of the cell (polarization) using ion pumps in the membrane that actively move ions across the membrane. **Figure 2**(**a**) presents the resting state of the electric organ.

An EOD is generated when the polarization is reversed (depolarization) in some part of the membrane due to the activation of spinal electromotor neurons that synapse onto electrocytes (**Figure 2**(**b**)). This asymmetric depolarization results in current flow across the electrocyte and generates approximately 80–150 mV. A large number are arranged serially and stimulated by command signals from the brain that fire all electrocytes in synchrony so that their individual potentials summate. The serially positioned electrocyte columns are often arranged in parallel giving rise to high current. The columns of electrocytes are wrapped with connective tissue with high electrical resistance funneling EOD current flow outside the fish.

The electrophysiological characteristics of electrocytes determine the wave form (time course of voltage change) of each EOD but nerve cells in the brain exclusively control its timing (**Figure 3**). These neurons fire in synchrony and each synchronous firing drives each EOD. In strongly electric fishes, which generate occasional EODs, and in pulse-type weakly electric fishes, which generate irregular EODs, this group of neurons is called the command nucleus because it gathers information from other parts of the brain and makes the decision to fire an EOD. The frequency of EODs in these fishes ranges from approximately 1 to 100 Hz. In wave species and pulse

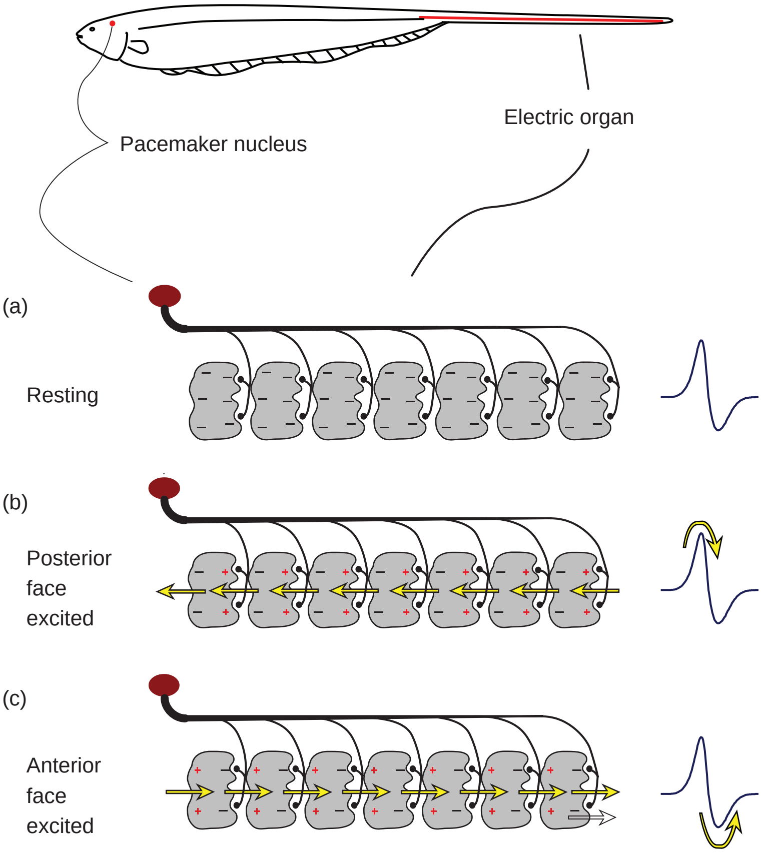


Figure 2 The mechanisms of electric organ discharges (EODs). The inside of electrocytes (gray) has negative charge relative to the outside in the resting state. The pacemaker or command nucleus generates a signal for each EOD. The signal is propagated in the axon of the relay cells that project down the spinal cord. There, the signal stimulates electromotor neurons whose axons innervate each electrocyte. The posterior faces of the electrocytes are excited causing electrical current to flow toward the head. This excitation propagates to the anterior faces causing electrical current to flow toward the tail and the sequential current flow produces the biphasic waveform shown on the right.

species with regularly firing EODs, the brain nucleus is termed the pacemaker nucleus because it intrinsically generates a periodic and regular rhythm of EODs. The frequency of EODs in these fishes ranges from several tens of hertz to as high as ~1500 Hz. A relay station in the brain called the relay nucleus relays the synchronous firing of the command or pacemaker nuclei. Nerve cells in the relay nucleus fire in synchrony following command/pacemaker input and send this information down the spinal cord where the cell bodies of the electromotor neurons further relay the signal to the electrocytes in the electric organ.

Weakly electric fishes that normally generate EODs with a regular rhythm modify their regularity for various behavioral functions. The patterns of modification include gradual increases and decreases in firing frequency, rapid increase of the frequency, brief interruptions (stopping) of the EOD, and random insertion of extra EOD pulses. While regularly discharging EODs are used for electrolocation, these modulations function as social communication signals in contexts such as courtship and aggression (**see also Sensory Systems, Perception, and Learning: Shocking Comments**: Electrocommunication in Teleost Fish) and as jamming avoidance for electrolocation (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems). Such modulations are realized by descending inputs to the command/pacemaker and relay nuclei that modify the regular rhythm of EODs. Several brain nuclei, each dedicated to a particular behavioral pattern, provide these inputs.

Physiological Mechanisms of Electric Organ Discharges

The fundamental mechanism of electrical generation in the electric organ is the movement of ions across the cell membrane of electrocytes. The gradient of chemical and electrical potential across the membrane is the force moving the ions. Membrane ion pumps maintaining the resting potential of the electrocytes create the gradients

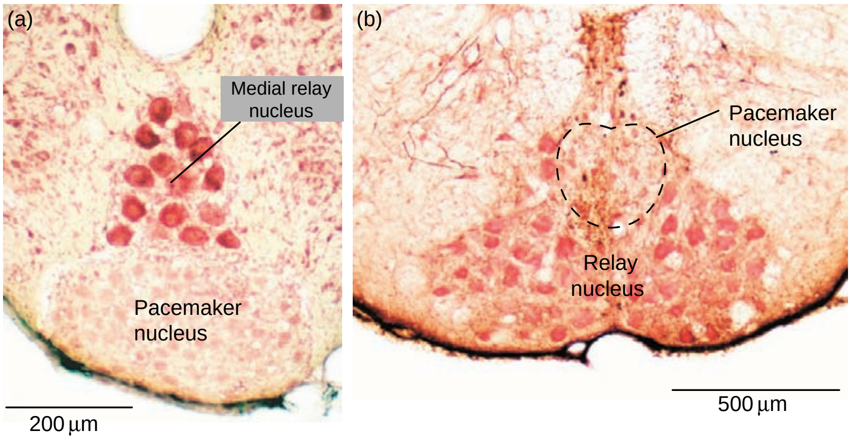


Figure 3 The pacemaker and relay nuclei in *Gymnarchus niloticus* (a) and *Electrophorus electricus* (b). The ventral end of a transverse brain section through the hindbrain is shown in each panel. Large somata (~50 μm in diameter) of relay cells are visible in the dorsal and ventral sides in *G. niloticus* and *Electrophorus electricus*, respectively. The pacemaker nucleus is packed with much smaller pacemaker cells. The lateral relay nucleus is outside of the section in (a).

(**Figure 2**). The axon terminals of electromotor neurons terminate in a part of the electrocyte where they form a chemical synapse. Neurotransmitter substance (acetylcholine) is released there and binds to postsynaptic receptor molecules in the membrane that have a pore through which ions flow. This creates postsynaptic potentials (PSPs) – a change in electrical potential difference between inside and outside the electrocyte at the synapse. PSPs are responsible for the EOD in *Torpedo, Raja*, and *Astroscopus*. In other electric fishes, the PSPs trigger an action potential that results in a short and larger EOD pulse.

Action potentials are a brief change in membrane potential whose time course and amplitude are fixed. They are mediated by voltage-sensitive ion channels for Na^+, Ca^{2+}, or K^+. PSPs or action potentials are created only in a part of electrocyte (active face) that receives innervation from an electromotor neuron (**Figure 2(b)**). The current flow occurring in this part of the electrocyte must exit from other parts of the cell (inactive face). The inactive face may have low resistance and/or high capacitance with a convoluted membrane. As a result of the inward current at the active face and the outward current at the inactive face, at most 150 mV of potential is generated across an electrocyte. The noninnervated face can also be active generating current in the opposite direction creating a negative phase of an EOD in many species (**Figure 2(c)**). A considerable degree of variation exists in the EOD waveform shape. The variation is due to the various morphological specializations of electrocytes, different patterns of innervation and propagation of action potentials within the electrocytes, and the spatial distributions of ion channels within the electrocytes (**see also Detection and Generation of Electric Signals**: Electric Organs).

Synchronous Firing of Electrocytes

A large number of electrocytes are arranged in series and in parallel in the electric organ. In *Torpedo nobiliana*, for example, approximately 1000 electrocytes are stacked in series in one column, and there are 500–1000 columns on each side of the fish. In serially connected electrocytes, the current exiting from the inactive face of a cell tends to enter the active face of the neighboring electrocyte. The direction of this current is inhibitory to the neighboring electrocyte and thus opposes synchronization. All electrocytes must be individually innervated and stimulated at once in order for potentials from all electrocytes to be summed. Synchronous firing of electrocytes is achieved by brain neurons that send a command signal to each electrocytes.

Generation of EOD Commands by Central Nervous System

The simplest command neurons are found in the electric catfish, *Malapterurus electricus*. The somata of two giant electromotor neurons in the anterior segment of the spinal cord are coupled with gap junctions. Each presynaptic fiber to the electromotor neurons bifurcates and terminates on either side for simultaneous stimulation. In most electric fishes, an unpaired command/pacemaker nucleus exists in the medulla (a hindbrain structure) that contains a large number of neurons that fire synchronously. The nucleus is called the command nucleus in mormyrid pulse-type fishes that emit EODs with irregular intervals. It is called the pacemaker nucleus in gymnotiform fishes and mormyriform *Gymnarchus niloticus*, which emit EODs with regular intervals (**Figure 3**). Because neurons in these nuclei show the earliest firing in the brain that is related one to one with EODs, these neurons are considered the decision makers. A short electrical pulse applied to the nucleus resets the phase of its pacemaking activity.

The demand for the precision of synchronization is higher in fishes with high EOD frequencies. In apteronotid electric fishes whose EOD frequencies can be as high as 1500 Hz, an error in timing of firing as little as 0.4–0.5 ms results in total cancellation of output EODs. Reflecting this, species with higher EOD frequencies show tighter electrical coupling and closer apposition among pacemaker neurons. High-frequency wave-type fishes in Gymnotiformes (*Eigenmannia virescens* and *Apteronotus albifrons*) and Mormyriformes (*G. niloticus*) show remarkably stable EOD intervals (jitter = 100 ns to 1 μs). Pacemaker/command signals from these nuclei are sent to the electric organ via one or two intermediate relay nuclei in the brain and electromotor neurons in the spinal cord.

Figure 4 shows the patterns of anatomical connections of the pacemaker/command complex in different fishes. In gymnotiform fishes, the neurons in the pacemaker project to the relay nucleus, an unpaired nucleus in the medulla adjacent to the pacemaker nucleus. Neurons in the relay nucleus extend their axons into the spinal cord where they synapse to the somata of electromotor neurons. The pacemaker neurons in *G. niloticus* project out bilaterally to the lateral relay nuclei, which in turn project to an unpaired midline nucleus, the medial relay nucleus. Although the pacemaker and medial relay nuclei are closely apposed, there is no direct projection from the pacemaker to the relay nucleus. In pulse-type mormyrid electric fishes, the command nucleus projects bilaterally to relay nuclei called the bulbar command-associated nucleus which in turn projects to the medullary relay nucleus as in *G. niloticus*. A difference is that the command neurons also directly connect to the medullary relay nucleus and this projection carries the command signal to the electric organ. The bulbar command-associated nucleus mediates corollary discharges to the upper parts of the brain for gating of sensory information (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems and Active Electrolocation). Neurons in the final relay nuclei of all species extend their axons into the spinal cord where they synapse onto the somata of electromotor neurons. The somata and the axons of the relay nuclei have large diameters for fast conduction of nerve impulses which is believed to contribute to the precise firing of EODs.

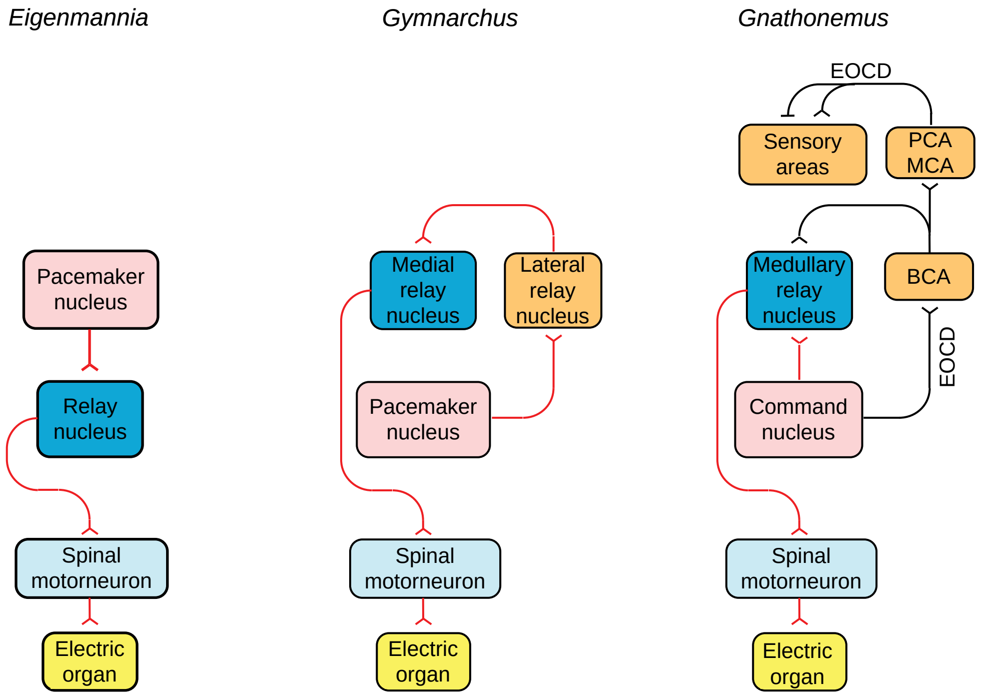


Figure 4 Comparison of central electromotor pathways that drive EODs. *Eigenmannia* (left) represents the pattern of all pulse- and wave-type gymnotiform fishes. A mormyriform pulse-type fish, *Gnathonemus* (right), involves a system for electric organ corollary discharges (EOCDs) that project to sensory areas in the brain. A mormyriform wave species, *Gymnarchus niloticus* (center), has an intermediate complexity. The pacemaker and command nuclei are the site of the EOD generation. Neurons in all the following structures fire one action potential for each EOD. The structures and connections shaded with orange color are actually bilateral but only one side is shown. Note the lack of projection from the pacemaker nucleus to the medial relay nucleus in *G. niloticus*, and the duplicated projections to the medullary relay nucleus in *Gnathonemus*. PCA, paratrigeminal command-associated nucleus; MCA, mesencephalic command-associated nucleus.

Central Control of EOD Time Patterns

Although the command and pacemaker nuclei are responsible for the basic rhythm of EODs at rest, they are by no means uncontrolled generators or oscillators. The time pattern of EODs at rest is modified in various ways for behavioral functions such as electrolocation, courtship, and aggression. The patterns include gradual rises or falls of EOD frequencies that occur over periods of seconds, rapid acceleration or deceleration of EOD timing that occurs within milliseconds, and total cessation of EODs (interruptions) (**Figure 5**). These modulations arise from neuronal inputs to the command/pacemaker and relay nuclei (**Figure 6(a)**). Physiology of the neuronal mechanisms for these modulations has been studied in many species of weakly electric fishes with the following techniques. First, input nuclei were identified by injection of backfill tracers into the pacemaker or relay nucleus. These tracers travel to brain nuclei projecting to the injection site. The nuclei projecting to the pacemaker/relay nuclei were then stimulated electrically or chemically to identify their physiological effects on the pacemaker/relay nucleus and EODs. The projection nuclei are generally found in the diencephalon and midbrain. **Table 1** shows EOD modulation patterns and the brain nuclei responsible for generating them in different genera of weakly electric fishes. The general features of these modulation mechanisms are: (1) the projection nuclei occurring in the diencephalon or the midbrain, (2) labeled line organization in which each projection nucleus serves a specific behavioral output pattern, (3) projections not only to the pacemaker nucleus but also to the relay nucleus, and (4) relatively conserved anatomical connections but functions varying among different species.

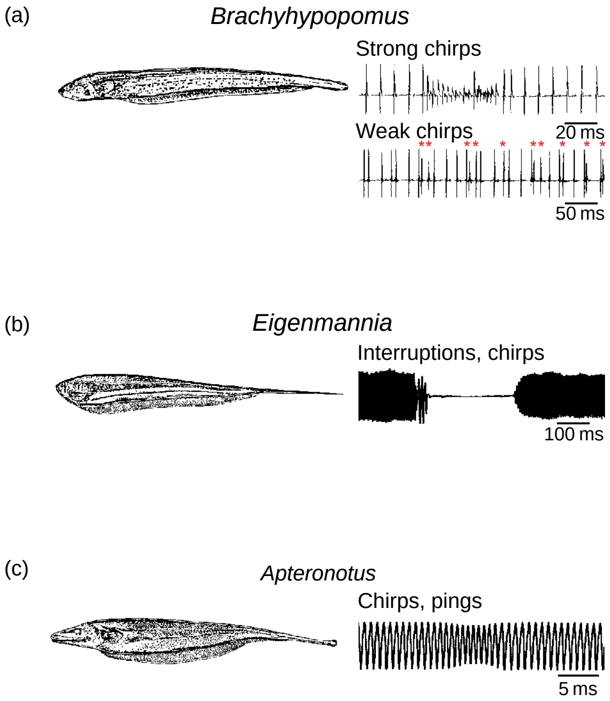


Figure 5 Examples of EOD modulations in gymnotiform electric fishes. (a) Chirps in *Brachyhypopomus brevirostris* consist of extra EOD pulses inserted in the regular train of EOD pulses. A strong chirp interrupts several regular pulses and consists of a sequence of high-frequency small-amplitude pulses. Weak chirps consist of extra pulses inserted without interruption of background EODs. Extra pulses are marked with asterisk. (b) *Eigenmannia* interrupts its wave-type EOD of several hundred hertz for 100–500 ms. The interruption occurs continuously a few tens times per minute in courting males. This behavior also is referred to as chirps. (c) *Apteronotus* rapidly increases its high-discharge frequency (~500–1000 Hz) by a few hundred hertz that lasts for a few tens to a few hundred milliseconds.

Gymnotiform Pulse Fishes

Figure 6(a) shows premotor brain nuclei and their behavioral functions in *Brachyhypopomus brevirostris*. Gradual frequency rises and falls are mediated by the subdivisions of the prepacemaker nucleus (PPn), the PPn-G and the PPn-I, respectively. Neurons from both nuclei project to the pacemaker nucleus and modulate its firing frequency. The inhibitory effects by the PPn-I are mediated by gamma-aminobutyric acid (GABA) and cause total cessation of the pacemaker activity which in turn results in total cessation of relay cells, electromotor neurons, and the electric organ (interruptions). This type of interruption is always preceded by a frequency fall. The sublemniscal prepacemaker nucleus (sPPn) projects to the relay nucleus and steadily depolarizes the membrane potential of relay cells. Due to the depolarization-blocking action potentials through the inactivation of Na^+ channels, the relay cell rejects inputs from the pacemaker nucleus and stops driving the electromotor neurons and electric organ. This type of interruption occurs without any preceding change in EOD frequency because the pacemaker activity is uninterrupted. Chirps are mediated by the PPn-C, which projects to the relay nucleus. Unlike the effects from the sPPn, the excitatory effects from the PPn-C are brief and cause additional spiking in relay neurons which is propagated down to the electric organ to produce extra pulses in between regular EODs (**Figure 5(a)**). Neurotransmitters and their postsynaptic receptor types are known for each of these pathways (**Figures 6(b)**, **6(c)**, and **7**).

Gymnotiform Wave Fishes

Wave-type gymnotiform fishes typically generate EODs at a stable frequency that remains within a few hertz over hours. However, when a fish encounters a neighbor whose EOD frequency is very close (within a few hertz) to the fish's own EOD frequency, it shifts the discharge frequency away from that of the neighbor. *Eigenmannia* increases and decreases the EOD frequency for lower- and higher-frequency neighbors, respectively. *Apteronotus* only increases the frequency in

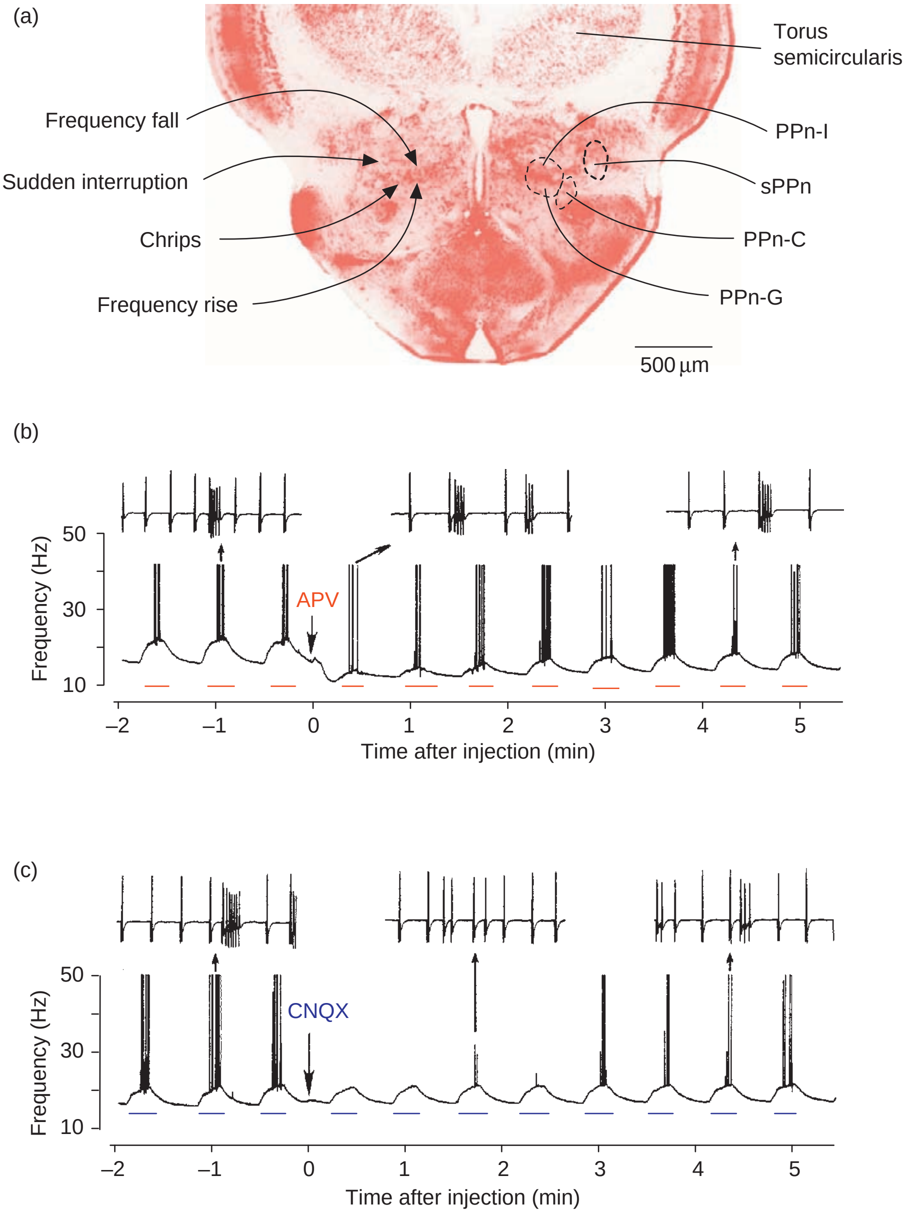


Figure 6 (a) A transverse section of the midbrain of *Brachyhypopomus brevirostris* showing brain nuclei controlling EOD modulation behaviors. Stimulation of small areas indicated with arrows using glutamate iontophoresis modulates the EODs. (b) Selective blocking of frequency-rising behavior by injection of the slow glutamate receptor blocker 2-amino-5-phosphonopentanoic acid (APV) into the pacemaker nucleus (red bars). The PPn-C and PPn-G were stimulated simultaneously to induce chirps and frequency rises, which are seen as spike-like transient increases in frequency and gradual increase in frequency. The three traces above are time-expanded EOD recordings showing chirps. Note that APV injection reduces the frequency-rising behavior but chirping responses are unaffected. (c) Similar experiment as in (b) but a neurotransmitter blocker for the fast type of glutamate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), was injected. CNQX injection abolishes chirps but the gradual frequency-rise responses to iontophoretic stimulation of the PPn are unaffected.

response to a low-frequency neighbor but does not respond to a high-frequency neighbor. These frequency changes, called the jamming avoidance responses (JARs), result in expanding frequency differences between the fish and function to avoid mutual jamming of their electrolocation systems (**see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems). The diencephalic nuclei, the nucleus electrosensorius up (the nE↑) and the nucleus electrosensorius down (nE↓), are involved in frequency-increasing and frequency-decreasing JARs, respectively. Stimulation/lesion of these nuclei affects frequency-increasing and -decreasing JARs, respectively.

The frequency-increasing JAR is mediated by excitatory neuronal input to the pacemaker nucleus from the PPn-G which in turn is stimulated by the nE↑ (**Figure 8**). The frequency-decreasing JAR, however, is not mediated by inhibitory inputs to the pacemaker like the

Table 1 Premotor nuclei and their projections in weakly electric fishes

Order	*Genus*	*EOD modulation pattern*	*Diencephalon midbrain nucleus*	*Primary projection target*	*Neutrotransmitter or receptor subtype*
	Brachyhypopomus (pulse)	Gradual frequency rises	PPn-G	P	NMDA
		Gradual frequency falls	PPn-I	P	GABA
		Sudden interruptions	sPPn	R	NMDA
		Interruptions	PPn-I	P	GABA
		Chirps	PPn-C	R	AMPA
		Tumultuous rise	NE	?	unknown
Gym	*Eigenmannia (wave)*	Gradual frequency rises	PPn-G	P	AMPA+NMDA
		Gradual frequency falls	sPPn	R	NMDA
		Chirps (interruptions)	PPn-C	R	AMPA
	Apteronotus (wave)	Gradual frequency rises	PPn-?	P	NMDA
		Gradual frequency falls (unnatural)	sPPn	R	NMDA
		Chirps	PPn-C	R	AMPA
	Sternopygus (wave)	Gradual frequency rises	PPn	P?	NMDA
		Interruptions	sPPn	R?	NMDA
	Gymnarchus (wave)	Interruptions	PPn	P	GABA
		Gradual frequency rise	Unknown	Unknown	Unknown
		Gradual frequency fall	PPn	P	GABA
Mor	*Brienomyrus (pulse)*	Seallops	PCN	C	glutamate?
		Accelerations	DP	C	glutamate?
		Rasps	DP an PCN	C	glutamate?
		Cesations (interruptions)	VPd	PCN/DP	GABA

Gym, Gymnotiformes; Mor, Mormyriformes; P, Pacemaker nucleus; C, Command nucleus; R, Relay nucleus.

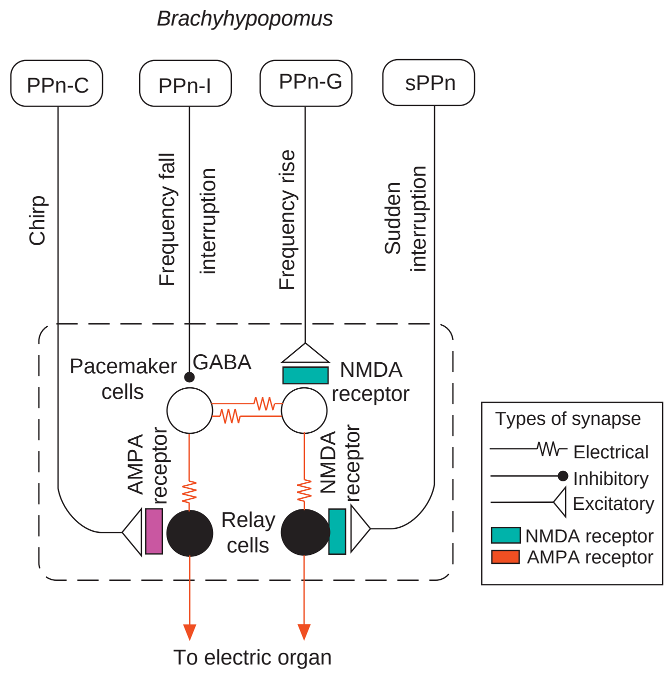


Figure 7 Organization of premotor nuclei and the pacemaker/relay cell complex in gymnotiform pulse fish, *Brachyhypopomus brevirostris*. Stimulation of each subdivision of the prepacemaker nucleus (PPn) induces the EOD behavioral patterns described along the projection lines. Pharmacological experiments as in **Figures 6(b)** and **6(c)** revealed the type of neurotransmitter and receptor subtypes in the pacemaker/relay complex. Red lines are involved in the generation of the basic rhythm; other lines are involved in behavioral modulations.

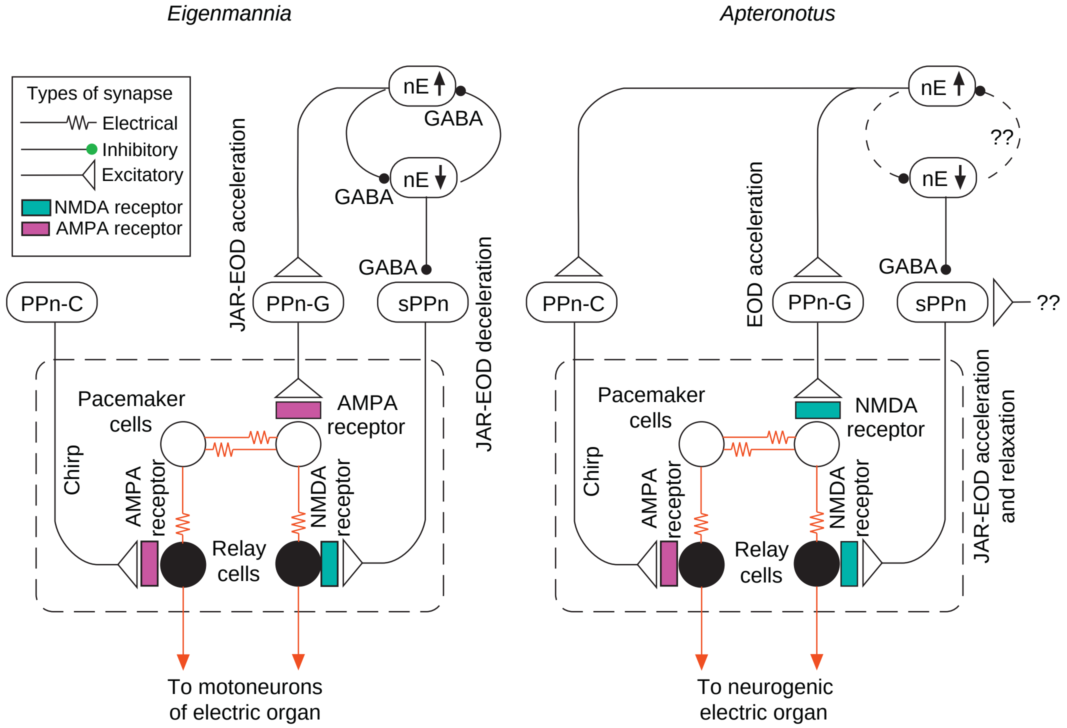


Figure 8 Organization of premotor nuclei and the pacemaker/relay cell complex in gymnotiform wave fishes, *Eigenmannia virescens* and *Apteronotus leptorhynchus*. Stimulation of each subdivision on the prepacemaker nucleus (PPn) induces the EOD behavioral patterns described along the projection lines. Pharmacological experiments as in **Figures 6(b)** and **6(c)** revealed the type of neurotransmitter and receptor subtypes in the pacemaker/relay complex. Red lines are involved in the generation of the basic rhythm; other lines are involved in the behavioral modulations.

frequency-falling behavior in pulse-type *Brachyhypopomus*. Instead, the frequency-decreasing JAR occurs by inhibition from the nE↓ of the sPPn that normally has a constant excitatory effect on the pacemaker nucleus. In *Apteronotus*, anatomical organization of premotor nuclei is similar to that of *Eigenmannia*; their physiological roles are, however, vastly different. The pathway through the nE↑ and PPn-G to the pacemaker nucleus is not involved in the frequency-increasing JAR in *Apteronotus*. Instead, an excitatory effect via sPPn is responsible for the behavior. The nE↑ and nE↓ do exist and have similar anatomical, physiological, and pharmacological connections with the PPn-G and sPPn in *Apteronotus*, but they are not involved in their JAR. Gymnotiform wave fishes show transient EOD modulations that occur within milliseconds during social interactions. These take the form of short interruptions of the EOD in *Eigenmannia*, and rapid frequency rises in *Apteronotus*. These EOD modulations are often called chirps and they are mediated by the PPn-C that projects to the relay nucleus in both genera (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish).

Mormyriform Pulse Fishes

Unlike gymnotiform fishes, mormyrid pulse fishes emit EODs with variable timing. The somewhat irregular EODs are modulated into different EOD patterns in different behavioral situations. The known patterns include cessations (interruptions), accelerations, scallops, and rasps. **Figure 9** shows the organization of premotor nuclei in *Brienomyrus brachyistius*. The command nucleus acts as an integrate-and-fire unit that receives excitatory inputs from various parts of the brain, including the midbrain precommand nucleus (PCN) and thalamic dorsal posterior (DP) nucleus. The output of the command nucleus, while driving the EODs via the relay nucleus, provides corollary discharges to the sensory system for gating of sensory information. The corollary discharge signal also projects to a part of a midbrain nucleus called the ventroposterior nucleus (VPd) that in turn projects to the PCN and the DP nucleus with inhibitory synapses. A simple central pattern generator for their basic EOD rhythm is suggested where the PCN and DP nucleus send constant excitatory inputs to the command nucleus and the corollary discharges

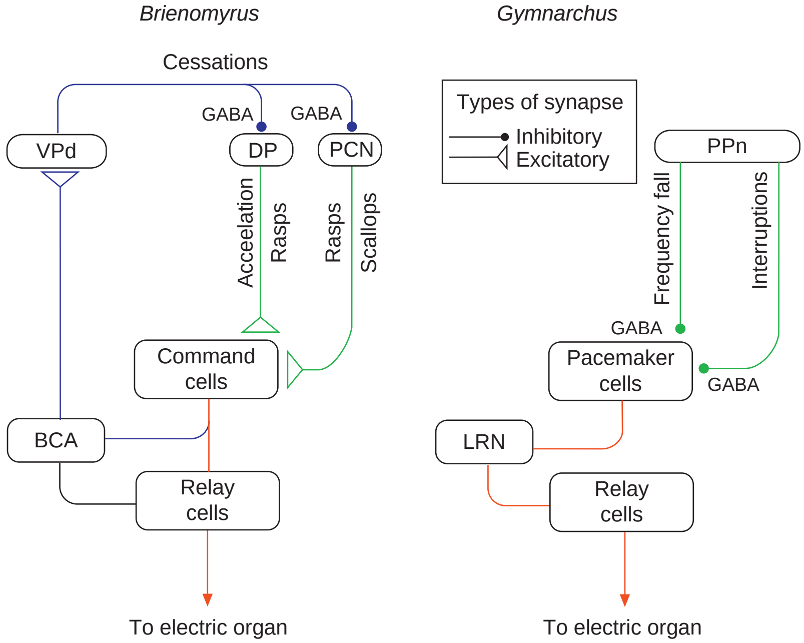


Figure 9 Organization of premotor nuclei and the pacemaker/relay cell complex in a mormyriform pulse fish, *Brienomyrus brachyistius* and a wave fish, *Gymnarchus niloticus*. Stimulation of each premotor nucleus induces the EOD behavioral patterns described along the projection lines (green). Pharmacological experiments as in **Figures 6(b)** and **6(c)** revealed the type of neurotransmitter and receptor subtypes in the pacemaker/relay complex. Red lines are involved in the generation of the basic rhythm; blue lines convey the corollary discharge information; and green lines show the projections for EOD pattern modulation. BCA, the bulbar command-associated nucleus; and LRN, the lateral relay nucleus.

via the VPd cast inhibition back to the DP nucleus and PCN. Stimulation of these premotor nuclei induces specific EOD modulation patterns (**Figure 9**).

Mormyrid Wave Fish *G. niloticus*

G. niloticus, the sole wave-type electric fish of the Mormyriformes, exhibit JARs similar to those of *Eigenmannia* and interruptions as a submissive behavior. Only one diencephalic nucleus has been identified. This PPn casts inhibition to the pacemaker nucleus (**Figure 9**). This projection mediates both frequency-decreasing JARs and interruptions. Premotor nuclei responsible for frequency-increasing JARs are unknown.

See also: **Detection and Generation of Electric Signals**: Active Electrolocation; Electric Organs; Physiology of Tuberous Electrosensory Systems. **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Bass AH (1986) Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In: Bullock TH and Heiligenberg W (eds.) *Electroreception*, pp.13–70. New York: Wiley.

Bennett MVL (1971) Electric organs. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 5, pp. 347–491. New York: Academic Press.

Caputi AA, Carlson BA, and Macadar O (2005) Electric organs and their control. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 410–451. New York: Springer.

Carlson BA (2002) Neuroanatomy of the mormyrid electromotor control system. *Journal of Comparative Neurology* 454: 440–455.

Carlson BA (2006) A neuroethology of electrocommunication: Senders, receivers, and everything in between. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes,* vol. 2, pp. 805–848. Enfield, NH: Science Publishers.

Carlson BA and Hopkins CD (2004) Central control of electric signaling behavior in the mormyrid *Brienomyrus brachyistius*: Segregation of behavior-specific inputs and the role of modifiable recurrent inhibition. *Journal of Experimental Biology* 207: 1073–1084.

Crampton WGR and Albert JS (2006) Evolution of electric signal diversity in gymnotiform fishes. In: Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) *Communication in Fishes,* vol. 2, pp. 647–731. Enfield, NH: Science Publishers.

Heiligenberg W, Metzner W, Wong C, and Keller C (1996) Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: Evolutionary changes of a behavior and its neuronal substrate. *Journal of Comparative Physiology* 179: 653–674.

Hopkins CD (1988) Neuroethology of electric communication. *Annual Review of Neuroscience* 11: 497–535.

Kawasaki M and Heiligenberg W (1989) Distinct mechanisms of modulation in a neuronal oscillator generate different social signals in the electric fish *Hypopomus*. *Journal of Comparative Physiology* 165: 731–741.

Kawasaki M and Heiligenberg W (1990) Different classes of glutamate receptors and GABA mediate distinct modulations in the firing pattern of a neuronal oscillator, the medullary pacemaker of gymnotiform electric fish. *Journal of Neuroscience* 10: 3896–3904.

Kawasaki M, Maler L, Rose GJ, and Heiligenberg W (1988) Anatomical and functional organization of the prepacemaker nucleus in gymnotiform electric fish: The accommodation of two behaviors in one nucleus. *Journal of Comparative Neurology* 276: 113–131.

Metzner W (1993) The jamming avoidance response in *Eigenmannia* is controlled by two separate motor pathways. *Journal of Neuroscience* 13: 1862–1878.

Moller P (1995) *Electric Fishes*. London: Chapman and Hall.

Zhang Y and Kawasaki M (2007) Interruption of pacemaker signals is mediated by GABAergic inhibition of the pacemaker nucleus in the African electric fish Gymnarchus niloticus. *Journal of Comparative Physiology – Part A* 193: 665–675.

Relevant Websites

http://www.fiu.edu – Florida International University; Philip Stoddard's Lab: Electric Field and Potential Animations.

http://nelson.beckman.illinois.edu – Mark E. Nelson, Beckham Institute, University of Illinois: Electrosensory Signal Processing Lab.

Development of Electroreceptors and Electric Organs

F Kirschbaum, Humboldt-Universität zu Berlin, Berlin, Germany

J-P Denizot, CNRS, Gif sur Yvette, France

© 2011 Elsevier Inc. All rights reserved.

Introduction
Electroreceptor Development
Development of EOs
Further Reading

Glossary

Ampullary electroreceptors Electroreceptors sensitive to weak electric fields up to about 50 Hz; there are primitive ampullary electroreceptors in cartilaginous and nonteleost fishes and derived ampullary electroreceptors in teleost fishes.

Dorsolateral placodes Localized epidermal thickenings that form within the inner layer of the cephalic ectoderm following neurulation. These placodes give rise to sensory organs of the lateral line system, neuromasts, and ampullary electroreceptor organs.

Epaxial muscle The part of the lateral muscle which is situated dorsal to the vertebral column and the horizontal myoseptum as opposed to the hypaxial muscle lying ventral to the horizontal myoseptum.

Larval electric organ An electric organ that develops during early development in mormyrids and some gymnotiforms; it degenerates later during ontogeny and is replaced by a more derived electric organ.

Myogenic electric organs Electric organs derived from muscle tissue.

Neurogenic electric organs Electric organs formed by the axons of motor neurons.

Tuberous electroreceptors Electroreceptors sensitive to weak electric fields above 50 Hz that occur in teleost fishes possessing electric organs. These receptors are capable of monitoring the weak electric fishes' own discharges and are therefore used in electrocommunication and orientation.

Introduction

Electroreception is the capacity to perceive weak electric fields. The corresponding sensory organs are electroreceptors, which evolved from mucous glands in the case of primitive mammals (Platypus), and from the lateral line system in amphibians, lampreys, and fishes (**Figure 1**; **see also Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs).

Superficial electroreceptive organs originated in the common ancestor of lampreys and gnathostomes early in the Palaeozoic and are found in extant lampreys. The electroreceptive organ of cartilaginous fishes and nonteleost bony fishes – lungfishes, coelacanths, bichirs, reedfishes, sturgeons, and paddlefishes – is the ampullary organ, often called the ampullae of Lorenzini. This primitive ampullary organ comprises a hair cell bearing an apical kinocilium, which, however, seems to have been lost with the origin of neopterygian bony fishes (gars, bowfins, and teleosts). The phylogeny of teleost fishes is characterized by repeated, independent evolution (re-invention) of electrosensory systems. Ampullary organs re-evolved three times independently in the African knifefishes (Xenomystinae) and elephantnoses (Mormyriformes), both members of the bony tongues (Osteoglossomorpha), as well as in the catfishes (Siluriformes) and South American knifefishes (Gymnotiformes), members of the Ostariophysi. In addition to these derived, low-frequency-sensitive (up to 50 Hz) ampullary organs with hair cells bearing apical microvilli, high-frequency-sensitive (50 Hz to 2 kHz) tuberous electroreceptors evolved independently in the two teleost taxa, Mormyriformes and Gymnotiformes, concomitantly with electric organs (EOs).

EOs that produce electric fields have been invented at least seven times independently in cartilaginous (Torpedinoids, Rajoids) and teleost fishes (Mormyriformes, Gymnotiformes, Siluriformes, and Uranoscopidae); their anatomy and histology are more diverse than those of electroreceptor organs (**see also Detection and Generation of Electric Signals**: Electric Organs). EOs are derived from muscle tissue or, in one case, nerve fibers (family Apteronotidae of the Gymnotiformes). The electric discharge in strongly electric fish is powerful enough to be used for defense or stunning prey. The weak EOs together

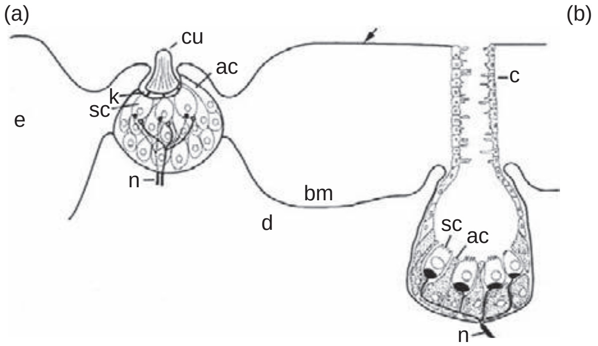


Figure 1 Schematic of a superficial neuromast (a) and a primitive ampullary organ (b). The cupula (cu) of the superficial neuromast comprising polysaccharides protects the sensory cell (sc) from the aquatic environment. The movements of the cupula are transferred to the sensory cells through the kinocils (k). In the ampullary organ, the sensory cells are found at the base of a canal (c) which is embedded in the dermis (d). The canal is filled with electrically conductive polysaccharides that protect the sensory cells from the outer environment. In both organs, the accessory cells (ac) surround the sensory cells. The base of the sensory cells is connected in both organs to afferent sensory nerve fibers (n). In addition, the sensory cells of the neuromast receive efferent input from a motor nerve fiber (dotted lines). bm, basement membrane; arrow, surface of the epidermis.

with the appropriate electroreceptors (electric system) are used for active electrolocation (**see also Detection and Generation of Electric Signals**: Active Electrolocation) and electrocommunication (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish).

Electroreceptor Development

Lampreys (Agnatha)

Ammocoete larvae of lampreys possess multivillous sensory cells, resembling sensory cells belonging to the octavolateralis system. They occur in small groups on the tail and in the gill-vent papillae. They might represent future end buds, the electroreceptor cells of adult lampreys.

Development of the Primitive Ampullary Organ

The primitive ampullary organs are often called the ampullae of Lorenzini. In Chondrichthyes (elasmobranchs and holocephalans), the ampullae of Lorenzini consist of a long canal at the end of which are found the receptor cells. The length and distribution of these canals vary significantly between species and probably reflect niche differences rather than taxonomic positions. The ampullae of many different receptor organs are grouped together in clusters beneath the skin with tubes radiating out from each cluster in many directions. In benthic feeding skates, most of the receptors are on the ventral body surface, whereas many sharks have equal numbers, dorsal and ventral.

The ampullary receptors of the bony fishes (Actinistians, Dipnoi, Polypteriformes, and Chondrostei) consist of a short canal leading to the receptor cells. These organs are predominantly found on the head region and their number varies from one (*Polypterus*) to about 75 000 in *Polyodon* (Chondrostei).

The development has been well studied in elasmobranchs, sturgeons, and paddlefish *(Polyodon spathula)*, and it is quite similar to the development described for amphibians. Early in development, localized epidermal thickenings on the trunk, so-called placodes, form within the inner layer of the cephalic ectoderm. In the beginning, they are covered by an overlying layer of ectoderm. A prominent otic placode gives rise to the inner ear. In general, six pairs of lateral-line placodes develop, three preotic and three postotic placodes that give rise to the lateral line and electrosensory cells located on the head and the trunk, respectively. Below these placodes, neuroblasts of the sensory ganglia of the lateral-line nerve develop and their fibers come into contact with the placodes and apparently induce mitotic activity and enlargement of the placodes. These are termed sensory ridges. The central part of the sensory ridges gives rise to neuromast primordia, and the lateral zones to ampullary primordia which thereafter differentiate into the final ampullary organ composed of sensory cells, supporting cells, and modified epidermal cells (**Figure 1**).

Development of the Derived Ampullary and Tuberous Electroreceptor Organs

Teleostei

Osteoglossomorpha

The osteoglossomorpha are an unusual group of teleost fishes comprising about 250 species of freshwater fishes, most of which are in one family, Mormyridae. The monospecific family Gymnarchidae comprises the large-growing piscivorous aba (*Gymnarchus niloticus*). All species of Mormyridae and Gymnarchidae are weakly electric fishes. They are monophyletic and are grouped into the superfamily Mormyroidea, sometimes considered an order Mormyriformes. They are related to members of the family Notopteridae, of which fishes of the genera *Xenomystus* and *Papyrocranus* (subfamily Xenomystinae) are electroreceptive. The fishes of the subfamily Xenomystinae possess ampullary electroreceptors; their development has not been described to date.

All members of Mormyroidea possess both derived ampullary receptors and two types of tuberous receptors, called knollenorgans and mormyromasts, in Mormyridae and gymnarchomasts in the case of *Gymnarchus*. The knollenorgans serve electrocommunication, whereas the mormyromasts serve active electrolocation. There are no data on the development of electroreceptors in *Gymnarchus*.

Detailed studies have been performed concerning the development of electroreceptors in various species of mormyrids. The electroreceptors start to differentiate at about day 6 after fertilization and they are functional at about day 8. Interestingly, these electroreceptors comprise two distinct larval tuberous electroreceptors, type A and type B (**Figures 2(a)–2(d)**), as well as a larval mormyromast called the promormyromast (**Figure 3**). The larval electroreceptors are predominantly found in the head region (**Figure 4**). In addition to these three types of larval tuberous electroreceptors, the knollenorgans and ampullary organs found in adults develop as well. The two kinds of larval tuberous organs (A and B) degenerate at the onset of the juvenile stage, whereas the promormyromast differentiates at the same time into the more complicated mormyromast found in adults (**Figure 4**). In mormyrids, the various electroreceptors do not seem to develop from placodes or sensory ridges; they apparently develop directly from basal cells of the epidermis.

Siluriformes

There are descriptions on the development of electroreceptors in several species of catfishes. The most detailed study deals with the channel catfish, *Ictalurus nebulosus*. By stage 24, the preotic lateral-line placodes can be recognized, whereas the postotic placodes are not recognized until stage 28 (larva about 2 days old). These placodes, with the exception of the posterior placode, form sensory ridges by stage 34. From these ridges and the posterior placode, neuromast primordia begin to form during stage 37. By stage 45, well-developed superficial neuromasts of the lateral line are present. Ampullary organ primordia develop

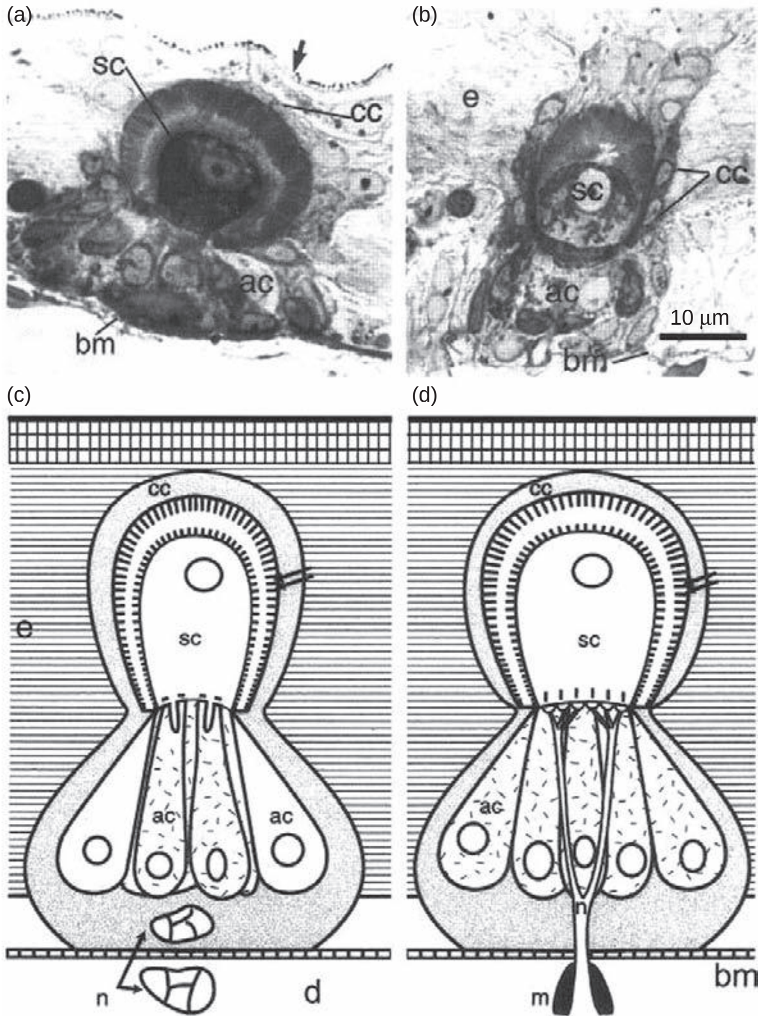


Figure 2 (a–c) Tuberous larval electroreceptors of type A (a, c) and B (b, d) in semithin sections (a, b) and in schematic drawing (c, d) in the epidermis (e) of the head of a 12-day-old larva of *Mormyrus rume proboscirostris*. Each type of organ consists of a single sensory cell (sc) surrounded by microvilli of the surrounding covering cells (cc); the sensory cell sits on a platform of accessory cells (ac); the outer surface of the epidermis is indicated either by a thick arrow (a) or a thick line (c, d). bm, basal membrane; n, nerve; m, myelin sheath. Scale = 10 μm.

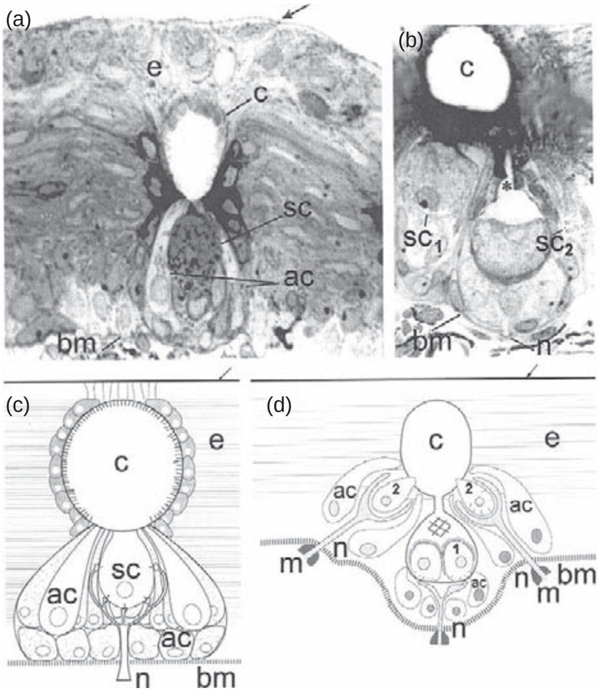


Figure 3 Larval mormyromast (promormyromast) (a, c), and mormyromast (b, d) of mormyrid fish in semithin sections (a, b) and in schematic drawing (c, d). The promormyromast develops into the mormyromast in particular through differentiation of a second type of sensory cell (sc_2 (b), 2 (d)). ac, accessory cells; e, epidermis; bm, basement membrane; n, nerve; sc_1, sensory cell of type 1; sc_2, sensory cell of type 2; m, myelin sheath.

from the sensory ridges around stage 43 (hatching) to 45. At stage 51, both mature ampullary organs and ampullary organ primordia are found on the head of the embryo.

Gymnotiformes

The only detailed study on the development of derived electroreceptors was performed on glass knifefish (*Eigenmannia lineata*). Four days after spawning, most of the lines of neuromasts are formed; the first electroreceptor primordia form on the lateral edges of the neuromast lines. The first differentiated tuberous organs occur on the head on day 7, and the first ampullary organs 1 day later. On the trunk, tuberous and ampullary organs occur 1 day later. The electroreceptor primordia apparently develop from general ectoderm (stratum germinativum) via induction of lateral-line fibers.

Development of EOs

Chondrichthyes

In cartilaginous fishes, EOs have evolved twice independently. In the superfamily Torpedinoidei, strong EOs evolved from branchial muscles and are found in the genera *Torpedo* and *Narcine*. The electric cells (electrocytes) represent disk-shaped structures which are oriented in the ventro-dorsal direction. The EO of electric ray (*Torpedo marmorata*) consists of about 500–1000 parallel columns of about 1000 disk-shaped cells. *T. marmorata* is a live-bearing fish. The EO is already functional before birth. Newborn electric rays (80–90-mm body length) can use their EO discharge (EOD) in prey capture. Differentiation of the electrocytes begins when embryos are 40 mm long, by the horizontal flattening of myotubes. Cell-shape transformation is finished by 55 mm embryo length; the electrocytes have by then acquired their disk-shaped structure. Discharges are first recorded in 60-mm embryos.

Several species of skates (Rajoids) emit weak electric discharges that are probably used for communication. The EO, comprising cup- or disk-shaped electrocytes, is located in the tail in the center of the lateral most bundle of longitudinally running muscle fibers. The EO is spindle shaped and runs most of the length of the tail. The

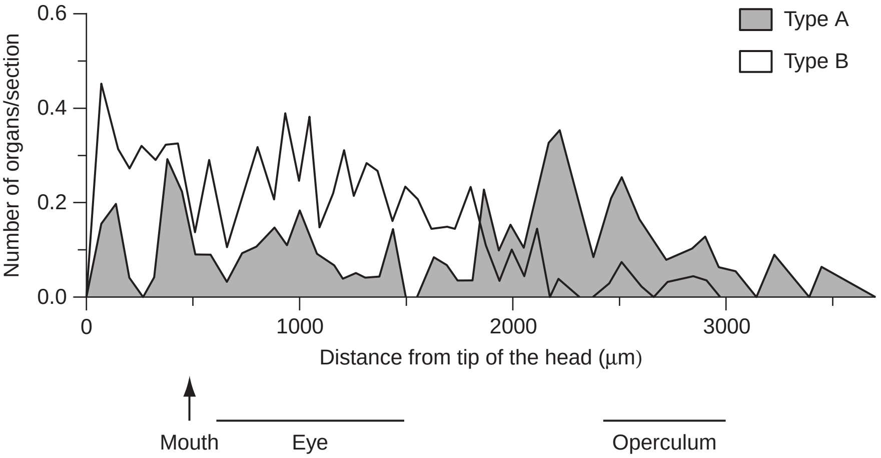


Figure 4 Diagram showing the distribution of the two types (A and B) of larval tuberous organs in the epidermis of an 11-day-old larva of *Campylomormyrus cassaicus* from the tip of the head to the end of the operculum, comprising a total length of 3700 μm, based on analysis of 2-μm-thick sections in the transverse plane. Note the difference in the distribution of the two types of organs.

development of these organs is known for several *Raja* species. The different species apparently represent different steps in the evolution of electrocytes from muscle fibers. In some stages during the differentiation of the electrocytes, myofibrils seem to be capable of contraction. Myofibrils in general are found in various degrees in all the EOs of myogenic origin. However, there has never been an indication of contractile fibers except for the case mentioned above.

Teleostei

Osteoglossomorpha

The development of EO has been described in members of both families of the order Mormyriformes. In *Gymnarchus niloticus*, the electrocytes develop from the medial part of the hypaxial and epaxial muscle. They are quite similar to muscle fibers and they develop directly into the electrocytes of the final organ, which consists of four spindle-like structures extending from the tip of the tail in the axial muscle up to at most the middle of the dorsal fin.

In mormyrids, the ontogeny of the EO has been described in several species. The most detailed description is in the study performed on elephantfish (*Pollimyrus isidori*). Surprisingly, mormyrids possess a larval EO in the axial muscle during the larval phase, which lasts for about 40 days. Thereafter, the adult EO in the caudal peduncle starts to become functional (**Figure 5**). The two organs are connected to the same pacemaker; however, they possess separate populations of electromotor neurons. The larval EO degenerates later during ontogeny. Larval and adult EOs produce different types of EODs. The larval organ possesses many features which indicate its origin from muscle; for example, the early electrocytes are difficult to distinguish from muscle fibers. During development, the adult organ quickly loses the characteristics indicating its origin from muscle, for example,

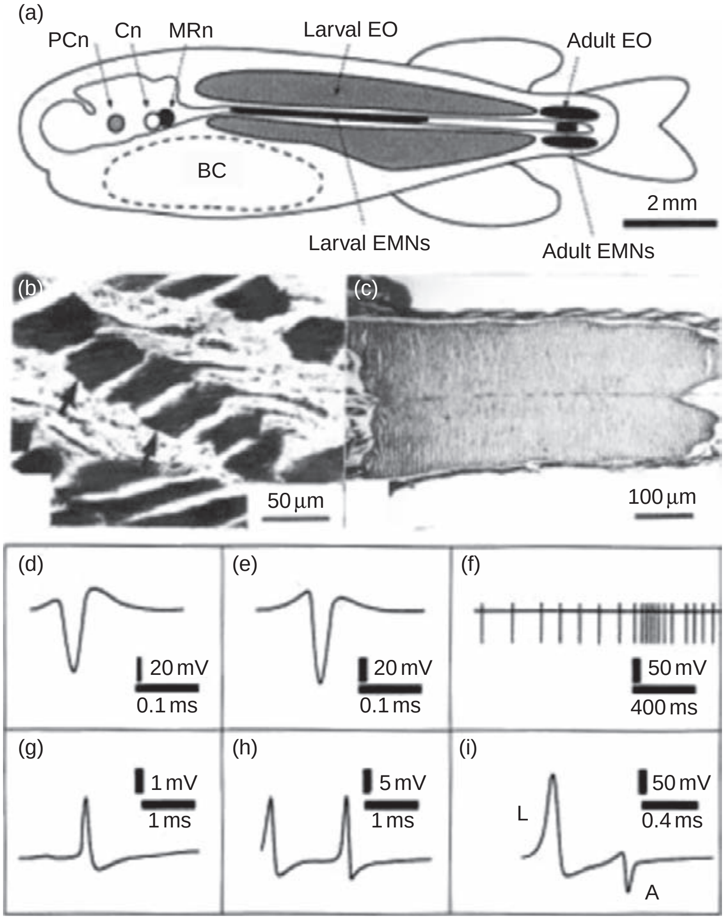


Figure 5 Topographical (a), histological (b, c), and physiological and ontogenetic data (d–i) on the larval and adult electric organs (EOs) in *Pollimyrus isidori*. (a) Topography of larval and adult EOs and electromotor neurons (EMNs) in a 15-mm larva. (b) Rows of larval electrocytes (arrows) from the epaxial muscle. (c) Sagittal section of the caudal peduncle showing the dorsal and ventral part of the adult organ with densely packed electrocytes (about 100 per column). (d–i) Discharge types: male (d), female and juvenile (e); sequence of pulse intervals recorded from an adult fish showing variable inter-pulse interval (f); larval type (g); larvae of approximately 10-mm length and juveniles about 20-mm long produce double pulses (h). In juveniles (i) from 15 mm on, the head-positive larval EOD, L, is followed and clearly separated by a head-negative adult EOD phase, A. BC, body cavity; PCn, pre-command nucleus; Cn, command nucleus; MRn, medullary relay nucleus.

the myotomic arrangement. These two organs therefore represent different steps in the evolution of the EO, interestingly being present in the same extant species.

Siluriformes

The African catfishes of the family Malapteruridae emit strong electric discharges of about 350 V. The EO is located just beneath the skin around the body; the origin of the electrocytes is uncertain. More recently, weak EOs have been recognized in some species of the African catfish families Mochokidae (*Synodontis* spp.) and Clariidae (*Clarias gariepinus*). The origin of the EO of *Synodontis* spp. seems to be the sonic muscle. The ontogenies of these organs are unknown.

Gymnotiformes

The South American electric knifefishes (gymnotiforms) are composed of five families with about 200 recognized species. These fish comprise a strong electric fish, the electric eel (*Electrophorus electricus*), whereas the remaining species all emit weak EODs. The EOs are very diverse in their anatomy, histology, and developmental origin. The EOs are of myogenic origin in species of the families Gymnotidae, Rhamphichthyidae, Hypopomidae, and Sternopygidae, and of neurogenic origin in all fish of the family Apteronotidae. A recent comparative ontogenetic study has demonstrated that there are two general types concerning the ontogenetic origin of the electrocytes. In species of the families Gymnotidae, Rhamphichthyidae, and Hypopomidae, the electrocytes originate below the hypaxial muscle, whereas in fish of the families Sternopygidae and Apteronotidae, the origin lies inside the axial muscle. The development of the organs is a long and complicated process, best exemplified by *Eigenmannia lineata* and (long nosed black ghost) *Apteronotus leptorhynchus* (**Figure 6**). In *Eigenmannia lineata*, the larvae start to emit

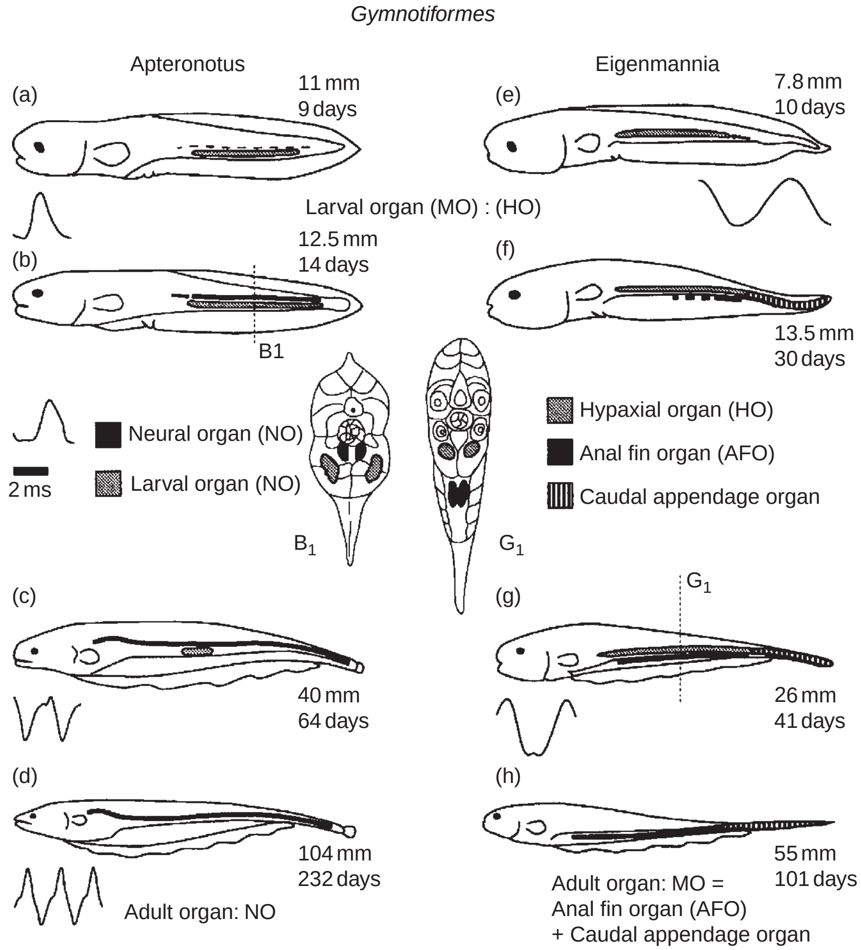


Figure 6 Comparison of the ontogenetic development of electric organs in two gymnotiform species that possess neurogenic (NO, *Apteronotus leptorhynchus* – (a)–(d)) and myogenic organs (MO, *Eigenmannia lineata* – (e)–(h)), respectively. The larval myogenic hypaxial organ is found in both species early during ontogeny; these organs are considered homologous structures. The larval organ eventually is replaced by the neurogenic organ in *Apteronotus*, and by the two myogenic organs in *Eigenmannia*. The larval organ is considered plesiomorphic (similar to that in the primitive ancestral clade), and the adult organs are considered apomorphic structures (structures that distinguish the group).

discharges just before the onset of exogenous feeding, at day 8 after fertilization when they are about 7 mm long. The electrocytes lie in the hypaxial muscle (**Figure 6(e)**) and extend caudally as the fish grows until they have reached the tip of the caudal appendage (**Figure 6(f)**). Then, additional electrocytes develop below the hypaxial muscle in between the muscles controlling movement of the anal fin (**Figure 6(g)**). The last step is the degeneration of the EO in the axial muscle (larval myogenic organ). The adult organ is fully formed by day 101, when juveniles are 55 mm long (**Figure 6(h)**).

Adult *Apteronotus leptorhynchus* possess a neurogenic organ (**Figure 6(d)**). In 9-day-old, 11-mm-long larvae, just at the onset of exogenous feeding, the larvae possess a myogenic organ in the hypaxial muscle (**Figure 6(a)**). This organ degenerates later on (**Figure 6(c)**). In 13-day-old larvae, a neurogenic organ starts to develop dorsal to the myogenic organ in the hypaxial muscle. This organ continuously extends rostrally and caudally (**Figure 6(c)**) and is the only organ present in the adult (**Figure 6(d)**). This ontogenetic sequence suggests that during evolution in apteronotids, first a myogenic and later a neurogenic EO developed. The EOD continuously changes during ontogeny and thus reflects the changing amplitudes of the discharges of both the myogenic and the neurogenic organs.

Comparative study has shown that the electrocytes in all species develop from mononucleated cells (electroblasts) directly into electrocytes. However, myofibrils can always be detected in the developing cells, indicating their myogenic origin. The electrocytes of wave-type species (families Sternopygidae and Apteronotidae) originating in the hypaxial muscle seem to represent more primitive organs compared with those originating below the hypaxial muscle (pulse-type species of the families Gymnotidae, Rhamphichthyidae, and Hypopomidae).

Astroscopidae

In the family Uranoscopidae, some species of the genus *Astroscopus* and *Uranoscopus* possess modified eye muscles emitting strong electric discharges of about 20–30 V. The development of these EOs has not been described to date.

See also: **Detection and Generation of Electric Signals**: Active Electrolocation; Electric Organs; Morphology of Electroreceptive Sensory Organs. **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish.

Further Reading

Albert JA and Crampton WGR (2006) Electroreception and electrogenesis. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 431–472. Boca Raton, FL: CRC Press.

Bennett MVL (1971) Electric organs. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 5, pp. 347–491. New York: Academic Press.

Bensouilah M, Schugardt C, Roesler R, Kirschbaum F, and Denizot JP (2002) Larval electroreceptors in the epidermis of mormyrid fish: I. Tuberous organs of type A and B. *Journal of Comparative Neurology* 447: 309–322.

Bullock TH and Heiligenberg W (1986) *Electroreception*. New York: Wiley.

Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) (2005) *Electroreception*. New York: Springer.

Denizot JP, Bensouilah M, Roesler R, Schugardt C, and Kirschbaum F (2007) Larval electroreceptors in the epidermis of mormyrid fish: II. The promormyromast. *Journal of Comparative Neurology* 501: 810–823.

Denizot JP, Kirschbaum F, Schugardt C, and Bensouilah M (1998) Larval electroreceptors indicate a larval electric system in mormyrids. *Neuroscience Letters* 241: 103–106.

Denizot JP, Kirschbaum F, Westby GWM, and Tsuji S (1978) The larval electric organ of the weakly electric fish *Pollimyrus* (*Marcusenius*) *isidori* (Mormyridae, Teleostei). *Journal of Neurocytology* 7: 165–181.

Denizot JP, Kirschbaum F, Westby GWM, and Tsuji S (1982) On the development of the adult electric organ in the mormyrid fish *Pollimyrus isidori* (with special focus on the innervation). *Journal of Neurocytology* 11: 913–934.

Kirschbaum F (1977) Electric organ ontogeny: Distinct larval organ precedes the adult organ in weakly electric fish. *Naturwissenschaften* 64: 387–388.

Kirschbaum F (1981) Ontogeny of both larval electric organ and electromotoneurones in *Pollimyrus isidori* (Mormyridae, Teleostei). In: Szabo T and Czech G (eds.) *Advances in Physiological Sciences, Vol. 31: Sensory Physiology of Aquatic Lower Vertebrates*, pp. 129–157. Budapest: Pergamon.

Kirschbaum F (1983) Myogenic electric organ precedes the neurogenic organ in apteronotid fish. *Naturwissenschaften* 70: 305–307.

Kirschbaum F and Schwassmann HO (2008) Ontogeny and evolution of electric organs in gymnotiform fish. *Journal of Physiology* 102: 347–356.

Moller P (1995) *Electric Fishes: History and Behaviour*. London, New York: Chapman and Hall.

Northcutt RG (2003) Development of the lateral line system in the channel catfish. In: Browman HI and Skiftesvik AB (eds.) *The Big Fish Bang: Proceedings of the 26th Annual Larval Fish Conference*, pp. 137–159. Bergen, Norway: Institute of Marine Research.

Northcutt RG (2005) Ontogeny of electroreceptors and their neural circuitry. In: Bullock TH, Hopkins CD, Popper AN, and Fay RR (eds.) *Electroreception*, pp. 112–131. New York, NY: Springer.

Northcutt RG, Catania KC, and Criley BB (1994) Development of lateral line organs in the axolotl. *Journal of Comparative Neurology* 340: 480–514.

Visher HA, Lanoo MJ, and Heilenberg W (1989) Development of the electrosensory nervous system in *Eigenmannia* (gymnotiformes): I. The peripheral nervous system. *Journal of Comparative Neurology* 290: 16–40.

MUSCLES, SKELETON, SKIN, AND MOVEMENT

Functional Anatomy of Bones, Muscle, and Skin: An Introduction

ED Stevens, University of Prince Edward Island, Charlottetown, PE, Canada

© 2011 Elsevier Inc. All rights reserved.

A Brief Historical Perspective on Functional Anatomy of Fishes
Functional Anatomy of Fishes Today
Further Reading

Glossary

Bioluminescence Light produced by a chemical reaction that originates in an organism.

Chromatophores A variety of pigmented cells involved in the coloration of the skin of fish and other vertebrates and invertebrates. In fish, these are melanophores (black or brown), xanthophores (orange or yellow), erythrophores (red), cyanophores (blue), leucophores (whitish), and iridophores (iridescent).

Counter-illumination A defensive measure used by bioluminescent midwater cephalopods, decapod crustaceans, and fishes that camouflages an animal being viewed from below by mimicking downwelling light and reducing their silhouette.

Homology Indicates similarities due to inheritance, and used for homologous structures (e.g., limbs) or homologous gene sequences.

Iridescent Display of lustrous colors, which may vary with the angle of viewing. It is produced by interference of light waves with phase differences, when reflected from outer and inner surfaces of a transparent structure such as a crystal or membrane.

Tessellated cartilage Hyaline-like cartilage ‘tiled’ (tessellated) on the outside with tesserae; a feature of the Elasmobranchii (in contrast with areolar cartilage).

A Brief Historical Perspective on Functional Anatomy of Fishes

The articles in this volume focus on the functional anatomy of fishes, that is, anatomy from the inside out (bones, muscles, and out to skin), with an emphasis on the physiological significance of these structures. Most biologists have at least a cursory knowledge of fish anatomy because most participated, as part of their undergraduate training, in a course in comparative vertebrate anatomy that involved the dissection of a fish, usually a dogfish shark. Moreover, many took a course such as the functional anatomy of fishes taught for many years by Karel Liem at Harvard and which is also taught at many other universities. Of course, many eat cooked fish muscle after first removing the skin and hopefully leaving behind the bones!

Presumably, even cavemen had an idea of the morphology of the animals they ate because some cave drawings illustrate animals including fish. However, it was not until Galen, a Greek physician, who worked in Rome between AD 165 and 200 that there was any substantial written record. Galen wrote a synthesis of what was known about anatomy, but, more importantly, he added to that knowledge by dissecting a variety of animals including fishes, with the intention of discovering their morphology. Many of the names given to bones and muscles by Galen persist to the present day. Galen’s views were essentially dogma until the 1500s. Belon dissected a variety of animals, including fishes, and noted the similarity in the limb bones in all vertebrates. Amazingly, three anatomists published works on fishes at almost the same time: Belon (1553), Rondelet (1554), and Salviani (1554). Lorenzini published a monograph on the anatomy of Torpedo about 100 years later (1678). However, Cuvier is usually considered to be the founder of comparative anatomy. Georges Cuvier (sometimes reported as Georges Leopold Chretien Frederic Dagobert Cuvier and other times as Jean Léopold Nicolas Frédéric Cuvier) Baron de Montbeliard (1769–1832) was an extremely prolific writer. His works include nine volumes on comparative anatomy and the 22-volume *Historie Naturelle des Poissons* (*Natural History of Fishes*). Cuvier focused on the function that different structures performed and how their structure is adapted to their function. His great contribution was that he stressed an important concept that still has resonance: the structure of each and every part of the body is related to the animal's lifestyle; that is, structure and function are interdependent.

In the years that followed Cuvier, there was a struggle between comparative anatomists, especially Richard Owen, who held the view that species were immutable, versus those who supported Darwin, especially T.H. Huxley. Thus, the focus of scientific discussions was distracted away from the actual comparative anatomy and toward the arguments for and against Darwin's ideas about natural selection. In addition, more recently, much of life sciences has been concerned with the role of functional genomics, making it difficult to fund research on functional morphology. The series of articles in the present section show that there remain fascinating questions related to functional anatomy and that important research in the area of functional morphology is still ongoing. The articles in this section show how functional anatomy is just as important as functional genomics in understanding how the bodies of fishes work; it may be perhaps not as topical, but is just as interesting and just as important.

Functional Anatomy of Fishes Today

The following section provides a synthesis of current knowledge concerning the functional anatomy of the skeleton, of muscles, and of skin of fishes.

Skeleton – Bones or No Bones

The Chondrichthyes are considered cartilaginous fishes because the long endoskeletal elements called bones in other vertebrates are made of cartilage instead. Scientists have recognized the gross differences in skeletal material between the Chondrichthyes and other vertebrates for more than two centuries and yet, we have made only short inroads into answering the most basic questions surrounding the evolution and predominance of cartilage in the skeletons of these fishes. Most adult vertebrate animals have bony skeletons, with cartilage mostly restricted to joints and flexible structures. In contrast, the Chondrichthyes (sharks, batoids, and chimaeras) have endoskeletons made entirely of cartilage (**see also The Skeleton**: Cartilaginous Fish Skeletal Anatomy). Moreover, in sharks and batoids, most of the skeletal cartilage is tessellated, covered with mineralized blocks called 'tesserae'. There are several other forms of cartilage found in the bodies of these fishes that likely serve distinct functional and metabolic roles; these are described in a separate article (**see also The Skeleton**: Cartilaginous Fish Skeletal Tissues).

In contrast to the chondrichthyan fishes, most of the skeleton of bony fish becomes calcified during development. The skeleton of bony fishes is composed of hundreds of individual bony and cartilaginous elements that vary considerably in form (**see also The Skeleton**: Bony Fish Skeleton). The details of this variation are the product of functional demands generated by the life history, ecology, and behavior of individual species. Coupled to this are the constraints set in place from the phylogenetic history of each species. It is this combination – phylogeny and function – that makes an understanding and appreciation of the skeleton of fishes central to so many aspects of the evolutionary and ecological biology of fishes.

Muscle

The two articles in this section on muscle compare the cranial muscles of the cartilaginous fishes (**see also The Muscles**: Cartilaginous Fishes Cranial Muscles) with those of the bony fishes (**see also The Muscles**: Bony Fish Cranial Muscles). The cranial muscles of fishes are the actuators of their feeding and respiratory mechanisms, and are thus of great interest regarding vertebrate evolutionary history and functional diversification. The article **The Muscles**: Cartilaginous Fishes Cranial Muscles is a guide to the gross anatomy and function of these muscles in the three main lineages of cartilaginous fishes: sharks (spiny dogfish), batoids (southern guitarfish), and holocephalans (spotted ratfish), and includes a video of these jaw muscles in action. The article **The Muscles**: Bony Fish Cranial Muscles provides a much generalized description of the more common functions performed by or within the head, and those muscles responsible for these functions, including buccal pumping (ventilation), suction feeding, premaxillary protrusion, biting and tearing, and pharyngeal jaw prey processing. Videos of these muscles in action are available in **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics.

Skin

The integument or skin is the envelope that not only separates and protects a fish from its environment, but also provides the means through which most contacts with the outer world are made (**see also The Skin**: The Many Functions of Fish Integument). It is a large organ and is continuous with the linings of all body openings, and also covers the fins. Fish integument is a multifunctional organ, and its components may serve important roles in protection, communication, sensory perception, locomotion, respiration, ion regulation, excretion, and thermal regulation. The integument consists of two layers (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes): the outer layer and the inner layer. The outer layer, the epidermis, is essentially cellular in structure, comprised of a multilayered epithelium that usually includes specialized cells. The many types of these cells are described in the very well illustrated article **The Skin**: Functional Morphology of the Integumentary System in Fishes. The inner layer, the dermis, is primarily a fibrous structure with relatively few cells, although it may contain scales, nerves, blood vessels, adipose tissue, and pigment cells.

Skin coloration and patterns, and their capacity for change, have important roles in the life of fish, such as camouflage and social signaling (**see also The Skin**: Coloration and Chromatophores in Fishes). A variety of chromatophores (pigmented cells), and skin structural features, provide a physical basis for the wide chromatic variation between and within species. Short-term, physiological, and long-term, morphological, regulation of chromatophores involves the autonomic nervous system, and endocrine, and paracrine factors. Chromatophore-based patterns can result from local variations in the types of these cells, their numerical distribution, and differential control of intracellular pigment movement. Additionally, iridescent coloration may derive from skin structures such as collagen fiber arrays and surface microstructure.

In addition to pigments, many fish are bioluminescent (**see also The Skin**: Bioluminescence in Fishes). Marine teleost fish comprise one of the most diverse groups of luminescent animals with over 45 families and more than 190 genera containing luminescent species. Predominantly, blue light is emitted diffusely through translucent tissues that harbor symbiotic bioluminescent bacteria or discretely form complex photophores equipped with reflectors, lenses, and filters. Light displays can vary from quick flashes to long-lasting glows. Variety in form is matched by diversity in function with luminescence used for predation, defense, and intraspecific signaling. The article **The Skin**: Bioluminescence in Fishes is illustrated with some beautiful examples of bioluminescence in fishes.

Finally, **The Skin**: Hagfish Slime describes a very special function of skin that is present only in the hagfishes – that is, hagfish slime. Hagfish slime is very different from mucus. Hagfish slime is the ultra-dilute material that is formed when hagfishes eject the contents of their slime glands into seawater. The slime consists of two main components, mucin vesicles and thread skeins, which each arise from distinct cells within the slime gland. Deployment of the slime involves swelling of the mucin vesicles when they contact seawater, and their subsequent shearing into long mucin strands when they are acted upon by hydrodynamic mixing. The mucin strands attach to the thread skeins and transduce mixing forces to them, thereby effecting their unraveling. Hagfish slime is believed to function as a defense against gill-breathing predators.

In conclusion, the articles in this section describe how the structural components that make up the skeleton, the muscles, and the skin contribute to the physiology of fishes.

See also: **The Muscles**: Bony Fish Cranial Muscles; Cartilaginous Fishes Cranial Muscles. **The Skeleton**: Bony Fish Skeleton; Cartilaginous Fish Skeletal Anatomy; Cartilaginous Fish Skeletal Tissues. **The Skin**: Bioluminescence in Fishes; Coloration and Chromatophores in Fishes; Functional Morphology of the Integumentary System in Fishes; Hagfish Slime; The Many Functions of Fish Integument.

Further Reading

Cole FJ (1949) *A History of Comparative Anatomy from Aristotle to the Eighteenth Century*. London: Macmillan.

MUSCLES, SKELETON, SKIN, AND MOVEMENT

The Skeleton

Contents

Cartilaginous Fish Skeletal Anatomy
Cartilaginous Fish Skeletal Tissues
Bony Fish Skeleton

Cartilaginous Fish Skeletal Anatomy

KM Claeson, University of Texas at Austin, Austin, TX, USA

MN Dean, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Anatomy
Sharks
Batoids
Chimaeroids
Further Reading

Glossary

Anterior Toward the front or head. In fishes, the term rostral can be substituted for anterior.

Autostyly Jaw condition where palatoquadrate is strongly attached to the ethmoid region and directly fused to the auditory region of the chondrocranium. The hyomandibula does not participate in jaw suspension.

Ceratotrichia Thin, keratinous fin rays, originating from large collagen fibers, and forming the distal-most elements in the fins of cartilaginous fishes (contrast with actinotrichia).

Distal The furthest from the central nervous system, or in the direction away from the central brain. Receptors are the most distal retinal neurons.

Dorsal Toward the top or back of the fish (the dorsal surface).

Endoskeleton The skeletal system formed by all skeletal elements that are nondermal in developmental origin.

Euhyostyly Jaw condition when the palatoquadrate has no ligamentous connection to the chondrocranium and does not contact it anteriorly, but only posteriorly via the hyomandibula (as in batoids).

Extant A species or clade, etc., that is still in existence.

Holostyly Jaw condition when palatoquadrate is fused to chondrocranium (as in Holocephalii).

Hyostyly Jaw condition when the palatoquadrate articulates with the chondrocranium, but is not strongly attached or fused (as in most sharks).

Lateral In a direction away, or a position far from the body's midline (e.g., your eyes are lateral on your face and lateral to your nose).

Medial In a direction toward, or a position near the body's midline (e.g., your nose is medial on your face and medial to your eyes).

Neoselachii A clade of cartilaginous fishes that includes extant sharks and batoids, but not chimaeroids.

Posterior Toward the rear, or behind. In fishes, the term caudal can be substituted for posterior.

Proximal Nearest to the central nervous system, or in the direction toward the central brain. Ganglion cells are the most proximal retinal neurons.

Skeletal element An individual portion in a cartilaginous skeleton, analogous to individual bones in the skeleton of a bony vertebrate.

Spiracle An external opening near the eye in elasmobranch fishes that communicates to the throat, via a passage between the hyomandibula and cranium.

Suspensorium The chain of cartilages, including the hyomandibula that suspends the jaws from the chondrocranium.

Synarcual A series of fused vertebrae.

Ventral Toward the bottom or belly of the fish (the ventral surface).

Introduction

From the outside, for the most part, the members from each of the three major extant groups of the Chondrichthyes look unmistakably distinct; a reef shark could never be confused with a barndoor skate or a white-spotted chimaera. Of course, these differences are reflected broadly in the skeleton; however, certain aspects of skeletal design are conserved among these fishes (**Figures 1** and **2**). The article **The Skeleton:** Cartilaginous Fish Skeletal

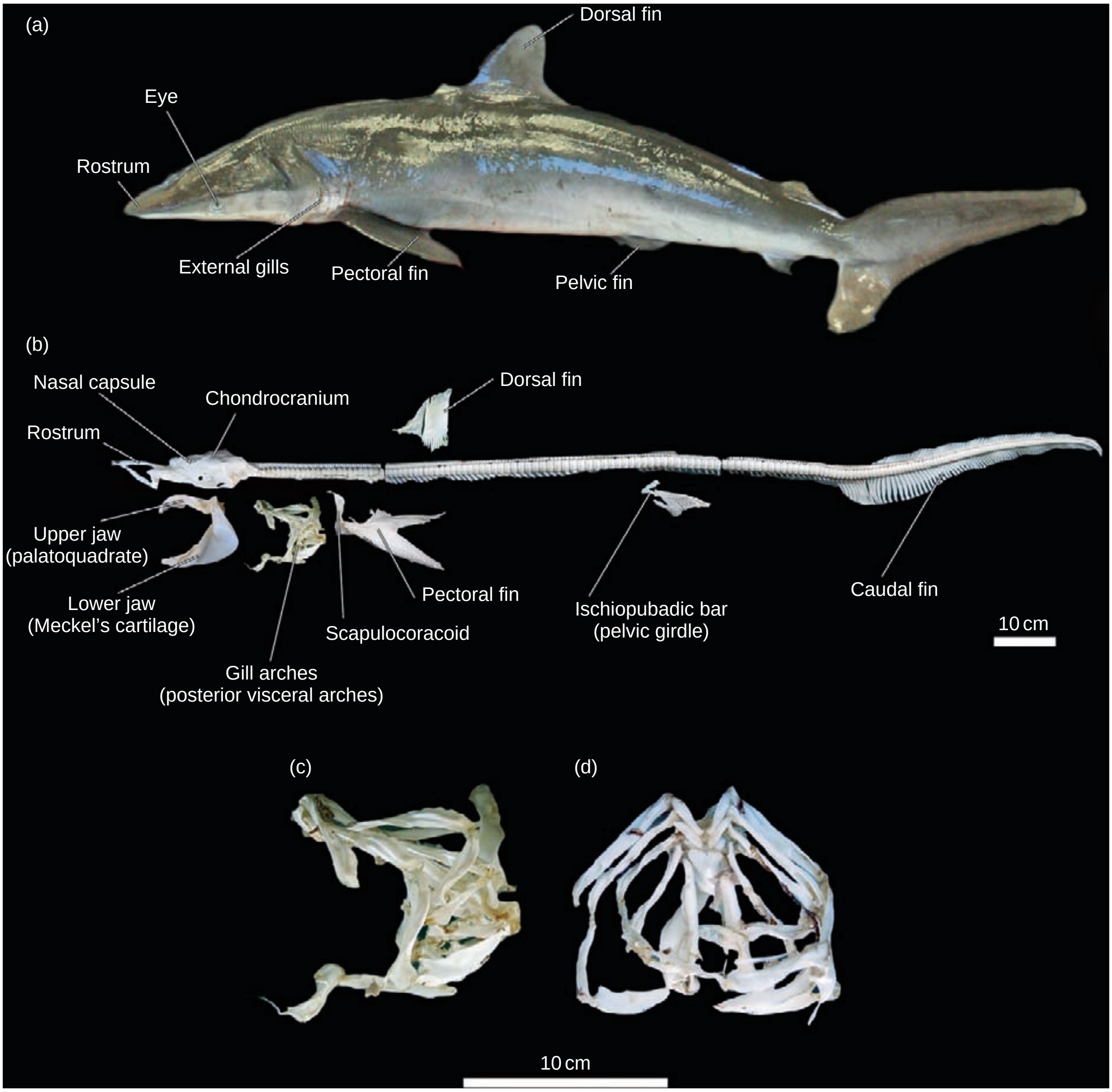


Figure 1 The body plan of sharks: (a) external anatomy of silky shark, *Carcharhinus falciformis*, in lateral view; (b) skeletal anatomy of silky shark, *Carcharhinus falciformis*, in lateral view; (c) closeup of branchial basket in lateral view; and (d) close-up of branchial basket in ventral view. Skeleton was prepared by dissection and maceration before it was dried and mounted. Branchial basket collapsed during drying and is slightly deformed.

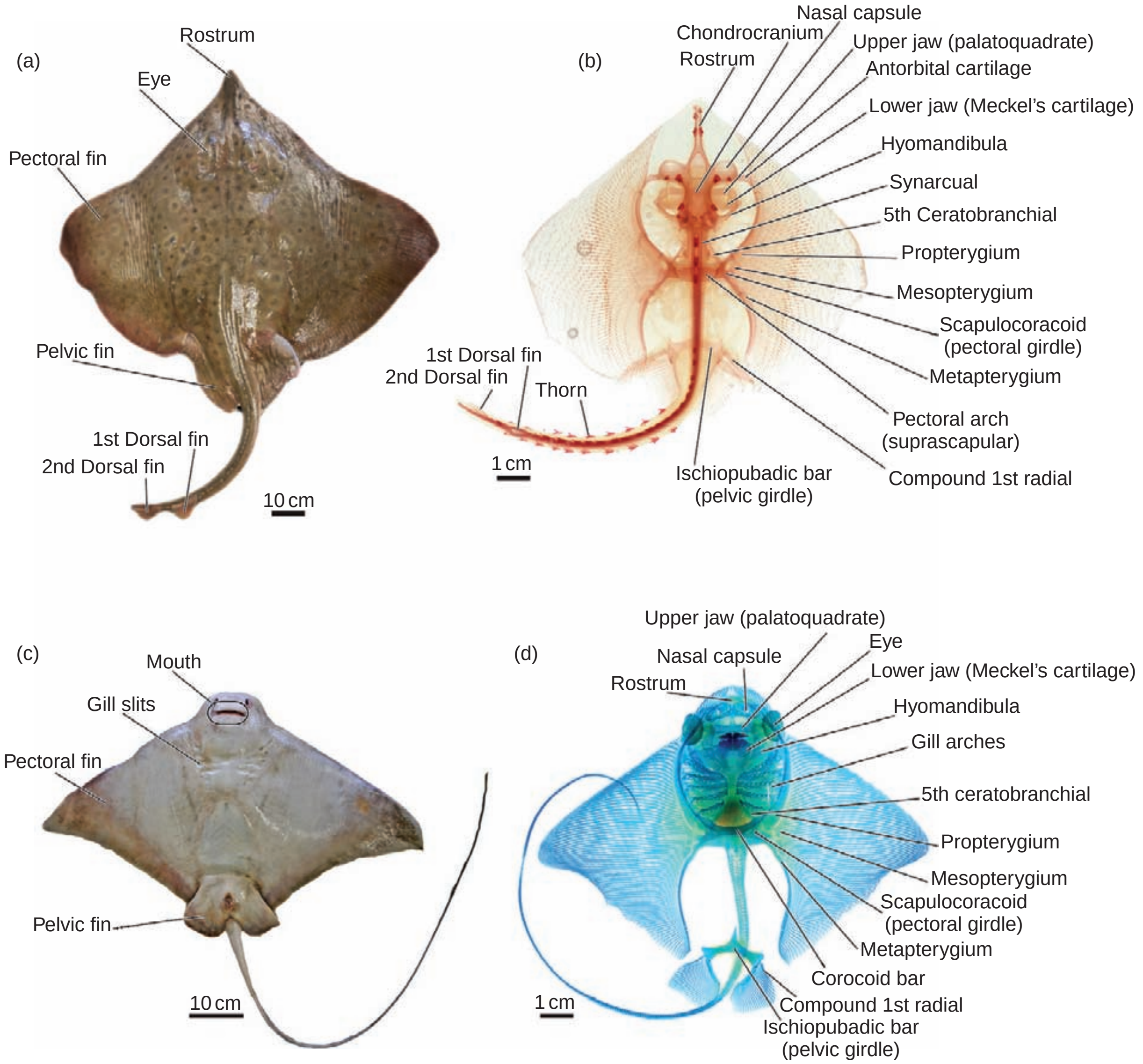


Figure 2 The body plan of batoids: (a) external anatomy of the barndoor skate, *Dipturus laevis*, in dorsal view; (b) skeletal anatomy of a skate, *Raja sp.*, in dorsal view. Skeleton was prepared by clearing and staining. Chemicals are used to digest the muscle fibers and make them clear. Alcian blue stains for uncalcified cartilage and Alizerin red stains for calcified (mineralized and hard) cartilage. The skeleton in this specimen is fairly well developed; (c) external anatomy of the cownose stingray, *Rhinoptera bonasus*; (d) skeletal anatomy of the eagle ray, *Myliobatis sp.* Skeleton was prepared by clearing and staining. This is a young specimen; the skeleton is not very well calcified yet, although all major parts have developed. (b) Courtesy of President and Fellows of Harvard College.

Tissues discusses conservation and variation at the tissue level; here, we show how these different types of cartilages are assembled to form the skeletons of chondrichthyan fishes.

A simplified chondrichthyan skeleton can be divided into three distinct parts – the axial, visceral, and the appendicular skeletons – that are discussed below. To understand the similarities and differences among taxonomic groups, it will help to think about the skeleton in pieces, just do as most anatomists, functional morphologists, and paleontologists. Researchers can compare these pieces through broad taxonomic surveys to gain insight into chondrichthyan evolution and how certain morphologies might be tied to certain ecologies.

General Anatomy

Axial Skeleton

The axial skeleton includes the skull, vertebral column, and ribs. The skull of all chondrichthyan fishes is called the chondrocranium and is essentially a cartilaginous box containing the brain and other important sense organs. Although the adult chondrocranium is a single piece of cartilage with definable regions, it is assembled early in embryonic development through the coalescence of separate cartilages. The embryonic basal and ethmoid plates form the anterior wall and floor of the chondrocranium; the occipital arches form the posterior wall of the chondrocranium; and the orbital cartilages contribute to the lateral walls of the chondrocranium. Although the complete chondrocranium varies in size and shape among species, there are generally identifiable regions that are conserved across the different groups of chondrichthyans and can be used to make broad comparisons among groups. From roughly rostral to caudal, these are the rostrum, nasal capsule, antorbital process, orbital region, otic capsules, and occipital condyles (**Figure 3**).

Posterior to the chondrocranium, the remainder of the axial skeleton is the vertebral column, composed of individuated vertebrae. There are three primary segments of a vertebra in chondrichthyans: dorsally, the paired basidorsals, contributing to the neural arches that protect the spinal cord; ventrally, the paired basiventrals, contributing to hemal arches that house blood vessels; and, in the middle, the centrum. The vertebral centrum is a spool-shaped structure that acts as the primary load-bearing structure and to which the neural and hemal arches that protect the important surrounding soft tissues are attached. Although the arches are made of tessellated cartilage, as in the rest of the skeleton, the centra are areolar cartilage; this is apparently the only place in the body where this tissue is found (**see also The Skeleton:** Cartilaginous Fish Skeletal Tissues). The centra are arranged so that the top and bottom of the spool face rostrally and caudally. Numerous centra are then strung together to form the spinal column, resembling many spools of thread strung together on a string. In the center of each centrum are the remains of the notochord, a stiff but flexible rod that serves as the embryonic foundation for the vertebral column. Among the different elasmobranch taxa, the shape of the persistent notochord in cross section is highly variable in fully developed centra, a result of differential folding as the areolar cartilage grows around it. That shape is consistent within certain groups of elasmobranchs and can be used diagnostically to distinguish between the vertebrae of different groups (e.g., those of sharks and batoids, **Figure 4**).

Visceral Arches

Beneath the chondrocranium is the visceral skeleton, which includes the upper and lower jaws (palatoquadrate and Meckel's cartilage), hyoid, and gill arch cartilages. These collected structures are called the visceral arches and can be thought of as jointed, symmetrical loops/semicircles of cartilaginous elements, arranged in rostrocaudal (anteroposterior) series, and supporting the breathing and feeding structures of the head. The joints

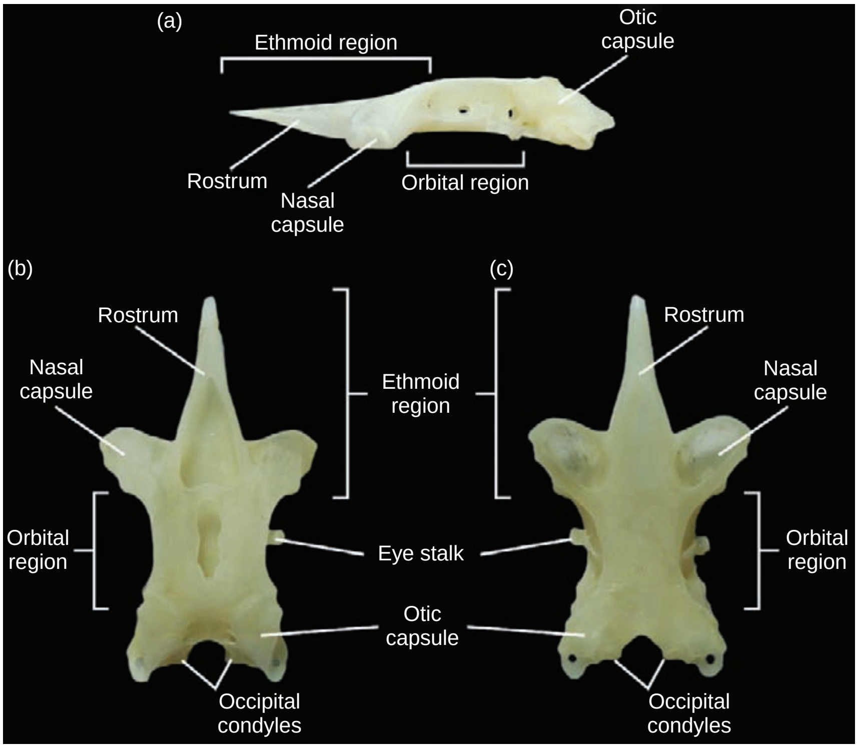


Figure 3 The chondrocranium of a skate, *Leucoraja ocellata*: (a) lateral view; (b) dorsal view; (c) ventral view.

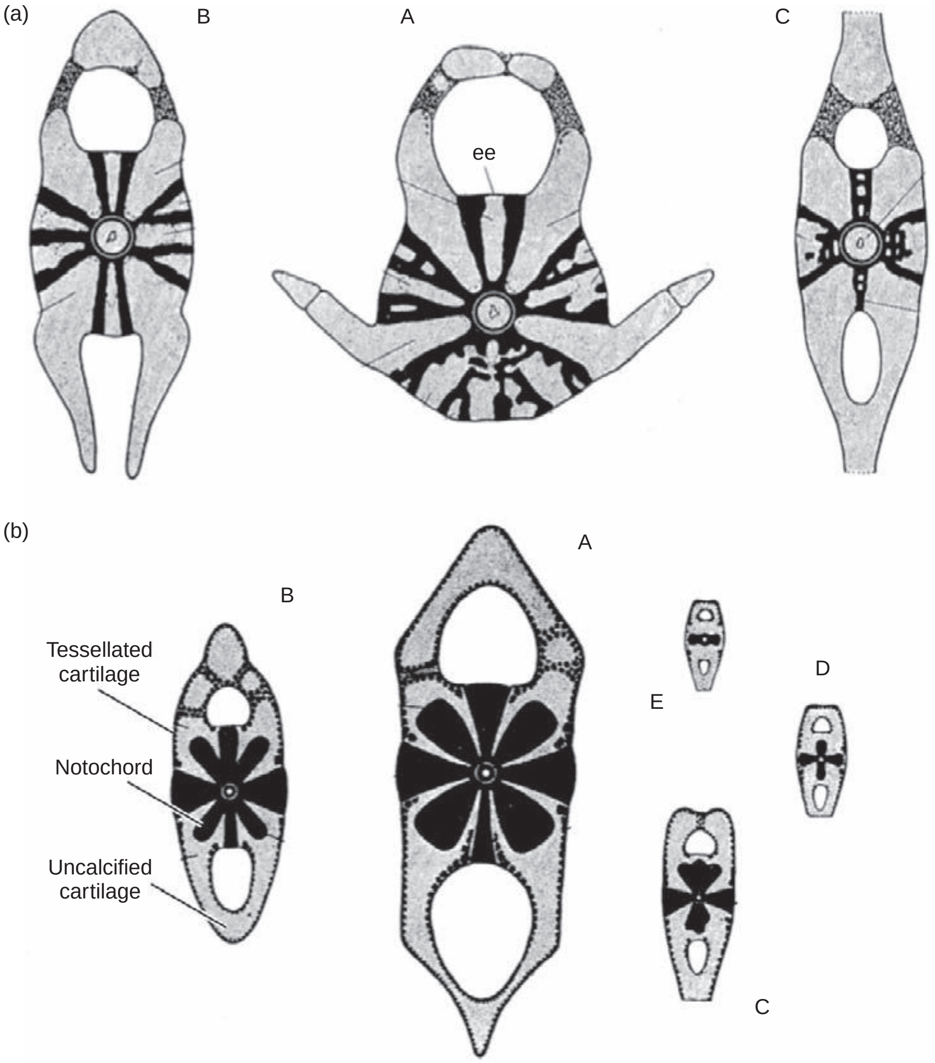


Figure 4 Cross section of vertebrae showing folding pattern of notochord in the centrum: (a) shark vertebrae, *Stegostoma tigrinum*, 660 mm long, ×6 (A, vertebra from region of pectoral girdle; B, vertebra from post-cloacal region; C, vertebra from region half-way along the caudal fin); (b) batoid vertebrae, *Rhinobatos granulatus*, ×3 (A, caudal vertebra, taken a short distance in front of the first dorsal fin; B, vertebra from the base of the caudal fin; C, from half-way between the base of the caudal fin and the extreme caudal end of the vertebral column; D, from half-way between C and the extreme hind end of the vertebral column; E, vertebra, a short distance behind D). Tessellated cartilage crust surrounds uncalcified cartilage. Notochord present as spokes (shark) or clover pattern (batoid). Modified from Ridewood (1921) On the calcification of the vertebral centra in sharks and rays. *Philosophical Transactions of the Royal Society of London, Series B* 210: 311–407.

in the arches allow them to expand and collapse, making breathing and feeding possible.

The mandibular arch (the first visceral arch) forms the jaw in chondrichthyans. It is divided into two parts, the dorsal palatoquadrate (upper jaw) and the ventral Meckel's cartilage (lower jaw). The palatoquadrate and Meckel's cartilage changed shape many times during the evolution of the Chondrichthyes. This is correlated to a change in the way the jaw is attached to the chondrocranium. The earliest chondrichthyans from the Paleozoic had a massive palatoquadrate that was firmly attached to the chondrocranium and was not mobile. In the modern Holocephalii, the jaws are also firmly in place, an arrangement is known as holostyly, which gave the group its name. The holostylic arrangement in Holocephalii is only convergent with the Paleozoic condition, and therefore these similar features are not derived from the same common ancestor. This Paleozoic type of jaw connection is known as autostylic. As chondrichthyans evolved, the palatoquadrate became less firmly connected with the chondrocranium and more mobile. There is evidence that these changes are correlated with the evolution of the variety of feeding styles seen in modern species.

The hyoid arch (the second visceral arch) is posterior to and closely associated with the mandibular arch. It is also divided into dorsal and ventral components. The hyomandibula is the sole dorsal element and the ventral components are the ceratohyal and basihyal. The remainder of the visceral arches are known collectively as the gill arches or branchial basket (**Figures 1**(**c**) and **1**(**d**)). The latter descriptor relates to the shape into which the series of U-shaped arches conform. As they are adjoined by ventral branchial cartilages, they form a structure that looks like the ribcage of a wooden ship, with the longer dorsal elements converging and connecting ventrally. The gill arches are the support system for the soft structures involved in gill ventilation and respiration.

With this knowledge of the relative arrangements of the skeletal structures that support the mouth, throat, and gills, we can now understand the general pathway of water in a breathing shark; this pathway is likely, in general, true for chondrichthyans; however, it has yet to be demonstrated in chimaeroids. The chondrichthyans (as well as other fishes) extract oxygen when they bring water in and pass it over their gills. Water enters the orobranchial chamber through the jaws (visceral arch 1) or the spiracle (bounded posteriorly by visceral arch 2), typically as the floor of the chamber is depressed through ventral movement of the ventral hyoid and branchial arches. This water is pushed or drawn over the gills, and oxygen and carbon dioxide are exchanged across a network of blood vessels found within the soft gill tissues. The water, then oxygen-depleted, passes out of the gill chamber by way of gill slit openings (the spaces between the branchial arches). In the sharks, the gill slits are lateral on the body, whereas in batoids the gill slits are ventral. The gill openings in chimaera are covered by a fleshy operculum with a single posteroventral exit (**see also The Muscles**: Bony Fish Cranial Muscles).

Appendicular Skeleton

The appendicular skeleton includes the cartilages of the paired fins and the limb girdles. In general, skeletal elements are smaller and more flexible the more distal they are in the appendicular skeleton. This is correlated with a tendency for proximal elements to be involved in supporting the more distal elements of the appendicular skeleton, and those distal elements to be more involved in locomotion.

The pectoral (forelimb) and pelvic (hindlimb) girdles are the support structures for the paired fins. The majority of taxa have a U-shaped pectoral girdle, with each arm of the girdle pointing dorsally and comprised of coracoids, scapulae, and suprascapulae. The coracoids and scapulae often fuse into a scapulocoracoid, a term sometimes used in place of pectoral girdle. Suprascapulae are often small and might be absent from some groups. In certain chondrichthyans, the pectoral girdle is tightly associated with the axial skeleton (i.e., the arms of the girdle articulate with the vertebral column). In contrast, the pelvic girdle is composed of a single ischiopubadic bar, is always completely free of the axial skeleton, and is closely associated with the cloaca.

The pectoral and pelvic fins are attached to the ventrolateral edges of their respective girdles. The components of these fins, from proximal to distal, are flat basal pterygial cartilages (two to four in the pectoral fin and two in the pelvic fin), rod-like radial cartilages, and long, parallel fin rays called ceratotrichia. Radial cartilages vary greatly in overall length and number depending on the taxon; for example, they form the majority of the pectoral fins in batoids, which tend to have reduced numbers of ceratotrichia. Fin rays form the jointed and flexible flapping portion of the fin, which are referred to as actinotrichia (a derivation of dermatotrichia) in bony ray-finned fishes. In chondrichthyans, the fin rays are known as ceratotrichia because they are made of elastoidin (not bone), which has properties that are a combination of collagen and elastin. Ceratotrichia are homologous to actinotrichia in bony fishes.

Sharks

The term shark is a common name that refers to a large taxonomic sampling of chondrichthyans (approximately 403 of 970 chondrichthyans, ~41.5%) that are neither batoids nor chimaeroids. In general, sharks have a long body, and the paired fins are relatively short compared with the body (**Figure 1**). However, there is a lot of variation in the shark skeleton. For instance, the goblin shark, *Mitsukurina*, has a skull with an enormous rostrum that protrudes far anteriorly over a highly protrusible mouth while the angel shark, *Squatina*, has a short snout and relatively fixed mouth, but its fins extend anteriorly and resemble wings. In this section, we base our description of a generalized shark skeleton on the common dogfish shark, *Squalus*.

Axial Skeleton

In the ethmoid region of sharks, a short, broad median rostrum is situated anteromedially to the paired nasal capsules, giving many sharks a bullet-shaped body. The nasal capsules are round and situated anteroventrally. Posterior to the nasal capsules are very large orbits, broad cavities in the chondrocranium that house the eyes. The boundaries of the orbit are defined by the antorbital process, supraorbital crest, and postorbital process of the chondrocranium. The orbit is open ventrally. The interorbital space (the space between the eyes on the dorsal surface of the chondrocranium) is slightly narrower than the broad ethmoid region and roughly the same size as the optic region.

Visceral Arches

In most modern sharks, the palatoquadrate articulates with the chondrocranium, but not firmly, a condition called hyostyly. The hyoid cartilages (hyomandibula and ceratohyal) attach to the palatoquadrate and Meckel's cartilage, forming a movable suspensorium. The third hyoid cartilage, a large median basihyal, articulates at its lateral edges with the distal ends of the left and right ceratohyals.

Appendicular Skeleton

The pectoral girdle in sharks includes fused coracoid and scapular cartilages (the scapulocoracoid) and suprascapular cartilages. The ventromedial portion of the scapulocoracoid is the coracoid bar. The scapular process projects posterodorsally from the lateral end of the coracoid bar. The boundary between the coracoid and scapula occurs roughly where the proximal portion of the basal ptergygia of the pectoral fin articulates with the scapulocoracoid. The pectoral fin skeleton is composed of three short basal pterygia: the proptergygium (anteriormost), mesopterygium (medial), and metapterygium (posteriormost). Long radial cartilages extend from the distal ends of all three basal pterygia. Cartilaginous ceratotrichia comprise the distalmost portion of the pectoral fin.

Batoids

Batoids represent over half of the extant species diversity of Neoselachii. Except in a small handful of cases, they are easily distinguished from other chondrichthyans by their flat bodies (**Figure 2**). Compared to the generalized shark body plan, the paired pectoral fins are immense and stretched anteriorly and posteriorly, then effectively joined to the sides of the head; this creates a dorsoventrally flattened body disk anterior to the pelvic girdle. However, within the distinctive depressiform body plan of batoids, there is considerable variation, especially in the shape of the body disk and the stoutness of the tail, which are often representative of swimming mode and ecology. For instance, electric rays are usually nearly circular, a function of the lateral displacement of the pectoral fins by the electric organs. This organization makes their pectoral fins less useful for locomotion; instead they swim by a more shark-like wagging of the tail. Many benthic stingrays also have round body disks, although they lack electric organs; these species locomote along the bottom by undulating the margins of their wings. Some of the largest stingrays, on the other hand, have diamond-shaped bodies with massive, powerful fins for swimming and whip-like, nonpropulsive tails.

Axial Skeleton

Batoids often possess elaborate rostral and/or antorbital cartilages projecting off the anterior ethmoid region of the chondrocranium (**Figure 3**). The rostral and antorbital cartilages are highly variable among batoids and play a large role in determining the overall shape of the body disk. In rajiforms (skates), rhinobatiforms (guitarfishes), and pristioforms (sawfish) there is an anteriorly projecting rostral cartilage that is continuous with the ethmoid region and articulates with one or two rostral appendages distally. In myliobatiforms (stingrays) and torpediniforms (electric rays), the projecting rostral cartilage is absent or miniscule. The antorbital cartilages of torpediniforms, however, are paired and greatly expanded compared to the antorbital cartilages of rajiforms. Antorbital cartilages articulate with the nasal capsule proximally. Nasal capsules are round to oval to kidney-bean shaped and project ventrolaterally. In some batoids, the pectoral fin articulates with the distal margins of the antorbital cartilage. Posterior to the nasal capsule is the orbit. The interorbital space is narrower than the internasal space. The otic capsules are posterior to the orbit and are only slightly wider than the interorbital space.

All batoids share a modified vertebral element known as the synarcual. This is not to be confused with the chimaeroid synarcual (discussed below). The batoid synarcual cartilage is a long tube-like element, hypothesized to be the fusion of the first several vertebrae. Little is known about the synarcual cartilage; however, new research indicates that only neural arches contribute to the tube-like portion in which the spinal cord sits. Anteriorly, the synarcual contacts the chondrocranium. Posteriorly, it is forked and is closely associated with isolated centra made of areolar cartilage. In stingrays, a second, posterior synarcual is present.

Visceral Arches

Batoids share a jaw morphology known as euhyostyly. In this condition, the proximal hyomandibula and distal ceratoyal are still present, as in sharks. However, euhyostyly permits more freedom of movement of the hyomandibula relative to the ceratohyal, and – due to the comparative lack of ligamentous associations – of the jaws relative to the cranium. This type of suspensorium allows a great range of motion in the mouth of rays, with many species able to protrude their jaws up to 50% of their head length and some capable of twice this. The ceratohyal is secondarily free from the suspensorium and contributes to the gill arches. The basihyal, which is large and easily identified in sharks, is fused with the first hypobranchial cartilages in batoids to create a horizontal bar on the floor of the throat, anteromedial to the remaining visceral cartilages. The gill arches are wedged between the pectoral fins and axial skeleton, and are sometimes tightly connected to the synarcual. External gill slits are ventral to the body in batoids.

Appendicular Skeleton

In most batoids, the pectoral girdle and fins form the dominant structures for locomotion; this is in contrast to those species with thick shark-like tails that swim by lateral movements of the tail (e.g., sawfishes, guitarfishes, and electric rays). The different cartilages of the girdle fuse together to create a sturdy U-shaped scapulocoracoid cartilage. Dorsally, the scapulocoracoid articulates with the vertebral column via the suprascapula. This means that the pectoral girdle is the shape of a ring, unlike in other chondrichthyans where the U is not completely closed dorsally. The length of the pectoral fins of *Raja* and other batoids is often half their total body length or more. The cartilages that create the base of the wing-like structure (pro-, meso-, and metapterygia) are expansive, especially the pro- and metapterygia. In some species, the propterygia is so long that it articulates with the antorbital cartilage anteriorly. Pectoral fins of skates and rays consist of a series of parallel radial cartilages that emanate from the basal pterygia of the pectoral girdle. The structural reinforcement of individual radials, as well as the arrangement and bracing of joints between them, is variable among different groups of batoids and is reflective of locomotory life style (e.g., oscillatory vs. undulatory swimming).

Chimaeroids

Chimaeroids have large heads, long snouts, and tube-like bodies that taper to small and sometimes whip-like tails. They are sister group to the elasmobranchs, but externally, they bear little resemblance to their shark and batoid relatives.

Axial Skeleton

If batoid heads are flat (depressiform), those of chimaeroids are narrow (compressiform). Paired lateral rostral rods that lie medial to the nasal capsules extend anteriorly from the ethmoid region. This entire area, anterior to the orbits and surrounding the rostral rods, is packed with soft tissues and as such, the rostrum is soft and flexible. A single median rostral rod is slightly dorsal to the lateral rostral rods, which supports the tip of the nose and is sometimes exceedingly long (e.g., in the Rhinochimaeridae). The nasal capsules are bulbous and project laterally from the anteroventral portion of the ethmoid region. The chimaeroid face is shorter than that of most of the other chondrichthyans: the orbit is posterior to the ethmoid region, anterior to the brain, and dorsal to the paired olfactory tracts. The orbit is bounded ventrally by a suborbital ridge, anteriorly by the antorbital crest, and posteriorly by the postorbital. The left and right orbits are separated by a thin wall of connective tissue, which forms the interorbital septum. Posterior to the orbit in all chimaeroids is the otic region. The semicircular canals of the ear (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays) bulge on the dorsal surface of the chondrocranium. Posteriorly, the otic region extends to the prominent occipital crest.

Posterior to the chondrocranium is the chimaeroid synarcual. The synarcual is loosely articulated with the occipital region. The synarcual is a cartilaginous plate formed by the fusion of the first 10 vertebral segments. On the dorsal edge of the synarcual is an articulation surface for the basal cartilage of the first dorsal fin and the fin spine. The chimaeroid synarcual is not tightly associated with the suprascapular cartilage and the pectoral girdle, which is in contrast to the condition in batoids.

Visceral Arches

The suspension and morphology of the jaws of chimaeroids is unique among extant Chondrichthyes. Unlike the looser jaw suspensions of the elasmobranch fishes, in chimaeroids the palatoquadrate is fused with the chondrocranium and the hyoid is nonsuspensory (i.e., it does not form a movable suspensorium for the upper and lower jaws). In addition, whereas all other chondrichthyans replace their teeth, the broad, hypermineralized tooth plates of chimaeroids are permanent. The Meckel's cartilage (lower jaw) is fused at the symphysis, forming a single, U-shaped element.

Appendicular Skeleton

The pectoral girdle is positioned just posterior to the neurocranium. The paired halves of the pectoral girdle are fused at the symphysis. The ventralmost portion of the pectoral girdle is the coracoid region. The dorsal portion is the elongate scapular process. This distal portion of the scapular process points anterodorsally to lie lateral to the base of the chimaeroid synarcual. The pectoral fins are dibasal (with two basal cartilages) and articulate with the glenoid fossa on the posterior edge of the coracoid by way of the basal cartilages (propterygium and metapterygium). These pterygia support the radials of the pectoral fin. The pelvic fins lie along the ventral surface of the body. The single basipterygium of the pelvic fin is a flat ovoid cartilage. The anterior margin of the pectoral fin is defined by the basipterygial process, which is formed by the fusion of the proximalmost radials and the basipterygium. Additional radials articulate with the basipterygium and are situated parallel to the basipterygial process.

See also: **Aquaculture**: Physiology of Fish in Culture Environments. **Brain and Nervous System**: Functional Morphology of the Brains of Cartilaginous Fishes. **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics; Paired Fin Swimming; Undulatory Swimming. **Design and Physiology of the Heart** Cardiac Anatomy in Fishes. **Detection and Generation of Electric Signals**: Electric Organs. **Hearing and Lateral Line**: Auditory System Morphology; The Ear and Hearing in Sharks, Skates, and Rays. **Integrated Function and Control of the Gut:** Barrier Function of the Gut. **Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes; Morphology of the Olfactory (Smell) System in Fishes. **The Muscles**: Cartilaginous Fishes Cranial Muscles. **The Skeleton**: Cartilaginous Fish Skeletal Tissues.

Further Reading

Claeson KM (2008) Variation of the synarcual in the California Ray, *Raja inornata* (Elasmobranchii: Rajidae). *Acta Geologica Polonica* 58: 121–126.

Daniel JF (1934) *The Elasmobranch Fishes*. Berkeley, CA: University of California Press.

Dean MN and Motta PJ (2004) Anatomy and functional morphology of the feeding apparatus of the lesser electric ray, *Narcine brasiliensis* (Elasmobranchii: Batoidea). *Journal of Morphology* 262: 462–483.

Didier DA (1995) Phylogenetic systematics of extant chimaeroid fishes. *American Museum Novitates* 3119: 1–86.

Garman S (1913) *The Plagiostomia (Sharks, Skates, and Rays),* Memoirs of the Museum of Comparative Zoology at Harvard College. Cambridge, MA: Harvard University.

Goodrich ES (1958) *Studies on the Structure and Development of Vertebrates*. New York: Dover.

Holmgren N (1941) Studies on the head in fishes embryological, morphological, and phylogenetical researches. Part II: Comparative anatomy of the adult selachian skull with remarks on the dorsal fins in sharks. *Acta Zoologica (Stockholm)* 22: 1–100.

Kemp NE (1977) Banding pattern and fibrillogenesis of ceratotrichia in shark fins. *Journal of Morphology* 154: 187–203.

Liem KF, Bemis WE, Walter WF, Jr., and Grande L (2001) *Functional Anatomy of the Vertebrates: An Evolutionary Perspective*, 3rd edn. Orlando, FL: Harcourt College Publishers.

Ridewood WG (1921) On the calcification of the vertebral centra in sharks and rays. *Philosophical Transactions of the Royal Society of London, Series B* 210: 311–407.

Schaefer JT and Summers AP (2005) Batoid wing skeletal structure: Novel morphologies, mechanical implications, and phylogenetic patterns. *Journal of Morphology* 264: 298–313.

Cartilaginous Fish Skeletal Tissues

MN Dean, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Tissues: *No Bones about It...*
Further Reading

Glossary

Appositional growth Growth by accretion of tissue at the margins (contrast with interstitial growth).
Areolar calcification/cartilage A weblike form of calcified cartilage found only in the vertebral centra in elasmobranch fishes (cf. tessellated cartilage).
Cap vs. body (prismatic vs. globular calcified cartilage) The two forms of calcified cartilage proposed to comprise tesserae: the prismatic cap is dense and on the perichondral surface, whereas the globular body is found on the underside of tesserae and comprised of coalesced blobs of calcification.
Cellular vs. acellular bone Bone with and without cells, respectively. Acellular bone is much more common in fishes than in mammals.
Chondrocyte Cell type in cartilage that produces extracellular cartilage matrix.
Elastic cartilage Cartilage type found in our epiglottis and ear cartilage that has a high resilience due to its constituent elastin fibers.
Endoskeleton The skeletal system formed by all skeletal elements that are nondermal in developmental origin.
Exoskeleton External support structure of an organism; mineralized exoskeletons were common and extensive in extinct fishes.
Extant A species or clade, etc., that is still in existence.
Extinct A species or clade, etc., that is no longer in existence and typically studied only from fossil forms.
Extracellular matrix (ECM) The polysaccharide- and fiber-rich material that surrounds cells in animal tissues and provides structural support. In hyaline cartilage, the ECM is the glassy, gel-like material that makes up the bulk of the tissue.
Fibrocartilage An intermediate between hyaline cartilage and dense fibrous tissue found in the annulus fibrosus of our intervertebral disks. Fibrocartilage is rich in collagen bundles but has smaller amounts of extracellular matrix.
Hyaline cartilage The most widespread of the three primary cartilage types in mammals, has a comparatively low fibrous- and high matrix-content, and acts most notably as a bearing surface in joints.
Interstitial growth Growth from within a tissue, typically characteristic of nonrigid tissues (contrast with appositional growth).
Intertesseral fibers Collagenous fibers that connect adjacent tesserae.
Lacunae Small space/pit in mineralized/unmineralized matrix in which cartilage/bone cells lie. Lacunae in tesserae are connected by tiny canals called canaliculi.
Neoselachii A clade of cartilaginous fishes that includes extant sharks and batoids, but not chimaeroids.
Perichondral bone A collar of bony tissue that forms in the outer fibrous layer of cartilage (the perichondrium).
Skeletal element An individual portion in a cartilaginous skeleton, analogous to individual bones in the skeleton of a bony vertebrate.
Supernumerary tesseral layers Extra layers of tesserae beneath the subperichondal layer; common in large sharks and species that eat hard prey.
Tessellated cartilage Hyaline-like cartilage tiled (tessellated) on the outside with tesserae; a feature of the Elasmobranchii (cf. areolar cartilage).
Tesserae Mineralized and mostly hexagonal blocks, forming the outer crust of tessellated cartilage.

Introduction

Chondrichthyan fishes – the sharks, rays, and chimaeras – often are described as an evolutionary success story. The group evolved over 400 million years ago and although extant chondrichthyans are not as speciose as bony fishes, they have diverse morphologies and ecologies. The skeletons of chondrichthyans changed tremendously from the earliest-known Paleozoic species to today's extant species. One of the most significant changes is in the material that builds the skeleton: in contrast to extinct forms, all modern chondrichthyan fishes possess endoskeletons fashioned almost entirely of cartilage. This is so characteristic of the group that sharks, rays, and chimaeras are known as the Chondrichthyes or cartilaginous fishes.

Scientists have recognized the gross differences in skeletal material between the Chondrichthyes and other vertebrates for more than two centuries and yet we have made only short in-roads into answering the most basic questions surrounding the evolution and predominance of cartilage in the skeletons of these fishes. How does chondrichthyan cartilage differ from the cartilage of other vertebrates? How many types of cartilage are found in the Chondrichthyes? How do the skeletons of sharks, rays, and chimaeras differ? Are cartilaginous fish skeletons only cartilaginous? By first examining the skeletal materials (this article) and then how these are organized into general body plans (**see also The Skeleton**: Cartilaginous Fish Skeletal Anatomy), we consolidate what is currently known about what it means to have a cartilaginous skeleton and also pinpoint topics demanding further study. This article's focus on cartilage only is a reflection of our scant knowledge of other chondricthyan skeletal tissues; although ligaments and tendons are typically also considered skeletal tissues, little is known of these materials in cartilaginous fishes.

Tissues: *No Bones about It...*

The Chondrichthyes are considered cartilaginous fishes because the long endoskeletal elements called bones in other vertebrates are made of cartilage instead. We are familiar with the distinction between cartilage and bone if we have ever eaten chicken and wrestled with the gristle (cartilage) at the ends of the long bones. The textural difference between these two tissues and the comparatively small amount of cartilage in the adult chicken skeleton is characteristic of nonchondrichthyan vertebrates. Most vertebrates with bony skeletons begin life with uncalcified, comparatively soft cartilaginous skeletons, scaffolds on which their bony version will be patterned. Much of the skeletal cartilage in embryos is systematically invaded by blood vessels, calcified, and then degraded as it is replaced by bone (a process called endochondral ossification). When most vertebrates reach adulthood, endoskeletal uncalcified cartilage typically persists only as articular surfaces in joints and as contour fillers (e.g., in our noses and ears). In this respect, cartilaginous fishes are impressive at the very least for the huge amount of cartilage that persists in the adult skeleton.

A natural question at this point is whether the Chondrichthyes ever possessed bone in their skeletons, and the answer is yes. Early Paleozoic sharks possessed osteonal dentine and acellular bone in association with dorsal spines and other dermal or exoskeletal elements. (The exoskeletal distinction here is important because early chondrichthyans and many other fishes possessed extensive dermal armors that are almost completely absent from the variety of extant groups.) Prismatic cartilage (see below) was preceded by perichondral bone in the endoskeleton, but it appears that by the time the hallmarks of the extant elasmobranch skeleton arose ~400 million years ago, most of the bone in the skeleton was gone. Although many authors debate the presence of bone in the extant chondrichthyan endoskeleton, it is largely accepted that the base of chondrichthyan placoid scales and teeth is comprised of acellular bone. However, no cellular bone has ever been found in any chondrichthyan fish.

Another key distinction between chondrichthyan skeletal cartilage and that of other vertebrates is in the forms that the cartilage takes. Cartilage, in general, is a stiff, gel-like tissue formed by cartilage cells (chondrocytes). The chondrocytes secrete an extracellular matrix rich in proteoglycans (macromolecules with hydrophobic domains that ensure the turgidity of cartilage) and type II collagen, which acts as the tissue's tensile component. Compared with bone, cartilage has a much lower metabolic rate and essentially no blood supply, meaning that cartilage grows slowly and cannot repair itself. In mammals, several primary types of cartilage are recognized, for example, hyaline, elastic, and fibrocartilage. These represent a gradient in the type and proportion of fibrous tissue they contain. Whereas elasmobranch cartilage shares some features with mammalian cartilages, there are several distinct, clade-specific tissue morphologies.

Tessellated Cartilage

In elasmobranch fishes (sharks, rays, and relatives) the majority of skeletal elements are made mostly of an uncalcified, hyaline-like cartilage. The proteoglycan, collagen, and water levels in this cartilage are roughly comparable with mammalian cartilage, although relative proportions of these components vary with species. In all elements except the vertebral centra (**Figure 1** – central schematic), the inner uncalcified tissue is wrapped with an outer layer of mineralization, sandwiched between the uncalcified cartilage core and the fibrous perichondrium that wraps the skeletal elements (**Figures 1**(**a**) and **1**(**b**); although sometimes a layer of uncalcified cartilage is seen intervening between tesserae and perichondrium). Under a microscope (and even sometimes with the naked eye, especially in dried specimens), the mineralized layer looks like the tiled floor of a bathroom: the outer crust of each skeletal element is comprised of thousands of abutting, hexagonal, mineralized blocks called tesserae (**Figure 1**(**a**)). As a result of this distinct outer patterning, this composite of mineralized and unmineralized cartilage

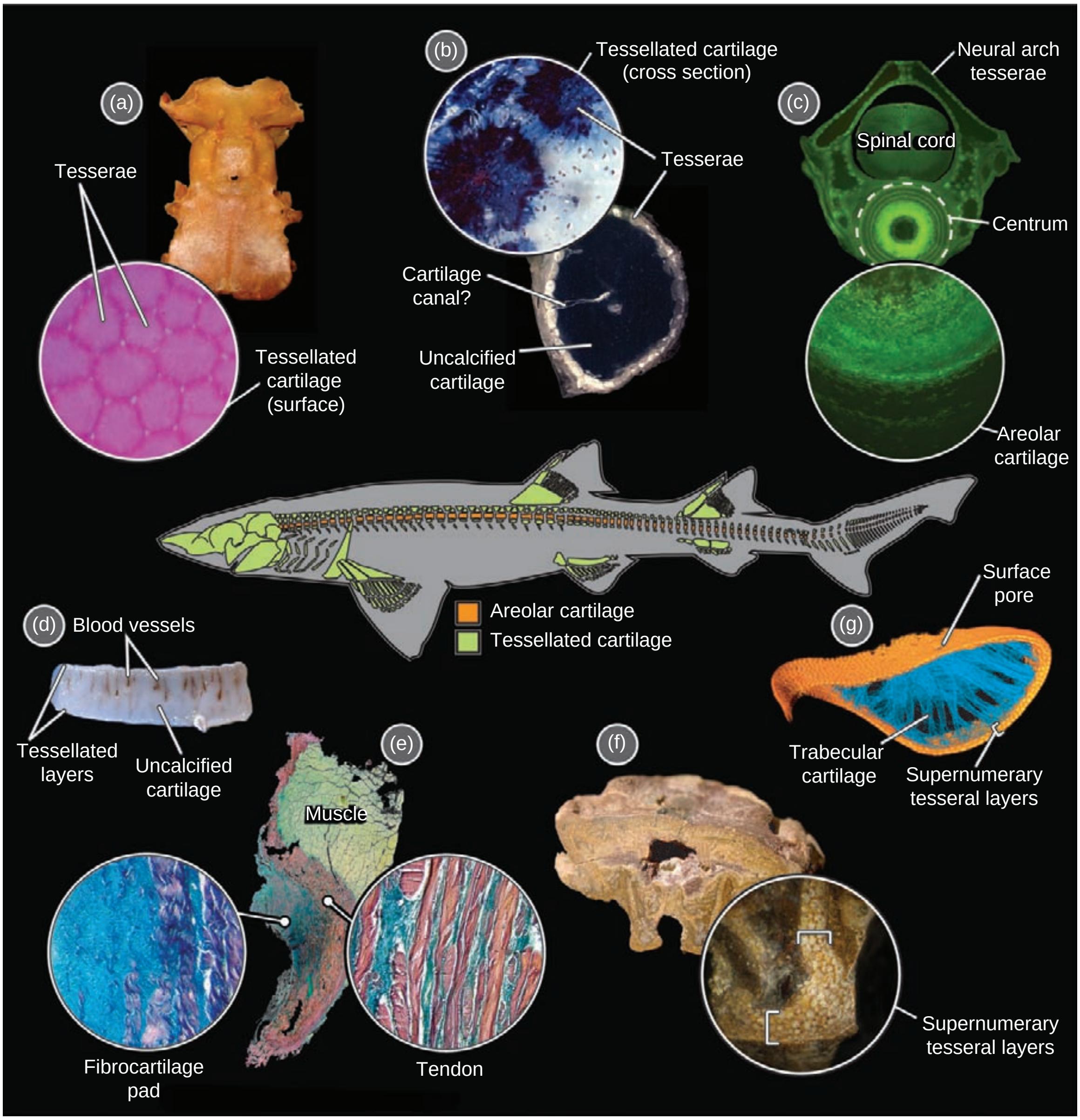


Figure 1 Cartilage types in the skeletons of elasmobranch fishes. The schematic skeleton in the center represents a generalized elasmobranch in lateral view; the images contained in circles are enlargements of the adjacent specimen. The majority of the skeleton is tessellated cartilage ((a) in surface view of a dried angel shark chondrocranium; (b) a cross section of a stingray pelvic girdle), comprised of uncalcified, hyaline-like cartilage tiled by mineralized blocks (tesserae). Areolar cartilage ((c) a cross section of a stingray vertebra, stained with OTC to label active mineral deposition) is found only in the vertebral centra, whereas all the surrounding cartilages are tessellated. Neural arch tesserae may exhibit some features similar to bony tissue. The tissues at the bottom of the figure are modifications of the two primary cartilage types and deserve further study. Although cartilage is considered avascular, some elasmobranch cartilages may exhibit intrinsic vascular tissue ((d) a cross section of a stingray pectoral girdle). Elasmobranch tendons may also be modified in areas of compression to form fibrocartilage ((e) stained stingray adductor muscle and tendon containing fibrocartilage pad). The tessellated layer may be thickened in some areas by supernumerary tesseral layers, especially in the chondrocranium ((f) cross section of a fossilized Paleozoic xenacanthid shark chondrocranium, brackets mark tessellated layers) and the jaws ((g) cross section of a volume-rendered microCT scan of a myliobatid stingray lower jaw). The jaws and other cartilages may also be reinforced by hollow, mineralized struts called trabeculae (g) colored in blue), oriented perpendicular to the tesseral mat. Photo in panel (b): Courtesy of Laura Macesic. All images in panel (e) are from Springer-Verlag; fossil specimen (AMNH7930). Photos in panel (f): Courtesy of the American Museum of Natural History.

is called tessellated (tiled) cartilage. Tessellated cartilage contains a large component of collagenous tissue as well. Each tessera is adjoined to its surrounding tiles by tufts of intertesseral fibers that merge with the overlying perichondrium. As mineralized tissues are typically patterned on fibrous networks, this mesh of collagen probably represents a part of the scaffold on which the whole of the mineralized tissue is organized, but this is yet to be demonstrated.

There are hardly any published data on elasmobranch skeletal material properties, so it is difficult to assess the functional advantages and disadvantages of cartilaginous versus bony skeletons. However, three-dimensional computer models of the jaws of whiteshark *(Carcharodon carcharias)*, modeled in both tessellated cartilage and bone (based on material property data for these tissues), suggest that performance (bite force) is not affected by a change in skeletal tissue. Moreover, although the models show that the cartilaginous jaws would exhibit more strain and deformation than the hypothetical bony ones, the tissue stress levels are considerably lower in tessellated cartilage.

Cells are distributed throughout all phases of the tissue, scattered throughout the unmineralized cartilage, linearly arrayed between the tufts of intertesseral fibers, and housed in mineralized lacunae (lacunar spaces) in the tesserae themselves. Until recently, it was unclear how the intratesseral chondrocytes entombed within tesserae were able to remain alive. High-resolution synchrotron X-ray imaging has revealed a network of tiny canaliculi that likely help distribute nutrients to cells. It is suspected that tesseral cells play a role in mineralization and tesseral growth; materials involved in mineralization may be somehow passed through the canalicular network to tesseral margins, which would explain how tesserae increase in size as animals age. It is important to note that unlike bone, tessellated cartilage cannot repair itself and, unlike mammalian calcified cartilage, elasmobranch calcified cartilage is apparently not ever resorbed. As a result, the mineral that is put into place is there to stay and tesserae can only grow appositionally (i.e., interstitial growth is not possible).

The mechanisms of initiation and regulation of the mineralization process in tessellated cartilage are entirely unknown, although there is evidence that uncalcified matrix proteoglycans are degraded and the enzyme alkaline phosphatase (which also is involved in mammalian cartilage mineralization) is upregulated in areas where tesserae are forming. It was suggested that tesserae are formed in two pieces: an outer cap portion made of very dense prismatic cartilage and an inner body formed by the coalescence of blobs of globular calcified cartilage. Based on these morphological differences and because the cap tends to stain red (like bone) and the body blue (like cartilage) when elasmobranch fishes are cleared and double stained, some authors posit that tesserae are actually blocks of calcified cartilage surmounted by a thin veneer of bone. This concept grew, in part, out of decades of fierce debate over whether elasmobranchs have retained any bone in their endoskeletons.

If this bi-partite tesserae theory is correct, we might expect to see the tiles organized around two different types of collagen, as bone and cartilage are characteristically patterned on type I and type II fibers, respectively. However, the few studies to attempt to type and label elasmobranch collagen have given conflicting results and the answer remains a mystery. These inconsistencies are likely due, in part, to researchers investigating cartilage from different regions of the skeleton; available evidence from numerous techniques suggests that tessellated cartilage in the neural arch of the backbone exhibits distinct structural differences from tessellated tissue elsewhere in the body and may contain a mineralized bone-like (type I-based) tissue (see below).

Immunohistochemical studies localizing and comparing elasmobranch collagens in vertebral and nonvertebral elasmobranch cartilage would be a very useful next step.

Areolar Cartilage

To the best of our knowledge, all calcified endoskeletal elements in elasmobranchs are tessellated with the exception of the centra in the vertebral column, which are comprised of areolar calcified cartilage (**Figure 1**(**c**)). The gross morphology of vertebrae will be considered in more detail in **The Skeleton**: Cartilaginous Fish Skeletal Anatomy – for now, think of the centrum as the central load-bearing part of each vertebra, like a section of the trunk of a tree. As the animal grows, each vertebra's centrum lengthens by adding material appositionally at both (rostral and caudal) ends. This fact has proved extremely convenient for researchers seeking to estimate the ages and growth rates of elasmobranch species: a sagittally sliced centrum reveals the animal's past history, like sedimentary layers in an archaeological dig (rings are visible in the centrum in **Figure 1**(**c**)). Remember that the calcified material in elasmobranch endoskeletons is never resorbed – the number of layers and the distance between them then provide clues to age, growth rate, water temperature, and even surrounding water chemistry.

Areolar cartilage is even less well described than tessellated cartilage. Despite the potential overlap in interests between morphological studies and aging studies (both consider vertebral micro-anatomy), no interdisciplinary study has investigated the ultrastructure of ontogenetic stages in areolar calcified cartilage or the mechanism by which layers are deposited. Morphological work shows areolar calcified cartilage – like the tesserae in tessellated cartilage – to be highly cellular, containing entombed cells in lacunae connected by short canalicular passageways. Cells are arranged concentrically about the centrum's center (where the notochord is found in developing animals; **see also**

The Skeleton: Cartilaginous Fish Skeletal Anatomy) creating laminated cellular rings, with each layer and the cells within layers separated by thin widths of calcified material. The persistent description of areolar cartilage as a web-like calcification likely arose from inorganic sample preparations that destroyed interstitial cells, leaving only this latticework of calcified tissue.

Areolar and tessellated cartilage are the primary cartilages found in the skeleton of elasmobranchs, but anecdotes and results from many studies provide evidence that there may be other types or subtypes. This would hardly be surprising given that vertebrates, and especially fishes, tend to exhibit many intermediate tissues that do not fit easily into the categories of cartilage and bone (see Hall's book for descriptions of the unappreciated variety of vertebrate skeletal tissues). Some options that warrant further organized investigation are as follows:

Neural Arch Cartilage

The cartilage of the neural arch (a skeletal arch on the dorsal side of vertebrae, arching over the spinal cord; **Figure 1**(**c**)) is tessellated, but apparently differs from that in other tessellated cartilage. Neural arch tesserae exhibit more bone-like characters such as tight packing of fibers with strong type I collagen immunoreactivity. Data combined from several sources suggest that neural arch tesserae grow faster than other tesserae; this seems to be the result of disproportionately fast perichondral mineralization in neural arch tesserae, probably related to the mineralization of the stout ligament that lines the spinal cord canal. We suspect that immunohistochemical and ultrastructural comparisons of tesserae from different regions of the body will reveal useful diagnostic differences.

Vascular Cartilage

Cartilage is described as essentially avascular because it contains considerably fewer vessels than bone, and yet cartilage often does contain some blood vessels (**Figure 1**(**d**)). In mammalian cartilage, these are contained in large vascular canals, hundreds of micrometers in diameter, which transport materials between perichondral regions and deep cartilage tissue. Analogous passages have been reported on in elasmobranchs, penetrating the areolar cartilage in vertebral centra and containing a variety of materials (chondrocytes, lymphatic and vascular tissues). Elasmobranch vascular canals are apparently similarly sized to those of mammals (up to 350 μm in diameter) and were found only in those shark species that attain a large maximum size; the small shark and two batoid species examined lacked canals. This phenomenon is likely widespread across cartilage types and species. Vascular canals have been observed in tessellated cartilage, extending beneath the teeth into the uncalcified cartilage of the lower jaw cartilage of blue sharks (*Prionace glauca*) and perforating the pectoral girdles of several batoid species (**Figure 1**(**d**); pers. obs.). Whereas elasmobranch cartilage canals likely play a similar role to those of mammals in nourishing and maintaining cartilage, their association with deep uncalcified cartilage suggests that, unlike mammalian canals, they do not help to mediate mineralization.

Fibrocartilage

The demonstration in cownose rays (*Rhinoptera bonasus*) of a tissue very similar to mammalian fibrocartilage is a further suggestion that elasmobranch skeletal tissue is more varied and plastic than previously believed (**Figure 1**(**e**)). The tissue is found in a tendon that serves to transmit forces between the upper and lower jaw portions of a jaw-closing muscle, which generates the crushing forces needed for eating hard-shelled organisms such as mollusks. Like all tendons, this tissue is loaded in tension, but where it wraps around a calcified process on the lower jaw, it is also subject to compressive forces. In this area, the tendon is modified to contain an uncalcified cartilaginous component that acts to manage the compressive portion of the tendon's load (**Figure 1**(**e**)). These likely represent a common elasmobranch tendon modification in areas of complex loading as cartilaginous plugs have also been observed in the tendons and ligaments connecting the hyomandibula and jaws in batoid species (e.g., those associated with jaw protrusion in the lesser electric ray, *Narcine bancroftii*).

Tessellated Cartilage Reinforcements

Tessellated cartilage can be reinforced in at least two ways: by adding additional layers of tesserae (supernumerary tesseral layers; **Figures 1**(**f**) and **1**(**g**)) or by adding struts (trabeculae; **Figure 1**(**g**)). Unlike maximum tesseral size, which is not associated with animal size – an adult sevengill shark *Notorhynchus* may weigh 100 times more than an adult round stingray *Urobatis*, but both exhibit tesserae up to half a millimeter in width, larger than those of an adult great white shark *Carcharodon* – additional tesseral layers in flat regions of the skeleton are more common in larger species, with some of the biggest sharks exhibiting up to four layers of tesserae. This has also been observed in the cranial structures of extinct sharks and rays. However, we should note that some large coldwater sharks (e.g., sleeper sharks *Somniosus*, sixgill sharks *Hexanchus*; pers. obs.; J. Maisey, personal communication) seem to exhibit the opposite condition; in these species the jaws and crania appear almost entirely unmineralized with only patches of tessellation (e.g., at the basal plate of the chondrocranium in *Hexanchus*). Supernumerary tesseral layers are also found in the jaws of hard prey specialists, such as myliobatid stingrays, supporting the notion that these layers provide mechanical support. In addition to these reinforcements in broad flat areas of the skeleton, the tesseral layer can also exhibit more localized thickening in curved regions, especially those with small radii of curvature (e.g., at the caudal and ventral edges of the jaws). This has been observed in species of all sizes and may represent a general structural constraint of tiling a curved surface.

The second type of tessellated cartilage reinforcement, trabecular cartilage, was first observed in the jaws of myliobatid stingrays, supporting the tissue directly beneath the flat tooth plates that are used to crush mollusks (**Figure 1**(**g**)). Trabecular cartilage is characterized by hollow, apparently tessellated struts (trabeculae) that extend from one tesseral surface through the uncalcified cartilage lumen of the jaws to the opposite tesseral surface. Trabeculae are open at their ends, creating obvious pores in the tesseral mat (**Figure 1**(**g**)); whether these were derived from cartilage canals (see above) or perhaps the intertesseral pores that have been observed in nonmyliobatid stingrays remains to be determined. Trabeculae have also been observed in the visceral arches of *N. bancroftii* – a species of electric ray that excavates buried prey with its jaws – and of some sharks (J. Ramsay, personal communication). The prevalence of trabecular cartilages suggests this type of reinforcement either evolved multiple times or is a plastic response to functional demands on the skeleton, although the latter was shown not to be the case for cownose rays.

It should be reiterated that the types of calcification mentioned above have been described or noted only in elasmobranchs. By comparison, almost nothing is known of the morphology or formation of calcified endoskeletal material in chimaeroids, sister group to the sharks and rays. As far as has been reported, holocephalans (chimaeroids) are the only nontessellated group among the extant Chondrichthyes. One study describes chimaeroid tissue mineralization as 'continuous cartilage calcification' comprised of smooth surface sheets of mineral, but this has, to the best of our knowledge, never been figured in a published article. It is reasonable, however, to assume that the endoskeletons of chimaeroids are much less mineralized than those of elasmobranchs: in CT scans of several species, the only cartilages that appear reinforced are those associated with the jaw joint, the tooth plates, and the occipital joint. Examinations of chimaeroid mineralization and skeletal reinforcement would provide integral knowledge to understand the evolution of chondrichthyan skeletons.

See also: **Aquaculture**: Physiology of Fish in Culture Environments. **Brain and Nervous System**: Functional Morphology of the Brains of Cartilaginous Fishes. **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics; Paired Fin Swimming; Undulatory Swimming. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes. **Detection and Generation of Electric Signals**: Electric Organs. **Hearing and Lateral Line**: Auditory System Morphology; The Ear and Hearing in Sharks, Skates, and Rays. **Integrated Function and Control of the Gut**: Barrier Function of the Gut. **Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes; Morphology of the Olfactory (Smell) System in Fishes. **The Muscles**: Cartilaginous Fishes Cranial Muscles. **The Skeleton**: Cartilaginous Fish Skeletal Anatomy.

Further Reading

Applegate SP (1967) A survey of shark hard parts. In: Gilbert PW, Mathewson RF, and Rall DP (eds.) *Sharks, Skates and Rays*, pp. 37–66. Baltimore, MD: Johns Hopkins Press.

Ashhurst DE (2004) The cartilaginous skeleton of an elasmobranch fish does not heal. *Matrix Biology* 23: 15–22.

Bordat C (1988) Les cartilages calcifiés de la petite roussette (*Scyliorhinus canicula* L., Chondrichthyens): Histologie et ultrastructure. *Canadian Journal of Zoology – Revue Canadienne De Zoologie* 66: 1432–1445.

Coates MI, Sequeira SEK, Sansom IJ, and Smith MM (1998) Spines and tissues of ancient sharks. *Nature* 396: 729–730.

Clement JG (1992) Re-examination of the fine structure of endoskeletal mineralization in Chondrichthyes: Implications for growth, ageing and calcium homeostasis. *Australian Journal of Marine and Freshwater Research* 43: 157–181.

Dean MN, Hale LF, Mull CG, Gorb SN, and Summers AP (2009) Ontogeny of the tessellated skeleton: Insight from the skeletal growth of the round stingray *Urobatis halleri. Journal of Anatomy* 215: 227–239.

Dean MN and Summers AP (2006) Cartilage in the skeleton of cartilaginous fishes. *Zoology* 109: 164–168.

Dingerkus G, Séret B, and Guilbert E (1991) Multiple prismatic calcium phosphate layers in the jaws of present-day sharks (Chondrichthyes; Selachii). *Experientia* 47: 38–40.

Donoghue PC and Sansom I (2002) Origin and early evolution of vertebrate skeletonization. *Microscopy Research and Technique* 59: 352–372.

Eames BF, Allen N, Young J, *et al.* (2007) Skeletogenesis in the swell shark *Cephaloscyllium ventriosum. Journal of Anatomy* 210: 542–554.

Egerbacher M, Helmreich M, Mayrhofer E, and Böck P (2006) Mineralisation of the hyaline cartilage in the small-spotted dogfish *Scyliorhinus canicula* L. *Scripta Medica (BRNO)* 79: 199–212.

Gelsleichter JJ, Musick JA, and van Veld P (1995) Proteoglycans from the vertebral cartilage of the clearnose skate, *Raja eglanteria* – inhibition of hydroxyapatite formation. *Fish Physiology and Biochemistry* 14: 247–251.

Hall BK (2005) *Bones and Cartilage: Developmental Skeletal Biology*. London: Elsevier.

Janvier P and Arsenault M (2002) Palaeobiology – calcification of early vertebrate cartilage. *Nature* 417: 609–1609.

Kemp NE and Westrin SK (1979) Ultrastructure of calcified cartilage in the endoskeletal tesserae of sharks. *Journal of Morphology* 160: 75–102.

Moss SA (1977) Skeletal tissues in sharks. *American Zoologist* 17: 335–342.

Ørvig T (1951) Histologic studies of placoderms and fossil elasmobranchs. I: The endoskeleton, with remarks on the hard tissues of lower vertebrates in general. *Arkiv för Zoologi* 2: 321–454.

Porter ME, Beltrán JL, Koob TJ, and Summers AP (2006) Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes). *Journal of Experimental Biology* 209: 2920–2928.

Porter ME, Koob TJ, and Summers AP (2007) The contribution of mineral to the material properties of vertebral cartilage from the smooth-hound shark *Mustelus californicus. Journal of Experimental Biology* 210: 3319–3327.

Summers AP (2000) Stiffening the stingray skeleton – an investigation of durophagy in myliobatid stingrays (Chondrichthyes, Batoidea, Myliobatidae). *Journal of Morphology* 243: 113–126.

Bony Fish Skeleton

EJ Hilton, Virginia Institute of Marine Science, Gloucester Point, VA, USA

© 2011 Elsevier Inc. All rights reserved.

A Hierarchical Approach to the Study of the Skeleton
Cranial Axial Skeleton
Postcranial Axial Skeleton
Appendicular Skeleton
Conclusion
Further Reading

Glossary

Chondral bone Bone that ossifies in association with a cartilage, through either ossification on the surface of a cartilaginous precursor (perichondral bone), as an ossification of the cartilaginous precursor (endochondral bone), or a combination of the two.

Compound bone A bone that is formed by two or more histologically distinct tissues, often the result of fusion during ontogeny.

Dermal bone Bone that ossifies in contact with the dermis.

Durophagy Eating hard-shelled organisms such as coral or mollusks.

Endoskeleton The skeletal system formed by all skeletal elements that are nondermal in developmental origin.

Exoskeleton External support structure of an organism formed by all skeletal elements developed from connection to the dermis, including all dermal bones, teeth, scales, and fin rays; mineralized exoskeletons were common and extensive in extinct fishes.

Membrane bone Bone that ossifies directly within ligaments or other tissue not in contact with the dermis and separate from cartilage.

The skeleton of bony fishes presents a seemingly endless diversity of forms that reflect the varied life history strategies of these organisms. The skeleton provides the architectural support for musculature, while at the same time providing protection for internal organs and sensory systems. The skeleton of bony fishes is composed of hundreds of individual bony and cartilaginous elements that can vary in form, reflective of the function of each element in an organism. A solid understanding of the skeleton and its diversity, therefore, is critical for studies of functional morphology and ecomorphology. In addition, because of the complex nature of the skeleton of bony fishes, it is rich in phylogenetic data and has been fundamental in the formulation of many compelling hypotheses regarding the evolutionary biology of fishes.

This article provides a brief overview of the skeletal structure of actinopterygians and fish-like sarcopterygians. By working from illustrations of three taxa (bowfin, *Amia calva*; goldeye, *Hiodon alosoides*; and Japanese jack mackerel, *Trachurus japonicus*), some of the osteological diversity of bony fishes generally is highlighted. These three taxa represent three phylogenetic levels (i.e., *Amia* is a nonteleostean actinopterygian, *Hiodon* is a basal teleost, and *Trachurus*, an acanthopterygian, is a derived teleost). Although these three taxa serve as exemplars for various phylogenetic levels, as well as different ecological guilds (*Amia* and *Hiodon* as temperate freshwater fishes and *Trachurus* as a temperate/tropical marine form), it must be kept in mind that these are only three out of more than 30 000 species and do not capture the range of variation found in the diversity of bony fishes.

A Hierarchical Approach to the Study of the Skeleton

Because of the complex nature of the skeleton of bony fishes, there are many ways in which one can approach the study of the skeleton. For instance, one can study the exoskeleton as distinct from the endoskeleton. Both the endoskeleton and exoskeleton can be further broken down into various parts, based on variation in the details of their development, for example. The approach adopted here is hierarchical and combines dividing the skeleton into topographic regions and functional units. The primary division of the skeleton is the axial and appendicular regions. Both of these are further divided into regional units (e.g., cranial vs. postcranial portions of the axial skeleton), which themselves are divided into more refined functional units. Other terminology exists for portions of the skull, including the chondrocranium or neurocranium (roughly equivalent to braincase as used here) and the dermatocranium (all dermal bones of the skull, including the skull roof, infraorbital bones, jaws, and the opercular series). However, the dermatocranium comprises elements from many different regions of the skull and is therefore somewhat unwieldy to discuss in the context of functional units. Another complicating factor is that the terminology for individual skeletal elements has changed dramatically over time and there are many synonyms for several elements that are in current use.

Cranial Axial Skeleton

Four major functional units form the skull of bony fishes (**Figure 1**): the braincase, skull roof and infraorbital series, opercular series, and splanchnocranium. Although these are recognizable as discrete units of the skull, the distinction between some of them may be obscured, particularly in derived representatives (e.g., the bones of the braincase and skull roof in teleostean fishes).

As the name suggests, the braincase of bony fishes (**Figure 2**) is the box surrounding the brain and sense organs of the head. The braincase may be divided into four regions (anterior to posterior): the ethmoid, orbital, otic and occipital regions. Early in ontogeny the braincase is entirely cartilaginous, but in most bony fishes the braincase becomes well ossified in the adult (exceptions may include some deep-sea fishes, in which the bony skeleton is reduced generally, and highly pedomorphic taxa, such as the gobioid *Schindleria*). In basal sarcopterygians (e.g., coelacanths), there is an intracranial joint between the ethmosphenoid and the otico-occipital units. Virtually, all of the bones of the braincase are chondral, with the exception of the parasphenoid and vomer, two dermal bones that are tightly associated with the ventral surface of the braincase, and the intercalar, which is a membrane bone in extant taxa. Both the vomer and parasphenoid are primitively toothed bones, although only nonteleostean actinopterygians, osteoglossomorph and elopomorph teleosts, and a few other more derived teleosts have parasphenoid dentition (i.e., in most actinopterygians the parasphenoid is edentulous). In most basal actinopterygians and some basal teleosts, the vomer is a paired element (e.g., in *Hiodon*, during early ontogeny the vomer is paired but is fused to form a median element in the adult, **Figure 2(d)**). The parasphenoid is often the largest bone of the braincase, covering much of its ventral surface. The parasphenoid bears lateral projections (the so-called ascending rami) that often tightly embrace the braincase posterior to the orbit.

The ethmoid region forms the bones and cartilages that surround the nasal capsules. The ethmoid region also forms the articulation point for the anterior portions of the splanchnocranium (in particular the upper jaws and the anterior portions of the palatoquadrate). In lungfishes, the palatoquadrate (forming the upper jaw) is fused to the ethmoid region, forming the so-called autostylic condition of jaw suspension that is reflective of durophagy (other taxa, including holocephalans and tetrapods independently evolved autostyly). The nasal capsule is typically a large concavity in the lateral surface of the ethmoid region that supports the nasal lamellae. The largest bone of the ethmoid region is typically the mesethmoid, which forms in the cartilage separating the left and right nasal capsules. In basal actinopterygians and sarcopterygians, there are sometimes dermal bones that lie anterior or dorsal to the mesethmoid. For instance, the rostral bone forms in the anterior midline of the snout in taxa such as *Polypterus* (bichirs), *Amia*, and the basal teleost genus *Elops* (ladyfishes), and this bone supports the anteriormost portion of the cephalic sensory canal system. In other basal teleosts, there is another dermal bone, the supraethmoid, which develops dorsal to the mesethmoid and does not contain any part of the sensory canal; these bones may fuse to form a compound element (e.g., *Hiodon*). Ventral to, and often tightly sutured to, the mesethmoid is the vomer. Anteriorly, the mesethmoid and (sometimes) the vomer form the articulation surface for the upper jaws. Forming the posterior wall of the nasal capsule and the articulation surface for the palatoquadrate is the lateral ethmoid bone. In nonteleostean actinopterygians, there is a second more anterior pair of ethmoid bones, the preethmoids (e.g., in *Amia*).

The orbital region (which is associated with the eye) is sometimes also called the sphenoid region, in reference to the sphenoid bone of humans, which lies posterior to the ethmoid region and is associated with the orbit. The dominant bone of this region in relatively basal taxa is the orbitosphenoid, which ossifies in the cartilage that forms the septum between the eyes. This bone is paired in basal actinopterygians (e.g., sturgeons, *Amia*), but may be median in the adult (e.g., basal teleosts) or absent (most acanthopterygians, which lack a bony or cartilaginous septum). The other elements that may be considered part of the orbital region include the pterosphenoid, sphenotic, and basisphenoid, all of which are positioned between the orbitosphenoid (if present) and the more posterior otic region. As is the case with the orbitosphenoid, these bones may be lost as independent elements or altogether, particularly in more derived groups of bony fishes.

The otic region is formed by the bones that are in contact with the semicircular canals and ampullae of the inner ear. This region also forms much of the lateral wall of the braincase posterior to the orbit. The largest bone of the otic region typically is the prootic, which forms in association with the foramen or foramina for portions of the trigeminal and facial cranial nerves, in some taxa forming the so-called trigeminofascialis chamber. The pterotic lies dorsal to the prootic, and in most teleosts is

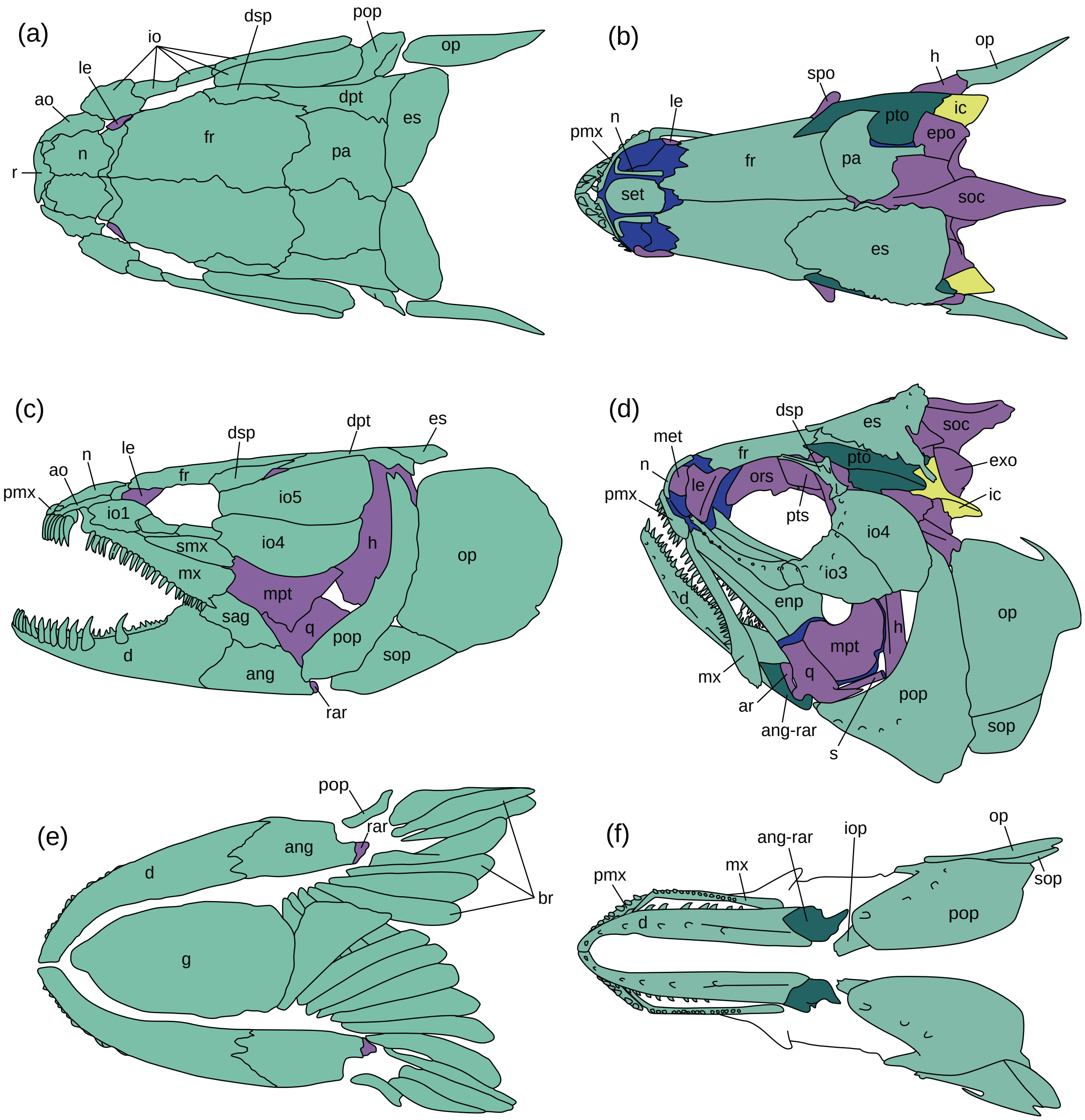


Figure 1 Skulls of *Amia* (a, c, e) and *Hiodon* (b, d, f) in dorsal (a, b), lateral (c, d), and ventral (e, f) views. Cartilage is shown in dark blue, chondral bone in purple, dermal bone in light green, compound bone in dark green, and membrane bone in yellow. Abbreviations: ang, angular; ang-rar, anguloretroarticular; ao, antorbital; ar, articular; br, branchiostegal; dpt, dermopterotic; dsp, dermosphenotic; enp, endopterygoid; epo, epioccipital; es, extrascapular; fr, frontal; g, gular; h, hyomandibula; ic, intercalar; io, infraorbital; iop, interopercle; le, lateral ethmoid; met, mesethmoid; mpt, metapterygoid; mx, maxilla; n, nasal; op, opercle; ors, orbitosphenoid; pa, parietal; pmx, premaxilla; pop, preopercle; pto, pterotic; q, quadrate; r, rostral; rar, retroarticular; s, symplectic; sag, surangular; set, supraethmoid; smx, supramaxilla; soc, supraoccipital; sop, subopercle. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; and Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142.

incorporated into the surface of the skull roof. In basal actinopterygians there is an autogenous dermopterotic that is a true dermal skull roofing bone that carries a sensory canal, and is separate from the chondral autopterotic. In teleosts, the autopterotic and dermopterotic are fused to form a compound bone, which is often called the pterotic. In at least some taxa, these two bones are never seen as separate elements during ontogeny, and it is often held as an example of so-called phylogenetic fusion, in which plesiomorphically separate elements form a single

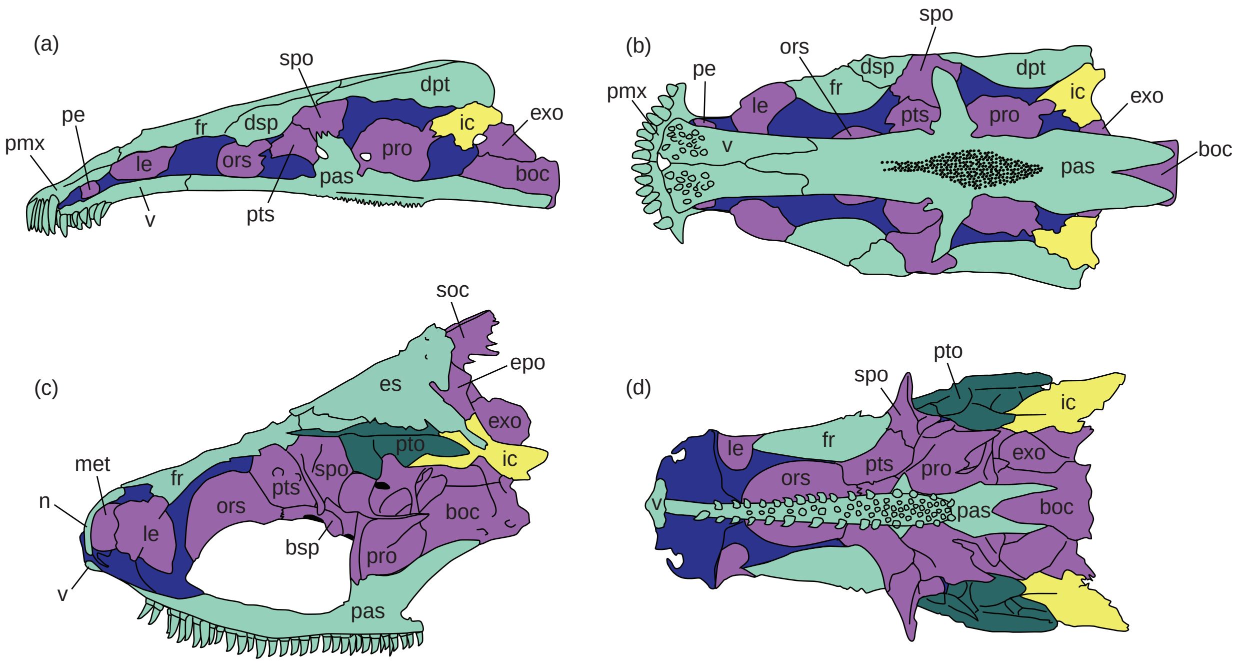


Figure 2 Bones of the skull roof and braincase of *Amia* (a, b) and *Hiodon* (c, d) in lateral (a, c) and ventral (b, d) views. Cartilage is shown in dark blue, chondral bone in purple, dermal bone in light green, compound bone in dark green, and membrane bone in yellow. Abbreviations: boc, basioccipital; bsp, basisphenoid; dpt, dermopterotic; dsp, dermosphenotic; epo, epioccipital; es, extrascapular; exo, exoccipital; fr, frontal; ic, intercalar; le, lateral ethmoid; met, mesethmoid; n, nasal; ors, orbitosphenoid; pas, parasphenoid; pe, preethmoid; pmx, premaxilla; pro, prootic; pto, pterotic; pts, pterosphenoid; soc, supraoccipital; spo, sphenotic; v, vomer. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690 and Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142.

element in derived taxa (this hypothesis however can rarely, if ever, be distinguished from phylogenetic loss).

The bones that interface with the postcranial axial skeleton and its associated musculature comprise the occipital region of the braincase. The median basioccipital lies in the ventral midline of the occipital region. In basal forms, the basioccipital is posteriorly concave, and often resembles a vertebral centrum. This overall similarity in form led to the idea that the occipital region of actinopterygians incorporated the first vertebral centrum into its structure. However, there is little structural or ontogenetic evidence that a centrum is incorporated into the skull in most actinopterygians. A notable exception is in adult *Amia*, in which two centra are fused to the basioccipital during ontogeny. In some teleosts (e.g., *Hiodon*), in particular the acanthomorphs, the articulation between the skull and vertebral column is formed by the basioccipital and the exoccipitals, a pair of bones that form on either side of the foramen magnum. In lungfishes, the exoccipital is the only chondral bone of the braincase. The exoccipital can be readily identified across taxa because it typically contains or defines the foramen for the vagal nerve. Dorsolateral to the exoccipital are two paired bones, the epioccipital and the intercalar. The epioccipital is often called the epiotic because it houses the posterior semicircular canal of the inner ear. However, it has been shown that at least in extant teleosts, this bone forms in association with cartilage derived from the occipital arch of early developmental stages. Nonteleostean actinopterygians (e.g., sturgeon, gars, and *Amia*) do, however, have an epiotic bone. The intercalar is found ventral to the epioccipital (in teleosts) or epiotic (in *Amia*) and in older literature this bone is termed the opisthotic. In *Amia* and extant teleosts, the intercalar is composed only of membrane bone. The remaining bone of the occipital region in teleostean fishes is the supraoccipital (not present in nonteleostean actinopterygians or sarcopterygians). This chondral bone forms in the posterior midline on the dorsal surface of the braincase and may form part of the skull roof. The supraoccipital often bears a median crest that extends posteriorly.

The skull roof of bony fishes is formed by those bones visible in dorsal view of the skull. By this definition bones such as the supraoccipital and the mesethmoid would be considered as part of the skull roof. Plesiomorphically, the skull roof comprises only those dermal bones that form the dorsal surface of the skull, and that cover the cartilage and chondral bones of the braincase. There is a trend across the evolution of fishes toward the sinking of the skull roofing bones (i.e., in most basal taxa the bones of the skull roof are at the surface and are covered by only a thin layer of skin, whereas the bones are deeper and covered by muscle and skin in more derived fishes). In virtually all actinopterygian fishes, these dermal bones include (anterior to posterior) the nasals (which may be reduced to mere tubes surrounding the sensory canal), the frontals, the parietals, and the extrascapulars (as with the nasals, these may be little more than tubular bones). The frontal typically is the largest of the skull roofing bones, followed by the parietal. In fish-like sarcopterygian fishes, there is an additional pair of bones, which has, in part, led to controversy regarding the identity of the skull roofing bones between actinopterygians and sarcopterygians. In sarcopterygians, there are paired frontals, parietals, and postparietals. Based on the pattern and association of the bones to the sensory canals and their topographic position (relative to the pineal organ), it has been proposed that the bones commonly termed (and which is followed here) the frontals and parietals in actinopterygians are homologous to the parietals and postparietals, respectively, of sarcopterygians. A change of terminology for actinopterygians has been suggested but has not yet been widely adopted.

The infraorbital series of bones (=circumorbital bones) is associated with the skull roof by housing the infraorbital sensory canal, a ventral branch from the supratemporal sensory canal (contained in the pterotic). The infraorbital bones define the orbit and are numbered sequentially (anterior to posterior), although certain elements of the series are given particular names (e.g., infraorbital 1, which is often enlarged, is called the lachrymal, and the posteriormost infraorbital, which contains the branching between the infraorbital canal and the dorsal supraorbital canal, is called the dermosphenotic). In addition, the elements that are positioned under the eye are sometimes termed the suborbitals and those posterior to the eye are the postorbitals. Basal actinopterygians also have an antorbital bone, which lies anterior to the orbit. There is variably a supraorbital bone, which lies dorsal to the orbit, although this bone does not generally have a connection to the sensory canal system.

The opercular series forms the bones of the gill-cover and functions in both feeding and respiration. In virtually all bony fishes, the largest bone of the opercular series is the opercle, which is typically also the dorsal-most of the series. The only exception is in extant sturgeons and paddlefishes in which the opercle is lost and the large bone forming the support for the gill cover is the subopercle (the opercle is present in close fossil relatives, but is greatly reduced in size compared to the subopercle). Typically, the subopercle is smaller and positioned ventral to the opercle, and bridges the gap between the opercle and the branchiostegals, which form the supports for the ventral portion of the opercular flap. In basal actinopterygians, including the basal teleosts of the family Elopidae (ladyfishes and tarpons), there is a gular plate, which forms between the left and right lower jaws. This gular is medial in most actinopterygians in which it is present, but is paired in *Polypterus* and coelacanths. The interopercle develops in the interoperculomandibular ligament and is found only in *Amia* and teleosts among extant fishes. This bone, which forms a linkage between the opercular series and the lower jaw, is important for transmission of force during jaw opening. The preopercle carries the dorsal cephalic sensory canal to the lower jaw. In sarcopterygians (e.g., coelacanths), there are also other bones of the cheek that carry sensory canals (e.g., the squamosal).

The splanchnocranium includes all the bones and cartilages of the visceral arches, which include the mandibular, hyoid, and branchial arches (oral jaws, hyoid, and gill arches, respectively) as well as the palatoquadrate (the roof of the mouth). Therefore, although they are all part of an integrated unit, they are all treated as distinct portions of this region of the skull. The oral jaws (upper and lower) most frequently bear teeth, although entire orders of fishes (e.g., Cypriniformes) may be edentulous. The form of the teeth, as that of the bones of the jaws themselves, reflects the ecology and diet of fishes (e.g., piscivorous fishes often have sharply pointed teeth set in relatively narrow jaws, whereas durophagous fishes have more robust jaws and often have molariform teeth). The upper jaw (**Figures 1** and **3**) is

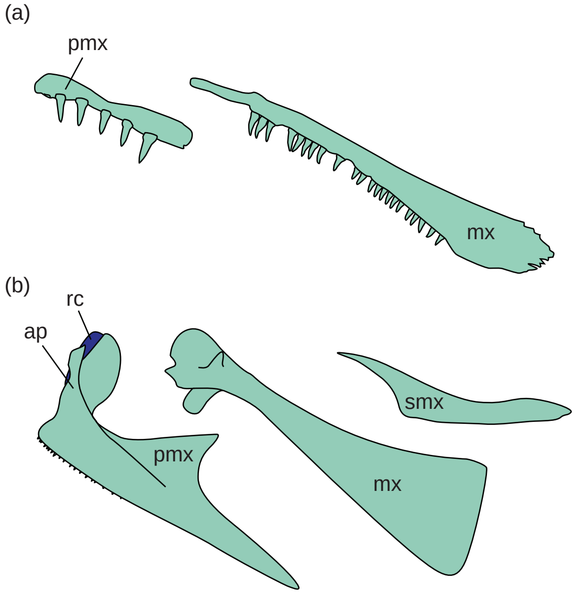


Figure 3 Bones of the upper jaw of *Hiodon* (a) and *Trachurus* (b) in lateral view. Cartilage is shown in dark blue and dermal bone in light green. Abbreviations: ap, ascending process; mx, maxilla; pmx, premaxilla; rc, rostral cartilage; smx, supramaxilla. Modified from Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142 and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

formed by a series of bones: the premaxilla, maxilla, and supramaxilla (there are sometimes more than one supramaxillae positioned along the posterodorsal margin of the maxilla). The premaxilla is the anteriormost of the series and articulates with the ethmoid region of the braincase. In basal forms (e.g., nonteleostean actinopterygians and basal teleosts), the premaxilla is rigidly articulated with the braincase and is relatively small in comparison to the maxilla. In these basal forms, the maxilla is the main tooth-bearing bone of the upper jaw, while the premaxilla is restricted to the anterior margin of the jaw. Also in many early actinopterygians (e.g., *Polypterus*, palaeoniscoids) and sarcopterygians, the maxilla is firmly attached to the other bones of the cheek. In other nonteleostean actinopterygians and basal teleosts, the maxilla is freed from the cheek and rotates forward during feeding, forming a mouth that is more round in cross section. Across the evolution of the teleosts, the premaxilla becomes the dominant bone of the upper jaw, and the maxilla becomes an edentulous bone that is excluded from the margin of the jaw. With the premaxilla becoming as the primary bone of the upper jaw, exemplified by Acanthomorpha, also comes the development of a large ascending process of the premaxilla. The left and right premaxillae meet in the midline of the jaw, and the ascending processes surround the rostral cartilage, a median cartilage that articulates with the ethmoid region of the braincase and serves to support the upper jaw during jaw protrusion. In fact, all of the modifications of the upper jaw seen across the evolution of teleosts can be correlated to the development of jaw protrusion, which is extremely well developed in acanthomorph teleosts.

The lower jaw of bony fishes is a complex of dermal, chondral, compound and membrane-bone elements (**Figures 1** and **4**). The chondral bones include ossifications of Meckel's cartilage, which runs along much of the length of the jaw; in some taxa, it is regressed in adults. These chondral bones include the mentomeckelian, near the anterior tip of the jaw, and the articular and retroarticular bones at the posterior end of the jaw (there is typically a single articular; *Amia* is unique in having two articular bones). The articular forms the articulation surface for the lower jaw with the quadrate, and the retroarticular serves as the attachment site for the interoperculomandibular ligament, which contributes to the jaw opening mechanism. On the dorsal surface of Meckel's cartilage is the coronomeckelian, which is a membrane bone that forms in the tendon of the adductor mandibulae muscle where it attaches to the lower jaw. Surrounding the chondral portions of the lower jaw is a series of dermal elements. Two of these, the angular (posterior) and the dentary (anterior), are sensory canal-bearing bones. In most extant teleosts, the angular is fused to either the articular or the retroarticular bone. In most extant fishes, the dentary is the only tooth-bearing bone and is typically the largest bone of the lower jaw. In nonteleostean fishes, there are additional dermal bones, which include the surangular on the external surface, the prearticular on the internal surface (covering much of the posterior region of Meckel's cartilage), and a series of coronoid bones, which are small tooth-bearing elements. Therefore, there is a general trend toward loss of bones in the lower jaw across actinopterygian fishes.

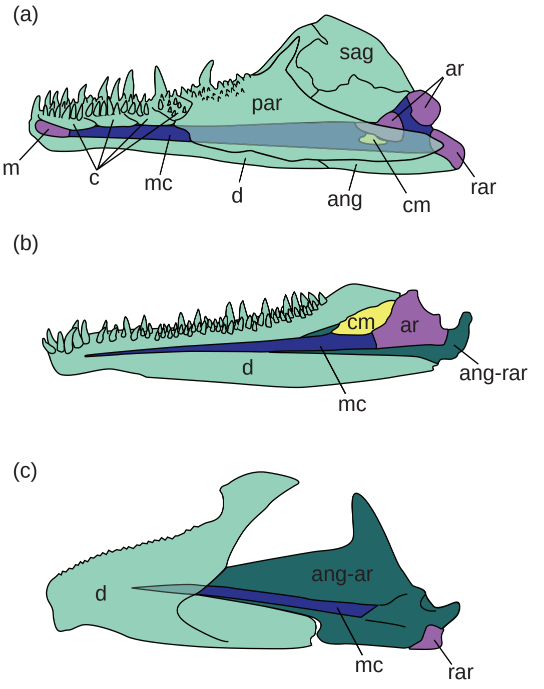


Figure 4 Lower jaw of *Amia* (a), *Hiodon* (b), and *Trachurus* (c) in medial view. Cartilage is shown in dark blue, chondral bone in purple, dermal bone in light green, compound bone in dark green, and membrane bone in yellow. Abbreviations: ang, angular; ang-ar, anguloarticular; ang-rar, anguloretroarticular; ar, articular; c, coronoid; cm, coronomeckelian; d, dentary; m, mentomeckelian; mc, Meckel's cartilage; par, prearticular; rar, retroarticular; sag, surangular. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142; and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

The dorsal portions of the hyoid arch (i.e., the hyosymplectic cartilage and its ossifications, the hyomandibila and the symplectic), together with the palatoquadrate and its ossifications (palatine, ectopterygoid, endopterygoid, metapterygoid, and quadrate), form the suspensorium or the roof of the mouth (**Figure 5**). The palatine, ectopterygoid, and endopterygoid may bear teeth, particularly in relatively basal forms. In nonteleostean and basal teleostean actinopterygians, there are

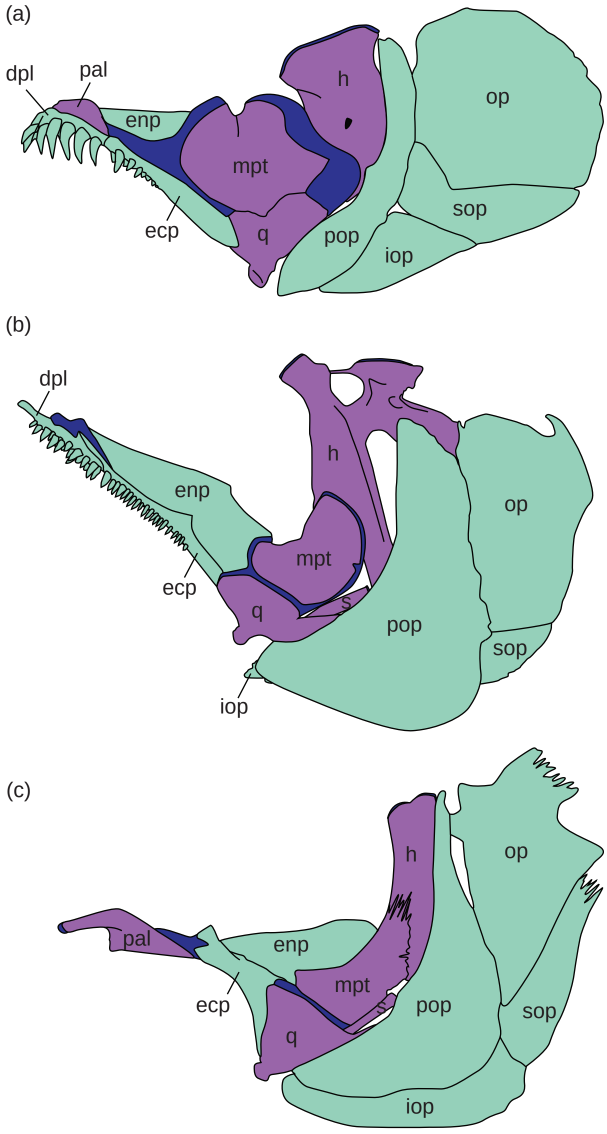


Figure 5 Suspensorium and opercular bones of *Amia* (a), *Hiodon* (b) and *Trachurus* (c) in lateral view. Cartilage in dark blue, chondral bone in purple, and dermal bone in light green. Abbreviations: dpl, dermopalatine; ecp, ectopterygoid; enp, endopterygoid; h, hyomandibula; iop, interopercle; mpt, metapterygoid; op, opercle; pal, palatine; pop, preopercle; q, quadrate; s, symplectic; sop, subopercle. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142; and and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

toothed dermopalatines of varying number. These are distinct from the palatine (also called the autopalatine), which is a chondral bone at the anteriormost extent of the palatoquadrate cartilage that forms the anterior articulation of the suspensorium with the ethmoid region of the braincase. The ectopterygoid and endopterygoid are dermal bones that form in association with the palatoquadrate, and form the major bones of the roof of the mouth. The metapterygoid and quadrate are posterior ossifications of the palatoquadrate cartilage. The quadrate bears a distinct head for the articulation with the lower jaw. In teleosts, it also bears a distinct posteroventral process that is often tightly associated with the symplectic, which is often a stick-like or wedge-like bone that forms in the ventralmost part of the hyosymplectic cartilage. The hyomandibula is the dorsal ossification of the hyosymplectic cartilage and is typically the largest bone of the hyoid arch. This bone forms the posterior articulation of the suspensorium with the neurocranium as well as the articulation point for the opercle.

The ventral portions of the hyoid arch bridge the elements of the suspensorium with the branchial arches (**Figure 6**). Articulating directly with the suspensorium (typically near the symplectic) is the interhyal, which also articulates with the posterior certatohyal. The anterior and posterior ceratohyal ossify in a single cartilage, and serve to support the branchiostegals. One (basal taxa) or two (most teleosts) hypohyal bones lie anteriorly, and these articulate with the bones and cartilages of the branchial arches. In *Polypterus* and teleosts (convergence), there is an urohyal bone, which is a membrane bone that forms in the tendon of the sternohyoideus muscle. The urohyal serves to strengthen the attachment of this muscle to the branchial arches, which contributes to the rapid jaw opening that is necessary for suction feeding.

In the midline of the mouth is a series of median elements: the basihyal, which is in series with the hyoid arch, and the basibranchials, which are in series with the branchial arches. The basibranchials ossify in an anterior basibranchial cartilage (copula) and are numbered anterior to posterior based on their associated branchial arch; a separate posterior cartilage found in most actinopterygians represents the fourth basibranchial. All of these elements may bear toothplates. The ventral portions of the branchial arches follow the same pattern as the hyoid arch. There is a series of hypobranchial elements that articulate directly with the basibranchials, and a more distal series of five certaobranchials. There are five ceratobranchials. The fifth ceratobranchial often does not have a dorsal counterpart (i.e., in most fishes there are only four epibranchials) and often exhibits extreme modifications in association with the development of the so-called pharyngeal jaws, including hypertrophy and robust molariform dentition in durophagous fishes (e.g., some members of the family Sciaenidae). In many fishes, these modifications of the fifth ceratobranchial are matched by similar modifications of the dorsal branchial elements as well.

In some nonteleostean (e.g., sturgeons) and some basal teleostean fishes (e.g., *Elops*), there are three series of

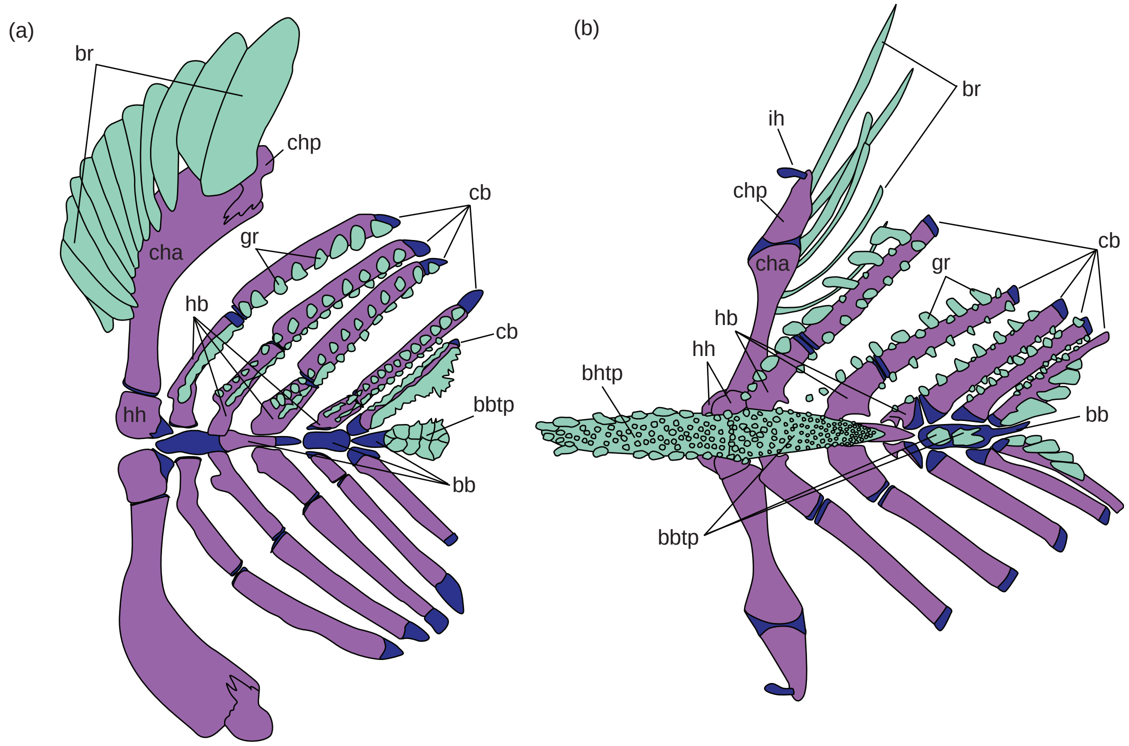


Figure 6 Ventral portion of the hyoid and branchial arches of *Amia* (a) and *Hiodon* (b) in dorsal (oral) view. Cartilage is shown in dark blue, chondral bone in purple, and dermal bone in light green. Abbreviations: bb, basibranchial; bbtp, basibranchial toothplate; bhtp, basihyal toothplate; br, branchiostegal; cb, ceratobranchial; cha, anterior ceratohyal; chp, posterior ceratohyal; gr, gill raker; hb, hypobranchial; hh, hypohyal; ih, interhyal. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690 and Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142.

elements in the dorsal portions of the branchial arches: the suprapharyngobranchials, infrapharyngobranchials, and the epibranchials, which articulate distally with the ceratobranchials. In these taxa, the supra- and/or infrapharyngobranchials articulate with the neurocranium. In most extant teleosts (**Figure** 7), the suprapharyngobranchial

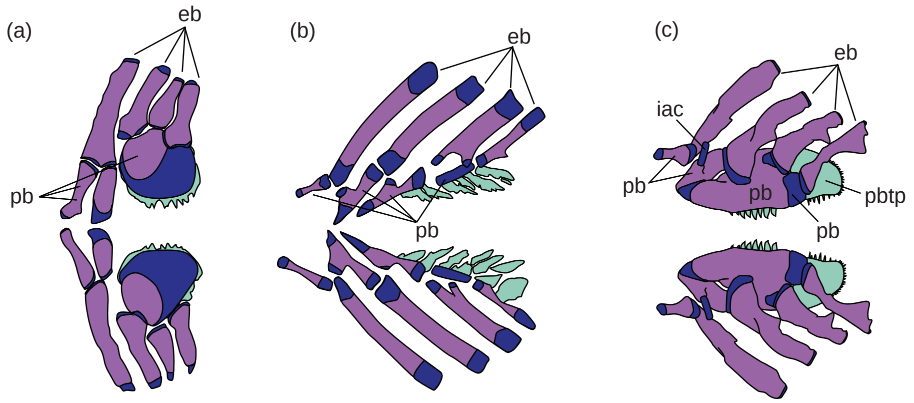


Figure 7 Dorsal portion of the branchial arches of *Amia* (a), *Hiodon* (b), and *Trachurus* (c) in dorsal view. Cartilage is shown in dark blue, chondral bone in purple, and dermal bone in light green. Abbreviations: eb, epibranchial; iac, interarcual cartilage; pb, pharyngobranchial; pbtp, pharyngobranchial toothplate. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142; and and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

series is absent, and the infrapharyngobranchials (often termed simply 'pharyngobranchials') suspend the branchial basket from the neurocranium.

All elements of the branchial arches may support toothplates and gill-rakers, which form series on both the leading and trailing edges of the arch and function to keep the gill tissue clear, and they serve to sieve the water column to collect food in some filter-feeding taxa (e.g., many Clupeidae). The number and arrangement of the toothplates, particularly on the dorsal branchial arch skeleton, often have significant phylogenetic and functional significance. The structure of the branchial arches generally can inform greatly about the ecology of fishes and is rich in phylogenetically informative character data.

Postcranial Axial Skeleton

The postcranial axial skeleton of fishes comprises three basic units – the scale jacket, the vertebral skeleton, and the median fins and their supporting skeletal elements. The scales of fishes are widely varied in their structure. These range from the large, bony cosmoid scales of most fossil fish-like sarcopterygians, which contain a layer of cosmine (lost in extant sarcopterygians), to the thin, flexible cycloid scales found in most teleostean fishes. Some teleosts have so-called ctenoid scales, which are similar to cycloid scales, but which have small spines (cteni) on the exposed posterior field of the scale. Basal sarcopterygians and basal actinopterygians (e.g., *Polypterus*, gars) have thick, interlocking ganoid scales that are coated with ganoine (enamel and dentine). Scales (or in some cases other dermal ossifications) support the lateral sensory canal (if present) and serve to protect the fish. If the scales are reduced in size, there may be other functional protecting layers that are developed (e.g., the greatly thickened dermis of the ocean sunfish, *Mola mola*). Scales and the dermis itself may also store energy used during locomotion and respiration. More broadly speaking, the scale jacket may also include other bony structures, such as scutes (bony plates serially arranged along the body, as in sturgeons, some catfishes, and carangid fishes) and fulcra (scales typically associated with the leading edge of fins).

The vertebral column can be divided into abdominal and caudal regions, with the caudal region further divided into the preural and ural regions. The number, form, and modifications of the vertebrae (e.g., zygapophyses, which may lock together sequential vertebrae in groups such as some billfishes) greatly affect the flexibility of the body, and therefore have significant functional correlations. The abdominal region is defined as that region posterior to the skull with vertebrae bearing parapophyses (**Figures 8**(**a**) and 8(**b**)). The parapophyses are ossifications of paired cartilages (basiventrals) found ventrally along the length of the notochord in early stages of development. The parapophyses are matched by the neural arches, which are ossifications of the basidorsals (which form on either side of the spinal cord). In basal forms (e.g., sturgeons), irregularly shaped cartilaginous elements – interdorsals and interventrals – are intercalated between adjacent basidorsals and basiventrals, respectively. Both neural arches and parapophyses may fuse to the centra, which typically ossify within the notochordal sheath (although there is substantial variation in the mode of ossification of centra, e.g., some parts of a centrum ossify in conjunction with the basidorsals and basiventrals in addition to the notochord). In some basal actinopterygians (e.g., sturgeons) and fish-like sarcopterygians (coelacanths and lungfishes), the centra do not ossify (the so-called unconstricted notochord), although the other elements of the vertebrae are present.

In actinopterygians, the neural arches extend dorsally, forming the neural spine. In derived teleosts, most neural and haemal spines are not preformed in cartilage but are membrane bone. The left and right neural spines typically fuse forming a median structure, although the two may remain separate in basal actinopterygians. Intercalated between the neural spines anterior to the dorsal fin is a series of supraneural bones. The centra and neural arches of the abdominal region continue into the caudal region (**Figures 8**(**c**) and 8(**d**)). The parapohyses, on the other hand, end at the abdominal–caudal division and are replaced by the haemal arches and spines. Like the parapophyses, these structures develop from the basiventrals, but are similar in form to the neural arches. The hemal arches surround the major blood vessels of the caudal region.

Intermuscular bones are associated with the other elements of the vertebrae of teleosts, and these bones are widely regarded as a synapomorphy for the Teleostei. These bones form in the ligaments of the myosepta, and are thought to help stiffen the body and provide more attachment sites for transmission of force. Only relatively basal teleosts (e.g., elopomorphs and clupeomorphs) have a complete set of intermuscular bones (i.e., epineurals, epicentrals, epipleurals, and myorhabdoi, a naming based on which fold of the myosepta they are found within), and their presence, absence, and positioning are phylogenetically informative.

The elements of the vertebral column continue posteriorly into the ural region (defined as the region posterior to the vertebra that includes the parhypural, which is the last element that has a hemal arch surrounding the blood vessels; i.e., the first ural vertebrae is associated with hypural 1), which contains most of the supports for the caudal fin (**Figure 9**). The caudal fin and its supports are the source of many phylogenetically informative

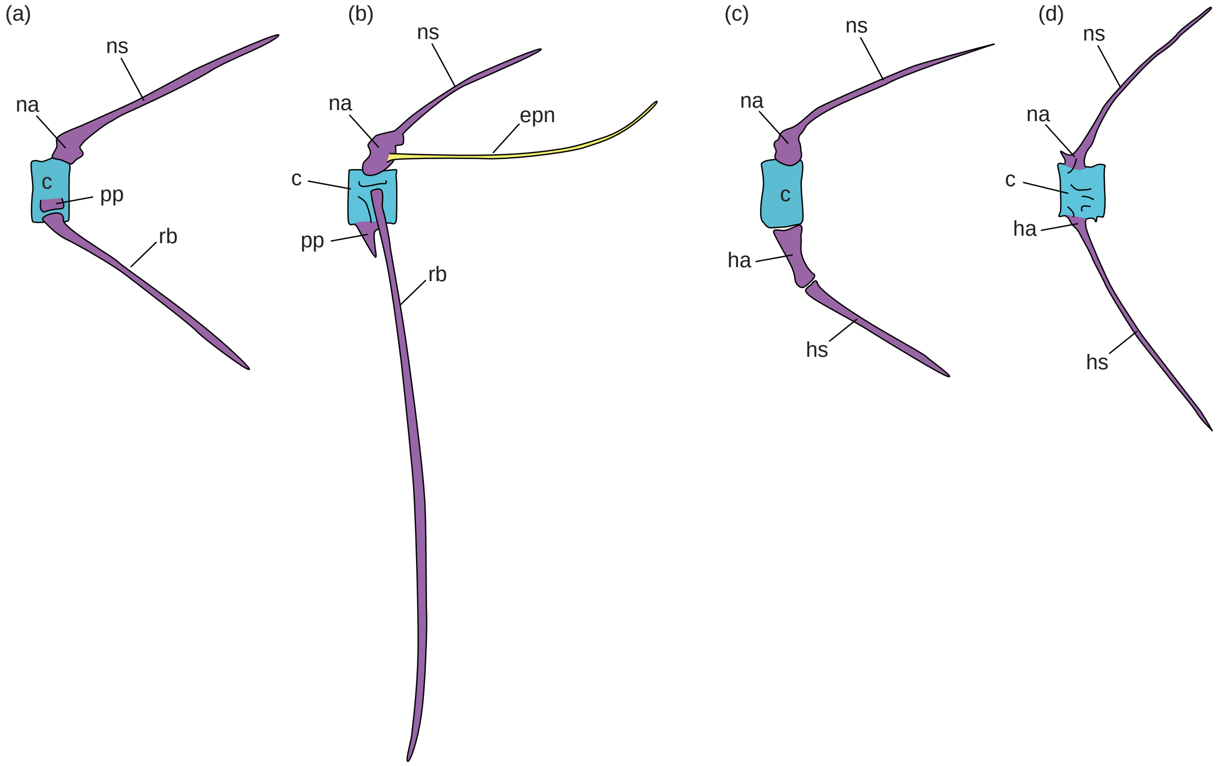


Figure 8 Abdominal (a, b) and caudal (c, d) vertebrae of *Amia* (a, c) and *Hiodon* (b, d) in lateral view. Chondral bone is shown in purple, membrane bone in yellow, and perichordal bone in light blue. Abbreviations: c, centrum; epn, epineural intermuscular bone; ha, hemal arch; hs, hemal spine; na, neural arch; ns, neural spine; pp, parapophysis; rb, rib. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; and Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142.

characters and is perhaps the most-studied region of the skeleton.

In basal sarcopterygians and nonteleostean actinopterygians, the caudal fin is heterocercal, in which the vertebral supports bend slightly dorsally, and the ventral skeletal supports (the hypurals, the serial homologues of the haemal spines) extend to the very tip of the notochord. The fin rays are almost entirely hypaxial, and produce an internally and externally asymmetrical, shark-like caudal fin. Nonteleostean actinopterygians (e.g., gars, *Amia*) have an abbreviated heterocercal caudal skeleton. This is in contrast to the condition seen in teleosts, in which the notochord has a sharp bend at its posterior end, causing the hypurals to be directed posteriorly. The fin itself is symmetrical, although the internal supporting elements are asymmetrical, forming the so-called homocercal caudal fin. Most notable in the asymmetry of the internal skeleton is the presence of uroneurals, which are the homologs to the neural arches of ural vertebrae that have become separated from their vertebrae and modified into stabilizing struts that serve to stiffen the vertebrae of the posterior caudal skeleton. The presence of uroneurals is a key synapomorphy of the Teleostei. In derived teleosts, the uroneurals may fuse to the terminal centrum (which is thought to incorporate preural centrum 1 and all ural centra; **Figure 9(c)**). There are significant functional consequences to the various patterns found in the caudal skeletons and fins of fishes, although both heterocercal and homocercal tails appear to function asymmetrically (perhaps through active control by species with homocercal tails). Therefore, even fishes with a broad diversity of tail forms all may produce asymmetrical patterns of thrust and lift, which are matched to ecological and behavioral necessities.

The dorsal and anal fins have a similar overall structure and arrangement (**Figure 10**). In both fins the skeletal supports for the fin rays, collectively called the pterygiophores, are composed of a series of radial elements termed the proximal, middle, and distal radials. The proximal radials are typically the longest of the series. In most derived teleosts, the proximal and middle radials are fused, forming the so-called proximal-middle radial. The distal radials are typically the smallest elements of a pterygiophore, and are embraced by the bases of the fin rays. In neopterygians (gars, bowfins, and teleosts), the fin rays are about equal in number to the number

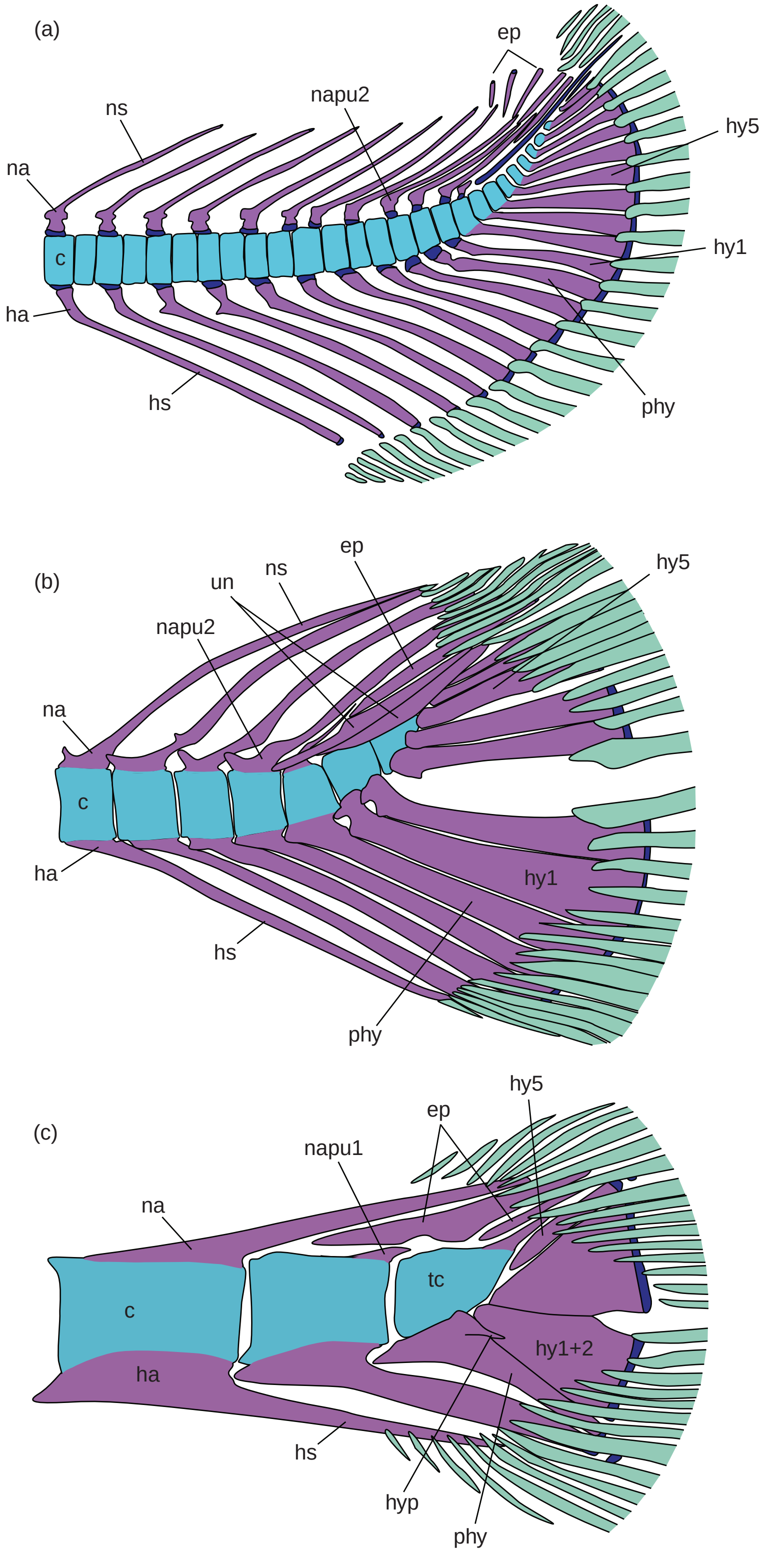


Figure 9 Caudal skeletons of *Amia* (a), *Hiodon* (b), and *Trachurus* (c) in lateral view. Cartilage is shown in dark blue, chondral bone in purple, perichordal bone in light blue, and dermal bone in green. Abbreviations: ep, epural; ha, hemal arch; hs, hemal spine; hy, hypural; hyp, hypurapophysis; na, neural arch; napu2, neural arch of preural 2; ns, neural spine; phy, parhypural; tc, terminal centrum; un, uroneural. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142 and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

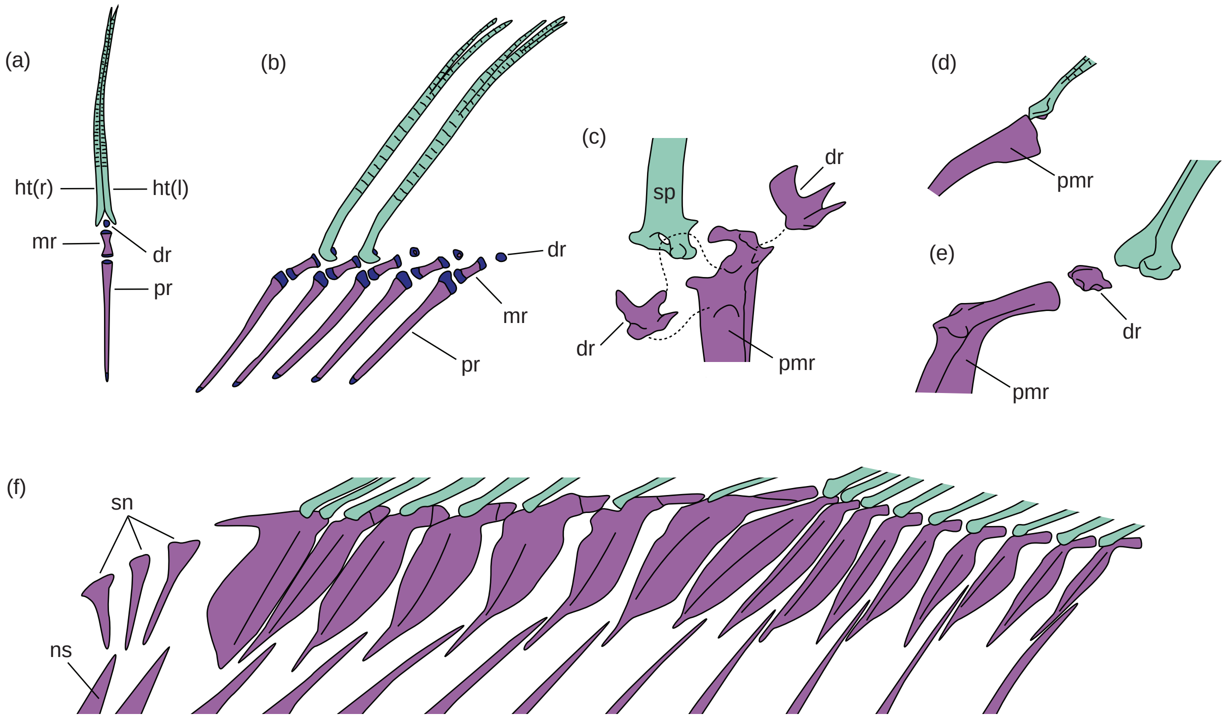


Figure 10 Median fin supports of *Amia* (a, b) and *Trachurus* (c–f). (a) Anterior view of a single fin ray and supporting elements. (b) Lateral view of five pterygiophores; fin rays removed from last three to show distal radials. (c) Oblique view of third dorsal spine. Elements are separated to show complex structures (dashed lines indicate articulations). (d) Articulated and (e) disarticulated soft fin rays and their support. (f) Pterygiophores from the spinous and anterior portion of the soft dorsal fin. Cartilage is shown in dark blue, chondral bone in purple, and dermal bone in green. Abbreviations: dr, distal radial; ht(l), left hemilepidotrichia; ht(r), right hemilepidotrichia; mr, middle radial; ns, neural spine; pmr, proximal–middle radial; pr, proximal radial; sn, supraneural; sp, spine. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690 and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

of pterygiophores, which stands in contrast to more basal actinopterygians (e.g., sturgeons), in which the fin rays greatly outnumber the pterygiophores. The fin rays (lepidotrichia) of nonacanthomorph teleosts are all soft rays formed by paired hemilepidotrichia, with the exception of catfishes and some cyprinids, which have leading spine-like fin rays. In Acanthomorpha, a series of true, unsegmented median spines precede the soft rays of both the dorsal and anal fins, and may form a separate spinous dorsal fin.

Appendicular Skeleton

The pectoral girdle of most actinopterygian fishes (**Figure 11**) is unique among vertebrates in having a direct and often strong attachment to the posterior region of the skull. This connection is achieved through the posttemporal bone. The posttemporal most often bears dorsal limb that contacts the posterodorsal portion of the skull roof or the braincase (e.g., with the extrascapular or the epioccipital) and a ventral limb that contacts or braces against the skull (often at the intercalar) through ligament or direct bony articulation. Ventral to the posttemporal is the supracleithrum, which is attached to the axial skeleton (either the basioccipital or an anterior vertebra) through the so-called Baudelot's ligament. The supracleithrum and the posttemporal carry the trunk lateral sensory canal to the skull. The largest dermal bone of the pectoral girdle is the cleithrum, which is typically a J-shaped bone that provides much of the surface area for muscular attachment in the pectoral girdle. In basal actinopterygians and in sarcopterygians, there is also a clavicle that is positioned anteroventral to the cleithrum. The clavicles are lost in gars and all teleosts. In the bowfin, *Amia*, there are two small plates that are superficial to the sternohyoideus muscle that have been homologized with the clavicles of more basal actinopterygians. Also in basal actinopterygians and sarcopterygians, there is a median dermal interclavicle intercalated between the clavicles; this bone is lost in Neopterygii. Posteriorly, there is a series of postcleithra (often one to three elements, but

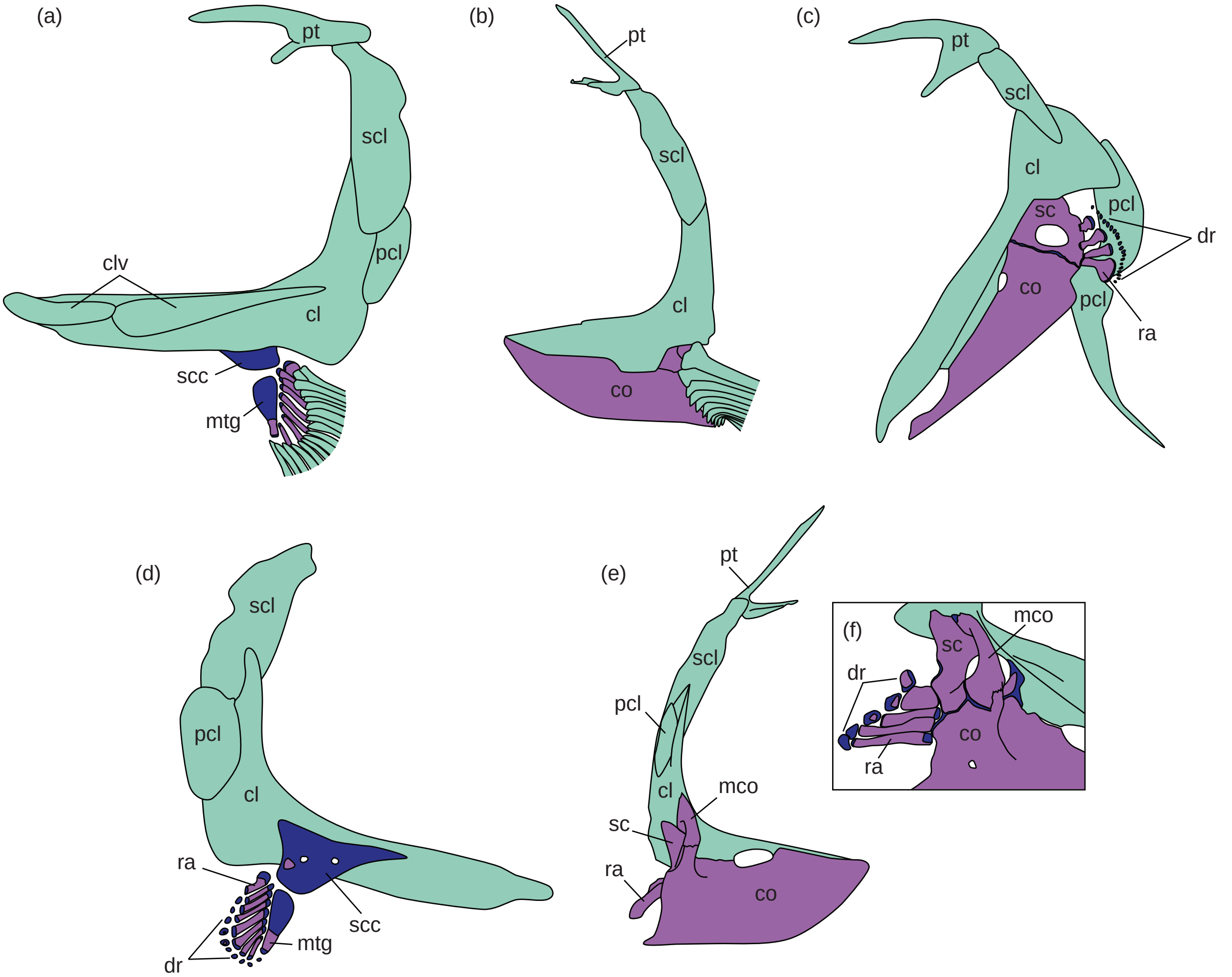


Figure 11 Pectoral girdle of *Amia* (a, d), *Hiodon* (b, e, f), and *Trachurus* (c) in lateral (a–c) and medial (d–f) views. Cartilage is shown in dark blue, chondral bone in purple, and dermal bone in green. Abbreviations: cl, cleithrum; clv, clavicle; co, coracoid; dr, distal radial; mco, mesocoracoid; mtg, metapterygium; pcl, postcleithrum; pt, posttemporal; ra, radial; sc, scapula; scc, scapulocoracoid cartilage; scl, supracleithrum. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142; and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus*. *Bulletin of Marine Science* 58: 438–493.

sometimes more or less), which extend posteroventrally from the supracleithrum–cleithrum joint. In many teleosts the posteriormost postcleithrum is greatly elongated and rib-like in its form. In sarcopterygians, the shoulder girdle is often separate from the skull (e.g., coelacanths and lungfishes), but may have additional dermal bones (e.g., the anocleithrum and extracleithrum of coelacanths).

The internal chondral portion of the pectoral girdle of bony fishes is formed by the scapulocoracoid cartilage and its ossifications, and the radial elements that directly support the fin rays. In some taxa, there may be additional neomorphic elements that are synapomorphies for these groups (e.g., the supracleithral cartilage of sturgeons, family Acipenseridae). Three endochondral bones are present in nonteleostean and basal teleostean actinopterygians: the scapula, the coracoid, and the mesocoracoid. The scapula ossifies dorsally, surrounding the scapular foramen, whereas the coracoid ossifies ventrally within the scapulocoracoid cartilage. The mesocoracoid ossifies in a medial arch of the scapulocoracoid cartilage that braces against the medial surface of the cleithrum. The mesocoracoid is lost in advanced teleostean fishes. In Sarcopterygii, a single element articulates with the scapulocoracoid, forming the so-called monobasal fin. The proximal radials of the pectoral fin of most actinopterygians articulate with the scapula and the coracoid and extend distally to support the fin rays. Typically, there are four or more proximal radials (=actinosts) that ossify and a single propterygium that often becomes fused to the leading fin ray. In nonteleostean actinopterygians, there is

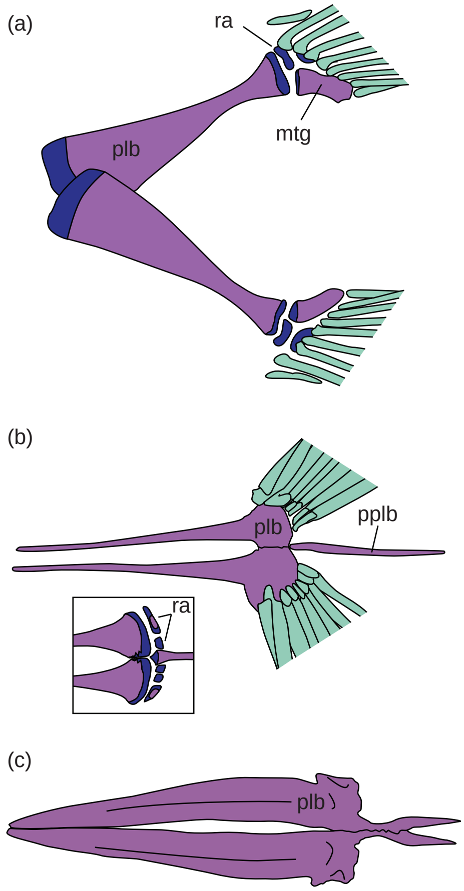


Figure 12 Pelvic girdle of *Amia* (a), *Hiodon* (b), and *Trachurus* (c) in dorsal view (inset in (b) shows base of pelvic bone and radials with fin rays removed). Cartilage is shown in dark blue, chondral bone in purple, and dermal bone in green. Abbreviations: mtg, metapterygium; plb, pelvic bone; pplb, postpelvic bone; ra, radial. Modified from Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690; Hilton EJ (2002) Osteology of the extant North American fishes of the genus Hiodon Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142; and Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus. Bulletin of Marine Science* 58: 438–493.

also a posteriorly positioned, elongated metapterygoid that contributes to the support of the proximal radials. Finally, there is a series of distal radials, which are entirely surrounded by the bases of the fin rays of the pectoral fin. In sturgeons, there is a pectoral fin spine that is formed by the fusion of adjacent fin rays. In catfishes, the pectoral fin also has a pectoral fin spine formed by the fusion of the segments of the leading fin ray. In these spines, the posterior margin may be serrated.

The pelvic fin of basal taxa is positioned posteriorly on the ventral portion of the abdominal region. In derived teleostean fishes (e.g., Acanthomorpha), the position of the pelvic fin is shifted forward on the body, and in many acanthomorphs, the pelvic fins may be anterior to the pectoral fins. The migration of the pelvic fin has both phylogenetic (e.g., occurs as a derived state) as well as functional significance, and seems to be correlated with increased maneuverability and control during locomotion. In Acanthopterygii the soft fin rays of the pelvic fin are preceded by a stout fin spine.

The largest element of the pelvic girdle of most bony fishes is the pelvic bone (basipterygium), which forms a large plate-like element that serves to support the pelvic fin musculature (**Figure 12**). In nonteleostean fishes, the anterior ends of the left and right basipterygia contact or slightly overlap one another. In sturgeons and paddlefishes, the left and right pelvic girdles are widely separated, each embedded in the hypaxial musculature of the body wall. In most teleosts, the posterior regions of the basipterygia are in contact with the midline. Pelvic radials are lost in the adults of derived acanthomorphs.

Conclusion

Collectively, the skeletons of fishes display a complex and diverse array of forms. The details of this variation, which are merely hinted at in this chapter, are the product of functional demands generated by the life history, ecology, and behavior of individual species. Coupled to this are the constraints set in place from the phylogenetic history of each species. It is this combination – phylogeny and function – that makes an understanding and appreciation of the skeleton of fishes central to so many aspects of the evolutionary and ecological biology of fishes.

See also: **The Muscles**: Bony Fish Cranial Muscles.

Further Reading

Arratia G (2008) Actinopterygian postcranial skeleton with special reference to the diversity of fin ray elements and the problem of identifying homologies. In: Arratia G, Schultze H-P, and Wilson MVH (eds.) *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 49–101. München: Verlag Pfeil.

Arratia G (2009) Identifying patterns of diversity of the actinopterygian fulcra. *Acta Zoologica (Stockholm)* 90(supplement 1): 220–235.

Arratia G and Schultze H-P (1991) Palatoquadrate and its ossifications: Development and homology within osteichthyans. *Journal of Morphology* 208: 1–81.

Arratia G and Schultze H-P (1992) Reevaluation of the caudal skeleton of some actinopterygian fishes: III. Salmonidae. Homologization of caudal skeletal structures. *Journal of Morphology* 214: 187–249.

Arratia G, Schultze H-P, and Casciotta J (2001) Vertebral column and associated elements in dipnoans and comparison with other fishes: Development and homology. *Journal of Morphology* 250: 101–172.

Forey PL (1998) *History of the Coelacanth Fishes*. London: Chapman and Hall.

Grande L and Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Society of Vertebrate Paleontology, Memoir* 4: 1–690.

Hilton EJ (2002) Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Fieldiana (Zoology) new series* 100: 1–142.

Jessen H (1972) Schultergürtel und Pectoralflosse bei Actinopterygiern. *Fossils and Strata* 1: 1–101.

Nelson GJ (1969) Gill arches and the phylogeny of fishes, with notes on the classification of vertebrates. *Bulletin of the American Museum of Natural History* 141: 475–552.

Patterson C (1975) The braincase of pholidophorid and leptolepid fishes, with a review of the actinopterygian braincase. *Philosophical Transactions of the Royal Society* 269: 275–579.

Patterson C (1977) Cartilage bones, dermal bones, and membrane bones, or the exoskeleton versus the endoskeleton. In: Andrews SM, Miles RS, and Walker AD (eds.) *Problems in Vertebrate Evolution*. pp. 77–121. London: Academic Press.

Patterson C and Johnson GD (1995) The intermuscular bones and ligaments of teleostean fishes. *Smithsonian Contributions to Zoology* 559: 1–85.

Rosen DE, Forey PL, Gardiner BG, and Patterson C (1981) Lungfishes, tetrapods, paleontology and plesiomorphy. *Bulletin of the American Museum of Natural History* 167: 159–276.

Schultze H-P (1996) The scales of Mesozoic actinopterygians. In: Arratia G and Viohl G (eds.) *Mesozoic Fishes: Systematics and Paleoecology*, pp. 83–93. München: Verlag Pfeil.

Schultze H-P (2008) Nomenclature and homologization of cranial bones in actinopterygians. In: Arratia G, Schultze H-P, and Wilson MVH (eds.) *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 23–48 München: Verlag Pfeil.

Schultze H-P and Arratia G (1986) Reevaluation of the caudal skeleton of some actinopterygian fishes: I. *Lepisosteus and* Amia. Journal of Morphology 190: 215–241.

Schultze H-P and Arratia G (1988) Reevaluation of the caudal skeleton of some actinopterygian fishes: II. *Hiodon, Elops, and* Albula. Journal of Morphology 195: 257–303.

Shadwick RE and Lauder GV (eds.) (2006) *Fish Biomechanics*. San Diego: Elsevier Academic Press.

Suda Y (1996) Osteology and muscular attachments of the Japanese jack mackerel, *Trachurus japonicus. Bulletin of Marine Science* 58: 438–493.

MUSCLES, SKELETON, SKIN, AND MOVEMENT

The Muscles

Contents

Cartilaginous Fishes Cranial Muscles
Bony Fish Cranial Muscles

Cartilaginous Fishes Cranial Muscles

DR Huber, University of Tampa, Tampa, FL, USA

MC Soares and MR de Carvalho, Universidade de São Paulo, São Paulo, Brazil

© 2011 Elsevier Inc. All rights reserved.

Introduction
Shark Musculature
Batoid Musculature
Holocephalan Musculature
Further Reading

Glossary

Branchiomeric Pertaining to muscles originating between the dorsal and ventral elements of the visceral arches of the skeleton.

Chondrocranium Cartilaginous cranium of the chondrichthyan fishes.

Epibranchial Pertaining to muscles lying dorsal to the branchial arches.

Hypobranchial Pertaining to muscles lying ventral to the branchial arches.

Meckel's cartilage Lower jaw of the chondrichthyan fishes.

Palatoquadrate Upper jaw of the chondrichthyan fishes.

Raphe A seam-like union between two halves of an anatomical structure having a sheet of connective tissue at the septum.

Scapulocoracoid Pectoral girdle of the chondrichthyan fishes.

Introduction

Study of the cranial musculature of the cartilaginous fishes has a long history owing to both the phylogenetic position of this clade and the dynamic nature of their predatory endeavors. Basal gnathostomes diverged from a common ancestor with the Teleostomi over 400 million years ago and thus the study of their cranial musculature promises insight into the proximate mechanisms underlying the transition of the anterior visceral arches from respiratory structures into those involved in active predation. While early studies focused on describing the cranial muscles and their relation to the visceral arches as a means of codifying the evolutionary history of jawed vertebrates, later studies focused on the range of function enabled by the diverse morphologies of the various chondrichthyan feeding (**see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays, **Food Acquisition and Digestion:** Energetics of Prey Capture: From Foraging Theory to Functional Morphology, and **The Reproductive Organs and Processes:** Anatomy and Histology of Fish Testis) and respiratory mechanisms (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics).

Although taxonomically few, cartilaginous fishes possess a remarkable diversity of feeding mechanisms (six jaw suspension types, ram/biting/suction/filter feeding, jaw protrusion). While their respiratory mechanisms are not as diverse, they represent both predominant systems found in aquatic vertebrates, the suction-force pump and ram ventilation. The cranial muscles serve as the actuators of these mechanisms. Thus, studying their functional diversity provides critical links in our understanding of the relationship between the morphology, behavior and ecology in cartilaginous fishes, and the reconstruction of vertebrate evolutionary history. The following information is a basic guide to the cranial musculature of the three major radiations of extant cartilaginous fishes. Among the elasmobranchs, sharks are represented by the spiny dogfish *Squalus acanthias* and batoids by the southern guitarfish *Rhinobatos percellens*. The holocephalans are represented by the spotted ratfish *Hydrolagus colliei*. Hypobranchial and pharyngeal muscles are described in addition to cranial muscles, as these structures are also involved in feeding and respiration.

Shark Musculature

The muscles involved in feeding and respiration in cartilaginous fishes can be broadly categorized as epibranchial, branchiomeric, or hypobranchial. The epibranchial muscles form a cranial extension of the epaxial musculature, and as such retain the myomeric structure typical of the trunk. The epibranchial muscles originate on the epaxial muscle mass, insert onto the otic capsule of the chondrocranium, and elevate the cranium during prey capture (**Figures 1** and **2**, and **Table 2**).

The branchiomeric muscles adduct the dorsal and ventral elements of the visceral arches. As the visceral arches (I–VII) differentiated from respiratory structures into those suited for respiration and feeding (mandibular, hyoid, and branchial arches I–V), a concomitant differentiation of the branchiomeric muscles occurred. Branchiomeric muscles associated with the mandibular arch include the adductor mandibulae, preorbitalis, levator palatoquadrati, intermandibularis, and spiracularis. The adductor mandibulae originates on the palatoquadrate (upper jaw), inserts onto Meckel's cartilage (lower jaw), has multiple divisions, and is the primary jaw adductor in all cartilaginous fishes (**Figures 1–5** and **Table 2**). The preorbitalis originates on the chondrocranium, posterior to the nasal capsule, and inserts onto the median raphe, separating the dorsal and ventral divisions of the adductor mandibulae (**Figures 1** and **2**, and **Table 2**). It elevates the lower jaw, protrudes the upper jaw, and depresses the cranium.

The levator palatoquadrati originates on the otic capsule of the chondrocranium and inserts onto the palatoquadrate (**Figures 1**, **2**, and **4**, and **Table 2**). It elevates the upper jaw into its resting position during the recovery phase of the gape cycle after the upper and lower jaws have been adducted. The intermandibularis is a thin sheet of muscle lying between the two halves of the lower jaw, with its right and left portions inserted upon the midventral raphe. It elevates the floor of the mouth during feeding and respiration, facilitating hydraulic transport through the oropharyngeal cavity (**Figure 3** and **Table 2**). Lastly, the spiracularis originates on the lateral wall of the chondrocranium, posterior to the levator palatoquadrati, and inserts onto and closes the spiracle (**Figures 1**, **2**, and **4**).

Branchiomeric muscles of the hyoid arch include the interhyoideus, dorsal and ventral hyoid constrictors, hyoid trematic constrictor, and levator hyomandibulae. The interhyoideus is a thin muscle sheet lying dorsal to the intermandibularis. It originates on the ceratohyal cartilages of the hyoid arch, inserts onto the midventral raphe, and elevates the floor of the mouth (**Figures 2**

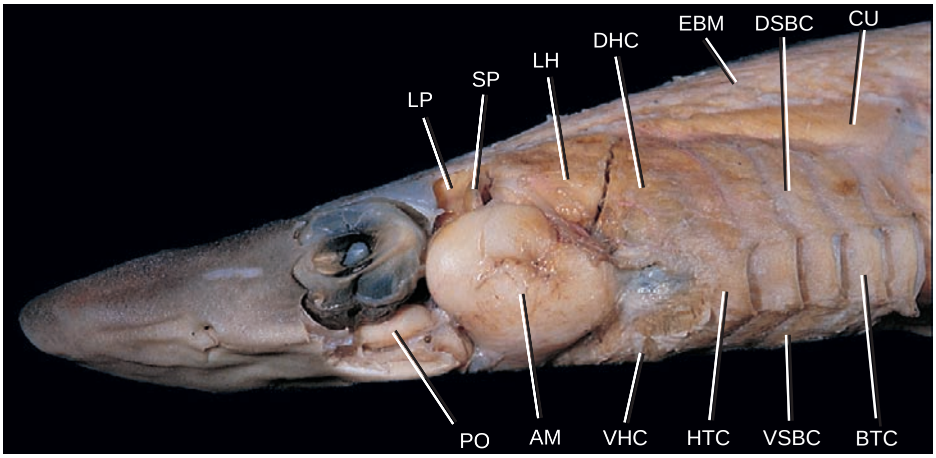


Figure 1 Epibranchial and branchiomeric cranial muscles of the spiny dogfish *Squalus acanthias* in lateral view. See **Table 1** for abbreviated terms. Modified from Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

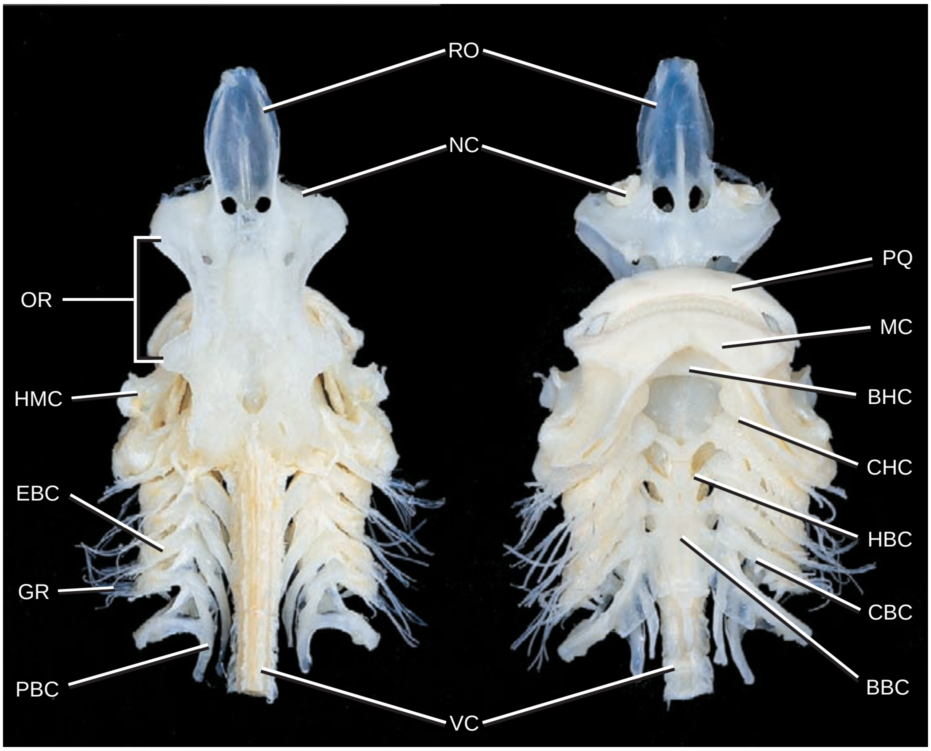


Figure 2 Dorsal and ventral views of the visceral arches and chondrocranium of the spiny dogfish *Squalus acanthias*. See **Table 1** for abbreviated terms. Modified from Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

Table 1 List of terms and abbreviations of the cranial musculature of cartilaginous fishes

AM	Adductor mandibulae	HTC	Hyoid trematic constrictor
AMA	*Adductor mandibulae anterior*	HMC	Hyomandibular cartilage
AMLI	*Adductor mandibulae lateral 1*	HYP	*Hypaxialis*
AML2	*Adductor mandibulae lateral 2*	HBC	Hypobranchial cartilage
AMM	*Adductor mandibulae medial*	IB	*Interbranchial*
AMP	*Adductor mandibulae posterior*	IH	*Interhyoideus*
BBC	Basibranchial cartilage	IM	*Intermandibularis*
BHC	Basihyal cartilage	LA	*Labialis anterior*
BA	*Branchial adductor*	LI	*Lateral interarcual*
BTC	*Branchial trematic constrictor*	LR	*Lateral rectus*
CBC	Ceratobranchial cartilage	LAOA	*Levator anguli oris anterior*
CHC	Ceratohyal cartilage	LAOP	*Levator anguli oris posterior*
CC	*Coracoarcualis*	LVH	*Levator hyoideus*
CB	*Coracobranchialis*	LH	*Levator hyomandibulae*
CH	*Coracohyoideus*	LP	*Levator palatoquadrati*
CHM	*Coracohyomandibularis*	LVR	*Levator rostri*
CM	*Coracomandibularis*	MC	Meckel's cartilage
CU	*Cucullaris*	MR	*Medial rectus*
CP	*Cucullaris profundus*	NC	Nasal capsule
CS	*Cucullaris superficialis*	OR	Orbit
DM	*Depressor mandibularis*	PQ	Palatoquadrate
DPR	*Depressor rostri*	PBC	Pharyngobranchial cartilage
DHC	*Dorsal hyoid constrictor*	POP	Post-orbital process
DI	*Dorsal interarcual*	PO	*Preorbitalis*
DO	*Dorsal oblique*	RO	Rostrum
DOC	*Dorsal opercular constrictor*	SCC	Scapulocoracoid cartilage
DR	*Dorsal rectus*	SPV	Spiracular valve
DSBC	*Dorsal superficial branchial constrictor*	SP	*Spiracularis*
EP	*Epaxial*	VHC	*Ventral hyoid constrictor*
EBM	*Epibranchial*	VOC	*Ventral opercular constrictor*
EBC	Epibranchial cartilage	VSBC	*Ventral superficial branchial constrictor*
EHC	Epihyal cartilage	VC	Vertebral column
GR	Gill rays		

Table 2 Origins, insertions, and actions of the cranial muscles of sharks

Muscle	*Origin*	*Insertion*	*Action*
Adductor mandibulae	Palatoquadrate cartilage	Meckel's cartilage	Adduct lower jaw
Branchial adductor	Epibranchial cartilage	Ceratobranchial cartilage	Adduct gill arch
Branchial trematic constrictor	Tendinous sheath of gill slit	Gill slit	Compress gill pouch
Constrictor hyoideus dorsalis	Chondrocranium, cucullaris	Tendinous sheath of hyomandibular cartilage	Compress first gill pouch
Constrictor hyoideus ventrails	Raphe of first gill slit	Tendinous sheath of hyomandibular cartilage	Compress first gill pouch
Coracoarcualis	Scapulocoracoid cartilage	Coracomandibularis, corachyoideus	Expand oropharyngeal cavity
Coracobranchialis	Coracoarcualis, pectoral girdle	Basihyal, hypobranchial, and basibranchial cartilages	Abduct hyoid and branchial arches
Coracohyoideus	Coracoarcualis	Basihyal cartilage	Abduct hyoid arch
Coracomandibularis	Coracoarcualis	Meckel's cartilages	Abduct lower jaw
Cucullaris	Epibranchial	Pectoral girdle	Elevate pectoral girdle
Dorsal interarcual	Pharyngobranchial cartilage	Pharyngobranchial cartilage	Adduct gill arch
Dorsal oblique	Chondrocranium	Eyeball	Rotate eyeball
Dorsal rectus	Chondrocranium	Eyeball	Rotate eyeball
Dorsal superficial branchial constrictor	Cucullaris	Tendinous sheaths of gill slits	Compress gill pouches
Epibranchial	Epaxialis	Chondrocranium	Elevate chondrocranium
Hyoid trematic constrictor	Tendinous sheath of hyomandibular cartilage	First gill slit	Compress first gill pouch
Interbranchial	Gill rays	Gill rays	Adduct gill arches, move interbranchial septa
Interhyoideus	Ceratohyal cartilage	Midventral raphe	Elevate floor of mouth
Intermandibularis	Meckel's cartilage	Midventral raphe	Elevate floor of mouth
Lateral interarcual	Pharyngobranchial cartilage	Epibranchial cartilage	Adduct gill arch
Lateral rectus	Chondrocranium	Eyeball	Rotate eyeball
Levator hyomandibulae	Chondrocranium, epibranchial muscles	Hyomandibular cartilage	Elevate hyomandibular cartilage
Levator palatoquadrati	Chondrocranium	Palatoquadrate cartilage	Elevates upper jaw
Medial rectus	Chondrocranium	Eyeball	Rotate eyeball
Preorbitalis	Chondrocranium	Adductor mandibulae	Adduct lower jaw, protrude upper jaw
Spiracularis	Chondrocranium	Spiracle	Close spiracular valve
Ventral oblique	Chondrocranium	Eyeball	Rotate eyeball
Ventral rectus	Chondrocranium	Eyeball	Rotate eyeball
Ventral superficial branchial constrictor	Coracoarcualis	Tendinous sheaths of gill slits	Compress gill pouches

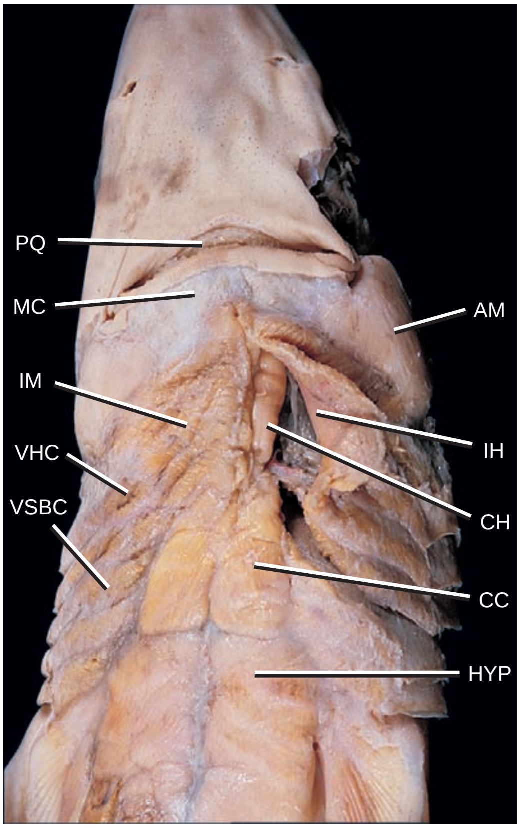


Figure 3 Branchiomeric and hypobranchial cranial muscles of the spiny dogfish *Squalus acanthias* in ventral view. The intermandibularis and interhyoideus have been partially reflected to show the coracohyoideus. See **Table 1** for abbreviated terms. Modified from Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

and **3**, and **Table 2**). The dorsal and ventral hyoid constrictors as well as the hyoid trematic constrictor compress the first gill pouch, assisting in hydraulic transport during respiration and feeding. Both the dorsal and ventral hyoid constrictors insert onto the tendinous sheath overlying the hyomandibular cartilages. The former originates from the otic capsule of the chondrocranium and cucullaris muscle, whereas the latter originates adjacent to the interhyoideus along the raphe of the first gill slit (**Figures 1–4** and **Table 2**). The hyoid trematic constrictor originates on the tendinous sheath overlying the hyomandibular cartilages and inserts onto the first gill slit (**Figure 1** and **Table 2**). The levator hyomandibulae originates on the otic capsule of the chondrocranium and epibranchial musculature, inserts onto the hyomandibular cartilages, and elevates these cartilages following a gape or respiratory cycle (**Figures 1** and **2**, and **Table 2**).

The branchiomeric muscles of the branchial arches include the dorsal and ventral superficial branchial constrictors, branchial trematic constrictors, dorsal and lateral interarcual, branchial adductor, interbranchial, and cucullaris muscles. The dorsal and ventral superficial branchial constrictors lie above and below the gill slits in positions comparable to the respective hyoid constrictors. The dorsal superficial branchial constrictor originates on the cucullaris and extends downward to meet its ventral counterpart at a series of tendinous intersections on each gill slit. The ventral division originates on the fascia of the coracoarcualis muscle (**Figures 1** and **3–5**, and **Table 2**). The branchial trematic constrictor muscles originate on the

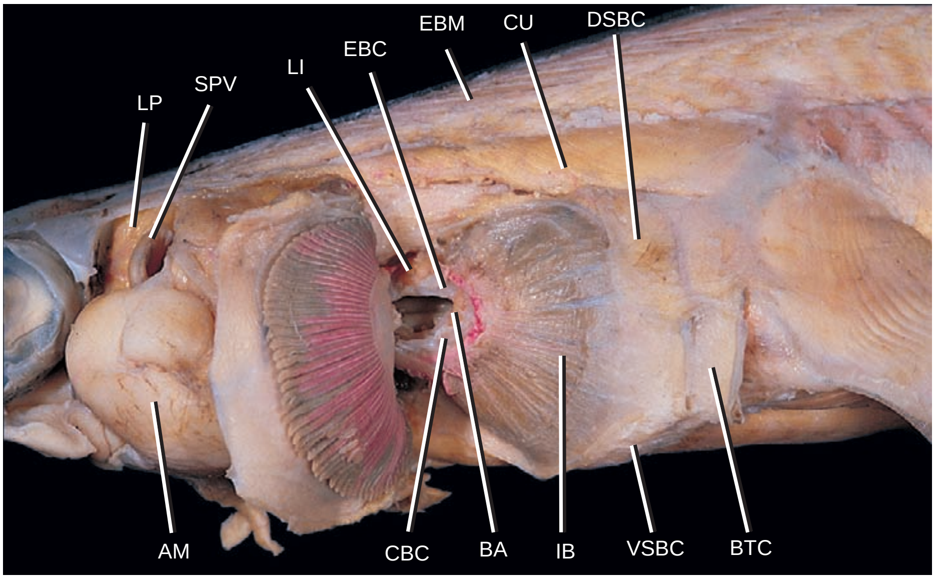


Figure 4 Epibranchial and branchiomeric cranial muscles of the spiny dogfish *Squalus acanthias* in lateral view with the gills reflected to show the deep branchial muscles. See **Table 1** for abbreviated terms. Modified from Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

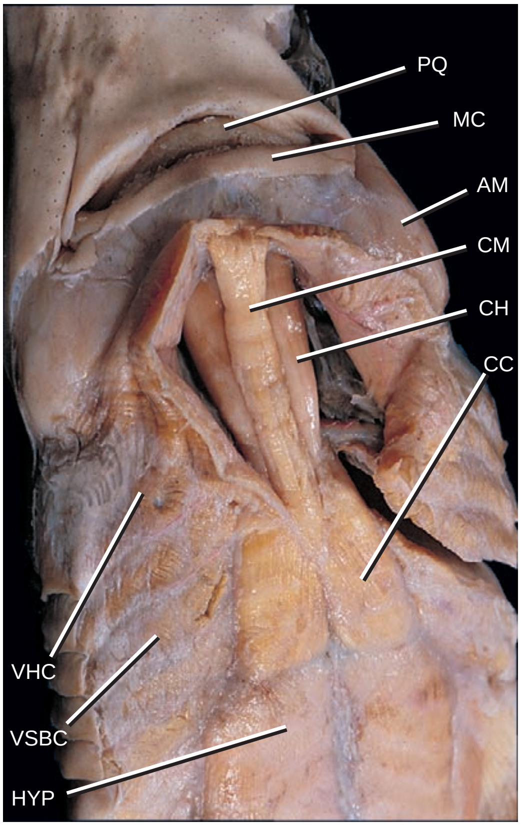


Figure 5 Branchiomeric and hypobranchial cranial muscles of the spiny dogfish *Squalus acanthias* in ventral view. The intermandibularis and interhyoideus have been removed to show the coracomandibularis and coracohyoideus. See **Table 1** for abbreviated terms. Modified from Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

tendinous intersections of the dorsal and ventral superficial branchial constrictors, and they insert onto each of the gill slits (**Figures 1** and **4**, and **Table 2**). Collectively these muscles compress the gill pouches.

The gill arches are moved by the dorsal and lateral interarcual, branchial adductor, and interbranchial muscles. The dorsal interarcual muscles are located between the pharyngobranchial cartilages of adjacent branchial arches and control the distances between these elements. The lateral interarcual muscles originate, and insert upon, the pharyngobranchial and epibranchial cartilages of the same branchial arch. They adduct these elements. The branchial adductors originate, and insert upon, the epibranchial and ceratobranchial cartilages of the same branchial arch. They adduct these elements as well. The interbranchial muscles are an extensive series of circumferential fiber bundles that span between adjacent gill rays on each branchial arch (**Figures 2** and **4**, and **Table 2**). They control the orientation of the interbranchial septa and adduct the branchial arches. Lastly, the cucullaris originates on the epibranchial musculature, and inserts onto, the scapulocoracoid cartilage of the pectoral girdle, which it elevates (**Figures 1** and **4**, and **Table 2**).

The hypobranchial muscles are largely associated with the abduction of the visceral arches, resulting in expansion of the oropharyngeal cavity. The coracomandibularis and coracoarcualis lie in series in the floor of the mouth, with the former immediately deep to the interhyoideus (**Figures 3** and **5**, and **Table 2**). The coracohyoideus is a paired muscle lying deep to the coracomandibularis, both of which originate on the fascia of the coracoarcualis. The coracomandibularis and coracohyoideus insert onto, and abduct, the lower jaw and basihyal cartilage of the hyoid arch, respectively. The paired, segmented coracoarcualis muscles originate upon the scapulocoracoid cartilage. They assist the coracomandibularis and coracohyoideus in their functions (**Figures 2**, **3**, and **5**, and **Table 2**). The segmented coracobranchialis muscles originate on the coracoarcualis and coracoid bar, and insert onto and abduct the ventral elements of the hyoid and branchial arches.

The positions of the eyes are controlled by six extrinsic muscles, all of which originate on the chondrocranium. The lateral, medial, dorsal, and ventral rectus muscles insert onto the eyeball orthogonal to the viewing plane, while the dorsal and ventral oblique muscles insert onto the eyeball from an acute angle. The same arrangement of extrinsic eye muscles is found among all cartilaginous fishes (**Figure 6** and **Table 2**).

Coordinated Muscle Activity in Feeding and Respiration

Like other aquatic vertebrates, sharks typically capture prey by expansive, compressive, and recovery phases of the gape cycle (**see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays, **Food Acquisition and Digestion:** Energetics of Prey Capture: From Foraging Theory to Functional Morphology, **Ventilation and Animal**

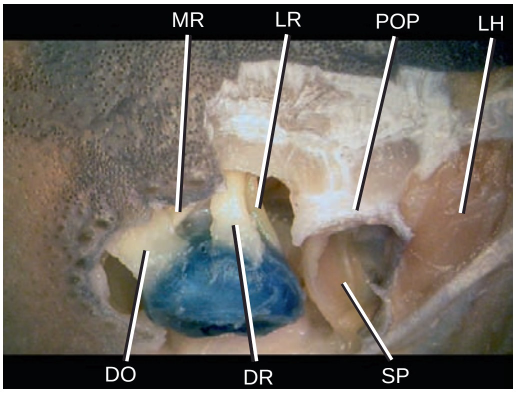


Figure 6 Extrinsic eye muscles of the southern guitarfish *Rhinobatos percellens* in dorsal view (rostrum toward the left). See **Table 1** for abbreviated terms.

Respiration: Gill Respiratory Morphometrics, **Buoyancy, Locomotion, and Movement in Fishes:** Feeding Mechanics, and **The Muscles:** Bony Fish Cranial Muscles). The expansive phase is characterized by cranial elevation via the epibranchial musculature and abduction of the ventral elements of the visceral arches. The coracomandibularis, coracohyoideus, coracoarcualis, and coracobranchialis are activated in rapid succession resulting in a posteriorly directed wave of abduction, which facilitates hydraulic transport of food and water (**Figure 7**, **Video Clip 1**). The compressive phase is characterized by contraction of the adductor mandibulae followed by the preorbitalis, which adducts both jaws while protruding the upper. Head depression occurs via relaxation of the epibranchial musculature. The palatoquadrate and hyomandibular cartilages are returned to their resting positions during the recovery phase via the levator palatoquadrati and levator hyomandibulae muscles (**Figure 7**, **Video Clip 1**).

Respiration in *S. acanthias* occurs via a suction-force pump. While experimental evidence of muscle activity patterns during shark respiration is lacking, the plausible mechanism is described as follows. The external gill slits are closed via the branchial trematic constrictors, and then water is drawn into the oropharyngeal cavity and parabranchial chambers through the mouth and spiracles as the hypobranchial muscles expand the floor of the mouth (suction pump). The mouth and spiracles are then closed via the adductor mandibulae and spiracularis muscles. The branchial chambers are then compressed via the dorsal and ventral branchial constrictors, dorsal and lateral interarcual, and branchial adductor muscles as the external gill slits open, thereby forcing water out (force pump; **Figure 8**). In addition to the suction-force pump, many sharks rely upon ram ventilation in which the mouth is held open during forward movement, resulting in water flow across the gills.

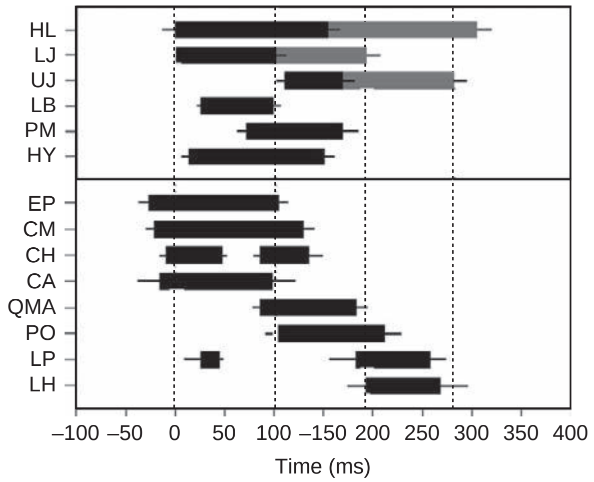


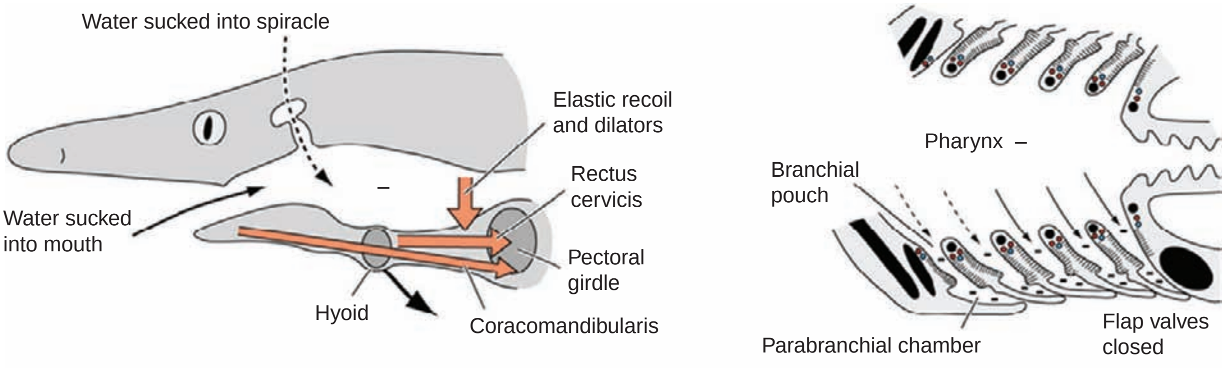
Figure 7 Kinematic (top) and motor (bottom) patterns during prey-capture in the spiny dogfish *Squalus acanthias.* Black and gray bars for kinematic events indicate onset, peak, and end of activity. Black bars for motor events indicate onset and duration of muscle activity. Dashed vertical lines indicate the onset of the expansive, compressive, and recovery phases and the end of the recovery phase. HL, head lift; HY, peak hyoid depression; LB, labial extension; LJ, lower jaw movement; PM, prey movement; QMA, quadratomandibularis anterior (subdivision of the adductor mandibulae); UJ, upper jaw movement. **Table 1** provides information on those terms not listed in the figure caption. From Wilga CD and Motta PJ (1998) Conservation and variation in the feeding mechanism of the spiny dogfish *Squalus acanthias*. *Journal of Experimental Biology* 201: 1345–1358.

Batoid Musculature

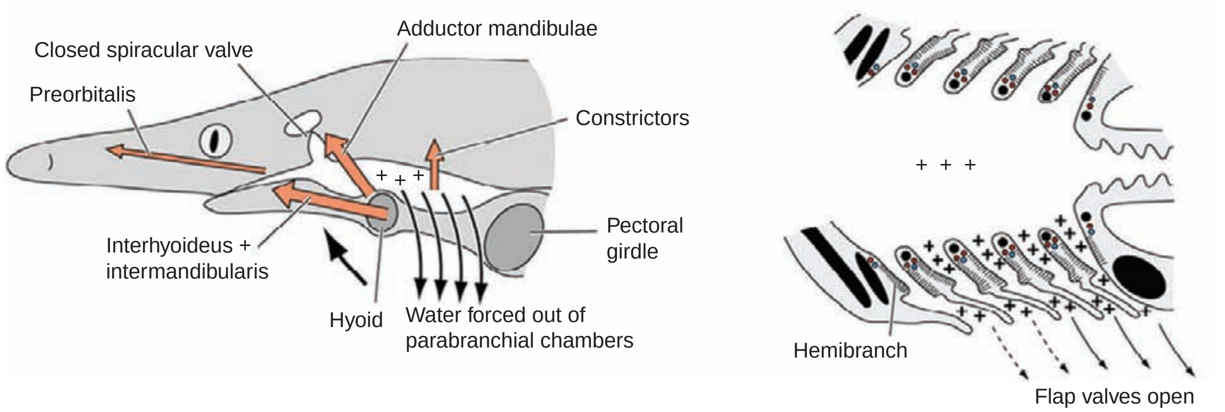
Phylogenetic relationships among the different groups of sharks and rays (Batoidea) are still debated, although little doubt exists that elasmobranchs are monophyletic. Molecular data suggest that sharks and rays are sister groups (i.e., sharks are monophyletic), whereas morphological data suggest that rays are derived sharks (i.e., sharks are not monophyletic) and part of the Squalomorphii, a large group including the six- and seven-gill sharks, bramble sharks, dogfishes, and the superorder Hypnosqualea (sawsharks, angelsharks, and rays). Patterns in the cranial muscles corroborate the latter hypothesis because there are many subdivided muscles among species of Batoidea that are derived from the patterns seen in squalomorph sharks (e.g., in the adductor mandibulae complex).

In batoids, the epaxial musculature inserts directly onto the chondrocranium and elevates the head (**Figure 9** and **Table 3**). The branchiomeric muscles of the mandibular arch include the adductor mandibulae complex, preorbitalis, levator palatoquadrati, and spiracularis muscles. The adductor mandibulae complex adducts the jaws and is composed of three divisions, all of which originate on the palatoquadrate (upper jaw) and insert onto Meckel's cartilage (lower jaw). The medial division surrounds the mouth opening, with lateral divisions 1 and 2 adjacent to it along the mandibular arch (**Figures 10** and **11**, and **Table 3**). There is great variation in the adductor mandibulae complex among batoids, with both fusions and further subdivisions common among these divisions.

The adductor mandibulae complex is partially overlain by the preorbitalis, which originates on the nasal capsule and inserts onto the lower jaw via a tendon that joins the adductor mandibulae complex (**Figures 10** and **11**, and **Table 3**). The preorbitalis both adducts the lower jaw and protrudes the upper. The levator palatoquadrati and the spiracularis originate on the otic region of the chondrocranium. The levator palatoquadrati inserts onto and elevates the upper jaw. The spiracularis inserts onto the spiracular cartilage and distal tip of the hyomandibular cartilage to form the anterior


(a) Suction pump

(b) Inspiration


(c) Force pump

(d) Expiration

Figure 8 Mechanics of gill ventilation via the suction-force pump in the spiny dogfish *Squalus acanthias* seen in lateral (a, c) and frontal (b, d) sections of the pharynx. Relative pressures are indicated by + and –. Narrow black arrows indicate the flow of water that entered the pharynx through the mouth, while dashed black arrows indicate that which has entered through the spiracle. Orange arrows indicate muscle activity and wide black arrows depict movements of the hyoid. From Liem KF, Bemis WE, Walker WF, Jr., and Grande L (2001) *Functional Anatomy of the Vertebrates: An Evolutionary Perspective*, 3rd edn. Belmont, CA: Brooks/Cole-Thomson Learning.

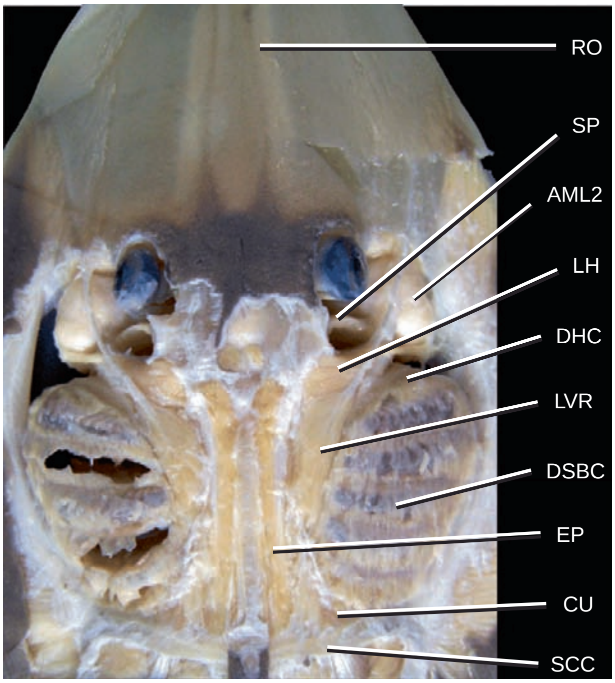


Figure 9 Epaxial and branchiomeric cranial muscles of the southern guitarfish *Rhinobatos percellens* in dorsal view. See **Table 1** for abbreviated terms.

Table 3 Origins, insertions, and actions of the cranial muscles of batoids

Muscle	*Origin*	*Insertion*	*Action*
Adductor mandibulae lateral 1	Palatoquadrate cartilage	Meckel's cartilage	Adduct lower jaw
Adductor mandibulae lateral 2	Palatoquadrate cartilage	Meckel's cartilage	Adduct lower jaw
Adductor mandibulae medial	Palatoquadrate cartilage	Meckel's cartilage	Adduct lower jaw
Branchial trematic constrictor	Propterygium of pectoral fin	Gill slits	Compress gill pouch
Coracoarcualis	Scapulocoracoid cartilage	Coracomandibularis, corachyoideus	Expand oropharyngeal cavity
Coracohyoideus	Coracoarcualis	Basihyal cartilage	Abduct hyoid arch
Coracohyomandibularis	Hypobranchial muscles and raphe	Hyomandibular cartilage	Abduct hyomandibular cartilage
Coracomandibularis	Coracoarcualis	Meckel's cartilages	Abduct lower jaw
Cucullaris	Scapulocoracoid cartilage	Propterygium of pectoral fin	Elevate pectoral fin
Depressor hyomandibularis	Hypobranchial muscles and raphe	Hyomandibular cartilage	Abduct hyomandibular cartilage
Depressor mandibulae	Hypobranchial muscles and raphe	Meckel's cartilage	Abduct lower jaw
Depressor rostri	Hypobranchial muscles and raphe	Rostrum	Depress rostrum
Dorsal hyoid constrictor	Dorsal superficial branchial constrictor	First Gill pouch	Compress gill pouch
Dorsal oblique	Chondrocranium	Eyeball	Rotate eyeball
Dorsal rectus	Chondrocranium	Eyeball	Rotate eyeball
Dorsal superficial branchial constrictor	Cucullaris	Dorsal hyoid constrictor	Compress gill pouches
Epaxialis	Scapulocoracoid cartilage	Chondrocranium	Elevate chondrocranium
Interbranchial	Hypobranchial muscles	Gill arch	Adduct gill arch
Lateral rectus	Chondrocranium	Eyeball	Rotate eyeball
Levator hyomandibulae	Chondrocranium	Hyomandibular cartilage	Elevate hyomandibular cartilage
Levator palatoquadrati	Chondrocranium	Palatoquadrate cartilage	Elevate upper jaw
Levator rostri	Chondrocranium, epibranchial musculature	Rostrum	Elevate rostrum
Medial rectus	Chondrocranium	Eyeball	Rotate eyeball
Preorbitalis	Chondrocranium	Meckel's cartilages	Adduct and elevate jaws, protrude upper jaw
Spiracularis	Chondrocranium	Spiracular and hyomandibular cartilages	Close spiracular valve
Ventral hyoid constrictor	Ventral superficial branchial constrictor	First Gill pouch	Compress gill pouch
Ventral oblique	Chondrocranium	Eyeball	Rotate eyeball
Ventral rectus	Chondrocranium	Eyeball	Rotate eyeball
Ventral superficial branchial constrictor	Propterygium of pectoral fin	Ventral hyoid constrictor	Compress gill pouches

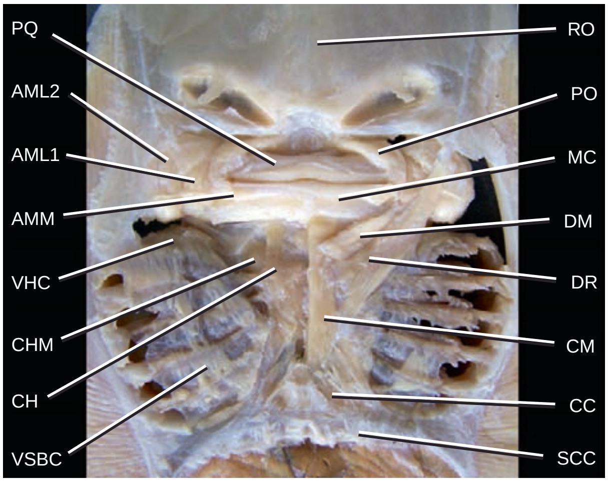


Figure 10 Branchiomeric and hypobranchial cranial muscles of the southern guitarfish *Rhinobatos percellens* in ventral view. See **Table 1** for abbreviated terms.

wall of the spiracle, which is closed via contraction of this muscle (**Figures 6** and **9**, and **Table 3**).

The branchiomeric muscles of the hyoid arch include the dorsal and ventral hyoid constrictors, levator hyomandibulae, depressor rostri, levator rostri, depressor mandibularis, and depressor hyomandibularis. The dorsal and ventral hyoid constrictors originate on the respective superficial branchial constrictors, and they unite medially to form the anterior wall of the branchial chamber, which they compress (**Figures 9** and **10**, and **Table 3**). The levator hyomandibulae originates on the otic region of the chondrocranium and inserts onto the hyomandibulae, which it retracts following a gape cycle (**Figures 6** and **9**, and **Table 3**). The rostrum is moved by the depressor and levator rostri muscles. Dorsally, the levator rostri originates on the epaxial musculature and otic capsule, and it inserts via a stout tendon between the nasal capsule and rostral cartilage. The depressor rostri originates along the ventral surface of the hypobranchial musculature and hypobranchial raphe, and it inserts onto the rostrum via a long tendon (**Figures 9–11** and **Table 3**). The depressor mandibularis appears to be a division of the depressor rostri, which inserts onto and assists in the abduction of the lower jaw (**Figures 10** and **11**, and **Table 3**). The depressor hyomandibularis originates along the ventral surface of the hypobranchial musculature, inserts onto the distal tips of the hyomandibular cartilages, and abducts these structures.

The branchiomeric muscles of the branchial arches include the dorsal and ventral superficial branchial constrictors, branchial trematic constrictor, interbranchial, and cucullaris muscles. The dorsal and ventral superficial branchial constrictors surround the gill arches. The ventral division originates on the propterygium of the pectoral fin, whereas the dorsal division originates on the cucullaris. These muscles insert onto each other and their respective hyoid constrictors, and they compress the gills (**Figures 9–11** and **Table 3**). Laterally, the branchial trematic constrictor originates under the propterygium, inserts onto the superficial branchial constrictors, and also compresses the gills. The interbranchial muscles originate on the coracohyoideus and coracohyomandibularis, and insert onto and adduct the gill arches. The cucullaris originates on the scapulocoracoid, inserts on the fifth dorsal superficial branchial constrictor, and elevates the pectoral fin (**Figure 9** and **Table 3**). Although not found in *R. percellens*, many batoids possess branchial adductor muscles that originate and insert upon the epibranchial and ceratobranchial cartilages, and adduct these structures.

The hypobranchial muscles include the coracoarcualis, coracohyoideus, coracomandibularis, and coracohyomandibularis. The coracoarcualis originates on the scapulocoracoid cartilage, and provides support for the origin of the coracohyoideus and coracomandibularis muscles, assisting these muscles in abducting the oropharyngeal

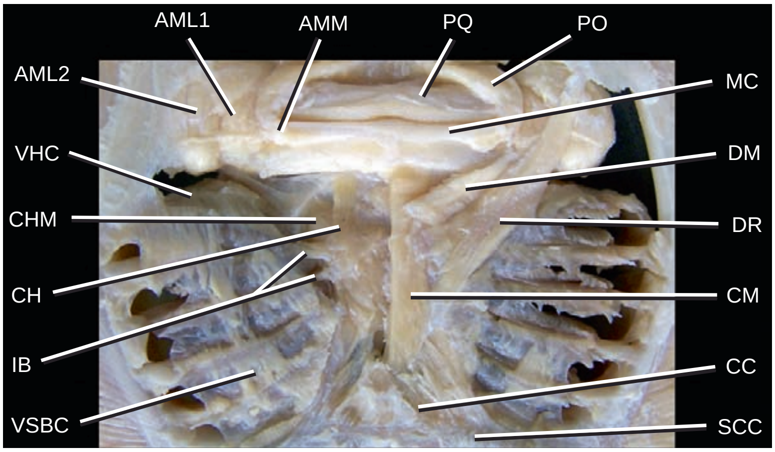


Figure 11 Close-up of the branchiomeric and hypobranchial cranial muscles of the southern guitarfish *Rhinobatos percellens* in ventral view. See **Table 1** for abbreviated terms.

cavity (**Figures 10** and **11**, and **Table 3**). The coracohyoideus and coracomandibularis insert onto, and abduct, the basihyal cartilage and lower jaw, respectively (**Figures 10** and **11**, and **Table 3**). The coracohyomandibularis originates along the hypobranchial musculature and raphe ventral to the gill arches, and it inserts through a tendon onto the hyomandibulae, further aiding the depressor hyomandibularis in abducting the structures (**Figures 10** and **11**, and **Table 3**).

Holocephalan Musculature

The epibranchial muscles of holocephalans fishes originate upon the epaxial muscle mass and insert onto the chondrocranium (**Figures 12–14** and **Table 4**). They contribute minimally to cranial elevation owing to the relatively akinetic cranial skeleton of holocephalans. The branchiomeric muscles of the mandibular arch include the labialis anterior, intermandibularis, levator anguli oris anterior and posterior, and adductor mandibulae anterior and posterior. The labialis anterior originates, and inserts upon, the premaxillary and superior maxillary cartilages, respectively. The intermandibularis originates upon the inferior maxillary cartilage and inserts onto the premandibular cartilages, a pair of fibrocartilaginous masses located at the lower jaw symphysis (**Figure 12** and **Table 4**). Contraction of these muscles protracts the upper and lower sets of labial cartilages. The levator anguli oris anterior and posterior muscles originate upon the antorbital crest of the chondrocranium and insert onto the superior maxillary cartilage, from where they retract the labial cartilages via a series of ligamentous attachments (**Figure 12** and **Table 4**). Deep to the levator anguli oris muscles, the adductor mandibulae anterior has a broad origin across the surface of the preorbital region of the chondrocranium. It is the primary jaw adductor and inserts via a stout tendon that wraps beneath the lower jaw. The adductor mandibulae posterior originates upon the suborbital ridge of the chondrocranium and its tendon merges with that of the adductor mandibulae anterior (**Figures 13** and **14**, and **Table 4**).

Branchiomeric muscles of the hyoid arch include the dorsal and ventral opercular constrictors, levator hyoideus, and interhyoideus muscles. The dorsal opercular constrictor originates from the vertebral column and pectoral girdle. The ventral opercular constrictor originates via the midventral raphe on the ventral surface of the pharyngeal cavity and inserts with the dorsal division onto the tendinous sheath overlying the opercular flap from where they compress the pharyngeal cavity (**Figure 12** and **Table 4**). The levator hyoideus originates from the ventral surface of the chondrocranium beneath the orbit and inserts onto the epihyal cartilage (**Figures 13** and **14**, and **Table 4**). The interhyoideus originates upon the symphysis of the lower jaw and inserts onto the ceratohyal cartilages (**Figure 13** and **Table 4**). These muscles abduct the hyoid arch. The

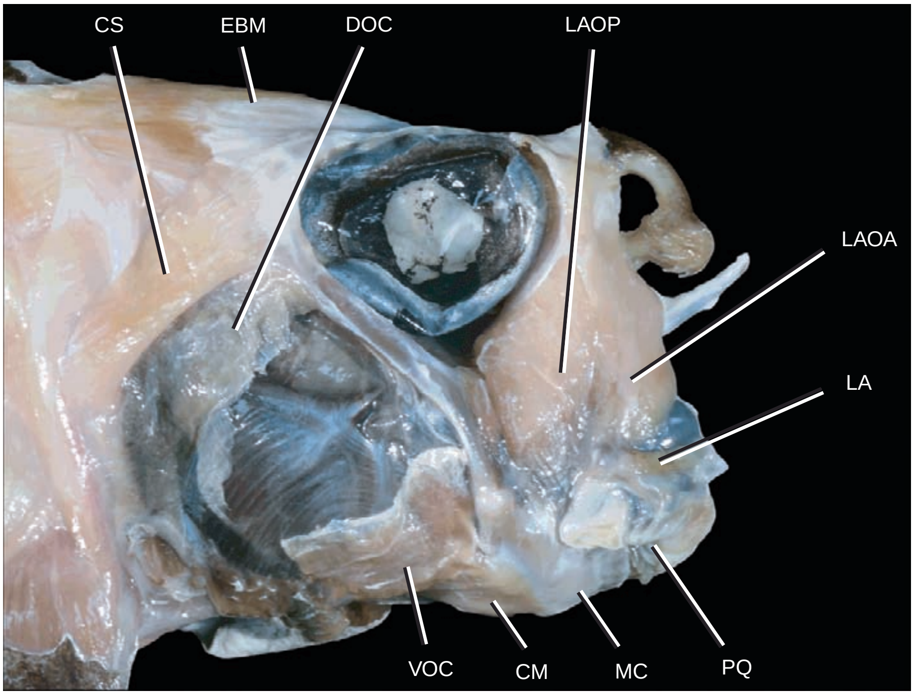


Figure 12 Epibranchial and superficial branchiomeric cranial muscles of the spotted ratfish *Hydrolagus colliei* in lateral view. See **Table 1** for abbreviated terms.

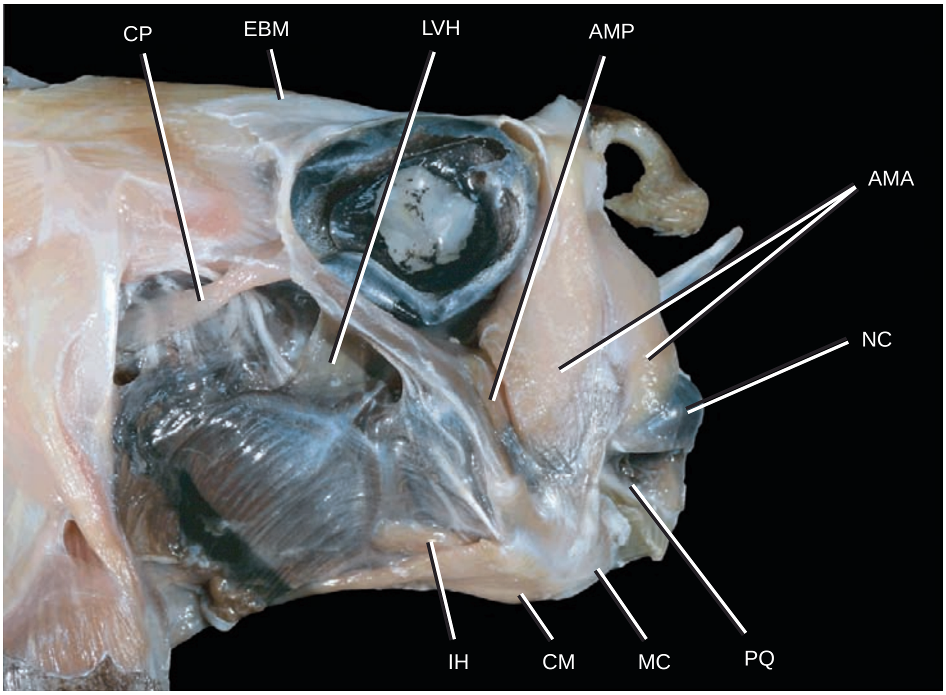


Figure 13 Epibranchial and deep branchiomeric cranial muscles of the spotted ratfish *Hydrolagus colliei* in lateral view. See **Table 1** for abbreviated terms.

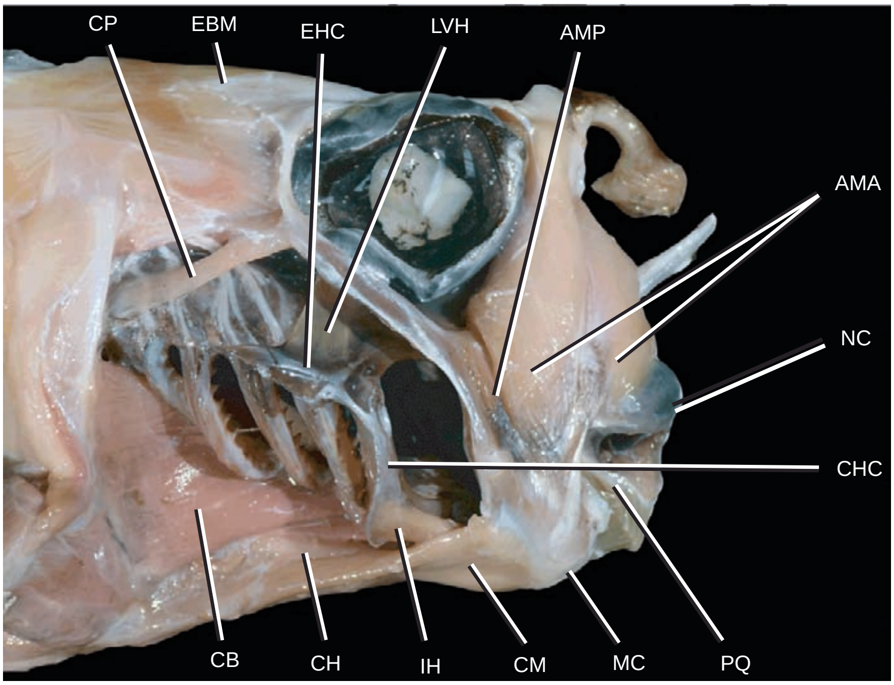


Figure 14 Epibranchial, deep branchiomeric, and hypobranchial cranial muscles of the spotted ratfish *Hydrolagus colliei* in lateral view. See **Table 1** for abbreviated terms.

levator hyoideus may assist in returning the hyoid arch to its resting position following a gape or respiratory cycle.

Branchiomeric muscles of the branchial arches include the constrictor branchialis, adductor arcuum branchialium, cucullaris superficialis, and cucullaris profundus. The constrictor branchialis originates, and inserts upon, the epibranchial and ceratobrancial cartilages of the first three branchial arches, respectively. The adductor

Table 4 Origins, insertions, and actions of the cranial muscles of Holocephalans

Muscle	*Origin*	*Insertion*	*Action*
Adductor arcuum branchialium	Epibranchial, pharyngobranchial cartilages	Ceratobranchial cartilage	Adduct gill arch
Adductor mandibulae anterior	Chondrocranium	Meckel's cartilage	Adduct lower jaw
Adductor mandibulae posterior	Chondrocranium	Meckel's cartilage	Adduct lower jaw
Constrictor branchialis	Epibranchial cartilag	Ceratobranchial cartilage	Adduct gill arch
Constrictor operculi dorsalis	Vertebral column, pectoral girdle	Tendinous sheath of operculum	Compress phayrngeal cavity
Constrictor operculi ventralis	Midventral raphe	Tendinous sheath of operculum	Compress phayrngeal cavity
Coracobranchialis	Pectoral girdle	Ceratobranchial cartilages	Abduct branchial arches
Coracohyoideus	Coracomandibularis	Basihyal cartilage	Abduct hyoid arch
Coracomandibularis	Pectoral girdle	Meckel's cartilage	Abduct lower jaw
Cucullaris profundus	Chondrocranium	Pectoral girdle	Elevate and rotate pectoral girdle
Cucullaris superficialis	Chondrocranium	Pectoral girdle	Elevate and rotate pectoral girdle
Dorsal oblique	Chondrocranium	Eyeball	Rotate eyeball
Dorsal rectus	Chondrocranium	Eyeball	Rotate eyeball
Epibranchial	Epaxialis	Chondrocranium	Elevate chondrocranium
Interhyoideus	Meckel's cartilage	Ceratohyal cartilage	Abduct hyoid arch
Intermandibularis	Inferior maxillary cartilage	Premandibular cartilage	Protract labial cartilages
Labialis anterior	Premaxillary cartilage	Superior maxillary cartilage	Protract labial cartilages
Lateral rectus	Chondrocranium	Eyeball	Rotate eyeball
Levator anguli oris anterior	Chondrocranium	Superior maxillary cartilage	Retract labial cartilages
Levator anguli oris posterior	Chondrocranium	Superior maxillary cartilage	Retract labial cartilages
Levator hyoideus	Chondrocranium	Epihyal cartilage	Elevate epihyal cartilage
Medial rectus	Chondrocranium	Eyeball	Rotate eyeball
Ventral oblique	Chondrocranium	Eyeball	Rotate eyeball
Ventral rectus	Chondrocranium	Eyeball	Rotate eyeball

arcuum branchialium has a complex origination from the epibranchial and pharyngobranchial cartilages, and inserts onto the ceratobranchial cartilages of the first four branchial arches. Both of these muscles adduct the branchial arches. The cucullaris superficialis and profundus originate upon the postorbital region of the chondrocranium, and insert onto the scapular process of the pectoral girdle, which they elevate and rotate (**Figures 12–14** and **Table 4**).

The hypobranchial muscles of holocephalans include the coracomandibularis, coracohyoideus, and coracobranchialis. The coracomandibularis originates on the coracoid bar of the pectoral girdle, and inserts onto, and abducts the lower jaw (**Figures 12–14** and **Table 4**). The coracohyoideus originates on the coracomandibularis and inserts onto the basihyal cartilage, which it abducts. The coracobranchialis has multiple divisions that originate on the coracoid bar, insert onto the ceratobranchial cartilages, and abduct the branchial arches (**Figure 14** and **Table 4**).

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **The Muscles**: Bony Fish Cranial Muscles. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Dean M and Motta PJ (2004) Anatomy and functional morphology of the feeding apparatus of the lesser electric ray, *Narcine basiliensis* (Elasmobranchii: Batoidea). *Journal of Morphology* 262: 462–483 (doi:10.1002/jmor.10245).

Didier DA (1995) Phylogenetic systematic of extant chimaeroid fishes (Holocephali, Chimaeroidei). *American Museum Novitates* 3119: 1–86.

Fishbeck DW and Sebastiani AS (2008) *Manual of Vertebrate Dissection: Comparative Anatomy*, 2nd edn. Englewood, CO: Morton Publishing.

González-Isáis M and Domínguez HMM (2004) Comparative anatomy of the superfamily Myliobatoidea (Chondrichthyes) with some comments on phylogeny. *Journal of Morphology* 262: 517–535 (doi: 10.1002/jmor.10260).

Huber DR, Dean MN, and Summers AP (2008) Hard prey, soft jaws and the ontogeny of feeding mechanics in the spotted ratfish *Hydrolagus colliei. Journal of the Royal Society Interface* 5: 941–952 (doi:10.1098/rsif.2007.1325).

Huber DR, Eason TG, Hueter RE, and Motta PJ (2005) Analysis of the bite force and mechanical design of the feeding mechanism of the durophagous horn shark *Heterodontus francisci. Journal of Experimental Biology* 208: 3553–3571 (doi: 10.1242/jeb.01816).

Liem KF, Bemis WE, Walker WF, Jr, and Grande L (2001) *Functional Anatomy of the Vertebrates: An Evolutionary Perspective,* 3rd edn. Belmont, CA: Brooks/Cole-Thomson Learning.

Liem KF and Summers AP (1999) Gross anatomy and functional morphology of muscles. In: Hamlett WC (ed.) *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*, pp. 93–114. Baltimore, MD: Johns Hopkins University Press.

Mallatt J (1997) Shark pharyngeal muscles and early vertebrate evolution. *Acta Zoologica* 78(4): 279–294.

Miyake T (1988) The Systematics of the Stingray Genus *Urotrygon* with Comments on the Interrelationships within Urolophidae (Chondrichthyes: Myliobatiformes). PhD Thesis, Texas A&M University.

Miyake T, McEachran JD, and Hall BK (1992) Edgerworth's legacy of cranial muscle development with an analysis of muscles in the ventral gill arch region of batoid fishes (Chondrichthyes: Batoidea). *Journal of Morphology* 212: 213–256.

Motta PJ (2004) Prey capture behavior and feeding mechanics of elasmobranchs. In: Carrier J, Musick J, and Heithaus M (eds.) *Biology of Sharks and Their Relatives*, pp. 165–202. Boca Raton, FL: CRC Press.

Motta PJ and Wilga CD (1995) Anatomy of the feeding apparatus of the lemon shark, *Negaprion brevirostris. Journal of Morphology* 226: 309–329 (doi:10.1002/jmor.1052260307).

Motta PJ and Wilga CD (1999) Anatomy of the feeding apparatus of the nurse shark. *Ginglymostoma cirratum. Journal of Morphology* 241: 1–29. doi: 10.1002/(SICI)1097-4687(199907)241:1<33::AID-JMOR3>3.0.CO; 2-1.

Nishida K (1990) Phylogeny of the suborder Myliobatidoidei. *Memoirs of the Faculty of Fisheries of Hokkaido University* 37: 1–108.

Wilga CD (2005) Morphology and evolution of the jaw suspension in lamniform sharks, *Journal of Morphology* 265: 102–119 (doi:10.1002/jmor.10342).

Wilga CD and Motta PJ (1998) Conservation and variation in the feeding mechanism of the spiny dogfish *Squalus acanthias. Journal of Experimental Biology* 201: 1345–1358.

Wilga CD and Motta PJ (1998) Feeding mechanism of the Atlantic guitarfish *Rhinobatos lentiginosus:* Modulation and kinematic motor activity. *Journal of Experimental Biology* 201: 3167–3184.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Bony Fish Cranial Muscles

LA Ferry, Arizona State University, Phoenix, AZ, USA

LP Hernandez, George Washington University, Washington, DC, USA

© 2011 Elsevier Inc. All rights reserved.

Overview
Buccal Pumping
Suction Feeding
Premaxillary Protrusion
Biting/Tearing
Pharyngeal Jaw Processing
Further Reading

Glossary

Buccal pumping A two-stage cycle of expansion and compression used to generate water flow into the mouth and over the gills for respiration.

Jaw protrusion The anterior movement of the upper jaw or premaxilla. Premaxillary protrusion contributes to the expansive phase of suction feeding and is thought to enhance suction generation compared with species that cannot protrude the jaws.

Key innovation The evolutionary novelties that have a disproportionate effect (overwhelmingly positive) on the subsequent radiation of the species that possess them.

Prey capture The act of entrapping prey within the jaws or buccal cavity such that it will subsequently be ingested; the beginning is kinematically defined as the moment of jaw opening.

Prey processing Those behaviors that follow prey capture that mechanically break down or reposition the food prior to chemical digestion.

Overview

The cranial muscles of bony fishes can be divided into several groups based largely on their anatomical location: cheek muscles, body muscles, ventral cranial muscles, pharyngeal jaw muscles, and muscles associated with the pectoral girdle. This categorization is convenient for describing muscles based on their origins and insertions, but is not terribly informative for understanding functional units. Below are very generalized descriptions of the more common functions performed by or within the head, and those muscles responsible for these functions, including buccal pumping (ventilation), suction feeding, premaxillary protrusion, biting and tearing, and pharyngeal jaw prey processing.

Buccal Pumping

Of central importance to fish survival is respiration. In order for oxygen to be delivered to the tissues, most fishes, excepting those that possess various forms of air breathing organs (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction), must somehow bring water into the mouth and move it over the gills, facilitating the diffusion of oxygen from the water and into the blood. This is often termed gill ventilation.

Some species are able to utilize what is commonly referred to as ram ventilation to ventilate the gills. In ram ventilation, the animal uses forward propulsion and water passively enters the mouth, which is held open. Water continues over the gills, which are similarly abducted to avoid impediments to flow, and exits the opercular openings. A small handful of fishes, such as the tunas and other constantly active species, are obligate ram ventilators and rely on ram ventilation exclusively. This includes some shark species. However, contrary to popular myths, most sharks do not rely on ram ventilation and, therefore, do not need to swim constantly for this purpose.

The vast majority of fish species use buccal pumping for gill ventilation, whereby a current of water is drawn into the mouth and over the gills via actively generated movements of skeletal elements within the head. Benthic and sedentary species often rely exclusively on buccal pumping. Many species can readily alternate between buccal pumping and ram ventilation as conditions and swimming speeds allow.

Buccal pumping is usually described as a two-stage cycle of expansion and compression. Expansion must occur gradually, from anterior to posterior, for a current to be generated in this direction. Thus, buccal pumping can be thought of as an abbreviated form of suction generation as used for capturing aquatic prey (described next). The head of the fish is effectively divided into two chambers divided by the respiratory structures, the gills. The anterior chamber is the oral or buccal chamber, whose volume is controlled largely by mouth opening and hyoid depression. The posterior chamber is the opercular chamber that lies posterior to the gills, controlled by abduction of the gill bars and opercular opening.

During active buccal pumping, mouth opening and hyoid depression are facilitated largely by the sternohyoideus muscle (**Figure 1** and **Table 1**). This is the first stage in the two-stage cycle, and water is drawn into the mouth during this stage. Stage 2 consists of opercular expansion caused by contraction of the levator operculi and the dilatator operculi (**Figure 1**). During this second stage, water flows over the branchial arches and into the opercular region where it then exits. Note that the branchial arches are expanded as water flows from the anterior chamber of the mouth into the branchial apparatus and out the operculi. Much of the expansion of the branchial apparatus is thought to be passive, facilitated by the water itself. However, during very active ventilation, the pectoral girdle (via contraction of the hypaxial musculature) and ventral branchial muscles may be actively engaged to assist in expanding this region more fully (**Figure 2**).

During mouth opening, or stage 1, the branchial arches must be actively compressed and the operculi held closed in

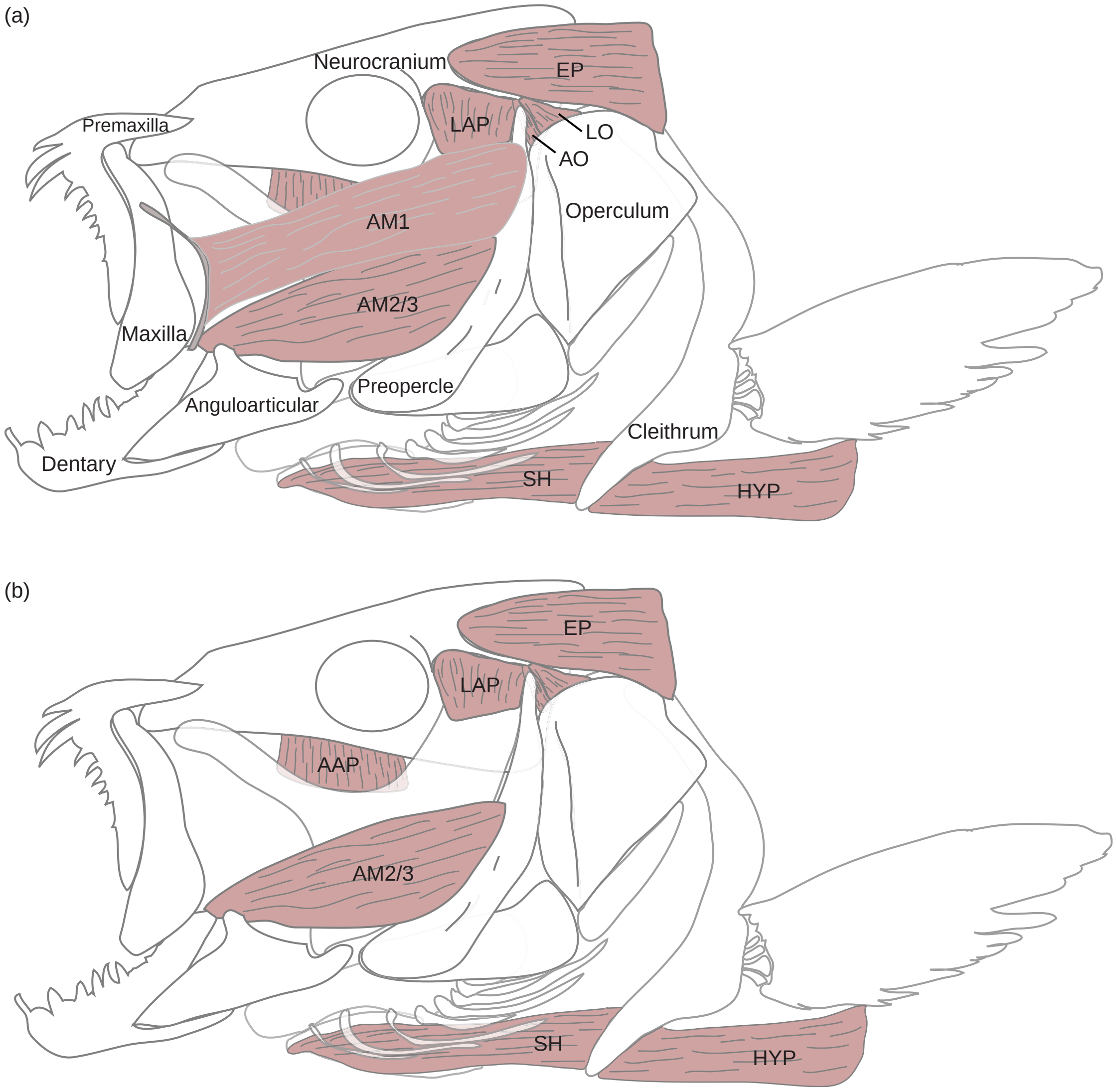


Figure 1 Musculature of the body and cheek region for a highly generalized teleostean fish. (a) The more superficial muscles. (b) The AM1 has been removed to reveal more clearly the more deeply situated AAP. Key bony elements are labeled for orientation. Muscle abbreviations present in the figure are explained in **Table 1**.

Table 1 Generalized list of muscles typically found in the heads of bony fishes, or that contribute to behaviors associated with the head, such as nearby body musculature

Muscle group	*Muscle (abbreviation as in figures)*	*Origin*	*Insertion*	*Function*
Body muscles				
	Epaxials (EP)		Back of neurocranium	Cranial elevation, as in the expansive phase of suction feeding and in some forms of premaxillary protrusion.
	Hypaxials (HYP)		Pectoral girdle/cleithrum	
Cheek muscles				
	Adductor mandibulae 1 (AM1)	Preopercle	Lower jaw and sometimes the upper jaw	Jaw closure, as in the compressive phase of suction feeding, biting, or oral jaw processing.
	Adductor mandibulae 2 (AM2)	Preopercle and suspensorium	Dentary and/or anguloarticular	Jaw closure, as in the compressive phase of suction feeding, biting, or oral jaw processing.
	Adductor mandibulae 3 (AM3)	May be shared with AM2	Medial side of the articular	Jaw closure, as in the compressive phase of suction feeding, biting, or oral jaw processing.
	Levator arcus palatini (LAP)	Posterior neurocranium (e.g., sphenotic and frontal)	Hyomandibula	Lifts the hyoid complex, as in the expansive phase of suction feeding
	Levator operculi (LO)	Postorbital region of the neurocranium	Opercle	Opercular expansion, as in during ventilation and in the expansive phase of suction feeding.
	Dilatator operculi (DO)	Posterolateral edge of neurocranium	Opercle	Opercular expansion, as in during ventilaton and in the expansive phase of suction feeding
	Adductor arcus palatini (AAP); Adductor hyomandibula	Parasphenoid and prootic	Hyomandibula and metapterygoid	Head compression, as in the preparatory and compressive phases of suction feeding.
	Adductor operculi (AO)	Auditory region of the neurocranium	Opercle	Opercular compression, as in the preparatory phase of suction feeding.
Ventral muscles				
	Intermandibularis (IM)	Ventral midline between dentaries	Dentaries	Slight medial adduction of dentaries
	Geniohyoideus (GH)/Protractor hyoideus (PH) (considered synonyms)	Hyoid arch	Dentaries	Primarily expansive role during suction feeding. May show division of labor and contribute to both mouth opening and closing, as in during ventilation.
	Hyohyoideus (HYH)	Ventral midline or urohyal	Hyoid arch	Maintains tension on the hyoid arch.
Branchial arch muscles				
	Levators interni and externi (LI, LE)	Posterolateral edge of neurocranium	Epibranchials	Brace and lift branchial arches
	Transversi ventrales (TV)			Connect the right and left sides of the branchial arches ventrally for stabilization.
	Adductorbranchialis (AD)	Epibranchials	Ceratobranchial	Connects dorsal and ventral portions of the branchial arches for compression

(*Continued*)

Table 1 (Continued)

Muscle group	*Muscle (abbreviation as in figures)*	*Origin*	*Insertion*	*Function*
Pharyngeal Jaw muscles				
	Levator internus 3 (LI)	Neurocranium	Upper pharyngeal jaw, typically pharyngobranchial 3	Elevates and moves the upper pharyngeal jaw anteriorly, serving to protract the jaws.
	Levator externus 4 (LE 4)	Neurocranium	Epibranchial 4	In most conformations causes EB4 to rotate, thereby depressing the upper pharyngeal jaw.
	Levator posterior (LP)	Neurocranium	Generally epibranchial 4; ceratobranchial 5 in some species	Action supplements levator externus 4
	Obliquus dorsalis (OD)	Pharyngobranchial 3	Epibranchial 4	Connects PB3 and EB4 dorsally, allowing flexion at the joint between PB3 and EB4
	Obliquus posterior (OP)	Epibranchial 4	Ceratobranchial 5	Pulls EB4 ventrally
	Retractor dorsalis (RD)	Anterior vertebrae	Epibranchial 4	Retracts entire pharyngeal jaw apparatus posteriorly
	Pharyngocleithralis internus (PCI)	Pectoral girdle	Posterioventral portion of the lower pharyngeal jaw, ceratobranchial 5	Rocks the lower pharyngeal jaw posteriorly during prey processing.
	Pharyngocleithralis externus (PCE)	Pectoral girdle	Anteroventral portion of ceratobranchial 5	Rocks the lower pharyngeal jaw anteriorly during prey processing, serving to open the jaws.
Muscles associated with the pectoral girdle				
	Sternohyoideus (SH)	Cleithrum	Urohyal	Hyoid and lower jaw depression, as in ventilation and the expansive phase of suction feeding.
	Protractor pectoralis (PP)	Hypaxial musculature	Cleithrum	Protraction of the pectoral girdle, assists in hyoid and lower jaw depression

The typical conformation is provided, though interesting and/or common deviations may be noted.

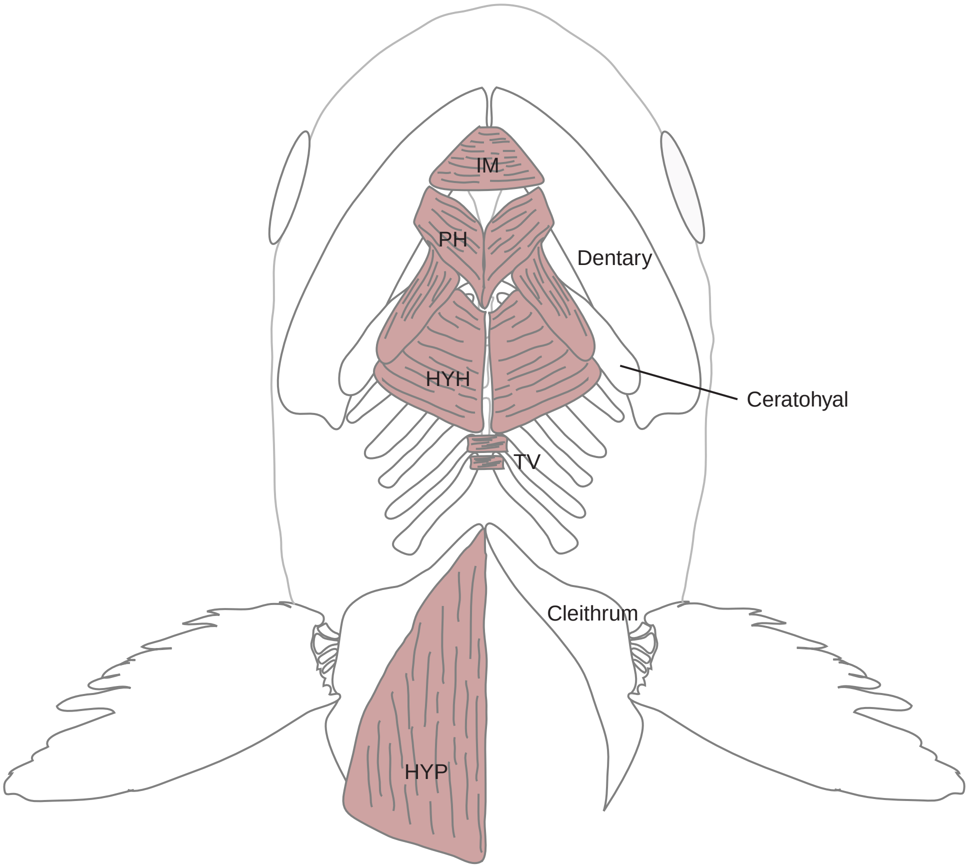


Figure 2 Ventral musculature for a highly generalized teleostean fish. Key bony elements are labeled. Muscle abbreviations present in the figure are explained in **Table 1**.

order to prevent water from entering at the gills and flowing in the wrong direction, from posterior to anterior. Such movement of the gills is due to activation of the levators externus, internus, and posterior (**Figure 3**(**a**)), which pull and contract the branchial basket dorsally. Moreover, the adductors bring the ventral portion of the basket toward the dorsal portion. This action is assisted by the muscles that compress the buccal chamber by moving the operculi medially and essentially holding them closed, mainly the adductor operculi. Similarly, during stage 2, when the branchial and opercular regions are open and expanded, the mouth must be closed again so that water moves posteriorly and does not exit the open mouth. Mouth closure is the role of the adductor mandibulae complex (see **Figure 1**).

Though the above describes the dominant mechanism among fishes, there are extensive variations on this theme. For example, during periods of shallow or very calm ventilation, the muscles associated with jaw opening are scarcely active or not active at all. Relaxation of the adductor mandibulae complex will cause the mouth to open slightly, as anyone who has held a recently anesthetized or euthanized fish can attest, and this degree of opening is sufficient for generating a small current of water into the mouth when oxygen demand is suitably low. Similarly, while the maintenance of an anterior to posterior current of water through the head is necessary for maximizing oxygen extraction at the gills, many species show periods where this current is slowed or even reversed, particularly elasmobranchs. This is also almost certainly linked with periods of low oxygen demand.

Fishes will also cough to clear the gills of noxious substances or irritants, or simply to remove excess particles or mucus. A fish cough is essentially the movements outlined here in reverse.

Suction Feeding

Suction feeding is the primary mode of prey acquisition for aquatic species. Suction feeding consists of the generation of currents of water into the head, hopefully with a prey item or items entrained in the flow (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics). Suction is a highly flexible mode of aquatic prey capture that works on a wide variety of unattached prey items, and even some attached prey. It is

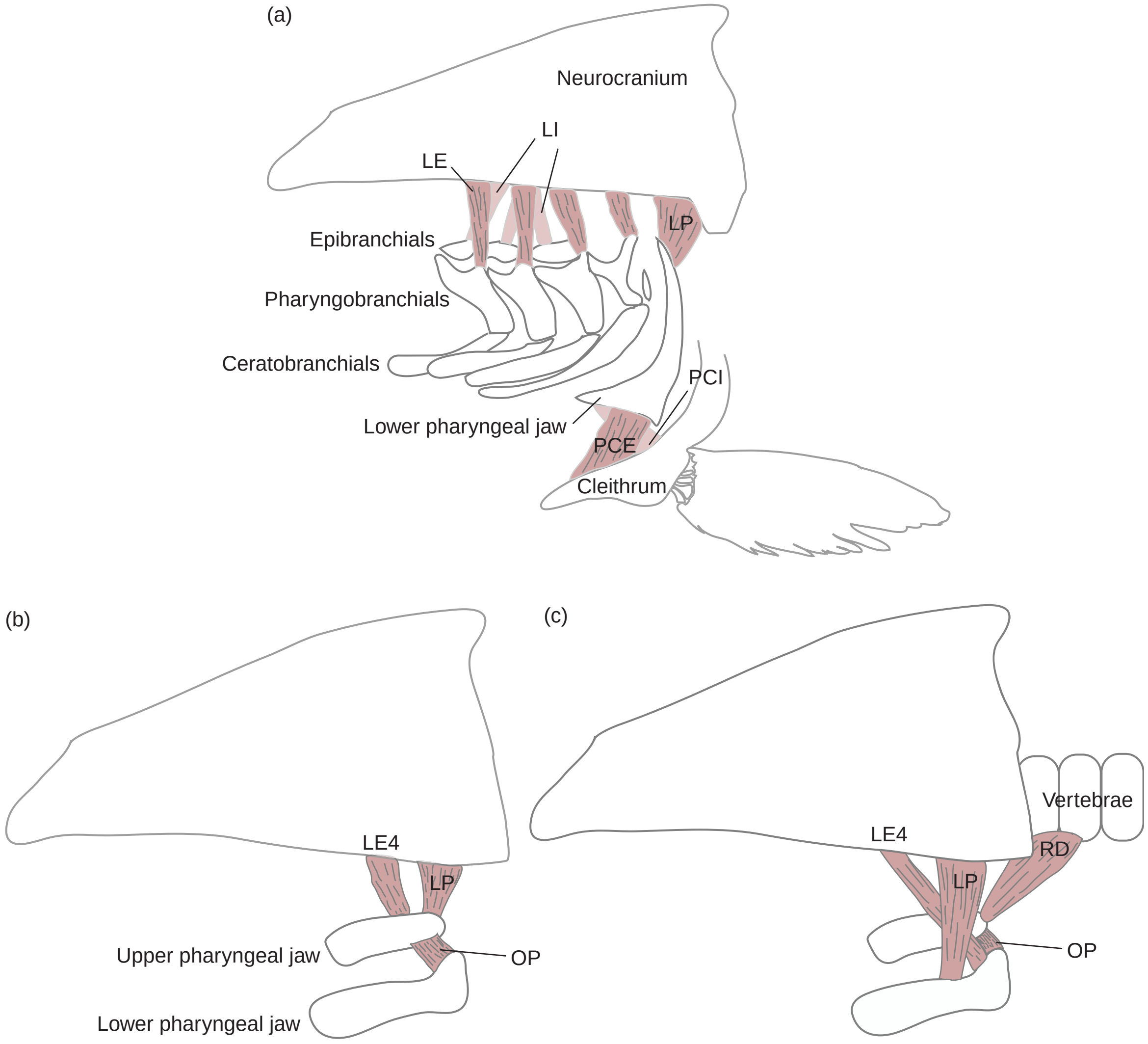


Figure 3 Branchial and pharyngeal jaw muscles. (a) The pharyngeal jaw configuration of a typical cyprinid is shown, and the muscles associated with the remainder of the branchial apparatus are also included (which are broadly consistent among teleosts). Note the pharyngeal sling, composed of the levator posterior muscle, associated with most cyprinids. (b) A stereotypical pharyngognath condition is indicated with upper and lower pharyngeal jaws shown. (c) The specialized pharyngeal sling is shown, formed by the LP and LE4, as found in the labrid fishes. The positions of other muscles in (c) are fairly general among pharyngognath fishes. Key bony elements are labeled. Muscle abbreviations present in the figure are explained in **Table 1**.

especially useful for compensating for forward locomotion of the predator. In the dense and viscous aquatic medium, forward movement creates a bow wave in front of the predator that serves to inadvertently push the desired prey item farther away from the mouth. The alternative to suction feeding is ram feeding, whereby the predator swims forward with an open mouth sufficiently large to engulf the prey and to redirect or overcome any bow wave effects. Suction is only effective a short distance from the mouth, again, because of the physical properties of the aquatic medium. Therefore, suction is often used in combination with ram feeding, biting, scraping, or any number of other feeding behaviors that essentially place the mouth close enough to the intended food item for suction to be utilized.

The hallmark of suction generation is the creation of a current of water moving through the head in an anterior to posterior direction. This behavior is massive and rapid, sometimes described as ballistic, and therefore there are typically none of the inefficiencies described above for ventilation. The generation of this current is broken into four phases, each characterized by a suite of muscle activity. These are the preparatory, expansive, compressive, and recovery phases.

The preparatory phase of suction feeding can essentially be thought of as a phase during which the head is compressed slightly. This phase is present in many, but not all feeding events, and is performed in order to expel any water remaining in the buccal and opercular cavities in preparation for the massive expansion that will occur next. The jaws are closed via the adductor mandibulae complex (**Figure 1**), the head is compressed medially via the adductor arcus palatini, the opercular region is similarly compressed via the adductor operculi. This compression is used to create a relatively negative space, compared with ambient pressure, such that when the mouth is opened during the expansive phase, water will rush in.

The massive and rapid expansive phase is typically characterized by contraction of the sternohyoideus to depress the lower jaw, and the levator arcus palatini to expand the hyoid apparatus (**Figure 1**). As in ventilation, activity may be seen in the hypaxial musculature as well as the protractor hyoideus (also referred to as the geniohyoideus) and the hyohyoideus (**Figure 2**). While these muscles are working to open and expand the mouth and ventral region of the head, the epaxial (body) musculature is commonly recruited for rotating the neurocranium dorsally, thereby expanding the head even further (**Figure 1**). The head is expanded laterally via the contribution of the levator operculi as well as the dilatator operculi. Moreover, the levator operculi has been implicated in mouth opening via the linkage of several opercular elements. Expansion at the opercular region tends to occur slightly later than mouth opening or cranial rotation in order to maintain the anterior to posterior wave of expansion so prevalent in suction feeding, and putatively required to generate the anterior to posterior flow of water into the head. The prey item is effectively captured, or not, during the expansive phase.

The compressive phase follows the expansive phase and during this time the prey item is retained in the oral cavity, while the water that was taken in with the prey is slowly ejected. Therefore, this phase is much like the preparatory phase. The jaws are closed via the adductor mandibulae complex on or around the prey and the head is compressed medially via the adductor arcus palatini. Water may exit the mouth if the food item is held in the jaws and mouth closure is prevented. However, most of the water is directed out of the operculi and the dilatator operculi holds the operculi open (**Figure 1**).

The recovery phase is a period of time characterized by little to no muscular activity, as all of the functional units within the feeding apparatus are returning to their prefeeding positions. This stage may contain prolonged or residual activity of the dilatator operculi as the operculi are being held open for the continued expulsion of water within the head. This stage may be abbreviated depending on the degree to which subsequent transport events are used for moving the prey out of the mouth and into the esophagus. Prey transport is not treated specifically here; however, many studies note that it is a full-body behavior engaging the epaxial and hypaxial musculature of the body (**Figures 1** and **2**).

Premaxillary Protrusion

Premaxillary protrusion is an important element of the expansive phase of suction feeding present in many teleostean lineages. However, it has received so much attention in the literature that it will be treated separately here from the standpoint of the different muscles necessary to create this movement. Indeed, premaxillary protrusion is thought to be a key innovation in fishes responsible, at least in part, for the incredible radiation of fishes into the most diverse group of vertebrates. Various studies suggest that this mechanism has evolved independently a number of times. All of the mechanisms commonly employed result in the protrusion of the premaxilla anteriorly or antero-ventrally, and this is thought to enhance suction production through the creation of a smaller, tubular mouth opening (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics for the specific biomechanical and hydrodynamic consequences of this).

The primary mechanism of premaxillary protrusion relies on lower jaw depression as the input force, and therefore activation of the sternohyoideus (**Figure 1**). Forces from the lower jaw are transferred largely through ligamentous connections to the maxilla, and from there to the premaxilla. The twisting-maxilla model is similar with the addition of rotation or twisting of the maxillae along their long axes in response to lower jaw depression. This places tension on the heads of the maxillae, where they articulate with the premaxillae, and the premaxillae are effectively projected anteriorly. The forces created by depression of the lower jaw are likely supplemented by contributions from cranial elevation, therefore activation of the epaxials. As the cranium is rotated dorsally and posteriorly, ligamentous tension on the premaxilla causes it to effectively slide off the cranium and be protruded anteriorly. The cranial elevation model may work in lieu of, or more typically in combination with, the lower jaw depression model.

Biting/Tearing

Biting and tearing behaviors are often employed to obtain food items that are attached or otherwise part of items too large to be taken into the mouth fully. As mentioned above, biting or tearing may occur in lieu of suction production, as food items are not affected by suction. Or, suction may be subsequently employed to move the dislodged or detached bit of food into the mouth. In species specialized for biting jaw closing muscles are often hypertrophied. These changes represent a trade-off in that the same muscles cannot simultaneously be optimized for force and speed.

Muscles with a broad cross-sectional area are good for force production (because force that can be produced by a muscle area is proportional to its cross-sectional area), while elongate muscles are good for speed. Given that suction is a rapid movement, many species specialized for biting are no longer capable of producing notable suction (though a marginal amount of suction seems possible by virtually any species just by virtue of opening the mouth under water). Bite strength strongly depends on those muscles associated with mouth closure, or the adductor mandibulae complex (**Figure 1**). Activity of the adductor mandibulae complex will tend to alternate with those muscles associated with jaw opening and head expansion: the epaxials, the levator operculi, and the sternohyoideus.

An interesting suite of modifications has arisen in a number of biting lineages, and several of these have evolved multiple times in independent lineages suggesting that they are adaptations to specific feeding challenges posed by different forms of biting. For example, a number of biting and scraping species possess a novel joint within the lower jaw called an intramandibular joint (IMJ). We have yet to determine what specifically controls this joint, be it portions of the adductor mandibulae or perhaps the protractor hyoideus (geniohyoideus), or even if movement at the joint is active or passive. Anatomical studies suggest that there is enough variation among groups that possess this joint, such as some scarids, some pomacanthids, some kyphosids, and some poeciliids, that there is likely more than one mechanism. Similarly, biting species that show complex behaviors for prey processing within the oral jaws, such as winnowing or chewing, sometimes show muscle duplications of the adductor mandibulae complex, as in the case of the tetraodontiform fishes. These duplications may allow for finer control of the mechanism when the muscles are activated individually, as well as a stronger bite when they are activated synchronously.

Pharyngeal Jaw Processing

Most perciform fishes, as well as a handful of other teleostean lineages (notably the cypriniforms), possess modified branchial elements co-opted as a second set of jaws, the pharyngeal jaws (for additional reading see Wainwright). These pharyngeal jaws consist of tooth plates fused typically to pharyngobranchial 3 (the upper pharyngeal jaw, with epibranchial 4) and ceratobranchial 5 (the lower jaw). The pharyngeal jaw apparatus (PJA) is largely responsible for prey-processing behaviors such as chewing and raking, which follow prey capture and mechanically break down the food prior to chemical digestion. When the PJA is present, the oral jaws tend to give up this role and thus the pharyngeal jaws can represent a second, decoupled source of evolutionary innovation related to feeding. Alternatively, the oral jaws can become specialized for prey acquisition while the pharyngeal jaws become specialized for prey processing.

In most fishes it is the upper pharyngeal jaw that is mobile through the action of the pivoting epibranchial bone. It can move in a circular or even figure-8 pattern, with motion in all three dimensions, as it is protracted and retracted, contacting the more (although not entirely) immobile lower pharyngeal jaw as it sweeps past. The protraction of the upper pharyngeal jaw, typically pharyngobranchial 3, is caused by the levator internus associated with this pharyngobranchial (**Figure 3**). Depression is facilitated by the levator externus (4) and the levator posterior (**Figure 3**(**b**)). The levator externus (4) causes the modified epibranchial 4 to pivot, thereby placing force on pharyngobranchial 3 and depressing it. Epibranchial 4 and pharyngobranchial 3 form a joint via the connection of the obliquus dorsalis, and the pivot motion of epibranchial 4 is aided by the obliquus posterior. Retraction of the upper jaw is facilitated largely by the retractor dorsalis (**Figure 3**(**c**)). The lower jaw is typically more restricted in its movement, as it is often more strongly associated with the pectoral girdle. The pharyngocleithralis internus serves to move the lower pharyngeal jaw posteriorly, useful for raking and scraping, and the pharyngocleithralis externus moves the lower jaw anteriorly, thereby contributing to jaw opening.

There is great variation in the elaboration of the PJA. Oddly, cypriniforms have lost the upper pharyngeal jaws and generally use a greatly hypertrophied ceratobranchial 5 (the lower pharyngeal jaw) to masticate against an enlarged basioccipital process (as shown in **Figure 3**(**a**)). Moreover, they have evolved a very large muscular sling (largely composed by the levator posterior whose insertion is believed to have changed) that suspends the lower pharyngeal jaws. Notably, the labrids have convergently evolved a muscular sling that supports the lower pharyngeal jaw and allows for extensive movement (shown in **Figure 3**(**c**)).

See also: **Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics.

Further Reading

Graham JB (1997) *Air-Breathing Fishes: Evolution, Diversity, and Adaptation,* 299pp. San Diego, CA: Academic Press.

Wainwright PC (2006) Functional morphology of the pharyngeal jaw apparatus. In: Shadwick R and Lauder GV (eds.) *Fish Biomechanics*, ch. 3, pp. 77–99. San Diego, CA: Academic Press.

Westneat MW (2006) Skull biomechanics and suction feeding in fish. In: Shadwick R and Lauder GV (eds.) *Fish Biomechanics*, ch. 2, pp. 29–76. San Diego, CA: Academic Press.

Winterbottom R (1974) A descriptive synonymy of the striated muscles of the teleostei. *Proceedings of the Academy of Natural Sciences of Philadelphia* 125: 225–317.

MUSCLES, SKELETON, SKIN, AND MOVEMENT

The Skin

Contents

The Many Functions of Fish Integument
Functional Morphology of the Integumentary System in Fishes
Coloration and Chromatophores in Fishes
Bioluminescence in Fishes
Hagfish Slime

The Many Functions of Fish Integument

DG Elliott, Western Fisheries Research Center, US Geological Survey, Seattle, WA, USA

Published by Elsevier Inc.

Introduction
Protection
Communication and Sensory Perception
Locomotion
Respiration, Ion Regulation, and Excretion
Other Functions
Further Reading

Glossary

Bioactive substance A substance that has an effect on living tissue.
Integument The organ system that covers the external surfaces of a fish's body, including the fins. The integument is comprised of the epidermis and dermis of the skin (including scales, when present), and also includes skin extensions such as barbels and flaps.
Keratinization Process in which the cytoplasm of the outermost epidermal cells is replaced by keratin, a fibrous structural protein that is tough and insoluble when assembled in bundles. Keratinization of the outer skin layers is common in terrestrial vertebrates but uncommon in fishes.
Mucus (adj. mucous) A slippery secretion produced by, and covering, the skin and other mucous membranes of fish. Mucus is elaborated by goblet cells and other cells in fish epidermis, and is comprised of glycoproteins (mucins) and other substances.
Pheromone A secreted or excreted chemical factor that triggers a social response in members of the same species.

Introduction

The integument or skin of fishes has both important similarities to and important differences from the integument of other vertebrates (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes). For fish, as for other vertebrates, the integument is the body envelope that not only separates and protects the animal from its environment, but also provides the means through which most of the contacts with the outer world are made. Fish integument is a large organ that is continuous with the lining of all the body openings, and also covers the fins. In addition to its protective functions, fish skin may serve important roles in communication, sensory perception, locomotion, respiration, ion regulation, excretion, and thermal regulation.

Protection

Numerous integumentary adaptations have evolved for protecting fishes in their aqueous environment. For example, mucous secretions (**Figure 1**) help to keep the skin surface free of pathogens by means of continual sloughing

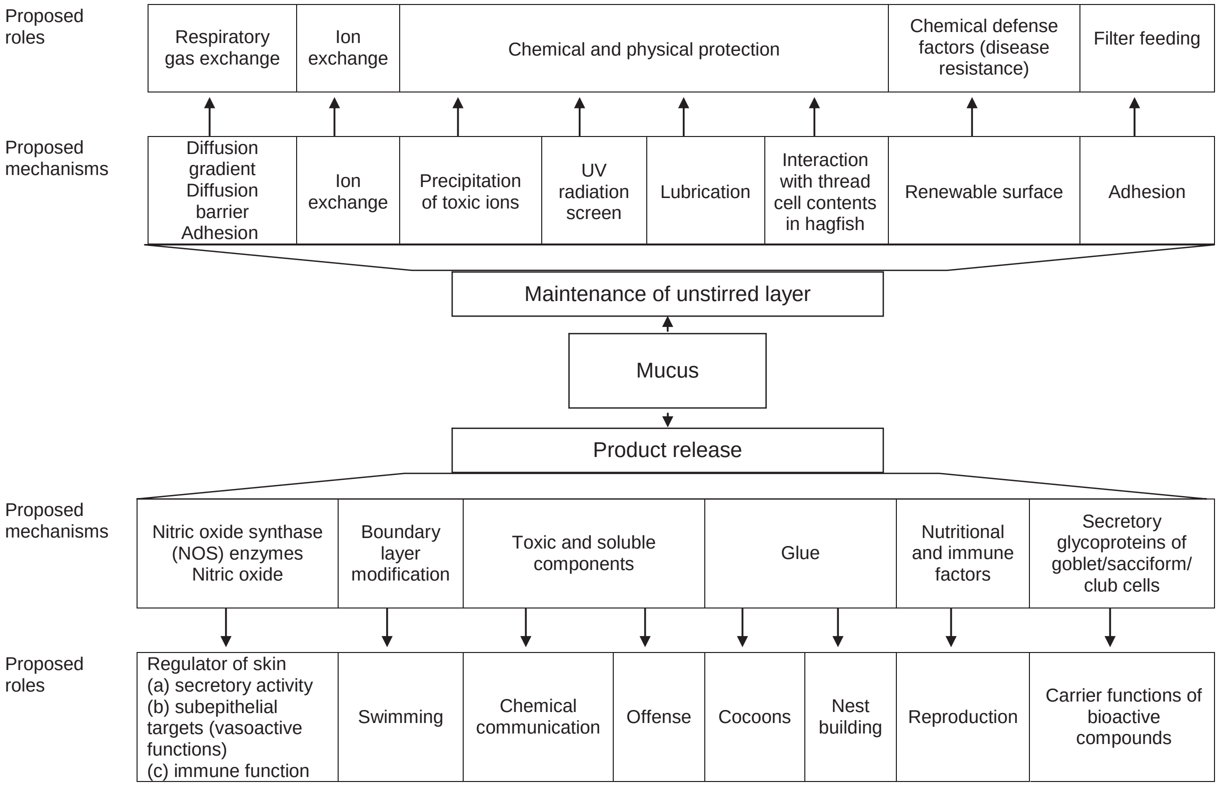

Proposed roles
Respiratory gas exchange
Ion exchange
Chemical and physical protection
Chemical defense factors (disease resistance)
Filter feeding
Proposed mechanisms
Diffusion gradient
Diffusion barrier
Adhesion
Ion exchange
Precipitation of toxic ions
UV radiation screen
Lubrication
Interaction with thread cell contents in hagfish
Renewable surface
Adhesion
Maintenance of unstirred layer
Mucus
Product release
Proposed mechanisms
Nitric oxide synthase (NOS) enzymes
Nitric oxide
Boundary layer modification
Toxic and soluble components
Glue
Nutritional and immune factors
Secretory glycoproteins of goblet/sacciform/club cells
Proposed roles
Regulator of skin
(a) secretory activity
(b) subepithelial targets (vasoactive functions)
(c) immune function
Swimming
Chemical communication
Offense
Cocoons
Nest building
Reproduction
Carrier functions of bioactive compounds

Figure 1 Some of the numerous roles suggested for fish mucus, divided into roles that involve the production and maintenance of a layer of mucus surrounding the fish, and roles that involve the release of mucus and associated substances into the surrounding water. Mucus components are produced by a variety of cells in fish epidermis (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes). Modified from Fig. 1 in Shephard KL (1994) Functions for fish mucus. *Reviews in Fish Biology and Fisheries* 4: 401–429, with permission from Springer; and from Fig. 10 in Zaccone G, Kapoor BG, Fasulo S, and Ainis L (2001) Structural, histochemical and functional aspects of the epidermis of fishes. *Advances in Marine Biology* 40: 253–348.

and renewal, and the presence of antimicrobial substances such as fatty acids, immunoglobulins, complement components, lectins, lysozyme, proteolytic enzymes, and other antimicrobial peptides and proteins. Through precipitative action, mucus may also help to clear suspended material from the gills and skin of fish in highly turbid or polluted waters. Research suggests that mucus of tropical reef fishes contains compounds that can absorb ultraviolet (UV) radiation and may function in screening out both UVA and UVB radiation. Certain tropical parrotfish and wrasse species (Labridae) secrete a mucous cocoon that surrounds their bodies at night; this envelope may provide protection against predators that hunt by smell or touch and may also have antimicrobial properties. In addition, mucous secretions and thickened (or rarely, keratinized) outer skin layers assist in protection against abrasion in burrowing or semiterrestrial fishes, for example, the cocoon of aestivating lungfishes (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction and **The Skin**: Hagfish Slime).

By means of their motility, nonkeratinized epithelial cells (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes) serve as a key component of the unique wound repair mechanism of fish integument. Within seconds or minutes after an injury occurs, epithelial cells at the edges of the wound begin to migrate toward the wound cavity in groups, retaining contacts between the cells as they migrate. These cells quickly cover the wound and provide a mechanical barrier against infection by opportunistic pathogens from the surrounding water during the early stages of wound repair. Closure of a wound by migrating epithelial cells occurs at a rate as fast as $5-12\,\mu\mathrm{m\,min}^{-1}$ in teleosts, with complete closure occurring within a few hours to a few days. The actual rate of wound closure depends on various factors including water temperature, the size and severity of the wound, and perhaps the fish species. Nevertheless, *in vitro* studies indicate that fish epithelial cells are among the fastest migrating cells known, moving at a speed 10–20 times faster than mammalian fibroblast cells.

In addition to providing wound coverage, migrating epithelial cells may help to protect the skin surface from pathogen invasion by means of phagocytic activity. These cells may help to eliminate foreign matter from the integument by a process in which cells that become filled with foreign matter subsequently are sloughed from the skin surface. In addition, uptake and sequestration of foreign material, such as pathogens, by epithelial cells may play a role in the development of local immunity in the integument.

Epithelial cell proliferation (hyperplasia) that results in epidermal thickening is another protective response frequently observed in fish. Epidermal hyperplasia has been reported in fish infested with skin parasites or exposed to pollutants, but may also occur at times that fishes are exposed to increased skin abrasion, as during spawning activity. Although not considered part of the integument proper, the specialized gill epithelium of teleost fishes appears to be particularly adapted to mounting this type of protective response (**see also Ventilation and Animal Respiration**: Plasticity in Gill Morphology).

Scales (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes) provide mechanical protection for deeper tissues, and bony encasements and spines discourage attacks by predators. Noxious substances elaborated by specialized cells in the skin (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes) and venoms produced by glands associated with spines on the fins or opercula can also help to dissuade such attacks (**Figure 1**).

Wandering leukocytes (white blood cells) are also present in the skin. These cells are important components of both the innate and adaptive immune systems of fishes (**see also Blood**: Cellular Composition of the Blood). Leukocyte numbers in the skin may increase significantly during the healing of skin injuries, certain infectious diseases, or during exposure to pollutants or other stressors.

Protection can also be afforded by patterns of skin pigmentation (**see also The Skin**: Coloration and Chromatophores in Fishes) that help to conceal a fish via cryptic coloration, countershading, or disruptive coloration. Among deep-sea fishes, multiple bioluminescent organs or photophores (**see also The Skin**: Bioluminescence in Fishes) distributed over the body surface can provide camouflage through countershading or by blurring the silhouette of the fish in the glow of spontaneous bioluminescence.

Communication and Sensory Perception

Integumentary pigmentation can also play an important role in intraspecies communication. For example, bright and conspicuous skin coloration may serve to advertise rather than conceal. In fact, most color patterns are a compromise between the necessity to communicate with other members of a species and the need to avoid becoming prey. Chromatophores (pigment cells) in the skin are responsible for much of the remarkable variation in coloration seen in fishes (**see also The Skin**: Coloration and Chromatophores in Fishes). In deep-sea fishes, the photophores in the skin may substitute for color patterns for species and mate recognition. Photophores may also serve as illumination in the darkness of the deep sea, and large light organs on the heads of some fishes may function as lures to attract prey (**see also The Skin**: Bioluminescence in Fishes).

Communication among fishes can be facilitated by substances produced in the integument (**Figure 1**). For example, fright pheromones released into the water from damaged club cells in the skin of some fish species can alert other fish of the same species to the presence of predators (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes and **Hormones in Communication**: Hormonal Pheromones). Several types of specialized sensory structures are located in the integument, including the mechanosensory lateral line system (**see also Hearing and Lateral Line**: Lateral Line Neuroethology), chemoreceptory structures or taste buds (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction), and electrosensory receptors (**see also Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction).

Locomotion

Integumentary features of fish can assist in locomotion. The slippery mucus of some fishes has marked friction-reducing properties, enabling them to move at greater speed with reduced expenditure of energy (**Figure 1**). In general, mucus taken from faster swimming predatory fishes has been shown to have better drag-reducing properties than mucus from slower moving species, although there are exceptions. The shape and distribution of scales on a fish may also contribute to drag reduction. In addition, the passive flexural stiffness that the integument provides to the body assists in the generation of the undulatory waves that propel most fishes during swimming.

Respiration, Ion Regulation, and Excretion

The skin is an important accessory organ to the breathing apparatus of some fish species. Gas exchange across the integument plays a significant role in the respiration of larval fish (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions). In addition, certain adult fishes that are adapted to survive temporary emersion or short terrestrial migrations can utilize atmospheric oxygen via diffusion through a well-vascularized skin (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). For most adult fish species that have been studied in freshwater or marine environments, cutaneous respiration has been found to match the oxygen demand of the skin itself, indicating that the skin functions as an oxygen exchanger primarily for its own benefit.

The role of the integument in maintaining the balance of water and minerals inside the body of a fish is not well understood. Whereas the skin of the primitive hagfishes and perhaps lampreys is highly permeable to water, the skin of higher fishes is generally believed to have low permeability to both water and ions. Nevertheless, a few studies with teleosts (higher bony fishes) have shown significant transfer of certain ions across the skin surface. The specialized cells (ionocytes) responsible for transepithelial ion transfer in both freshwater and marine species are especially abundant in the gill epithelium and are also found in extrabranchial locations such as the opercular lining skin and external skin (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes).

The function of the integument in excretion has received relatively little attention, but it may have a significant role in the excretion of nitrogenous wastes, principally ammonia and urea. Limited experimentation has suggested that the skin is a more important site of ammonia excretion in marine fishes than in freshwater fishes, although the skin of certain larval freshwater fishes may play a significant role in ammonia excretion (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). Some evidence also suggests that the integument of marine teleost fishes may be an important site of urea excretion (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia).

Other Functions

Although most fishes are obligate poikilotherms, they display a variety of adaptations to reduce the impact of temperature on physiological function, and some of these adaptations involve the skin. Certain tunas can maintain muscle temperature several degrees above the ambient water temperature through vascular heat exchangers, which include lateral networks of blood vessels originating in the skin (**see also Design and Physiology of Arteries and Veins**: The Retia). Antifreeze proteins are produced by fishes living in the polar oceans; some of these are abundant in the integument and appear to be important for protecting outer tissues from freezing.

In addition to other functions, mucus plays roles in feeding and reproduction of certain fishes (**Figure 1**). Epidermal mucus secreted by parent discus fish (*Symphysodon*) and certain other cichlids provides nutrients for fry that feed on the mucus by nipping at the skin surface of their parents (**see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care). For some suspension-feeding fishes that filter large volumes of water to extract minute prey such as bacteria and plankton, entrapment of food particles by mucus associated with the gill rakers and mucus-covered oral surfaces is an important component of the feeding mechanism. For certain nest-building fish species, mucus contributes glue for nest construction.

As research on fish integument has progressed, a number of bioactive substances have been identified in mucous secretions (**Figure 1**) or epidermal cells (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes), although the functions of many of these substances in fish are speculative. For example, fish epidermal mucus contains certain forms of nitric oxide synthase (NOS), the enzyme responsible for the production of nitric oxide (NO). NO is a prominent vascular and neuronal messenger that regulates many epithelial functions. The possible regulatory functions of NO in fish integument have not been elucidated, but roles for NO in the regulation of mucus release and blood flow in mammalian epithelia have been suggested.

Research has shown that the scales of teleost fishes represent an important internal reservoir of calcium and that scales can be reabsorbed under certain physiological conditions such as starvation and sexual maturation. Calcium is essential for a number of vital processes such as bone formation, clotting of blood, regulation of enzymatic processes, and modulation of permeability, and excitability of plasma membranes. Mobilization of calcium from fish scales is under hormonal regulation.

In summary, fish skin is a multifunctional organ and its components serve important roles in protection, communication, sensory perception, locomotion, respiration, ion regulation, excretion, and thermal regulation.

See also: **Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Blood**: Cellular Composition of the Blood. **Design and Physiology of Arteries and Veins**: The Retia. **Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction. **Hearing and Lateral Line**: Lateral Line Neuroethology. **Hormones in Communication**: Hormonal Pheromones. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction. **The Skin**: Coloration and

Chromatophores in Fishes; Functional Morphology of the Integumentary System in Fishes; Hagfish Slime. **Ventilation and Animal Respiration**: Plasticity in Gill Morphology; Respiratory Gas Exchange During Development: Respiratory Transitions.

Further Reading

Blake RW (1983) *Fish Locomotion*. Cambridge: Cambridge University Press.

Elliott DG (2000) Integumentary system: Gross functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 95–108. London: Academic Press.

Elliott DG (2000) Integumentary system: Microscopic functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 271–306. London: Academic Press.

Ellis AE (2001) Innate host defense mechanisms of fish against viruses and bacteria. *Developmental and Comparative Biology* 25: 827–839.

Jones SRM (2001) The occurrence and mechanisms of innate immunity against parasites in fish. *Developmental and Comparative Biology* 25: 841–852.

Kapoor BG and Khanna B (2004) *Ichthyology Handbook*. New York: Springer.

Lagler KF, Bardach JE, Miller RR, and Passino DRM (1977) *Ichthyology*, 2nd edn. New York: Wiley.

Long JH Jr., Hale ME, McHenry MJ, and Westneat MW (1996) Functions of fish skin: Flexural stiffness and steady swimming of longnose gar *Lepisosteus osseus*. *The Journal of Experimental Biology* 199: 2139–2151.

Rakers S, Gebert M, Uppalapati S, *et al.* (2010), Fish matters': The relevance of fish skin biology to investigative dermatology. *Experimental Dermatology* 19: 313–324.

Raschi W and Tabit C (1992) Functional aspects of placoid scales: A review and update. *Australian Journal of Marine and Freshwater Research* 43: 123–147.

Shephard KL (1994) Functions for fish mucus. *Reviews in Fish Biology and Fisheries* 4: 401–429.

Whitear M (1986) The skin of fishes including cyclostomes: Epidermis, dermis. In: Bereiter-Hahn J and Matoltsy AG (eds.) *Biology of the Integument – Vertebrates,* vol. 2 pp. 8–64. New York: Springer.

Zaccone G, Kapoor BG, Fasulo S, and Ainis L (2001) Structural, histochemical and functional aspects of the epidermis of fishes. *Advances in Marine Biology* 40: 253–348.

Zamzow JP and Losey GS (2002) Ultraviolet radiation absorbance by coral reef fish mucus: photo-protection and visual communication. *Environmental Biology of Fishes* 63: 41–47.

Functional Morphology of the Integumentary System in Fishes

DG Elliott, Western Fisheries Research Center, US Geological Survey, Seattle, WA, USA

Published by Elsevier Inc.

Introduction
General Morphology
Microscopic Features
Integumentary Extensions
Further Reading

Glossary

Club cell A secretory cell present in the middle layers of the epidermis of certain ray-finned fishes that releases its contents (e.g., pheromones) upon epidermal injury and cell membrane rupture.

Cuticle An acellular layer on the skin surface of a fish formed by secretions from epithelial cells, goblet cells, and other cells in the epidermis. The cuticle may contain a variety of protective substances including toxins and antimicrobial compounds.

Dermis The inner layer of the integument, derived from embryonic mesoderm, and consisting largely of fibrous connective tissue with collagen proteins comprising the major component, and also containing blood vessels, nerves, scales, pigment cells, and adipose (fat) tissue.

Epidermis The outer cellular layer of the integument, a multilayered epithelium derived from the ectoderm of the embryo. The basic cellular element common to the epidermis of all fish is the epithelial cell, although a variety of other cell types may be present depending on the fish species.

Elasmoid scale The scale type found on most higher bony fishes (teleosts). The elasmoid scale usually is a thin, flexible transparent structure consisting of a basal plate of collagenous tissue, with superficial mineralization. Elasmoid scales are commonly grouped into two general types, cycloid or ctenoid, based on surface sculpture. Cycloid scales lack surface projections, whereas ctenoid scales have surface projections of various types in the posterior area.

Ganoid scale An ancestral scale type of bony fishes, still present in some primitive ray-finned fishes. In ganoid scales that are least modified from the ancestral type, the scale consists of an osseous (bony) plate covered by a layer of calcified dentine (dentin) and capped by a layer of enamel-like ganoine; the scale is penetrated by canals carrying capillary blood vessels to supply the tissues exterior to the scale.

Goblet cell Unicellular mucin-secreting exocrine gland (also called mucous cell) common to most animal groups, including most fishes. Goblet cells, so-named because of their shape, are found in the epidermis of the skin of fishes as well as in mucous membranes of such organs as the intestine. Integumental goblet cells of fish exhibit holocrine secretion, with cell membrane rupture and release of the cell contents on the skin surface, followed by cell death.

Placoid scale The scale type found on cartilaginous sharks, rays, and chimaeras (Chondrichthyes). The typical placoid scale consists of a flattened, rectangular basal plate and a spine or cusp, with a structure resembling that of a tooth.

Sacciform cell Unicellular gland containing complex secretions, usually with a protein moiety. Unlike club cells, sacciform cells discharge their contents on the skin surface. Sacciform cells of some fish species may secrete alarm pheromones (see club cell), whereas sacciform cells of other species may secrete toxic or repellent substances for antipredator defense.

Introduction

The morphology of fish skin is highly adapted to carry out its multiple functions (**see also The Skin**: The Many Functions of Fish Integument). As in other vertebrates, the integument of all fishes consists of two layers: an outer epidermis and an inner dermis or corium (**Figures 1–4**). The two layers differ in origin, structure, and function. The epidermis is essentially cellular in structure, comprised of a multilayered epithelium derived from the embryonic ectoderm. For the most part, the dermis is a fibrous structure with relatively few cells, and is derived from embryonic mesenchyme of mesodermal origin.

General Morphology

Fish epidermis may vary in thickness depending on species (**Figures 2–4**), age, region of the body, and environmental conditions. In most fish species, the

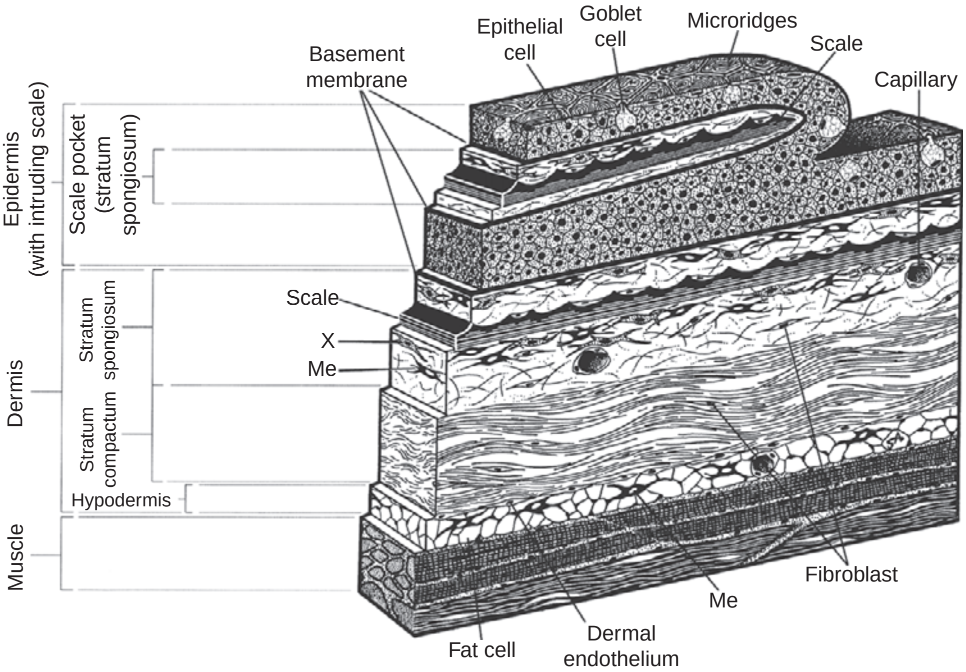


Figure 1 Three-dimensional section of the skin of a teleost fish, the coho salmon *Oncorhynchus kisutch*, showing some of the major morphological features and cell types of the epidermis and dermis. Me, melanophore; X, xanthophore (both are chromatophores or pigment cells). Reproduced from figure 17.1 in Elliott DG (2000) Integumentary system: Microscopic functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 271–306. London: Academic Press, artist Stewart Alcorn; partly modified from figure 15 in Hawkes JW (1974) The structure of fish skin. II. The chromatophore unit. *Cell and Tissue Research* 149: 159–172.

epidermis is thinner than the dermis. Keratinization of the epidermis to create horny surface layers, characteristic of land vertebrates, is rare among fishes. Instead, fish epidermis is generally metabolically active throughout all its layers. Because the epidermis of most fishes contains little or no pigment and therefore appears largely transparent by visual inspection, the morphological features of this tissue are generally viewed with the aid of a microscope.

The dermis of skin contains blood vessels, nerves, scales, pigment cells, and adipose (fat) tissue, but the bulk of the typical dermal layer is comprised of fibrous connective tissue (**Figure 1**). When a fish is being skinned, the collagenous fibers of connective tissue that bind the skin to the underlying muscle and bone are very obvious. With the exception of scales, morphological characteristics of most dermal features are best observed microscopically.

Scales (**Figures 1**, **3**, and **4**) are an important component of the dermal skeleton, and are the dermal structures that are generally most prominent by visual inspection, although they can vary in size from microscopic structures to huge bony plates. Scales usually are covered completely by epidermal tissue (**Figures 1** and **4**), although portions of scales may protrude from the epidermal surface in certain species such as sharks (**Figure 3**).

Fish scales differ in structure and origin from the horny scales occurring on the skin of reptiles, birds, and some mammals, despite a superficial resemblance to these other scale types. The scales of these other vertebrates (as well as appendages such as feathers, hair, hooves, nails, claws, and quills) consist largely of epidermal tissue of ectodermal origin that has become keratinized. These hard but nonmineralized features are largely comprised of keratins, a family of tough, insoluble fibrous structural proteins. In contrast, fish scales are mineralized dermal structures that are primarily of mesenchymal origin.

The type, number, and size of scales can reveal much information about a fish's lifestyle. On a bony fish, scale patterns can range from a heavy coating of mail-like armor to a few large bony plates on the back, to a dense covering of thin, flexible scales, to a few localized prickles or no scales at all. Large scales modified into bony plates function as protective armor on a number of slow-moving, bottom-oriented fishes such as sturgeons (Acipenseridae), many South American catfishes (Loricariidae, Callichthyidae, and Doradidae), poachers (Agonidae), and pipefishes and seahorses (Syngnathidae). Conversely, the bodies of free-swimming fishes are often covered with typical scales, which provide some protection against mechanical damage without adding too much weight. Fishes that are fast swimmers and those that live in fast-flowing water tend to have numerous fine scales

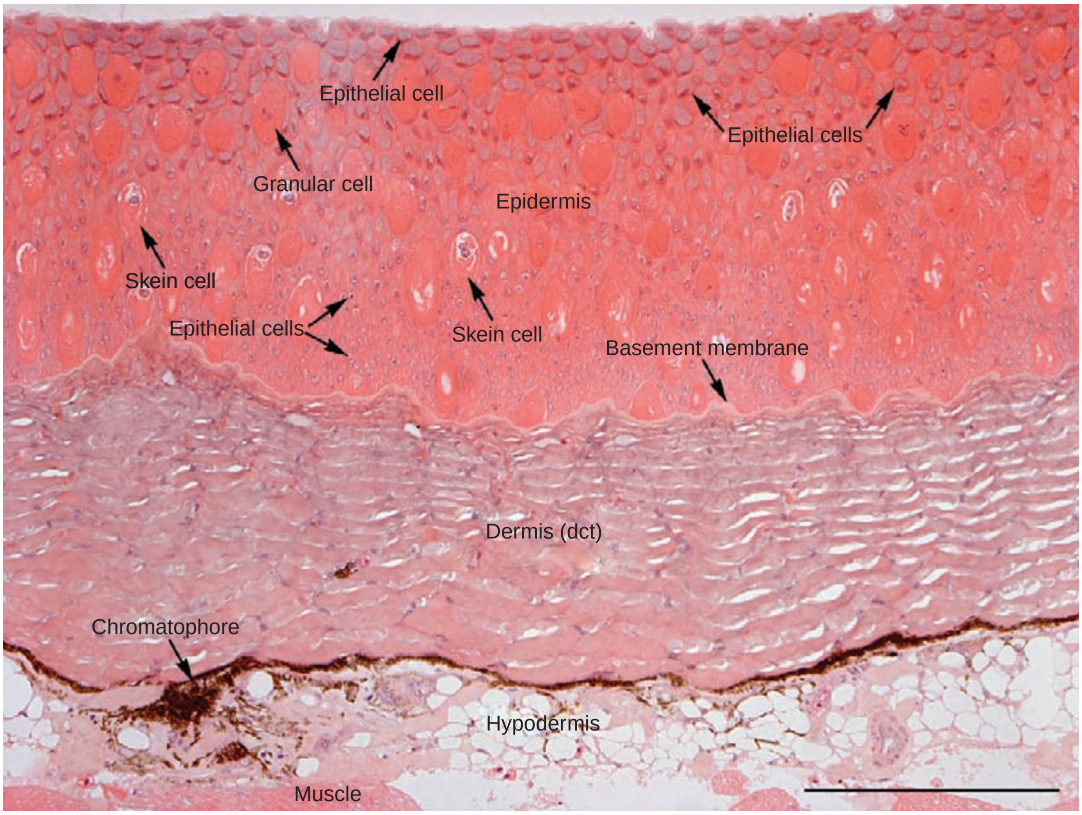


Figure 2 Histological section showing skin morphology of a primitive jawless fish, a spawning adult Pacific lamprey, *Lampetra tridentata*. The outer layer is a thick epidermis, which is separated from the dermis by an acellular basement membrane. Principal epidermal cell types are epithelial cells, secretory granular cells, and modified uninucleate or binucleate cells called skein cells, which may play a role in epidermal tissue support. The dermis of lamprey skin consists of dense collagenous tissue (dct) and lacks scales. The dermis is separated from the underlying skeletal muscle by the hypodermis, which is comprised largely of adipose (fat) tissue. Chromatophores (pigment cells) are visible in the upper hypodermis. Hematoxylin and eosin stain. Scale = 100 μm.

(e.g., trout, **Figure 4**), whereas fishes that live in quiet water and do not continuously swim at high speeds often have rather coarse scales (e.g., perch and carp). Fishes that lack scales are often bottom-dwellers in moving water (e.g., sculpins), fish that frequently hide in tight places such as caves and crevices (e.g., many catfish and eels), or fast-moving pelagic fish (e.g., swordfish and some mackerels). The primitive agnathans or jawless fishes (hagfish and lampreys) are also scaleless (**Figure 2**). However, some fishes that appear scaleless in fact have numerous deeply embedded scales (e.g., most tunas and anguillid eels). This fact emphasizes the importance of microscopy in study of fish skin.

Microscopic Features

There are many features of fish integument that can only be observed microscopically. Because there are almost 28 000 living fishes, mostly teleosts, a great diversity exists in the microscopic morphology of the integument.

Epidermis

The number of cell layers in fish epidermis may vary from two in larval fishes to 10 or more in adults. In pelagic (open-water) fish species, the epidermis is frequently thickest in the dorsal areas of the body, but in benthic (bottom-dwelling) species, the epidermis covering ventral surfaces is often thicker. In some species such as salmonids, the epidermis is often thicker in nonscaled areas, such as the top of the head and the fins, than in scaled areas of the body. The thickness and structure of the epidermis and the types of cells present can also be influenced by the size, condition, sex, and degree of sexual maturation of a fish. In addition, other factors, such as changes in ambient conditions or exposure to adverse environmental conditions, dietary deficiencies, and the presence of pathogens or other stressors, can affect the structure and cellular composition of the epidermis. Such changes may be particularly evident among fish held in captivity.

Principal epidermal cell types

As in the epidermis of other vertebrates, the fundamental structural unit of fish epidermis is the epithelial cell. This

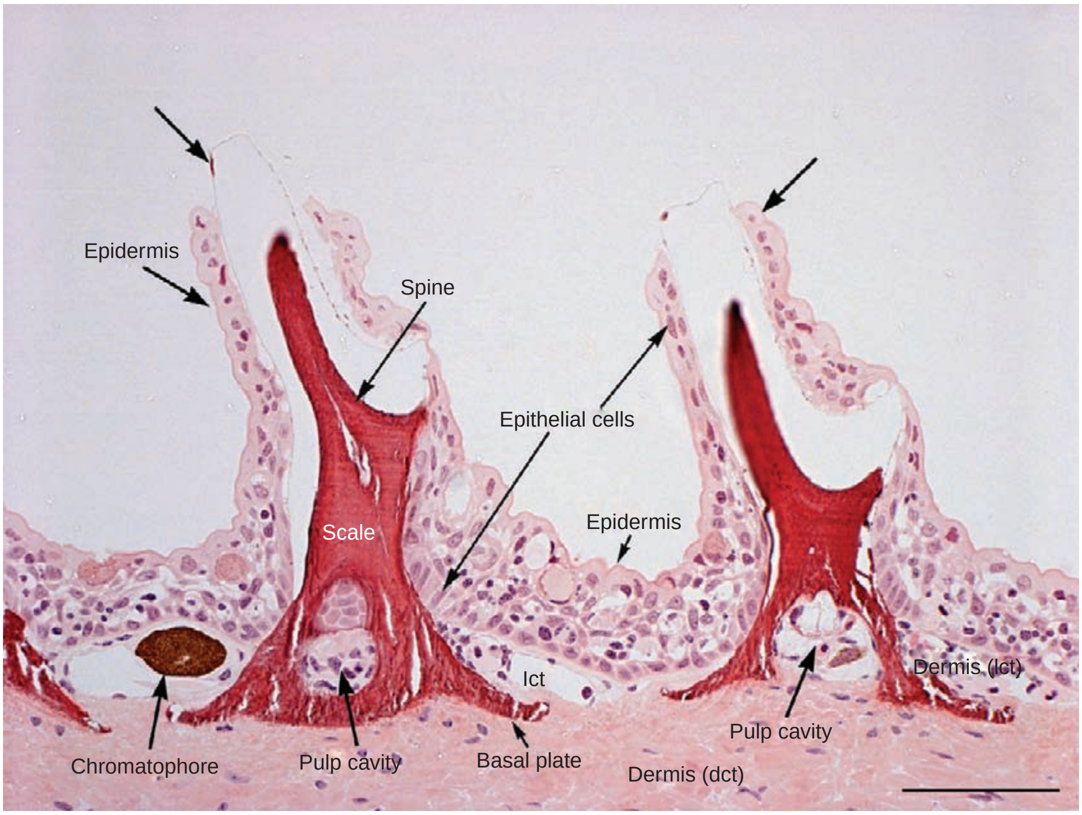


Figure 3 Histological section of fin tissue from a chondrichthyan, an adult female smooth dogfish shark, *Mustelus canis*. Tooth-like placoid scales or odontodes are visible beneath the epidermis, and show the characteristic structure including a basal plate (anchored in the dermis), a spine or cusp, and a pulp cavity. The remnants of epidermis (arrowed), possibly damaged during sampling, suggest that these scales may have been completely covered by epidermis in the living fish, but the spines of placoid scales may protrude through the epidermis in other areas of the skin. The visible dermis of the fin includes loose connective tissue (lct) of the stratum spongiosum, including a chromatophore (pigment cell), and dense connective tissue (dct) of the stratum compactum. Hematoxylin and eosin stain. Scale = 100 μm.

is the only constant feature, because a great diversity of cell types exists in the various fish taxa. Some of these include holocrine mucous cells and a variety of other secretory cells, ionocytes, sensory cells, and wandering cells such as leukocytes (white blood cells).

Epithelial cells

The basic cellular element of fish epidermis, the epithelial cell (**Figures 1–3** and **5**), has been known by many names, including malpighian cell, epidermal cell, filament-containing cell, filamentous cell, polygonal cell, polyhedral cell, keratocyte, keratinocyte, principal cell, and common cell. In primitive jawless fishes, homologous cells often have been termed 'mucous' cells, even though this adjective generally implies a goblet cell in other fishes.

Unlike the mammalian epidermal epithelial cell, the corresponding cell in most fishes is metabolically active throughout all layers of the epidermis. In teleost fishes, epithelial cells in all epidermal layers are capable of mitotic division, although active cell division is most common in the lower layers. Among the jawless fishes, dividing epithelial cells appear to be confined to the lower layers. Dead cells are regularly sloughed from the surface of fish epidermis and replaced by living cells beneath. One study showed that translocation of epithelial cells from the lower epidermal layers to the surface layer and subsequent sloughing of those cells occurred within a span of about 4 days. In comparison, a similar process of epithelial cell translocation and shedding in humans requires about 33–37 days. More recent studies of the epithelium on the gills of certain fish suggest that significant cell shedding can occur in a matter of hours under hypoxic conditions, but the gill respiratory epithelium is a highly specialized surface tissue with a single layer of epithelial cells (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy, **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics, Plasticity in Gill Morphology, **Bony Fishes**: Crucian Carp, **Role of the Gills**: Morphology of Branchial Ionocytes, and **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes).

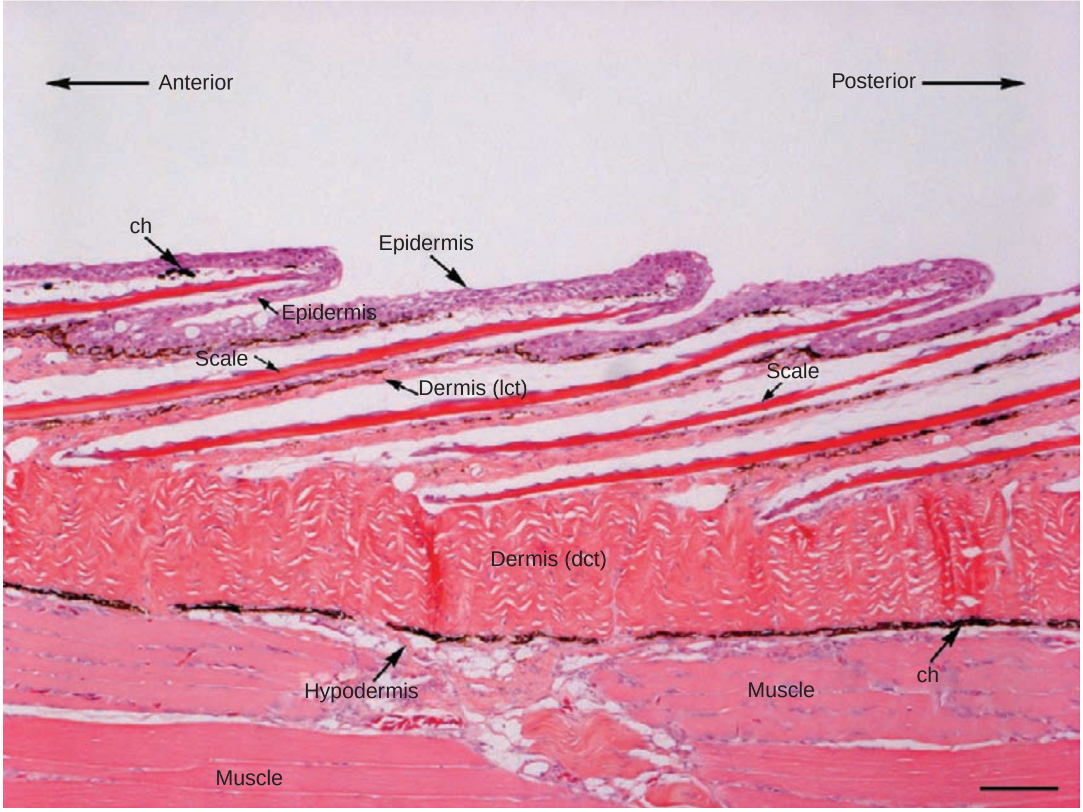


Figure 4 Histological section of skin layers of a juvenile steelhead trout, *Oncorhynchus mykiss*, showing overlapping (imbricated) scales. The thin epidermis covers and wraps around the posterior (nonoverlapped) portion of each scale. The scales lie in pockets of loose connective tissue (lct) of the stratum spongiosum of the dermis. The dense connective tissue (dct) of the stratum compactum of the dermis lies beneath the scales, separated from the skeletal muscle by a thin hypodermis comprised of adipose (fat) tissue and an upper layer of chromatophores or pigment cells (ch). Hematoxylin and eosin stain. Scale = 100 μm.

Epithelial cells typically are small relative to many other epidermal cell types, and may vary in shape depending on their position in the epidermis. The basal layer cells adjacent to the acellular basal lamina or basement membrane are cuboidal (cube-shaped) or columnar (column-shaped), whereas the superficial cells are often flattened (squamous) (**Figure 5**). The shape of the epithelial cell nucleus roughly corresponds to the shape of the cell. The presence of tonofilaments (intermediate filaments) is a distinctive cytoplasmic feature of the epithelial cell that is visible only by electron microscopy. Tonofilaments are about 7–8 nm in diameter, arranged in bundles (tonofibrils) or randomly distributed, and comprise an important component of the cytoskeleton of individual cells. Additionally, the attachment of tonofibrils to the desmosomal plaques that join adjacent epithelial cells enables the epidermis to respond as a whole to mechanical stress.

The exterior surface of superficial epithelial cells of teleosts is characterized by microridges or micropapillae that often form regular fingerprint-like patterns (**Figures 1** and **6(a)**). The function of the surface microridges is unknown, but they may provide some mechanical protection against trauma and assist in holding mucous secretions to the skin surface. Microridges also increase the absorptive surface area of epithelial cells, alter the boundary layer conditions, and may be a factor in enabling the skin to function in gas exchange. They may also scatter light differentially resulting in iridescent color patterns (**see also The Skin**: Coloration and Chromatophores in Fishes).

In a few fishes, keratinization occurs in the surface epithelial layers. Examples of keratinized external epithelium include nuptial tubercles or contact organs in breeding males of some species of Salmoniformes, Cypriniformes, and certain other teleost orders, the sometimes bizarrely shaped horny projections (unculi) on attachment organs on the lips and fins of fishes in some ostariophysan genera, and the polyhedral keratinized plaques on the skin of the Asian catfish *Bagarius*, which are believed to help protect the fish from abrasion.

The mucous secretions produced by superficial epithelial cells assist in protecting fish from pathogens and other insults (**see also The Skin**: The Many Functions of Fish Integument). These secretions form a

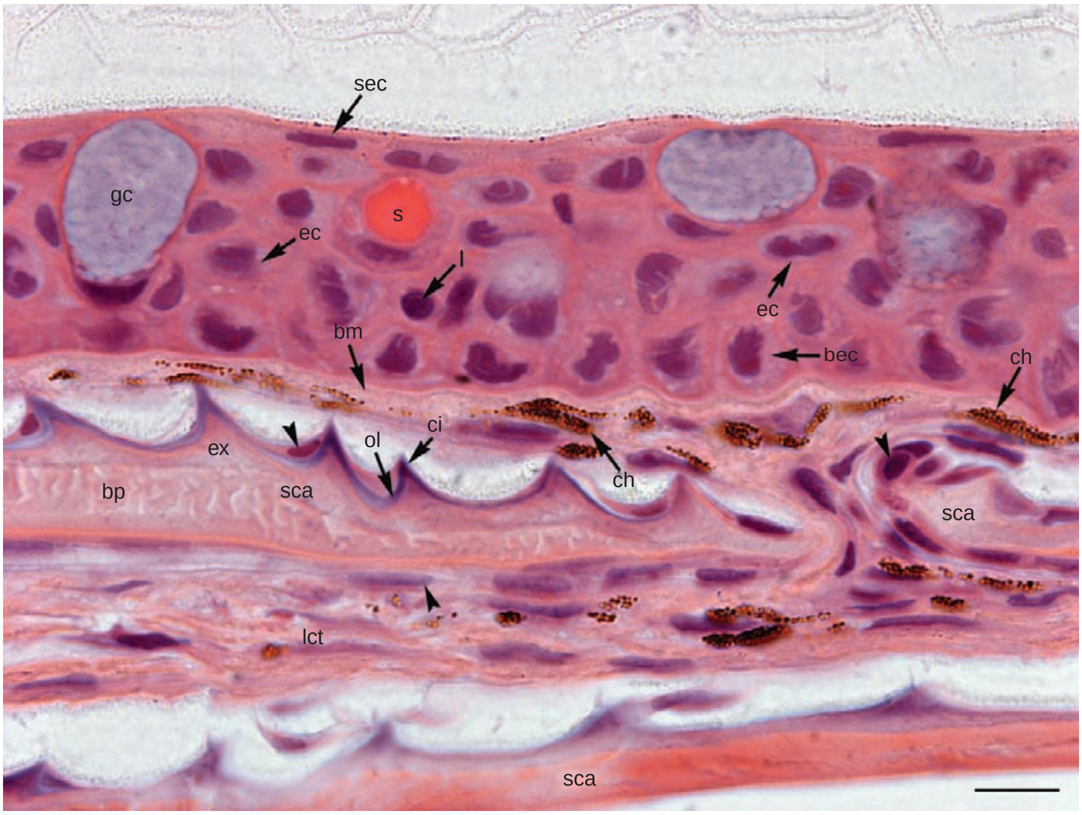


Figure 5 Histological section of the skin of a juvenile Chinook salmon, *Oncorhynchus tshawytscha*, showing the epidermis and upper dermis. Epithelial cells visible in the epidermis include basal layer epithelial cells (bec) with rounded nuclei, mid-layer epithelial cells (ec) with rounded to elongated nucli, and surface layer epithelial cells (sec) with elongated nuclei. Secretory cells in the epidermis include mucous goblet cells (gc) and an unidentified secretory cell (s) that is either a serous goblet cell or sacciform cell. Electron microscopy would be necessary for definitive identification of this cell. A wandering lymphocyte (l) is also visible in the epidermis. The epidermis is separated from the loose connective tissue (lct) of the dermis (stratum spongiosum) by an acellular basement membrane (bm). Chromatophores or pigment cells (ch) with cytoplasmic brown-black melanin granules (melanophores) are present in the dermis. The basal plate (bp), external layer (ex), outer limiting layer (ol), and circuli (ci) of one cycloid-type elasmoid scale (sca) are visible; dermal cells involved in scale growth are shown at arrowheads. Hematoxylin and eosin stain. Scale = 10 μm.

layer called the cuticle on the skin surface, where they are mixed and modified by secretions from other cells (**Figure 6**(**b**)). The cuticle layer is continually sloughed and renewed.

Goblet cells

The goblet cell (**Figure 5**) is a unicellular exocrine gland common to most animal groups. This cell is the second category of secretory cell in the fish skin, and occurs in the internal epithelia (mucous membranes) of fish as in other vertebrates. The goblet cell also is almost universally present in the skin of fishes, with a few exceptions, and is even found in the specialized gill epithelium (**see also Role of the Gills**: Morphology of Branchial Ionocytes). Lamprey epidermis contains no goblet cells; the production of mucus in lampreys is entirely from epithelial cells, which are sometimes called 'mucous cells'. Goblet cells are also lacking in the relict bony fish *Polydon* (paddlefish) and the teleost *Periophthalmus* (mudskipper).

Goblet cells are most frequently recognized in the middle to outer layers of epidermis, although in a very thin epidermis, the base of a mature goblet cell may be adjacent to the basement membrane. Evidence suggests that goblet cells differentiate from epithelial cells in the lower layers of the epidermis, and that differentiated goblet cells do not undergo further mitotic division.

Immature goblet cells are rounded but become flattened laterally and generally increase in size as they move toward the surface of the epidermis. As mucous secretions are elaborated in the maturing cell, the nucleus and organelles usually become displaced basally. Upon reaching the skin surface, the goblet cell emerges (usually between adjacent epithelial cells (**Figure 6**(**a**)), its cell membrane ruptures at the apical point, the cell contents are released, and then the cell dies.

The abundance and size of goblet cells may vary in different body regions of a fish. A specific distribution of goblet cells may help to ensure an even layer of mucus over the surface of a moving fish, or to reduce friction

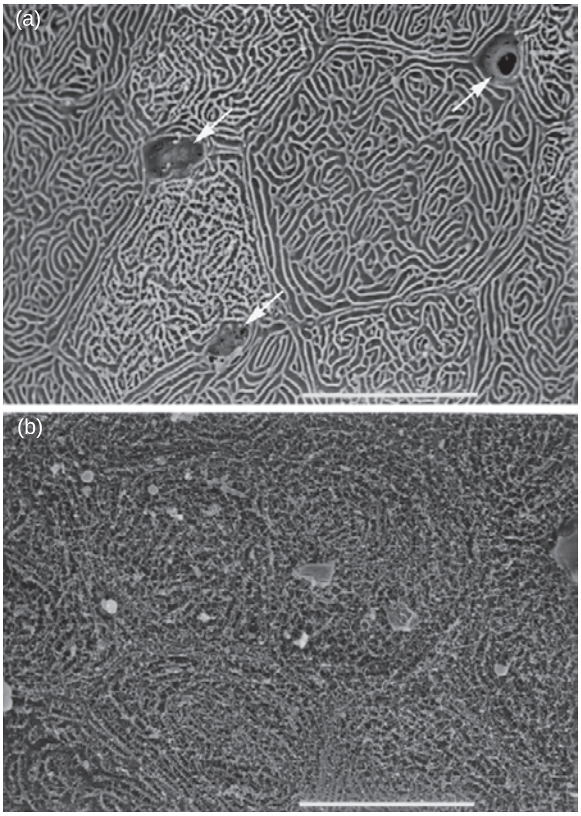


Figure 6 Scanning electron micrographs of the epidermal surface of a juvenile Chinook salmon (*Oncorhynchus tshawytscha*). (a) Individual surface epithelial cells showing 'fingerprint' microridge patterns; apical ends of superficial goblet cells (arrowed) are visible between epithelial cells. (b) Skin surface fixed to preserve the mucous cuticle on the surface of the epidermis. Microridge patterns of superficial epithelial cells are faintly visible beneath the mucous cuticle, which has trapped some surface debris. Scale = 10 μm. (b) Reproduced from figure 17.12(b) in Elliott (2000) Integumentary system: Microscopic functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 271–306. London: Academic Press.

between overlapping scales during flexion of the fish's body during swimming. In certain fish species, goblet cells may aggregate in patches on various areas of the body to form multicellular holocrine glands. Goblet cell abundance also may differ between male and female fish of the same species, as in certain salmonid fishes. The number of goblet cells also can change seasonally and during processes such as larval metamorphosis, sexual maturation, or adaptation to seawater.

Alterations in environmental conditions, such as sudden changes in temperature or exposure to ultraviolet radiation or contaminants, can also affect epidermal goblet cell numbers. For example, acute exposure to an irritant can cause rapid holocrine secretion by goblet cells and a consequent reduction in their numbers, whereas chronic exposure to an irritant may result in an increase in goblet cell numbers as new cells differentiate and move to the epidermal surface. In some instances, even a mild stressor, such as a single incidence of capture in a net, can promote a subsequent increase in epidermal goblet cells. Exposure to toxicants or other stressors can also alter the size or morphology of goblet cells, and can result in changes in the chemical composition of the secretion produced.

In some species such as salmonids, the greatest volume of epidermal mucous secretions is from goblet cells. The secretions of most epidermal goblet cells are glycoproteins, but a wide array of components have been demonstrated in different fishes. A variety of roles have been attributed to goblet secretions, including lubricating, protective, and possibly regulatory functions (**see also The Skin**: The Many Functions of Fish Integument).

Other epidermal cell types

In addition to epithelial and goblet cells, the epidermis of fish may include other secretory cell types. Principal among these are the club cells and sacciform cells. Club cells are present in certain taxa of ray-finned (actinopterygian) fishes, including the primitive Polypteriformes and eels of the order Anguilliformes. The largest group of fishes with club cells is the superorder Ostariophysi, which includes over one-quarter of all known fish species and three-fourths of all freshwater species. Club cells are usually large and round or oval to club-shaped, with one or two centrally located nuclei with prominent nucleoli (**Figure 7**). Club cells characteristic of eels possess a secretory vacuole in the cytoplasm, whereas club cells characteristic of Ostariophysi do not. These cells are generally located in the middle epidermal layers and lack openings to the epidermal surface. The club cells of ostariophysan fishes contain an alarm pheromone that is released into the surrounding water only when the club cells are broken, which frequently happens even with a minor injury to the fragile epidermis. Other ostariophysans detect the alarm pheromone by smell, and perform species-specific antipredator defensive reactions such as gathering into a tight school, diving for cover, or becoming motionless. Polypterids and eels do not show an alarm reaction, however, and epidermal club cells may have other functions in these species as well as in ostariophysans. Research has identified a variety of bioactive substances within club cells, including anti-pathogenic agents, toxins, and specific factors that may be involved in epidermal cell regulation (**see also The Skin**: The Many Functions of Fish Integument).

Sacciform cells have been reported in cartilaginous fishes such as rays and chimaeras, and in relict bony fishes such as polypterids. Among teleosts, sacciform cells are characteristic of the superorder Acanthopterygii (spiny-rayed fishes) and Paracanthopterygii (a group including the Gadiformes or cods), and also occur in some fishes of other teleost superorders such as Ostariophysi and

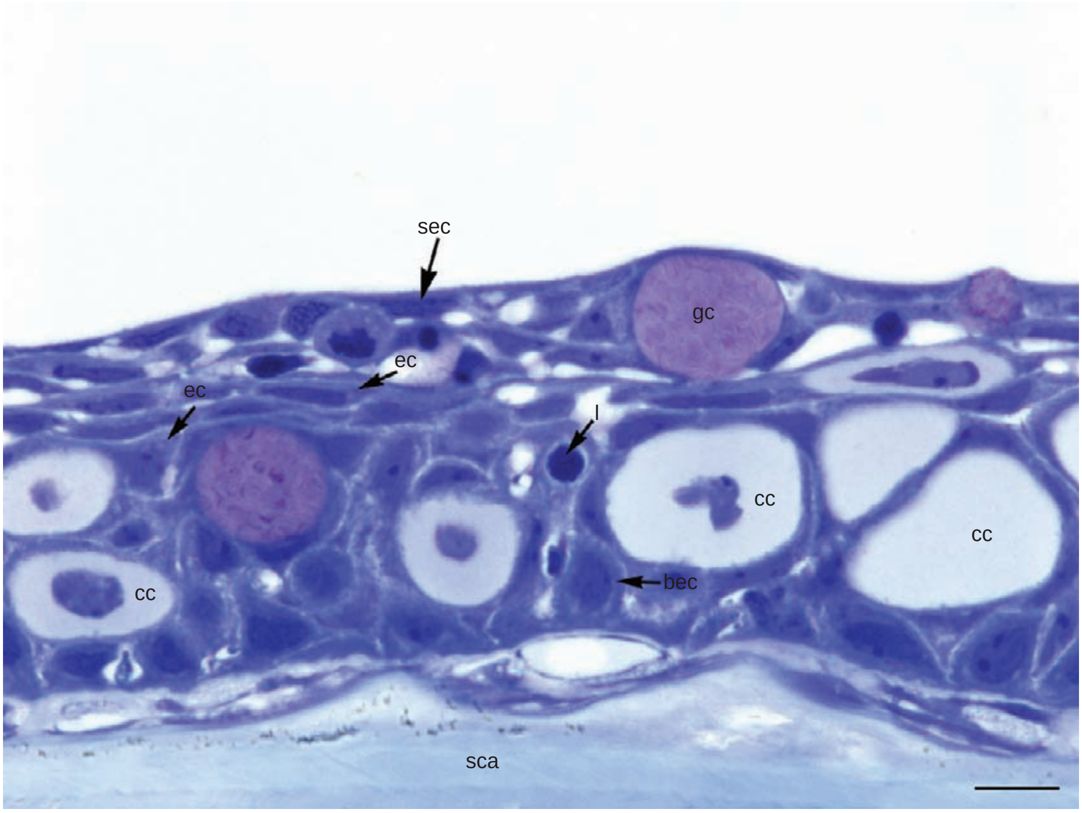


Figure 7 Histological section of the epidermis of a goldfish, *Carassius auratus*, showing typical ostariophysan-type club cells (cc) with a central nucleus (when visible) but no secretory cytoplasmic vacuole. Other epidermal cells include basal epithelial cells (bec) with rounded nuclei, mid-level epithelial cells (ec) with rounded to flattened nuclei, surface epithelial cells (sec) with elongated nuclei, mucous goblet cells (gc), and a wandering lymphocyte (l). A portion of an elasmoid scale (sca) is visible in the dermis beneath the epidermis. Methylene blue-azure II stain. Scale = 10 μm.

Protocanthopterygii. By light microscopy, sacciform cells have basally located nuclei and homogeneous to granular cytoplasm that stains readily with acid dyes (**Figure 5**). In some fishes, sacciform cells can be distinguished from certain types of goblet cells only by electron microscopy. Like goblet cells, sacciform cells of some species appear to differentiate deep in the epidermis and increase in size as they move toward the skin surface. Most mature sacciform cells open at the skin surface by an apical pore, and evidence suggests that the usual mode of secretion is holocrine. In the Gadiformes, sacciform cells are enormous and swollen, often appearing cyst like. Among pelagic gadoid fishes, sacciform cells appear not to open at the epidermal surface, whereas among benthic gadoids the typical surface openings are present. The precise functions of sacciform cell secretions are unknown, and the secretions may vary even within a species. Sacciform cell secretions usually include a protein moiety, and various enzymes, serotonin, cholesterol, and other lipids have been identified. Research has suggested that in some fish species, sacciform cells may be analogous to the granular glands of amphibian skin, producing secretions that are toxic or repellent to predators (**see also The Skin**: The Many Functions of Fish Integument). In other fishes, sacciform cells may produce regulatory peptides or intraspecific alarm pheromones (**see also The Skin**: The Many Functions of Fish Integument).

Additional secretory cell types are present in the epidermis of certain fishes. Included among these is the elongate oval thread cell of hagfish, which releases an impressive protein thread when the animal is stressed. The protein threads released from thread cells mix with the contents of mucous goblet cells to form the thick and copious slime that has given the hagfish the nickname 'slime eel' (**see also Hagfishes and Lamprey**: Hagfishes, and **The Skin**: Hagfish Slime, and The Many Functions of Fish Integument). Venomous fishes have multicellular nonducted holocrine glands associated with spines to form defensive organs. Release of venom into a wound made by the spine generally follows rupture of the fine epidermal covering of the tip of the spine. The granular cell in the middle to upper layers of lamprey epidermis (**Figure 2**) is a large cell with a centrally located nucleus and prominent nucleolus, numerous small granules in the cytoplasm, and appendages penetrating deep into the epidermis. Granular cells release their contents when lamprey skin is damaged, but the function of the proteinaceous secretion is unknown.

Epidermal paraneurons are receptosensory cells that possess a receptive site and a secretory site on their plasma membranes. These cells release signal substances that are common to neuronal secretions, and may have regulating functions. The spectrum of bioactive substances synthesized by paraneurons, and the physiological functions of these cells, vary among vertebrate species. Ultrastructural and immunohistochemical markers enable easy recognition of paraneurons by their polarization, but the paraneurons of fish have received relatively little attention. Merkel cells are paraneuronal cells that have been found in the epidermis of teleosts, dipnoans (lungfish), and lampreys, although their functions are unknown. Studies in mammals suggest that Merkel cells may play a role in a variety of functions, including touch perception, nerve growth, and proliferation of certain cell types. Additional studies in fish have demonstrated putative paraneurons among mechanoreceptor cells, electroreceptor cells, and chemosensory cells in fish integument.

Further discussion of certain epidermal cell types can be found elsewhere in the encyclopedia. For links to these articles, **see The Skin**: The Many Functions of Fish Integument.

Dermis

In primitive vertebrates, as in annelid worms, the dermis consists essentially of two sets of collagen fibers arranged in opposing spirals around the body, creating a bias sleeve that has the ability to bend without wrinkling. Most fishes require the accommodation of lateral flexions only, but the hagfishes are so flexible that they can tie themselves in knots (**see also Hagfishes and Lamprey**: Hagfishes). The overall thickness of the dermis varies with species, position on the body, and life stage.

The dermis of most fishes is divided into two major layers. The upper (outer) layer is a loose network of collagenous connective tissue (**Figures 1** and **3–5**) and is called the stratum spongiosum or stratum laxum, whereas the lower layer is a dense layer consisting primarily of orthogonal collagen bands (**Figures 1**, **3**, and **4**) and is called the stratum compactum. The relative thickness of the two layers depends on the presence or absence of scales, and the separation between dermal layers may be indistinct in nonscaled areas. In fin tissue, the dense connective tissue layer may be reduced. In lampreys and some primitive actinopterygians, the dermis consists primarily of dense collagenous tissue (**Figure 2**).

The dermis is separated from the epidermis by an acellular basement membrane (also called the basal lamina or adepidermal membrane; **Figures 1**, **2**, and **5**). The deep face of the dermis is bounded by a single layer of cells termed the dermal endothelium (**Figure 1**). Immediately below the dermal endothelium, and separating the dermis from the underlying skeletal musculature, is a layer of well-vascularized loose connective tissue called the hypodermis or subcutis (**Figures 1**, **2**, and **4**).

Specialized dermal elements

Chromatophores

Integumentary colors are primarily dependent on the presence of chromatophores (pigment cells) in the skin (**Figures 1–5**). In all fishes from the primitive cyclostomes to higher teleosts, these cells occur in the dermis, where they may be found in the stratum spongiosum, in the hypodermis, or both (**see also The Skin**: Coloration and Chromatophores in Fishes).

Scales

As evidenced by their fundamental differences in structure, scales evolved independently in cartilaginous and bony fishes (**Figures 8** and **9**). The cartilaginous sharks, rays, and chimaeras (Chondrichthyes) possess placoid scales (also called odontodes). A typical placoid scale consists of a flattened, rectangular basal plate embedded in the upper layer of dermis, and a spine or cusp that projects backward and often through the epidermal surface (**Figures 3**, **8**(**a**), and **9**(**a**)). The protruding spines of placoid scales give shark skin its characteristic rough texture. The structure of the placoid scale resembles that of a tooth (**Figure 9**(**a**)). The spine is comprised of a cap or cone of dentine that is covered with a layer of hard, transparent enameloid, and encloses a pulp cavity. Blood vessels, nerves, and presumptive lymph channels enter the pulp cavity through one or more openings that perforate the basal plate. Placoid scales show modifications in shape for a variety of functions, including protection from predators and ectoparasites, reduction of mechanical abrasion, accommodation of bioluminescent and sensory organs, and reduction of frictional drag. These scales may fit close together or be set apart, but they usually are nonoverlapping except where they protect the lateral line canal. Placoid scales do not exhibit continuous, indefinite growth; they may be replaced when old, worn out, or lost.

In contrast to the scales of Chondrichthyes, the scales of bony fishes (Osteichthyes) are layered plates, with bone as one of the layers. The ganoid scale, an ancestral scale type of bony fishes, is still present in some primitive actinopterygian (ray-finned) fishes. Ganoid scales are rigid plates that are often rhomboidal or diamond-shaped (**Figure 8**(**b**)). They may slightly overlap and articulate with one another by peg-and-socket joints at the margins, and frequently are bound together by connective tissue bundles called Sharpey's fibers. In the bichirs (Polypteridae), ganoid scales are least modified from their ancestral form (**Figure 9**(**b**)). In these fishes, a thick osseous basal plate lies beneath a stack of thin, incompletely mineralized collagenous layers

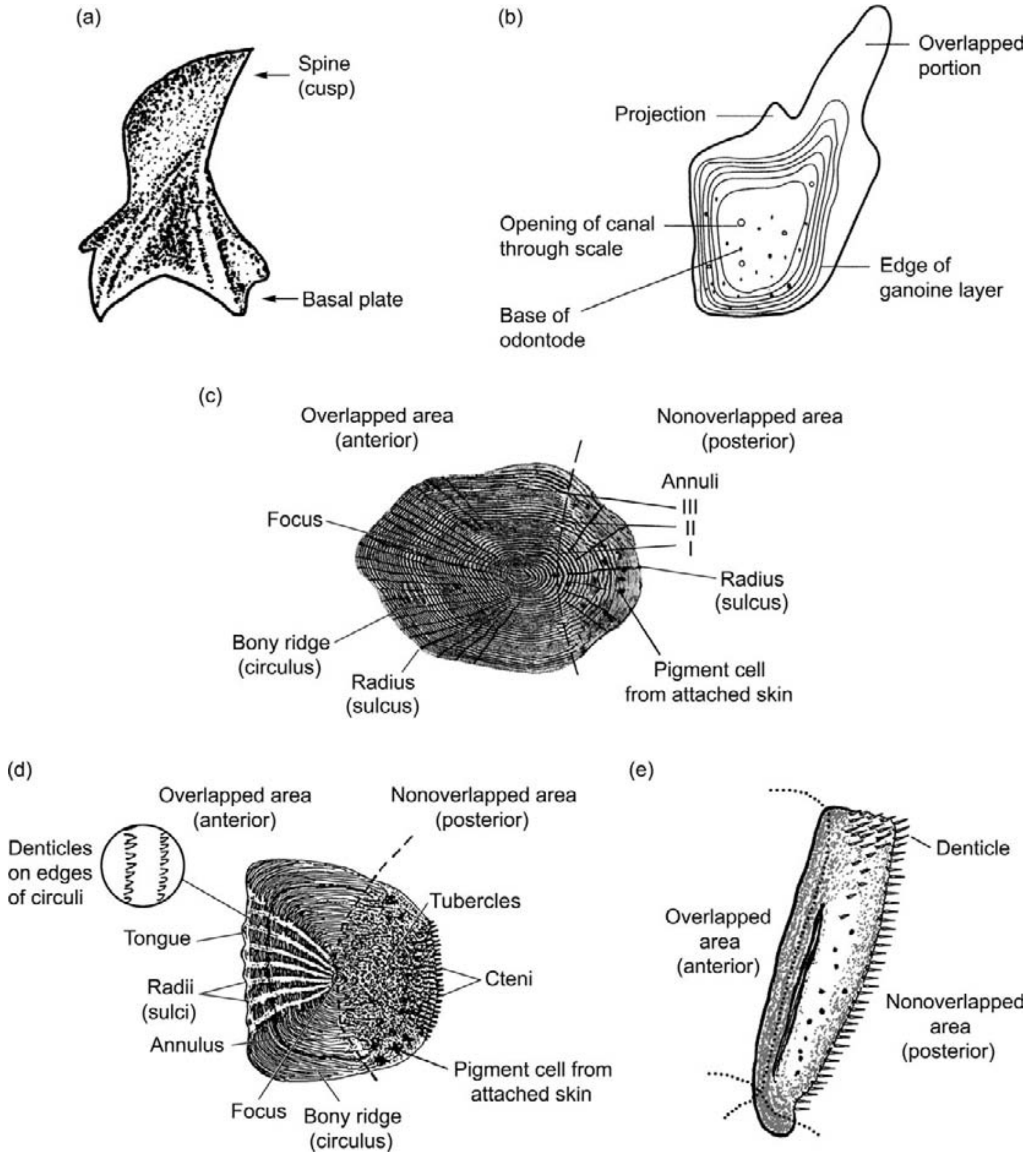


Figure 8 Individual fish scales showing some morphological features of major scale types. (a) Placoid scale (odontode) from the ventral body of a spiny dogfish shark, *Squalus acanthias*. (b) Ganoid scale of a longnose gar (*Lepisosteus osseus*) shown as a transparency. (c) Elasmoid scale (cycloid type) of a teleost fish. (d) Elasmoid scale (ctenoid type) of a teleost fish. (e) Scute (bony plate) of a teleost, the armored catfish *Corydoras aeneus*. (a) Reproduced from figure 5.6(c) in Elliott (2000) Integumentary system: Gross functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 95–108. London: Academic Press; adapted from figure 2 in Sayles LP and Hershkowitz SG (1937) Placoid scale types and their distribution in *Squalus acanthias*. *Biological Bulletin* 73: 51–66. (b) Adapted from figure 3(b) in Kerr T (1952) The scales of primitive living actinopterygians. *Proceedings of the Zoological Society of London* 122: 55–78. (c) Reproduced from figure 5.8(a) in Elliott (2000) Integumentary system: Gross functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 95–108. London: Academic Press; adapted from a drawing courtesy of the Michigan Institute for Fisheries Research. (d) Reproduced from figure 5.8(e) in Elliott (2000) Integumentary system: Gross functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 95–108. London: Academic Press; modified from figure 1 in Sire J-Y (1986) Ontogenic development of surface ornamentation in the scales of *Hemichromis bimaculatus* (Cichlidae). *Journal of Fish Biology* 28: 713–724 and from figure 1 in Kuusipalo L (1998) Scale morphology in Malawian cichlids. *Journal of Fish Biology* 52: 771–781. (e) Adapted from figure 32(e) in Sire J-Y and Huysseune A (1996) Structure and development of the odontodes in an armoured catfish, *Corydoras aeneus* (Siluriformes, Callichthyidae). *Acta Zoologica* 77: 51–72.

(elasmodine) arranged in a plywood-like pattern. The elasmodine is covered by a layer of vascular dentine that is capped by a layer of enamel-like, hypermineralized ganoine. Each scale is penetrated by canals carrying capillary blood vessels to supply the tissues exterior to the scale. The capillaries are part of the secondary circulation that contains very few red blood cells and is unique to fishes (**see also Design and Physiology of Capillaries**

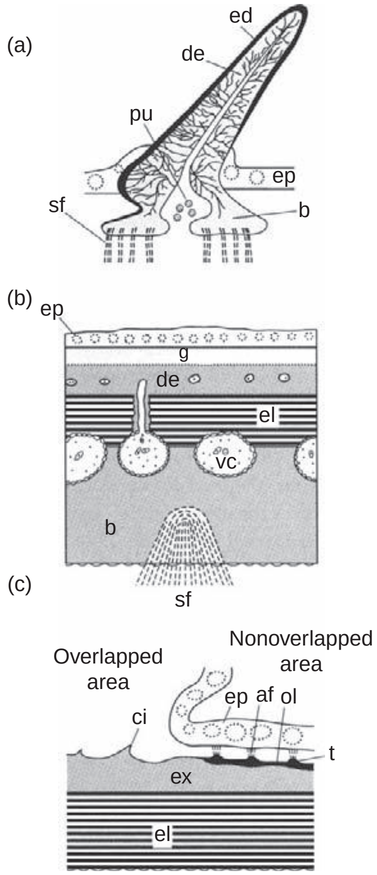


Figure 9 Schematic drawings comparing major structural layers and components of fish scales shown in cross section (not drawn to scale). (a) Placoid scale (odontode) of a chondrichthyan. (b) Portion of a ganoid scale of a polypterid. (c) Portion of an elasmoid scale of a teleost (cichlid). af, anchoring collagen fibers; b, bone; ci, circulus; de, dentine; ed, enameloid; el, elasmodine; ep, epidermis; ex, external layer; g, ganoine; ol, outer limiting layer; pu, pulp cavity; sf, Sharpey's (collagen) fibers; t, tubercle; vc, vascular canal. (a–c) Reproduced from figures 17.24(a)–17.24(c) in Elliott (2000) Integumentary system: Microscopic functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 271–306. London: Academic Press; (a) adapted from figure 311 in Bertin L (1958) Denticules cutanés et les dents. In: Grassé P-P (ed.) *Traité de Zoologie. Anatomie, Systématique, Biologie, Vol. 13: Agnathes et Poissons*, part 1, pp. 505-531. Paris: Elsevier-Masson; (b) adapted from figure 7(e), and (c) adapted from figures 1 and 2 in Sire J-Y (1990) From ganoid to elasmoid scales in the actinopterygian fishes. *Netherlands Journal of Zoology* 40: 75–92.

and Secondary Circulation: Secondary Circulation and Lymphatic Anatomy). The scales of gars (Lepisosteidae) have no dentine layer but still have a ganoine layer (**Figure 8**(**b**)). Small tooth-like odontodes may project from the surface of the scales of some bichirs and gars, although they may be only transiently present in juvenile or regenerating scales of gars (**Figure 8**(**b**)). The dermal ossification of sturgeons (Acipenseridae) and paddlefish (Polydontidae) is reduced to bony denticles or scales without dentine or ganoine. These structures are present only as isolated vestigial denticles in paddlefish, but exist as rows of large articulated bucklers and small denticles in sturgeon. One primitive actinopterygian, the bowfin (*Amia*), does not have ganoid scales; its scales are elasmoid scales of the cycloid type, very similar to those of teleosts.

Most living bony fishes are teleosteans, and the majority of these possess elasmoid (bony-ridge) scales (**Figures 1**, **4**, **5**, **8**(**c**), **8**(**d**), and **9**(**c**)). The elasmoid scale lacks dentine and ganoine and is usually reduced to a relatively thin, flexible, transparent structure consisting of: (1) a basal plate (the thickest layer) comprised of elasmodine that is partially mineralized and consists of several layers of collagen organized into a plywood-like structure (2) the external layer, a thin well-mineralized layer comprised of a network of interwoven collagen fibrils; and (3) the limiting layer (or outer limiting layer), a hyper-mineralized layer that lacks collagen fibrils; and is deposited at the scale surface in the region close to the epidermis (**Figures 5** and **9**(**c**)). Each elasmoid scale is usually marked by calcified concentric ridges (bony ridges) called circuli or striae (**Figures 5**, **8**(**c**), **8**(**d**), and **9**(**c**)). The circuli and the backward-facing denticles (**Figure 8**(**d**)) that are often present on their edges may assist in the mechanical anchoring of the scale into the covering dermis. Uncalcified grooves called sulci or radii converge on the scale center (focus) and allow flexibility (**Figures 8**(**c**) and **8**(**d**)).

On the basis of their surface sculpture, the elasmoid scales of teleosts are commonly grouped into two general types: cycloid and ctenoid. The cycloid scale is generally a round, thin, flat scale (**Figure 8**(**c**)). Ctenoid scales differ from cycloid scales in that they have stiff comb-like projections (cteni or ctenii), soft projections (ciliated cteni), or short tubercles or grains in the posterior area (**Figure 8**(**d**)). The function of the cteni or other posterior projections (collectively called granulation) is not well understood, but some scientists have hypothesized that they may improve the hydrodynamic efficiency of swimming and help to reduce drag by affecting the profile of the overlying epidermis.

Cycloid scales are characteristic of the more primitive teleosts with soft-rayed fins, including fishes such as trout and salmon (Salmonidae) (**Figures 4** and **5**), minnows (Cyprinidae) (**Figure 7**), and herring (Clupeidae), whereas ctenoid scales are found on many spiny-rayed fishes (Acanthopterygii) such as perch (Percidae), most mullets (Mugilidae), and scorpionfishes (Scorpaenidae). Nevertheless, certain soft-rayed fishes, such as a few of the characins (Characidae), have ctenoid-like contact organs on their scales, and conversely, some spiny-rayed fishes such as the brook silverside (*Labidesthes*) and wrasses (Labridae) have only cycloid scales. In certain species such as the mud dab (*Limanda*) and the freshwater basses (*Micropterus*), both cycloid and ctenoid scales may be present on the same individual. Furthermore, the scale types observed may differ depending on the age or sex of a fish.

Elasmoid scales are most often arranged in an overlapping (imbricated) pattern like shingles on a roof (**Figure 4**), with the free margin directed posteriorly to minimize friction with the water. The cteni of ctenoid scales are located on the posterior area that is not overlapped by other scales, and cycloid scales often have less distinct circuli in the posterior nonoverlapped area than in the anterior area. In certain fishes, the posterior edge of each scale approaches the epidermis, which forms only a slight indentation or tuck beneath the scale, while in other (usually pelagic) species, both the scale and the covering epidermis protrude and overlap the epidermis that covers the scale behind (**Figure 4**). Certain fishes such as burbots (*Lota*) and immature adult freshwater eels (*Anguilla*) have mosaic patterns of scalation; the scales are minutely separated or meet adjacent scales only at the margins rather than overlapping one another.

Growth of the formed elasmoid scale generally continues throughout life, with circuli marking successive stages in the growth of the scale. Closely spaced circuli discernible on magnified scales represent periods of slow growth. When these occur on an annual basis they are termed annuli (**Figures 8**(**c**) and **8**(**d**)). Annuli or accessory annuli (discontinuity of circuli that do not represent an annual cycle) may form as a result of decreased metabolism and appetite in cold seasons, fasting periods associated with spawning or unavailability of food, partial scale decalcification and resorption in females with developing eggs or young, or mechanical injury. Specific patterns of scale growth and morphology are used in fish life history and growth studies. Distinctive marks can also be induced on fish scales by manipulation of water temperature and feeding regimes; these are used for batch marking of hatchery-reared fish for later identification of specific groups. An advantage of using scales rather than certain other hard structures such as otoliths (ear bones) or vertebrae for such studies is that scales can be sampled without killing the fish. Scales may also store the history of environmental exposure, whether it be seawater versus freshwater or certain toxicants. Laser ablation coupled with ionic analysis with mass spectrometry of scales can be used to trace this history. For example, the ratio of strontium and calcium is often used to trace previous movements between freshwater and seawater.

Most teleost fish lose their scales at least once during their lifetimes, but both the lost scales and the overlying epidermis that is torn when the scales are shed usually regenerate quickly unless the integumental injury is severe. Some fishes lose their scales more easily than others, and excessive scale loss may be lethal among bycatch of certain fisheries.

Modified scales

A variety of scale modifications exist in fishes; most of these serve protective functions. Placoid scales of Chondrichthyes may be enlarged or fused to form defensive weapons that are particularly effective when associated with venom glands. Examples of such modifications include the tail spine or 'stinger' of stingrays (Dasyatidae), and the dorsal fin spines of the spiny dogfish shark (*Squalus*) and chimaeroids (*Chimaera*, *Hydrolagus*).

Many modifications of cycloid and ctenoid scales have evolved in teleost fishes as well. Specially perforated and sometimes tubulated scales provide protection and surface outlets for the lateral line sensory canal. The two knife-like spines that are carried in sheaths of skin at the base of the tail of surgeonfish (*Acanthurus*) are modified scales that swing out in an attack. Spinous scales are also present in teleosts such as triggerfishes (*Balistes*), filefishes (*Monacanthus*), the batfish (*Ogocephalus*), and frogfish (*Antennarius*). The numerous sharp bony spines that stand erect when a porcupine fish (Diodontidae) or puffer (Tetraodontidae) inflates its body are modified scales. In certain fishes such as seahorses and pipefishes (Syngnathidae), a dermal skeleton of bony plates is arranged segmentally and forms a protective semirigid case. For South American armored catfishes (e.g., Callichthyidae), protection is provided by large bony plates or scutes bearing tooth-like structures (dermal denticles) (**Figure 8**(**e**)). Among fishes, the most extreme examples of protective armor may be the rigid encasements of trunkfishes (Ostraciidae), which rival those of the most completely boxed turtles.

Fin rays

Dermal rays or spines support the fins of fishes. The fin supports in lampreys and hagfishes are unsegmented cartilaginous rods that provide only weak support. In both elasmobranchs and bony fishes, the dermal fin rays are known collectively as dermotrichia. These include several kinds of collagenous structures with varying types and degrees of mineralization. Dermotrichia may be segmented or unsegmented, branched or unbranched, biserial (two lateral elements paired on the midline) or uniserial. The fin rays (lepidotrichia) present in all Osteichthyes except dipnoans are comprised of mineralized bone and are believed to have derived from modified scales in early vertebrates.

Integumentary Extensions

Fishes have evolved a wide variety of integumentary modifications, including extensions of the skin. For example, barbels are integumentary extensions that have developed independently in many taxa as accessory feeding structures carrying sensory organs. Barbels that differ in structure and location are present on sturgeons

(Acipenseridae), goatfishes (Mullidae), marine and freshwater catfishes (Siluriformes), and some Cypriniformes such as carp (*Cyprinus carpio*) and loaches (Cobitidae).

The most frequently cited examples of the extension of skin into flaps are those of the sargassum fish (*Histrio*) and seadragon (e.g., *Phyllopteryx*). The function ascribed to the exaggerated skin flaps in these fishes is protective resemblance to the seaweed in which they hide. Although the skin extensions in certain other fishes, such as scorpionfishes (*Scorpaena*), are less prominent, these extensions, combined with mottled coloration, also likely aid in camouflage.

Certain less permanent skin extensions assist in reproduction, and may be reduced in size or shed completely after the breeding season. Keratinized breeding (nuptial) tubercles or pearl organs of Cypriniformes and certain other fishes function primarily to facilitate contact between spawning individuals. However, the antler-like breeding tubercles of breeding male stonerollers (*Campostoma anomala*) are used in defense of nests against intruding males. Outgrowths of abdominal skin or fin tissue of skin-brooding fishes enable the attachment of eggs to the skin.

Acknowledgments

The author thanks Carla Conway and Barry Egenes (Western Fisheries Research Center) for preparation of the figures.

See also: **Bony Fishes**: Crucian Carp. **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy. **Hagfishes and Lamprey**: Hagfishes. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes. **Role of the Gills**: Morphology of Branchial Ionocytes. **The Skin**: Coloration and Chromatophores in Fishes; Hagfish Slime; The Many Functions of Fish Integument. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics; Plasticity in Gill Morphology.

Further Reading

Bereiter-Hahn J and Zylberberg L (1993) Regeneration of the teleost fish scale. *Comparative Biochemistry and Physiology: A Comparative Physiology* 105: 625–641.

Elliott DG (2000) Integumentary system: Gross functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 95–108. London: Academic Press.

Elliott DG (2000) Integumentary system: Microscopic functional anatomy. In: Ostrander GK (ed.) *The Laboratory Fish*, pp. 271–306. London: Academic Press.

Farrell AP, Hodaly AH, and Wang S (2000) Metal analysis of scales taken from Arctic grayling. *Archives of Environmental Contamination and Toxicology* 39: 515–522.

Ferguson HW (2006) Skin. In: Ferguson HW (ed.) *Systemic Pathology of Fish,* 2nd edn., pp. 64–89. London: Scotian Press.

Huysseune A and Sire J-Y (1998) Evolution of patterns and processes in teeth and tooth-related tissues in non-mammalian vertebrates. *European Journal of Oral Sciences* 106(supplement 1): 437–481.

Kapoor BG and Khanna B (2004) Integument; dermal skeleton; coloration and pigment cells; epidermal breeding tubercles and bony contact organs; biotoxins; alarm pheromones. *Ichthyology Handbook*. New York: Springer.

Le Guellec D, Morvan-Dubois G, and Sire J-Y (2004) Skin development in bony fish with particular emphasis on collagen deposition in the dermis of the zebrafish (*Danio rerio*). *International Journal of Developmental Biology* 48: 217–231.

Meunier FJ and Brito PM (2004) Histology and morphology of the scales in some extinct and extant teleosts. *Cybium* 28: 225–235.

Raschi W and Tabit C (1992) Functional aspects of placoid scales: A review and update. *Australian Journal of Marine and Freshwater Research* 43: 123–147.

Sire J-Y (1990) From ganoid to elasmoid scales in the actinopterygian fishes. *Netherlands Journal of Zoology* 40: 75–92.

Sire J-Y and Akimenko M-A (2004) Scale development in fish: A review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). *International Journal of Developmental Biology* 48: 233–247.

Sire J-Y, Donoghue PCJ, and Vickaryous MK (2009) Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *Journal of Anatomy* 214: 409–440.

Sire J-Y and Huysseune A (2003) Formation of dermal skeletal and dental tissues in fish: A comparative and evolutionary approach. *Biological Reviews of the Cambridge Philosophical Society* 78: 219–249.

Wells BK, Bath GE, Thorrold SR, and Jones CM (2000) Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus)* scales reflects water chemistry. *Canadian Journal of Fisheries Aquatic Science* 57: 2122–2129.

Whitear M (1986) The skin of fishes including cyclostomes. Epidermis, dermis. In: Bereiter-Hahn J and Matoltsy AG (eds.) *Biology of the Integument – Vertebrates,* vol. 2, pp. 8–64. New York: Springer.

Witten PE and Huysseune A (2007) Mechanisms of chondrogenesis and osteogenesis in fins. In: Hall BK (ed.) *Fins into Limbs: Evolution, Development and Transformation*, pp. 79–92. Chicago: University of Chicago Press.

Zaccone G, Kapoor BG, Fasulo S, and Ainis L (2001) Structural, histochemical and functional aspects of the epidermis of fishes. *Advances in Marine Biology* 40: 253–348.

Coloration and Chromatophores in Fishes

D Burton, Memorial University of Newfoundland, St. John's, NL, Canada

© 2011 Elsevier Inc. All rights reserved.

Color: An Important Characteristic in Fish Integumentary Function
Pigmentary and Structural Coloration
Chromatophores
How Do Fish Change Color?
Physiological and Morphological Color Changes
The Regulation of Color Changes
Integumentary Patterns in Fish
Further Reading

Glossary

Chromatophores A variety of pigmented cells involved in the coloration of the skin of fish and other vertebrates and invertebrates. In fish, these are melanophores (black or brown), xanthophores (orange or yellow), erythrophores (red), cyanophores (blue), leukophores (whitish), and iridophores (iridescent).

Chromatosomes Pigmented organelles found within chromatophores. Their movements result in physiological color changes of fish.

Countershading A color scheme used by many fishes; the dorsal (upper) surface is a dark color to conceal the fish when viewed from above, and the ventral (lower) surface is a very pale color to conceal the fish when viewed from below.

Hue Variety of color or tint.

Iridescent Display of lustrous colors, which may vary with the angle of viewing. It is produced by interference of light waves with phase differences, when reflected from outer and inner surfaces of a transparent structure such as a crystal or membrane.

Melanin-concentrating hormone (MCH) A cyclic peptide neurosecretory hormone synthesized in the hypothalamus, stored in the pars nervosa of the pituitary, from which it is secreted, producing intracellular aggregation of melanosomes, paling skin in teleosts.

Melanophore-stimulating hormone (MSH) A peptide hormone synthesized in and secreted by the pars intermedia of the pituitary. It induces intracellular dispersion of melanosomes, resulting in skin darkening, and dispersion of xanthophore pigments, as well as longer-term morphological increases in melanophores and their melanogenesis.

Mononeuronic regulation Control of teleost melanophore physiological responses by a single nerve type (adrenergic) mediating bidirectional movements of melanosomes through α-adrenoceptors and either β-adrenoceptors or an ATP co-transmitter.

Morphological color changes Changes in the amount of pigmentation, or in the number of chromatophores, in the skin, including prolonged adaptations to background coloration and seasonal development of nuptial colors associated with breeding.

Physiological color change Rapid and slower changes in skin color due to chromatophore intracellular pigmentary movements.

Pigmentary coloration The color of melanophore, xanthophore, erythrophore, and cyanophore pigments depends on their selective absorption of incident light.

Structural coloration The microscopic structure of fish skin and the presence of iridophores and leukophores can reflect and scatter light to produce white, silvery, and, often, iridescent structural colors.

Color: An Important Characteristic in Fish Integumentary Function

The skin is the boundary between fish and its environment. It has special qualities, including a diversity of colors, often due to pigmented cells termed 'chromatophores', which serve vital roles in the survival of species. For example, varying abilities to change color may aid in the need to become obscure, or to advertise socially.

Such color changes have generated curiosity over a long historical period; in Roman times, they were reported by Pliny. At major aquaria, the enormous diversity in coloration and patterns is instantly apparent to an interested public. Many species display spectacular colors, others may be brown, gray, or black. Stripes and spots can be attributes of coloration and some species have the capacity to change pattern texture as well as color. Regardless of public and scientific interest, coloration has been studied in detail in very few of the over 20 000 extant fish species. Such studies have made significant contributions to fish ethology, physiology, cell biology, and our understanding of vertebrate autonomic neural and hormonal regulatory systems, including their interaction at the peripheral level.

Cyclostomes, elasmobranchs, and many teleosts are often dark gray, brown, or black. However, even darkly colored skin can display patterns as in some marine and freshwater rays. The actinopterygii (ray-finned teleosts) include the most brightly colored species, particularly those inhabiting coral reefs and other shallow tropical waters. Often, there is countershading in which a dark upper surface provides camouflage when viewed from above; a pale lower surface blending with bright surface water. This differentiation may be absent as in wolfish, *Anarrhichas lupus*, while in scribbled leatherjacket, *Aluterus scriptus*, both upper and lower surfaces are patterned dark and blue. Peritoneal melanophores also occur; their function invites investigation, although they can darken the stomach to conceal bioluminescent ingested prey.

The capacity of cyclostome, elasmobranch, and teleost species to change color in response to different types of stimuli varies and the extent of this phenomenon is unknown. Some actinopterygii, such as European minnow, *Phoxinus phoxinus*, and flatfish (order Pleuronectidae) inhabiting shallow clear water can change color rapidly on different backgrounds. Photocycle illumination periodicity may evoke chromatic change in various species, including cyclostomes; even brightly colored neon tetra, *Paracheirodon innesi*, and regal tang fish, *Paracanthurus hepatus*, dim at night. During breeding, Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus* sp.), and stickleback (*Gasterosteus* sp.) display gender-related nuptial color change, but this is not so in *Phoxinus* and *Pleuronectes*, which do respond chromatically to background. Stress is another factor that evokes color change in various species.

Possible relationships between skin coloration and fish color vision raise interesting questions. Deep-sea fish are generally black; those in blue-water depths (100–1000 m), including redfish such as the golden redfish, *Sebastes marinus*, appear black or gray in wavelengths penetrating this depth, but appear red at the surface.

Pigmentary and Structural Coloration

Integumentary colors can be pigmentary or structural, or a combination of both, resulting in the enormous intraspecific and interspecific range of hues and patterns. Pigmentary colors are caused by selective absorption of specific wavelengths of incident light by pigments contained within organelles. The perceived hue is the product of any residual reflected wavelengths. Structural coloration is associated with the physical nature of intracellular and extracellular surfaces that are reflective and cause interference or scattering of light. Iridescence depends on reflected wavelength phase shifts and phase interactions, perceived colors changing with the angle of viewing; in contrast, scattering involves shorter wavelengths and is caused by very small particles, producing colors which are not influenced by the angle of viewing.

Chromatophores

The pigmented cells that give coloration to the skin of fish and other vertebrates and invertebrates are called ‘chromatophores’. Therefore, chromatophores can be subdivided into those that absorb light (pigmentary) and those that reflect light (structural), and then further subdivided according to the pigment they contain (**Table 1**).

Origin and location of chromatophores

In fish, as in other vertebrates, all chromatophores originate from embryonic neural crest cells, differentiating during migration, or after distribution. The current view is that the various types of chromatophores are replenished from stem cells, or chromatoblasts, within the skin.

Table 1 Fish chromatophores

Chromatophore	*Color*	*Organelle*	*Pigment*
Light absorbing			
Melanophore	Black, brown	Melanosome	Melanins
Xanthophore	Yellow, orange	Xanthosome	Carotenoids
		Erythrosome	Pteridines
Erythrophore	Red, orange, yellow	Xanthosome	Carotenoids
		Erythrosome	Pteridines
Cyanophore	Blue	Cyanosome	
Light reflecting			
Leukophore	Whitish	Leukosome	Purine, uric acid
Iridophore	Iridescent		Purine platelets

The melanophores, xanthophores, erythrophores, cyanophores, and leukophores are dendritic cells associated with their shared embryonic origins with neurons and are categorized as 'paraneurons'; their pigmented organelles are collectively called 'chromatosomes'.

Fish chromatophores occur mainly in the upper layers of the dermis. In elasmobranchs and some teleosts, for example, flatfish species, both dermal and epidermal melanophores occur (**Figures 1(a)–1(d)**). The presence of epidermal xanthophores in the emerald rockcod, *Trematomus bernacchii*, suggests that other epidermal chromatophores may be found in fish in future. Dendritic processes of dermal melanophores have a stellate (star-shaped) or reticulate (net-like) arrangement, with intra- and interspecific variation and are more irregular in epidermal melanophores (**Figures 1(c)** and **1(d)**). The dendritic processes of dermal melanophores have a two-dimensional distribution, parallel to the skin surface; those of epidermal melanophores are three dimensional, adapted to being between tightly compressed cells. Dendritic processes have an intracellular organization of radiating cytoskeletal actin filaments and microtubules. Iridophores are usually round or oval, but in the dark sleeper, *Odontobutis obscura*, dendritic iridophores have been described.

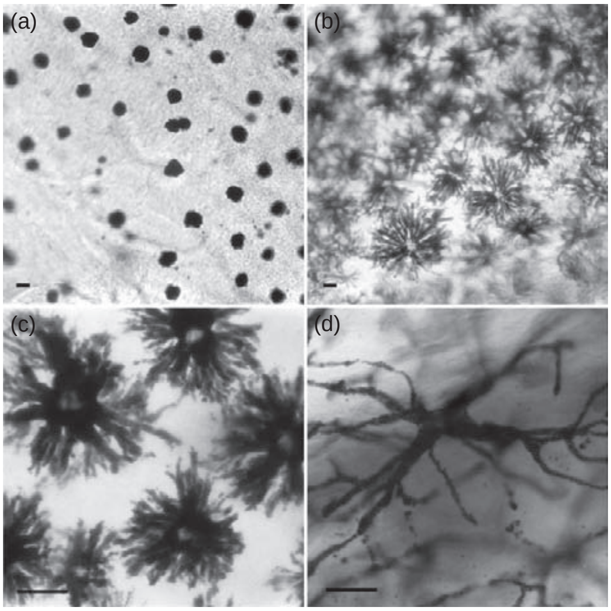


Figure 1 Dendritic chromatophores represented by melanophores of winter flounder, *Pseudopleuronectes americanus*. Dermal melanophores with melanosomes (a) aggregated into the cell center, (b) dispersed into the dendritic processes. (c) Dermal melanophores under higher magnification demonstrating the morphology of the melanosome-filled dendritic processes. (d) Epidermal melanophores with dispersed melanosomes demonstrating the morphological difference in the dendritic processes compared with dermal melanophores in (c). Scale = 20 μm. Photographic assistance: G. Hillier and C. Hawkins.

The colors

Without chemical analysis, it is safest to call yellowish organelles as xanthosomes and reddish organelles as erythrosomes. Cyanophores (blue) have recently been described in two callionymid species: the mandarin fish, *Synchiropus splendidus*, and psychedelic fish, *Synchiropus picturatus*. Leukophores (white) are known in few genera, including *Fundulus*, *Oryzias*, and *Lebistes*.

Melanins (brown and black) are derived from the amino acid tyrosine by melanogenesis, involving initial oxidation to dopa and dopaquinone, catalyzed by tyrosinase, followed by polymerization and decarboxylation to produce eumelanin in fish. Melanins are insoluble in water and organic solvents, but soluble in alkalis.

Carotenes (yellow and orange) are hydrocarbons and xanthophylls are oxygen-containing derivatives, such as astaxanthin, predominant in red marine fish. Carotenoid color is influenced by the number of double bonds, occurrence of certain radicals, and concentration. Carotenoids are insoluble in water, but soluble in oils and organic solvents, and are also known as 'lipochromes'. Animals cannot synthesize carotenoids, being dependent on plants for their supply, but can modify them into pigments found only in animals. Pteridines are heterocyclic nitrogenous catabolic products, with a pyrimidine ring and a secondary pyrazine ring, chemically and metabolically related to the purines. Their solubility in water is variable.

Although purines are colorless, they produce opaque whiteness or glistening silvery and iridescent colors in fish skin. They have limited solubility in water; ultrastructure studies reveal that they occur as uniformly spaced crystalline platelets in parallel stacks in iridophores. In general, blue colors are produced by iridophores and by the structure of the integument, including ordered dermal collagen arrays producing extracellular differential light scatter. Whitear and Mittal have shown that the ultrastructure of the epidermis of the pogge, *Agonus cataphractus*, and shanny, *Blennius pholis*, reveals a capacity for its superficial cells to develop labyrinthine patterns of microridges; the possible differential light-scattering potential of these, and of the cuticular composition, in brightly colored species calls for investigation.

Individual species may possess some or all types of chromatophores. Their relative frequencies can change during prolonged exposure to background, or in seasonal color changes during the reproductive cycle. The skin color is a summation of separate pigmentary and structural factors. In blue-green damselfish, *Chromis viridis*, such summation produces a characteristic green by means of chromatophore units, in which several small iridophores and sometimes small xanthophores, in that order, occur above a melanophore in the dermis.

How Do Fish Change Color?

Color change in fish chromatophores results from moving the pigment within a cell that stays in the same shape and size. This intracellular chromatosome, or crystalline platelet, motility producing color changes is in response to neural or hormonal stimuli. Such movement in dendritic chromatophores occurs along the radiating microtubules. Bidirectional movements are based on interactions between microtubular tubulin and various motor proteins with adenosine triphosphatase (ATPase) activity. Melanosome aggregation (**Figure 1**(**a**)) pales dendritic processes (not visible) and skin, whereas their dispersion (**Figures 1**(**b**)–**1**(**d**)) darkens dendritic processes and skin. In leukophores, the direction of leukosome movements is opposite to that in light-absorbing chromatophores, consistent with their contrasting contribution to skin coloration.

Iridophore platelets are very refractive and light reflective compared with the cytoplasm separating them (**Figure 2**).

The alternating layers are thin relative to light wavelength. A stack of platelets increases reflectivity considerably, the interference hue being influenced by the refractive index and thickness of the platelets and cytoplasmic layers. In leukophores, light is scattered in all directions by the leukosomes. Iridophores are densely distributed in pale and silvery areas, particularly the lower surface, which may be devoid of other chromatophores. Iridophore reflecting platelets do not necessarily move. However, the platelets can simultaneously change their inclination in *Paracheirodon innesi*, or move apart in some other species, in response to neural stimuli. This changes interference coloration by varying platelet spacing and might involve the cytoskeleton tubulin–dynein system within these cells.

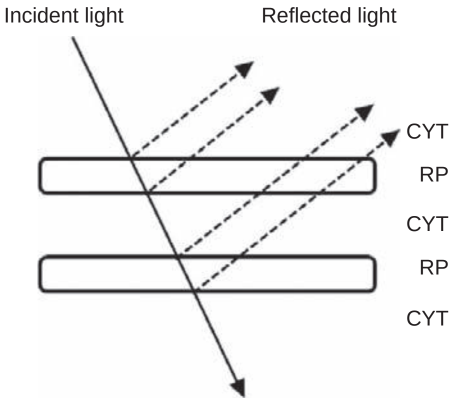


Figure 2 Simplified schematic of light reflection from two platelets of a stack within an iridophore. Different distances traveled by reflected light of each wavelength (not shown) result in a range of phase shifts, interference, and iridescent colors. CYT, cytoplasmic layers; RP, reflecting purine platelets. Graphics assistance: C. Short.

Color changes and pigment movements can be estimated or recorded at macroscopic or microscopic levels. *In vivo*, recording may involve visual comparisons with Munsell or Ostwald gray scales. Both *in vivo* and *in vitro* recording can employ a photocell, or a scale such as the Hogben melanophore index in which 1.0 represents complete melanosome aggregation (appearing pale) and 5.0 complete dispersion (appearing dark). Some accounts do not include a microscopic description of the chromatophores present, making it difficult to determine whether data represent the activity of one or more types. The variety of chromatophores present and the objectives of the experiment determine the suitability of a method. The often-quoted statement by Parker that "no single method has such superiority than it can be said to enjoy exclusive possession of the field" still holds today.

Physiological and Morphological Color Changes

Color changes may be physiological responses to background change (**Figure 3**), stressors, and photocycle and are rapid (seconds/hours), involving intracellular chromatosome movements as in the freshwater European minnow and marine flatfish. Physiological color changes can be primary responses of chromatophores directly to incident light, or, secondary, mediated through nervous and/or endocrine control. These criteria represent the current position in a prolonged debate on the two responses. Primary responses occur in those larval stages in which chromatophores are not yet under neural and/or endocrine control. There have also been reports of primary responses of chromatophores, both innervated and denervated, in adults of a few species, such as the dark chub, *Zacco temmincki*, and medaka, *Oryzias latipes*.

Morphological longer-term (days/weeks) responses to background or diet are due to changes in pigmentation within chromatophores or in the number of chromatophores. Thus, tilapia, *Sarotherodon mossambicus*, displays extensive increases and decreases in dermal melanophore numbers within 10 days' exposure to black and white backgrounds, respectively. However, the distinction between physiological and morphological color change is becoming blurred as our knowledge increases. In general, physiological color change precedes background-related morphological responses; both can involve the same hormones, neurotransmitters, and paracrine agents. Background-related morphological color change is now

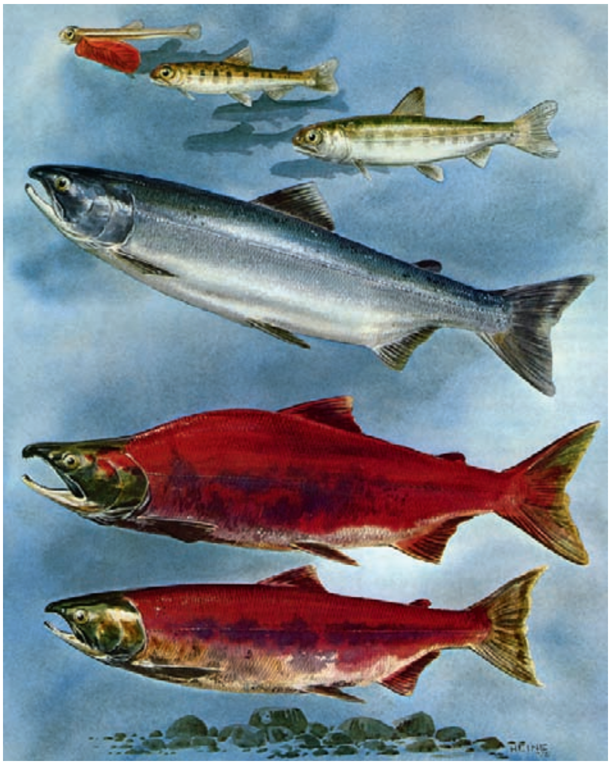


Figure 3 Sockeye salmon life-history stages. (Top to bottom) Alevin, fry, smolt, ocean phase, spawning male, and spawning female. Illustration courtesy of the estate of Harry Heine. Reproduced from plate 4 in Burgner RL (1991) Life history of sockeye salmon (*Oncorhynchus nerka*). In: Groot C and Margolis L (eds.) *Pacific Salmon Life Histories*, pp. 1–117. Vancouver: UBC Press, with permission from UBC Press.

considered a physiological phenomenon, representing a balance between differentiation of chromatophores from stem cells and their apoptosis (cell death), which is as an example of tissue homeostasis. Cytoplasmic cyclic adenosine monophosphate (cAMP) signaling pathways are involved in physiological color change and appear to regulate chromatophore survival and apoptosis, as well as their differentiation and dendricity (the number of processes).

Skin coloration may not be a reliable criterion in fish taxonomy, or a reliable discriminator of stock structure. Diet can influence xanthophore pigmentation. For example, the golden cod of Labrador is a population of Atlantic cod, *Gadus morhua*, in which skin color is influenced seasonally by an invertebrate diet that is rich in carotenoids. Morphological color changes can be associated with specific life-cycle stages, as in salmonids (**Figure 3**). The parr markings (vertical bars) of young salmonids are replaced by silvery sides and a darker dorsal surface during smoltification (**see also Hormonal Control of Reproduction and Growth:** Endocrinology of Fish Growth). When salmon return to breed in freshwater, nuptial red coloration is observed. The red pigment deposits in the fins and skin, as well as the pink muscle tissue of salmonids, come from dietary carotenoids, including the pigment astacene from oceanic crustacean.

The Regulation of Color Changes

In cyclostomes and elasmobranchs, neural control is unlikely. Instead, cyclostome melanophores are under agonistic pineal and pituitary regulation and elasmobranch melanophores are under pituitary control.

In teleosts, background-related physiological color change is mediated through rapid sympathetic adrenergic nervous regulation and/or slower hormonal control. There is considerable interspecific variation in the extent of neural and hormonal regulation of teleost physiological chromatic change. In paradise fish, *Macropodus opercularis*, rapid responses of melanophores to black and white background changes involve the nervous system alone; in *Phoxinus*, there is rapid neural initial control and slower pituitary secondary control; and in eel, *Anguilla anguilla*, there is predominantly slower pituitary control. Both neural and pituitary controls are essential in attaining final chromatic equilibrium in *Phoxinus*.

Background-related color changes involve photic stimulation of the eyes; subsequent central processing is only partly understood. The dorsal retina receives light reflected from the substrate, direct light from above being received by the ventral retina; the ratio between the two, or albedo, determines responses. There is evidence for excitatory and inhibitory synapses at different levels within the brain, including the optic tectum and medulla oblongata (**see also Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes and Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern). A melanosome-aggregating spinal cord pathway originates from a paling center in the medulla oblongata and has a local outflow, varying between species, to the sympathetic chain. The neural pathways then follow anterior and posterior sympathetic routes, connecting through spinal nerves to an adrenergic neural plexus associated with the dermal melanophores.

It has been demonstrated that, in *Phoxinus*, the spinal pigmentomotor tracts have a dorsomedial distribution within the substantia gelatinosa Rolandi and corpus commune posterius. Microsurgical lesions, including unilateral ones, in the spinal pigmentomotor tracts produce bilateral dark mottled patterns over the entire dorsal and lateral skin in white-adapted *Phoxinus*. This indicates a diffuse neural distribution to the melanophores, including some contralateral decussation. Individual melanophores can display degrees of postsurgical asymmetrical dispersion, demonstrating their multiple neural associations with the dermal plexus. Similar asymmetrical responses, along margins of surgically produced caudal bands, lead to the same conclusion. Functionally, cutaneous pigmentomotor axons appear to retain their integrity rather than synapse within the dermal nerve plexus; a conclusion supported histologically in *Phoxinus*.

Dineuronic control in teleosts (i.e., two separate types of control neurons), involving melanosome-aggregating and -dispersing neurons, as opposed to mononeuronic control involving a single neuron type (adrenergic), has been debated extensively. Currently, there is no support for cholinergic neural control of melanosome dispersion. Alternatively, spinal pigmentomotor neuron activity could be regulated by excitatory and inhibitory centers within the brain, providing a basis for mononeuronic control of bidirectional responses, dependent on melanophore adrenoceptor physiology.

One mechanism for mononeuronic bidirectional regulation involves the presence of α- and β-adrenoceptors on the melanophore plasma membrane. Such adrenoceptors can facilitate mononeuronic control, since concentration level of a single neurotransmitter changes the bias between α- and β-adrenoceptor activity. α-Adrenoceptors mediate melanosome aggregation; α_2-adrenoceptors are the effective subtype, but α_1-adrenoceptors are functional in at least some teleosts. β-Adrenoceptors mediate melanosome dispersion; both β_1- and β_2-adrenoceptors coexist on melanophores of two gobies, *Tridentiger trigonocephalus* and *Chasmichthys gulosus*, and winter flounder, *Pseudopleuronectes americanus*. Melanophore α-adrenoceptors have a higher stimulation threshold. In *Pseudopleuronectes*, melanosome dispersion in response to lower concentrations of catecholamine is reversed over a transition range as α-adrenoceptor activity overrides that of β-adrenoceptors at higher concentrations. An alternative mononeuronic mechanism occurs in melanophore control in tilapia, guppies, and siluroid catfish. ATP is released from adrenergic nerves as a co-transmitter; adenosine resulting from its dephosphorylation reverses aggregation by the clearing catecholamine.

The major intracellular second messenger in teleost melanophores is cAMP, which mediates melanosome dispersion. Stimulation of β-adrenoceptors increases adenylate-cyclase activity, raising cAMP levels. α_2-Adrenoceptor stimulation reduces adenylate-cyclase activity and cAMP levels. When α_1-adrenoceptors are present on chromatophores, they mediate more rapid pigment aggregation through another second messenger, inositol 1,2,5-trisphosphate (IP_3), by release of intracellular Ca^{2+}.

Pituitary hormones control slower (hours) physiological and prolonged (days/weeks) morphological color changes. Melanophore-stimulating hormone (MSH), synthesized in the pars intermedia, increases chromatophore intracellular cAMP levels, triggering chromatosome dispersion on a black background. Darkening is associated with increased plasma MSH and pituitary MSH cell activation. During morphological color change, this hormone stimulates melanogenesis through cAMP signaling to trigger activation of tyrosinase. MSH, continually released from an implanted minipump, can stimulate melanophore development *in vivo* in tilapia on a white background. Another hormone, melanin-concentrating hormone (MCH), hypothalamic in origin but stored in the pars nervosa, aggregates melanosomes on a white background by decreasing melanophore cAMP signaling. Long-term MCH treatment on a black background inhibits melanin increase. MCH also inhibits MSH secretion. It is not clear whether MCH is involved in melanophore apoptosis during prolonged adaptation to a white background, although neuronal noradrenaline is considered important.

Chromatophore physiological responses can involve the pineal body, and its hormone melatonin (*N*-acetyl-5-methoxytryptamine), in nighttime blanching associated with circadian rhythms. There are diurnal changes in plasma melatonin in flounder, *Platichthys flesus*, in continuous darkness, indicating the presence of an intrapineal oscillator. This contrasts with direct regulation of melatonin by light intensity acting on the pineal in rainbow trout, *Oncorhynchus mykiss*. Melatonin aggregates melanosomes and erythrosomes in some species, but others are refractory, which may relate to ethological differences.

Additional hormones implicated in chromatic physiology include adrenaline, adrenocorticotropic hormone, and gonadal steroids, so also are paracrine factors. Melanization may be influenced locally by melanization-stimulating factor (MSF) and melanization-inhibiting factor (MIF) within the skin. A few reports indicate that prostaglandins, endothelins, and nitric oxide influence melanophore physiological responses.

Integumentary Patterns in Fish

Integumentary coloration ranges from a single hue to chromatic patterns, which may undergo short-term or longer seasonal change. Skin patterns are important in the lives of fish; they may have blending or disruptive cryptic roles, be epigamic or associated with stress and excitement. Causal ethological analysis has been used, with variable success, to determine whether particular patterns, and their intensity, represent indicators for motivational states, which control the motor performances of fish. Such analysis may provide a bridge toward physiological research on mechanisms controlling particular behavioral attributes. Detailed accounts of the basis of patterns are available for relatively few fish species. These involve local variations in the chromatophore types, their relative size, and numerical distribution. In addition, rapid pattern changes may involve differential physiological responsiveness of chromatophores.

Seasonally, iridescence, coupled with erythrophore-based red coloration and zigzag dancing behavior, in male sticklebacks (*Gasterosteus aculeatus*), attracts females. This nuptial coloration is a secondary sexual characteristic dependent on testicular androgen levels. *Phoxinus* has lateral vertical stripes with macromelanophores distributed differentially, resulting in a disruptive pattern. Macromelanophore patterns in *Xiphophorus* have been studied for evidence of a genetic factor in human melanoma formation. Hybrids of platyfish, *Xiphophorus maculatus*, and swordtail, *Xiphophorus helleri*, develop spontaneously, malignant melanoma induced by genes clearly defined by classical genetics. In paradise fish (*Macropodus opercularis*), alternate red and green vertical banding is associated with a numerical distribution of different types of chromatophores. They also display a gradient of physiological responses of melanophores, at various depths of the skin, to background changes. In flatfish (Pleuronectiformes), the patterns have morphological attributes, but changes involve differential physiological responses. Local variations in α- and β-adrenoceptor transition ranges facilitate simultaneous paling and darkening of different pattern areas of flatfish by adrenergic neurons alone. These fish are exceptional among animals in their sensitivity to both substrate texture and color (**Figure 4**).

Intrapattern scale autografts demonstrate that in yoshinobori, *Rhinogobius brunneus*, differential activity is a neural function rather than a chromatophore function. In green sunfish, *Lepomis cyanellis*, and bluegill, *Lepomis macrochirus*, localized electrical stimulation of the anterior medulla oblongata produces banding patterns, while stimulation of its posterior region elicits paling. In pencil fish, *Nannostomas trifasciatus*, melatonin may be involved in circadian rhythms of patterns; treatment with it during the daytime induces night patterns. It aggregates melanosomes in most of the pattern, while macromelanophores in dark spots display pigment dispersion, implicating two types of melatonin receptors.

In cichlids, pattern displays can depend on differentially responding superficial and deep melanophores. In the cichlid *Haplochromis burtoni*, territorial males possess a dark eyebar with melanophores differentially controlled during social signaling by an eyebar nerve. There are intermorph differences; eyebar melanophores of barless males have less pigment and are more responsive to noradrenaline. A wide variety of color pattern polymorphisms occur in cichlid fish in Africa; Lake Victoria being remarkable for its diversity of both interspecific and intraspecific color morphs. How this diversity is maintained is a problem that continues to be addressed.

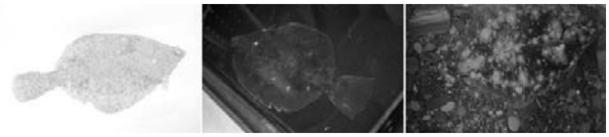


Figure 4 Color of *Pseudopleuronectes americanus* on (left to right) white, black, and pebble backgrounds. On pebbles, responses vary between different areas of skin producing a disruptive camouflaging pattern. Photographic assistance: G. Hillier and C. Hawkins.

During pattern metamorphosis in zebrafish, *Danio*, selective survival and apoptosis of melanophores are essential for stripe formation; larval and adult patterns being different. In channel catfish, *Ictalurus punctatus*, MIF inhibits melanization in white ventral skin; MSF stimulates melanization in black dorsal skin. Local variations in activity of these two factors may be a major influence in developing fish pattern morphology.

Within the ancient taxa represented by fish, chromatic cellular and regulatory processes have undergone widespread adaptations, resulting in diverse integumentary patterns and their control, related to habitat and behavioral requirements. Historically, individual research programs on fish coloration have dwelled either on chromatophores and their control, or on ethological aspects; greater emphasis needs to be placed on combining both approaches.

See also: **Brain and Nervous System:** Autonomic Nervous System of Fishes; Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern. **Hearing and Lateral Line:** Vestibular System Anatomy and Physiology. **Sensory Systems, Perception, and Learning:** Circadian Rhythms in Fish; Communication Behavior: Visual Signals. **The Pituitary:** Pituitary Gland or Hypophysis. **The Skin:** Functional Morphology of the Integumentary System in Fishes. **Vision:** Color vision and Color Communication in Reef Fish.

Further Reading

Bagnara JT and Hadley ME (1973) *Chromatophores and Color Change, the Comparative Physiology of Animal Pigmentation*. Englewood Cliffs, NJ: Prentice Hall.

Baker BI (1991) Melanin-concentrating hormone: A general vertebrate neuropeptide. *International Review of Cytology* 126: 1–47.

Burton D (2009) Flatfish (Pleuronectiformes) chromatic biology. *Reviews in Fish Biology and Fisheries* 20: 31–46 (doi:10.1007/s11160–009–9119–0).

Cott HB (1940) *Adaptive Coloration in Animals*. London: Methuen.

Dijkstra PD, Seehausen O, and Groothuis TGG (2008) Intrasexual competition among females and the stabilization of a conspicuous color polymorphism in a Lake Victoria cichlid fish. *Proceedings of the Royal Society B* 275: 519–526.

Fox HM and Vevers G (1960) *The Nature of Animal Colors*. London: Sidgwick and Jackson.

Fujii R (1969) Chromatophores and pigments. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 3, pp. 307–353. New York: Academic Press.

Fujii R (1993) Coloration and chromatophores. In: Evans DH (ed.) *The Physiology of Fish*, pp. 535–562. Boca Raton, FL: CRC Press.
Fujii R (2000) The regulation of motile activity in fish chromatophores. *Pigment Cell Research* 13: 300–319.
Grove DJ (1994) Chromatophores. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 331–352. Chur, Switzerland: Harwood.
Parker GH (1948) *Animal Color Changes and Their Neurohumors*. London: Cambridge University Press.
Schartl M and Adam D (1992) Molecular cloning, structural characterization, and analysis of transcription of the melanoma oncogene of *Xiphophorus*. *Pigment Cell Research Supplement* 2: 173–180.
Schliwa M (1984) Mechanisms of intracellular organelle transport. In: Skay JW (ed.) *Cell and Muscle Motility*, vol. 5, pp. 1–82. New York: Plenum.
Sugimoto M and Oshima N (eds.) (2002) *Special Issue: Biology of Pigment Cells in Fish* (11 articles). *Microscopy Research and Technique* 58: 433–523.

Bioluminescence in Fishes

AF Mensinger, University of Minnesota Duluth, Duluth, MN, USA

© 2011 Elsevier Inc. All rights reserved.

Light Organs
Light Environment
Luminescence Observation
Flash Kinetics
Counter-illumination
Bioluminescent Fish
The Future
Further Reading

Glossary

Bathypelagic Aphotic ocean-depth zone from 1000 to 4000 m.

Bioluminescence Light produced by a chemical reaction that originates in an organism,

Counter-illumination A defensive measure by bioluminescent midwater cephalopods, decapod crustaceans, and fishes that camouflages an animal being viewed from below by mimicking downwelling light and reducing its silhouette.

Isolume A line joining points of equal light intensity.

Luminescence Light emission that does not derive energy from the temperature of the emitting body, as in bioluminescence.

Mesopelagic A biogeographic region of open oceans between 200 and 1000 m depth and not near the ocean floor.

Photophore Light-emitting organ in bioluminescent animals.

Many people's experience with bioluminescence is limited to firefly displays or observing their own ghostly outline while swimming through dinoflagellates during nocturnal forays into the ocean. Yet, beneath the ocean's surface, creatures spanning the scale from bacteria to jellyfish to sharks are capable of a dazzling array of luminescent displays. However, the diversity of bioluminescent organs reaches its apex in teleosts with approximately 190 genera in over 45 families containing bioluminescent individuals. Most luminescence is produced by endogenous chemical reactions involving a substrate (luciferin) catalyzed by either luciferase or a photoprotein. Bioluminescence has clearly evolved many times independently, although the substrates are relatively conserved and only four principal luciferins are responsible for most marine bioluminescence. Moreover, the majority of fish luminescence is based on Cypridina or Coelenterazine luciferin. A number of fish species harbor symbiotic bioluminescent bacteria that provide light and these prokaryotes use a different biochemical pathway.

Light Organs

Light organs or luminescent tissues come in a diverse array of shapes and sizes, ranging from simple photophores to complex protuberances. A number of species culture bioluminescent bacteria that can be located in a large organ under the eye (flashlight fish), in luminescent lures at the end of long stalks (anglerfish), or internally in organs that transmit light through translucent tissue (pony fish). As the bacteria appear to luminesce constantly, some species have developed shutter or filter mechanisms, to regulate the light. Although the bioluminescence from these organs can be diffused over large areas, there is rarely more than one site where the bacteria are cultured.

In contrast, other fish have evolved hundreds of small, circular dermal photophores that are scattered on their ventral and/or lateral surfaces, are neurally controlled, and emit variable flashes. Many deep-water species have evolved pairs of orbital organs. Other families, such as the myctophidae (lanternfish), have streaks of luminous tissue on their head or caudle peduncle in additional to photophores. The tube shoulders (platytroctidae) squirt luminescent ink into the environment from anterior-situated glands situated on their shoulders. In his 1978 review, John Buck postulated at least 20 functions of luminescence spanning three main areas: predation, defense, and communication (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals). Virtually every example is found among teleost fish with organ, tissue, and/or photophore combinations allowing multiple functions.

Light Environment

The brighter the environment, the less effective is luminescence. Therefore, the majority of luminescent fish are black-water creatures and live where there is little or no light that could interfere with their displays (**see also Vision**: Visual Adaptations to the Deep Sea, Physiological Optics in Fishes, Photoreceptors and Visual Pigments, and Adaptations of Photoreceptors and Visual Pigments). These habitats include dark caves or crevices in shallow-water reefs, the variable twilight of the mesopelagic ocean, or the constant darkness (except for bioluminescence) of the abyssal plain. A number of species undergo diurnal migration, following dim isolumes and ascending in the water at night before returning to the darker depths during the day. The majority of luminescent fishes are meso- or bathypelagic, inhabiting an environment where spatial refuge is scarce and a premium is placed on remaining inconspicuous. Yet, by their very nature, light displays can compromise the emitter's location and expose it to predators. Therefore, luminescent animals must balance the advantage of luminescent displays against the risk of detection.

As light originates from astral sources, the prevailing light direction is downward. Seawater filters both the intensity and the spectral composition of the downwelling light relatively quickly with blue light the dominant wavelength at depth. The majority of fish luminescence is blue or blue-green, with emission maxima rarely exceeding 515 nm, which is consistent with wavelengths that would be maximally transmitted through the water column. Midwater visual systems show a general trend toward trading visual acuity for visual sensitivity to maximize photon capture and many retinas are dominated by rods and/or possess a reflective tapetum. Upward-looking eyes, best suited for discerning faint silhouettes illuminated by the downwelling light, are common and the visual pigments of most luminescent fish are maximally sensitive to blue-green light. It is estimated that visual adaptations allow the detection of daylight in midwater animals at depths up to 1000 m in the open ocean.

Luminescence Observation

As the vast majority of luminescent fishes inhabit the deep ocean, only a few shallow water species or hardy midwater dwellers have been captured in sufficient condition to study the physiology and function of luminescent organs. More often than not, mesopelagic fish are moribund, and while some remain alive long enough to observe flash patterns and quantify light intensities, one must wonder how representative these displays are to the natural condition. While submersibles and remote-operated vehicles (ROVs) have provided great insight into the midwater world, their bright lights scare or blind luminescent fish and make *in situ* observations of natural luminescence impossible. Therefore, much information on luminescent fish is inferred and based on the morphology, size, types, and body position of luminescent organs.

Flash Kinetics

The flash kinetics of the light displays is highly variable. Symbiotic bioluminescent bacteria emit a continuous glow that can be blocked by shutter mechanisms or perhaps modified by oxygen regulation to the colony. However, many bacterial symbionts are used for defense, so that the continuous glow may not increase the risk of predation. Photophores or other luminous tissue that are neurally controlled can regulate flash duration, frequency, and intensity, resulting in irregular single flashes to trains of high-frequency flashes. However, the majority of these displays are brief (<1 s), and as pharmacological manipulation of these organs can produce long-lasting glows, it does not appear that the displays are substrate limited. Rather, it could represent the continual balance of display versus detection.

Counter-illumination

The prevailing downward direction of light has been a strong evolutionary driving force in selecting the pigmentation of near-surface fish resulting in dark backs and lighter ventral surfaces. A lighter underside results in less-visible silhouette to visual predators lurking below. In contrast, the majority of luminescent fish are uniformly black, presumably an adaptation to minimize light reflection and remain unseen. Forward-facing or upwardly directed eyes, combined with the darker depths, result in ventral blind spots for fish. The lateral line only provides short-range detection, so predators that discern a faint silhouette can close in from below with little chance of detection. For defensive purposes, fish have evolved ventral counter-luminescence organs that match the intensity and angular distribution of downwelling light. The luminescence can originate from internal bacterial organs that diffuse light through translucent tissue or serially arranged ventral photophores. The long-term glow from these organs is consistent with a need for extended periods of counter-illumination.

Bioluminescent Fish

Hatchetfish

While ventrally directed light has been hypothesized for counter-illumination, few studies have empirically demonstrated it. Hatchetfish, such as *Sternoptyx* and *Argyropelecus*, are small, laterally compressed fish with rows of photophores lining the ventral portion of their thin silhouette (**Figure 1**). Moribund on capture, the ventral photophores normally will emit long-lasting glows; however, *Argyropelecus* can emit brief (<100 ms), high-intensity flashes. *Sternoptyx* has two large luminescent organs in the roof of its mouth that produce longer flashes (~1 s). Large tubular reflector channels are associated with the ventral photophores and match the angular distribution of luminescence with the downwelling light. A preorbital organ in *Argyropelecus* shines directly into the eye and is hypothesized to control ventral photophore intensity by acting as a reference for ambient light. Whether the oral organs are used in a similar fashion or used to lure prey remains to be determined.

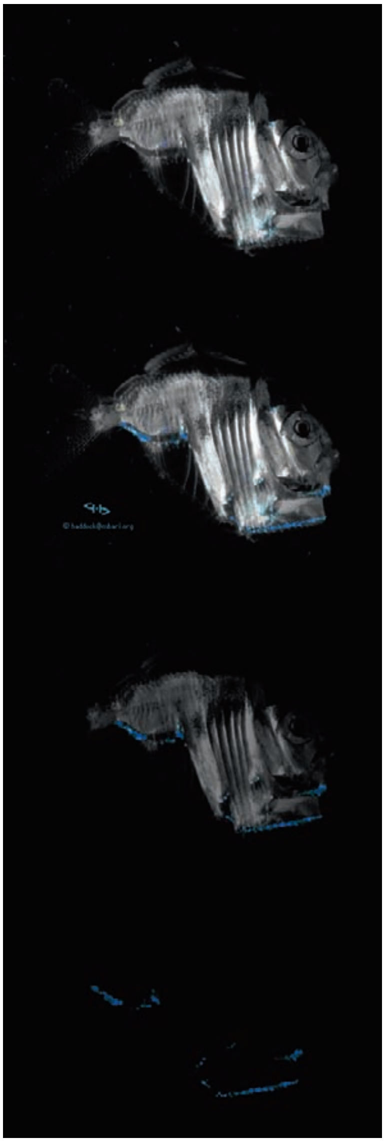


Figure 1 The hatchetfish, *Sternoptyx*, has many photophores beneath its body and contains the substrate luciferin which will fluoresce under ultraviolet (UV) light. Under normal circumstances, bioluminescent light would be emitted from these same fluorescent photophores. The glow is directed downward through light guides, presumably to function as counter-illumination (fish length, approximately 3 cm). Photograph by Steve Haddock.

Midshipman

Perhaps, the best example of counter-illumination is in the midshipman *Poricthys notatus*. Its name originates from the 800 ventral photophores that resemble the buttons on a midshipman's coat (**Figure 2**). The fish is found along the Pacific coast of the continental US, and probably is best known for its relatively loud and incessant hum generated during the spring mating season, which disrupts the sleep of houseboat owners. Fish are buried in the sand during the day, but ascend the water column at night to feed. They migrate from offshore depths to the intertidal habitat to breed. The male fish acoustically attract females and guard the developing young until they detach from the nest. Although the juvenile fish obtain luciferin from the egg yolk, the fish need to acquire dietary sources of luciferin to remain luminescent. The main source of exogenous luciferin in the wild appears to be the luminescent ostracod, *Vargula tsujii*; however, cannibalism of luminescent conspecifics also restores luminescence.

The ventral photophores, which are neurally controlled, approximate the angular distribution and intensity of downwelling irradiance rendering the silhouette of *P. notatus* invisible to dark-adapted observers. Counter-illumination was also effective in reducing predation by larger conspecifics. Interestingly, the counter-illumination was only initiated when a hydrodynamic stimulus was paired with an appropriate light presentation. Thus, luminescence is only initiated when the fish sense a disturbance such as a potential predator. This is in contrast to the continuous glows exhibited in other counterilluminating fish, and may conserve luciferin and/or energy. The fish also preferentially preys on organisms that have been illuminated by dinoflagellate luminescence in one of the few demonstrated examples of the luminescent burglar-alarm theory.

Flashlight Fish

The most widely known group of luminescent fishes to both science and the public are the flashlight fish

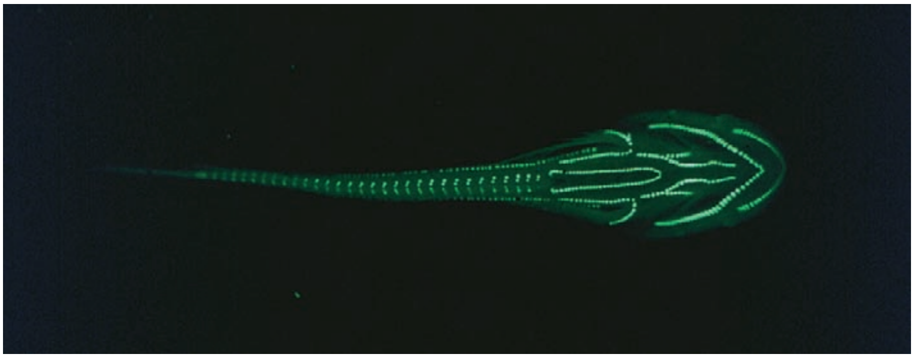


Figure 2 Ventral view of the midshipman, *Porichthys notatus*. Its photophores are stimulated to fluoresce under ultraviolet light and function for counterillumination.

(Anomalopidae), which are often displayed in public aquaria and are amenable for laboratory studies. Although almost exclusively nocturnal, they will depart from daytime hiding spots in reef caves and venture to shallow water during the night. A single, kidney-shaped light organ lies beneath each eye, and harbors dense concentrations of continuously luminescent bacteria. The light can be turned on or off by a black curtain that is raised from the lower border of the socket or by rotating the organ downward into a black pocket. The fish have exquisite control over the shutter, and can flash over 100 times min^{-1}. Jim Morin has studied the fish in the field and laboratory and has postulated that the lights are used to attract or illuminate prey, avoid predators, and for intraspecific signaling.

Anglerfish

Anglerfish also culture bacteria in an esca, a fleshy growth at the end of a modified dorsal fin spine (**Figure 3**). The spine is movable and the esca is used as a luminescent lure to attract prey to the fish. The bacteria appear to glow continually. However, flashes or pulses of light have been observed from live fish. The control mechanism is not understood, but blood flow and concomitant oxygen availability to the organ may be involved in regulation. Only female anglerfish are luminescent, which is consistent with their unusual natural history. The much smaller, nonluminescent males permanently attach to the female, who provides nutrients to the male throughout its life. Esca are species specific and range from simple bulbs to

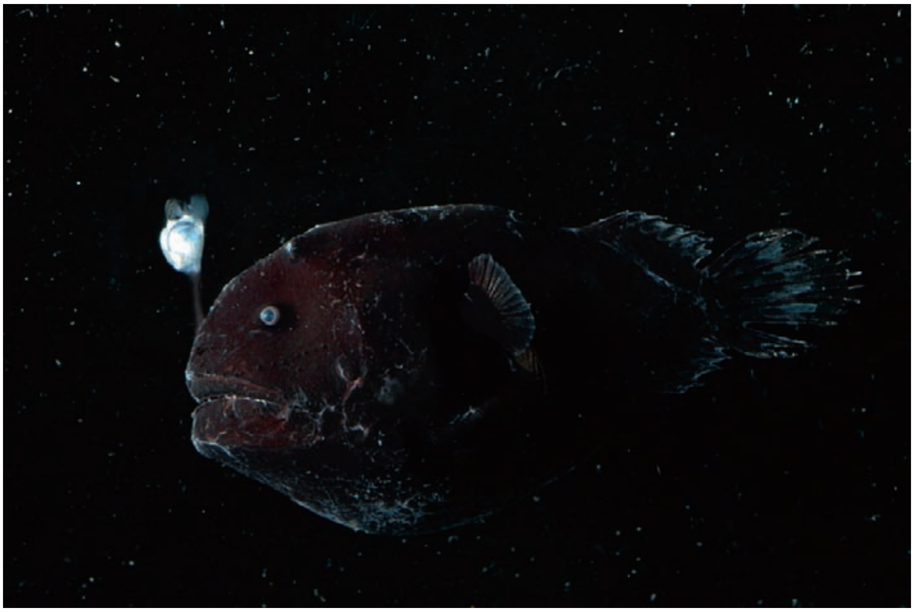


Figure 3 The anglerfish *Chaenophryne longiceps.* Anglerfish culture bacteria in an esca, which is a fleshy growth at the end of a modified dorsal fin spine. The spine is movable and the esca is used as a luminescent lure to attract prey to the fish (fish length approximately 4 cm). Photograph by Steve Haddock.

elaborate protuberances that may assist in the male locating the correct mate.

Cardinal Fish

Another group of relatively shallow-water species, the cardinal fishes (Apogonidae), have evolved visceral light organs. The light organs can be undifferentiated portions of the intestinal tract or specialized out-pockets of the alimentary canal. The unique aspect of this group is that luminescence has evolved independently within the family, with some species housing luminescent bacteria while others rely on endogenous biochemical reactions. Fish in the genus *Siphamia* have an organ containing luminescent bacteria that sits on top of the ventral musculature that is covered with chromatophores. Recent findings indicate that at least two species of *Siphamia* harbor an additional oral bacterial light organ that may be used to lure prey into the mouth. Endogenous light organs are formed from out-pockets of the intestine or pyloric ceca with some fish having an additional pair of anal light organs. The glow from either the bacterial or the glandular organs is projected through transparent keel muscles that contain chromatophores (**see also The Skin**: Coloration and Chromatophores in Fishes). The muscles act to diffuse the light with the intensity perhaps regulated by the chromatophores, and is presumably used for counter-illumination.

Ponyfish

Ponyfish (Leiognathidae) also project light through translucent tissue. These fish are found in coastal areas of the tropical and subtropical Indo-Pacific, and have evolved a light-organ system (LOS) that contains a circumesophageal bacterial light organ complete with reflectors, chromatophores, shutter, translucent and reflective swimbladder tissue, and translucent bone, muscle, and skin. An intense but diffuse blue-green light can be emitted ventrally for counter-illumination. Translucent patches on the flanks, opercular margin, and/or buccal cavity can emit rapid flashes of light, and the strong sexual dimorphism of the LOS combined with *in situ* observations of schooling fish suggests sex-specific signaling. The flashing may also function in prey attraction, predator avoidance, or schooling.

Barreleye Fish

Barreleye fish (Opisthoproctidae) are named for their unusual barrel-shaped, tubular eyes with symbiotic bacterial bioluminescence reported in three genera. The ventral surface of the mirror belly Opisthoproctus is characterized by a flattened and projecting sole that may act to reflect the luminescence downward from a single rectal organ for counter-illumination. The eyes were originally considered to be directed permanently upward to search for prey, but recent observations show that eyes can pivot forward.

Tubeshoulders

Tubeshoulders are a family (Platytroctidae, formerly Searsiidae) of mid- to deep-water fish that possess multiple luminescent organs. Ventrally directed photophores and patches of white tissue are found on the body. However, they can also eject a blue-green luminous fluid from a black sac located under the pectoral girdle. The secretion is bright and long lasting and may be analogous to squid ink, blinding, or confusing predators. The ink is composed of modified cells that form sheets or balls, which may stick to potential predators that could turn the hunter into the hunted.

Lanternfish

The lanternfish or myctophids are very abundant midwater fish found throughout the world's oceans, consisting of approximately 300 species, many of which undergo diurnal vertical migrations. The lanternfish account for over 60% of all deep-sea fish biomass and play an important ecological role as prey for larger organisms; thus, there is strong selective pressure to remain inconspicuous. The fish possess species-specific serial photophores and/or luminous tissue. The mostly ventral primary photophores are well developed and contain lenses, while smaller, less-developed secondary photophores are distributed over the body. Streaks or patches of luminous tissue may appear on the flanks and heads, but more often are found on the caudal peduncle as supra- and infracaudal organs (**Figure 4**). Although moribund on capture, specimens can remain alive for several hours allowing estimation of flash intensity and kinetics. Serial photophores produce a dim glow for hours, and can also increase light intensity during rapid flickering events.

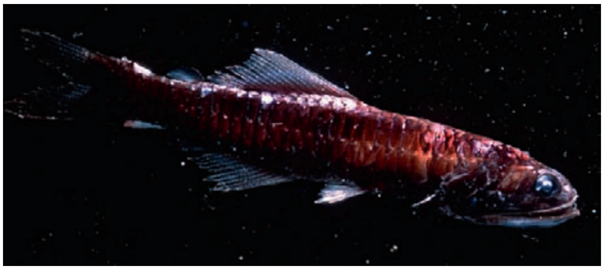


Figure 4 Some lanternfish (myctophids) have very bright light organs near their tail (white caudal organs). These stern chasers produce a blinding flash at the instant that the animal darts away, leaving a confused predator in its wake. Photograph by Steve Haddock.

The intensity of these photophores can be modulated by ambient light intensity, suggesting a role in counter-illumination. Luminous tissue displays much quicker (<100 ms) and intense flashes with frequencies up to 30 Hz. Cephalic tissue in Diaphus, producing flashes of 300–400 ms, appears suited for prey illumination; however, species-specific signaling is also possible. The caudal organs have been characterized as stern chasers, whose quick flashes combined with tail flips could confuse trailing predators. Although photophore patterns were hypothesized originally to serve for species recognition, the minute differences in photophore location within genera and the tendency of midwater animals to sacrifice visual acuity make identification unlikely at the species level.

Dragonfish

The dragonfish (family Stomiidae) comprise 27 genera of midwater fish that all contain a prominent postorbital photophore. As with the myctophids, many of these fish survive capture for a short period of time, allowing bioluminescence to be observed. The typical member of this family is an elongated fish, equipped with impressive detention and possessing diverse luminescent organs. Most bear well-developed, serial ventral photophores in addition to minute photophores scattered over the rest of the body. Many have two orbital photophores, glandular patches of luminous tissue, and/or luminous bodies found among fin rays. The barbels are quite diverse, ranging in length from stubby chin protrusions to filaments longer than the fish, and in morphology from simple stalks to elaborate fleshy protrusions (**Figure 5**). The luminous tissue may extend the whole length or be confined to the terminal end of the barbel. The glandular patches can emit trains of high-intensity light, and are thought to deter predators. Barbels may act for species recognition or to lure prey.

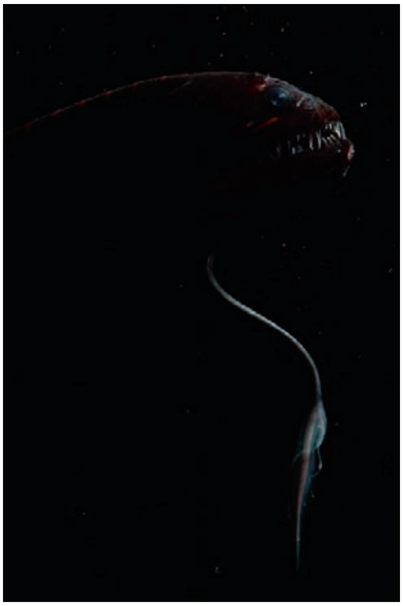


Figure 5 The Pacific Black Dragonfish (*Idiacanthus antrostomus*) is one of the most amazingly bioluminescent animals in the sea. It is covered with photophores along lower and upper surfaces, and has photophores under its eyes and at the end of a long barbel. When disturbed, it lights up all over, even down the lengths of its fins. Photograph by Steve Haddock.

The postorbital organs emit intense blue-green light and flash for 1–2 s. Many of the organs can be rotated and may act as headlights, although the effect of these high beams on dark-adapted eyes needs to be studied. Sexual dimorphism in organ size is common; so, perhaps, they have evolved to attract mates. Three genera, Malacosteus, Aristostomias, and Pachystomias, have evolved a suborbital organ that emits red light, which appears to be a stealth form of luminescence (**Figure 6**). Long-wave luminescence was a surprising discovery because red light is rapidly attenuated and has a much shorter range than blue light. The subsequent discovery of red-sensitive photopigments in the family confirmed that the fish can detect their own light (**see also Vision**: Photoreceptors and Visual Pigments and Adaptations of Photoreceptors and Visual Pigments). While limited in distance, few midwater animals have red-sensitive visual pigments, which may allow these animals to operate their luminescence without fear of detection, which is consistent with the long flashes (~4 s) from the red organs. Additionally, many midwater species have evolved bright red carapaces that, like black pigmentation, would function to absorb most ambient and bioluminescent wavelengths. The red bioluminescent may be an adaptation to illuminate prey that would reflect the long wavelengths.

The Future

With the advent of submersibles and ROVs, scientists are moving forward in understanding many of the mysteries of the midwater environment. New species of luminescent fish are discovered yearly but the function and physiology of the light organs remain more speculation than scientific fact. Although the stress of capture probably does not distort the emission spectra of the light, flash intensity and kinetics of moribund specimens may not adequately represent the natural signaling of the animals. The frequency of spontaneous or evoked luminescence from midwater fish remains completely unknown. While *in situ* observations could advance the field in quantum leaps, the task is daunting: how does one noninvasively observe the behavior of small, black fish swimming in a dim, deep, depauperate environment. The advent of long-endurance autonomous gliders fitted with image-intensifying and infrared cameras that could

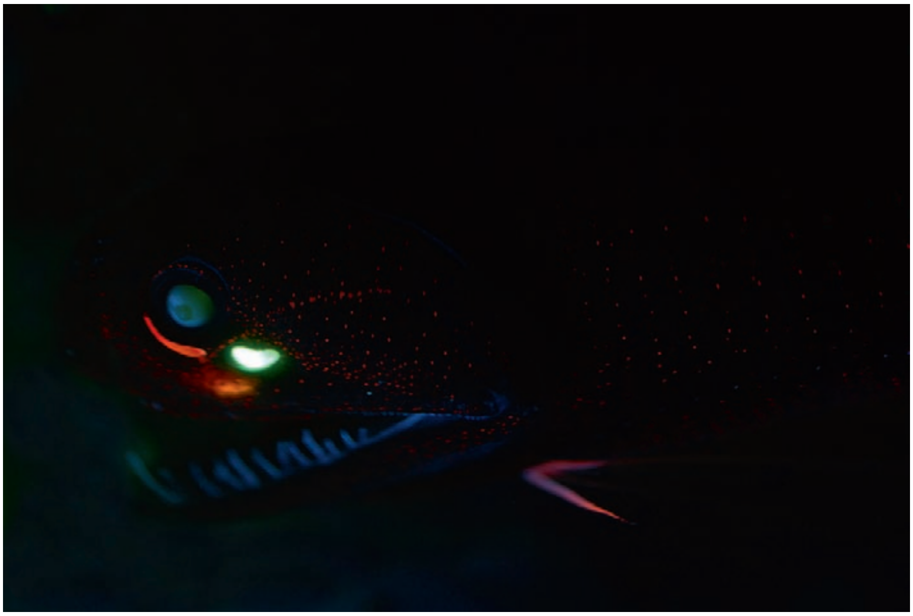


Figure 6 Close-up of the head of *Aristostomia* under fluorescent light. The postorbital photophore produces blue light; the crescent-shaped suborbital photophore emits red wavelengths. The small red dots on the fish's dermis are primitive photophores. Photograph by Steve Haddock.

sample the water columns for extended periods of time might be the best approach. While the field has moved incrementally forward since Harvey's 1952 monograph, *in situ* observations and controlled laboratory experiments remain infrequent. Access difficulties, expensive ship and submersible coast, and low funding have hamstrung efforts and our knowledge of bioluminescent fish lags behind all other areas of marine ecology. Hopefully, novel *in situ* observation instruments coupled with twenty-first-century technology will shed new light on the ecology and physiology of bioluminescent fishes.

See also: **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals. **The Skin**: Coloration and Chromatophores in Fishes. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Photoreceptors and Visual Pigments; Physiological Optics in Fishes; Visual Adaptations to the Deep Sea.

Further Reading

Haddock SHD, Moline MA, and Case JF (2010) Bioluminescence in the seas. *Annual Review of Marine Science* 2: 443–493.

Harvey EN (1952) *Bioluminescence*. New York: Academic Press.

Herring PJ (1978) *Bioluminescence in Action*. London: Academic Press.

Herring PJ (1982) Aspects of the bioluminescence of fishes. *Oceanography and Marine Biology: An Annual Review* 20: 415–470.

Herring PJ (1987) Systematic distribution of bioluminescence in living organisms. *Journal of Bioluminescence and Chemiluminescence* 1: 147–163.

Mensinger AF and Case JF (1990) Luminescent properties of deep sea fish. *Journal of Experimental Marine Biology and Ecology* 144: 1–15.

Mensinger AF and Case JF (1997) Luminescent properties of fishes from nearshore California basins. *Journal of Experimental Marine Biology and Ecology* 210: 75–90.

Ruxton GD (2005) Combining motility and bioluminescent signaling aids mate finding in deep-sea fish: A simulation study. *Marine Ecology Progress Series* 293: 253–262.

Ruxton GD, Speed MP, and Kelly DJ (2004) What, if anything, is the adaptive function of countershading? *Animal Behavior* 68: 445–451.

Turner JR, White EM, Collins MA, Partridge JC, and Douglas RH (2009) Vision in lanternfish (Myctophidae): Adaptations for viewing bioluminescence in the deep-sea. *Deep-Sea Research: Part I – Oceanographic Research Papers* 56: 1003–1017.

Warrant EJ and Locket NA (2004) Vision in the deep sea. *Biological Reviews* 79: 671–712.

Relevant Websites

http://www.mbari.org – Monterey Bay Research Institute.
http://lifesci.ucsb.edu – The Bioluminescence Web Page.

Hagfish Slime

DS Fudge, JE Herr, and TM Winegard, University of Guelph, Guelph, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Hagfish Slime Gland
GMCs and Mucin Vesicles
Gland Thread Cells
Volume and Concentration
Mechanics and Function
Slime Deployment and Maturation
Structure and Function
Further Reading

Glossary

Exocytosis The process by which neurotransmitters or hormones, which are stored in secretory vesicles or granules, are released from a nerve or endocrine cell when these storage granules fuse with the plasma membrane

Gland mucus cell Cell within hagfish slime glands that give rise to the mucus component of hagfish slime.

Gland thread cell Cell within hagfish slime glands that give rise to the fibrous component of hagfish slime.

Hagfish slime The material that is formed when hagfishes forcefully release the glandular contents of their numerous slime glands into seawater.

Hagfish slime exudate The thick white fluid that is ejected from hagfish slime glands and that interacts with seawater to form hagfish slime. The exudate contains numerous mucin vesicles and thread skeins.

Holocrine secretion Mode of secretion in which entire cells are ejected from a gland.

Intermediate filaments The 10-nm-diameter cytoskeletal filaments that make up hagfish slime threads.

Mucin vesicles Small, membrane-bound structures containing condensed mucus that are released when gland mucus cells are ejected from hagfish slime glands.

Slime threads Fine protein fibers that permeate hagfish slime and arise from the deployment of ejected thread skeins.

Thread skein Intricately coiled intermediate filament bundle that develops within gland thread cells and deploys after ejection from the slime glands to form the fibrous component of the hagfish slime.

Introduction

Hagfishes are best known for their ability to produce alarming amounts of slime when they are stressed or provoked (**see also Hagfishes and Lamprey**: Hagfishes). In fact, many of their common names include the word slime, such as slime eel and slime hag. The Atlantic hagfish, *Myxine glutinosa*, is twice named for its slime (myx = slime, glutin = glue). Many animals release slime under stress, but hagfish slime is unique because of the large volumes that are produced (up to several liters from a single hagfish) (**Figure 1**), the impressive speed with which it is made, and the fact that it contains tens of thousands of fine protein fibers (**Figure 2**).

Hagfish slime can be defined as the material that is formed when hagfishes forcefully release the glandular contents of their numerous slime glands into seawater. Many marine animals, including hagfishes, make some sort of epidermal mucus or slime that coats their body, but hagfish slime is different from these secretions in many ways. For one, epidermal mucus is secreted at a slow and relatively constant rate to replace the dispersal of mucus from the outer surface (**see also The Skin**: Functional Morphology of the Integumentary System in Fishes), whereas hagfish slime is released infrequently from specialized glands. Another difference is that hagfish slime exudate is released via holocrine secretion in which entire cells are ejected from the glands, whereas epidermal mucus is secreted via exocytosis of mucin granules at the apical plasma membrane of goblet cells.

The Hagfish Slime Gland

The slime originates within numerous slime glands located along both sides of the hagfish's body (**Figure 3**). *Eptatretus stoutii*, the Pacific hagfish, typically possesses about 150–200 slime glands. The glands are dominated by two cell types: gland mucus cells (GMCs) and gland thread cells (GTCs), which are responsible for the mucus

Figure 1 Hagfishes are able to produce vast amounts of slime when they are provoked. Here, a Pacific hagfish (*Eptatretus stoutii*) has produced liters of slime in response to being handled in an aquarium. Photo courtesy of Andra Zommers.

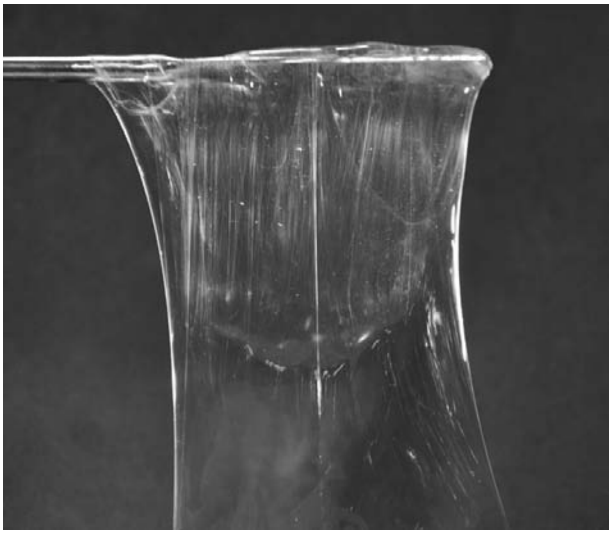


Figure 2 Hagfish slime contains tens of thousands of fine protein fibers that are over 10 cm long and about 2 μm in diameter. From Fudge DS, Winegard T, Ewoldt RH, *et al*. (2009) From ultra-soft slime to hard alpha-keratins: The many lives of intermediate filaments. *Integrative and Comparative Biology* 49: 32–39.

and fibrous components of the slime, respectively. GMCs and GTCs originate in the germinal layer of the gland and are pushed toward the gland lumen as they mature. The glands are approximately spherical in shape and are encased by a capsule of connective tissue, which is, in turn, surrounded by a thin layer of striated muscle fibers. When these muscle fibers contract, mature GMCs and GTCs are ejected from the gland via the holocrine mode into seawater through the gland pore.

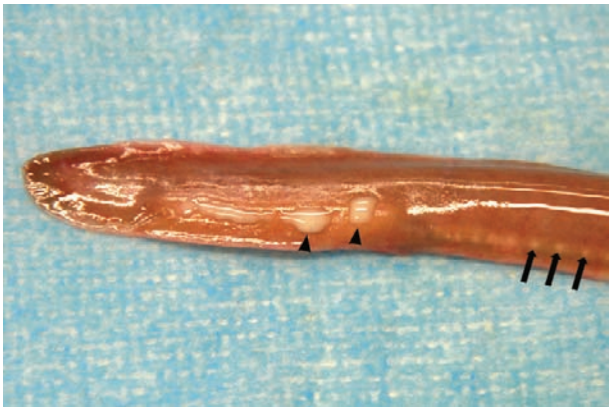


Figure 3 Hagfish slime is produced in numerous slime glands (arrows) that line both sides of the animal's body. Here, slime exudate (arrowheads) has been expressed from several slime glands via mild electrical stimulation of the muscle layer that surrounds the gland capsule. Photo courtesy of Timothy Winegard.

GMCs and Mucin Vesicles

GMCs are distinct from the small and large mucus cells found in hagfish epidermis. The sole function of GMCs is to package condensed mucins into disk-shaped membrane-bound vesicles that are about 7 μm in diameter (**Figure 4**). When mature GMCs are ejected from the slime gland, they lose their plasma membrane (presumably via shearing within the gland duct) and release numerous vesicles that swell and rupture upon contact with seawater. Compared with GTCs, GMCs and their secretion products are poorly studied. The genes for the molecules that make up the mucus component of hagfish slime have yet to be characterized, but histological staining and immunolabeling suggest that they are mucins. Mucins are very large (0.5–30 MDa), heavily glycosylated proteins in which up to 85% of the dry weight is carbohydrate. Hagfish mucins are unique in that they comprise only 12% carbohydrate and contain less serine and more sulfate than typical mucins. Hagfish slime also has been shown to contain higher levels of lysozyme, alkaline phosphatase, cathepsin B, and proteases relative to hagfish epidermal mucus.

Hagfish slime mucin vesicles are believed to have an internal osmolarity of about 897 mOsm based on the fact that they rupture in magnesium sulfate solutions with osmolarities lower than this, and are stable in magnesium sulfate solutions with higher osmolarities. The vesicles are stable in 1 M solutions containing the anions citrate, sulfate and phosphate, acetate, or tartrate, but rupture in similar solutions containing chloride, bicarbonate, or nitrate ions. These experiments suggest that the vesicle membrane is permeable to univalent anions but

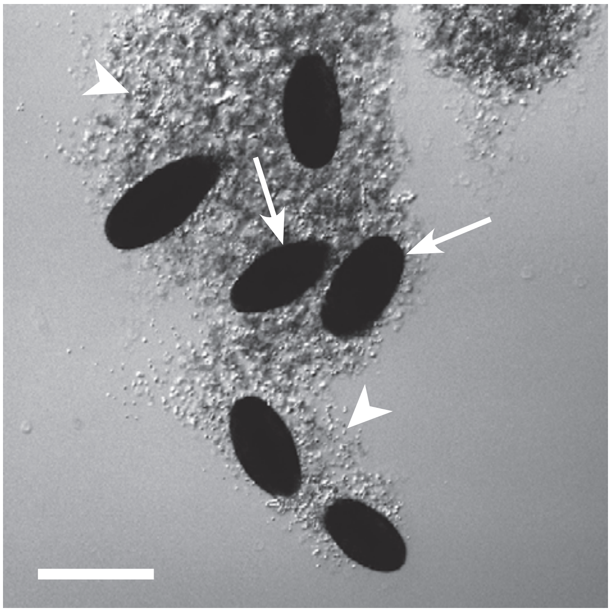


Figure 4 A small blob of exudate collected from a slime gland and pipetted into a drop of seawater. Hagfish slime gland exudate contains two main solid components – thread skeins (arrows) that consist of a single coiled intermediate filament bundle, and mucin vesicles (arrowheads), which appear as the granular material between the thread skeins. The material shown represents about 5 nl of exudate. From Herr JE, Winegard TM, O'Donnell MJ, Yancey PH, and Fudge DS (2010) Stabilization and swelling of hagfish slime mucin vesicles. *Journal of Experimental Biology* 213: 1092–1099.

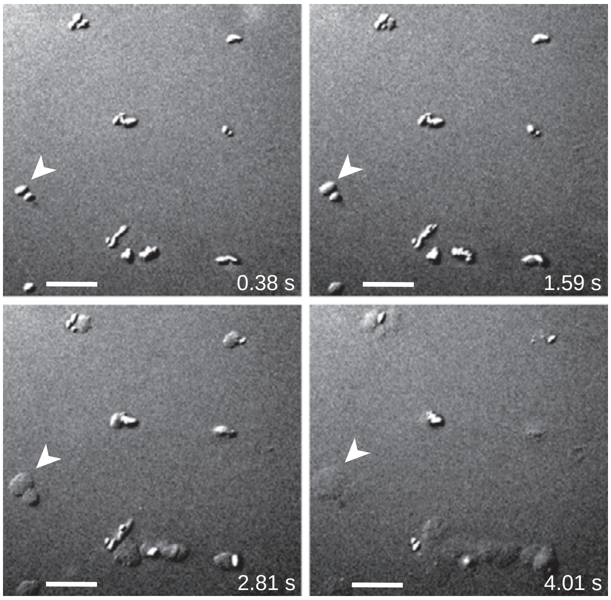


Figure 5 A composite of stills from a video of isolated mucin vesicles in the slime of *Myxine glutinosa* rupturing on exposure to seawater. Timestamps in the lower right corner indicate time following contact with seawater. Scale = 20 μm. Arrowheads indicate a single mucin vesicle as it changes over time. From Herr JE, Winegard TM, O'Donnell MJ, Yancey PH, and Fudge DS (2010) Stabilization and swelling of hagfish slime mucin vesicles. *Journal of Experimental Biology* 213: 1092–1099.

impermeable to polyvalent anions. Vesicle rupture likely occurs not via a simple osmotic influx of water (as the vesicle is about the same osmolarity as seawater if not slightly hypoosmotic), but rather via the influx of ions such as chloride across the vesicle membrane, which then causes a subsequent secondary osmotic influx of water molecules. The water causes the vesicle to swell and then rupture, (**Figure 5**), resulting in the release of the mucin molecules into the external environment, where they are then free to interact with the fibrous component of the slime. This model predicts the presence of anion channels in the vesicle membrane; however, at this point, there is no direct evidence for them.

The fact that the mucin vesicles rupture readily in such a wide range of solutions raises the question of how they might be stabilized within the gland mucous cells prior to release from the slime gland. Chemical analysis of the fluid component of slime exudate has revealed high concentrations of methylamines such as betaine, trimethylamine oxide (TMAO), and dimethylglycine (DMG) (**Table 1**). Although these compounds are known osmolytes in other organisms, neither TMAO nor betaine (the two most abundant compounds) is capable of stabilizing the vesicles,

Table 1 The concentration of identified inorganic and organic osmolytes in the fluid component (supernatant) of fresh slime exudate

Osmolyte	*Concentration (mmol L^{-1})*
Cl^-	191.5 ± 6.6
K^+	143.0 ± 3.0
Na^+	41.2 ± 2.6
Mg^{2+}	2.15 ± 0.76
Ca^{2+}	0.000 45 ± 0.000 09
pH	7.31 ± 0.02
Total inorganic	379
Betaine	218 ± 7
TMAO	101.3 ± 4.8
Glycine	79.9 ± 7.5
Dimethylglycine	68.6 ± 6.0
Creatine	15.0 ± 1.4
Inositol	2.30 ± 0.68
b-alanine	2.17 ± 0.68
Taurine	2.13 ± 0.42
Glucose	1.23 ± 0.22
Total organic	490 ± 10

The total osmolarity of the supernatant is approximately 888 mOsm.
Values are means ± SEM, *N* = 5.
Inorganic ions were measured using ion-specific electrodes.
Organic solutes were analyzed using HPLC, and TMAO was analyzed using ferrous sulfate and EDTA.
TMAO, trimetheylamine oxide.

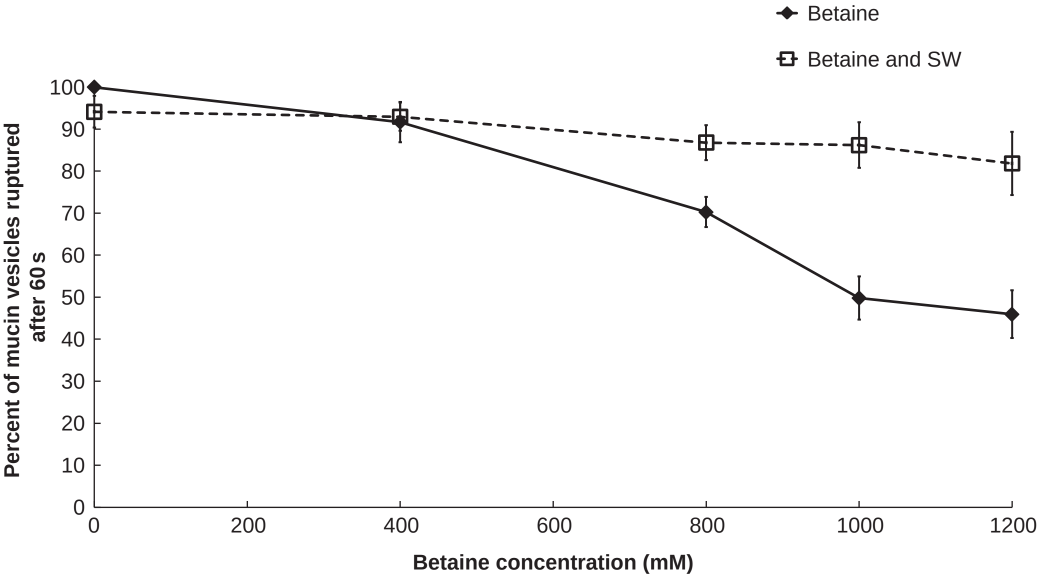


Figure 6 The percentage of isolated mucin vesicles from the slime of *Myxine glutinosa* that ruptured after 60 s of exposure to varying concentrations of betaine in 5.0 mM Tris, pH 8.0 (solid line). The dotted line represents the effect of changing betaine concentration in the presence of sea salts (33‰). Values are means ± SEM ($N = 6$ in each treatment). Two-way ANOVA showed significant effects of betaine concentration and the presence or absence of sea salts ($P < 0.05$). From Herr JE, Winegard TM, O'Donnell MJ, Yancey PH, and Fudge DS (2010) Stabilization and swelling of hagfish slime mucin vesicles. *Journal of Experimental Biology* 213: 1092–1099.

even at concentrations exceeding the internal osmolarity of the vesicle itself (**Figure 6**). Even more intriguing is the fact that the clear supernatant that is obtained when fresh slime exudate is centrifuged also is not stabilizing (**Figure 7**). This suggests that stabilization of vesicles within the gland may depend upon the chemical microenvironment within GMCs or another mechanism such as elevated hydrostatic pressure.

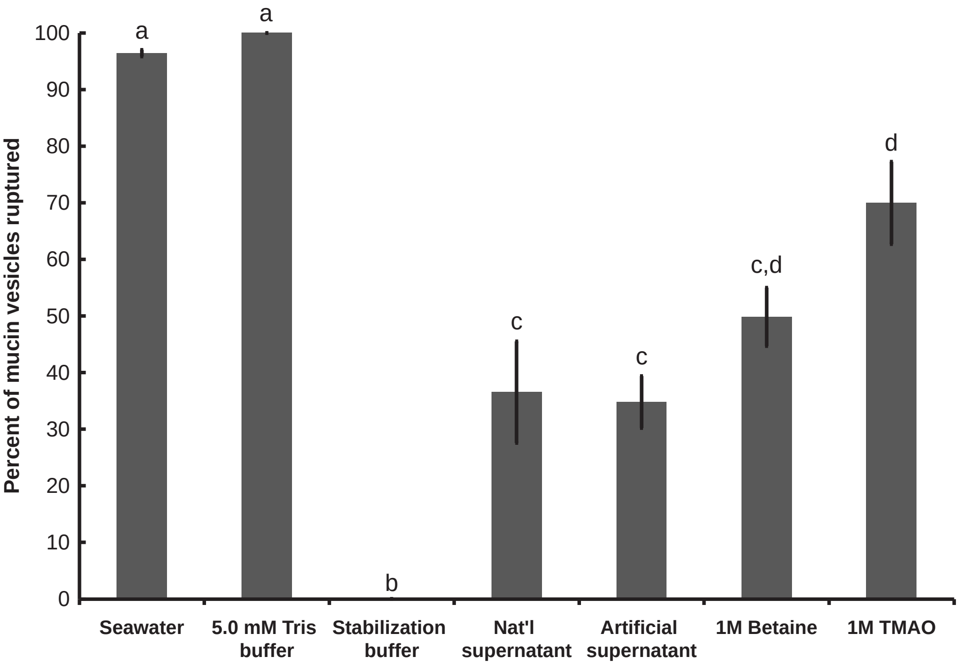


Figure 7 The percentage of mucin vesicles that ruptured after 60 s of exposure to different solutions. Values are means ± SEM ($N = 6$ in each treatment). Different lowercase letters denote significant differences between means ($P < 0.05$). From Herr JE, Winegard TM, O'Donnell MJ, Yancey PH, and Fudge DS (2010) Stabilization and swelling of hagfish slime mucin vesicles. *Journal of Experimental Biology* 213: 1092–1099.

Gland Thread Cells

The fibrous component of hagfish slime originates in the GTCs. The function of these cells is to package 10-nm-diameter cytoskeletal elements known as 'intermediate filaments' into a single, continuous, intricately coiled protein thread that, in mature cells, occupies the vast majority of the cell volume. GTCs are large and roughly ellipsoidal in shape, with distinctive blunt and pointed ends (**Figure 8**). Estimates in the literature of slime thread length vary between 6 and 60 cm, and this value certainly depends on the size of the GTC and presumably the species. More direct measurements of unraveled thread skeins suggest that slime threads are between 11 and 17 cm long, and can be stretched to 34 cm before they break.

How the cell assembles a thread that is about 100 000 times longer than its width and 1000 times longer than the cell itself is not well understood. Some clues about the mechanisms of thread assembly and packaging have come from a detailed study of GTC maturation within the slime gland. This work suggests that a short primal thread arises near the blunt end of the cell near the nucleus. The primal thread is believed to be capped at one end and increases in length via the addition of intermediate filaments at the other. Thread diameter increases by the addition of intermediate filaments laterally. This model is consistent with the changes in thread morphology that occur as immature GTCs develop into mature thread cells, but does not explain how the cell regulates thread diameter and quality and achieves such exquisite packing.

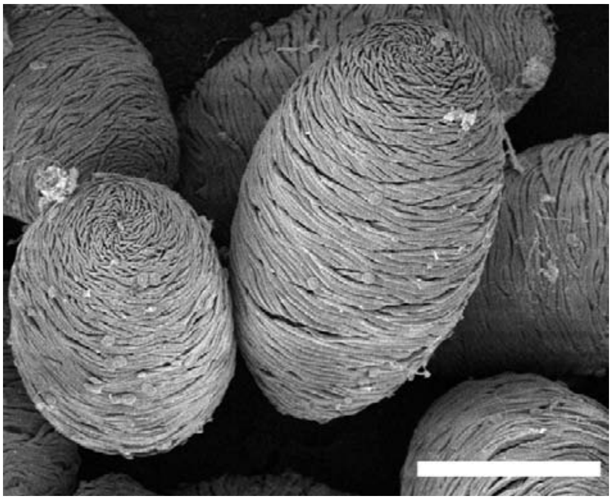


Figure 8 Scanning electron micrograph of gland thread cells that have lost their plasma membranes during ejection from the slime gland. These ball-of-yarn-like structures are referred to as thread skeins. Scale = 50 μm. Image courtesy of Douglas Fudge.

The mechanism of assembly aside, simply describing the anatomy of the thread within mature GTCs, is not trivial and has attracted the attention of several investigators. The majority of the thread is organized as if it were laid down in staggered loops within an egg-shaped barrel. Within these loops, the thread takes on three main trajectories – an ascending trajectory toward the pointed end of the cell, a curved trajectory in which the thread winds circumferentially around the cell periphery for about 60°, and a descending trajectory toward the blunt end. Because of the 60° peripheral runs, the loops are staggered around the longitudinal axis of the cell, and about six of these three-dimensional loops define a cone-like structure that points toward the pointed end of the cell. Although it is meaningless to delineate the beginning of one cone and the end of another, for the purpose of this description, successive cones are neatly nested on top of each other, and occupy the majority of the cell volume. The packing of the loop segments in the peripheral runs gives a cabled appearance to the surface of intact slime thread skeins, but this cabling effect is an illusion, which is sensible because twisting of the thread around itself would hinder effective unraveling. At the blunt end of the cell, the hollow space inside the cones is filled by a series of loops that are oriented parallel to the longitudinal axis of the cell. This staggered loop packing of the thread within GTCs has been compared with the faking of a rope on the deck of a ship to prevent its fouling as it is paid out. Thread diameter within the GTC is not constant, with the largest diameter occurring in the middle of the thread (~3 μm) and the ends tapering down to less than 1 μm.

Volume and Concentration

Estimates of the sliming capacity of a single hagfish vary widely in the literature, but most agree that, in a typical sliming event, a hagfish can produce a mass of slime that is many times larger than itself. For a Pacific hagfish, a typical sliming event induced by a pinch on the tail produces about 900 mL of slime. As for the total amount of slime that a hagfish can produce, estimates in the literature vary from 7 to 100 L. The most recent estimate puts this number at about 25 L for the Pacific hagfish. This number is based in part on the mass of stored slime exudate in the glands, which is about 3–4% of body weight.

One reason that hagfishes are able to produce so much of the slime is that it is remarkably dilute, containing only about 20 mg of threads and 15 mg of mucins per liter, which is about 1000× more dilute than typical mucus such as gastric mucus. These measurements suggest that the vast majority of the volume of hagfish slime is seawater.

Mechanics and Function

Recent work has begun to characterize the mechanical properties of hagfish slime and its components. One aspect of the behavior of the slime that is fairly well documented is its tendency to contract, especially when it is agitated. Slime volume decreases by a factor of 50 when it is stirred, and this presumably occurs due to a collapsing of the mucin and thread components of the slime and egress of the bulk seawater that makes up so much of the slime volume just after it is formed. Isolated slime threads are soft and extensible in water, but ultimately quite strong (**Figure 9**). Their high extensibility means that they can stretch to a length greater than 3 times their original length before they break. At low strains, the threads are elastic, but when they are stretched to strains greater than 0.35, they become stiffer and recover less (**Figure 10**). This change in properties has been demonstrated to occur due to a conformational change in the constituent proteins in which α-helices are stretched out to form β-sheets (**Figure 11**). Although there is little information on the mechanical properties of the mucus component of the slime, hagfish slime mucin solutions have been shown to exhibit low

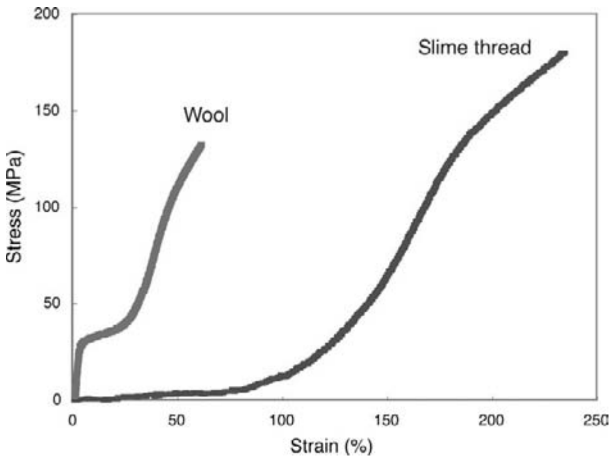


Figure 9 Stress–strain curve for a slime thread tested in water compared to a wool fiber in water. Note the low stiffness of the slime thread at low strains, the extreme strain stiffening that happens at higher strains, and the high breaking stress. From Fudge DS, Gardner KH, Forsyth VT, Riekel C, and Gosline JM (2003) The mechanical properties of hydrated intermediate filaments: Insights from hagfish slime threads. *Biophysical Journal* 85: 2015–2027.

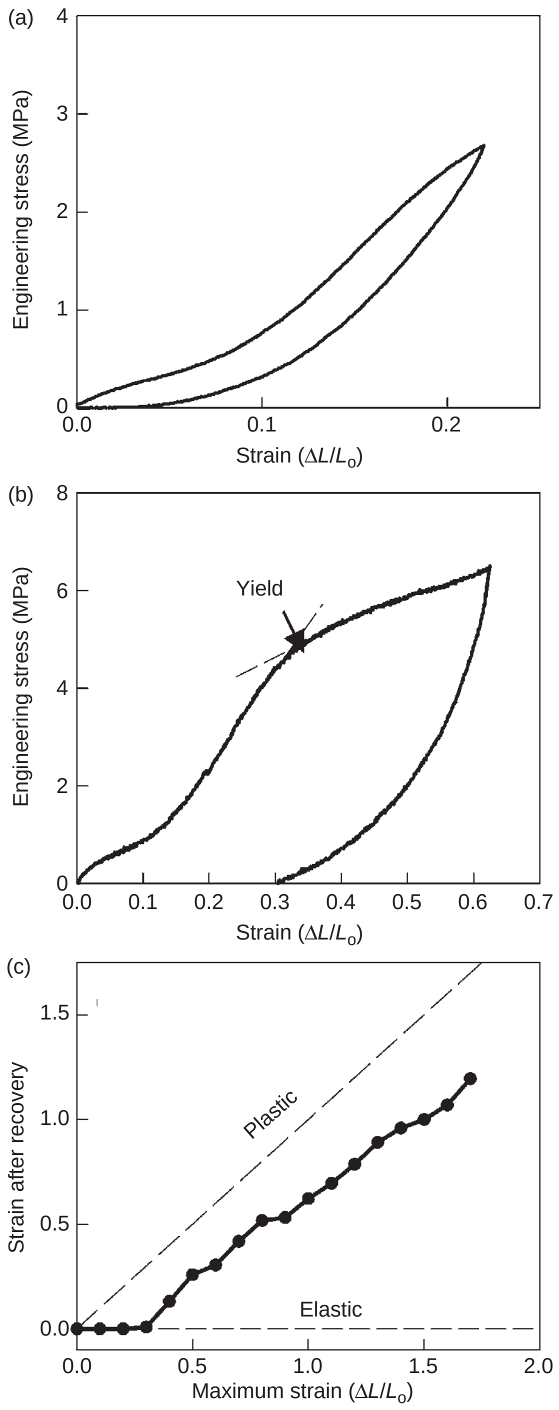


Figure 10 Recovery behavior of hagfish threads in seawater. (a) Typical load cycle at low strains, showing completely reversible deformation. (b) Typical load cycle at higher strains, showing that deformation past the yield point is mostly plastic. (c) Results from trials in which threads were extended to a given strain, held, and allowed to recover. Note that deformation is elastic up to a strain of 0.35 and plastic thereafter. From Fudge DS, Gardner KH, Forsyth VT, Riekel C, and Gosline JM (2003) The mechanical properties of hydrated intermediate filaments: Insights from hagfish slime threads. *Biophysical Journal* 85: 2015–2027.

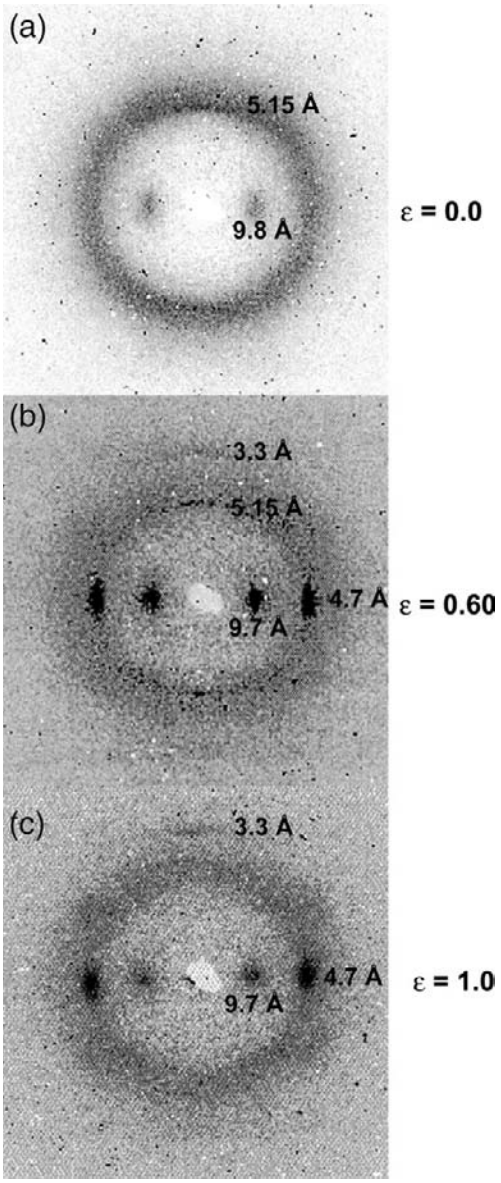


Figure 11 X-ray diffraction patterns for bundles of hagfish threads as a function of prior strain. (a) Unstrained threads exhibit a typical α-pattern, whereas threads extended to a strain of 1.0 exhibit a typical β-pattern (c). Threads extended to a strain of 0.60 exhibit a mixed pattern, suggesting the presence of both α-helix and β-sheet structure (b). Diffraction maxima (dark spots) are labeled according to the molecular spacings (in Angstroms, Å) to which they correspond. From Fudge DS, Gardner KH, Forsyth VT, Riekel C, and Gosline JM (2003) The mechanical properties of hydrated intermediate filaments: Insights from hagfish slime threads. *Biophysical Journal* 85: 2015–2027.

viscosity similar to seawater, even at mucin concentrations many times higher than natural concentrations. Recent work on the mechanical response of fresh slime suggests that it is an ultra-soft material with material properties that are unlike any other polymer.

Slime Deployment and Maturation

One of the more remarkable aspects of hagfish slime is the speed at which it expands from a thick glandular exudate into an ultra-dilute material with a volume over a 1000 times greater. High-speed video has revealed two behavioral phenomena that aid in the rapid deployment of the slime. The first is the fact that the exudate is forcefully ejected from the slime glands (**Figure 12**). The second is the convective mixing that occurs as the hagfish struggles to escape whatever has provoked it (**Figure 13**). Exudate transferred into still seawater in a beaker does not form competent slime, but simply sinks as a coherent blob, whereas modest amounts of mixing allow the slime to fully deploy (**Figure 14**).

Perhaps the most impressive aspect about deployment of the slime is that it involves the unraveling of slime threads from a condensed form within GTCs to an unraveled form that is 1000× longer (150 μm GTC→150 mm slime thread). Early hagfish slime researchers hypothesized that the thread skein was assembled under significant pressure within the GTC, with deployment triggered by osmotic rupture of the GTC membrane upon contact with seawater. Later it was shown that the thread skeins lose their plasma membrane during ejection from the slime gland, which precludes any mechanism involving osmotic swelling across a membrane. More recent work has shown that disruption of the mucin

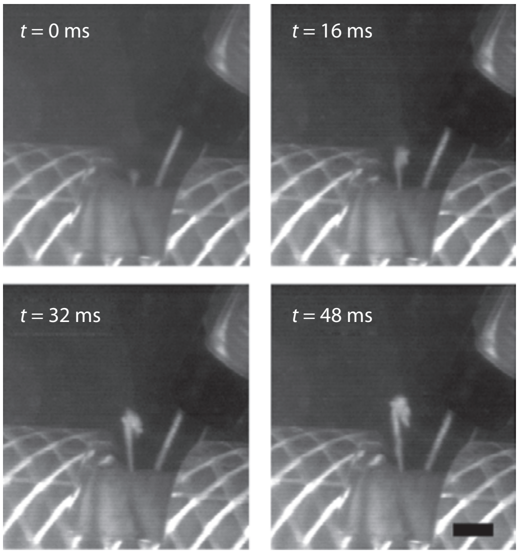


Figure 12 Close-up of slime release from a single slime gland of a hagfish constrained in a tube with a window cut in it. Note that the slime is released as a coherent jet. These events were filmed at 125 frames s^{-1}, and the mean jet velocity was 0.17 m s^{-1}. Scale = 5 mm. From Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209:702–710.

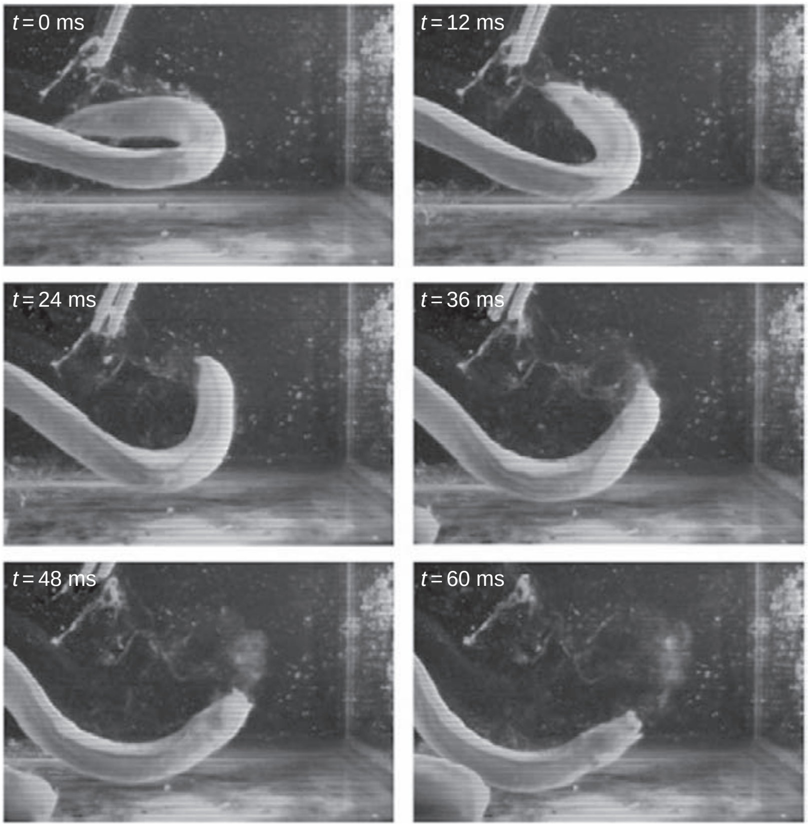


Figure 13 High-speed video (125 frames s^{-1}) of a sliming event demonstrating that released slime rarely envelops the hagfish and often is dispersed by an evasive maneuver that mixes the exudate with seawater. From Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209: 702–710.

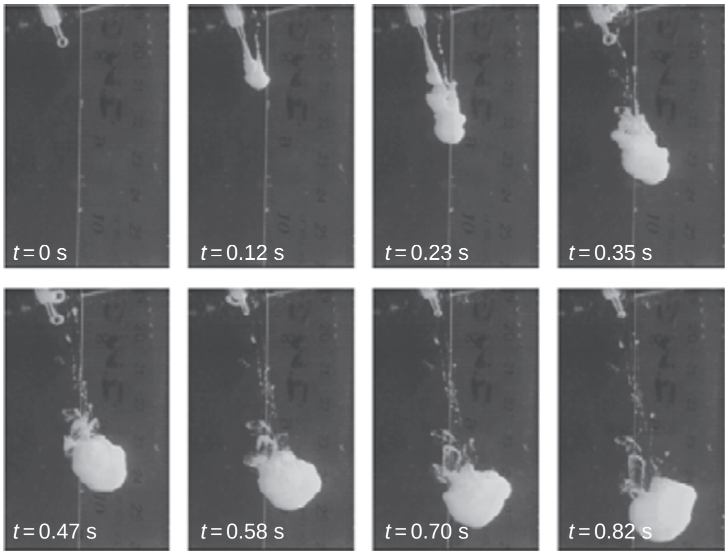


Figure 14 Slime exudate introduced into still seawater from a syringe fails to hydrate as it does *in vivo*. From Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209: 702–710.

network with dithiothreitol (DTT) has an inhibitory effect on whole slime formation, and these effects are mediated at least in part by an inhibition of thread skein unraveling (**Figure 15**). Further exploration of this phenomenon revealed that mucin vesicles shear into long mucus strands (**Figure 16**) that attach to the thread skeins and initiate unraveling via the transmission of hydrodynamic mixing forces to them (**Figure 17**; **Video Clip 1**). The subsequent entanglement between threads and mucin strands further unravels the thread skeins until the slime has fully deployed. These findings provide evidence for a unique biological process in which the thread skeins, which are immune to turbulent mixing forces because of their small size, are critically dependent on the mucin vesicles for their deployment. This suggests that mucins preceded the thread skeins in the evolution of the slime in hagfishes.

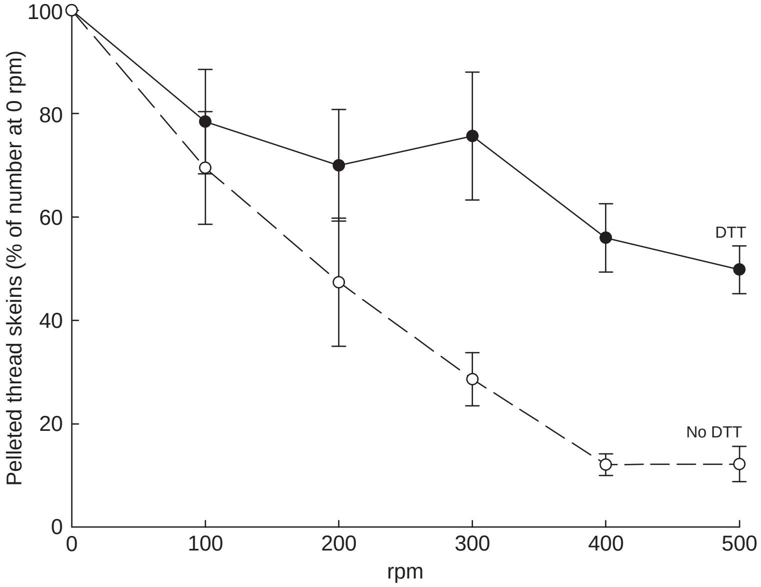


Figure 15 Mean (±SEM) number of condensed thread skeins as a function of mixing rate expressed as the percentage of the number of skeins at 0 rpm. These data provide an inverse measure of the degree of thread skein unraveling in the whole slime. Solid line indicates data for trials in which mucins were disrupted with 10 mM dithiothreitol (DTT). The data demonstrate that the effects of DTT on whole slime formation are mediated at least in part by an inhibition of thread skein unraveling. These data also suggest that mucins play a critical role in the unraveling of the threads. From Winegard TM and Fudge DS (2010) Deployment of hagfish slime thread skeins requires the transmission of mixing forces via mucin strands. *Journal of Experimental Biology* 213: 1235–1240.

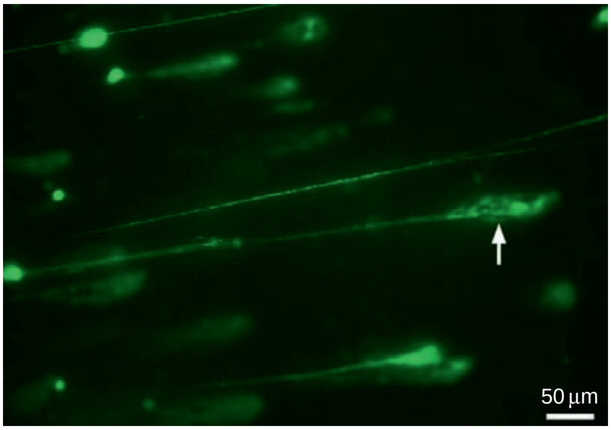


Figure 16 Fluorescent tagging of mucins using a fluorescein-labeled lectin dye illustrates the formation of mucin strands from elongated mucin vesicles exposed to shear in a flow-through chamber. Arrow indicates an aggregation of ruptured mucin vesicles that have begun elongating to form strands. From Winegard TM and Fudge DS (2010) Deployment of hagfish slime thread skeins requires the transmission of mixing forces via mucin strands. *Journal of Experimental Biology* 213: 1235–1240.

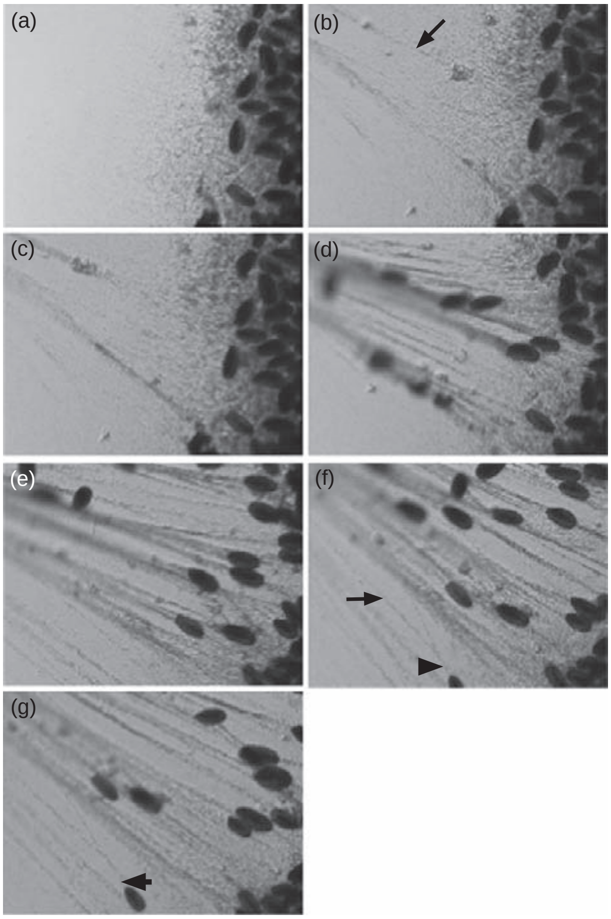


Figure 17 Slime exudate exposed to flow created by a syringe pump. (a) Condensed exudate puddle prior to flow. Note the condensed thread skeins on the far right and the mucin vesicle boundary layer to the left. (b, c) Mucin strands and chains begin to form as flow is initiated. (d, e) Mucin strand attachment to thread skeins and movement of thread skeins. (f, g) Unraveling of thread skeins initiated. Arrowheads indicate an unraveling thread skein; arrows indicate aggregations of mucin strands. From Winegard TM and Fudge DS (2010) Deployment of hagfish slime thread skeins requires the transmission of mixing forces via mucin strands. *Journal of Experimental Biology* 213: 1235–1240.

Structure and Function

Early models of slime structure hypothesized that swollen mucin vesicles decorated deployed slime threads. However, visualization of the mucin network with fluorescently labeled lectins disproved this hypothesis and suggested that mucins form a separate network of fibers that interacts with the thousands of deployed slime threads in mature slime (**Figure 18**). The current model of slime structure suggests that the vast majority of the volume of mature slime must be bulk seawater. This idea is supported by the fact that hagfish slime that is lifted out of seawater into air will decrease in volume as water runs out of it, leaving only about 5% of its original volume in a matter of minutes. These observations suggest that the slime is adapted not to bind water irreversibly, but rather to entrain a large volume of seawater within countless microchannels defined by the intermingling networks of slime threads and mucin strands.

Many functions have been proposed for the slime, including defense against predators, feeding, localization of eggs, and avoidance of competition while scavenging, but few of these hypotheses have been tested experimentally. Videos of hagfishes scavenging on baited traps show that they sometimes release slime while feeding, which may serve to discourage competitors from approaching too closely. One group of researchers tested the hypothesis that hagfish slime functions as a defense against gill-breathing predators by measuring the hydrodynamic resistance across the gills in isolated fish heads with and without slime. They found that the slime is superbly suited to catching on the gills and increasing the resistance to flow through them (**Figures 19** and **20**). Regardless of the exact function of hagfish slime, it is a

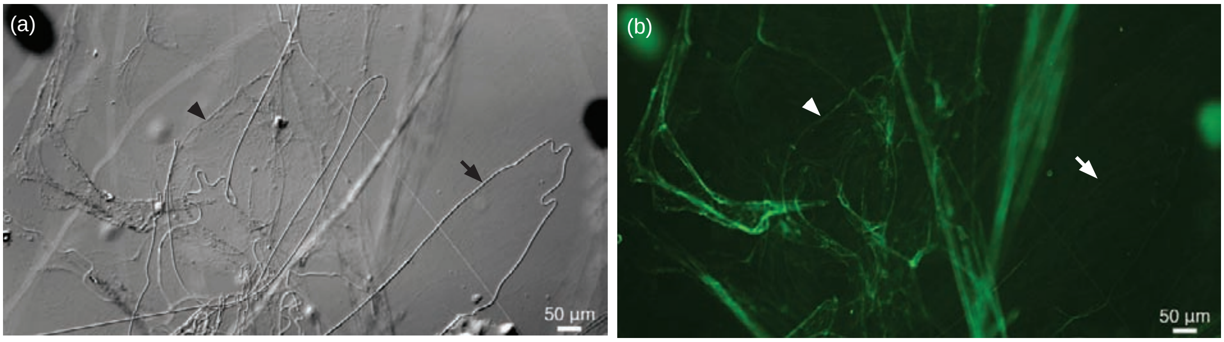


Figure 18 Whole slime formed in seawater containing a fluorescent lectin dye that binds to hagfish slime mucins. (a) Differential interference contrast (DIC) image of whole slime network depicting unraveled threads and mucin strand network. Arrowhead indicates mucin strands connecting slime threads, and the arrow indicates a slime thread. (b) Same image viewed in fluorescence highlights the complexity of the mucin network. From Winegard TM and Fudge DS (2010) Deployment of hagfish slime thread skeins requires the transmission of mixing forces via mucin strands. *Journal of Experimental Biology* 213: 1235–1240.

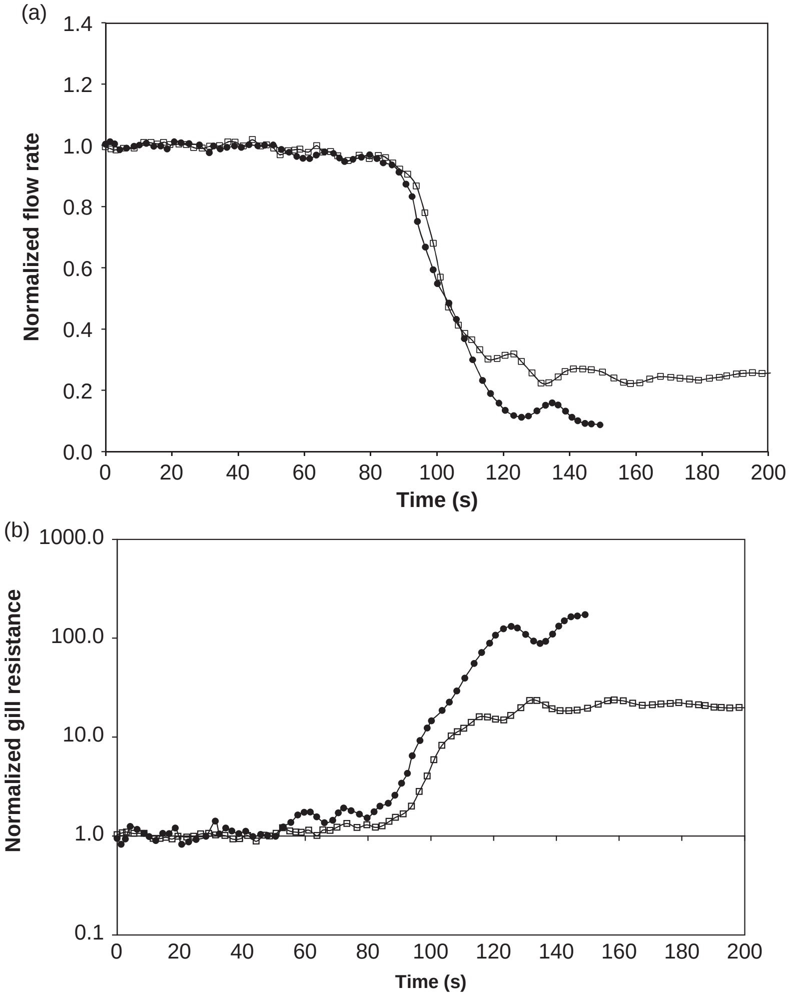


Figure 19 The effects of hagfish slime on (a) water flow rates and (b) gill resistance in the gills of an isolated rockfish head. Slime release occurred at ~95 s; results from two fish heads are shown separately, and the data have been normalized to their pre-slime values. Note the log scale for normalized resistance. From Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209: 702–710.

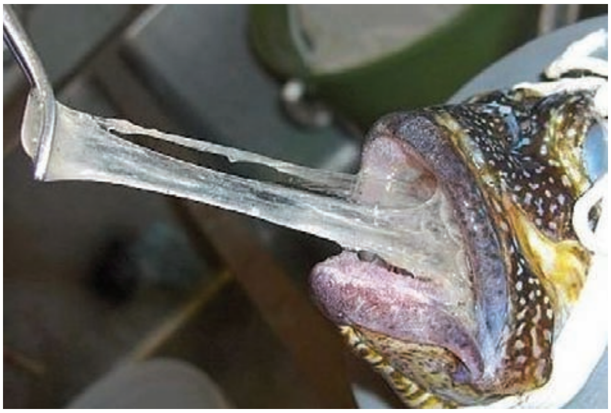


Figure 20 Hagfish slime is very effective at catching on the gills of fishes like this rockfish. From Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209: 702–710.

complex and fascinating material that has likely played an important role in the evolutionary staying power of the hagfishes.

See also: **Hagfishes and Lamprey**: Hagfishes. **The Skin**: Functional Morphology of the Integumentary System in Fishes.

Further Reading

Downing SW, Spitzer RH, Koch EA, and Salo WL (1984) The hagfish slime gland thread cell. I. A unique cellular system for the study of intermediate filaments and intermediate filament–microtubule interactions. *Journal of Cell Biology* 98: 653–669.

Downing SW, Spitzer RH, Salo WL, *et al.* (1981) Hagfish slime gland thread cells: Organization, biochemical features, and length. *Science* 212: 326–328.

Fernholm B (1981) Thread cells from the slime glands of hagfish (Myxinidae). *Acta Zoologica* 62: 137–145.

Ferry JD (1941) A fibrous protein from the slime of the hagfish. *Journal of Biological Chemistry* 138: 263–268.

Fudge DS, Gardner KH, Forsyth VT, Riekel C, and Gosline JM (2003) The mechanical properties of hydrated intermediate filaments: Insights from hagfish slime threads. *Biophysical Journal* 85: 2015–2027.

Fudge DS, Levy N, Chiu S, and Gosline JM (2005) Composition, morphology and mechanics of hagfish slime. *Journal of Experimental Biology* 208: 4613–4625.

Fudge DS, Winegard T, Ewoldt RH, *et al.* (2009) From ultra-soft slime to hard alpha-keratins: The many lives of intermediate filaments. *Integrative and Comparative Biology* 49: 32–39.

Herr JE, Winegard TM, O'Donnell MJ, Yancey PH, and Fudge DS (2010) Stabilization and swelling of hagfish slime mucin vesicles. *Journal of Experimental Biology* 213: 1092–1099.

Koch EA, Spitzer RH, and Pithawalla RB (1991) Structural forms and possible roles of aligned cytoskeletal biopolymers in hagfish (slime eel) mucus. *Journal of Structural Biology* 106: 205–210.

Koch EA, Spitzer RH, Pithawalla RB, Castillos FA, 3rd, and Parry DA (1995) Hagfish biopolymer: A type I/type II homologue of epidermal keratin intermediate filaments. *International Journal of Biological Macromolecules* 17: 283–292.

Koch EA, Spitzer RH, Pithawalla RB, and Downing SW (1991) Keratin-like components of gland thread cells modulate the properties of mucus from hagfish (*Eptatretus stouti*). *Cell and Tissue Research* 264: 79–86.

Koch EA, Spitzer RH, Pithawalla RB, and Parry DA (1994) An unusual intermediate filament subunit from the cytoskeletal biopolymer released extracellularly into seawater by the primitive hagfish (*Eptatretus stoutii*). *Journal of Cell Science* 107: 3133–3144.

Lim J, Fudge DS, Levy N, and Gosline JM (2006) Hagfish slime ecomechanics: Testing the gill-clogging hypothesis. *Journal of Experimental Biology* 209: 702–710.

Luchtel DL, Martin AW, and Deyrup-Olson I (1991) Ultrastructure and permeability characteristics of the membranes of mucous granules of the hagfish. *Tissue and Cell* 23: 939–948.

Newby WW (1946) The slime glands and thread cells of the hagfish *Polistotrema stouti*. *Journal of Morphology* 78: 397–409.

Salo WL, Downing SW, Lidinsky WA, *et al.* (1983) Fractionation of hagfish slime gland secretions: Partial characterization of the mucous vesicle fraction. *Preparative Biochemistry* 13: 103–135.

Spitzer RH, Downing SW, and Koch EA (1979) Metabolic–morphologic events in the integument of the Pacific hagfish (*Eptatretus stoutii*). *Cell and Tissue Research* 197: 235–255.

Subramanian S, Ross NW, and MacKinnon SL (2008) Comparison of the biochemical composition of normal epidermal mucus and extruded slime of hagfish (*Myxine glutinosa* L.). *Fish and Shellfish Immunology* 25: 625–632.

Verdugo P, Deyrupolsen I, Aitken M, Villalon M, and Johnson D (1987) Molecular mechanism of mucin secretion. 1. The role of intragranular charge shielding. *Journal of Dental Research* 66: 506–508.

Winegard TM and Fudge DS (2010) Deployment of hagfish slime thread skeins requires the transmission of mixing forces via mucin strands. *Journal of Experimental Biology* 213: 1235–1240.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

MUSCLES, SKELETON, SKIN, AND MOVEMENT

Buoyancy, Locomotion, and Movement in Fishes

Contents

Buoyancy, Locomotion, and Movement in Fishes: An Introduction
Buoyancy in Fishes
Swimbladder Function and Buoyancy Control in Fishes
Experimental Hydrodynamics
Undulatory Swimming
Functional Properties of Skeletal Muscle: Work Loops
Paired Fin Swimming
Maneuverability
Stability and Turbulence
Fast Start
Feeding Mechanics
Biomimetics: Robotics Based on Fish Swimming

Buoyancy, Locomotion, and Movement in Fishes: An Introduction

ED Stevens, University of Prince Edward Island, Charlottetown, PE, Canada

© 2011 Elsevier Inc. All rights reserved.

A Short Historical Perspective on Buoyancy and Locomotion
Buoyancy and Locomotion Today
Further Reading

Glossary

Biomimetics The analytical and synthetic process by which biologists and engineers duplicate biological structure, function, or both.

Buoyancy, **positive and negative** A positively buoyant organism is less dense than the surrounding fluid, so tends to rise to the surface. A negatively buoyant one is denser than the fluid and tends to sink.

Electromyography (EMG) A technique for measuring the electrical activity associated with muscle contraction. Sensing electrodes implanted in the muscle tissue detect timing of muscle action as a high-frequency burst of voltage spikes.

Fast start A sudden acceleration from resting or swimming. It can be an escape response or a predator strike, or a maneuver observed during social interactions.

Lift A hydrodynamic force acting at right angles to a body's direction of motion through a fluid. For example, the force on the wings of airplanes that keeps them airborne.

Mauthner cells Two giant neurons located in the hindbrain of most fish species and usually control escape responses. However, non-Mauthner cell escapes have also been reported, and the Mauthner cells may be involved in other behaviors such as post-feeding turns.

Particle image velocimetry (PIV) A technique for revealing complex patterns of flow in a fluid by automatically tracking the motion of suspended particles.

Reynolds number The ratio of inertial to viscous forces in a flow.

Root effect An extreme pH sensitivity of the hemoglobin; at low pH Root hemoglobin remains in a deoxygenated state even at very high oxygen partial pressures.

Swimbladder A gas-filled sac present in the abdominal cavity of most teleost fishes.

Vortex A mass of fluid in whirling or circular motion. Vortices always appear where hydrodynamic lift is being generated.

A Short Historical Perspective on Buoyancy and Locomotion

The extraordinary and, insofar as we are aware, the only historical account of the development of theory, ideas, and devices to test these ideas concerning biomechanics in fishes is given by Alexander in the book *Fish Biomechanics*. Alexander presents a historical account from the time of Aristotle up to 1983 and the brief summary and figures presented here concerning buoyancy and locomotion are taken from his account.

The idea that the swimbladder may play a role in buoyancy – based on the Archimedes principle a submerged body displaces a volume of water equal to its volume – was expanded by Rondelet in 1554 to include the idea that swimbladder in fishes was a buoyancy organ. In 1662, Robert Boyle published Boyle's law showing that the volume of a gas is inversely proportional to the pressure at a constant temperature (i.e., while one increases, the other decreases). Hence, when a fish descends in the water column resulting in an increase in ambient pressure, the volume and thus the buoyant force of a swimbladder decrease. A few years later, workers at the Accademia del Cimento reduced the pressure in a container containing a fish and showed that the fish swelled, floated to the surface, and could not swim down. **Figure 1** is well suited for the present series of articles because the three panels on the left concern the role of the swimbladder in buoyancy and the panel on the right shows how a fish swims using its tail rather than paired fins. Confirmation (or more exactly, partial confirmation) of the application of Boyle's law to swimbladders occurred with the classic studies by Alexander. However, the mechanisms of gas secretion were the subject of much debate. It was not until Pelster (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes) and Berenbrink (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems) resolved the important problems at the biochemical and cell level that a relatively complete understanding of the mechanisms involved was achieved.

Borelli (1680) (**Figure 1**) was apparently the first to argue that fish swim with force generated by the tail rather than rowing with the paired fins. An idea supported by his observation that a fish could still swim after the paired fins had been removed (the precursor of similar experiments on pared paired fins subsequently carried out by Paul Webb in 1977). Although there was very little experimental work on how fish swim for 200 years following Borelli's suggestion, the theoretical groundwork was established by various workers during this period. It was not until the early 1800s that the ideas relating body shape to streamlining and drag appeared. Even so, these theoretical underpinnings were understood by a very few workers in the field until Steve Vogel made them accessible to all biologists in 1981 with the publication of his book *Life in Moving Fluids*.

The ability to take photographs in rapid succession was a major key to gaining some understanding of all aspects of animal locomotion, including fish swimming. Marey, in 1894, published a series of photographs of an eel swimming and showed that there is a wave that passes down the body and that the wave pushes the water, resulting in the fish moving forward (**Figure 2**). Little progress was made on the problem until the series of studies by Gray. His

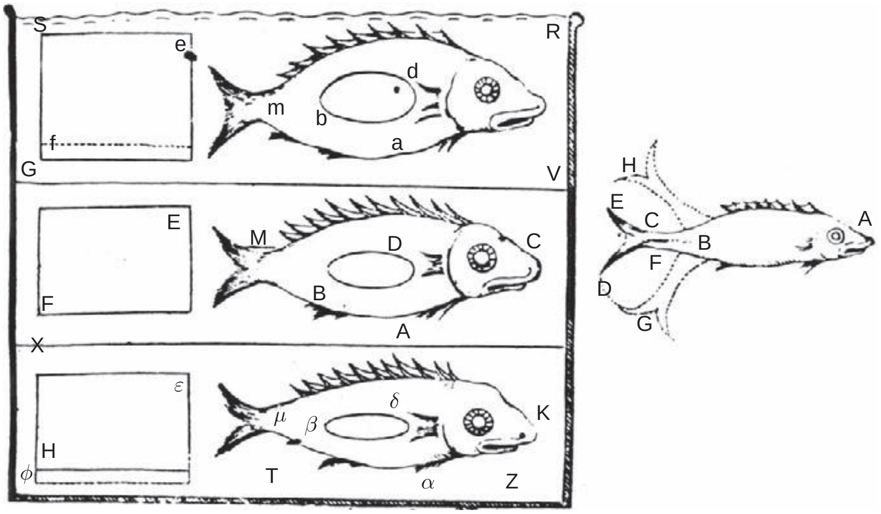


Figure 1 Fish with swimbladders of different size in water with different densities on the left. Diagram on the right illustrates how fish swim by moving the tail back and forth rather than rowing with their paired fins. From Borelli GA (1680) De motu animalium pars prima. Romae. http://books.google.ca/books?id=A48PAAAAQAAJ&pg.

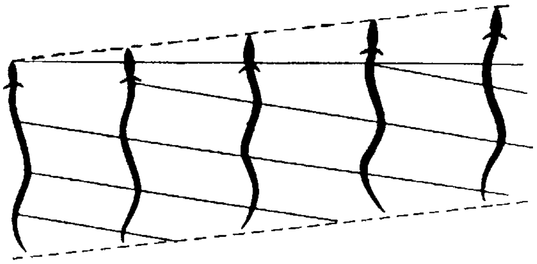


Figure 2 Films of an eel (*Anguilla anguilla*) swimming. Lines illustrate that the propulsive waves of muscle contraction progress from head to tail as the eel swims forward. From Marey EJ (1894) *Le mouvement*. Paris: Masson.

detailed analysis related the waves passing down the body in response to sequential contraction of muscle in different segments at different times. Harris, a colleague of Gray, using a model of a shark in a wind tunnel, showed that movement of fins could account for stability and maneuverability. Many studies of fish locomotion have followed along as technology has allowed for higher frame rates. As discussed in many of the articles in the present section, combining high frame rate with a laser to illuminate a slice of water and adding reflective particles to the water (PIV or DPIV, digital particle image velocimetry) has provided an extremely important window to understanding how fish swim.

Buoyancy and Locomotion Today

This section provides a synthesis of current knowledge concerning two related areas: buoyancy (largely concerned with movement in the vertical direction) and locomotion (largely concerned with movement in the horizontal direction).

Buoyancy

Some fish have no adaptations for buoyancy and are denser than both freshwater and seawater (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). Many have a gas-filled swimbladder and some have deposits of low-density organic compounds large enough to match their density to that of the water. As a fish swims deeper, its swimbladder is compressed by hydrostatic pressure. To maintain buoyancy when it swims deeper, fish must secrete gas into the swimbladder (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes). Similarly, it must absorb gas or expel gas from the swimbladder during ascent. Gas secretion into the swimbladder requires the activity of gas gland cells (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes), which acidify the blood and thus induce a decrease in its gas-carrying capacity. The decrease in gas-carrying capacity is due to the evolution of hemoglobins with special properties (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems) so that their affinity for oxygen markedly decreases in the presence of acid, the so-called Root effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). The second step in the process of gas secretion, the increase in gas partial pressure, is multiplied by back-diffusion and countercurrent concentration of gas molecules in a countercurrent system, the retia mirabilia (**see also Design and Physiology of Arteries and Veins**: The Retia). Thus, the functional swimbladder required the evolution of several likely independent features that were subsequently combined to allow the secretion of gases at very high partial pressures into the swimbladders at a water depth of several thousand meters.

Locomotion and Movement

Understanding how fish swim requires an understanding of the way water moves around swimming fishes because the water motion provides information about the forces that fishes generate as they interact with their environment (**see also Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics). Viscosity and viscous forces are much more important for a fish swimming in water than for locomotion of a land animal. Consequently, animal size is very important in water because Reynolds numbers, the ratio of inertial forces to viscous forces, scale with length. When fish move their fins or tail, they create vortices in the water and this rotation is important because it is strongly related to force. A technique called PIV is the most important experimental technique used for hydrodynamic measurements; it reveals how all the water molecules are moving around the fish as the fish swims or feeds.

Many fish swim using lateral movements of the tail, undulatory swimming (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). Waves of curvature progress along the axis from the head to the tail by progressive waves of muscle activity driving sequential contraction of the myotomal muscle blocks (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). Regions of active muscle contraction on one side of the body cause local shortening, whereas muscles on the opposite side are stretched while inactive. A great deal has been learned about how fish muscles contract and power swimming by studying isolated muscles and having them contract and relax in a fashion analogous to that which occurs during swimming – the so-called work-loop method (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops).

Some fish swim using their paired fins especially at low speeds (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming), but the paired fins also have many other functions including maneuvering (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability), stability (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence), display, protection, and reproduction. About 20% of living fishes rely on pectoral fin swimming as their primary mode of propulsion and all fish use these fins for maneuverability and stability. Maneuvers underlie most behaviors of fishes and are executed by all body and fish surfaces that steer or power rotational changes in posture or swimming trajectory (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability). Agility is greater for maneuvers powered by the body and tail. The other side of this coin is that in turbulent water or when swimming at high speeds, it is important for the fish to maintain stability (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence). The body as well as median and paired fins act as trimmers or power control surfaces to minimize and correct displacements due to turbulence in the water.

A different type of locomotion, the fast start, is unique to fishes (**see also Buoyancy, Locomotion, and Movement in Fishes**: Fast Start). Fast starts are unsteady maneuvers performed during predator–prey encounters or social interactions, usually of very short duration. Interestingly, they are carried out by both the predator in trying to capture the prey and the prey in trying to escape predation. Escape responses are usually controlled by Mauthner cells (**see also Brain and Nervous System**: Physiology of the Mauthner Cell: Discovery and Properties) and **Brain and Nervous System**: Physiology of the Mauthner Cell: Function) and usually consist of the fish bending into a C-shape and so are called C-starts.

The final type of movement discussed in this collection of articles concerns feeding in fishes (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics). Despite the considerable diversity in feeding morphology and ecology among fishes, suction feeding is the most common mode of capturing prey. It involves the rapid expansion of the buccal (mouth) cavity that generates a negative pressure inside the mouth relative to the surrounding fluid and thus draws the water and the food into the mouth. The evolution of jaws that can protrude was an important additional feature that enhances suction feeding performance.

The last articles in this section concern the development of robotic fish, an area of study referred to as biomimetics (**see also Buoyancy, Locomotion, and Movement in Fishes**: Biomimetics: Robotics Based on Fish Swimming) and applied aspects of fish swimming performance (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance). Fish robots are built for three reasons: (1) to test biological hypotheses, (2) to engineer novel designs for underwater vehicles, and (3) to entertain. First, biologists and engineers formulate the intended function or behavior of the robot, the subprocesses that will create the function or behavior, and the specific design plans. Robots are built to model individual structures, particular species, or whole populations of fish. Fish robots may or may not be self-propelled, behaviorally autonomous, powered by on-board batteries, or controlled by computers. Many robotic fish projects study propulsion, with body and fins built to produce bio-realistic locomotion.

In summary, here the collection of articles provides an up-to-date account of what we know about how fish move and the methods that scientists use to gain this knowledge.

See also: **Buoyancy, Locomotion, and Movement in Fishes:** Biomimetics: Robotics Based on Fish Swimming; Buoyancy in Fishes; Experimental Hydrodynamics; Fast Start; Feeding Mechanics; Functional Properties of Skeletal Muscle: Work Loops; Maneuverability; Paired Fin Swimming; Stability and Turbulence; Swimbladder Function and Buoyancy Control in Fishes; Undulatory Swimming. **Design and Physiology of Arteries and Veins**: The Retia. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance. **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Alexander RMcN (1983) History of fish biomechanics. In: Webb PW and Weihs D (eds.) *Fish Biomechanics*, pp. 1–35. New York: Preeger.

Berenbrink M (2007) Historical reconstructions of evolving physiological complexity: O_2 secretion in the eye and swimbladder of fishes. *Journal of Experimetnal Biology* 210: 1641–1652.

Borelli GA (1680) De motu animalium pars prima. Romae.http://books.google.ca/books?id=A48PAAAAQAAJ&pg (accessed 17 June 2010).

Marey EJ (1894) *Le mouvement*. Paris: Masson.

Vogel S (1981) *Life in Moving Fluids*. Boston, MA: W. Grant Press.

Webb PW (1977) Effects of median-fin amputation on fast-start performance of rainbow trout (*Salmo gairdneri)*. *Journal of Experimental Biology* 68: 123–135.

Buoyancy in Fishes

RMcN Alexander, University of Leeds, Leeds, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Densities of Fishes
Density and Lifestyle
Buoyancy Strategies
Further Reading

Glossary

Benthopelagic Habitually swimming close to the bottom at great depths.

Bohr and Root effects Two related effects whereby acids tend to release oxygen from hemoglobin.

Buoyancy, positive and negative A positively buoyant organism is less dense than the surrounding fluid, so tends to rise to the surface. A negatively buoyant one is denser than the fluid and tends to sink.

Deep-scattering layer A layer in the sea with a sufficiently rich population of small fish and other plankton to be detected by echo-sounding. It is much nearer the surface at night than by day.

Ground effect A hydrodynamic effect that reduces the energy cost of generating lift when an aircraft flies close to the ground, or a negatively buoyant organism swims close to the bottom.

Kilopascal (kPa) SI unit of pressure, 1000 N m^{-2}. Atmospheric pressure at sea level is about 100 kPa. Used for blood pressure, hydrostatic pressure, and partial pressure of gases.

Leptocephalus The leaf-like larva of an eel.

Lift A hydrodynamic force acting at right angles to a body's direction of motion through a fluid. For example, the force on the wings of airplanes that keeps them airborne.

Particle image velocimetry A technique for revealing complex patterns of flow in a fluid by automatically tracking the motion of suspended particles.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Squalene A hydrocarbon present in large quantities in the livers of some sharks.

Swimbladder A gas-filled sac present in the abdominal cavity of most teleost fishes.

Triglycerides Compounds of glycerol and fatty acids, including the typical fats and oils found in animals.

Vortex A mass of fluid in whirling or circular motion. Vortices always appear where hydrodynamic lift is being generated.

Wax esters A class of low-density oily compounds present in large quantities in the coelacanth and some lantern fishes (Myctophidae).

Introduction

Visitors to an aquarium may notice that some fishes can remain almost motionless in mid-water, while others sink as soon as they stop swimming. The former must be very close to the density of the water they live in, and the latter are obviously denser than the water. This article is about the densities of fishes, about the differences of body composition that affect density, and about the associated costs and benefits. It may seem obvious that the ability to hang effortlessly in mid-water can save fish energy, but it will be argued that, for some life styles, a density greater than water may be preferable.

Densities of Fishes

Density is mass divided by volume. Very small differences of density between fish and water are of interest in studies of fish buoyancy. Fish densities must therefore be measured accurately. It is of course easy to measure mass accurately by weighing. The most obvious way to measure the volume of a fish is to put it into a container filled to the brim with water, and measure the volume of water that overflows. This is very difficult to do accurately. **Figure 1** illustrates a much better method. The fish is dried with a towel and weighed (a). Then it is weighed again, totally immersed in water (b). If its density is very close to that of the water, it may be necessary to hang a

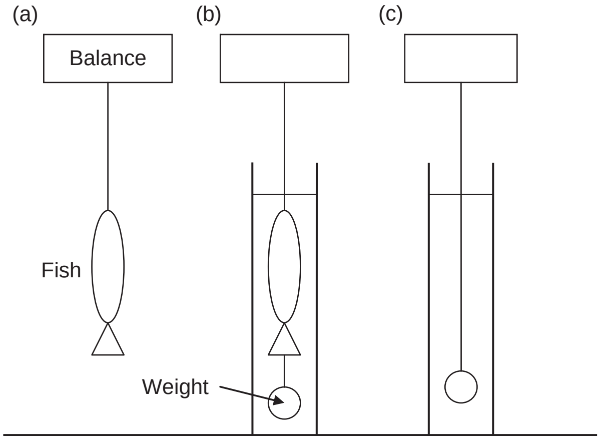


Figure 1 A method for measuring the densities of fishes.

small weight from its tail as shown. In this case, a third weighing is necessary, of the weight (without the fish) immersed (c). The difference (b, c) is the weight of the fish in water. By Archimedes' principle, an upward force equal to (fish volume × water density × gravitational *g*) acts on the immersed fish, so

$$\text{Weight in air} - \text{weight in water} = (\text{fish volume} \times \text{water density} \times g)$$

Also,

$$\text{Weight in air} = \text{mass} \times g$$

Hence,

$$\begin{aligned}\text{Density} &= \text{mass/volume}\\ &= \text{weight in air}\\ &\quad \times \text{water density}/(\text{weight in air} - \text{weight in water})\end{aligned}$$

Freshwater has a density of 1000 kg m^{-3}, and seawater about 1026 kg m^{-3}. These densities are affected only slightly by differences of temperature, or by differences of pressure corresponding to depths of 1 km or less. Differences of salinity between different seas have a larger (but still small) effect.

Most of the essential constituents of fish bodies are denser than either freshwater or seawater. For example, three typical marine teleost species had muscle of density 1055 kg m^{-3}, guts of density 1038 kg m^{-3}, skin 1054–1066 kg m^{-3}, and bone 1300–1500 kg m^{-3}. The densities of their livers, however, ranged from 986 kg m^{-3} (less even than freshwater) to 1050 kg m^{-3}. Triglyceride oils have densities around 930 kg m^{-3}, so oily livers have low densities. The muscles of freshwater teleosts and selachians have about the same density as those of marine teleosts, and selachian cartilage has a density of 1060–1180 kg m^{-3}.

Because most of their tissues are denser even than seawater, fish lacking buoyancy adaptations are denser than the water they swim in, with densities around 1075 kg m^{-3}. Many fish, however, have adaptations that reduce their density to match that of the water. A large proportion of teleosts have a swimbladder, a gas-filled cavity that reduces their densities by increasing their volumes without adding significantly to their masses. Some sharks contain large volumes of the low-density hydrocarbon squalene (860 kg m^{-3}), as does the chimeroid *Hydrolagus*. Some lantern fishes (Myctophidae), the castor oil fish *Ruvettus* and the coelacanth *Latimeria*, contain large quantities of wax esters (density also about 860 kg m^{-3}). Some benthopelagic fish (fish that swim close to the bottom at great depths) are brought close to neutral buoyancy by gelatinous muscle containing less protein and more water than ordinary fish muscle and by poorly ossified bone. They include the bristlemouth *Gonostoma* and the smooth-head *Xenodermichthys*. Similarly, the leptocephalus larvae of eels are neutrally buoyant due to their bodies consisting largely of dilute, low-density jelly.

Table 1 Fractional volumes of low-density materials required to match the densities of typical fish to those of freshwater or seawater

		Fractional volume required in	
	Density ($kg\ m^{-3}$)	*Freshwater*	*Seawater*
Gas	Negligible	0.07	0.05
Squalene or wax esters	860	0.35	0.23
Triglycerides	930	0.52	0.34

To calculate the effects of these and other low-density materials, consider a fish that is initially denser than water, but acquires a quantity of low-density material (referred to for brevity as a float). Its density is then

$$\text{Density} = (\text{initial mass} + \text{float mass})/(\text{initial volume} + \text{float volume})$$

This equation has been used in **Table 1** to calculate the fractional float volume (float volume/total volume) required to match the density of a fish of initial density 1075 kg m^{-3} to that of freshwater (1000 kg m^{-3}) or seawater (1026 kg m^{-3}). It shows that a small volume of gas would be sufficient, but that large volumes of squalene or wax esters, and enormous volumes of triglycerides, would be required. In the extreme case, adding enough triglyceride for neutral buoyancy in freshwater would double the volume of the fish.

Density and Lifestyle

Dense Fishes

Fish that spend much of their time resting on the bottom tend to have no buoyancy adaptations, and to be denser than the water they live in. They include selachians such as

Scyliorhinus and *Raia* and flatfishes (Pleuronectiformes), which have densities of around 1075 kg m^{-3}. It may seem surprising that some fish that swim perpetually also lack buoyancy adaptations, and are denser than the water they live in. They include most pelagic selachians (but not the Basking shark *Cetorhinus*) and tunnies such as *Euthynnus* and *Katsuwonus* (which have densities of 1080–1100 kg m^{-3}).

A very large majority of teleost fish have swimbladders. In most cases this occupies about 7% (freshwater teleosts) or 5% (marine teleosts) of the volume of the body, giving the fish a density very close to that of the water they live in. In some bottom-living teleosts, however, the swimbladder has been reduced but not eliminated. For example, some species of *Gobius* which inhabit marine rock pools have swimbladders occupying only 1–2% of body volume, and are denser than the water. Other examples include bottom-living catfish such as *Clarias*, with tiny swimbladders that make only a negligible contribution to buoyancy but still perform a valuable function as part of the Weberian apparatus which enhances the auditory acuity of them and of other ostariophysan fish.

Changing Depth

Dependence on a swimbladder for buoyancy gives rise to a problem for species that use a substantial range of depths. The swimbladder wall is flexible, so the swimbladder is compressed to a smaller volume as the fish moves to greater depths, where the pressure is greater. It expands as the fish moves up toward the surface again. In most cases, the volume changes are very close to the predictions of Boyle's law. Pressure increases by about 1 atm (100 kPa) for every 10 m of descent, so descent from the surface to 10 m halves the volume of the swimbladder, and descent to 90 m (where the pressure is 10 atm) reduces it to one-tenth of the surface volume. The effects on the fish's buoyancy are thus very large. (Ostariophysan fish are less sensitive to small pressure changes because their swimbladder gases are a little above ambient pressure, making the swimbladder wall taut.)

To change the depth at which it has neutral buoyancy, a fish must add gas to the swimbladder or remove gas from it. The more primitive groups of teleosts, notably the protacanthopterygians (e.g., salmon) and the ostariophysans (e.g., carps), have an open connection between the swimbladder and the gut. This enables them to release gas to adapt to shallower depths, but they cannot of course gulp air in to adapt to a greater depth except when they are at the surface. More advanced teleosts, the paracanthopterygians (e.g., cod) and the acanthopterygians (e.g., perch), have closed swimbladders and can change the quantity of gas in the swimbladder only by secretion from the blood or by resorption into the blood. These processes generally occur through specialized parts of the swimbladder wall that are much more richly supplied with blood vessels than the rest of the wall (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes; **The Skin**: Functional Morphology of the Integumentary System in Fishes).

Resorption is passive. The partial pressures of dissolved gases at the surfaces of seas, lakes, and rivers are generally in equilibrium with the atmosphere, about 20 oxygen and 80 kPa nitrogen. At substantial depths, the partial pressure of nitrogen is the same, but that of oxygen may be greatly reduced by the respiration of organisms. The blood tends to equilibrate with the water as it passes through the gills, so the total of the partial pressures of gases in the blood is 100 kPa or less. The pressure of the gases in the swimbladder, however, increases with depth; it is 100 kPa at the surface, 200 kPa at 10 m, and so on. Except at the surface, gases will tend to diffuse out of the swimbladder into the blood. Gas is lost from the swimbladder continuously, and must be replaced by secretion if buoyancy is to be maintained. Unwanted losses are minimized by a layer of tightly packed plates of guanine, which greatly reduce the permeability of the swimbladder wall to diffusion of gases. Specialized regions of the swimbladder wall, however, enable the fish to make an opening in this diffusion barrier to allow gas to diffuse out quite rapidly when required. For example, cod and their relatives have a pocket called the oval in the swimbladder wall. The oval can be opened or closed like a drawstring purse, and the flow of blood to it can be adjusted, to allow gas to diffuse out when required.

Another structure in the swimbladder wall, the gas gland, is the site of secretion of gas into the swimbladder. To secrete gas, the flow of blood through it is increased, and the gland cells secrete lactic acid into the blood. This increases the partial pressures of oxygen by the Bohr and Root effects, and of all gases by the salting-out effect (the effect that makes gases less soluble in seawater than in freshwater). These increases of partial pressure are amplified in a rete by countercurrent multiplication (**see also Design and Physiology of Arteries and Veins**: The Retia). When, as a result, the partial pressures of gases in the blood of the gas gland rise above their partial pressures in the swimbladder, gas diffuses from the blood into the swimbladder. Some deep-sea teleosts have very large retia, with capillaries up to 25 mm long, which enable them to raise the blood gases to the enormous pressures required to secrete into their swimbladders. Fish with gas-filled swimbladders have been captured at depths exceeding 2000 m. The pressure at 2000 m is 20.1 MPa (200 atm).

The rates at which teleosts can adjust the quantity of gas in the swimbladder vary greatly. For example, while salmon can release swimbladder gas instantaneously through the connection to the gut, they need more than 10 days to refill an empty swimbladder by secretion. They could adjust volume immediately to accommodate reduced depths, but would need at least a day to secrete enough gas to adjust to a 1 m increase of depth. Cod (which have closed swimbladders) are slower to adjust volume to reduced depths but can secrete gas much faster. Experiments in which depth changes were simulated by changing the pressure in a pressure tank showed that cod could compensate for depth reductions at the rate of only 1 m h^{-1} at a depth of 5 m, but at 20 m h^{-1} at a depth of 65 m. They could resorb gas faster at the greater depth because of the greater differences of partial pressure between the gases in the swimbladder and in the blood. They could compensate for depth increases at a rate of 1 m h^{-1} at 12 °C, or less at lower temperatures, at all depths in the above range. The bluefish *Pomatomus* can secrete gas more rapidly, fast enough to compensate for depth increases at a rate of 2.5 m h^{-1}.

Many teleosts make rapid, substantial changes of depth, too fast for compensation to be possible. In one set of experiments, chum salmon (*Oncorhynchus*) making their homing migration were fitted with data loggers and returned to the sea. Recovered data loggers showed that as the fish continued their journey they made vertical movements of 10 m or more several times per hour, frequently visiting the surface and occasionally diving to 100 m or more. Descents were faster than ascents but required lower tail beat frequencies, suggesting that the fish were denser than seawater through most of their vertical range. It seems that the quantity of gas in the swimbladder gave them approximately neutral buoyancy at the surface but was compressed, making them denser, as they dived.

In another set of experiments, cod (*Gadus*) caught at 25–110 m were brought slowly to the surface. They lost much of their swimbladder gas, leaving them reasonably close to neutral buoyancy at the surface. They were fitted with data loggers and returned to the sea. They made frequent short vertical excursions of 50 m or more but the general trend was downward until they reached their preferred habitat near the bottom, at depths up to 150 m. They appeared to adapt to increasing depth at an average rate of 10 m d^{-1}. When their descent was complete, their behavior suggested that they were neutrally buoyant at their preferred depth.

Some marine fish make daily vertical migrations, spending the night near the surface and the day much deeper. In the oceans, an abundant community of small fishes, crustaceans, and siphonophores swim near the surface at night but spend the day at depths of several hundred meters. They are so abundant that they appear quite conspicuously in echo-sounder traces, as the deep scattering layer. The most numerous of the fishes are lantern fishes (Myctophidae), some of which have been observed from submersible vehicles moving between the top 50 m of the sea at night and around 300 m by day. Some of them depend on swimbladders for buoyancy. These seem to be close to neutral buoyancy at their nighttime depths, but their swimbladders must be so greatly compressed at their day time depths as to be almost useless as buoyancy aids. Other lantern fishes have lost their swimbladders and depend on wax esters for buoyancy. Their buoyancy can be affected very little by depth changes, but the wax esters need to be very much bulkier than a swimbladder (**Table 1**).

Buoyancy Strategies

The preceding section showed that fish with different ways of life tend to have different buoyancy adaptations. This one looks for possible explanations, by examining the relative merits of different buoyancy strategies for different ways of life.

Life on the Bottom

We have seen that fish that spend much of their time resting on the bottom tend to have no buoyancy adaptations, but to be denser than the water they live in. For them, negative buoyancy may be advantageous, in two related ways. It results in frictional forces between fish and bottom that will tend to prevent displacement of the fish by water currents. Also, it tends to counteract any lift forces due to water flowing over the convex dorsal surface of the fish, which might raise the fish from the bottom and result in its being washed away by the current. Experiments in a flume showed that rays (*Raia*) and plaice (*Pleuronectes*) were liable to be lifted from the bottom and dislodged by currents as slow as 0.6 m s^{-1}.

Keeping Afloat

For fish that spend much of their time hovering in mid-water, neutral buoyancy can save energy. A neutrally buoyant fish can remain almost motionless in mid-water; this can be confirmed by watching fish in aquaria. If it has a swimbladder, however, it cannot remain completely motionless but must make occasional fin movements. This is because its equilibrium is unstable. Any slight upward displacement results in expansion of the swimbladder making the fish less dense. Unless it makes a correcting fin movement, it will rise further and eventually be carried to the surface. Similarly, an accidental downward displacement will make the fish denser and it will sink to the bottom, unless it takes corrective action.

To remain stationary in mid-water, a fish that is denser than water must generate a supporting force by driving water downward. This can be done by fin movements.

The principle is the same as that of helicopters, which support themselves by using the rotor to drive air downward. The theory of helicopters tells us that, for geometrically similar fish of equal density, the energy cost of hovering should be proportional to (body mass)$^{1.14}$. The maximum aerobically sustainable metabolic rates of fishes are roughly proportional to (body mass)$^{1.0}$, so hovering is easier for a small dense fish than for an equally dense large one. Nevertheless, hardly any fish that spend much time hovering lack buoyancy aids. The mandarin fish (*Synchiropus*) is a striking exception to the general rule. This small tropical marine fish has an unusually high density of 1120 kg m^{-3}, but spends much of the time hovering just above the sea bottom where the energy cost is much reduced by ground effect.

The energy cost of excess density has been investigated by measuring the metabolic rates of sea bass (*Dicentrarchus*) with lead weights attached. Weights that increased the effective density of the fish by 4% increased the nocturnal metabolic rate by about 33%. (The fish were less active at night than by day, but the proportions of time spent hovering and swimming were unfortunately not measured.)

A fish that is denser than water needs more power for forward swimming than a neutrally buoyant fish because it must generate lift to support its excess weight. It can get this lift either by using fins as hydrofoils, or by swimming with the body tilted at an appropriate angle so that the body itself generates lift. **Figure 2(a)** shows the forces on a negatively buoyant fish getting the required lift from fins. The drag acting backward on the body is counteracted by the forward thrust due to the fish's swimming action. The fish's weight acts downward at the center of mass and is largely counteracted by the upthrust due to Archimedes' principle which acts at the center of buoyancy (the geometrical center of the fish). The centers of mass and buoyancy are generally slightly separated because tissues of different densities are unevenly distributed in the fish. The upthrust is a little less than the weight if the fish is denser than the water, so lift is required from fins. In the diagram, the required lift is provided partly by the tail and partly by the pectoral fins, in the proportions needed to balance the moments about the center of mass. Until recently, negatively buoyant sharks such as the dogfish *Scyliorhinus* were believed to generate lift in these ways. It was known that their asymmetrical (heterocercal) tails drove water downward (generating lift) as well as backward, and it was believed that the pectoral fins were tilted at an angle of attack so as to provide lift in the same way as the wings of an aeroplane. Experiments with a leopard shark showed that this was not the case. The flow of water behind the pectoral fins was observed by particle image velocimetry as the fish swam in a water tunnel. In level swimming, there were no vortices there, showing that the pectoral fins were not generating lift. Indeed, the fins were held at an angle of attack at which no lift could be expected. The body, however, was tilted as shown in **Figure 2(b)**, and must have generated lift.

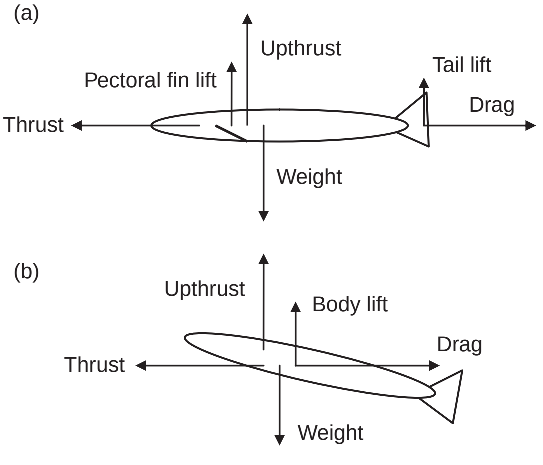


Figure 2 Forces acting on a swimming fish that is denser than the water. The fish obtains the required lift from: (a) fins and (b) its tilted body.

Hydrofoils generate lift by driving water downward. The power needed to do this, known as the induced power, depends on the span of the hydrofoil (the distance from one wing tip to the other). It is proportional to (lift/span)^2/speed. The body of a fish such as the dogfish is in effect a hydrofoil of very small span, much less than the span of the pectoral fins. Consequently, the induced power is much higher if the fish uses body lift as in **Figure 2(b)** than if it used lift from fins as in **Figure 2(a)**. The dogfish's use of the less economical option has not been explained.

Tunnies such as *Euthynnus* and *Katsuwonus* are denser than seawater and have to swim perpetually to avoid sinking. Like dogfish, they swim with their pectoral fins extended. It is possible that, unlike dogfish, they use these fins to generate lift. The experiments that would be needed to find out have not been performed yet.

The induced power can be avoided if the fish has a buoyancy organ that matches its density to that of the water, but this incurs other energy costs. Any buoyancy organ increases the size of the fish and so the profile power, the power that is required to overcome the drag on the body when it is not generating lift. This power is proportional to (cross-sectional area) × speed^3 × (drag coefficient). The drag coefficient depends on the Reynolds number, a function of the fish's length and speed. In the range of Reynolds numbers relevant to the swimming of most fish, it is approximately proportional to (length × speed)$^{-0.5}$. For geometrically similar fish, cross-sectional area is proportional to length^2. Thus, the profile power required by a swimming fish and the additional profile power due to a buoyancy organ are both about proportional to length$^{1.5}$ × speed$^{2.5}$.

Thus, a swimming fish has the option of using its fins or body to generate hydrodynamic lift and prevent sinking, at an energy cost proportional to $(\text{lift/span})^2/\text{speed}$; or of using a buoyancy organ at a cost proportional to length$^{1.5}$ × speed$^{2.5}$. As speed increases, the cost of hydrodynamic lift falls and the cost of using a buoyancy organ rises. This argument suggests that a buoyancy organ will be the more economical option for fish that habitually swim slowly, and hydrodynamic lift for fish that spend much of their time swimming fast. The critical speed at which it becomes more economical to do without a buoyancy organ will be greater if that organ is a swimbladder than if it is a denser organic compound, because an adequate swimbladder is smaller and adds less to drag. It will be less for a fish that uses fins to generate lift than for one that depends on body lift, because a larger span will provide the required lift for less profile power. A rough calculation for marine fish with the options of lift from pectoral fins or buoyancy from a swimbladder led to the conclusion that the critical speed would be about $0.5\,\text{m}\,\text{s}^{-1}$ for a 0.1 kg fish, and $1.4\,\text{m}\,\text{s}^{-1}$ for a 10 kg fish. It must be emphasized that this calculation was rough and depended on some doubtful assumptions. As already noted, most pelagic selachians, and tunnies such as *Euthynnus* and *Katsuwonus*, swim perpetually but have no buoyancy organs. These fish are noted for fast swimming, and it may well be that hydrodynamic lift is the more economical option for them.

So far in this discussion of energy costs related to buoyancy, we have considered only the work required of muscles. There are other energy costs that may be significant. First, consider the energy cost of secreting gas into the swimbladder. The work that must be done on the gas is easily calculated; it is the work needed to compress the gas from its partial pressure in the blood, to the (higher) partial pressure in the swimbladder. Unfortunately, however, the efficiency of the secretion process is unknown, so we cannot confidently calculate the metabolic energy cost. For fish that live at moderate, near-constant depths and need to only secrete enough gas to replace losses by diffusion through the swimbladder wall, the energy cost is probably small. For fish that live at great depths, and so have to secrete against very large differences of partial pressure, it may perhaps be large. It may be sufficiently large to make gelatinous muscle a better option than a swimbladder for benthopelagic fishes.

Additional energy is needed for gas secretion when a fish compensates for increased depth by adding gas to the swimbladder, as a cod did in an experiment described earlier. This energy cost seems never to have been measured, but maintenance of constant swimbladder volume seems not to be an option for fish that make substantial daily depth changes, because they cannot secrete gas fast enough.

Triglycerides, wax esters, and squalene are energy-rich compounds, so their synthesis as the fish grows requires substantial investment of energy. This should be recognized in comparison of their merits with those of other buoyancy strategies.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. **Design and Physiology of Arteries and Veins**: The Retia.

Further Reading

Alexander RMcN (1993) Buoyancy. In: Evans DH (ed.) *The Physiology of Fishes*, pp. 75–97. Boca Raton, FL: CRC Press.

Alexander RMcN (2003) *Principles of Animal Locomotion*. Princeton, NJ: Princeton University Press.

Blake RW (1979) The energetics of hovering in the mandarin fish (*Synchropus picturatus*). *Journal of Experimental Biology* 82: 25–33.

Denny MW (1993) *Air and Water*. Princeton, NJ: Princeton University Press.

Jones FRH and Scholes P (1985) Gas secretion and resorption in the swimbladder of the cod *Gadus morhua*. *Journal of Comparative Physiology B* 155: 319–331.

Lapennas GN and Schmidt-Nielsen K (1977) Swimbladder permeability to oxygen. *Journal of Experimental Biology* 67: 175–196.

Lefrançois C, Odion M, and Claireaux G (2001) An experimental and theoretical analysis of the effect of added weight on the energetics and hydrostatic function of the swimbladder of European sea bass (*Dicentrarchus labrax*). *Marine Biology* 139: 13–17.

Pelster B (2001) The generation of hyperbaric oxygen tensions in fish. *News in Physiological Science* 16: 287–291.

Strand E, Jørgensen C, and Huse G (2005) Modelling buoyancy regulation in fishes with swimbladders: Bioenergetics and behaviour. *Ecological Modelling* 185: 309–327.

Tanaka H, Takagi Y, and Naito Y (2001) Swimming speeds and buoyancy compensation of migrating adult chum salmon *Onchorhynchus keta* revealed by speed/depth/acceleration data logger. *Journal of Experimental Biology* 204: 3895–3904.

Tsukamoto K, Yamada Y, Okamura A, *et al.* (2009) Positive buoyancy in eel leptocephali: An adaptation for life in the ocean surface layer. *Marine Biology* 156: 835–846.

Van der Kooij J, Righton D, Strand E, *et al.* (2007) Life under pressure: Insights from electronic data-storage tags into cod swimbladder function. *ICES Journal of Marine Science* 64: 1293–1301.

Webb PW (1989) Station holding by three species of benthic fish. *Journal of Experimental Biology* 145: 303–320.

Wilga CD and Lauder GV (2000) Three-dimensional kinematics and wake structure of the pectoral fins during locomotion in Leopard sharks *Triakis semifasciata*. *Journal of Experimental Biology* 203: 2261–2278.

Swimbladder Function and Buoyancy Control in Fishes

B Pelster, University of Innsbruck, Innsbruck, Austria

© 2011 Elsevier Inc. All rights reserved.

Introduction
Morphology
Metabolic Activity and the Single Concentrating Effect
The Single Concentrating Effect
Countercurrent Concentration
Resorption of Gas
Failure of Swimbladder Inflation
Control of Swimbladder Inflation
Further Reading

Glossary

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Gas partial pressure The pressure exerted by a single gas with respect to total gas pressure.

Root effect An extreme pH sensitivity of the hemoglobin; at low pH, Root hemoglobin remains in a deoxygenated state even at very high oxygen partial pressures.

Rete mirabile (pl. retia mirabilia) A vascular network, usually consisting of a parallel array of both arterial and venous vessels with countercurrent flow. Retia facilitate transfer of heat or oxygen between the arterial and venous vessels. Rete is Latin for 'net or network', mirabile for 'wonderful' or 'miracle'.

R-state hemoglobin Relaxed (oxy) hemoglobin, at high oxygen tensions hemoglobin assumes relaxed state which has higher oxygen affinity than T-state hemoglobin.

Salting-out effect A decrease in physical solubility of gases in fluid due to an increase in salt concentration.

T-state hemoglobin Tense (deoxy) hemoglobin is a more stable hemoglobin form in the absence of oxygen (e.g., organic phosphates bind preferentially to T-state hemoglobin).

Introduction

Depending on their composition, fish are usually somewhat denser than the surrounding water, largely because of their skeleton and protein masses (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). Neutral buoyancy, however, is advantageous to any object in water, including a submarine or an animal, because it saves energy used to prevent sinking or rising. An air-filled chamber like the fish swimbladder is an excellent device to reduce the density of fish without adding much weight. In fact, the density of air is only $0.00125\,\mathrm{g\,cm^{-3}}$ at 1 atm, and still only $0.7\,\mathrm{g\,cm^{-3}}$ at 700 atm, a pressure experienced in the sea at a depth of 7000 m below the surface. Thus, by using a gas-filled swimbladder the status of neutral buoyancy may be achieved by adding very little volume to the overall volume of a fish compared with other materials like lipids (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). Nonetheless, there are problems using a swimbladder as a floating device, especially when the fish changes water depth.

A given swimbladder volume will grant the fish neutral buoyancy only at one particular depth, provided the gas-filled swimbladder obeys Boyle's law, which it apparently does. Swimming upward will result in expansion of the swimbladder gas, and the increased swimbladder volume will add additional lift. By the same token, any descent from the level of neutral buoyancy will reduce swimbladder volume, creating a sinking force, which increases with depth. Thus, like a Cartesian diver, the level of buoyancy constitutes a labile equilibrium, any deviation upward lifting the fish with increasing speed, and vice versa.

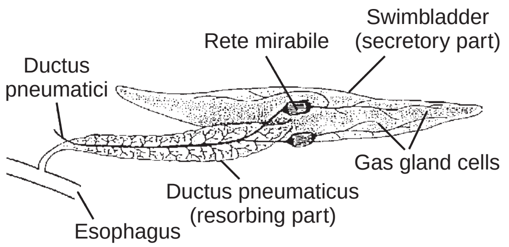
Pelagic fish behaviorally vary their depth, partly following a diurnal rhythm. Accordingly, changes in swimbladder volume can at least in part be compensated either by secretion of gas during descent or by resorption of gas during ascent. In addition, gas must be secreted continuously into the swimbladder in order to compensate for the diffusional loss of gas to the environment, because the total gas pressure inside the swimbladder exceeds gas pressure of the physically dissolved gases in the water, which hardly increases with increasing water depth.

Morphology

Physostome and Physoclist Fishes

The swimbladder originates as an unpaired dorsal outgrowth of the foregut (**Figure 1(a)**). In so-called physostomous fishes the embryonic connection to the gut may persist as the

(a) Physostome fishes (Eel)



(b) Physoclist fishes (Perch)

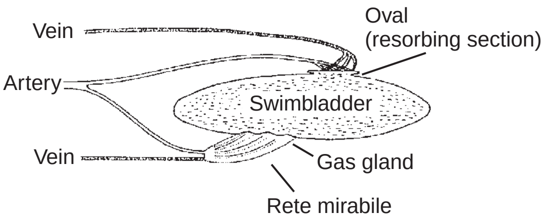


Figure 1 (a) Swimbladder structure of a physostome fish (*Anguilla anguilla*) with a persisting ductus pneumaticus. In the eel, the ductus pneumaticus is converted to a resorbing section of the swimbladder. (b) Swimbladder structure of a physoclist fish (*Perca fluviatilis*). In perch the rete mirabile is unipolar and in intimate contact to the gas gland. The oval represents the resorbing section of the swimbladder. From (a) Modified from Dorn E (1961) Über den Feinbau der Schwimmblase von Anguilla vulgaris L. Licht- und elektronenmikroskopische Untersuchungen. *Zeitschrift für Zellforschung und mikroskopische Anatomie* 55: 849–912. (b) Modified after Denton E (1960) The buoyancy of marine animals. *Scientific American* 203: 119–129.

pneumatic duct. The pneumatic duct can be used to transfer air, which can be released to the water or gulped at the water surface and pushed into the swimbladder. In the great majority of teleosts, however, this connection is completely lost at an early stage during development and the swimbladder is a closed gas cavity (physoclist fishes; **Figure 1(b)**). Deep-sea fish usually are physoclist.

Secretory and Resorbing Sections of the Swimbladder

The structural diversity in general swimbladder morphology is remarkable, but some features can be identified in all swimbladders. The two physiological phenomena associated with swimbladder function, namely gas secretion and gas resorption, do not occur at the same location. Therefore, every swimbladder must have a secretory section and a resorbing section that can be separated temporarily from the secretory part of the bladder. In cyprinids and wrasse, for example, the swimbladder consists of two chambers, a thick-walled section in which gas is deposited, and a thin-walled chamber in which gas is resorbed. In other fish, a special section of the secretory bladder, the oval (**Figure 1(b)**), can be closed off by muscular activity and is used as the resorbing part of the swimbladder (e.g., gadoid fishes). In the physostome eel, the pneumatic duct is transformed into a resorbing part of the swimbladder. It is separated from the secretory part of the swimbladder by a sphincter, and the opening to the foregut is functionally closed (**Figure 1(a)**). This means, the eel cannot gulp air at the surface in order to fill the swimbladder.

In many species, the arterial and venous blood vessels to and from the secretory section of the swimbladder are arranged in a countercurrent fashion (**see also Design and Physiology of Arteries and Veins**: The Retia). The arterial supply transiently splits off forming arterial capillaries, running parallel to venous capillaries returning from the swimbladder epithelium. This countercurrent system has also been named red body or rete mirabile, which can be easily seen in the European eel (**Figure 2**). Blood supply to the resorbing part of the swimbladder is separate and bypasses this rete mirabile.

Layers of the Swimbladder Wall

The wall of the secretory bladder consists of a number of thin tissue layers, the inner swimbladder epithelium, muscularis mucosae, submucosa, and tunica externa. The tunica externa represents a dense connective tissue capsule. The submucosa usually is impregnated with guanine crystals or may include layered lipid membranes, rendering the swimbladder wall relatively impermeable to gases in order to reduce diffusional loss of gases out of the secretory section. The muscularis mucosa mainly consists of smooth muscle cells which on contraction transfer gas from the secretory section of the swimbladder to the

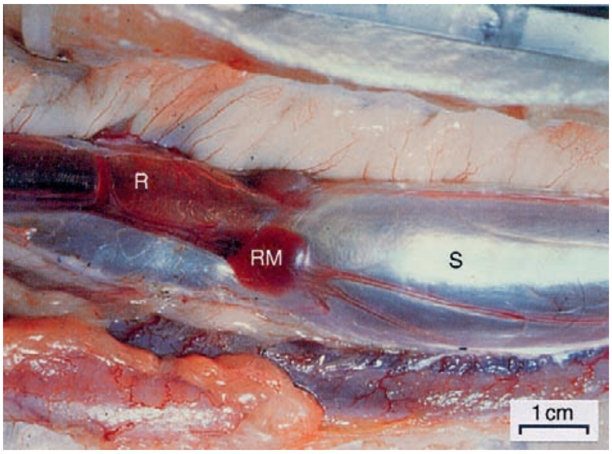


Figure 2 The swimbladder of the European eel with a secretory section (S) and the pneumatic duct, modified into a resorbing section (R). Two retia mirabilia (RM) are located next to the sphincter muscle separating the secretory and the resorbing section. The ductus pneumaticus is functionally closed, so that the eel cannot gulp air at the water surface to fill the swimbladder. From Pelster B (1993) Die Schwimmblase als hydrostatisches Organ. *Biologie in Unserer Zeit* 23: 254–258.

resorbing part. The resorbing part of the swimbladder consists of thin membranous structures with an extensive network of blood vessels (**Figure 2**).

Important for the secretion of gas into the swimbladder are the so-called gas gland cells in the swimbladder epithelium. Gas gland cells are specialized in the production of acidic metabolites. In the eel (*Anguilla*), a model species for the analysis of swimbladder function, the whole internal epithelium of the secretory bladder consists of gas gland cells. In *Perca*, *Gadus*, and many other species, gas gland cells are clustered together, forming a massive complex of several cell layers. A compact gas gland may also result from an extensive secondary folding of a single layer of epithelial cells (*Gobius*, *Syngnathus*). A characteristic of gas gland cells is its intimate contact with an extensive capillary vascular system.

Structure of Gas Gland Cells

Gas gland cells are cubical or cylindrical ranging in size from 10–25 μm to giant cells of 50–100 μm or even more. Gas gland cells are polarized with some small microvilli on the luminal side, while the basal side often is more densely vacuolated and shows a large number of infoldings (**Figure 3**) similar to other secretory or resorbing tissues. Gas gland cells are characterized by the presence of very few filamentous or elongated mitochondria with few tubular cristae.

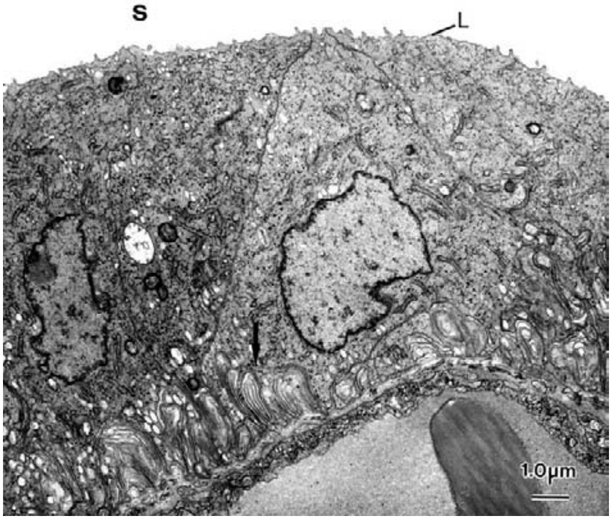


Figure 3 Histology of swimbladder gas gland cells of the European eel *Anguilla anguilla*. Extensive membrane foldings (indicated by an arrow) mark the contact site to blood vessels, while only a few small protrusions are seen at the swimbladder surface (S); L, luminal membrane. From Pelster B (1997) Buoyancy at depth. In: Randall DJ and Farrell AP (eds.) *Fish Physiology*, vol. 16, pp. 195–237. Academic Press.

Metabolic Activity and the Single Concentrating Effect

Gas Secretion: A Diffusional Process

Although the term ‘gas secretion’ suggests that secretory events (exocytosis and adenosine triphosphate (ATP)-dependent gas movements) are required to fill the swimbladder, gas is transferred into the swimbladder lumen only by passive diffusion along a gas partial pressure gradient. The high gas partial pressure necessary to establish the diffusion gradients between the blood and the swimbladder lumen is established by two mechanisms, the reduction of the effective gas-carrying capacity of blood and subsequent countercurrent concentration of gases in a countercurrent system, the rete mirabile.

The Single Concentrating Effect

The reduction of the effective gas-carrying capacity of swimbladder blood is achieved by the metabolic activity of the epithelial gas gland cells. Although the swimbladder typically is exposed to a high P_{O_2}, gas gland cells produce acidic metabolites anaerobically and release these metabolites into the blood. Blood glucose is the main fuel; internal glycogen stores do not appear to be of major importance. Gas gland tissue of various species *in vitro* and *in vivo* has been shown to produce large amounts of lactate, even at hyperbaric oxygen pressures. A quantitative analysis of lactate and glucose metabolism of the active, gas-depositing swimbladder of the European eel revealed that about 75–80% of glucose taken up from the blood is converted into lactate.

The low mitochondrial density and comparatively low activities of enzymes of the citric acid cycle and of the respiratory chain suggest that aerobic glucose metabolism is of minor importance. A comparison of glucose metabolism and of O_2 and CO_2 exchange in the swimbladder of the European eel suggests that only 1% of the glucose removed from the blood is oxidized. Nevertheless, gas gland cells produce significant amounts of CO_2, but most of this CO_2 is formed by decarboxylation of glucose in the pentose phosphate shunt.

A summary of the metabolic pathways involved in glucose metabolism, based on results obtained from European and American eels, is shown in **Figure 4**. End products of glucose metabolism are lactic acid and CO_2, both of which are released into the blood. Due to the generation of significant amounts of CO_2 in gas gland cells, the P_{CO_2} in these cells is expected to exceed P_{CO_2} values in the blood and also in the swimbladder lumen. Therefore, CO_2 readily diffuses down its partial pressure gradient into the blood, lowering blood pH, and also into the swimbladder lumen, filling the swimbladder with CO_2.

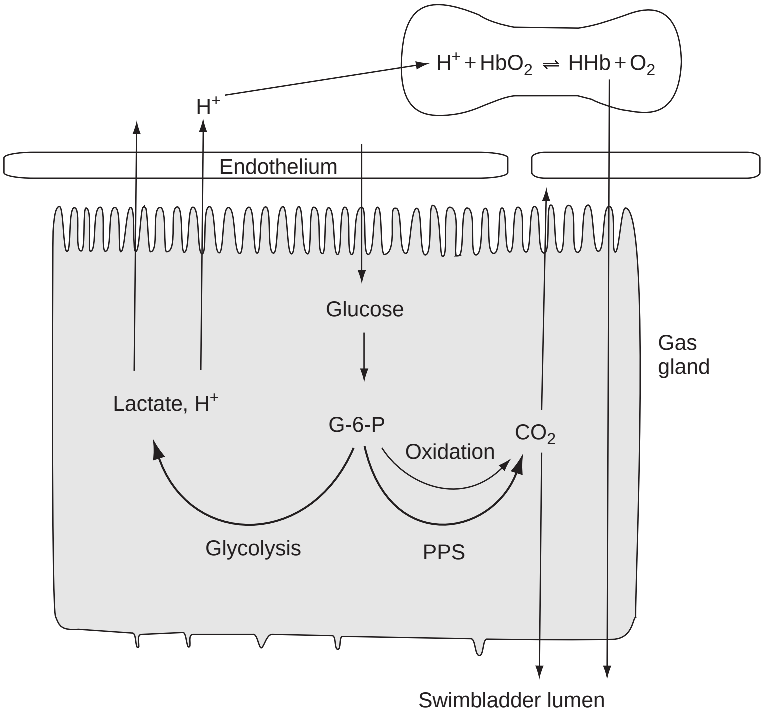


Figure 4 Present concept of glucose metabolism in swimbladder gas gland tissue. Glucose is taken up from the blood, and mainly converted into lactate, in spite of the fact that gas gland tissue typically is exposed to high oxygen partial pressures. A fraction of the glucose in converted to CO_2 in the pentose phosphate shunt. Only a very small fraction of the glucose is oxidized by aerobic metabolism. The CO_2 produced in the cell diffuses down the partial pressure gradient into the swimbladder lumen as well as into the blood, where it contributes to blood acidification. Protons are secreted into the blood, acidify the erythrocytes, and thus reduce the oxygen-carrying capacity of the hemoglobin. Oxygen is released from the hemoglobin and diffuses down the partial pressure gradient through the gas gland cells into the swimbladder lumen. Lactate is released into the blood and contributes to the salting-out effect. From Pelster B (2004) pH regulation and swimbladder function in fish. *Respiratory Physiology and Neurobiology* 144: 179–190.

Lactic acid enters the blood in the ionic state; lactate and protons are released separately. Pathways for the release of protons from gas gland cells include Na^+-dependent carriers, such as Na^+/H^+ exchange and Na^+-dependent anion exchange, and a proton ATPase, a V-ATPase (**Figure 5**).

Acidification of the blood via CO_2 and proton release from gas gland cells plus the increase in blood lactate concentration reduce the effective gas-carrying capacity of the blood perfusing the gas gland cells and thus induce an increase in gas partial pressures in the blood. This initial increase in gas partial pressures in the blood by acidification is called the single concentrating effect. The increase in blood lactate concentration causes a decrease in physical solubility of all gases due to the salting-out effect. Lactate release from gas gland cells is expected to be in the millimolar range, which decreases physical gas solubility no more than 1% or 2%, with a concomitant increase in gas partial pressures of the same magnitude. At present, this is the only way to induce an increase in gas partial pressures for inert gases such as nitrogen and argon.

A much larger increase in gas partial pressure can be expected for CO_2. The acidification of the blood during passage of the gas gland cells shifts the equilibrium of the HCO_3^-/CO_2 reaction toward formation of CO_2, and this reaction is speeded up by the activity of carbonic anhydrase within the red blood cells and within the gas gland cell membrane. A significant increase in P_{CO_2} has been recorded in swimbladder blood as it passes through the gas gland tissue in the European eel.

Oxygen, however, is expected to show the largest increase in gas partial pressure. The hemoglobin of many fishes is characterized by the presence of the Root effect, that is, by a decrease in hemoglobin oxygen-carrying capacity with decreasing pH (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect and Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). All types of hemoglobin have two stable states: a high affinity R-state and a low affinity T-state (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion and Red Blood Cell Function). The Root effect appears to be due to a number of amino acid substitutions destabilizing the oxygenated R-state or stabilizing the T-state of hemoglobin at low pH, so that it no longer binds oxygen even at very high oxygen partial pressures.

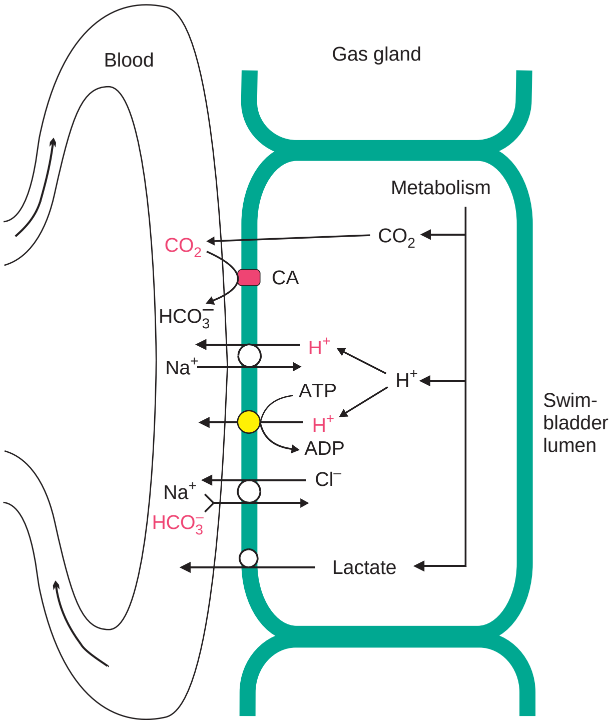


Figure 5 Hypothetical model showing the various proton translocating mechanisms identified so far in swimbladder gas gland cells of the European eel. Acidic metabolites (lactic acid (i.e., lactate and H^+) and CO_2) are produced in the glycolytic pathway and in the pentose phosphate shunt. The mechanisms of lactate transport have not yet been characterized in detail. CA, membrane bound carbonic anhydrase. Modified from Pelster B (2004) pH regulation and swimbladder function in fish. *Respiratory Physiology and Neurobiology* 144: 179–190.

Fishes that have a swimbladder usually also have Root-effect hemoglobin, and the acidification of the blood passing through the gas gland induces the transition from the oxygenated R-state to the deoxygenated T-state of their hemoglobin (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems). For the European eel, it has been estimated that about 40% of the hemoglobin can be deoxygenated in the swimbladder by acidification of the blood resulting in a large increase in P_{CO_2} in swimbladder blood.

The release of lactic acid and of CO_2 from gas gland cells thus causes an increase in the gas partial pressure of all gases in blood, and gases will diffuse into the swimbladder by diffusion following their partial pressure gradients. In addition, gas partial pressures in venous blood returning to the countercurrent system (rete mirabile) are higher than those in the arterial blood supplying the swimbladder epithelium. Thus, back-diffusion of gas from the venous to the arterial capillaries of the rete mirabile results in a countercurrent concentration (sometimes called countercurrent multiplier) of gases in the swimbladder.

Countercurrent Concentration

A countercurrent system is characterized by very close contact of arterial blood vessels, ideally capillaries, with venous vessels returning from the tissue. In a swimbladder rete mirabile, each arterial capillary is surrounded by several venous capillaries and vice versa. The diffusion distance between venous and arterial vessels is small (in the μm range), and the length of the vessels within the rete and the number of vessels determine total contact area. If the gas partial pressure in venous blood returning from the tissue to the heart is higher than in the arterial blood flowing to the tissue, gas molecules will diffuse back from the venous vessel to the arterial side, causing an increase in gas partial pressures on the arterial side of the rete and a decrease in gas partial pressures on the venous side. In a swimbladder with active, acid-secreting gas gland cells, the acidification of the blood will switch on the Root effect, causing a significant increase in blood P_{O_2}. Accordingly, oxygen will diffuse into the swimbladder, but the P_{O_2} in the venous blood returning to the rete mirabile will still be higher than in the arterial blood. Oxygen molecules will diffuse back along the partial pressure gradient from the venous to the arterial capillaries of the rete, causing an increase not only in P_{O_2} but also in the oxygen concentration in arterial blood. Thus, oxygen is concentrated at the swimbladder end of the rete mirabile by back-diffusion and countercurrent concentration. **Figure 6** illustrates the effect of proton and CO_2 release on oxygen and CO_2 concentrations as well as on P_{O_2} and P_{CO_2} in swimbladder capillaries. The resulting partial pressure gradients will drive subsequent countercurrent concentration of both gases in the rete mirabile.

On the other hand, if P_{O_2} is higher in the arterial inflow of the rete than in venous vessels, oxygen will diffuse from the arterial side to the venous vessels. In this case, oxygen would be shunted away from the swimbladder and immediately returned to the central circulatory system. A countercurrent system thus concentrates molecules at one end of the rete. This is the basic mechanism of countercurrent concentration, and it is not only useful for the concentration of gas molecules as discussed in the example above, but also useful for the countercurrent concentration of salts and metabolites in the kidney, or for the countercurrent concentration of heat (**see also Design and Physiology of Arteries and Veins**: The Retia).

Figure 7 shows a theoretical plot of the concentrating ability of a countercurrent system for an inert gas (i.e., without chemical binding of gas). The concentrating ability of a countercurrent system depends on the diffusing capacity of the barrier between venous and arterial capillaries in the rete mirabile and on blood perfusion. The diffusing capacity depends on the diffusion distance and

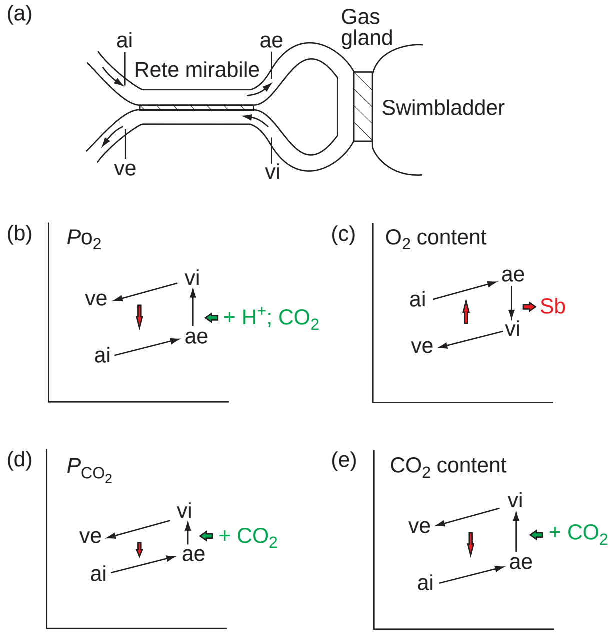


Figure 6 (a) Schematic drawing of a swimbladder with gas gland tissue and a rete mirabile. Secretion of protons and release of CO$_2$ from gas gland cells acidifies the blood, resulting in a switching on of the Root effect and an increase in blood P_{O_2} (b). The diffusion of oxygen into the swimbladder reduces oxygen content in swimbladder blood. Due to the elevated P_{O_2} in blood returning to the rete mirabile in the rete, oxygen diffuses back to the arterial side and is concentrated in the countercurrent system (c). Release of CO$_2$ from gas gland cells and acidification of the blood due to the release of protons increases blood P_{CO_2} (d), combined with an increase in CO$_2$ concentration (e). Due to the elevated P_{CO_2} in blood returning to the rete mirabile in the rete, CO$_2$ diffuses back to the arterial side and is concentrated in the countercurrent system (e).

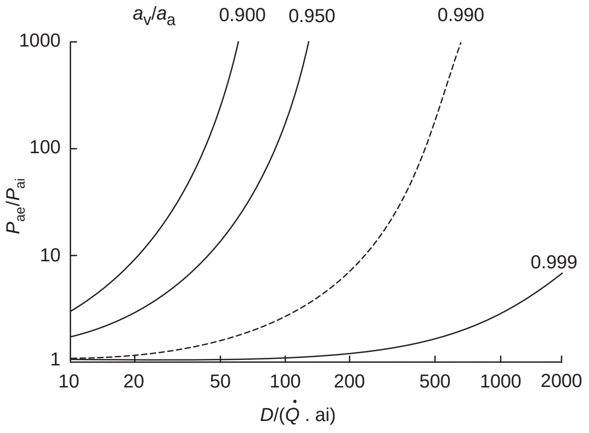


Figure 7 Efficiency of the rete in enhancing inert gas partial pressure, calculated as the ratio of partial pressure in the arterial efflux and influx (P_{ae}/P_{ai}). The efficiency is given by the conductance ratio ($D/Q{\cdot}\alpha$), the magnitude of the salting-out effect (α_v/α_a). For illustration a salting-out effect of up to 10% is assumed, although it can only be expected in the range of 1 or 2% according to our current knowledge. Adapted from Kobayashi H, Pelster B, and Scheid P (1989a) Solute back-diffusion raises the gas concentrating efficiency in counter-current flow. *Respiration Physiology* 78: 59–71.

the diffusivity or permeability, and on total contact area. Thus, a longer rete has a greater concentrating ability. Another crucial factor is the magnitude of the salting-out effect, that is, the change in physical solubility between venous and arterial blood. The higher the initial increase in gas partial pressure during passage of the gas gland cells, the higher the maximal gas partial pressures achieved in the rete.

For the swimbladder rete mirabile, it has been shown that the endothelial lining of the blood vessels is permeable not only to gases but also to metabolites, and that this permeability significantly augments countercurrent concentration. This is because solute back-diffusion in the rete initiates the salting-out effect in arterial capillaries and thereby causes an increase in gas partial pressure in addition to the increase induced by back-diffusion of gas molecules. Similarly, arterial PO$_2$ in the rete mirabile is enhanced not only by back-diffusion of oxygen, but also by back-diffusion of CO$_2$. Back-diffusion of CO$_2$ in the rete acidifies the arterial blood and increases arterial P_{O_2} by the Root effect. In an active, gas-depositing swimbladder, a P_{CO_2} gradient for back-diffusion of CO$_2$ inevitably exists, because CO$_2$ is produced as a byproduct of metabolism of gas gland cells. Thus, CO$_2$ production in the pentose phosphate shunt and CO$_2$ back-diffusion in the countercurrent system both appear to play pivotal roles in swimbladder function.

Based on lactate and acid secretion of the gas gland cells, the largest initial increase of gas partial pressures (single concentrating effect) in swimbladder blood vessels occurs with oxygen (due to the Root effect) and with CO$_2$ (due to the production of CO$_2$ in the pentose phosphate shunt and blood acidification). Accordingly, in the rete mirabile the highest gas partial pressures are achieved by these two gases, and oxygen and CO$_2$ are the main gases diffusing into and filling the swimbladder. Calculations using data collected from the European eel show that gas partial pressures of several hundred atmospheres can be achieved high enough to explain the occurrence of fish with a gas-filled swimbladder at depths of several thousand meters.

Compared with oxygen and inert gases, CO$_2$ has the highest diffusivity, and therefore diffusional loss out of the swimbladder is highest for CO$_2$, leaving behind oxygen and inert gases. Therefore, under steady-state conditions, the swimbladder contains mainly oxygen and inert gases.

Resorption of Gas

To reduce total gas volume in the swimbladder during ascent, gas has to be transferred from the secretory part of the swimbladder to the resorbing bladder or to the oval. In the resorbing section of the swimbladder, or in

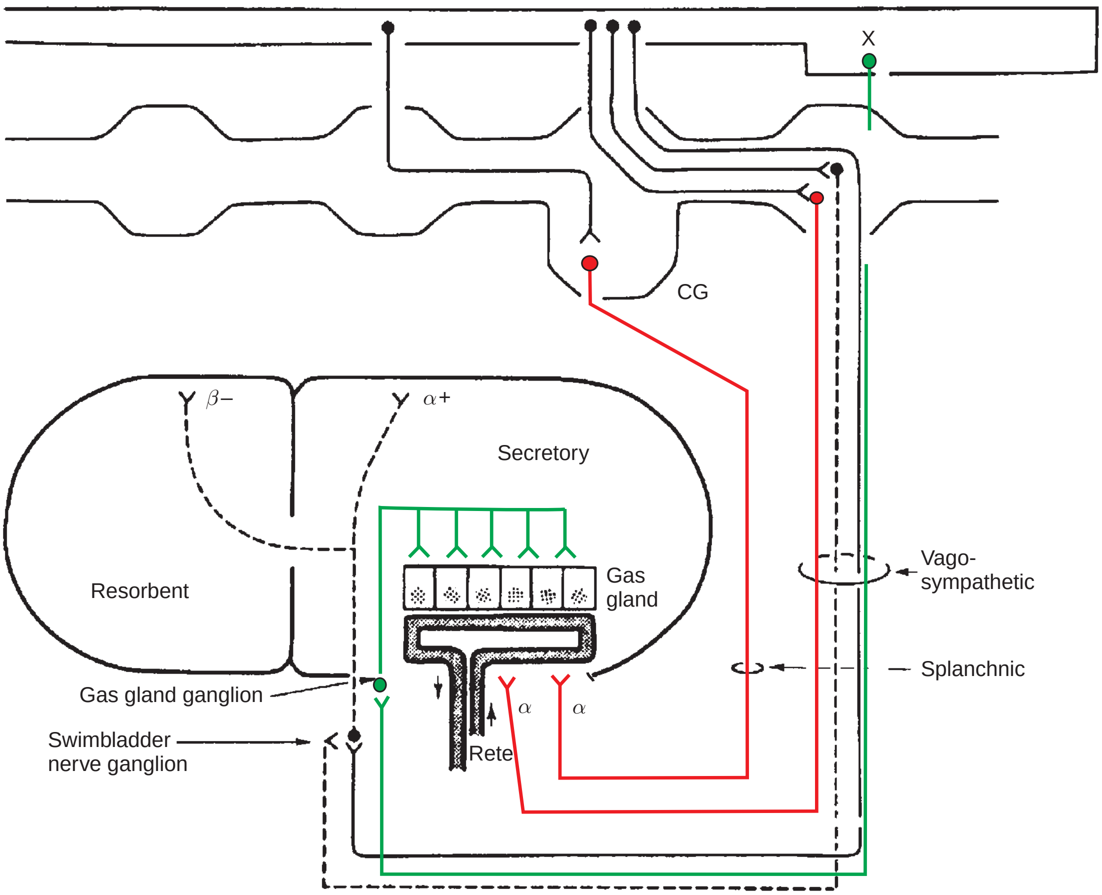


Figure 8 Schematic drawing summarizing our current knowledge of swimbladder innervation for a labrid swimbladder, which most likely can be transferred to other types like the eel swimbladder. α-Adrenergic innervation has been reported for the rete mirabile and for muscle cells in the secretory part of the bladder, while ß-adrenergic responsiveness has been observed in the resorbing section. Muscarinic cholinoceptors have been demonstrated in gas gland tissue. CG, coeliac ganglion. Modified from Nilsson S (2009) Nervous control of fish swimbladders. *Acta Histochemica* 111: 176–184.

the oval, gases are resorbed into the blood by diffusion along the partial pressure gradient. Total gas partial pressure in arterial blood after passing through the gills is close to ambient (i.e., close to or below 1 atm), and gas partial pressures in the swimbladder therefore are higher than in blood. In the oval or in the resorbing section of the swimbladder, gases diffuse into the richly vascularized epithelium lining this section of the bladder and into the blood. When the blood containing these resorbed gases enters the gills, the gases diffuse into ambient water.

However, different gas solubilities result in different rates of removal, and this not only applies to the leakage of gases through the swimbladder wall but also applies to the resorption of gases into the blood. As a result, the more soluble gases, mainly CO_2 and to some extent also O_2, are more rapidly resorbed, leaving behind the less-soluble inert gases. Consequently, CO_2 makes up some 20–30% of newly deposited gas, but, under steady-state conditions, contributes no more than 1% or 2% to swimbladder gas in the European eel.

Failure of Swimbladder Inflation

Failure to initially inflate the swimbladder can have a severe impact. In many species, there is a short time window during early development available for swimbladder inflation. If the initial filling does not occur within this window, the window is closed by atrophy of the pneumatic duct and/or of the swimbladder, or by further differentiation of the foregut. In fish farming, a malfunctioning swimbladder represents a serious problem, because it reduces growth rate and increases mortality. Lack of swimbladder inflation often is associated with a deformation of the spine (lordosis) and a decrease in viability of the larvae.

There may be several reasons for noninflation of the swimbladder. In fish farming, a thin oil or lipid film on the water surface, originating from lipid containing food, may not allow proper surfacing activity of the larvae, which is required for the initial filling of the swimbladder in some species. A bacterial infection or genetic defect may also be responsible for failure to inflate the swimbladder. Another potential reason could be the diet. For example, increasing the triiodothyronine level in female striped bass, *Morone saxatilis,* just prior to spawning increases swimbladder inflation and survival rates of the offspring.

Control of Swimbladder Inflation

With a flexible-walled swimbladder, the status of neutral buoyancy can only be maintained if swimbladder volume can be kept constant in the face of changing hydrostatic pressures. This means that during descent, gas must be deposited, and during ascent, gas must be resorbed. Accordingly, a sophisticated control system is required to adjust the swimbladder volume to the actual position and the swimming activity of the fish in the water column.

As already mentioned, two parameters appear to be crucial to adjust gas deposition: metabolic activity of the gas gland cells and blood flow through the swimbladder. The exchange characteristics of the rete mirabile and the hemoglobin oxygen-binding characteristics (e.g., the magnitude of the Root effect) are crucial for gas secretion. However, these parameters cannot be modified easily or rapidly and therefore cannot be changed in the short term. In addition, the process of gas resorption must be regulated by controlling the smooth muscle cells that guard the connection between the secretory and the resorbing parts of the swimbladder and thus control the transfer of gas from one part of the swimbladder to the other.

In adult fish, the swimbladder tissue is autonomically innervated, mainly by the splanchnic nerve, originating from the celiac ganglion, and the vagosympathetic trunk (**Figure 8**). Vagotomy or cholinergic blocking agents impair gas deposition. On the other hand, cholinergic stimulation increases lactate formation in gas gland cells, which enhances blood acidification and thus gas secretion. Adrenergic effects on gas gland cells have been postulated, but adrenergic nerves have not been demonstrated near gas gland cells. Thus, gas gland cells appear to be under cholinergic, parasympathetic control.

Adrenergic effects have been shown to be important for the control of blood flow to the secretory part of the bladder; α-adrenergic vasoconstriction significantly reduces blood flow to the rete mirabile. In addition, vasoactive intestinal polypeptide (VIP), nitric oxide, adenosine, and protons all exert some vascular control in the eel. In fact, several nonadrenergic, noncholinergic (NANC) mechanisms appear to contribute to the control of swimbladder vessels and the muscularis mucosae.

The swimbladder mucosa is under adrenergic control. There are α-adrenoceptors in the secretory muscularis mucosae and β-adrenoceptors in the resorbing parts of the bladder. In the eel, the secretory swimbladder contracts as a result of α-adrenoceptor activation, while the pneumatic duct, representing the resorbing section, relaxes on stimulation of the β-adrenoceptors. This suggests that during adrenergic stimulation, the swimbladder responds by decreasing secretory activity and shifting gas into the resorbing section of the bladder.

Acknowledgments

Parts of the study were supported financially by the Austrian Fonds zur Förderung der wissenschaftlichen Forschung (FWF).

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Design and Physiology of Arteries and Veins**: The Retia. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Evolution of the Bohr Effect; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M, Koldkjaer P, Kepp O, and Cossins AR (2005) Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Berg T and Steen JB (1968) The mechanisms of oxygen concentration in the swim-bladder of the eel. *Journal of Physiology* 195: 631–638.

Bonaventura C, Crumbliss AL, and Weber RE (2004) New insights into the proton-dependent oxygen affinity of Root effect haemoglobins. *Acta Physiologica Scandinavica* 182: 1–14.

Brittain T (2005) Root effect hemoglobins. *Journal of Inorganic Biochemistry* 99: 120–129.

Fänge R (1983) Gas exchange in fish swimbladder. *Reviews of Physiology, Biochemistry and Pharmacology* 97: 111–158.

Kobayashi H, Pelster B, and Scheid P (1989) Solute back-diffusion raises the gas concentrating efficiency in counter-current flow. *Respiration Physiology* 78: 59–71.

Kobayashi H, Pelster B, and Scheid P (1990) CO_2 back-diffusion in the rete aids O_2 secretion in the swimbladder of the eel. *Respiration Physiology* 79: 231–242.

Nilsson S (2009) Nervous control of fish swimbladders. *Acta Histochemica* 111: 176–184.

Pelster B (2001) The generation of hyperbaric oxygen tensions in fish. *News in Physiological Sciences* 16: 287–291.

Pelster B (2004) Development of the swimbladder: Structure and performance. In: Govoni JJ (ed.) *The Development of Form*

and Function in Fishes and the Question of Larval Adaptation, pp. 37–46. Bethesda, MD: American Fisheries Society.
Pelster B (2004) pH regulation and swimbladder function in fish. *Respiratory Physiology and Neurobiology* 144: 179–190.
Phleger CF (1998) Buoyancy in marine fishes: Direct and indirect role of lipids. *American Zoologist* 38: 321–330.
Robertson GN, McGee CAS, Dumbarton TC, Croll RP, and Smith FM (2007) Development of the swimbladder and its innervation in the zebrafish, *Danio rerio. Journal of Morphololology* 268: 967–985.
Steen JB (1970) The swimbladder as a hydrostatic organ. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, 4th edn., pp. 413–443. New York: Academic Press.

Experimental Hydrodynamics

ED Tytell, Johns Hopkins University, Baltimore, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Basic Fluid Dynamic Principles
Measuring Fluid Velocities
Interpreting Flow Fields
Future Developments
Further Reading

Glossary

Acceleration reaction A synonym for the added mass effect. See added mass.

Added mass The effect in which accelerating bodies in a fluid appear to have additional mass. It is also called the acceleration reaction. The body can be a solid immersed in the fluid, or a coherent region of the fluid itself (see Lagrangian coherent structure).

Added mass coefficient The amount of added mass relative to the body's own mass (units: dimensionless).

Circulation A measure of the total amount of vorticity in an area of fluid bounded by a loop (units: $m^2\ s^{-1}$).

Flow tunnel Also called a flume. A device that produces a constant, even fluid motion. It is often used for studying steady swimming in fishes, so that a fish can swim at a steady speed against the flow, but remain stationary relative to a camera or other measuring devices.

Impulse The total force applied over a time period. The integral of force with respect to time (units: $kg\ m\ s^{-1}$).

Kinematic viscosity The ratio of viscosity and density (μ/ρ) (units: $m^2\ s^{-1}$). At 20 °C, water's kinematic viscosity is $1.004 \times 10^{-6}\ m^2\ s^{-1}$.

Lagrangian coherent structure A region of fluid that tends to remain together. There may be fluid motion inside and outside of the region, but fluid moving inside the region tends to stay inside, while fluid outside tends to stay outside.

Laminar flow Flow in which parallel portions of the fluid (laminae) move steadily past each other. Laminar flow does not preclude the presence of vortices (see turbulent flow).

Particle image velocimetry A technique for revealing complex patterns of flow in a fluid by automatically tracking the motion of suspended particles.

Reynolds number The ratio of inertial to viscous forces in a flow.

Shear Differences in parallel flow velocity between two points. For example, if fluid is moving from left to right parallel to a wall at one speed and at a different speed (but still parallel to the wall) further from the wall, then there is shear between those two points.

Turbulent flow Complex, disordered flow that contains eddies or vortices of many different sizes and structures. Vortex flow is not necessarily turbulent (see laminar flow).

Viscosity The ease with which a liquid will flow under an applied pressure; molasses is more viscous than water.

Vortex A mass of fluid in whirling or circular motion. Vortices always appear where hydrodynamic lift is being generated.

Vortex filament An imaginary line around which fluid rotates, produced by tracing along the vorticity vector. Vortex filaments cannot have free ends; they must terminate on solid boundaries or loop around back onto themselves.

Vortex ring A three-dimensional fluid structure in which a vortex forms a loop.

Vorticity A point measure of how much the fluid at a point is rotating. In three dimensions, vorticity is a vector that defines the axis around which the fluid is rotating; the length of the vector is proportional to the angular velocity. Generally, vorticity is only produced when a force is applied to a fluid (units: s^{-1}).

Wake The moving fluid left behind after an object moves through a region of fluid. It is only present at high Reynolds number. Flow patterns in wakes can be used to estimate forces and power around an object.

Introduction

Fish physiologists use experimental hydrodynamics techniques to visualize and quantify the way water moves around swimming fishes. The water motion provides information about the forces that fishes generate as they interact with their environment, for instance, during locomotion (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming) and feeding (**see also Buoyancy, Locomotion, and Movement in**

Fishes: Feeding Mechanics). These flows and associated pressure waves also provide information to other aquatic animals (**see also Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays), and are therefore important for group behaviors such as schooling and for predator–prey interactions. Finally, environmental flows, such as eddies in a stream or wave motions around reefs, also produce forces to which fishes must adjust, either compensating for them or, in some cases, extracting energy from them (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence). This article introduces basic principles of fluid dynamics, some standard techniques for measuring fluid flows, and several methods for interpreting flow fields (**See also Buoyancy, Locomotion, and Movement in Fishes:** Buoyancy, Locomotion, and Movement in Fishes: An Introduction).

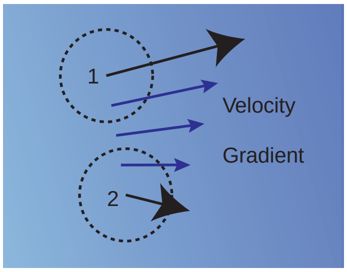


Figure 2 Definition of Reynolds number. Two blobs of fluid with different velocities (black arrows labeled 1 and 2). Viscosity will tend to equalize the velocity gradient between them (blue arrows), while the blobs' inertia will tend to keep their velocities different. Reynolds number is the ratio of inertial forces to viscous forces.

Basic Fluid Dynamic Principles

A fluid is different than a solid because it has no defined shape. Consider a block of rubber. If the bottom was fixed and a force was applied parallel to the top (called shearing the block), the block would resist the force, and the force would be proportional to how far you sheared it (θ in **Figure 1(a)**). Now imagine doing the same thing to a block of fluid. The fluid can be deformed forever, so the amount of shear is irrelevant, but the speed at which you shear the block matters. The fluid velocity would vary throughout the block from 0 at the bottom to V at the top (**Figure 1(b)**). The amount a fluid resists shear rate is called its viscosity, denoted μ. Fluids such as honey and motor oil are highly viscous, while water and alcohol are less viscous.

Thus, viscosity tends to make fluids stick together. If two blobs of fluid (1 and 2 in **Figure 2**) are moving at different speeds, viscosity will resist the shear between them and tend to equalize their speeds. The opposite effect is inertia, the mass of the blobs of fluid. If the blobs are relatively large or moving quickly, then the viscous forces on them will have a small effect, and they will carry on moving at their separate velocities. The relative importance of inertia and viscosity is called the Reynolds number *Re* (the symbols used in this article are explained in **Table 1**):

$$Re = \frac{\rho l U}{\mu} \tag{1}$$

where ρ is the density of the fluid, l a length, and U a velocity. The length and velocity are intentionally vague. The proper choice depends on the specific situation, but, in general, high Reynolds number ($Re >> 1$, which, in practice, means greater than 100 or so) means that viscosity is not very important. Reynolds number is also often written using the kinematic viscosity ν as

$$Re = \frac{lU}{\nu} \tag{2}$$

Re is only useful as an order of magnitude. One must choose an approximate length l and velocity U that are representative of lengths and velocities of interest. For instance, for a 10-cm fish swimming at 1 body length (L) per second, the scale of fluid motions is approximately the length of the fish and velocities are near the swimming speed. *Re* is thus $[(1000\ \mathrm{kg\ m}^{-3}) \times (0.1\ \mathrm{m}) \times (0.1\ \mathrm{m}^{-1}\ \mathrm{s})]/(1 \times 10^{-3}\ \mathrm{Pa\ s}) = 10\ 000$. If the fish were swimming at $1.5\ L\ \mathrm{s}^{-1}$, it would not be much more informative to write $Re = 15\ 000$, because the order of magnitude is still the same.

High Reynolds number flow has several important consequences, listed as follows:

1. Fluid motions persist for some time. If a force starts a blob of fluid moving, then the inertia of the blob will keep it moving for a while, even after the force has stopped. Thus, at high *Re*, one can learn about forces on

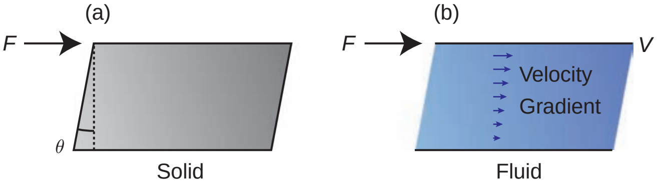


Figure 1 Differences between solids and fluids. Solids resist the amount of shear (θ), while fluids resist the shear rate ($\dot{\theta}$), which sets up a velocity gradient between the top and bottom.

Table 1 Symbols used in the article

Γ,	Circulation
δ,	Spacing between fluid velocity measurements
μ,	Viscosity
ρ,	Density
Σ,	A surface in a fluid
ω,	Vorticity vector
ω_z,	Component of the vorticity vector in the *z*-direction
a,	The thickness of a vortex ring along its central axis
A,	An area, usually the wetted surface area or the cross-sectional area
C,	A contour surrounding a vortex along which velocity is integrated to calculate circulation
d,	Diameter of a vortex loop in the horizontal plane
$\mathbf{C}_A$,	Added mass coefficient tensor
C_A,	Added mass coefficient for acceleration and force along the same axis
C_F,	Force coefficient. Normalized force relative to the swimming speed and body size
$\mathbf{I}$,	Impulse vector
$\mathbf{F}$,	Force vector
$\mathbf{F}_{\text{mean}}$,	Mean force vector
h,	Height of a vortex loop or a wake in the vertical plane
l,	Length (generally body length)
l,	Tangent vector along the contour *C*
L,	Body length of a fish
P_{thrust},	Thrust power
P_{wake},	Wake power; change in kinetic energy in the wake
Re,	Reynolds number
S,	Volume of an LCS
T,	Time period, usually half the tail beat period
$\mathbf{u}$,	Velocity vector
U,	Speed (generally swimming speed)
U_V,	Velocity of an LCS as a group
u,	Component of the velocity vector in the *x*-direction
v,	Component of the velocity vector in the *y*-direction
w,	Component of the velocity vector in the *z*-direction
Wa,	Wake vortex ratio; ratio of unsteady effects to steady forces for an LCS

a body, such as a fish, by studying its wake, the moving fluid left behind after the body has moved past. In contrast, at low *Re*, any fluid motion requires force, and as soon as the force stops, the fluid stops too.

2. Vortices may be present. A vortex is a rotating piece of fluid that usually places shear stress on the fluid. Vortices are directly related to force in a fluid, and, at the limit of infinitely high *Re*, they persist forever. Thus, they are useful for studying forces produced in a fluid.
3. Flow may be turbulent. Turbulence is a poorly understood phenomenon in which flow becomes complex and irregular. Turbulence changes many of the standard rules of thumb of fluid dynamics, such as drag and lift coefficients, so one must be careful when studying turbulent flows.

Vorticity

Vorticity is a measure of how much a very small blob of fluid is rotating. Rotation is important because it is strongly related to force. In general, a force must be applied to a fluid to produce vorticity.

Vorticity is a point measure. Imagine putting a very thin rod into a fluid, and consider whether it will rotate, independently of its translational motion. Vorticity is non-intuitive: a vortex usually, but not always, has vorticity, and vorticity may be present in flow that does not appear to be rotating, such as a shear flow (**Figure 3**). Mathematically, vorticity ω is the curl of the velocity field:

$$\omega = \nabla \times \mathbf{u} \tag{3a}$$

$$= \left(\frac{\partial u_z}{\partial y} - \frac{\partial u_y}{\partial z}, \frac{\partial u_x}{\partial z} - \frac{\partial u_z}{\partial x}, \frac{\partial u_y}{\partial x} - \frac{\partial u_x}{\partial y} \right) \tag{3b}$$

written concisely using the curl operator in eqn (3a), or explicitly in eqn (3b). Vorticity ω is itself a vector, and defines the axis around which fluid is rotating, according to the right-hand rule. For example, the vorticity vector in **Figure 3(a)** is pointing out of the page toward the reader, while the vector in **Figure 3(c)** is pointing into the page away from the reader. For two-dimensional (2D) flows, such as those pictured in **Figure 3**, the first two components of ω are 0; figures therefore often depict only the z-component as a contour map.

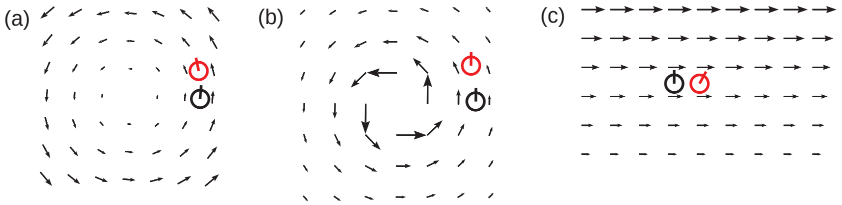


Figure 3 Examples of flow patterns with and without vorticity. In each case, the black circle represents an imaginary rod inserted into the **flow**, and the red circle indicates its position and orientation after some time has passed. (a) Swirling flow with vorticity. The red circle is rotated relative to the black circle. (b) Swirling flow without vorticity. The flow velocities decrease with radius so that the imaginary rod translates around, but does not rotate. Vorticity is zero at all points in this flow except for single point at the center. (c) Shear flow with vorticity. The imaginary rod translates and rotates, indicating vorticity.

One must remember, though, that real vorticity is 3D. In particular, if one follows the vorticity vectors, they define a vortex filament – a line around which fluid rotates. Vortex filaments cannot have free ends; they must either loop back on themselves or end at a solid boundary. The most common real 3D vortex structure, therefore, is a vortex ring, a circular vortex filament (**Figure 4**).

Added Mass

Fluids have one more strange effect that is prominent at high *Re*: the phenomenon of added mass, also termed the acceleration reaction. Accelerating bodies in a fluid behave as if they have some additional mass. In essence, added mass is the consequence of the fact that the fluid itself has mass. Accelerating a body in a fluid requires force to accelerate the body, and also some additional force to accelerate some fluid that surrounds the body. The acceleration reaction does not only affect solid bodies; it can also become important in unsteady flows without any solid bodies. If a blob of fluid accelerates, it also has added mass, which must be accounted for if one wants to estimate the force required to accelerate the blob.

Measuring Fluid Velocities

At any point in a moving fluid, the fluid has a speed and a direction in three dimensions. Generally, the motion at that point is represented by a vector (u, v, w), where u, v, and w are the speeds of the motion along the x, y, and z axes, respectively. The (u, v, w) vector is defined at every (x, y, z) coordinate in the fluid; therefore, the flow velocity is called a vector field. The magnitude of the vector $\left(\sqrt{(u^2 + v^2 + w^2)}\right)$ is the flow speed.

Techniques used for experimental hydrodynamics experiments fall into several classes, based on whether they make measurements at a single point or at many points in a plane or a volume, and what they measure at the point or points (i.e., the speed of the flow, or two or three components of the velocity vector). 1D techniques include hot wire anemometry and acoustic Doppler velocimetry, but neither of these are commonly used for studying fish. The most common technique is particle image velocimetry (PIV), a planar (2D) technique. Several fully volumetric (3D) methods are becoming more common.

Once velocities are estimated, derivative quantities such as vorticity can be estimated using standard

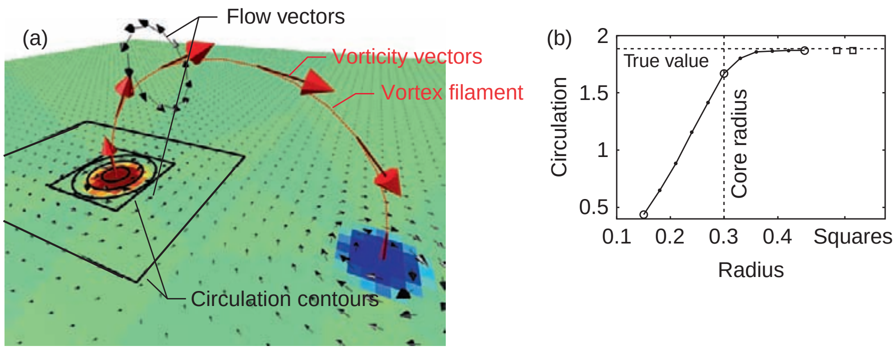


Figure 4 (a) 3D view of a vortex ring. Velocity vectors are shown in black and vorticity vectors are shown in red. Velocity vectors are shown only in the horizontal plane and in one example loop. The horizontal colored plane shows the z-component of the vorticity vector ω; red and blue indicate positive and negative values for ω_z, respectively. The central vortex filament is a red dashed line and it continues in a loop around under the horizontal plane. Several contours for estimating circulation are shown with thick black lines. (b) Circulation calculations. Circles show estimates for increasingly large circular contours, while squares indicate square contours. Open symbols indicate contours shown in (a).

numerical techniques. For planar measurements, such as PIV, with evenly spaced measurements of u and v on a grid with spacing δ between the measurements, out-of-plane vorticity can be approximated using a standard central difference technique as

$$\omega_z^{i,j} = \frac{1}{2\delta}\left[\left(v^{i,j+1} - v^{i,j-1}\right) - \left(u^{i+1,j} - u^{i-1,j}\right)\right] \qquad (4)$$

where $\omega_z^{i,j}$ is the z-component of vorticity at point (i, j), with i being the index that varies over the y values and j being the index that varies over the x values.

Particle Image Velocimetry

The technique called PIV is the most important experimental technique used for hydrodynamic measurements. It is a planar (2D) technique that can resolve the two components of the velocity vector that are in the plane. An important extension of the technique, called stereo PIV, can resolve all three velocity components but is still restricted to a planar measurement region.

Figure 5 shows a drawing of a typical PIV configuration. The water is seeded with small, reflective particles, and a strong light source, usually a laser, is used to illuminate a plane. A laser beam can be spread out to produce a plane using a cylindrical lens (shaped like half a cylinder) or a Powell lens (shaped like a house). One or more cameras are used to film the motion of the particles, and a computer algorithm is used to estimate the distance the particles have moved between two frames of the video.

For PIV to estimate fluid velocities accurately, the particles must not move too little or too much between frames. For a given average flow velocity, there are two main methods to ensure that the particle displacement is appropriate.

1. Use a continuous laser and adjust the frame rate of the camera (**Figure 6**(**a**)). Often this method requires a high-speed camera to achieve high-enough frame rates so that the particles do not move too far between frames. Because of the constant laser illumination, particle images may blur if the camera exposure time is too long. This is the most common method used for biological flows.
2. Use a pulsed laser and adjust the time between pulses (**Figure 6**(**b**)). Many of these systems use a double-pulsed laser, which can emit two pulses in rapid succession, but then have a relatively long delay before the next pair of pulses can be emitted. Pulsed lasers can often deliver large amounts of light in short amounts of time, which can be helpful to prevent particle blur. This method is more often used in engineering situations in which the flow is highly repeatable.

In recent years, a new generation of high-speed pulsed lasers have been developed, which combine the advantages of the two types of PIV systems.

Once the images are acquired, they must be processed to estimate the vector fields. The PIV algorithm does not track individual particles, because there are typically too many particles to identify specific ones reliably. Instead, it uses a technique called cross-correlation to estimate the average displacement of groups of particles (**Figure 7**). Each image is divided up into a grid of many small interrogation windows, and those windows are compared between two frames. The cross-correlation matrix indicates how well the images in the two windows match up when there is a slight offset between them (**Figure 7**(**b**)). The offset with the strongest correlation represents the

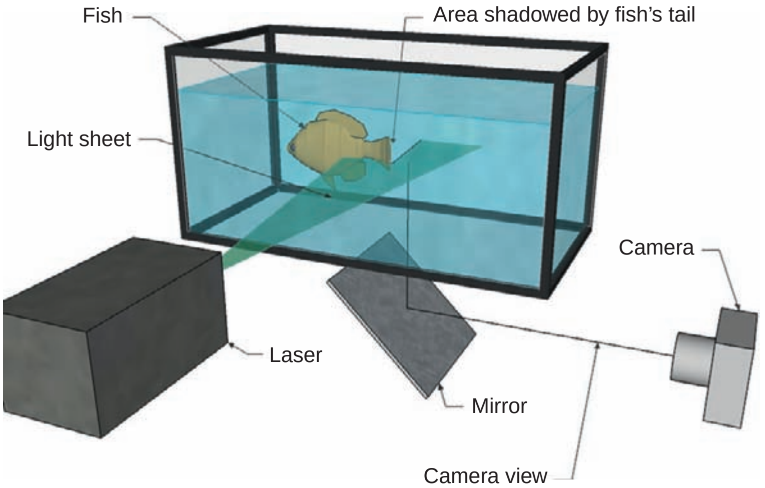


Figure 5 Schematic of a typical particle image velocimetry setup. A laser produces a horizontal light sheet, and a camera films the light sheet and fish from below. When the fish's tail is in the light sheet, it results in a shadow. These experiments are often performed in a flow tunnel, so that the fish swims against a flow (from left to right in this image), maintaining position in the laboratory frame of reference.

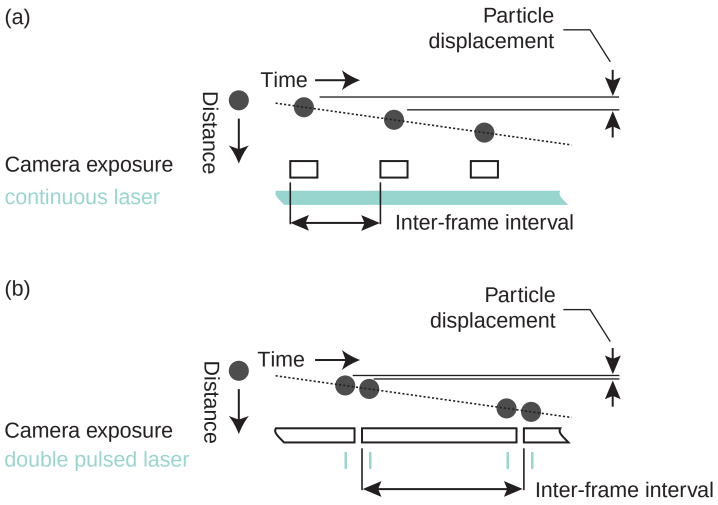


Figure 6 Schematic of the timing for two different PIV configurations. Panels show a particle (gray circle) as it moves over time along the dotted line. The open boxes indicate the camera exposure and the green bars indicate when the laser is on. Particles are shown only when the camera shutter is open and the laser is on. (a) With a high-speed camera and a continuous laser, the particle displacement may be relatively large, but the time between frames (inter-frame interval) is short. (b) With a normal camera and a double-pulsed laser, the particle displacement can be very small, but the inter-frame interval is often large.

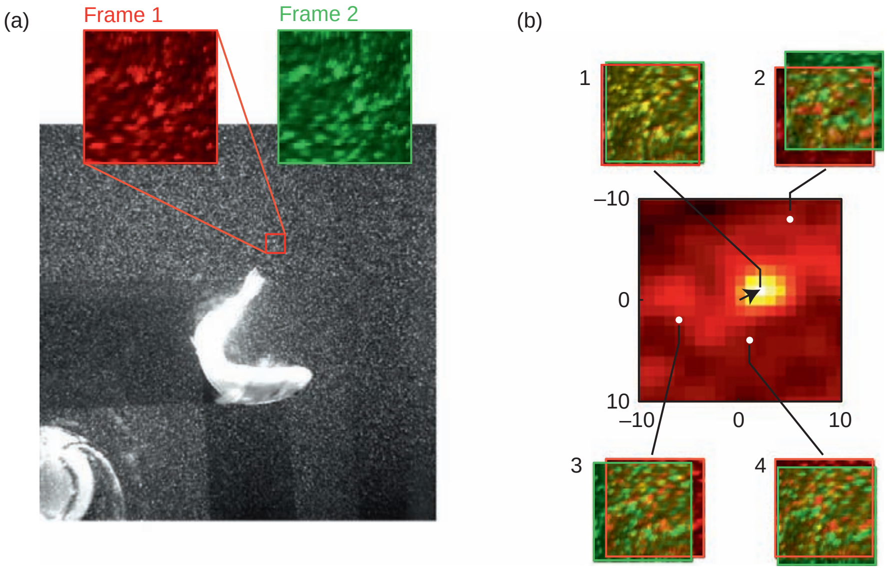


Figure 7 Example of the cross-correlation technique used to estimate velocity vectors for PIV. (a) Typical PIV image, with an interrogation window identified in red. The same window from the next frame is shown in green. (b) Example cross-correlation of the two windows. The center image shows the cross-correlation matrix, where hotter colors indicate greater correlation. The peak correlation is offset from the center by 3 pixels right and 2 pixels up, which is the best estimate of the average motion of particles in that window between frames. Around the cross-correlation are examples of the overlap between the two interrogation windows at different offsets. The windows are again in red and green, and regions where they overlap well show up as yellow.

average displacement of the particles (**Figure** 7(**b**1)). Most modern PIV systems now use a technique called super-resolution PIV, in which the cross-correlation technique runs several times, starting first with a fairly coarse grid, then repeating the algorithm with a finer grid that uses the previous results as a starting estimate. In this manner, very large numbers of vectors can be estimated.

An extension to the standard PIV technique is stereo-PIV, which uses two cameras at an angle to the light sheet to estimate the two in-plane velocity components, as well as the through-plane component. Besides the fact that it produces 3D velocity estimates, stereo-PIV has several other advantages. First, it is often able to correct errors better than normal PIV. Second, stereo-PIV calibration usually implicitly corrects lens distortion from the cameras.

Because fluid moves in three dimensions, planar PIV techniques require measurements in multiple planes or assumptions about the flow structure to generate 3D approximations of the flow. For example, counter-rotating vortex pairs are often assumed to be parts of the same vortex ring (**Figure 4**). **Figure 8** shows two examples of horizontal planes behind a swimming American eel (*Anguilla rostrata*) and a bluegill sunfish (*Lepomis macrochirus*), along with the simplest possible 3D reconstruction. Wakes of American eels are consistent with two unlinked vortex rings shed per tail beat (Fig. 8C), while the wakes of bluegill sunfish appear to have two linked loops per tail beat (Fig. 8D). One must be careful about these simple reconstructions. The bluegill sunfish's wake does have two linked vortex loops, as hypothesized in Fig. 8D, but the loops are not circular, and they interact in a complex way with vortices shed off the dorsal and anal fins. **Figure 9** shows a reconstruction of the bluegill sunfish's wake based on measurements made in multiple transverse planes.

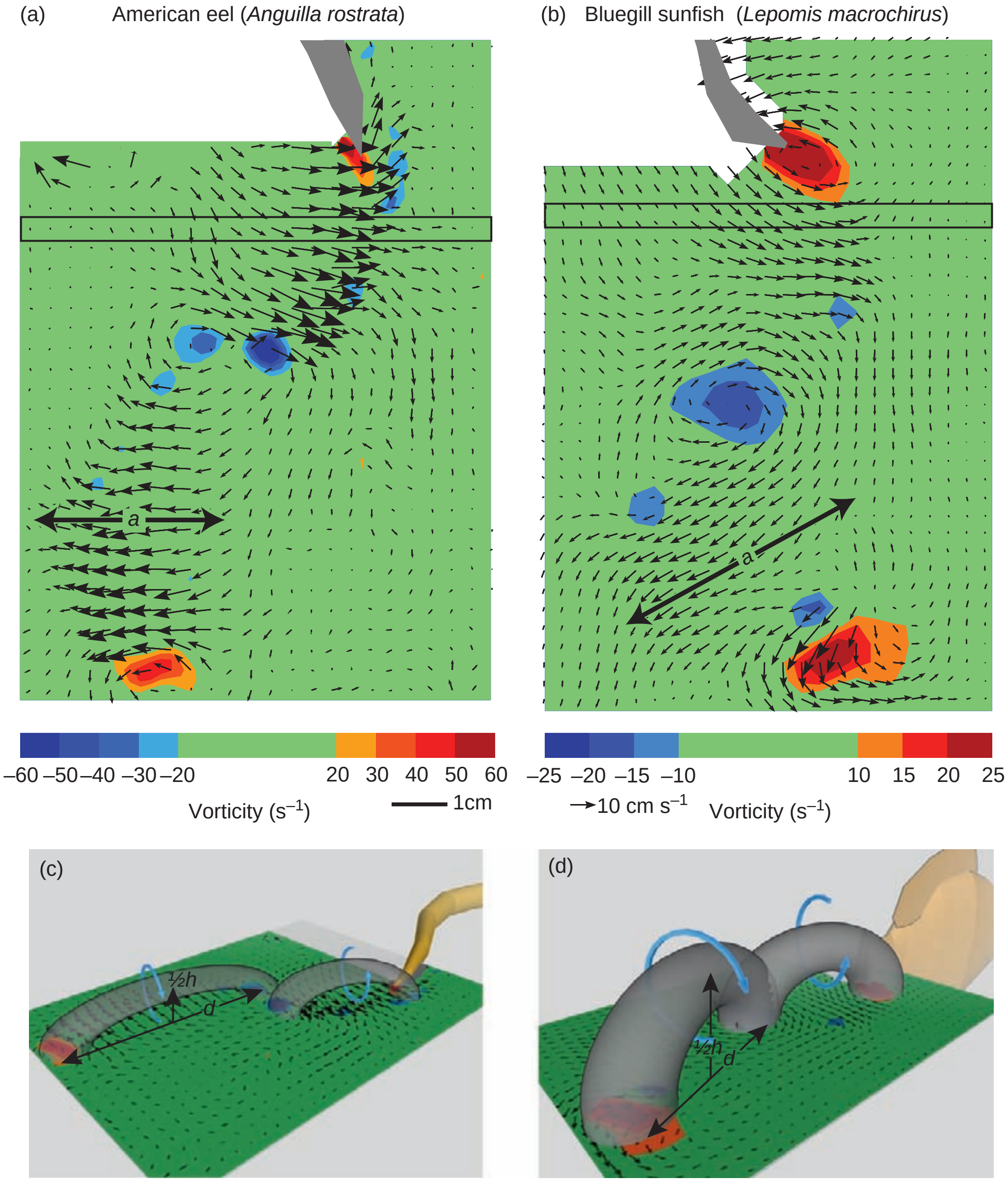


Figure 8 Examples of wake flow in horizontal planes behind an American eel and a bluegill sunfish, each swimming at approximately 1.9 Ls^{-1}. (a, b) Flow patterns near the end of a tail beat cycle. Velocities are given with black arrows, and vorticity is shown in color. Note that length and vector scales are the same, but the vorticity scales are different. The black box indicates the region used for power calculations. (c, d) Simplest 3D flow structure consistent with the wakes shown. Transparent gray tubes indicate vortex loops, and light blue arrows show 3D flow. Thick arrows show the diameter and half height of the vortex loops. (a, b) Modified from Tytell ED (2007) Do trout swim better than eels? Challenges for estimating performance based on the wake of self-propelled bodies. *Experiments in Fluids* 43(5): 701–712.

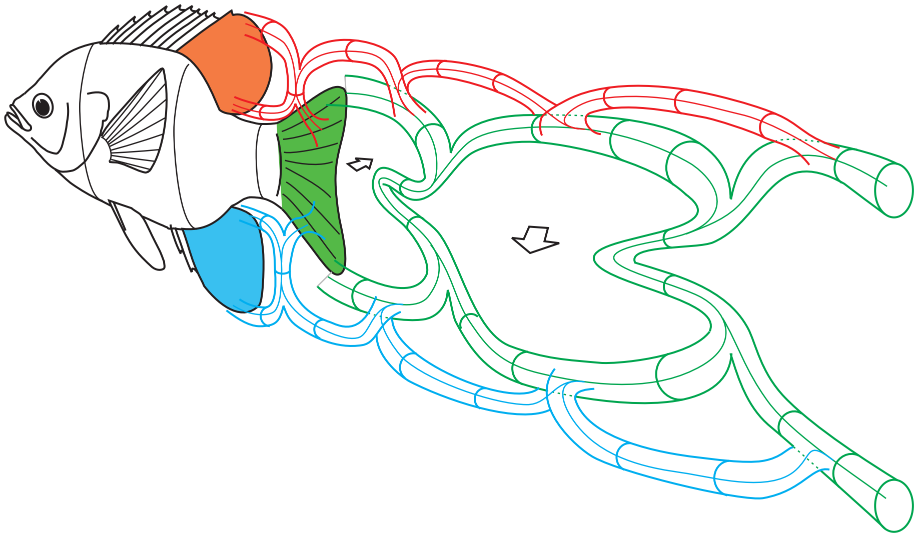


Figure 9 Schematic of 3D flow in the wake behind a bluegill sunfish, while the fish is swimming at 1.2 Ls^{-1}. Green loops show vortices shed off the caudal fin, while red and blue show those shed off the dorsal and anal fins, respectively. Note that the green loops are not circular. Modified from Tytell E (2006) Median fin function in bluegill sunfish. *Lepomis macrochirus*: Streamwise vortex structure during steady swimming. *Journal of Experimental Biology* 209: 1516–1534.

Volumetric Techniques

The oldest technique, dye flow visualization, is a volumetric technique, meaning that it resolves flow structures in a volume. Dye is injected into the flow and filmed as it follows the local fluid motion. Generally, dye tends to become concentrated near the center of vortices, but not always, which means that results need to be interpreted carefully. Dye images provide qualitative results about flow structure; for quantitative measurements of flow velocity, one must use other techniques. In addition, the fact that dye can move in three dimensions throughout a volume can make it challenging to interpret the images, particularly if only one camera is used. Some groups have used a planar light source, like those used for PIV, to illuminate a single plane of dye, which simplifies interpretation, but means that measurements are only available in that plane. Despite these limitations, dye can be quite useful for identifying the general structure of a flow or showing how two regions of flow mix.

Recently, two quantitative volumetric techniques, holographic PIV and defocusing PIV, have become feasible for biological fluid dynamics. Both of these techniques require a laser or lasers to illuminate a volume brightly and evenly. Holographic PIV relies on the interference between two beams of light to reconstruct 3D images of particles or bodies in the fluid. From these images, flow velocities can be estimated in a variety of ways. One method is to use computational techniques to estimate planar slices of the volume, which can then be analyzed using similar methods as 2D PIV. One form of defocusing PIV uses a single camera with a special lens with three apertures aligned in a triangle. A single particle thus produces three images in a triangle; the center of the triangle represents the position of the particle parallel to the camera, and the size of the triangle represents the particles distance away from the camera. The equipment and software necessary to implement defocusing PIV is now available commercially.

Interpreting Flow Fields

Force

In most cases related to fish locomotion, researchers who study fluid flow are interested in using their measurements to estimate forces on the fish's body. There are two general ways to estimate forces in a fluid, one based on fluid momentum that is best suited to rapidly changing or unsteady flows, and one based on fluid vorticity that is best for steady flows and time-averaged forces.

In both cases, one first estimates impulse **I**, the integral of force **F** applied to the fluid over time, or, more usefully, the mean force $\mathbf{F}_{\text{mean}}$ applied to the fluid multiplied by the total time T over which it was applied:

$$\mathbf{I} = \int_0^t \mathbf{F}\,\mathrm{d}t \tag{5}$$

$$= \mathbf{F}_{\text{mean}} T \tag{6}$$

One can then divide **I** by T to obtain mean force, or take the derivative to estimate instantaneous force:

$$\mathbf{F} = \partial \mathbf{I}/\partial t \tag{7}$$

Forces can be compared across different size or shape animals by estimating a force coefficient C_{F}:

$$C_F = F/[\frac{1}{2}\rho AU^2] \tag{8}$$

where A is the area of the body, usually either the wetted surface area or the cross-sectional area, and U the swimming speed. This normalization takes into account the fact that larger or faster animals generally produce more force, and allows one to ask the question, "If the body shapes and swimming speeds were the same, would these two animals produce the same forces?"

Vortex analysis

The amount of vorticity in an animal's wake is also related to the impulse on the animal. To estimate impulse, researchers often do not use vorticity directly, but use circulation, another measure of rotation strength. It is the total vorticity within an area circumscribed by a closed loop, such as the black loops shown in **Figure 4**.

For a steadily moving vortex ring, the procedure to estimate the force that generated it is quite straightforward. Any sufficiently large loop that passes through the center of a vortex ring has a constant circulation (**Figure 4(b)**) that is proportional to the force required to generate the ring:

$$F_{\text{mean}} = \frac{1}{4}\frac{\pi\rho\Gamma d h}{T} \tag{9}$$

where ρ is the density of the fluid, Γ the circulation, d the distance between the two vortex centers in the plane, and h an estimate of the out-of-plane height of the loop (see **Figure 8** for examples of h and d). For example, a good rough estimate for h for a ring shed by the caudal fin is the height of the fin itself. T is the time over which the two counter-rotating vortices were shed, generally half of the tail or fin beat cycle. Mathematically, circulation Γ can be written in two equivalent ways:

$$\Gamma = \int_{\Sigma} \omega \cdot \mathrm{d}\Sigma \tag{10}$$

$$= \oint_C \mathbf{u} \cdot \mathbf{dl} \tag{11}$$

where $\sum$ is a surface in the fluid, C the closed contour around the edge of the surface (thick black lines in Fig. 4), and l the tangent vector along the contour. The second form of the expression for circulation is important experimentally, because it allows one to estimate circulation without taking any derivatives of the velocity field. Taking derivatives tends to introduce numerical error, so the second form (eqn (11)) is often more accurate.

Momentum analysis

Newton's laws tells us that force is equivalent to change in momentum, and that, for any force on the body, there is an equal and opposite force on the fluid. Thus, in principle, one could simply measure the change in momentum in the fluid, which should be equal and opposite to the force on the animal.

For a solid body whose mass is not changing, this procedure is the familiar $F = ma$. In a fluid though, the volume of fluid affected may change, and thus m is not constant. To estimate change in fluid momentum, therefore, one must use three steps: (1) determine the volume of fluid affected by the force, (2) find the mean fluid velocity in that volume, and (3) include the effect of added mass.

1. Determine the volume. Some analyses have performed this step manually, but recently, a technique has been developed to identify what are called Lagrangian coherent structures (LCSs): volumes of fluid that tend to stay together. For example, if a fish's fin accelerated some fluid, one can track the volume of accelerated fluid as it grows and moves over time by using an LCS analysis.
2. Mean LCS velocity. Once the volume **S** has been determined, estimate the velocity U_v that the LCS itself is moving. Note that this velocity is not necessarily equal to the mean velocity of the fluid in the volume.
3. Added mass. Estimate the added mass coefficient tensor $\mathrm{C_A}$ for the volume, which will depend on the shape of the fluid volume, but not its velocity. For linear forces, the added mass tensor is a 3 × 3 matrix giving the added mass effect along each axis for acceleration along the same or a different axis. For complex bodies, the acceleration reaction may produce counter-intuitive forces, not necessarily parallel to the acceleration itself. However, we will simplify and consider only force and acceleration along the same axis, giving a scalar added mass coefficient C_A. For symmetric bodies or volumes of fluid, this approximation is appropriate.

The force on the animal corresponding to the changing fluid momentum is thus the opposite of the time derivative of the impulse required to produce the motion of the volume S:

$$F = -\frac{\partial}{\partial t}[\rho S(1 + C_A)U_V] \tag{12}$$

Figure 10 shows an example of this force calculation. The LCS around the bluegill sunfish's pectoral fin is estimated during one fin beat (**Figure 10(a)**). The vertical (lift) force is estimated, and the relative importance of the changes in velocity, volume, and added mass of the LCS are given (**Figure 10(b)**). Early in the cycle, changes in U_v are the dominant effect, while later in the cycle, the added mass coefficient C_A decreases, reducing the force.

Choosing vortex or momentum analyses

Since the tail and fins move back and forth, swimming is inherently unsteady; nevertheless, for swimming at a

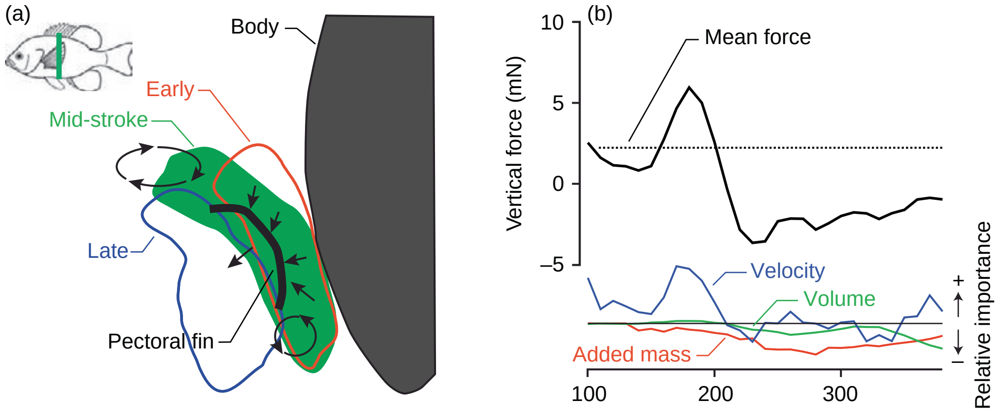


Figure 10 Example of a momentum analysis for the bluegill sunfish's pectoral fin beat. (a) Cross section through the fish body and pectoral fin. Schematics of the fin and flow are shown at mid-stroke with a thick black line and black arrows, respectively. The volume of fluid affected by the fin is shown in green for mid-stroke (same time as the fin and flow schematic), red for early in the stroke, and blue for late. Inset shows the position of the cross section. (b) Force estimates. Top panel shows the instantaneous force estimate based on the LCS calculations in (a), along with a mean force estimate (dotted line). Bottom panel gives the relative importance of the changes in LCS velocity, volume, and added mass to the force estimate. Early in the stroke, changes in velocity are the most substantial effect, contributing to positive force, whereas later in the stroke, changes in added mass become more influential, decreasing the total force. Modified from Peng J, Dabiri JO, Madden PG, and Lauder GV (2007) Non-invasive measurement of instantaneous forces during aquatic locomotion: A case study of the bluegill sunfish pectoral fin. *Journal of Experimental Biology* 210(4): 685–698 and Drucker EG and Lauder G (1999) Locomotor forces on a swimming fish: Three-dimensional vortex wake dynamics quantified using digital particle image velocimetry. *Journal of Experimental Biology* 202: 2393–2412.

steady average velocity, the vortex analysis described above is often sufficient and is much simpler to apply. For instance, in **Figure 10(b)** the time-averaged mean force matches the vortex analysis.

The relative importance of unsteady effects can be determined by the wake vortex ratio Wa as described by Dabiri:

$$Wa = \frac{C_A S U_V}{\Gamma} \tag{13}$$

which relates the observed vortex motion to the motion that one would expect if there were no unsteady forces. When Wa is very large or very small, the added mass forces are either speeding up or slowing down the vortex and an unsteady, momentum-based analysis is best. When Wa is near a critical value, either approach is correct, but the time-averaged vortex analysis may be simplest. For a vortex analysis (eqn. (9)) to be valid, the following condition serves as a rule of thumb:

$$Wa_{\text{crit}} = \frac{C_A}{1 + C_A} \tag{14}$$

If Wa is substantially above or below Wa_{crit}, then the momentum-based analysis is most appropriate.

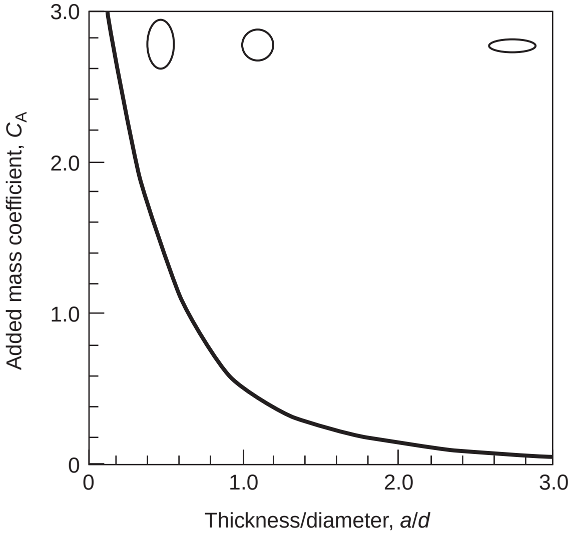


Figure 11 Theoretical added mass coefficients as a function of the thickness/diameter ratio of prolate spheroids, which approximate those of the LCS surrounding a vortex ring. Thickness *a* is the axis parallel to the motion of the ring, while diameter *d* is the largest diameter between vortex cores. Reproduced form Daniel TL (1984) Unsteady aspects of aquatic locomotion. *American Zodogist* 24: 121–134.

For the purposes of estimating Wa, the added mass of a vortex ring can be approximated by that of a solid prolate spheroid. Choose the largest diameter d_{max} (b or d in **Figure 8**, whichever is larger) and estimate the thickness of the loop (a in **Figure 8**). **Figure 11** shows added mass as a function of the thickness/diameter ratio a/d_{max}. Then estimate the mean circulation of the two vortex cores and the speed U_v that the whole loop is moving along its axis and use eqns (13) and (14) to determine the importance of unsteady effects. For example, in **Figure 10**. *Wa* is between 0.2 and 0.4, while $C_A/(1+C_A)$ is about 0.4. Thus, the vortex analysis is appropriate.

Power

Another useful metric for analyzing a wake is the hydrodynamic power contained in the wake, P_{wake}. This power is one component of the total propulsive power, $P_{\text{total}} = P_{\text{thrust}} + P_{\text{wake}}$. A perfectly efficient swimmer would somehow manage to devote all of its power to thrust and would leave no wake. Real swimmers are not perfectly efficient, and therefore they leave a wake. The thrust power P_{thrust} is difficult to estimate, particularly for steadily swimming fishes, but P_{wake}, which can be quantified in a fairly straightforward manner, can be used as a proxy measure for inefficiency. Higher P_{wake} values are indicative of low efficiency, though they are not conclusive.

Because power is change in kinetic energy; wake power is estimated by adding up the kinetic energy of the fluid that enters and leaves a volume that surrounds the fish. For a wake measurement, such as those in **Figure 8**, one must have a sufficiently wide cross section across the wake that the fluid momentum added by the animal decreases to zero at the edges. The large width means that we can assume that there is no flow through planes parallel to the swimming direction of the fish. Ideally, one would also have a cross section upstream of the fish, although this can usually be estimated from the mean swimming speed (equal to the flow speed in a flow tunnel). The planes form a control volume. The net power that the fish adds to the fluid is the rate kinetic energy enters the upstream cross section, minus the rate it leaves the downstream cross section.

$$P_{\text{wake}} = \frac{1}{2}\rho h U \int (2Uu + u^2 + v^2)\,\mathrm{d}y \qquad (15)$$

where h is the approximate height of the wake (usually equal to the height of a fin or the body), U the mean swimming speed or mean flow speed, u and v the fluid velocities added by the fish in the x and y directions, and where it is assumed that the upstream flow has a constant velocity U. Power can be normalized in a similar way as force (eqn (8)):

$$C_P = P/[\tfrac{1}{2}\rho A U^3] \qquad (16)$$

Figure 12 shows an example comparing wake power coefficients for American eels, rainbow trout (*Oncorhynchus mykiss*), and bluegill sunfish. Note that sunfish have the most costly wake, suggesting low swimming efficiency compared with American eels swimming slowly (0.5 Ls^{-1}) or rainbow trout swimming rapidly (~2 Ls^{-1}).

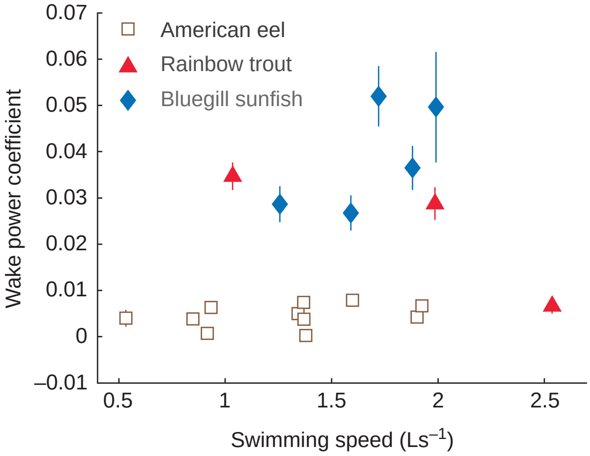


Figure 12 Wake power coefficients plotted against swimming speed for American eels, rainbow trout, and bluegill sunfish. Error bars indicate standard error; where they are not visible the symbol is larger than the error range. Modified from Tytell ED (2007) Do trout swim better than eels? Challenges for estimating performance based on the wake of self-propelled bodies. *Experiments in Fluids* 43(5): 701–712.

Future Developments

Much of this article has dealt with 2D flow fields, because these are currently the most common and easiest to measure. In the future, 3D measurements will become more common. Techniques such as defocusing PIV and holographic PIV are starting to be available commercially. In interpreting 3D flow fields is substantially more difficult than 2D fields. Nevertheless, the force and power analyses detailed above are applicable to 3D flows. In some ways, they are simpler to apply with 3D data, because one does not need to make assumptions about the 3D structure of the flow (e.g., the wake height h). At the same time, the flow data become much harder to visualize and understand. To help with this problem, it is likely that a class of techniques called dimensionality reduction methods will become much more important. These include principal orthogonal decomposition (POD) and biorthogonal decomposition (BOD), both of which are similar to principal components analysis, a technique commonly used to compare many different morphological measurements across fish species. Both techniques produce estimates of flow patterns that are the most coherent and energetically strongest. Then understanding the flow becomes easier, in that one can examine just these coherent modes.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy, Locomotion, and Movement in Fishes: An Introduction; Feeding Mechanics; Stability and Turbulence; Undulatory Swimming. **Hearing and Lateral Line**: The Ear and Hearing in Sharks, Skates, and Rays.

Further Reading

Adrian R (1991) Particle-imaging techniques for experimental fluid dynamics. *Annual Review of Fluid Mechanics* 23: 261–304.

Batchelor G (1973) *An Introduction to Fluid Dynamics*. Cambridge: Cambridge University Press.

Dabiri JO, Colin SP, and Costello JH (2006) Fast-swimming hydromedusae exploit velar kinematies to form an optimal vortex wake. *Journal of Experimental Biology* 209(11): 2025–2033.

Daniel TL (1984) Unsteady aspects of aquatic locomotion. *American Zodogist* 24: 121–134.

Denny MW (1993) *Air and Water: The Biology and Physics of Life's Media*. Princeton, NJ: Princeton University Press.

Drucker EG and Lauder G (1999) Locomotor forces on a swimming fish: Three-dimensional vortex wake dynamics quantified using digital particle image velocimetry. *Journal of Experimental Biology* 202: 2393–2412.

Epps B and Techet A (2010) An error threshold criterion for singular value decomposition modes extracted from piv data. *Experiments in Fluids* 48(2): 355–367.

Faber T (1995) *Fluid Dynamics for Physicists*. Cambridge: Cambridge University Press.

Fish FE and Lauder GV (2006) Passive and active flow control by swimming fishes and mammals. *Annual Review of Fluid Mechanics* 38: 193–224.

Holmes P, Lumley J, and Berkooz G (1996) *Turbulence, Coherent Structures, Dynamical Systems, and Symmetry*. Cambridge: Cambridge University Press.

Katz J and Sheng J (2010) Applications of holography in fluid mechanics and particle dynamics. *Annual Review of Fluid Mechanics* 42: 531–555.

Peng J and Dabiri JO (2008) An overview of a Lagrangian method for analysis of animal wake dynamics. *Journal of Experimental Biology* 211(2): 280–287.

Peng J, Dabiri JO, Madden PG, and Lauder GV (2007) Non-invasive measurement of instantaneous forces during aquatic locomotion: A case study of the bluegill sunfish pectoral fin. *Journal of Experimental Biology* 210(4): 685–698.

Tytell ED (2006) Median fin function in bluegill sunfish. *Lepomis macrochirus*: Streamwise vortex structure during steady swimming. *Journal of Experimental Biology* 209: 1516–1534.

Tytell ED (2007) Do trout swim better than eels? Challenges for estimating performance based on the wake of self-propelled bodies. *Experiments in Fluids* 43(5): 701–712.

Vogel S (2003) *Comparative biomechanics: Life's Physical World*. Princeton, NJ: Princeton University Press.

Undulatory Swimming

DJ Ellerby, Wellesley College, Wellesley, MA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Undulatory Swimming Modes
Myotomal Muscle Anatomy
Muscle Length Change and Activity
Muscle Mechanical Performance
Force and Power Transmission
Roles of Myotomal Muscle in Addition to Power Production
Further Reading

Glossary

Electromyography A technique for measuring the electrical activity associated with muscle contraction. Sensing electrodes implanted in the muscle tissue detect timing of muscle action as a high-frequency burst of voltage spikes.

Kinematic analysis Quantification and analysis of body movements.

Myoseptum The collagenous connective tissue forming the boundary between successive myotomal muscle blocks. In addition to forming the origins and insertions of myotomal muscle fibers, the myosepta are typically anchored to the skin and axial skeleton. Regions of the myotome that act as primary routes of force transmission are reinforced to form tendon-like structures.

Myotome A block of segmental muscle tissue. In fish, these consist of short (typically in the order of a few mm to a few cm) muscle fibers stretched between sheets of connective tissue, the myosepta, that separate adjacent muscle blocks.

Muscle action potential The wave of electrical depolarization that spreads across a muscle cell membrane in response to a stimulus from a motoneuron.

Sonomicrometry A technique that uses transmitted ultrasound as the basis for measuring length changes in muscle tissue. Pairs of ultrasound transducers are implanted parallel to the muscle fibers in the myotomal muscle. As the muscle tissue cyclically lengthens and shortens with each tail beat, this length change is indicated by the change in ultrasound transit time between the transducers.

Work loop A plot of muscle force production against muscle length. During contractions with a regular length-change cycle, the plot forms a closed loop. The area within the loop has units of work and represents the net work done by the muscle.

Introduction

All swimming animals move by transferring mechanical power generated by their muscles to the surrounding water. This accelerates the water, increases its kinetic energy and momentum, and creates a wake around and behind the animal. Through the conservation of momentum, transfer of power to the wake creates reaction forces that are the basis for movement through the water (**see also Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics). Many swimming animals transfer momentum using rostrocaudal waves of curvature that pass along their body axis. This article will focus on how body undulations of this type are generated in fish by contraction of the segmented myotomal musculature and the transfer of muscle force to the axial skeleton.

Undulatory Swimming Modes

Fish adopt a wide variety of swimming styles, utilizing fins and/or body movements to transfer muscle mechanical power to the water. Within the broader diversity of swimming styles, there are many species that swim using lateral undulations of their body axis. These body undulations, and the lateral movements of the caudal and unpaired dorsal and ventral fins they create, accelerate the surrounding water, generating lift, thrust, and stabilizing forces.

Undulatory swimmers are phylogenetically diverse. This swimming style is exhibited by petromyzontids, myxinids, and gnathostomes. Most of the data concerning kinematics and muscle dynamics have been obtained from gnathostomes. Within this group, the vast majority of data are from teleosts, with a relative handful of studies focusing on elasmobranch swimming. Undulatory swimmers also span a size range encompassing larval fish a few millimeters in length and whale sharks with body lengths in excess of 10 m. Despite this, the basic mechanics of swimming are similar in most undulatory swimmers. Lateral undulations are generated by sequential contraction of the segmented, myotomal muscle created by motor outputs from the spinal cord. Waves of muscle contraction pass along the body axis from anterior to posterior creating a wave of local muscle shortening and lateral bending of the body axis (**Figure 1**). The timing of the waves is offset between opposite sides of the body, resulting in an alternating wave of curvature moving along the body axis from anterior to posterior.

Undulatory swimmers have typically been broken down into a series of categories based on the two-dimensional kinematics of their midline during steady swimming. Categories are defined based on the number of waves of curvature present on the body and the proportion of the body axis that undulates (**Figure 2**). Under this scheme, the modes are named for genera that typify a particular swimming style. At one extreme, the anguilliform swimmers undulate the majority of their body axis. Subcarangiform and carangiform swimmers undulate progressively less of their body axis and have increasingly long mechanical wavelengths (so a smaller portion of a complete waveform is present on the body axis). Thunniform swimmers have a uniquely specialized muscle and connective tissue anatomy that sets them apart from the majority of other swimmers that transfer momentum to the water using axial movements. These result in the confinement of lateral movement to the caudal region rather than a clear axial progression of lateral undulation *per se.* In light of these differences, discussion is largely confined to more typical anatomies and the modes of undulatory swimming they generate.

Recent kinematic, morphological, and hydrodynamic analyses suggest that a kinematics-based scheme may have limitations in terms of clearly distinguishing between swimming modes. Amplitudes of undulation are actually quite similar in species typically separated into anguilliform and subcarangiform modes. Species in these groups also share a similar myotomal muscle and connective tissue anatomy that is distinct from the pattern seen in some carangiform swimmers. In contrast, visualization of wake structure has revealed an anguilliform wake pattern distinct from that shared by subcarangiform and carangiform swimmers. A comprehensive classification scheme will likely need to integrate kinematic, morphological, physiological, and hydrodynamic data. Unfortunately, at present, data from all these categories are available for very few species and a kinematics-based scheme remains the best available option for describing swimming modes.

Regardless of size, anatomical variation, hydrodynamic factors, phylogenetic affinities, or body midline kinematics, all undulatory swimmers are alike in one respect: the relationship between tail-beat frequency and

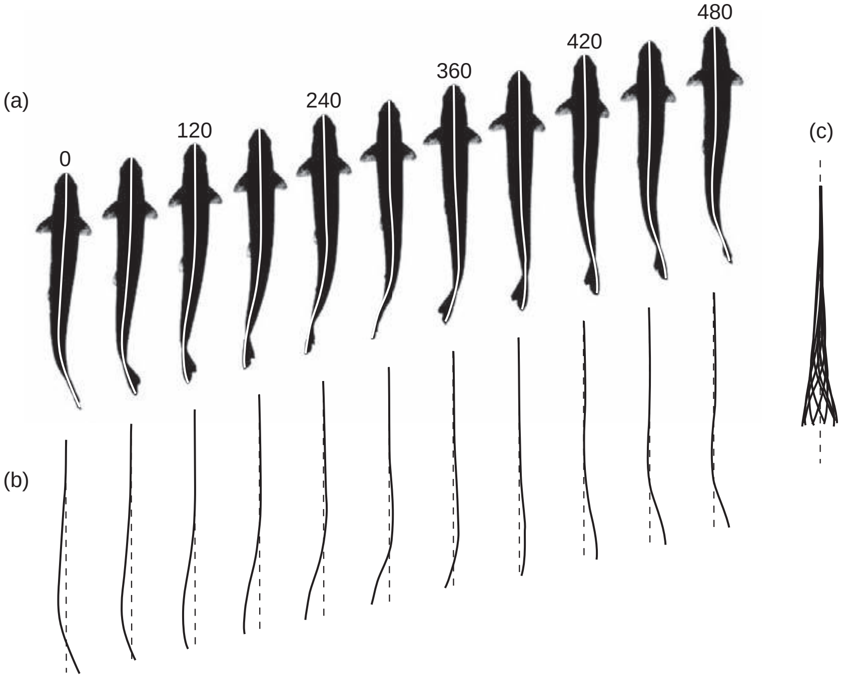


Figure 1 Axial undulations in a gnathostome fish. (a) Sequential images during a single tail beat. Numbers denote time in milliseconds. Axial midline is highlighted in white. (b) Midlines of sequential images shown in isolation. Dotted lines show a straight path parallel to the swimming direction for reference. (c) Superimposed sequential midlines indicating the amplitude envelope for lateral movement during a tail beat.

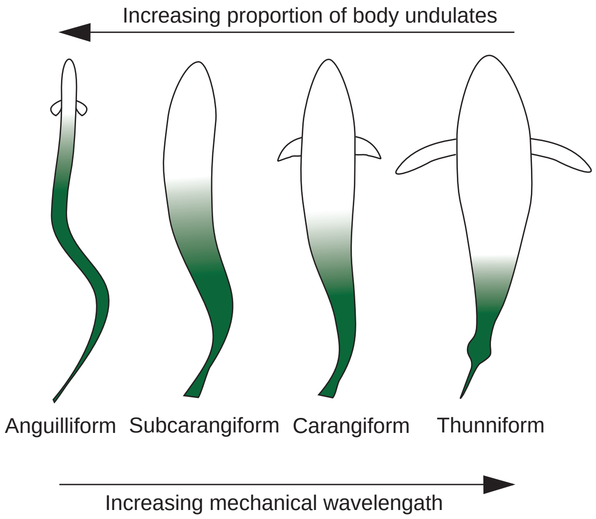

Increasing proportion of body undulates
Anguilliform
Subcarangiform
Carangiform
Thunniform
Increasing mechanical wavelengath

Figure 2 Undulatory swimming modes. Traditional categorizations of swimming mode based on two-dimensional midline kinematics.

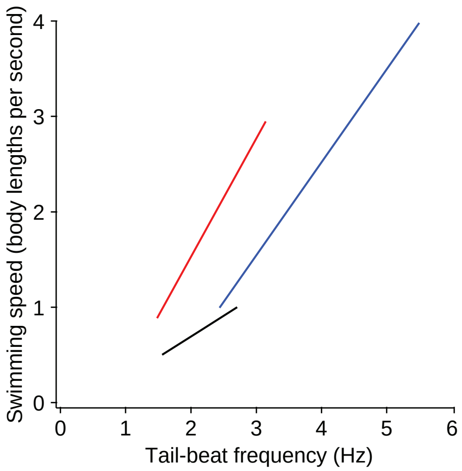


Figure 3 Relationships between undulatory swimming speed and tail-beat frequency. Representative data are presented for species spanning the range of swimming modes defined by midline kinematics. The data presented are as follows: black, American eel, *Anguilla rostrata*, anguilliform; blue, rainbow trout, *Oncorhynchus mykiss*, subcarangiform; red, bonito, *Sarda chiliensis*, carangiform. American eel: from Gillis GB (1998) Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: Kinematics in water and on land. *Journal of Experimental Biology* 201: 949–961. Rainbow trout: from Webb PW (1988) 'Steady' swimming kinematics of tiger musky, an esociform accelerator, and rainbow trout, a generalist cruiser. *Journal of Experimental Biology* 138: 51–69. Bonito: from Dowis HJ, Sepulveda CA, Graham JB, and Dickson KA (2003) Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae): II. Kinematics. *Journal of Experimental Biology* 206: 2749–2758.

swimming speed is approximately linear (**Figure 3**). That is, it can be described by a simple linear equation in the form, $U = fS + I$, where U is the swimming speed, f the tail-beat frequency, S the gradient or slope of the linear relationship, and I the zero speed intercept. The absence of a zero intercept for the relationships is unexpected, and appears to be due to modulation of undulatory kinematics at low speeds, particularly with regard to tail-beat amplitude. Despite this, for most of the speed range, speed control is primarily achieved via alteration of the tail-beat frequency. The gradient, S, is a useful measure of performance, as it represents a 'stride length', the distance traveled per tail-beat cycle.

The precise pattern of axial undulation is dictated by complex interactions between active and passive components of the musculoskeletal system, overall body shape, fin morphology and mechanical properties, and external hydrodynamic forces. This complexity, coupled with a tendency for any given study of undulatory swimming to focus on a subset of the system components, means that the mechanics of axial undulation are not fully understood. An increasing focus on integrating physiological, anatomical, mechanical, and hydrodynamic data, however, is leading to improved understanding of how muscle activity is translated into axial bending and ultimately the transfer of momentum to the surrounding water.

Myotomal Muscle Anatomy

The mechanical power for undulatory swimming is produced by the segmented, myotomal muscles that constitute a large portion of the total skeletal muscle mass in myxinids, petromyzontids, and gnathostomes. The myotomes are a series of blocks of muscle tissue separated by connective tissue, the myosepta (**Figure 4**). The myotomes are most easily visible as the flakes in a cooked fish filet when heat breaks down the connective tissue, allowing separation of the muscle blocks. Each myotome consists of many short muscle fibers that run between successive myosepta. The three-dimensional shape as well as the arrangement of the muscle fibers within a myotome are complex. The basic structure in most gnathostomes and myxinids consists of a series of nested cones of muscle tissue. In a cross section of a fish these are visible as a series of concentric circles of muscle alternating with connective tissue (**Figure 4**). A series of anterior-pointing cones are located dorsal and ventral to the midline of the fish. Dorsal and ventral to these are a series of posterior-pointing cones and at the dorsal and ventral extremes of the myotomes are a series of anterior-pointing arms of muscle tissue. Moving axially from the rostral to caudal myotomes, there is a trend for increased elongation of the cones and arms. Few of the muscle fibers, except for those in the most superficial and laterally placed muscle, are arranged in parallel with the long axis of the fish. Fiber angles change markedly with position within the myotomes. In the anterior myotomes of

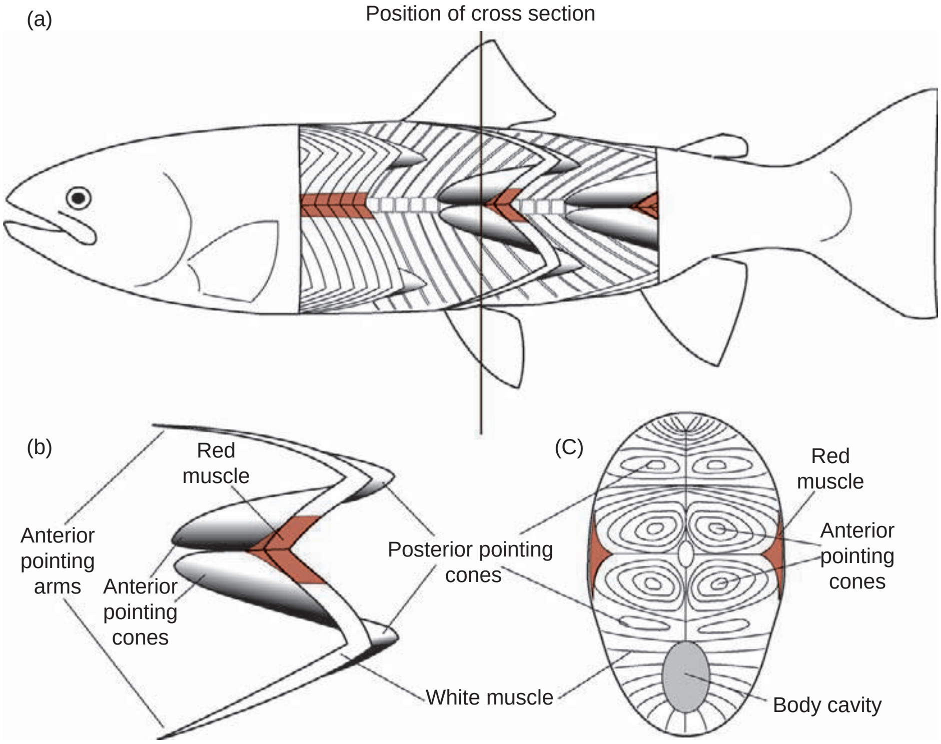


Figure 4 Myotomal muscle anatomy. (a) Lateral view of a typical gnathostome. A single myotome at the midpoint of the body axis is shown *in situ* with adjacent tissue removed to reveal its three-dimensional structure. (b) Component parts of a myotome. (c) Body cross section to reveal distribution of muscle fiber types and myosepta. Pink muscle is not shown as this typically represents a minimal component of the total muscle cross section.

gnathostome fish, arched trajectories of fibers spanning several myotomes have been identified. Starting anteriorly, close to the vertebral column, exterior arches run laterally outward from the midline of the body before curving back to the midline posterodorsally. Additional interior arches run under the midpoint of the outer arches, possibly having a supportive, pulley-like role during contraction of the fibers in the outer arches. The arrangement of these helix-like muscle trajectories and their associated connective tissue strongly suggests that adjacent myotomes interact in producing axial bending.

Muscle Fiber Types

The skeletal muscles of many vertebrates contain a mixture of muscle fiber types. These differ in terms of their mechanical performance and the metabolic pathways by which they obtain metabolic energy. Fish myotomal muscle is unusual in that different fiber types are anatomically separated. In most fish, the myotomal muscle contains three types of muscle fiber: red, pink, and white, named for their superficial appearance. White muscle accounts for the majority (typically >90%) of the myotomal muscle mass. Red muscle accounts for the most of the remainder. In most fish, red muscle is located laterally and has a wedge-shaped cross section (**Figure 4**). Pink muscle, if present, forms a thin layer between the red and white fibers. The color differences reflect underlying differences in their structure, biochemistry, and, as a consequence of these, their mechanical performance.

Regional Variation in Myotomal Muscle Properties

Myotomal muscle contractile properties vary between fiber types, with position on the body axis and among developmental stages. Force production and shortening by skeletal muscle are caused by myosin cross-bridge cycling. This requires adenosine triphosphate (ATP) energy and the binding and release of Ca^{2+} from troponin C, a protein component of thick myosin filament. Muscle shortening velocities decrease in the order white > pink > red in concert with a decreasing myosin-ATPase content. Factors affecting the Ca^{2+} occupancy of troponin-binding sites and the rates of cross-bridge attachment and detachment change the rates of muscle activation, shortening, and relaxation. Differences in the rates of activation, shortening, and relaxation can occur independently as they are influenced by separate muscle proteins.

Both red and white muscle fibers in a number of teleost species have slower rates of activation and/or relaxation moving along the body axis from anterior to posterior.

These differences have been correlated with longitudinal changes in the expression of three muscle proteins: parvalbumin, troponin T, and the myosin light chain (MLC; specifically MLC2 the regulatory light chain). Regional differences have been found in the relative amount of these proteins and/or in the relative proportions of alternate protein isoforms:

- Binding of Ca^{2+} to troponin C is an essential step in triggering cross-bridge cycling and muscle contraction. Parvalbumin binds free Ca^{2+} in the myoplasm, competing with troponin C. Parvalbumin, therefore, influences muscle relaxation by reducing the concentration of free Ca^{2+} in the myoplasm. High parvalbumin concentrations should be associated with rapid muscle relaxation. Parvalbumin content declines from anterior to posterior in trout (*Oncorhynchus mykiss*), sheepshead (*Archosargus probatocephalus*), and kingfish (*Menticirrhus americanus*) red band white muscle, and in cod (*Gadus morhua*) and largemouth bass (*Micropterus salmoides*) white muscle, likely being a factor in increased relaxation times moving from anterior to posterior in these species.
- Troponin T is a component of muscle thin filaments (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element). It is thought to affect the rate at which Ca^{2+} dissociates from troponin C, and therefore the rate at which muscle relaxes. The relative proportions of two troponin T isoforms shift from anterior to posterior in cod (*G. morhua*) and largemouth bass (*M. salmoides*), with the dominant anterior form likely having faster kinetics than the alternate isoform.
- MLC2 is a thick filament component that may modulate cross-bridge kinetics by controlling sensitivity to Ca^{2+}. The amounts of the slow MLC2 isoform increase, moving from anterior to posterior in both the slow and fast muscle of rainbow trout (*O. mykiss*).

The list of regionally variable proteins that influence myotomal muscle contractile properties is likely to grow as more data become available. Fewer data are available for elasmobranchs, but similar regional differences in contractile properties have not yet been detected.

In addition to regional mechanical differences, there is also variation in the extent to which fish muscle fibers rely on either aerobic or anaerobic metabolic pathways to supply energy for contraction. The volume fraction of red fibers occupied by mitochondria is typically greater than 25% compared with less than 10% in white muscle. Accordingly, the activities of mitochondrial enzymes associated with aerobic metabolism, such as cytochrome oxidase and citrate synthase, are higher in red than in white muscle. Pink muscle shows an intermediate level of activity for these enzymes. White muscle relies primarily on anaerobic glycolysis for its energy supply, as indicated by higher levels of glycolytic enzymes, such as phosphofructokinase, than found in red or pink muscle.

Muscle Length Change and Activity

The interaction between mechanical properties and the metabolic pathways that fuel this performance dictates the speed and sustainability of the movements that a particular muscle type can power. Steady, sustained, undulatory swimming is powered by the red, aerobic muscle fibers. Pink fibers supply additional mechanical power at the upper end of the aerobically supported speed range. The anaerobic white muscle has limited involvement in undulatory swimming in most species, except during brief, rapid sprints. These fibers are primarily active during rapid, asymmetrical, maneuvers used to capture prey and avoid predators. Accordingly, the majority of data for muscle function during undulatory swimming have been obtained from red and pink muscle.

The relationship between force production and length change is the major determinant of muscle mechanical performance. Direct recordings of muscle activity and length change patterns have been made in a number of fish species during undulatory swimming (**Figure 5**). These have been obtained using two techniques: electromyography and sonomicrometry. Force production by skeletal muscle is triggered by a series of chemical and electrical signals. A key step is the generation of a muscle action potential (MAP). This is a wave of electrical depolarization propagated along the cell membrane of muscle cells that is generated by changes in membrane permeability, primarily to sodium ions. Electromyography uses recording electrodes adjacent to muscle cells to detect the MAP.

Body undulations are driven by waves of muscle activity and contraction proceeding sequentially through the myotomes from anterior to posterior (**Figure 6**). In most species, activity coincides with local shortening of the myotomal muscle. This creates curvature of the body axis of the fish with the active muscle on the inside of the curve. The timing of the waves of activity on opposite sides of the body is offset. Muscle contralateral to the active muscle is inactive and being lengthened by the active muscle tissue. This throws the body axis into a series of curves that proceed along the body from anterior to posterior along the body.

These recordings reveal a number of similarities, irrespective of differences in swimming mode or taxonomic group:

- The muscle length change pattern is an approximately sinusoidal wave form.
- Muscle activity starts just before peak muscle length.
- Muscle activity persists during the majority of the shortening phase.

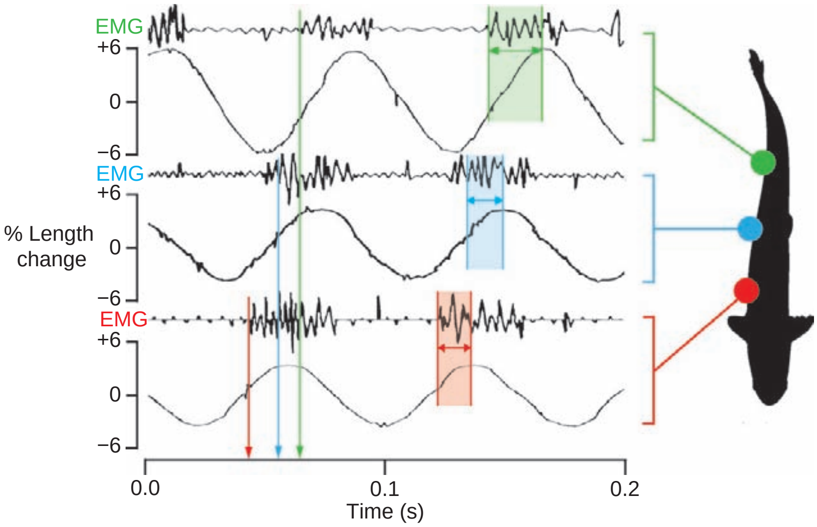


Figure 5 Myotomal muscle activity and length change at three points along the body axis. Vertical arrows show the relative timing of EMG onset at the three points. Shaded areas and horizontal arrows show the shift in EMG onset relative to peak muscle length. Adapted with permission from Ellerby DJ and Altringham JD (2001) Spatial variation in the fast muscle of rainbow trout *Oncorhynchus mykiss* during fast starts and sprinting. *Journal of Experimental Biology* 204: 2239–2250.

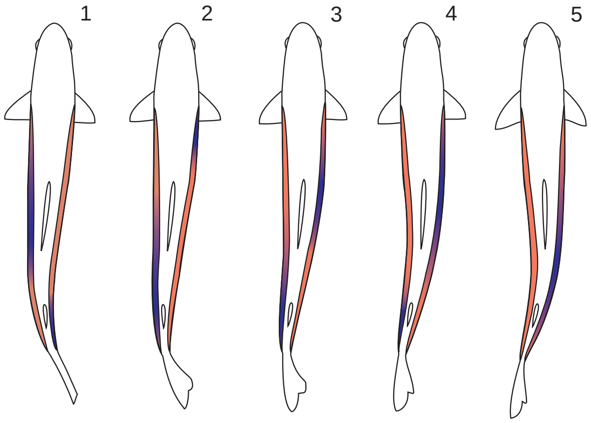


Figure 6 Timing of muscle activity along the body axis. Dark shading denotes the presence of EMG activity. Numbers show a sequential progression through the tail beat.

There are also a number of trends within teleost undulatory swimmers that are increasingly apparent as the mechanical wavelength of the axial undulations increases; that is, they are more marked in carangiform, than in subcarangiform or anguilliform swimmers:

- The amplitude of muscle fiber length change increases moving from anterior to posterior myotomes.
- The timing of muscle activity relative to the length change cycle shifts earlier, moving from the anterior to posterior myotomes.
- The relative duration of muscle activity decreases moving from anterior to posterior myotomes.
- The speed of axial progression of the electromyogram (EMG) activity increases from anterior to posterior.

As for muscle contractile properties, fewer data are available for undulatory elasmobranchs, but shifts in the relative timing of muscle activity have yet to be detected.

Muscle Mechanical Performance

EMGs are useful as indirect indicators of muscle force production. There are, however, limits to what can be learnt from EMG data for two reasons: first, there is a time lag between EMG activity and muscle force production and this is likely to change along the body axis due to regional differences in contractile properties; and, second, the EMG signal cannot indicate the magnitude of the force produced. Therefore, in the absence of direct measures of muscle force, EMG and sonomicrometry data alone cannot supply information about muscle work and power output.

The complexity of the myotomal anatomy means that direct measurements of myotomal muscle force are not possible in a swimming fish. Muscle force and work output can be measured in muscle fibers removed from the myotomes and maintained *in vitro* in oxygenated, physiological saline. Muscle work and power have been determined in this way for a number of species using the work loop technique (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops).

Applying this technique, patterns of length change and activation measured *in vivo* using sonomicrometry and electromyography are played back to the *in vitro* muscle fibers by an ergometer and the resulting force, work, and power outputs measured. For a muscle to do mechanical work on its surroundings, it must exert force primarily while shortening. As undulatory swimming requires work to be done on the surrounding water by the myotomal muscle then the majority of the active muscle mass should operate in this mode. This has generally been found to be the case; with the majority of force production is confined to the shortening phase of the length change cycle. A limitation in interpreting data from work loop experiments is that the muscle fibers are no longer connected to the connective tissue that transmit force and power to the axial skeleton.

Force and Power Transmission

In order to create axial undulations and ultimately to transfer power to the water, muscle force and power must be transmitted to the axial skeleton of the fish. There are a number of potential routes for force transmission from myotomal muscle via connective tissue and skeletal elements. Recently, detailed anatomical investigations of the myoseptal anatomy have led to a reevaluation of how they transmit force. These structures were typically viewed as relatively homogenous sheets of connective tissue. It is now clear that within the overall structure there are reinforced regions of connective tissue with a tendon-like structure. The orientation of these structures with regard to the muscle fascicles and axial skeleton strongly suggests that they are playing a major role in force transmission during undulatory swimming. In many teleosts, this force transmitting capacity is enhanced by ossification of the tendons to form the epineural and epipleural bones. The primary role of these tendons is the posterior transmission of forces from anterior muscle (**see also The Skeleton**: Bony Fish Skeleton).

In gnathostomes and myxinids, myoseptal connective tissue is also directly linked to the stratum compactum, a fibrous layer of connective tissue in the skin (**see also The Skin**: The Many Functions of Fish Integument). This layer is a likely route for transmission of force directly from superficial myotomal muscle fibers, and indirectly from muscle inserting on the myosepta.

Roles of Myotomal Muscle in Addition to Power Production

Power Transmission

The typical onset of muscle activity during muscle lengthening means that for part of the length change cycle myotomal muscle is stretched by adjacent muscle while exerting force. This means that the muscle is not a pure producer of work and power. In addition to doing work on the water, work also must be done on the muscle to stretch it while active. This has two mechanical consequences: first, the active prestretch enhances force production and power output during the subsequent shortening phase of the cycle; and, second, muscle that is stretched while active has high stiffness (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). On this basis, it has been hypothesized that the muscle tissue itself could act as a transmitter of force and power from adjacent muscle tissue in addition to doing positive work later in its length change cycle. The trend for increasingly early onset of activity moving from anterior to posterior along the body axis means that this later role is likely to be more pronounced in the posterior myotomes. In some species, peak positive power production in the anterior myotomes also appears to coincide with high stiffness in the poster myotomes. Given the robustness of the tendon-like myoseptal structures in many species, however, the role of muscle as power transmitter is likely to be of limited importance.

Stability Control

Work loop experiments suggest that the power output from anterior red myotomal muscle is suboptimal under *in vivo* conditions (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). In addition, at low swimming speeds in particular, the anterior myotomal muscle is intermittently active. This is a surprising operating mode for a power producing muscle. At a constant swimming speed, a power producing muscle would be expected to show a relatively constant level of activity and produce power at a near optimal level. Intermittent, suboptimal contractions suggest an alternate role. A likely explanation is that the anterior muscle is assisting in making small stabilizing and maneuvering corrections to control the heading of the fish (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence).

Modulation of Body Stiffness

It has been hypothesized that fish dynamically alter their body stiffness in order to match the natural frequency of the body axis to their tail-beat frequency. This may potentially reduce the mechanical work required to undulate the body axis. Stiffness modulation could be achieved by the action of muscle on the stratum compactum of the skin placing the connective tissue fibers in this layer in tension. Stiffness may also be affected by changes in intramuscular pressure during myotomal muscle contraction. Unconstrained muscle undergoes radial expansion as it shortens during contraction. Resistance of radial expansion by myoseptal connective tissue would increase intramuscular pressure and axial stiffness.

In summary, carefully timed activation of blocks of muscle on either side of the body plus having different muscle blocks contracting and relaxing at different rates results in waves of contraction or undulation of the body and ultimately forward swimming.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics; Functional Properties of Skeletal Muscle: Work Loops; Stability and Turbulence. **Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element. **The Skeleton**: Bony Fish Skeleton. **The Skin**: The Many Functions of Fish Integument.

Further Reading

Altringham JD and Ellerby DJ (1999) Fish swimming: Patterns in muscle function. *Journal of Experimental Biology* 202: 3397–3403.

Blake RW (2004) Fish functional design and swimming performance. *Journal of Fish Biology* 65: 1193–1222.

Coughlin DJ (2002) Aerobic muscle function during steady swimming in fish. *Fish and Fisheries* 3: 63–78.

Donley JM, Sepulveda CA, Konstantinidis P, Gemballa S, and Shadwick RE (2004) Convergent evolution in mechanical design of lamnid sharks and tunas. *Nature* 429: 61–65.

Gemballa S and Vogel F (2002) Spatial arrangement of white muscle fibres and myoseptal tendons in fishes. *Comparative Biochemistry and Physiology A* 133: 1013–1037.

Müller UK and VanLeeuwen JL (2006) Undulatory fish swimming: From muscles to flow. *Fish and Fisheries* 7: 84–103.

Schultz WW and Webb PW (2002) Power requirements of swimming: Do new methods resolve old questions? *Integrative and Comparative Biology* 42: 1018–1025.

Shadwick RE and Gemballa S (2006) Kinematics and muscle dynamics in swimming. In: Shadwick RE and Lauder GV (eds.) *Fish Physiology Series, Vol. 23. Fish Biomechanics*, pp. 241–280. San Diego, CA: Academic Press.

Syme DA, Gollock M, Freeman MJ, and Gamperl AK (2008) Power isn't everything: Muscle function and energetic costs during steady swimming in Atlantic Cod (*Gadus morhua*). *Physiological and Biochemical Zoology* 81: 320–335.

Videler JJ (1993) *Fish Swimming*. London: Chapman and Hall.

Videler JJ (2001) Fish locomotion. In: Steele J, Thorpe S, and Turekian K (eds.) *Encyclopedia of Ocean Sciences*, pp. 938–946. London: Academic Press.

Wardle CS, Videler JJ, and Altringham JD (1993) Tuning into fish swimming waves: Body form, swimming mode and muscle function. *Journal of Experimental Biology* 198: 1629–1636.

Webb PW (1984) Form and function in fish swimming. *Scientific American* 251: 72–82.

Functional Properties of Skeletal Muscle: Work Loops

DA Syme, University of Calgary, Calgary, AB, Canada

© 2011 Elsevier Inc. All rights reserved.

Work, Swimming, and Muscles
Measuring Work
What We Have Learned Using Work-Loop Analysis
Further Reading

Glossary

Cycle frequency The number of complete strain cycles imposed on the muscle per second (Hz), often analogous to the tail-beat frequency of a swimming fish.

Duty cycle The fraction of the strain cycle period during which the muscle is activated. It is a measure of the duration of stimulation of the muscle relative to the strain cycle duration.

Lengthening work Sometimes referred to as negative work, it is the work required to lengthen a muscle and is energy absorbed by the muscle. While negative work *per se* is not defined (and is thus, perhaps, not the best choice of terminology), the negative notation arises from the calculation of work in work-loop analysis, where an increase in muscle length results in a negative value when calculated as initial length minus final length. It is equally acceptable to express lengthening work as a positive or negative value, because it is understood that the absolute value represents the energy required to lengthen the muscle.

Net work The mathematical difference between shortening and lengthening work, typically calculated over a complete cycle of shortening and lengthening. In this calculation, the lengthening work is subtracted from shortening work when both are assigned positive values alternatively, if lengthening work is defined as a negative quantity, then net work is the sum of shortening and lengthening work. A positive value of net work denotes the muscle doing more work while shortening than it absorbs while being lengthened. A negative value denotes more work being required to lengthen the muscle than it produces while shortening.

Phase A particular reference point or segment during an oscillation.

Power The rate of doing work (watts = joules per second). In work-loop analysis, it is usually calculated as the work (J) done during one complete cycle, multiplied by the cycle frequency (Hz).

Shortening work Sometimes referred to as positive work, is the work done by a muscle as it shortens, and is energy exerted by the muscle on the external world.

Stimulus frequency or pulse frequency The rate (Hz) at which stimulus pulses are applied to the muscle or nerve to cause muscle contraction.

Strain cycle In work-loop analysis, this refers to the pattern of length change imposed on the muscle, typically sinusoidal, but not necessarily, and sometimes called the strain trajectory, or excursion in earlier literature.

Strain or strain amplitude The magnitude of change in muscle length expressed as a fraction or percent of the resting or average length. It is referred to as excursion or excursion amplitude in earlier literature. Strain may be expressed as peak-to-peak amplitude or only peak; refer to the specific study for details.

Work A measure of mechanical energy (joules), calculated as the integral of force with respect to muscle-length change in work-loop analysis.

Work loop A plot of muscle force production against muscle length. During contractions with a regular length-change cycle, the plot forms a closed loop. The area within the loop has units of work and represents the net work done by the muscle.

Work, Swimming, and Muscles

Swimming requires work. For a fish, it requires a good deal of energy to move through the water, more so for some than others. Most fishes are neutrally buoyant or nearly so; thus, relatively little energy need be expended to support their body weight (**see also Buoyancy, Locomotion, and Movement in Fishes:** Buoyancy in Fishes). For what, then, is the energy required? At a minimum, a mass of water must be accelerated backward to propel the fish forward. Water is dense and viscous relative to air; thus, as the fish moves through water, considerable energy is required to push water out of the way and to escape its grip on the body surface. Energy is also needed to accelerate the body itself, both forward and laterally. Subtle and not-so-subtle changes in body shape, attitude in the water column, vortices, and turbulence, all require energy to create, whether intentional or not. All these considered, swimming requires work.

In fishes, like most multicellular animals, work for locomotion comes from skeletal muscles that transduce chemical energy from adenosine triphosphate into mechanical work. During relatively slow and sustained swimming, the slow, red myotomal muscles are active, while during rapid movements, the fast, white muscles carry the load (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). What is it about these muscles that makes them suitable under the circumstances in which they are used? How can we understand the abilities of different muscles to power various aspects of swimming and the effects of swim speed, temperature, oxygen availability, etc., on the ability of muscle to power movement? Mechanical work of muscle has been measured historically using relatively simple contractions from fully activated muscle at constant velocity of muscle shortening, or working against a constant load such as lifting a weight off the ground. However, this does not mimic how muscles function in living, moving animals. In 1985, Robert Josephson reported successful adaptation of a technique allowing measurements from muscles working much as they do in moving animals, and we have since gained many new insights into how muscles are used by animals to move. This is the work-loop technique.

Before discussing what work loops tell us about muscles and swimming, a brief introduction to some terminology is needed. Proper terminology should be understood and abided by, as casual use will cause confusion and have one appear as blissfully uninformed as advertisers touting powerful whiteners in their toothpaste and efficiency in their software solutions. This will not be dealt with in detail. Work, with units of joules (newton meters, Nm), is a measure of mechanical energy, an important energy currency in the world of physical movement. Mathematically, work done on an object is the product of the net force (*N*) exerted on the object (or load) and its displacement (*m*); that is, how hard something is pushed multiplied by how far it moves. The relevant force in calculating work is the vector acting in parallel with the vector of movement, and because force need not be steady while the object moves, it can be stated more generally that work is the dot product integral of force with respect to displacement. In practice, we will see that all or most force acts in parallel with the load in typical work-loop studies, and so, work is calculated simply by summing the products of average forces and displacements over many small intervals. Therefore, for those anxious about integral calculus and vector algebra, if one can, or a calculator can, multiply two numbers together, then one can do work-loop analysis.

As a product of force and displacement, work is a linear function of their magnitude. Moving a given load a given distance would require the same work as moving double the load over half the distance, etc. Further, if a large force is exerted against an object that does not move, or if an object moves steadily without any force being applied to it (coasting through space), then no work is done on the object; anything multiplied by zero is zero.

Power is the rate of doing work, expressed as joules of work done per second (watts, W). Moving a given load a given distance will require a given amount of work. Doing it faster requires the same amount of work (all else being equal), but the work is done over a shorter period of time, and so the power is greater. A bulldozer and a person will do the same amount of work in moving a load of earth (in simple terms), but the bulldozer can do it much faster than a human, because it is more powerful. As for toothpaste whiteners being powerful, well, that it is a load of another sort! The work done by fish muscle per cycle of shortening/lengthening is a useful measure of the energy made available during each tail beat to propel the fish forward. Power, as a measure of how fast that work is done, is a useful gauge of how fast a fish might swim; bearing in mind that swimming speed is not a linear function of power.

Measures of work incorporate both the ability to produce force and the ability to shorten while producing force, making work-loop analysis all the more informative. Isometric force is not always a good predictor of the ability of a muscle to do work, and sometimes is a dismal predictor. Further, movement of muscle has a marked effect on force, and thus on work, including classical force–velocity and length–tension properties of muscle, the poorly understood effects of stretch and shortening on cross bridges, effects of muscle length on titin, activation, calcium sensitivity and release, and perhaps other passive force-generating elements in muscle, and the effects of stretch and shortening on the time course of force generation and relaxation. All of these are important in influencing how much work is produced during muscle contraction, and all are accounted for only during contractions where the muscle moves similar to how it would in a living, moving animal. Alternatively, none of these is accounted for during isometric contractions, and isotonic or isovelocity shortening do not account for the important effects of stretch on muscle work and energy available for locomotion, nor do they usually allow relatively realistic patterns of muscle activation, making work-loop analysis all the more valuable as a tool to understand muscle function in moving animals.

Measuring Work

Ideally, we would measure work directly from muscle in a swimming fish. However, it is not yet technically feasible to measure simultaneously the force and strain of

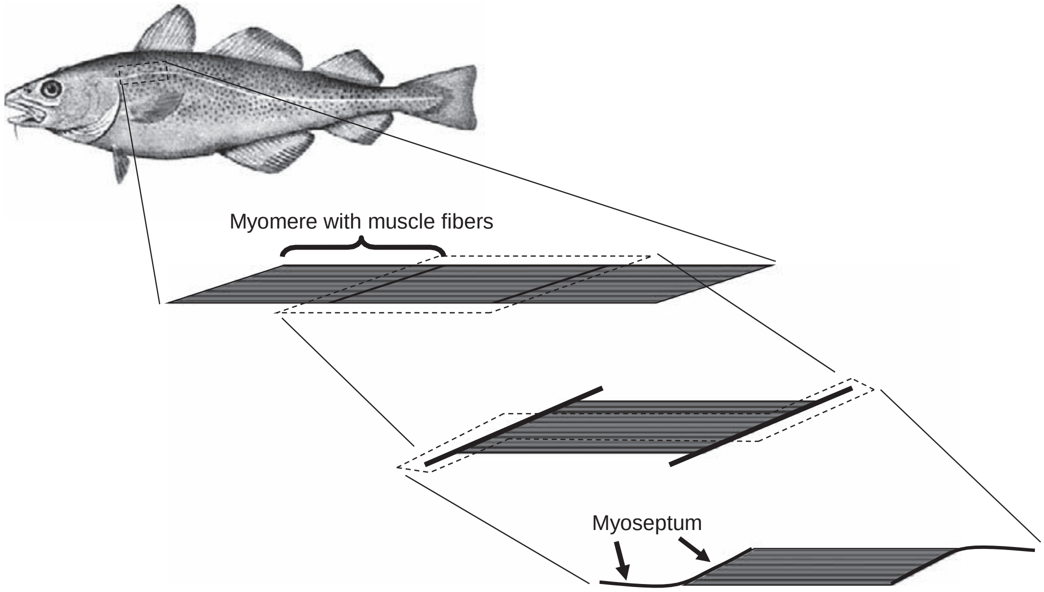


Figure 1 Isolation of a muscle preparation for work-loop experiments. In this example, a block of myotomal red muscle is first removed from the fish, typically directly beneath the lateral line. In this sketch, the muscle fibers are shown running horizontally, with dark myosepta separating the myomeres at an oblique angle. The initial block of tissue is several myomeres in length. This is then dissected to a preparation that is a single myomere in length with myosepta at either end, and finally pared down to the final preparation, which is relatively narrow in cross section and has segments of myoseptum extending from either end. These loose ends of the myosepta are secured to the apparatus (see **Figure 2**).

myotomal muscle in a swimming fish. The preparation of necessity is thus isolated muscle (**Figure 1**). For myotomal preparations, a small segment of muscle, usually several myotomes in length and several millimeters in width, is removed from a freshly killed fish and placed in cold physiological saline. The sample is then pared down via dissection, leaving normally one myotome in length and a width that is convenient for the experimenter's needs, ranging from a single fiber to about 1 mm in width. Short segments of myoseptum are left attached to either end of the preparation and used to secure it in the experimental apparatus. One end of the preparation is attached to the moveable arm of a servomotor, which is used to manipulate the length of the muscle. The other end is attached to a force transducer (**Figure 2**). In some instances, one end of the muscle is simply fixed to an immovable object and the other end to an ergometer, a device that can measure both muscle length and force, and thus work. It is named after the erg, a unit of energy ($\mathrm{g\,cm^2\,s^{-2}}$) equivalent to 100 nJ that is rarely, if ever, used today.

The preparation is immersed in physiological saline, usually with a means to maintain the pH, oxygen saturation, and temperature. As it is usually not practical or possible to maintain innervation of fish myotomal muscle, a pair of stimulating electrodes is placed alongside the muscle to activate the muscle directly.

The length of the muscle is oscillated by the servomotor with a trajectory that (usually) mimics that in a swimming fish. This strain trajectory is normally sinusoidal, as has been found to be a good approximation of how muscles stretch/shorten in swimming fish. Strain patterns other than symmetrical sine waves have been employed, including saw-tooth and modified sine waves, but the results do not differ greatly. The rate of cycling of the muscle length (cycle frequency) is set either to match the tail-beat frequency of the fish of interest, or a range of frequencies that can be explored to investigate the effects of cycling frequency on work and power output. The amplitude of the length change (strain) is likewise set to match some estimate in swimming fish or varied according to the experimenter's needs.

The final piece of the puzzle is muscle activation – precisely when (phase) and for how long (duration or duty cycle) the muscle is activated during each cycle of length change. Electromyogram (EMG) analysis along with measures of muscle strain in swimming fish have been used extensively to measure the phase and duration of muscle activation (**see also Buoyancy, Locomotion, and Movement in Fishes:** Undulatory Swimming). If known, these can be applied in work-loop analysis to obtain measures of work production that mimic those from a swimming fish (with due acknowledgment that muscles in swimming fish are activated via a complex barrage of synaptic depolarizations dispersed among the muscle fibers, not synchronously and maximally by a pulse of current from electrodes alongside the muscle). If such measures of EMG in swimming fish are not available, then the most common approach, and the one on

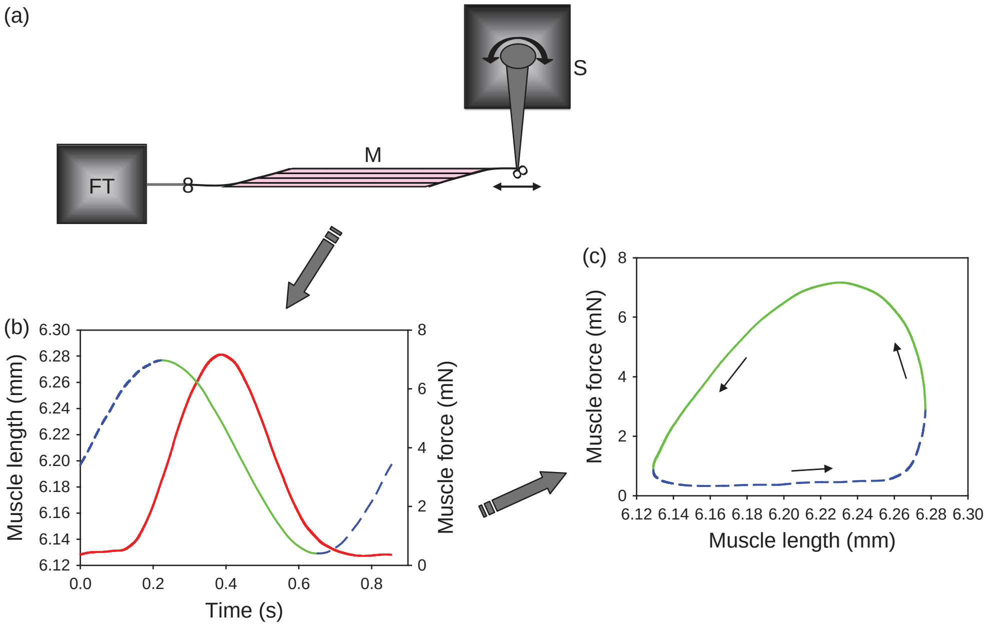


Figure 2 Work-loop experiments. (a) The loose myosepta of the muscle preparation (M) are typically tied to the arm of a servomotor (S) and force transducer (FT). The servomotor is used to impose cycles of oscillatory strain (length change) on the muscle fibers, and the force produced by the muscle is recorded with the force transducer. (b) In this example, a strain cycle with a sinusoidal trajectory has been imposed on the muscle (broken blue line is muscle lengthening and solid green line is muscle shortening). The resting or average length of the muscle was 6.2 mm, and the peak-to-peak strain amplitude was 2.4%. The muscle was stimulated near the point where force rises rapidly (about 0.1s), resulting in a transient rise and fall in force (red line). In this example, the stimulus phase was set to 10% of the sine cycle, or 36°, such that the majority of force occurred during the shortening portion of the strain cycle. (c) A work loop, formed by plotting muscle force against muscle length. Shortening (solid green line) and lengthening (broken blue line) segments of the loop are indicated, with the loop being traversed in a counterclockwise direction such that force during shortening is greater than force while lengthening. Work required to lengthen the muscle (lengthening or negative work) is the area beneath the lengthening portion of the loop, and work done by the muscle while shortening (shortening or positive work) is the area beneath the shortening portion of the loop. The net work, which is the difference between shortening and lengthening work, is the area within the loop, and would be considered a positive value in this example because more shortening work was done than lengthening work (i.e., the loop is counterclockwise, not clockwise).

which the vast majority of the literature is based, is simply to alter the stimulus phase and duration until net work output from the muscle is maximized.

The muscle's length is so oscillated and it is so stimulated, while length and force production are measured (**Figure 2**). A work loop is a graphical representation of the force–length relationship of the muscle during a complete cycle of lengthening and shortening. The trajectory of such a plot forms a closed loop. Work, being the integral of force with respect to muscle length, is readily gleaned from a force–length plot as the area beneath the curve. For lengthening work, it is the area beneath the lengthening arm of the loop (usually the lower arm), while for shortening work, it is the area beneath the shortening arm. The net work then, which is the difference between the shortening and lengthening segments, is the area inside the closed work loop. For a loop that is traversed in a counterclockwise direction, the area beneath the upper arm (shortening) is greater than the area beneath the lower arm (lengthening). Thus, more work is done by the muscle while it shortens than is required to lengthen the muscle and the net work would be a positive number. For a loop that is traversed in a clockwise direction, where force during lengthening is greater than the force during shortening, the net value is negative; the muscle absorbs more work in being lengthened than it produces while shortening.

Power, the rate of doing work, is typically calculated as the product of the work done during a single cycle and cycle frequency. Be mindful that this yields power averaged across the entire cycle, which includes the period when the muscle is being lengthened such that energy is being absorbed rather than produced. Thus, power from work-loop analysis will be considerably lower than the instantaneous, maximal power that might be measured using other techniques; but in the end, power from work-loop analysis best reflects the power available to swim.

The loop is an extremely useful and informative visualization of how the muscle behaves when working. If the phase and duration are adjusted to maximize net work output, the loop will typically be traversed in a counterclockwise direction. If phase and duration are not adjusted accordingly, the loops will have a different trajectory; they may run clockwise and may even form complex figure-eight patterns where one loop of the pair will have a net positive value and the other net negative, with the overall net work depending on the balance. Once experience is gained in understanding and interpreting the loops, one will know at a glance from the shape of the loops what the muscle is doing in terms of work output, activation, and even function in the swimming fish. It is a remarkably informative (though not powerful) construct.

What We Have Learned Using Work-Loop Analysis

Some of the first questions asked by physiologists studying work output of fish muscle included (1) how do strain (analogous to tail-beat amplitude in swimming fish) and cycle frequency (analogous to tail-beat frequency in swimming fish) impact the ability of muscles to produce work and power, (2) how do the abilities of the slower, oxidative red muscles differ from the faster, glycolytic white muscles, (3) what are the effects of temperature on work output, and (4) what are the functional roles of myotomal muscles in swimming fish? We will briefly consider what we have learned about each using work-loop analysis.

Effects of Strain and Cycle Frequency on Work and Power

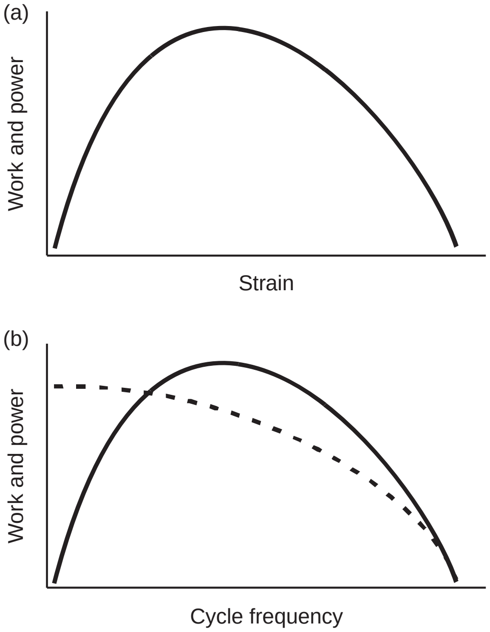


Figure 3 (a) Both net work and power show the same bell-shaped relationship with the amplitude of muscle strain. While the units of work and power differ, power is simply a scalar of work done per cycle. (b) Net work done per cycle (broken line) declines with increasing cycle frequency. Power, which is the product of work done per cycle and cycle frequency, shows a bell-shaped relationship with cycle frequency.

An increase in strain amplitude indicates that the muscle shortens and lengthens a greater distance. As work is the integral of force with respect to length change, we anticipate an increase in work with increased strain amplitude (**Figure 3**(**a**)). But this is true only up to a limit. All else remaining equal, the muscle must also move at a higher velocity (both peak and average) with increased strain amplitude. Force–velocity properties of muscle dictate that the faster a muscle shortens, the less force it is capable of producing. This limits the force and thus work done while shortening with increased strain amplitude, so that at relatively large strains, shortening and net work actually decline with further increases in strain amplitude. Changes in strain amplitude also influence the portion of the length–tension relationship over which the muscle operates, which may further impair force and thus work production at large strains. Other less-known impacts of stretch and shortening on force, activation, relaxation, etc., will also impact force and work done with changes in strain amplitude. The result is a bell-shaped relationship between net work done per cycle and strain amplitude, with work being maximized at some intermediate strain and falling as strain amplitude is either increased or reduced from this value. At zero strain (isometric contraction), work is zero, and at some large strain, net work would also be zero as the muscle shortens so fast that it can produce little to no force. In practice, muscles would likely be damaged before they reached strain amplitudes of this magnitude and in life would be restricted by the skeleton from experiencing such large extensions. The strain amplitude at which (net) work is maximized is in the neighborhood of 15% peak to peak (i.e., a 7.5% stretch above resting length and a 7.5% shortening below resting length). However, this value can vary considerably depending on the preparation, and factors such as temperature and cycle frequency. The relationship between power and strain amplitude is qualitatively identical to that for work, because power is simply a scalar of work (work per cycle × cycle frequency). Thus, there exists a tail-beat amplitude at which muscles would produce the most net work per cycle and power for swimming under a given regimen of temperature and tail-beat frequency. Do fish swim with these tail-beat amplitudes? Sometimes yes, and sometimes not. Should we expect them to? This is a fruitful area for further study.

It can be noticed that the word net is often sneaked in before the term work. This is to distinguish the relationship of net work with strain from those of shortening and lengthening work. In addition to force generated by cross bridges, work has components associated with elastic recoil and viscous resistance. Like stretching an elastic band and pulling on a fluid-filled plunger, lengthening work will rise monotonically with increasing strain in a relaxed muscle, and reach a maximum only as a result of damage at unphysiologically large strains. Shortening work will normally show a relationship with strain much like net work (bell-shaped), although not identical. The precise relationship between strain and net work is, of course, a function of the difference between the relationships between shortening and lengthening work with strain.

Cycle frequency, analogous to tail-beat frequency in a swimming fish, is the number of stretch/shorten cycles the muscle undergoes in a given period of time (usually per second, hertz). As the muscle shortens faster with increased cycle frequency, it will produce less force and thus tend to do less work during each cycle. Hence, at a given strain amplitude, shortening and net work per cycle decline with increasing cycle frequency (**Figure 3**(**b**), also see **Figure 5**). Net work per cycle is, in theory, maximal at a cycle frequency that approaches zero. In reality, such a slow tail-beat frequency would not be effective for swimming (as when moving a canoe paddle as slowly as possible through the water); so, the cycle frequency at which work is maximized is not particularly interesting to consider. Net work per cycle falls with increased cycle frequency, reaching zero near the frequency at which the muscle is moving so fast that it cannot produce force. Why is it near that frequency and not at it? Since we are talking about net work, the amount of work required to lengthen the muscle will also limit net work output. Force due to viscous resistance during lengthening increases with increasing cycle frequency. Further, at high cycle frequencies, the muscle typically does not relax fully between contractions, and so the muscle resists lengthening more and more as cycle frequency rises. Thus, it is not only the inability of the muscle to produce force while shortening at high cycle frequencies that limits net work output, but also the ever-rising work required to lengthen the muscle. A faster muscle, in terms of its ability to generate force and then relax, is able to cycle at higher frequencies while still producing positive net work. This can be seen later when we consider different types of muscle.

The relationship between power and cycle frequency is quite different from the relationship between work and cycle frequency (**Figure 3**(**b**)). Power is the product of work done per cycle and cycle frequency, the former which decreases and the latter which increases with increasing cycle frequency. The resulting power versus cycle frequency relationship is bell shaped, with power being zero at zero cycle frequency (even though work would theoretically be maximal), reaching a maximum at some intermediate frequency, and then falling at higher frequencies where the decrease in work per cycle exceeds the increase in cycle frequency. The power versus cycle frequency relationship has been used to predict the tail-beat frequency resulting in maximal power output for swimming, and would depend on the muscle, strain, temperature, etc.

Differences between Red and White Muscle

Simply put, red muscles tend to produce less power and operate at slower frequencies than white muscles. How much lesser? In the neighborhood of one quarter, but this can vary considerably. Why is this less? Not because red muscles lack the inherent ability to generate high forces, but because they cannot sustain high forces and hence high power at high shortening velocities, and they are not capable of rapid relaxation, which limits their ability to function at high cycle frequencies (**Figure 4**). As such, red muscles are typically used to power relatively slow, sustained, cruise swimming (**see also Buoyancy, Locomotion, and Movement in Fishes:** Undulatory Swimming). They possess characteristics that suit these activities, including a rich blood supply and high levels of myoglobin, relatively slow relaxation (slow rates of calcium uptake into the sarcoplasmic reticulum and slow rates of cross-bridge detachment), and they produce substantial amounts of force and power only when shortening at relatively slow velocities. Their power versus cycle frequency relationship thus has a peak in power at modest cycle frequencies (**Figure 4**), which tend to correspond with the tail-beat frequencies observed in a number of species of fishes swimming at their cruise speeds. Red muscles contribute little power if used at high tail-beat frequencies.

Conversely, the fast, white muscles have force–velocity characteristics that allow them to produce substantial amounts of force and hence power when shortening at relatively high speeds, and they relax rapidly (fast rates of calcium uptake into the sarcoplasmic reticulum, as well as high concentrations of calcium-binding proteins in the cytoplasm). Thus, their power versus cycle frequency relationship has a peak at high cycle frequencies (**Figure 4**), considerably faster than the tail-beat frequencies used by cruising fish. As fish tend not to swim steadily at high speeds, but rather burst and glide or only sprint for short periods, it is challenging to measure such behaviors in fish and to define clear relationships between muscle performance and swimming ability. Nonetheless, during high-speed movements, the white muscles shorten at velocities at which work-loop analysis clearly shows they can make substantial contributions to power.

Results from EMG studies convincingly show that red muscles are the only muscles recruited to power swimming at slower, cruise speeds. Available evidence suggests red muscles are either not used or make a limited contribution as swim speed increases toward burst and sprint speeds, or during escape maneuvers, and are then

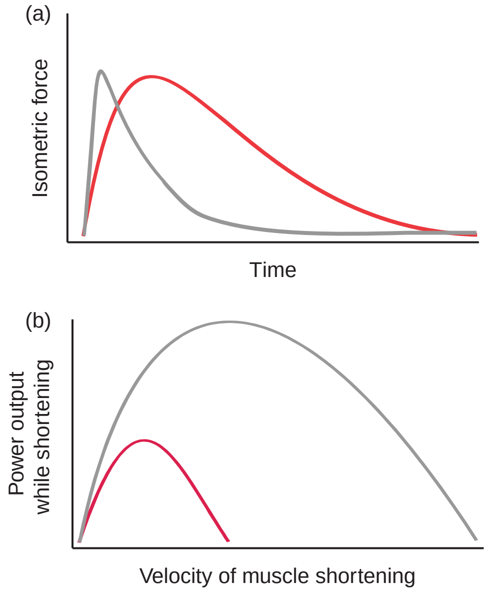


Figure 4 Other contractile properties of muscle can be used to estimate their ability to produce cyclic work and power. (a) Illustration of force produced during isometric twitches of red muscle (red trace) and white muscle (gray trace). The rates of force generation and relaxation are faster in white than red muscle. The shorter duration of the twitches in white muscle qualitatively predicts that the white muscle can endure faster rates of cycling during cyclic work activity than can red. (b) Illustration of the power output of fully activated muscle during steady shortening shows a bell-shaped relationship with the velocity of muscle shortening. This is the instantaneous power output of the muscle while shortening, and has both a higher maximum and faster velocity at that maximum for white muscle than red muscle. Such values of power can be used to predict qualitatively that white muscle will produce more power than red muscle during cyclic contractions, and sustain power output at higher cycle frequencies. However, the values of power obtained from such plots will differ considerably from those obtained during cyclic contractions (see text).

obviously supported by faster pink and white muscles. While EMG studies confirm which muscles are being recruited at different swim speeds, and while force–velocity, power–velocity, and twitch characteristics allow us to infer in a semiquantitative manner that red muscles should suffice to power slow movements while only white muscles would excel at powering fast movements, only measures of cyclic work using work-loop analysis have allowed us to confirm the real limits of their contributions to such movements. The as-yet poorly understood effects of stretch and shortening on the time course and extent of force production can only be fully accounted for on muscles that are activated and moving as they do in swimming fish.

If fast muscles can produce high power and continue to produce power at high cycle frequencies, why bother having the slow, red muscle at all? While there is likely more to answering this question than first meets the eye, measures of muscle work allow us to reason that differences in contractile properties and anatomical position of red and white muscle may result in white muscle moving so slowly at cruise swim speeds that they cannot produce enough power to sustain even slow swimming. Further, the slower red muscles are more efficient at producing work than the faster white muscles (i.e., red muscles tend to use less energy to perform a given amount of work than white muscles). For slow and sustained swimming, the economy of movement is likely an important consideration in recruiting a slower, less powerful muscle. Conversely, the faster, more powerful white muscles are recruited for rapid bursts and escape maneuvers, where performance is paramount and energy savings are not.

Effects of Temperature

In terms of the ability of fish muscles to produce work and power, cold muscles do not perform as well as warm muscles (**Figure 5**). With cooling, the rates of force generation and relaxation slow and the ability to sustain force production while shortening is reduced. These lead to less work done per cycle, reduced power output, and a slowing of the cycle frequencies at which power is maximal and at which the muscle can continue to produce work and power. The Q_{10} for these responses typically ranges from 2 to 3. For the fish, this means they must swim more slowly as temperature decreases and/or recruit more of their muscle or faster muscles to swim at a given speed in the cold. Yet, not all fish are equal in this regard, and the sensitivity to temperature depends heavily on whether the cold exposure is acute or long term. Fish that live in cold environments tend to possess muscles that are adapted to working in the cold. They produce more power and can function at higher cycle frequencies at a given, cool temperature than fish that live in warmer water and are acutely exposed to a cool temperature. This is true not only when comparing across species that inhabit arctic/temperate/tropical habitats, but likewise in fish acclimated to different temperatures. When exposed to cool temperatures, the muscles of a cold-acclimated fish can perform faster and with more power than the muscles of a warm-acclimated fish. There is thus a degree of compensation. However, the compensation is not complete. Cold-adapted or cold-acclimated muscles working in the cold still cannot produce as much power as warm muscles can.

So there might be an advantage in maintaining warm muscles when living in cold water, versus simply possessing cold adapted/acclimated muscles. A number of fish warm their muscles up, including tunas, the lamnid sharks, and some thresher sharks (**see also Design and Physiology of Arteries and Veins**: The Retia and **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks). Work-loop studies suggest the warmer muscles

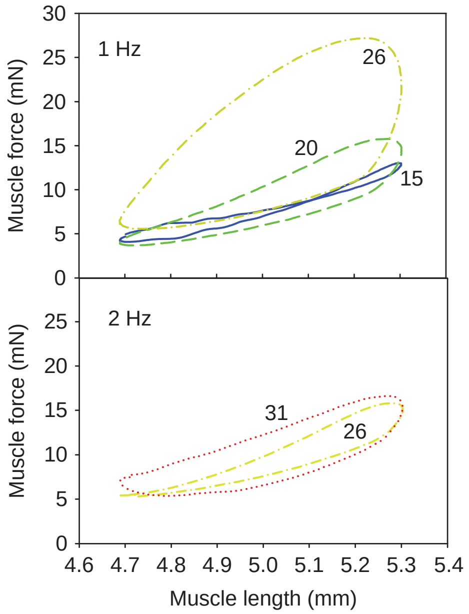


Figure 5 Work loops from salmon shark red muscle obtained at two different cycle frequencies (1 Hz upper panel and 2 Hz lower panel), and four different temperatures (15 °C solid blue, 20 °C broken green, 26 °C broken-dotted yellow, and 31 °C dotted red). Loops are traversed in a counterclockwise direction, except the 15 °C loop which has both clockwise and counterclockwise components. The area of the work loop (the net work done per cycle) increases with increasing temperature in salmon shark red muscle, which normally operates at warm temperatures. The net work done per cycle decreases with increasing cycle frequency (compare loops in upper and lower panels). Note that at 15 °C, the coolest temperature tested, the muscle is just capable of producing a small amount of net positive work at a 1-Hz cycle frequency, and that at the faster 2-Hz cycle frequency, only loops at 26 and 31 °C are shown because this warm-adapted muscle was not capable of producing net positive work at cooler temperatures. This highlights the temperature sensitivity of muscle contractile properties and the limitations imposed on the ability to produce work at different temperatures.

do very well if kept warm, but would do very poorly if these endothermic fish allowed their muscles to cool down, notably worse than cold-adapted muscles. We do not yet know much about these fishes, and research is underway to understand the costs and benefits of such strategies (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks).

Functions of Muscle during Swimming

How might we tell what a specific muscle is doing in a swimming fish? As noted earlier, for the most part, it is technically beyond our means to measure this directly. However, we can infer it with work-loop analysis. It is possible to measure the strain patterns of muscle in swimming fish, via either video analysis or sonomicrometry, and to measure electrical activity of muscles using EMG, which reflects muscle activity. Combining these two measures, we can quantify the strain amplitude and cycle frequency of the muscle and the phase and duration of muscle activation, all the ingredients required to perform work-loop analysis. The results of such experiments will approximate muscle performance in a swimming fish. This has now been accomplished in a number of species, and the results unearth a remarkable diversity in function and strategy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). Such studies confirm that slow muscles tend to be used to power slow, sustained swimming and that they cannot power sprint swimming, while fast muscles power fast, burst swimming, and likely supplement swimming in the cold. Providing even greater insight into muscle function, we can compare the work loops from muscles when used as they are in a swimming fish with results where work is maximized by optimizing parameters such as stimulus phase and duration in work-loop analysis. With this approach, we can begin to understand what the function of the muscles might be in contributing to movement (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). We have learned that some fishes appear to use muscles in the anterior region to produce power, while the more posterior muscles act as active struts to transmit this energy toward the tail. Conversely, some fishes use the more posterior muscles to produce power, with anterior muscles showing work that is highly variable from tail-beat to tail-beat, perhaps belying a role in steering and stabilizing the fish. Some fishes, such as eels, seem to use muscles along the entire length of the body to produce power for swimming. These are only examples of fishes that bend their bodies to swim. We have barely begun to explore the functions of muscles in labriform (pectoral fin)-based swimmers (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming).

An obvious hypothesis is that fish might be expected to use their muscles in a manner that allows them to produce maximal power for swimming. Work-loop analysis has allowed us to test this otherwise intractable hypothesis. In some instances, it seems to be supported, at least approximately, including slow swimming in carp, cruising in tunas, and, in some instances, in white muscle during fast starts. In cold-acclimated fish, there are changes in both the muscle and recruitment patterns that result in substantial improvements in power output over what would occur if such changes were not effected; a two- to ninefold increase has been reported in scup. However, a number of examples cast doubt on the notion of maximizing power output as a universal objective of muscle during swimming, including in red muscle during sustained swimming and in white muscle during fast swimming. Further, in acutely cooled fish, the recruitment patterns used by the fish (phase and duration of activation) do not change in the manner required to maximize power output and there is a marked reduction in power as a result. Indeed, in some instances, fish appear to recruit the faster pink muscle simply to sustain cruise swimming in the cold, not so much because the red muscles are incapable of producing enough power, but because they are not activated appropriately by the nervous system. Similarly, as noted above, even during swimming at normal temperatures, there are a number of reports suggesting the fish are not simply attempting to maximize power from their muscles. Other demands, such as stability, agility, and economy may well be dominant functions of muscle during cruise swimming. There remains much room for discovery.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Paired Fin Swimming; Undulatory Swimming. **Design and Physiology of Arteries and Veins**: The Retia. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks.

Further Reading

Altringham JD (1994) How do fish use their myotomal muscle to swim? *In vitro* simulations of *in vivo* activity patterns. In: Maddock L, Bone Q, and Rayner JMV (eds.) *Mechanics and Physiology of Animal Swimming*, pp. 99–110. Cambridge: Cambridge University Press.

Altringham JD and Ellerby DJ (1999) Fish swimming: Patterns in muscle function. *Journal of Experimental Biology* 202: 3397–3403.

Altringham JD and Johnston IA (1990) Modeling muscle power output in a swimming fish. *Journal of Experimental Biology* 148: 395–402.

Altringham JD and Shadwick RE (2001) Swimming and muscle function. In: Block BA and Stevens ED (eds.) *Tuna: Physiology, Ecology, and Evolution*, pp. 313–341. London: Academic Press.

Coughlin DJ (2003) Steady swimming by fishes: Kinetic properties and power production by the aerobic musculature. In: Val AL and Kapoor BG (eds.) *Fish Adaptations*, pp. 55–72. Enfield, NH: Science Publishers.

Curtin NA and Woledge RC (1993) Efficiency of energy conversion during sinusoidal movement of red muscle fibres from the dogfish *Scyliorhinus canicula. Journal of Experimental Biology* 185: 195–206.

Ellerby DJ and Altringham JD (2001) Spatial variation in fast muscle function of the rainbow trout *Oncorhynchus mykiss* during fast-starts and sprinting. *Journal of Experimental Biology* 204: 2239–2250.

James RS, Young IS, Cox VM, Goldspink DF, and Altringham JD (1996) Isometric and isotonic muscle properties as determinants of work loop muscle power output. *Pflugers Archive* 432: 767–774.

Johnson TP and Johnston IA (1991) Power output of fish muscle fibres performing oscillatory work: Effects of acute and seasonal temperature change. *Journal of Experimental Biology* 157: 409–423.

Josephson RK (1985) Mechanical power output from striated muscle during cyclic contraction. *Journal of Experimental Biology* 114: 493–512.

Josephson RK (1993) Contraction dynamics and power output of skeletal muscle. *Annual Reviews of Physiology* 55: 527–546.

Luiker EA and Stevens ED (1994) Effect of temperature and stimulus train duration on the departure from theoretical maximum work in fish muscle. *Canadian Journal of Zoology* 72: 965–969.

Rome LC (1994) The mechanical design of the muscular system. *Advances in Veterinary Science and Comparative Medicine* 38A: 125–179.

Rome LC (2007) The effect of temperature and thermal acclimation on the sustainable performance of swimming scup. *Philosophical Transactions of the Royal Society B* 362: 1995–2016.

Rome LC, Swank D, and Corda D (1993) How fish power swimming. *Nature* 261: 340–343.

Stevens ED (1993) Relation between work and power calculated from force–velocity curves to that done during oscillatory work. *Journal of Muscle Research and Cell Motility* 14: 518–526.

Syme DA (2006) Functional properties of skeletal muscle. In: Shadwick RE and Lauder GV (eds.) *Fish Physiology,* vol. 23 pp. 179–240. San Diego, CA: Academic Press.

Syme DA, Gollock M, Freeman MJ, and Gamperl AK (2008) Power isn't everything: muscle function and energetic costs during steady swimming in Atlantic cod (*Gadus morhua*) *Physiological and Biochemical Zoology* 81(3): 320–335.

van Leeuwen JL (1995) The action of muscles in swimming fish. *Experimental Physiology* 80: 177–191.

Wardle CS, Videler JJ, and Altringham JD (1995) Tuning in to fish swimming waves: Body form, swimming mode and muscle function. *Journal of Experimental Biology* 198: 1629–1636.

Paired Fin Swimming

EM Standen, McGill University, Montreal, QC, Canada

© 2011 Elsevier Inc. All rights reserved.

Diversity of Paired fins
Anatomy of Paired Fins
Fin Motion
Maneuvering
Fin Control
Hydrodynamics
Further Reading

Glossary

Angle of attack The angle made between the chord length of the fin and the direction of incident flow the fin encounters.

Aspect ratio The square of the fin span divided by the fin area.

Balistiform swimming Aquatic propulsion by means of undulatory movements of the dorsal and anal median fins.

Center of buoyancy The center of gravity of the volume of water which the animal's air-filled spaces or tissues of lower density than water displaces.

Center of mass The point in a body where the gravitational force may be taken to act.

Contralateral On the other or opposite side.

Labriform swimming Aquatic propulsion by means of beating the pectoral fins to produce lift and thrust.

How fishes use their fins for propulsion and maneuvering has become the focus of biomechanical study. Because up to 20% of living fishes rely on pectoral fin swimming as their primary mode of propulsion and all fish use these fins for stability and maneuverability, the majority of work has been done on paired pectoral fins. Only recently have the pelvic fins become the focus of study.

Diversity of Paired fins

Fish fins are folds of skin supported by skeletal elements controlled by fin musculature. Fins may be used for locomotion, reproduction, display, or protection. Generally, fish have two sets of paired fins and three median fins. The dorsal, anal, and caudal median fins are not paired and attach on the dorsal, ventral, and posterior aspects of the fish, respectively. Paired fins consist of the pectoral and pelvic fins, and attach to the fish's pectoral and pelvic girdles. The bony rays that support the fin membrane are made of either segmented (soft ray) or fused (spiny ray) bone.

Large variation exists in the size, shape, and location of paired fins among fishes. In extreme cases, some fish species have reduced or lost their paired fins entirely. A broad taxonomic review of the ray-finned fishes shows two trends in paired fin orientation and location:

1. pectoral fins in basal fishes lie ventral and with a near-horizontal attachment site, while in more derived fishes pectoral fins lie mid-dorsal with a vertical attachment site; and
2. pelvic fins in basal fishes are posterior of the fish's center of mass, while in more derived species they lie directly below or even in front of the center of mass (**Figure 1**).

The change in location and orientation of paired fins through fish evolution has changed fin function. Where fins are located relative to the fish's center of mass and center of buoyancy, the forces and torques each fin can produce will change (**Figure 2**).

Anatomy of Paired Fins

Generally, each set of paired fins is composed of a bony girdle that anchors the fin muscles within the fish's body and supports the fin. The external fin surface is made of a series of bony rays joined by a tissue membrane. For more information on musculo-skeletal anatomy and development, the reader is referred to **Brain and Nervous System**: Motor Control Systems of Fish, **The Skeleton**: Bony Fish Skeleton, and Cartilaginous Fish Skeletal Anatomy.

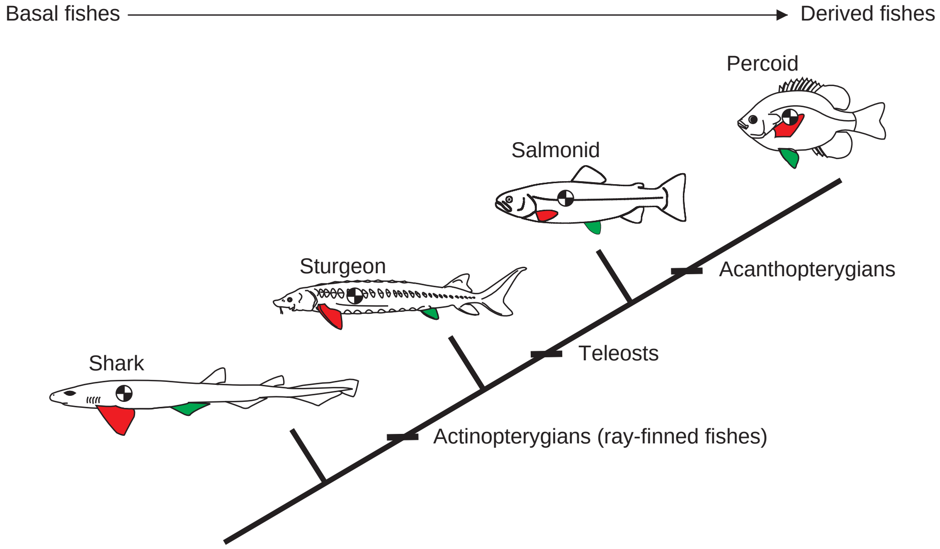


Figure 1 Paired fin position throughout fish evolution. A representative cladogram of selected fish groups. Black and white circles represent estimated location of fish centre of mass. From Standen EM (2008) Pelvic fin locomotor function in fishes: Three-dimensional kinematics in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 211: 2931–2942.

Each fin ray, called a lepidotrich, is composed of two halves called hemitrichia that are fused at their tip. Along their length, these two halves can slide relative to each other allowing the ray to bend. All bending of the rays is controlled by musculature attached to the hemitrichia on either side of the fin base (**Figure 3**).

Pectoral Fins

The pectoral girdle is homologous to the shoulder girdle and is composed of several bones (**Figure 4**). The fin skeleton consists of radials, located at the fin base and supporting the more distal fin rays. A cartilaginous pad at the end of the radials acts as a focal point for fin ray rotation. The first ray is generally thicker and shorter than the rest and joins with the scapula of the girdle. The rest of the rays are embedded into the cartilage and generally tend to be longer antero-dorsally and shorter ventrally.

The generalized teleost muscle morphology consists of pairs of muscles on either side of the pectoral girdle (**Figure 4**). Large arrectors, dorsal and ventral, attach to the marginal fin ray. Adductors, superficial and deep, attach to the rest of the fin rays in a sheet. Abductors, superficial and deep, lie to the lateral or ventro-lateral side of the pectoral girdle (see **Table 1** for detailed muscle origin and insertion points).

Pelvic Fins

The pelvic girdle is homologous to the pelvis and is composed of two plates that are thickened and fused together by a cartilaginous joint at their anterior most end (**Figure 5**). The thickening of the anterior plate provides a joint surface around which the fin ray bases can rotate. The lateral-most ray is often shorter than those adjacent to it and the base of the dorsal most hemitrich may be anchored into the body and skin of the fish.

The generalized teleost pelvic fin has six muscles associated with it, three on each side (**Figure 5**). Arrectors, dorsal and ventral, are not always present in more basal fishes. Adductors, superficial and deep, attach to the dorsal hemitrichia of each ray. Abductors, superficial and deep, attach to the ventral hemitrichia of each ray (see **Table 1** for detailed muscle origin and insertion points).

Fin Motion

Pectoral Fins

The diversity in pectoral fin morphology has led to diversity in how different fish use their fins for swimming. Fish can use their pectoral fins as nonoscillating passive control surfaces where fins are held stiff against oncoming flow. Pectoral fins may also be undulated or oscillated to actively produce forces. When a fin undulates a wave is passed from fin base to fin tip and usually there are multiple waves on the fin surface. When a fin oscillates waves are passed from fin base to fin tip but there is only one wave on the fin surface at a time; in this way, the fin appears to act as a solid plate. Oscillated fins can work like paddles producing drag-based propulsion, or they can work like flapping wings producing lift-based propulsion.

Passive control surfaces

Nonoscillating fins are found in sturgeons *(Acipenseridae)* and sharks *(Elasmobranchii)*. These fins have limited

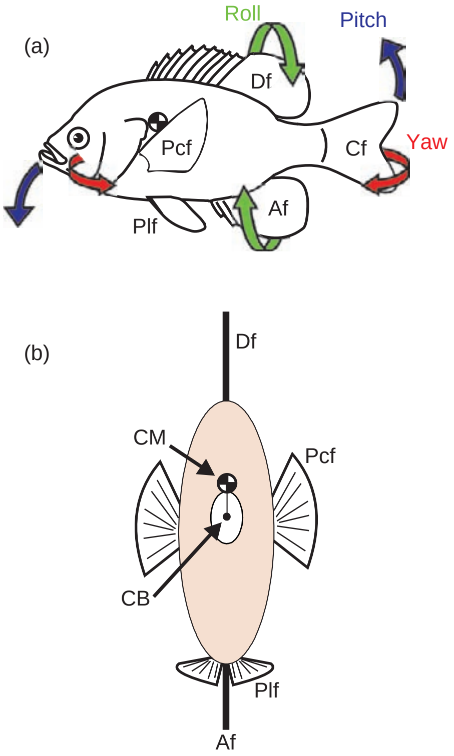


Figure 2 (a) Fins and axes of instability in bluegill sunfish *(Lepomis macrochirus)*. Paired fins in bluegill are represented by the pectoral (Pcf) and pelvic fins (Plf). Dorsal (Df), anal (Af), and caudal (Cf) fins are median fins. Fins work to control the forces that act on the fish in three major axes: pitch (head up and down movement), roll (body rotating along its longitudinal axis) and yaw (head side to side movement). (b) Schematic cross section showing center of mass (CM), center of buoyancy (CB) and fin placement. The white oval in the center of the fish represents the swimbladder cavity of the animal. The area of the fin surface as well as its location relative to the fish's center of mass and center of buoyancy will determine the amount of torque a fin can impose on the body at a given velocity. Modified from Standen EM and Lauder GV (2005) Dorsal and anal fin function in bluegill sunfish *Lepomis macrochirus*: Three-dimensional kinematics during propulsion and maneuvering. *Journal of Experimental Biology* 208: 2753–2763.

flexibility and they act as hydrofoils affecting lift and pitch during swimming.

Undulating fish fins

Animals that undulate their fins have three primary gait parameters that vary between species and swimming speed: undulatory wave frequency, wave speed, and length-standardized wavelength (essentially wavelength/chordlength). For example, as swimming speed increases in spotted stingrays (*Taeniura lymma*), the frequency and wave speed of undulatory waves on the pectoral fin increase, but their amplitude remains constant. Skates and rays *(Batoidea)* pass an undulatory wave rearward along their fin at twice the speed of their forward locomotion. The wings of skates closely approach the shape predicted by theoretical models to minimize cost of transport during swimming. Diversity among the batoid fishes (skates and rays) shows a continuum of locomotory motion of the pectoral fins from undulatory to oscillatory.

Fish such as the puffer fishes and burrfishes *(Tetraodontiformes)* also use undulatory pectoral fin motion to locomote. During fin abduction, an undulatory wave is passed from the anterior-most fin edge to the posterior edge. Left and right pectoral fins beat 180° out of phase in a contralateral oscillation so that when one fin is fully abducted the other is fully adducted. Puffer fish swim exclusively with their pectoral fins accompanied by dorsal and anal fin oscillation up to speeds of 3.0 BL s^{-1}, at which point they begin to beat their tails as well. Burrfishes appear to oscillate and undulate their fins and the undulation is hypothesized to function during maneuvers and slow-speed swimming rather than adding significantly to forward thrust production at higher-speed swimming.

Oscillating fish fins

Much work has been done to quantify and model forces produced by fin oscillations. Moving through three-dimensional space, there are three main planes in which a fin can be moved: the sagittal plane (paddling, where the fin attachment is ventral and the fin oscillates under the fish's body), the frontal plane (rowing, where the fin hinges more or less vertically and swings horizontally beside the body), or the transverse plane (flapping, where the fin is more or less horizontally hinged and swings vertically up and down beside the body; **Figure 6**).

Basal fishes, including some teleosts, demonstrate paddling fin motions. Rowing and flapping motions are seen throughout teleost evolution, and flapping appears to be derived from more basal paddling and rowing. Flapping is also seen in ratfish *(Chimaeriformes)* and batoids; the latter often combine flapping with undulation. Paddling and rowing fins have a distinct power and recovery portion to the stroke, whereas flapping fins are moved at angles that affect force production at all points in the stroke, eliminating the need for a distinct power and recovery portion of the stroke.

Gait transitions

Gait transitions occur when a fish changes its style of swimming to account for increasing or decreasing speed. In most acanthomorph fishes, slow-speed swimming is accomplished using pectoral fins only. As swimming speed increases, a transition speed is met where both pectoral fins and body caudal fin undulation contribute to thrust production. Finally, at the fastest speeds, the pectoral fins are tucked against the body to reduce drag and all thrust is produced by body caudal fin undulation.

Fin-beat frequency, fin-beat amplitude, and stroke plane can be measured during different speeds of swimming to quantify how fish change their fin motion to account for changing swimming speed. The swimming

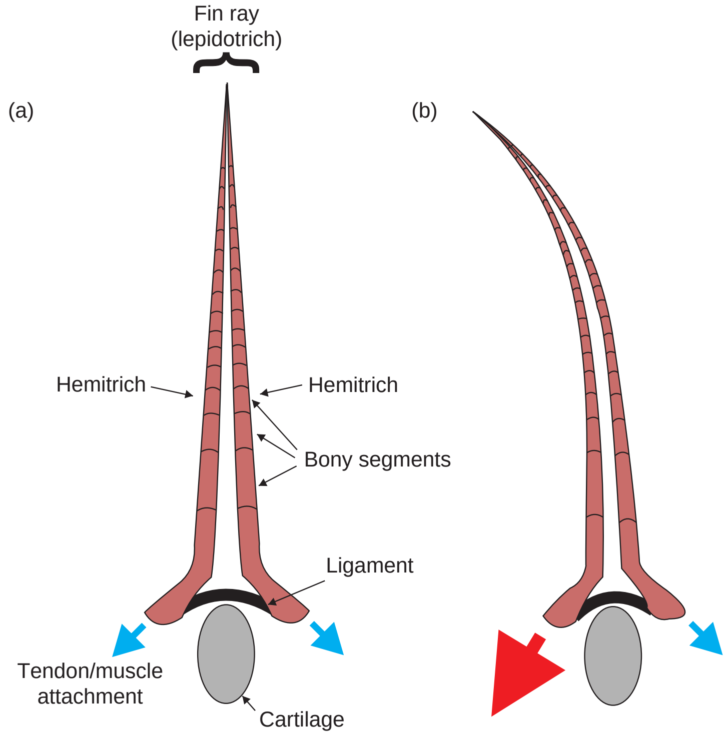


Figure 3 Fin ray structure. (a) Side view of one fin ray composed of two hemitrichia. Each hemitrich is made of bony segments. (b) Muscles attach to tendons at the base of each hemitrich and exert forces on each hemitrich, facilitating ray stiffening (symmetrical forces (a)) and bending (asymmetrical forces (b)). Modified from Figure 1d in Alben S, Madden P, and Lauder GV (2007) The mechanics of active fin-shape control in ray-finned fishes. *Journal of the Royal Society Interface* 4: 243–256.

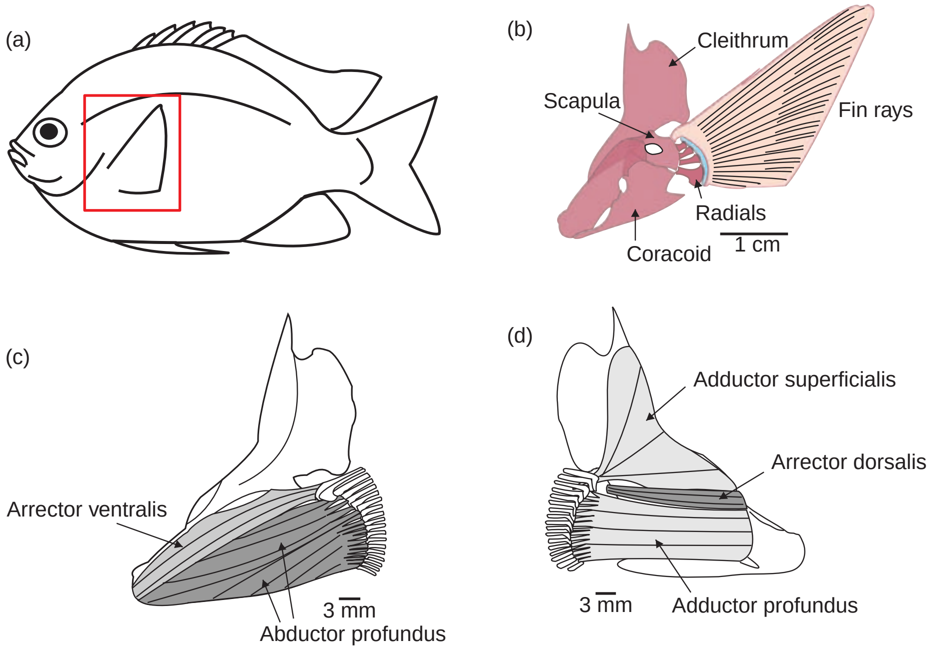


Figure 4 Pectoral fin anatomy. (a) The lateral view of labriform swimmer. (b) Skeletal anatomy of the pectoral girdle and fin. The cleithrum, coracoid, and scapula form the pectoral girdle supporting the radials. A cartilage pad (blue) rests at the distal end of the radials forming a surface for rotation for the fin rays. (c) Latero-medial view of the pectoral fin musculature. The abductor superficialis is removed from this image. (d) Medio-lateral view of the pectoral fin musculature showing the arrector dorsalis, adductor superficialis, and the adductor profundus. Modified from Figures 2a and 4a in Thorsen DH and Westneat MW (2005) Diversity of pectoral fin structure and function in fishes with labriform propulsion. *Journal of Morphology* 263: 133–150.

Table 1 Paired fin muscle location

Fin	*Muscle*	*Location*	*Origin*	*Insertion*
Pectoral	Abductor superficialis	Lateral or ventro-lateral of the pectoral girdle	Cleithrum (sometimes coracoid or even radials)	Diffuse or onto tendons attaching to anterior bases of fin rays
Pectoral	Abductor profundus	Lateral of the pectoral girdle but medial of superficialis	Lateral face of the coracoid (sometimes cleithrum and scapula)	Tendinous on the posterio-ventral flanges of fin rays
Pectoral	Arrector Ventralis	Well-developed lateral of most pectoral fin muscles	Anterolateral cleithrum (may include lateral faces of coracoid and scapula, inner face of cleithrum)	Tendinously on antero-medial base of medial half of marginal (first) fin ray
Pectoral	Adductor superficialis	Dorsomedial muscle mass located on the medial face of the pectoral girdle	Dorsomedial face of cleithrum (sometimes scapula, coracoid, or meso coracoid	By way of tendons attaching to the antero-dorsomedial faces of the fin rays distal to their bases and does not attach to the first fin ray
Pectoral	Adductor Profundus	Sometimes not entirely separate from superficialis lies medial of the pectoral girdle but lateral to the superficialis	Coracoid and postero-ventral portion of the cleithrum (sometimes scapula, radial, and mesocoracoid)	Tendinous on the postero-ventral flanges at the bases of all but the first fin ray
Pectoral	Arrector dorsalis	Dorsomedial of the pectoral fin musculature	Postero-ventromedial face of the cleithrum and adjoining coracoid (also scapula)	Tendinous on the base of the medial half of the first fin ray
Pectoral	Other muscles that may be present in the pectoral fin of fishes are the coracoradialis, adductor medialis, adductor radialis, and the interradialis pectoralis			
Pelvic	Abductor superficialis	Lies ventrally and medially	The ventral region of the pelvis or median septum between pelvic basals	Anterior (medial) rays but may serve all
Pelvic	Abductor profundus		Ventral face of the pelvis and the medial septum	Broadly on the bases of the soft fin rays
Pelvic	Arrector ventralis pelvicus		Ventrolateral face of the pelvis	Ventrolateral face of the pelvic spine or first pelvic ray, can be absent or not separate from other ventral pelvic musculature
Pelvic	Adductor superficialis pelvicus	Lies on the dorsal surface of the pelvis immediately above the profundus	Lateral surface of a median dorsal ridge on the pelvis or a midline fascia or the infracarinalis tendon	Fin rays distal of their bases
Pelvic	Adductor profundus pelvicus	Lies immediately above the pelvis	Dorsal side of pelvis	Anterolateral bases of fin rays
Pelvic	Arrector dorsalis pelvicus		Dorsolateral face of the pelvis	Dorsolateral surface of medial half of the first fin ray or pelvic spine when present
Pelvic	Extensor proprius	Dorsal most muscle of the pelvic musculature, lies above the adductor superficialis	Anterolateral surface of the dorsal part of the pelvis	Posteromedially to variable number of inner pelvic fin rays, when present.
Pelvic	Other muscles present in some teleosts are radialis ventralis, abductor accessorius, and arrector tertius			

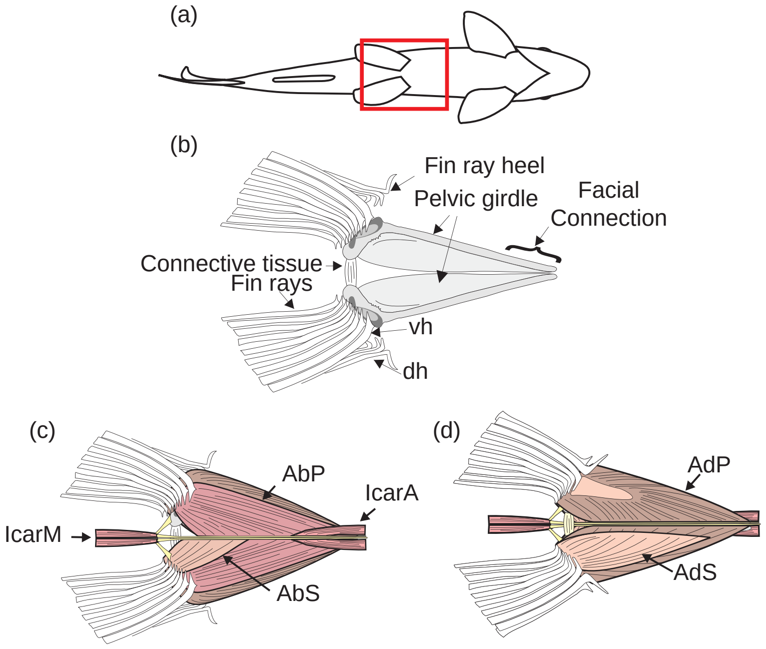


Figure 5 Pelvic fin anatomy. (a) Ventral view of trout. (b) The bony structure of the pelvic girdle. Ventral hemitrichia (vh) and dorsal hemitrichia (dh) make up the lepidotrichia of the fin rays. (c) Infracarinalis (Icar) muscles run midline along the ventral surface of the body attaching to the pelvic girdle and the infracarinalis anterior (IcarA). The abductor superficialis (AbS) and abductor profundus (AbP) muscles originate along the midline fascia and tendon of the Icar muscles and the pelvic girdle. The AbP attaches to the heel of the processes of all vh and the AbS attaches to the tips of the processes. (d) The adductor profundus (AdP) originates across the dorsal side of the pelvic girdle and along the midline fascia. This muscle is thicker on its medial and lateral edges. The adductor superficialis (AdS) lies in the cavity of the adductor profundus made by the thinning of the profundus muscle. The AbS originates on the pelvic girdle through the AdP muscle. Both adductor muscles insert on the processes of the dh. Modified from Standen EM (2010) Muscle activity and hydrodynamic function of pelvic fins in trout *(Oncorhynchus mykiss)*. *Journal of Experimental Biology* 213: 831–841.

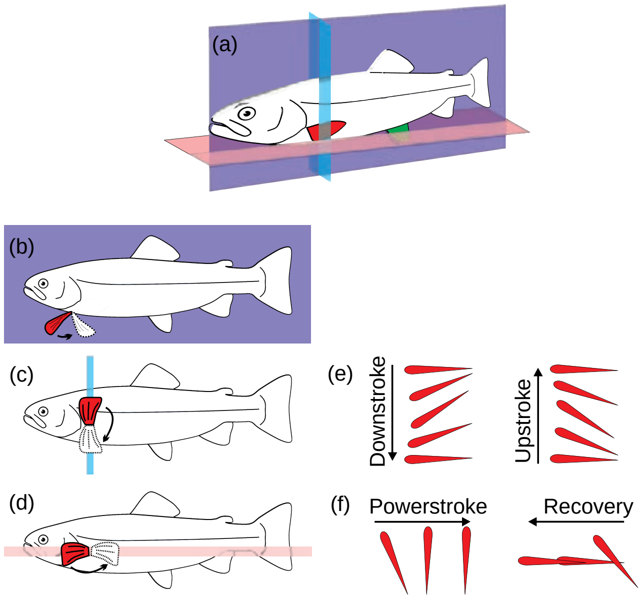


Figure 6 (a) Fish fin motion in three planes. Purple represents the fish's sagittal plane; (b) motion in this plane is considered paddling where the fin pivots under the body of the fish. Cyan represents the fish's transverse plane; (c) motion in this plane is considered flapping where the fin moves up and down at the side of the fish. Pink represents the fish's frontal plane; (d) motion in this plan is considered rowing where the fin moves back and forth at the side of the fish. (e) Subtle angle changes in the fin surface are present during flapping so that lift is created on both up- and downstrokes. (f) Rowing fins have a distinct power stroke where the fin is held perpendicular to the flow to maximize thrust and then feathered during recovery to minimize drag. Modified from Standen EM (2008) Pelvic fin locomotor function in fishes: Three-dimensional kinematics in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 211: 2931–2942.

speed at which an animal transitions from pectoral fin swimming to body caudal fin swimming is called $U_{\text{p-crit}}$ and can be used to compare swimming speeds among species of animals that have clear gait transitions. $U_{\text{p-crit}}$ varies among fish species and increases with body size.

Fin-beat frequency increases exponentially as speeds increase, peaking at gait transition. Some fish, such as the surfperch (*Embiotoca lateralis*), have a gait with a break between fin beats; fins produce thrust and then the fish glides for a portion of the stroke. In many fish studied, fin amplitude increases slowly at slow and intermediate speeds and plateaus at high speeds.

The stroke plane is the plane in which a fin moves during oscillation. Different species of fish tend to swim with different stroke-plane angles. A steep downstroke for thrust production has a large vertical force component. Shifts in stroke angle from negative angles of attack produce a downward backward force, while shifts toward a positive angle of attack create an upward forward force. Swimmers that combine flapping and rowing can decrease or increase the steepness of their stroke plane as swimming speed increases. Research on extreme flappers (*Gomphosus varius*) and extreme rowers (*Gasterosteus aculeatus*) has shown that stroke angle does not change with speed.

Switching gaits is useful to fish. It allows them to swim at different speeds by utilizing different types of muscle fibers, which makes swimming speed faster but not necessarily more efficient (reducing the amount of energy the fish uses to swim). Different fish species have different combinations of muscle fiber types in their pectoral fins: red fibers that are slow oxidative fibers, pink intermediate fibers, and white fast glycolitic fibers. Generally, slow oxidative fibers operate aerobically using less energy and can be used for endurance swimming. Fast-glycolitic fibers operate anaerobically, use a great deal of energy and give large amounts of power for very short bursts of speed; however, they quickly run out of energy and require rest periods.

Muscle fiber types in fish fins have not been extensively studied, but it appears that fish that swim with primarily a pectoral fin gait have pectoral fins made up of mostly red slow oxidative muscle fibers. In contrast to species of fish that swim aerobically using a body caudal fin gait, some labriform swimmers have only fast-glycolitic body musculature. Thus, when they change from a pectoral fin gait to a body caudal fin gait, they are also switching to swimming anaerobically and therefore cannot sustain this gait for a long time.

So, if one is a labriform swimmer and uses less energy to swim with aerobic muscle fibers only, why switch to body caudal fin swimming? Aerobic muscles (pectoral fin or otherwise) have a limit to the speed that they can lessen with peak mechanical output. This means that at a certain contraction speed the muscles do not provide enough force and the body muscle (usually in the form of white glycolitic fibers) needs to be recruited to swim faster. Work on parrot fish (*Scarus schlegeli*) shows that using body musculature gives them the power they need but it is more costly than if they could use their pectoral fins. A similar pattern is seen in bluegill sunfish (*Lepomis macrochirus*), where the maximum power output of the pectoral fin muscles occurs at the gait transition from pectoral fin swimming to body caudal fin swimming. Cost of transport, measured using respirometry, increases when bluegill switch from pectoral fin swimming to body caudal fin swimming. This suggests that using body caudal fin swimming is not more efficient but is simply required to reach maximum speeds.

Pectoral fins can also be used to aid in terrestrial locomotion of fishes. Tropical catfishes (e.g., *Clarias batrachus*) can use their pectoral fins to walk on land, a feat refined in the running behavior of mudskippers (*Gobiidae, Oxudercinae*) over mangrove flats during low tide.

Pelvic Fins

Pelvic fins, although little studied, are of great interest as they are one of the most morphologically plastic structures within the fishes. The function of the pelvic fins is as variable as their morphology between fish. They are used in swimming as an aerofoil, brake, propulsor, and rudder, for ground walking, and for hovering. At the same time, pelvic fins can be spined and used for protection, contain gases, and be used for floatation, sensory perception, camouflage, a holdfast organ, and for reproduction.

Although early studies that amputated pelvic fins and recorded no change in swimming ability concluded that pelvic fins had limited or no locomotory function, recent work has suggested otherwise. Slow-speed swimming experiments have shown that trout actively oscillate their fins in a contralateral pattern. This oscillation alters the flow conditions along the ventral aspect of the fish, possibly acting to stabilize the fish and influence the hydrodynamic function of downstream fins. Pelvic fins are also active during yawing maneuvers in trout species.

Like pectoral fins, pelvic fins are often tucked against the body during moderate- and high-speed steady swimming to reduce drag, possibly leading to their earlier reputation of having little to no locomotory function.

In some highly derived species, pelvic fins have developed into locomotory structures for punting along the ocean floor (skates and rays), walking on the substrate (epaulette sharks (*Hemiscyllium ocellatum*) frog and toad fishes (*Lophiiformes, Antennariidae*)), and slow-speed hovering oscillations (coelacanth), to name a few.

Maneuvering

Pectoral and pelvic fins are used by both cartilaginous and bony fishes for all types of maneuvers: yawing, pitching, acceleration, braking, backward swimming, and hovering. The versatility of paired fins becomes evident in the diversity and complexity of motion that these fins may undergo. Most of our understanding is of pectoral fins; however, recent work has given us new insight into pelvic fin motion as well.

Yawing

There are different turning strategies among fishes. Some fish (balistiform swimmers) suspend pectoral fin motion on the inside of the turn while continuing to steadily swim with the opposite side pectoral fin. Axial undulating fishes such as tetraodontiformes suspend the pectoral fin motion on the outside of the turn and hold the inside pectoral fin abducted for a pivot. Labriform, coelacanthiform, and ostraciiform swimmers move both pectoral fins, but in a distinct way compared with steady swimming. Rainbow trout (*Oncorhynchus mykiss*) initiate a turn by a series of rapid pectoral fin abductions and elevations on the inside of the turn followed by a similar but slower motion of the opposite fin to help complete the turn. Their pelvic fins also help control body position during yawing; the inside fin abducting and acting as an initial pivot to help turn the body with a later outside fin abduction to help realign the body with the flow.

Hovering

Fish have to balance forces during hovering; they must produce lift to stay elevated in the water column and balance fore and aft forces. During hovering, rainbow trout use an out-of-phase sculling pattern of both pectoral and pelvic fins to keep their body stationary. Both sets of paired fins contralaterally oscillate by adducting fins on one side and then the other. In addition, fish time the oscillation of fin pairs such that when the left pectoral fin is adducted, the right pelvic is adducting and vice versa. This somewhat synchronous motion appears to balance forces produced on either side of the body contributing to stability. Similarly, bass (*Centrarchidae*) and other acanthomorph fishes counter-oscillate pectoral fins for hovering. Balistiform swimmers use a dorso-ventral propagation of waves along the pectoral fins and this balances lift force and aft forces during hovering.

Braking

During braking, rainbow trout and more derived teleosts upturn the posterior edge of both pectoral fins simultaneously. This simultaneous motion maximizes drag and slows the forward progress of the animal without inducing a yawing moment; pitching moments tend to be counteracted by flaring of the pelvic fins. Although both pectoral fins abduct at the same time shorter fish tend to make symmetrical fin motions, while longer fish have asymmetrical fin motions. Both the rate of pectoral fin protraction and area of the fins have considerable impact on the braking performance of a fish. Fishes with partially ablated pectoral fins simply protract their fins faster to obtain a comparable level of performance, highlighting the importance of both behavior and morphology of the fins.

Fin Control

The muscles that control the fins are very diverse between species and only a fraction of fish species have been thoroughly described. Generally, adductors and abductors are used to affect the upstroke and downstroke of pectoral fins, respectively. Adductors and abductors not only have similar function on the pelvic fins, but also act in combination to produce a flaring of the fin. Fin muscle mass is proportional to force generation ability, and the ratio of muscle mass between adductors and abductors varies between species and changes the way the fins are used to produce forces.

Muscle motor activity of adductors and abductors in pectoral fins suggests that these muscles act antagonistically and alternate their activity through the stroke cycle (**Figure 7**). Pelvic fin adductor and abductor muscles are active in slowly swimming trout driving a contralateral fin oscillation. Interestingly, contrary to the antagonistic muscle action of the pectoral fins, the adductors and abductors of the pelvic fins are active at the same time, affecting fin abduction and arrection during the cycle (**Figure 7**).

Hydrodynamics

Thrust can be generated in many ways during swimming. Viscous stresses, bound circulation, flow separation, fin–vortex interaction, acceleration–reaction forces, and jet-like squeezing forces, all can produce thrust forces. Visualizing flow helps quantify and define forces produced by fins (**see also Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics, Maneuverability, Stability and Turbulence, and Undulatory Swimming).

Fins that are long from base to tip and narrow crosswise have a high aspect ratio and are effective at producing lift. Fins that are shorter from base to tip and wide crosswise have a lower aspect ratio and tend to be used for drag-based propulsion.

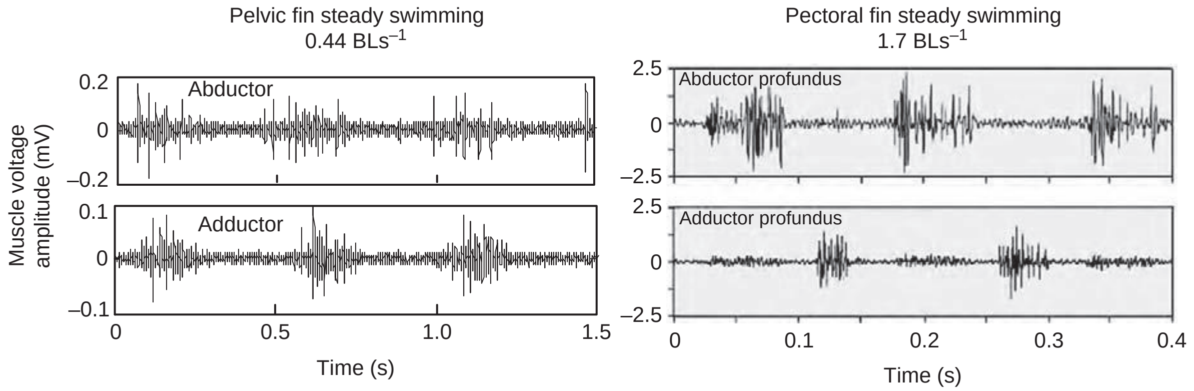


Figure 7 Paired fin muscle activity patterns. Pelvic fin abductors and adductors contract at the same time acting to abduct and arrect the fin during the oscillation cycle. This activation pattern suggests that adductor and abductor muscles work together to produce unique fin motion. In paired pectoral fins, the adductor and abductor operate one after the other, contributing to a clear adduction and abduction. Pelvic fin data from trout (*Oncorhynchus mykiss*) swimming at 0.44 BL s^{-1}, modified from Standen EM (2010) Muscle activity and hydrodynamic function of pelvic fins in trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 213: 831–841; pectoral fin data from the bird wrasse (*Gomphosus varius*), adapted with permission from Westneat MW and Walker JA (1997) Motor patterns of labriform locomotion: Kinematic and electromyographic analysis of pectoral fin swimming in the labrid fish *Gomphosus varius*. *Journal of Experimental Biology* 200: 1881–1893 (www.jeb.biologists.org).

Sharks and sturgeons have relatively passive pectoral fins that produce vortical wake structures from their trailing edges. Sharks can change the angle of their pectoral fins relative to the oncoming flow, thus changing the fin's angle of attack and the resulting fin wake structures. Because the pectoral fins are located in front of the center of mass, lift and drag forces produced by the fins result in pitching moments. Positive angles of attack result in an upward pitch in the water column and negative angles result in a downward pitch. If opposite fins are held with opposite angles of attack, the shark induces a rolling instability, facilitating a turning moment.

Rowing fins move in the sagittal plane and have a distinct thrust phase where the fin is held perpendicular to the water and adducted against the water (this is a drag-based method of producing thrust). The thrust phase is followed by a recovery stroke where the fin is abducted back to the initial position. Flapping fins move in the transverse plane and, by angling the fin relative to the flow, use lift to produce thrust in both the up- and downstrokes. Although it is useful to categorize motion in terms of rowing and flapping, the fish world does not have exclusively rowers or flappers; fish use both lift and drag (a combination of flapping and rowing) to produce forces during swimming. The relative contributions of lift and drag can change between species and within species depending on swimming speed.

In bluegill sunfish, a labriform swimmer, paired pectoral fin downstroke produces a wake structure composed of a vortex ring with a central jet pointing both laterally and downstream (**Figure 8**). As swimming

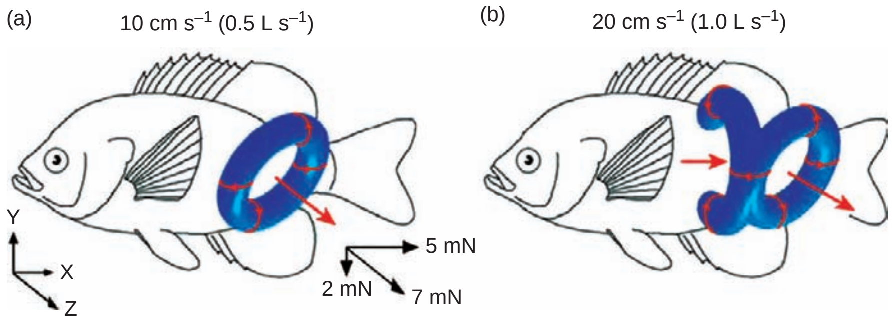

(a)
10 cm s^{-1} (0.5 L s^{-1})
(b)
20 cm s^{-1} (1.0 L s^{-1})
Y
X
Z
5 mN
2 mN
7 mN

Figure 8 Pectoral fin wake of bluegill sunfish (*Lepomis macrochirus*) reconstructed using quantitative flow visualization. (a) At low swimming speeds (e.g., 0.5 body length (L) per second), the pectoral fin wake consists of a single vortex ring produced per fin beat cycle. (b) At higher speeds, the wake is composed of one complete vortex ring generated on the downstroke of the pectoral fin and a linked vortex filament produced by the upstroke, which terminates on the flank of the fish to form a second, incomplete ring attached to the body. In these schematic reconstructions of wake morphology, curved red arrows represent centers of vorticity observed in perpendicular flow fields, and straight red arrows indicate the direction of jet flow through the center of each vortex ring. Locomotor forces (shown per fin) derived from wake momentum flux are resolved into thrust, lift, and laterally directed components. From figure 10.12 in Drucker E, Walker JA, and Westneat MW (2006) Mechanics of pectoral fin swimming in fishes. In: Shadwick RE and Lauder GV (eds.) *Fish Biomechanics*, vol. 23, pp. 369–424. London: Elsevier Academic Press.

speed increases, both downstroke and upstroke have associated jets (linked vortex rings). Wake size and vorticity strength increase with swimming speed in these labriform swimmers. These jets produce forces which counteract the drag associated with swimming at higher speeds. There are interspecies variations in wake structure as would be expected. Bluegills have a single vortex ring at 50% U_{pcrit} and as speed increases the jet orients more laterally. In contrast, *Embiotoca* sp. has two linked vortex rings and as speed increases they direct their jets downstream. To learn more about how modeling of hydrodynamics has helped design robotic fish fins, the reader is referred to **Buoyancy, Locomotion, and Movement in Fishes:** Biomimetics: Robotics Based on Fish Swimming.

Maneuvering Hydrodynamics

Fish have to balance three-dimensional hydrodynamic forces to control their body position. Depending on the location of the fish's center of mass relative to its center of buoyancy (**Figure 2**; **see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes and Swimbladder Function and Buoyancy Control in Fishes) different fish will experience different instabilities. Differences in the location of fins on a fish's body will determine how forces produced by those fins affect the fish's body.

Yawing

Lateral yawing turns are elicited by an asymmetrical production of forces around the fish's center of mass. Generally, greater thrust force is produced by the fin on the outside of the turn, while a braking force is produced by the fin on the inside of the turn. Depending on the species of fish, this combination of forces can pivot the animal in a very tight turn or a more gradual cruising turn. Studies have shown that fish with vertical pectoral fin placement produce greater lateral forces for turning than fish with ventrally located pectoral fins. For example, during slow turning, vertically oriented bluegill fins produce greater lateral yawing forces per unit of fin area compared with the horizontally oriented fins of rainbow trout.

Forces produced by fins during yawing are also greater than during steady swimming. The fin on the outside of the turn in yawing acanthomorph fishes produces 4 times greater lateral forces than during steady swimming and the inside fin generates posteriorly oriented vortex rings with a thrust force 9 times greater than steady swimming. This combination of forces pivots the fish and translates the fish toward the inside of the turn.

Hovering

Trout station hold by altering anterior–posterior directed wake flows. The forces cancel out over the cycle.

Braking

Both trout and bluegill produce anterior force during braking; the trout's line of action falls below the center of mass causing a downward pitching action, while the bluegill's braking line of action goes through the center of mass so there is little to no pitch destabilization.

See also: **Brain and Nervous System**: Motor Control Systems of Fish. **Buoyancy, Locomotion, and Movement in Fishes**: Biomimetics: Robotics Based on Fish Swimming; Buoyancy in Fishes; Experimental Hydrodynamics; Maneuverability; Swimbladder Function and Buoyancy Control in Fishes; Stability and Turbulence; Undulatory Swimming. **The Skeleton**: Bony Fish Skeleton; Cartilaginous Fish Skeletal Anatomy.

Further Reading

Alben S, Madden P, and Lauder GV (2007) The mechanics of active fin-shape control in ray-finned fishes. *Journal of the Royal Society Interface* 4: 243–256.

Arreola VI and Westneat MW (1996) Mechanics of propulsion by multiple fins: Kinematics of aquatic locomotion in the burrfish. *Proceedings of the Royal Society B London: Biological Sciences.* 263: 1689–1696.

Blake R (1983) Median and paired fin propulsion. In: Webb PW and Weihs D (eds.) *Fish Biomechanics*, pp. 214–247. New York: Praeger.

Daniel TL (1988) Forward flapping flight from flexible fins. *Canadian Journal of Zoology* 66: 630–638.

Drucker E and Lauder GV (2002) Wake dynamics and locomotor function in fishes: Interpreting evolutionary patterns in pectoral fin design. *Integrative and Comparative Biology* 42: 997–1008.

Drucker EG and Lauder GV (2000) A hydrodynamic analysis of fish swimming speed: wake structure and locomotor force in slow and fast labriform swimmers. *Journal of Experimental Biology* 203: 2379–2393.

Drucker EG and Lauder GV (2001) Wake dynamics and fluid forces of turning maneuvers in sunfish. *Journal of Experimental Biology* 204: 431–442.

Drucker E, Walker JA, and Westneat MW (2006) Mechanics of pectoral fin swimming in fishes. In: Shadwick RE and Lauder GV (eds.) *Fish Biomechanics,* vol. 23, pp. 369–424. London: Elsevier Academic Press.

Geerlink PJ and Videler JJ (1987) The relation between structure and bending properties of teleost fin rays. *Netherlands Journal of Zoology* 37: 59–80.

Higham TE, Malas B, Jayne BC, and Lauder GV (2005) Constraints on starting and stopping: Behavior compensates for reduced pectoral fin area during braking of the bluegill sunfish Lepomis macrochirus. *Journal of Experimental Biology* 208: 4735–4746.

Nelson JS (1989) Analysis of the multiple occurrence of pelvic fin absence in extant fishes. *Matsya* 15–16: 21–38.

Rosenberger L (2001) Pectoral fin locomotion in batoid fishes: Undulation versus oscillation. *Journal of Experimental Biology* 204: 379–394.

Standen EM (2008) Pelvic fin locomotor function in fishes: Three-dimensional kinematics in rainbow trout. (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 211: 2931–2942.

Standen EM and Lauder GV (2005) Dorsal and anal fin function in bluegill sunfish *Lepomis macrochirus:* Three-dimensional kinematics during propulsion and maneuvering. *Journal of Experimental Biology* 208: 2753–2763.

Thorsen DH and Westneat MW (2005) Diversity of pectoral fin structure and function in fishes with labriform propulsion. *Journal of Morphology* 263: 133–150.

Walker JA and Westneat MW (2002) Kinematics, dynamics, and energetics of rowing and flapping propulsion in fishes. *Integrative and Comparative Biology* 42: 1032–1043 (10.1093/icb/42.5.1032).

Westneat MW and Walker JA (1997) Motor patterns of labriform locomotion: Kinematic and electromyographic analysis of pectoral fin swimming in the labrid fish. *Gomphosus varius. Journal of Experimental Biology* 200: 1881–1893.

Wilga CD and Lauder GV (1999) Locomotion in sturgeon: Function of the pectoral fins. *Journal of Experimental Biology* 202: 2413–2432.

Wilga CD and Lauder GV (2004) Biomechanics of locomotion in sharks, rays and chimeras. In: Carrier JC, Musick JA, and Heithaus MR (eds.) *Biology of Sharks and their Relatives*,p. 616. Washington, DC: CRC Press.

Winterbottom R (1974) Descriptive synonymy of the striated muscles of the Teleostei. *Proceedings of the Academy of Natural Sciences Philadelphia* 125: 225–317.

Maneuverability

PW Webb, University of Michigan, Ann Arbor, MI, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Definitions of Maneuvers and Agility
Methodology
Effectors
Types of Maneuvers
Fish Size
Body Form
Further Reading

Glossary

Agility The rate of maneuver.

Gait Combinations of swimming motions and muscle-usage characteristic of swimming at various speeds.

Gait transitions The change from one swimming gait to another over a narrow range of speeds.

Heave A vertical movement.

Maneuvers Intentional changes in body posture and/or swimming trajectory controlled by a fish.

Minimum turning radius The smallest radius at which a fish can turn during a maneuver.

Pitch Vertical, head up/down rotation.

Reynolds number The ratio of inertial to viscous forces in a flow.

Roll Rotation about the head-to-tail longitudinal axis.

Routine or volitional swimming Locomotor behavior during normal activity in the absence of imposed controlling conditions.

Slip A lateral movement.

Speed–maneuver limit A boundary at which the mechanical energy for locomotion in a gait reaches a maximum, at which changes in speed and maneuvering must be inversely related.

Stability The control of posture and/or trajectory.

State The description of a system, here a fish, at some instant in terms of its position and velocity.

Surge An anterior–posterior movement.

Translational maneuver A maneuver involving change in speed without change in direction.

Yaw Left/right lateral rotation.

Introduction

The survival, growth, and reproductive success of fishes all hinge on successful interactions with a myriad of internal and external stimuli (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy, Locomotion, and Movement in Fishes: An Introduction). Responses to such stimuli usually involve some locomotor component, expressed in a variety of motions generally recognized as maneuvers. Turning maneuvers are common, and obviously essential, in spite of being energetically expensive, increasing swimming costs by over an order of magnitude compared with those for rectilinear swimming at the same average speed.

Studying maneuvers has proved to be challenging. Among ≈30 000 species of fishes, there is a wide range of maneuvers, many of which can be executed with different combinations of body and fin motions while swimming over a wide range of speeds. Recording maneuvers under controlled conditions is problematic. Consequently, information is incomplete and ideas on factors affecting maneuverability are often speculative.

Definitions of Maneuvers and Agility

Maneuvers are volitional whole-body changes in state. State is defined in terms of velocity. Changes in state result from combinations of rotational, linear, and centripetal accelerations, leading to one or more changes in posture, speed, or direction. Maneuvers are the opposite of stability. The same principles apply to both, with the difference that maneuvers are induced displacements while stability requires the correction of involuntary displacements (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence).

Changes in state are resolved about the center of mass with components for three rotational axes and for three translational planes (**Figure 1**). Rotational changes are pitch (vertical, head up/down rotation), yaw (left/right lateral rotation), and roll (rotation about the head-to-tail longitudinal axis). Translational changes in state are heave (vertical displacement), slip (lateral displacement), and surge (anterior–posterior displacement). Rotation results in changes in body posture, which, when combined

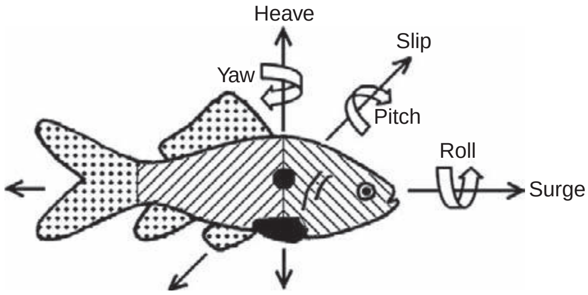


Figure 1 Maneuvers and stability control are mirror images of each other. Both maneuvers and displacements may occur along three translational axes (slip, heave, and surge) and three rotational axes (yaw, roll, and pitch), defined around the center of mass (filled circle). Control surfaces driving maneuvers and controlling stability involve the body (cross-hatched) with the anterior and head being destabilizing, and used for rapid steering turns. The pectoral fins (solid shading) anterior to the center of mass similarly can steer fast turns. The body and the median and paired fins (MPFs) (stippled) posterior to the center of mass can be used for steering and trimming. The body and fins may also beat to drive powered maneuvers and for powered stability control.

with translation, results in turns. Fish usually swim forward, but can also swim backward.

In addition to being changes of state, maneuvers are executed at various rates. Fishes that can execute maneuvers at higher rates are termed more agile. Rates of turning are usually described in terms of degrees per second, as described above for yawing rotations. Angular velocities are determined largely by swimming speed.

There is some debate as to the appropriate measure of size for comparing turning radii of maneuvers among fishes. The most common measure of size is a characteristic length – body length, depth, or width. Comparisons among fishes are usually made by normalizing performance with a fish total length. However, maneuvering performance is also expected to be affected by body shape. It has been suggested that body volume is a more useful measure of fish size, and volume is conveniently numerically equal to mass for neutrally buoyant fishes. Volume is considered limiting because it determines the amount of muscle that can be carried, space for digestive and other systems, and room to produce and store gametes. As such, volume$^{1/3}$ has also been used as a length scale. The ranking of maneuvering performance among species can be affected by the length scale used to normalize data.

Methodology

There are few common methods and no common protocols for measuring maneuverability. Maneuvers are usually analyzed from video or film records of routine swimming, especially during activities such as agonistic and predator–prey interactions that are expected to require high levels of maneuvering performance. Attempts to experimentally force fishes to maneuver in controlled and repeatable manners have proved to be challenging.

Maneuvering performance is quantified in various ways. Turning radius is calculated for turns, and performance is inversely proportional to turning radius. Agility is measured as rotation rates during hovering. During translocation, swimming speed is measured, which, with turning radius, determines angular velocities and centripetal acceleration. However, acceleration rates have not often been reported, except for fast-starts and braking. For these measures of maneuverability, performance is proportional to agility. The volume swept out by the body of a fish during a maneuver has also been considered as a measure of maneuvering performance.

Ideally, locomotor performance is evaluated at known physiological–mechanical limits occurring at gait transitions. Executing a maneuver diverts forces and energy from translocation, so that any maneuver at a gait transient is expected to reduce speed. Furthermore, the smaller the radius of a turn or the more agile the maneuver, the greater would be the reduction in the overall, net speed of swimming. Therefore, maneuvering performance should be expressed as functions of swimming speed for each gait for gait transitions; however, such a response surface is lacking for any maneuver.

Fishes may perform any maneuver up to the boundary set by the speed–maneuver limit. Many maneuvers are measured during routine swimming such as feeding and agonistic interactions presumed to require maximum performance, an assumption that is difficult to verify. Some studies hope to capture maximum performance by reporting results for the 20% highest-performance maneuvers during such activities.

Of all maneuvers, fast-starts or surging yawing turns have received most attention, and superficially would appear to be maximum, repeatable behaviors, whether induced during interactions such as predation situations or through artificial stimuli (**see also Buoyancy, Locomotion, and Movement in Fishes**: Fast Start). In practice, several methods have been used to induce fast-starts, and results for response variables, such as response duration, turning angle, turning radius, acceleration rate and speed, are affected by the method employed.

Effectors

Maneuvers are executed using the body and all the median and paired fins (MPFs) (**Figure 1**), with occasional contributions from respiratory water jets and hydrostatic forces. As with control systems for stability, the body and fins are dynamic effectors working in two ways. First, steering (trimming) effectors create maneuvering forces as a result of water flow over their surfaces during translation of the body to which the control surface is attached. Second, powered effectors generate flows with motions independent of body translation.

In steering, effectors anterior to the center of mass, notably the head and pectoral fins, can drive maneuvers at high rates (**Figure 1**). The forces acting on these surfaces increase rapidly with time, amplifying the maneuver. Control surfaces downstream from the center of mass – the posterior body, paired pelvic fins, dorsal, anal, and caudal fins – are not self-amplifying and hence tend to steer maneuvers, but with lower agility. The caudal fin is the most important of these steering surfaces being both large and most posterior to the center of mass.

For powered maneuvers, effectors are also propulsors, and all of them can power maneuvers with high agility. Powered maneuvers are executed by varying the stroke rate, the plane in which a propulsor beats, or by recruiting additional effectors. For example, a yawing turn can be executed by the caudal fin beating asymmetrically or by recruiting pectoral fin propulsors.

During swimming, steering and powered effectors often work together, especially at higher speeds. For example, extension of a pectoral fin may be used in a yawing turn while swimming with the caudal fin, or the caudal fin may help steer turns during swimming with the MPFs.

Types of Maneuvers

Many fishes rotate to change body posture while hovering using powered MPF effectors. Rotation while hovering always involves powered effectors. It is often associated with searching and vigilance maneuvers, for example, yawing and pitching to look for prey, especially when feeding on prey silhouetted against the water surface. Fish may roll to use refuges. Yawing rotation rates of 140–180° s^{-1} have been reported.

Powered effectors are also essential during slow swimming. The lower range of swimming speeds is powered by the MPF gaits (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming). Powered maneuvers are common because steering forces are proportional to the square of swimming speed and hence cannot be large at low speeds. Minimum yawing turning radii approach zero at low speeds with a value of 0.05 L (where L is the total length of the fish), at 107° s^{-1} being reported for a spotted boxfish, *Ostracion meleagris*. For other teleosts, yawing turning radii of 0.3–0.7 L at 100–2000° s^{-1} have been reported at maximum MPF swimming speeds of 0.7–2 L s^{-1}. Stiff-bodied fishes, such as flatfishes (order Pleuronectiformes) and rays (superorder Batoidea) that maneuver using long-based dorsal–anal and pectoral fins, respectively, have lower yawing turning performance, with mean radii ≈1 L and rates of 30–44° s^{-1}. Mean radii of pitching turns have been recorded of 0.08–0.16 L for some reef fishes.

Fishes may swim at low speeds with a pitched body posture called tilting. Such tilting is postulated to reduce net swimming costs. At low speeds, locomotor muscle would work at low strain rates when efficiency is small (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). Tilting increases drag allowing the muscle to work at high strain rates. The metabolic costs of overcoming the higher drag are offset by the savings in muscle efficiency.

Backward swimming is most commonly seen in bony fishes during MPF swimming with speeds reported from –0.4 to –3.9 L s^{-1}. Yawing turns while swimming backward have been described for angelfish, *Pterophyllum scalare*, with a radius of 0.7 L, very much larger than forward-swimming turns, and at a speed of 0.9 L s^{-1}, lower than typical routine forward-swimming speeds.

Higher swimming speeds are powered by body and caudal fin motions (BCF gaits) and it is likely that maneuvers combine powered forces and steering (**see also Buoyancy, Locomotion, and Movement in Fishes**: Stability and Turbulence). Faster turns are steered by the head and pectoral fins, while slower steered turns involve the posterior body and median fins. Body and fin effectors power smaller-radius maneuvers. Yawing turns are especially prominent during routine swimming reflecting the locations of median fins and body compression in the vertical plane. Yawing turns are usually made through large angles, sometimes even exceeding 360°. Pitching maneuvers involve depth changes and are usually steered with the pectoral fins typically through <20°. Rolls are least common among rotational maneuvers, probably because they reduce the effectiveness of camouflage and of sensory systems. Like pitching maneuvers, rolling maneuvers are usually of small magnitude, <20°. Rolls may be steered by asymmetrical deployment of the fins as fishes inspect refuges or prey and build nests, while more-rigid fishes bank in turns, combining yawing and rolling.

Most observations of turning maneuvers during BCF swimming are for the lower portion of the speed range powered by slow oxidative muscle. Reported radii in yawing turns range from 0.01 to 0.8 L (600–10 000° s^{-1}) for swimming speeds up to 6 L s^{-1}. Centripetal forces required for turning are high at higher speeds. However, BCF effectors develop large forces and torques because a large muscle mass powers large body and fin areas to generate the requisite forces and torques. Due to higher swimming speeds in BCF gaits, agility is higher than that in MPF swimming.

Elongate fishes swim backward during BCF swimming. Values reported for eels around $-0.8\,\mathrm{L\,s^{-1}}$ are comparable to routine forward speeds. Some fish swim backward by bending the caudal fin to beat while pointing forward, propelling a fish backward. Backward BCF swimming is much less efficient than forward BCF swimming.

Maneuvering usually conjures up images of turning, but translational maneuvers are major factors in behavior. The best-known translational maneuver is intermittent swimming, alternating a powered swimming phase with an unpowered phase (one of coasting, a decelerating surge at constant depth; gliding, a decelerating surge and heave by negatively buoyant fish sinking through the water column; and a jump through the air). The intermittent swimming pattern saves energy because the powered phase requires at least 3 times more power than that expended during the unpowered phase.

Surges also occur during braking. In routine swimming, braking is characteristic of saltatory search for food, a repeating sequence of swimming to an observation site, slowing to a stop, and hovering while visually searching for food. Fish can brake during active swimming by modulating propulsor beating rates and/or directions, or by reversing the direction of propulsive motions. Braking may also be unpowered, with drag being increased by extension of the fins. Unpowered braking rates have been reported from 0.1 to 1 × the acceleration of the Earth's gravity or -5 to $-155\,\mathrm{L\,s^{-2}}$.

Perhaps the best-studied maneuver is the fast-start, a large-amplitude tail beat with the body bending first to one side and then the other. Fast-starts are common in predator and prey interactions and in recovering from control-system failures, in rapid or turbulent flows, as well. Fast-starts are acceleration surges at rates from 1 to 15 × the acceleration of the Earth's gravity. They commonly include a yawing turn with average turning radii from 0.01 to 0.5 L (**see also Buoyancy, Locomotion, and Movement in Fishes**: Fast Start).

Rotational- and translational-state changes are often combined as fishes commonly change speed while turning. As noted, fast-starts are a combination of high rates of surge and a yawing turn. Agonistic interactions, courtship, and mating involve combined translational and rotational components in a variety of displays.

Fish Size

Size affects maneuverability. During ontogeny, fishes range in size from larvae, 2–3 mm in length, to adult tuna and sharks that can exceed 3 m in length. The mechanisms driving maneuvers depend on Reynolds number, *Re*, an estimate of the ratio of viscous forces to inertial forces (**see also Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics). Numerically, $Re = \rho L u / \mu$, where ρ is the density of water, L is a characteristic length, u is the swimming speed, and μ is the viscosity. At small *Re*, for small fishes swimming at low speeds, viscous drag forces dominate. At high *Re*, inertial forces dominate, and drag and lift forces used for steering and powered maneuvering can be large. Hence, options for maneuvering are reduced for larvae working at small *Re* compared with larger juveniles and adults.

The fins of larvae work at very small *Re*, and hence are unable to generate significant maneuvering forces. Therefore, maneuvering by larvae depends on a flexible tail that can generate forces to maneuver in any direction. Consequently, the body forms of larvae are most similar among species. Body-fin plans diverge during metamorphosis to juvenile and adult body-fin plans as fishes and their fins and function at larger *Re*. Larvae also cannot use intermittent swimming until they grow large enough to coast, a maneuver that is only possible at large *Re*.

Radii of turns tend to be proportional to fish size. In terrestrial and aerial animals, this is an important factor in predator–prey chases, when the typically smaller prey can escape a larger predator by maneuvering inside the predator's turning circle. The principle is theoretically applicable to fishes. In practice, turning radii are so small that such tactics are rarely an option. The timing of escape maneuvers may be more important than the spatial component of maneuvers.

Agility is also affected by fish size. Absolute swimming speeds increase with size, as will maximum centripetal acceleration. Size-specific swimming speeds, in $\mathrm{L\ s^{-1}}$, decrease with size so that for their size, small fishes are more agile, an observation equally well known by humans trying to retrieve small children. For large fishes, low size-specific maneuverability and agility affect feeding patterns, with predation in the largest of aquatic organisms involving group behaviors and/or diet changes to nonevasive foods.

Braking also becomes more difficult with increasing size. Drag that causes deceleration is proportional area of the body and fins while inertia sustaining a motion is a volume force. As a result, the inertia of a fish increases more rapidly with size than the drag force so that braking rates should vary with $\mathrm{L^{-1}}$. However, braking rates range from 0.1 to 1 × acceleration of the Earth's gravity for the fish over a range of sizes because the mechanisms used to brake also vary with fish size. Small fishes brake using median-fin extension to increase friction drag, while larger fish extend the median fins normal to the flow to generate much larger pressure drag including bending the body, and even bending the tail so that the tip projects forward.

Body Form

The shapes of fishes are extremely diverse, but all must drive a wide range of maneuvers. The earliest fishes, as with fish larvae, lacked control surfaces other than the tail, and hence a flexible posterior body must have provided the ability to orient control forces in any direction. Modern fishes, such as eels, with reduced fins still maneuver in this manner.

Control surfaces evolved early in fish evolution for stability. However, even a small ability to vary angles of attack of these stiff control surfaces would have converted them into steering effectors. This condition is seen in many modern elasmobranchs, which are typically less maneuverable than typical bony fishes. Such modern elasmobranchs with stiff fins retain high caudal-peduncle flexibility so that the tail continues as a major trimming and powered effector for maneuvers.

A major change in maneuverability occurred with the evolution of rayed fins. The highly flexible fin structure of actinopterygian fishes provides versatile control surfaces that can generate a large diversity of forces for trimming and powered maneuvers. Compared to less-derived fishes, the flexibility and distribution of actinopterygian MPFs provide multiple effectors that increase the range of maneuvers, especially at low speeds, while minimizing turning radii and the volume swept out. Truncated bodies are common among ray-finned fishes that minimize rotational resistance.

The importance of body-fin form for maneuverability can be shown across large systematic and morphological differences, such as among actinopterygians versus less-derived fishes, and it reflects the form of body and fins used for propulsion. Among teleosts, the highly flexible body form of eels is superior in maneuvering in burrowing-type situations such as holes and among dense macrophytes. In contrast, pelagic thunnids with stiff bodies and small-area caudal fins have the largest-reported turning radii for BFC maneuvers, and they require two or more tail-beats to make turns that less-stiff fishes achieve in a single beat. Stiff-body fishes also sweep out a larger volume while turning compared with more flexible fishes.

It proves harder to show differences in maneuverability when morphological differences are smaller, even for apparently large systematic and morphological differences seen in major divisions of actinopterygian fishes. Less-derived soft-rayed malacopterygians tend to have fusiform bodies, antero-ventral pectoral fins, and postero-ventral pelvic fins. The more recently evolved spiny-rayed acanthopterygians, in contrast, tend to have deeper bodies, lateral pectoral fins, and antero-ventral pelvic fins.

It has been considered that the more-derived acanthopterygian body-fin plan facilitates maneuverability, but current measures of performance for soft- and spiny-rayed fishes with different body-fin plans are often very similar. Current research shows that the fins of less-derived soft-rayed fins are functionally versatile, and often drive maneuvers similar to those of spiny-rayed fishes.

Maneuvering is clearly vital for key activities such as foraging and reproduction. Perhaps maneuvering similarities for a wide range of body-fin forms shows only that there are many ways to execute critical behaviors. However, natural history observations show differences between soft- and spiny-rayed forms in the directions expected. Numerous observations find deep-bodied spiny-rayed fishes in structurally complex habitats where maneuverability would be at a premium. Ethograms show more diverse maneuvering repertoires for deep-bodied spiny-rayed fishes, especially longer periods of hovering, swimming backward, braking, and maneuvering to follow prey in chases. Soft-rayed fishes swim through prey positions, overshooting if prey escapes. Spiny-rayed fishes are more likely to extend the pectoral fins laterally, brake rapidly, turn, and reorient the attack. Indeed, the evolutionary advantages of ray-finned forms may relate to increased stability and maneuverability at low swimming speeds and in braking.

The weakness of laboratory demonstrations of differences in maneuverability for some large differences in body-fin forms, such as those associated with evolution in ray-finned fishes, eventually returns to the question of methodology: Are protocols and methods adequate to describe maneuvering and agility? Are they sufficient to test differences in performance among different species? Clearly, there is great potential for further study and novel approaches to explore a mechanical basis for maneuvering.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy, Locomotion, and Movement in Fishes: An Introduction; Experimental Hydrodynamics; Fast Start; Functional Properties of Skeletal Muscle: Work Loops; Paired Fin Swimming; Stability and Turbulence.

Further Reading

Blake RW (1983) *Fish Locomotion*. Cambridge: Cambridge University Press.

D'Aout E and Aerts P (1999) A kinematic comparison of forwards and backward swimming in the eel *Anguilla anguilla. Journal of Experimental Biology* 202: 1511–1521.

Domenici P and Blake RW (1997) The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* 200: 1165–1178.

Drucker EG and Lauder GV (2001) Wake dynamics and fluid forces of turning maneuvers in sunfish. *Journal of Experimental Biology* 204: 431–442.

Gerstner CL (1999) Maneuverability of four species of coral-reef fish that differ in body and pectoral-fin morphology. *Canadian Journal of Zoology* 77: 1102–1110.

Harris JE (1953) Fin patterns and mode of life in fishes. In: Marshall SM and Orr P (eds.) *Essays in Marine Biology*, pp. 17–28. Edinburgh: Oliver and Boyd.

Krohn MM and Boisclair D (1994) Use of a stereo–video system to estimate the energy expenditure of free-swimming fish. *Canadian Journal of Fisheries and Aquatic Science* 51: 1119–1127.

Schrank AJ, Webb PW, and Mayberry S (1999) How do body and paired-fin positions affect the ability of three teleost fishes to maneuver around bends? *Canadian Journal of Zoology* 77: 203–210.

Standen EM and Lauder GV (2007) Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *Journal of Experimental Biology* 210: 325–339.

Walker JA (2000) Does a rigid body limit maneuverability? *Journal of Experimental Biology* 203: 3391–3396.

Walker JA (2004) Kinematics and performance of median and paired fins as control surfaces. *IEEE Journal of Oceanic Engineering* 29: 572–584.

Webb PW (2006) Stability and maneuverability. In: Shadwick RE and Lauder GV (eds.) *Fish Physiology*, pp. 281–332. San Diego, CA: Elsevier.

Webb PW and de Buffrénil VV (1990) Locomotion in the biology of large aquatic vertebrates. *Transactions of the American Fisheries Society* 119: 629–641.

Webb PW and Fairchild AG (2001) Performance and maneuverability of three species of teleostean fishes. *Canadian Journal of Zoology* 79: 1866–1877.

Webb PW and Gerstner C. (2000) Swimming behaviour: Predictions from biomechanical principles. In: Domenici P and Blake RW (eds.) *Biomechanics in Animal Behaviour*, pp. 59–77. Oxford: Bios Scientific Publishers Ltd.

Relevant Websites

http://www.people.fas.harvard.edu – Faculty of Arts and Science Harvard University, LauderLabVideos (htm – video of maneuvers).

http://www.youtube.com/watch?v=xBuCGDZWh0Q – Fish swimming on coral reef.

http://www.youtube.com/watch?v=QiGEmMPeQno – Four-view simulations of maneuvers of salmon.

http://www.usm.maine.edu – University of Southern Maine – boxfish, stiff-bodied fish maneuvering.

Stability and Turbulence

PW Webb and AJ Cotel, University of Michigan, Ann Arbor, MI, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Instabilities
The Nature of Turbulent Flows
The Importance of Scale
Response Latency
Fish–Eddy Interactions
Control Systems
Using Eddies
Further Reading

Glossary

Added mass A mass added to the inertia of an accelerating body because some volume of fluid is affected by that body.

Boundary layer A thin region of flow adjacent to any surface in which viscosity acts to create a velocity from that of the surface, where there is no slip between the water and the surface, to the velocity of the free stream.

Circulation Integral of vorticity over a given area.

Doppler effect The perceived change in frequency of a wave due to the motion of the sensor relative to the source of the wave.

Kármán gait The use of a Kármán vortex street to avoid active swimming.

Kármán vortex street A repeating pattern of alternating vortices shed by unsteady separation of flow over a bluff body.

Reynolds number The ratio of inertial to viscous forces in a flow.

Vortex strength The circulation calculated over a vortex tube, an area of constant vorticity.

Turbulence intensity The ratio of the standard deviation of turbulent flow variations relative to the mean velocity.

Vortex A mass of fluid in whirling or circular motion. Vortices always appear where hydrodynamic lift is being generated.

Vorticity A measure of angular velocity in a fluid or the local angular rate of rotation, that is, the tendency of a fluid to spin.

Introduction

Casual observation of aquatic habitats shows the ubiquity of turbulence, characterized by variations in flow velocity in space and time. Winds create surface waves, and currents flow over complex surfaces studded with projections that range from other animals and plants to inanimate rocks and boulders. Even apparently still waters regularly turn over.

Turbulence challenges stability of posture and of holding a desired trajectory during locomotion. Some stability challenges arise from a fish itself due to density differences among tissues and due to propulsion with periodic body and fin motions. However, external-flow perturbations associated with turbulence are larger and more likely to create problems for stability.

The study of fish interactions with turbulence is in its infancy. Most research has reported negative effects of turbulence on swimming speeds and increased metabolic costs. Other studies, however, show that fish can use some components in a turbulent flow as a booster, reducing energy costs and/or migration times. These impacts and opportunities from turbulence affect fish distribution and community structure.

Instabilities

Turbulence is characterized by variability in flow velocity. Any variation is a perturbation tending to create a displacement in postures and/or trajectory, and hence is a source of instability. Displacements are defined in a fish frame of reference in three translational directions and rotations about three axes (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability, **Figure 1**). Translations are slip (a lateral displacement), heave (a vertical displacement), and surge (a fore–aft displacement). Rotations are roll (rotation around the longitudinal axis), yaw, (rotation about the dorso-ventral axis), and pitch (rotation about the lateral axis).

Of the various displacements, fish appear to be especially sensitive to rolling instability. Control for rolling is continuously necessary for many fishes that are hydrostatically unstable in roll when the center of mass is above the center of buoyancy. Rolls are undesirable because they negate any dorso-ventral camouflage and could degrade sensory perception.

The Nature of Turbulent Flows

The natural world is a source of an essentially infinite range of types and magnitudes of stimuli. Organisms respond to a subset of stimuli having the largest impact on fitness. Only some perturbations in turbulent flow have potential to influence fish activities so that the fundamental questions for biologists are (1) what factors associated with turbulent velocity fluctuations affect fishes and (2) in what ways?

Turbulence may appear chaotic, because it is not possible to describe – or predict – velocity magnitude and direction at a given point. Nevertheless, predictable patterns occur within the flow in the form of eddies, or vortices. There are two major approaches to describing turbulence: a statistical approach describing velocity variation and a physical approach based on the properties of eddies (**Figure 1**).

Statistical descriptors of turbulence start with measures of velocity and its variation. Acoustic doppler velocimetry (ADV) is increasingly used in aquatic studies to measure instantaneous velocities in x, y, and z axes, from which mean velocity, $\bar{u}$, and its standard deviation, σ, are typically reported. These correlate with momentum and turbulent kinetic energy (TKE) associated with flow fluctuations, both of which contribute to displacement of fishes embedded in the flow. Stability problems increase, and control can fail, as momentum and TKE become large relative to the momentum and energy due to the motion of a fish.

Each of $\bar{u}$, σ, and TKE has been shown to correlate with reductions in one or more of posture, swimming speed, metabolic rate, habitat choice, and fish abundance. However, fish responses do not appear to be linear. Thus, salmonids tend to chose intermediate values of $\bar{u}$ or σ, while population abundance of salmonids is also higher at intermediate values of TKE.

Methods used to determine turbulence statistics usually measure velocity fluctuations in a small volume, essentially at a point. These measures are most useful in interpreting habitat locations for fishes, such as salmonids, that tend to hold station at a focal point. However, fish are exposed to spatial gradients when flow asymmetries will be experienced along various body dimensions. This would be expected to amplify destabilizing forces and torques. Using multiple measurements with flow probes, it has been found that spatial velocity and TKE gradients affect preferred locations for salmonids and salmonid density. Such spatially explicit data are essential in exploring how fish choose passages through turbulent flow fields.

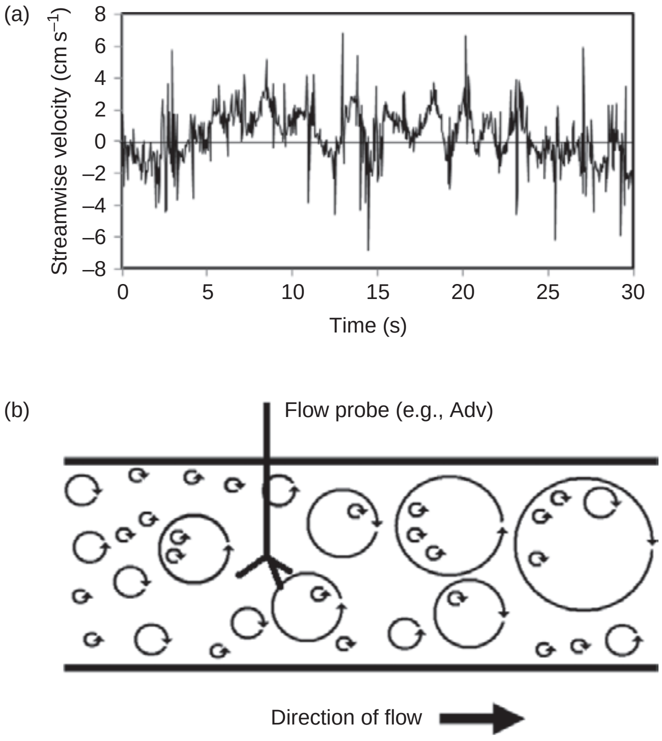


Figure 1 (a) Statistical and (b) physical representations of turbulent flow. (a) A record of streamwise (x-axis) velocity fluctuations measured at a point using a flow probe (ADV) in a trout stream. (b) A schematic of eddies of various sizes, rotations, and locations comprising the flow in a stream. A flow probe in the stream would record flow variations such as those in the upper panel.

The physical approach to turbulence considers structures in turbulent flow. Many images of structures in turbulent flow are found on the Internet, for example, eFluids website (see section Relevant Websites) that includes a gallery of videos and images of turbulence. Methods to determine turbulent-flow structures, such as ultrasound or particle imaging velocimetry (PIV), start with spatially explicit data. For example, PIV determines flow from the positions of particles in the fluid at successive small time intervals. PIV is currently the method of choice in the laboratory, and has been deployed in ocean and atmospheric applications. Small reliable instruments for river and shoreline applications are in development.

The physical approach recognizes that turbulence originates in gradients that cause streamlines to bend. The resulting curvature, or curl, in velocity vectors is defined as vorticity, and the structure in turbulent flows arises as curls roll up to create eddies or vortices. The limits of a vortex – and hence the diameter – can be found where vorticity declines to approach that of the surrounding water. From vorticity, an aerial measure of turbulence is obtained, and circulation is computed as the integral of vorticity over an area of the flow. When circulation is calculated over a vortex tube, that is an area of constant vorticity, the integral represents the strength of that vortex tube.

Circulation is a good measure of the potential impact of an eddy on fishes as it relates to the momentum and energy carried by that eddy. Real flows include numerous eddies of many different sizes and vorticities, and hence an area of flow could have a zero mean circulation. To avoid this problem, it has been suggested that turbulence should be measured as the absolute value of circulation. This correlates with fish densities at stream-reach scales, but has not been explored at finer scales. In practice, understanding how eddies affect fishes requires consideration of the sign of the rotation of eddies. For example, the ability to swim in energy-saving Kármán gait, slaloming without swimming in the alternating eddies of the Kármán vortex street, depends on both the size and direction of rotation of eddies. Examples of fish using the Kármán gait can be seen at the websites mentioned in the section Relevant Websites.

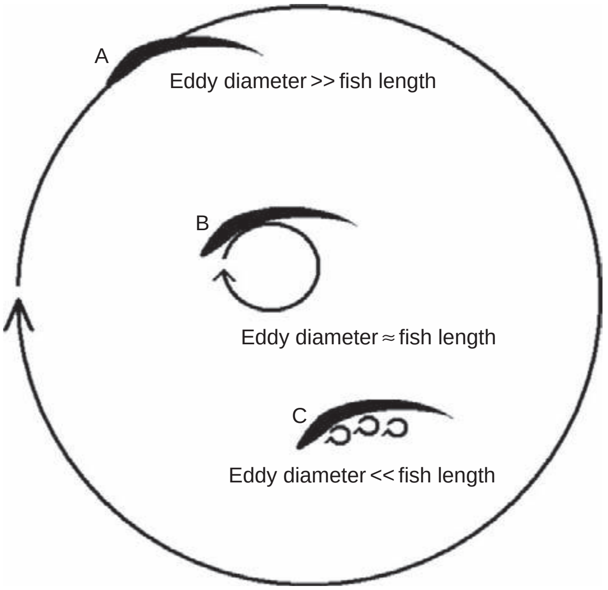

A
Eddy diameter >> fish length
B
Eddy diameter ≈ fish length
C
Eddy diameter << fish length

Figure 2 Eddies range in diameter, λ, from (a) $\lambda << L$, where L is fish total length, (b) $\lambda \approx L$, and (c) $\lambda << L$. When $\lambda << L$, a fish will treat the flow the same as a rectilinear flow. When $\lambda << L$, the small eddies are ignored. Stability problems occur when $\lambda \approx L$.

The Importance of Scale

There are several advantages to the physical approach to turbulence; for example, results are spatially explicit, results can generate all the statistical measures for flow and the spatial distribution of these measures. However, the effect of turbulence on fishes (and other organisms) depends on the size of eddies relative to the size of the organism. Therefore, the most important advantage of describing the structures in turbulent flow is that length scales of eddy structures can be estimated for comparison with fish size.

Initial consideration of eddy/fish scale focused on length scales (**Figure 2**), in which the measure of relative size is the diameter of an eddy, λ, relative to the total length of a fish, L. Early estimates suggested that flow-regime choices and negative effects of turbulence on swimming performance began to occur when $\lambda/L \approx 0.66$, with λ based on mean eddy diameter. In experimental studies, negative effects for creek chub, *Semolitus atromaculatus*, occur when $0.5 < \lambda/L < 1$ and similar values are found for rainbow trout, *Oncorhynchus mykiss*, using the Kármán gait.

Length scales provide a framework from which to evaluate fish responses to a given physical perturbation, but parameters based on Newton's laws, considering momentum or circulation related to forces and torques, would be more useful. For a fish fully embedded in an eddy, ratios of fish to eddy momentum and energy approach unity when stability failure occurs.

Response Latency

In addition to the spatial variations in velocity in turbulent flows, velocities also vary with time, for example, as wind or boat-generated waves travel over a shoreline. There is an eddy-specific rotation period, t_{eddy}, depending strongly on eddy size, vorticity, and the flow Reynolds number. Thus, typical records of flow variations (**Figure 1**(**a**)) are a mix of velocities peaking at various times.

Fishes cannot respond instantly to a displacement caused by an eddy, but rather there is a response latency, T, between a displacement growing to a level requiring a response and the corrective motor response. Fishes can respond to strong threatening stimuli in as little as 10 ms via the Mauthner cells. Response latencies for simple flow-induced displacements of the body are of the order of 60 ms for rolls, and 100–200 ms for other disturbances. The complex velocity stimuli of turbulent flows are likely to be associated with the latter longer-response latencies.

Response latency probably contributes to postural or trajectory failure when $T \approx 0.5 t_{\text{eddy}}$. Then the intended correction is in phase with a disturbance, so that the correction amplifies the disturbance. This promotes stability failure, a phenomenon known in the aeronautical industry as 'pilot-induced error'; data on t_{eddy} for eddies that just cause stability failure are lacking.

Fish–Eddy Interactions

Consideration of length scales has been instrumental in reaching current ideas on fish–flow interactions in turbulence. In general, it is recognized that when $\lambda << L$, eddies are sufficiently small to have only negligible effects and are ignored by a fish. Indeed, this is an assumption in the design of many flumes that induce microturbulence to obtain a rectilinear flow profile. Furthermore, those experiments that expected but did not show negative effects of turbulence on fishes have attributed this to λ/L being too small to create stability problems.

At the other extreme, with $\lambda << L$, eddy diameters can span oceans, as seen in various oceanic gyres. Fish will perceive the water velocity of large eddies as any other current. The eddy flow is likely to be ignored other than as a barrier to be breasted or more likely as a stream to be ridden in migration.

It is eddies with λ of the order of L, which have rotational rates, dimensions, momentum, and energy of sufficient magnitude relative to size, momentum, and energy of a fish, that cause major displacements and stability failure. Knowing the relative size for such eddies is important for understanding behavior, habitat choice, for exploring migration strategies to reduce energy costs, or to minimize passage times, etc. Such understanding has immediate utility for many fishway designs.

However, all the current considerations of eddy–fish interactions resolve eddy and fish characteristics at the scale of the whole eddy and fish cross sections. In practice, the impact of an eddy is expected to depend on local effects, especially the edge of an eddy acting on the anterior of a fish. Although simultaneous observations of turbulent flow and fish swimming in that flow are currently lacking, fish will experience maximum angular velocities as they enter an eddy. The shape of the anterior fish body, increasing in depth and width from zero at the nose to maxima at about 0.33 L from the nose reduces the rate at which forces and torques grow as a fish enters an eddy compared to more bluff shapes.

The degree to which a fish might penetrate an eddy depends on the speed of the fish. At typical cruising speeds of around 1 L s^{-1}, >20% of the fish body would enter an eddy within typical response times. Thus, the shape of the fish body is such that displacements should be small within likely response times, an interaction favoring a fish being able to execute stabilizing responses, reducing the chance of stability failure. Unusual body forms, such as the head of hammerhead sharks, *Sphyrna* sp., would clearly be at a disadvantage in high levels of turbulence, but their pelagic habits probably lack potentially disruptive turbulence.

Sprinting fishes could penetrate further into eddies. Migratory fishes seeking to minimize passage time and traverse obstacles, such as waterfalls, rapids, fish ladders, and turbines, would be expected to penetrate eddies with large circulation within typical response-latency times. Two factors reduce this problem. First, the momentum and energy of a fast-swimming fish is high so that threshold circulation for control failure will be associated with larger – and less common – eddies. Second, swimming fish do not experience the absolute size of an eddy but rather an apparent size, which, like the Doppler effect, is affected by speed. Thus at a high velocity, perceived eddy dimensions are reduced compared with absolute values. Then, the absolute size of eddies that are likely to challenge control systems are increased at higher swimming speeds.

The axis of rotation of eddies affects fishes. Vertical eddies have less impact than horizontal eddies for fish with compressed bodies. Although forces and torques will be increased for a vertical eddy in direct proportion to body compression, damping and corrective forces and torques are increased much more. Similar interactions occur for depressed forms experiencing horizontal eddies.

Control Systems

Damping Instability

Fishes stabilize posture and trajectories in a variety of ways. Turbulent perturbations can occur rapidly (**Figure 1**) so that the first factor promoting stability is damping. Damping the potential growth of a disturbance depends on resistance forces, momentum, and energy of a fish. Resistance forces, drag and inertia, for the fish plus an added mass of water that is dragged along with any body trying to move in that water, are larger for larger body spans at right angles to the direction of a disturbance slowing the rate of growth of a displacement. For example, body compression and deploying median fins promote damping of yaw and slip disturbances. Deployment of paired fins (**see also Buoyancy, Locomotion, and Movement in**

Fishes: Paired Fin Swimming) similarly damps heave, pitch, and roll.

Increased swimming speed (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming and Fast Start) also leads to greater damping. Instabilities arise from the differences between the mean and instantaneous velocity, the velocity component that is the signature of turbulence, quantified by σ. However, the momentum and energy of a fish, moving against the flow or holding position in the flow, slow down the growth of displacements. The smaller the ratio of σ to $\bar{u}$, the easier it is to control stability; an approximate analogy that illustrates the point is the well-known experience that it is easier to control stability while riding a bicycle at high speed than when moving slowly. The ratio of $\sigma/\bar{u}$ is defined as the turbulence intensity, TI, and fishes have been shown to avoid habitats with high TI. In addition, observations on reefs and in freshwater shoreline communities show that more powerful swimmers moving at higher speeds occur in more-turbulent locations.

Correcting Displacements

Damping only slows the growth of displacements. The body and all the median and paired fins provide additional hydrodynamic control mechanisms, in which the flow over control surfaces generates correction forces (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming). Body and appendage control surfaces can be functionally classified in two ways. First, trimming forces are created when the flow over an effector control surface results from translocation of the body. A fish can, of course, modify angles of attack to modulate these forces. Second, many control surfaces also act as propulsors, and hence can actively generate forces that can be harnessed for stability control. These can be described as powered effectors creating correction forces by their own motions. In practice, many effectors combine trimming and powered control, and powered forces are generally most important for stability.

The stabilizing functions of control surfaces depend on their location relative to the center of mass (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability, Figure 1). The head, embellishments such as the extensions of hammerhead sharks, and pectoral fins all anterior to the center of mass are generally destabilizing. A displacement increasing the angle of these surfaces to the flow amplifies the destabilizing forces acting on the surface, thereby tending to increase the initial displacement.

Failure of stability control appears to start with such positive feedback (**Figure 3**). As a fish approaches the control limits of its stabilizing systems, the fish tends to begin to slip and heave, and, to some extent, surge. Rotational displacements may increase to a small extent, but are largely actively minimized. Failure invariably starts with a yaw or pitch of the head, and as the head is anterior to the center of mass, the displacement continues to grow. Within 100 ms, the head, normally in line with the flow, can be bent so that it is normal to the flow!

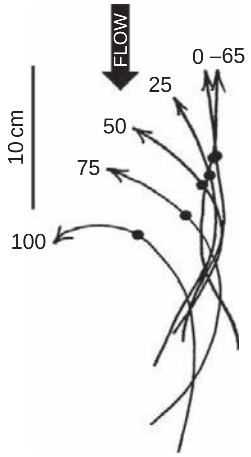


Figure 3 Kinematics of a stability failure in Atlantic salmon, *Salmo salar*, swimming in a flume at a flow speed of 10 L s^{-1}; numbers indicate time in milliseconds. The nose is indicated by the arrow head and the center of mass by the filled circle. Two outlines are shown at –65 and 0 ms as the fish swam steadily. The instability starts at the head after time 0 ms after which the nose begins to yaw as the fish could no longer sustain a head-into-flow posture. The head subtended an increasing angle to the flow, with increasing drag driving positive feedback causing the displacement to grow. The fish is no longer able to control posture as the center of mass moves laterally and downstream. The fish begins to respond after 100 ms with a turn downstream.

Fish may show two general types of responses as failure approaches. First, a fish may attempt to use a high-thrust fast-start to correct posture to keep the anterior of the body heading into the flow. However, the trajectory is displaced laterally from its initial path. Additional tail-beats are required to return the trajectory to its original path.

Second, when the initial displacement grows too fast to correct, the fish further amplifies the displacement into a turn downstream before attempting to turn back into the flow. In experimental flumes, short flume lengths give insufficient space and time for fishes to recover from such a failure, and this reduces the maximum speeds that can be attained compared to results in longer flumes. In streams, turning downstream not only provides space and time for recovery, but often there are flow refuges in which a fish can recover.

In contrast to control surfaces anterior to the center of mass, effectors posterior to the center of mass (the posterior body, median fins, and, usually, the pelvic fins) tend to be self-correcting. In this case, the displacement of a control surface is associated with a force that counters the displacement, reducing the growth or correcting the displacement.

Self-correcting control surfaces are especially important when t_{eddy}/T takes values that might promote pilot-induced error.

Powered control systems may also be self-correcting. This has been demonstrated for flying birds and the same principles apply to swimming fishes. Then the angle of attack of a powered fin effector is increased by a displacement with a force increase correcting the displacement.

The tail is an especially important trimming and powered effector, because its area is further downstream from the center of mass than other control surfaces. Hence, the tail can generate large corrective forces and torques. The earliest chordates lacked fins, but had a flexible tail, which presumably was able to bend and rotate to create corrective forces in any direction. Such flexibility is retained as part of the control system of aquatic vertebrates. In bony fishes, tail flexibility becomes of diminished importance, superseded by median and paired fins, with concomitant stiffening of the caudal skeleton.

Using Eddies

Both fishes and humans seek to use eddies for various purposes. Small eddies produced by the placoid scales of elasmobranchs may help reduce drag. The eddies increase the energy in the thin layer of flow adjacent to the fish – the boundary layer – which makes that boundary more resistant to separating from the body, a result that creates larger eddies and is a source of energy waste. The rigid carapace of boxfishes creates eddies that trail backward. These can vary in strength when the body posture is perturbed, and in this situation provide a self-correcting stabilizing mechanism. Schooling fish may use eddies created by the tails of school members ahead to give themselves a lift, reducing their costs of swimming.

Managing fishways also requires considering eddies (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance). In principle, the eddies in turbulent flow could be manipulated to promote various goals, although this is an area on which there are few studies to elaborate on potential mechanisms. Fish ladders that provide passageways for fishes around dams are commonly comprised of successive chambers through which fish pass. The openings between chambers, and sometimes structures within them, create eddies that may both serve as stimuli to promote passage and create low-flow areas as well, where fishes can rest. Alternatively, very high shear flows over dams and raceways will create substantial turbulence that may provide fish barriers, for example, at lamprey dams seeking to prevent these fishes from entering streams to breed. Raceways, recirculating tanks, and cages in rivers or tidal areas are used in aquaculture. The flow through such fish-culture systems will create eddies, and these are important for ensuring that the water is well oxygenated, the carbon dioxide and other wastes are removed, and the food is appropriately distributed. The eddies could also be sources of disturbance increasing metabolic rates. In principle, fish-culture designs could consider the nature of eddies they create to explore systems that might improve growth.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Fast Start; Maneuverability; Paired Fin Swimming; Undulatory Swimming. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance.

Further Reading

Castro-Santos T, Cotel AJ, and Webb PW (2008) Fishway evaluations for better bioengineering: An integrative approach. *American Fisheries Society, Symposium* 69: 557–575.

Cotel AJ, Webb PW, and Tritico H (2006) Do trout choose habitats with reduced turbulence? *Transactions of the American Fisheries Society* 135: 610–619.

Crowder DW and Diplas P (2002) Vorticity and circulation: Spatial metrics for evaluating flow complexity in stream habitats. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 633–645.

Enders EC, Boisclair D, and Roy AG (2005) A model of total swimming costs in turbulent flow for juvenile Atlantic salmon (*Salmo salar)* *Canadian Journal of Fisheries and Aquatic Sciences* 62: 1079–1089.

Hedrick TL, Cheng B, and Deng X (2009) Wingbeat time and the scaling of passive rotational damping in flapping flight. *Science* 324: 252–255.

Liao JC (2008) A review of fish swimming mechanics and behavior in perturbed flows. *Philosophical Transactions of the Royal Society, Biological Sciences* 362: 1973–1993.

Odeh M, Noreika JF, Haro A, Maynard A, Castro-Santos T, and Cada GF (2002) Evaluation of the effects of turbulence on the behavior of migratory fish. *Final Report 2002, Report to Bonneville Power Administration, Contract No., 00000022, Project No. 200005700,* pp. 1–55.

Pavlov DS, Lupandin AI, and Skorobogatov MA (2000) The effects of flow turbulence on the behavior and distribution of fish. *Journal of Ichthyology* 40(supplement 2): S232–S261.

Tritico HM, Cotel AJ, and Clarke JN (2007) Development, testing and demonstration of a portable submersible miniature particle imaging velocimetry device. *Measurement Science and Technology* 18: 2555–2562.

Webb PW (2002) Control of posture, depth, and swimming trajectories of fishes. *Integrative and Comparative Biology* 42: 94–101.

Webb PW, Cotel AJ, and Meadows L (2010) Waves and eddies: Effects on fish behavior and habitat distribution. In: Domenic P and Kapoor BG (eds). *Fish Locomotion: An Etho-Ecological Perspective*, pp.1–39. Enfield, NH: Science Publishers.

Weihs D (2002) Stability versus maneuverability in aquatic animals. *Integrative and Comparative Biology* 42: 127–134.

Relevant Websites

http://www.people.fas.harvard.edu – Faculty of Arts and Sciences, Harvard University, LauderLabVideos.htm – videos of control surface.

http://www.efluids.com – Gallery of videos and images of turbulence.

http://www.youtube.com/watch?v=XOyht5jGuwA – Lamprey building nests in turbulent flow.

http://www.biotactic.com – Video of fishes swimming in turbulence.

Fast Start

P Domenici, CNR – IAMC, Torregrande (Or), Italy

© 2011 Elsevier Inc. All rights reserved.

Introduction
Neural Control
Muscle Activity
Hydrodynamics
Performance and Kinematics
Body Design
Environmental Factors
Future Directions
Further Reading

Glossary

C-start A fast start in which the body of the fish is bent into a C-shape at the end of stage 1. It is used mainly in escape responses, but it also has been observed in predator strike in certain species (e.g., in archerfish).

Double-bend response A C-start including both stages 1 and 2.

Escape latency The time between stimulus onset and the fish's reaction.

Escape response A sudden acceleration maneuver (i.e., a fast start) as a response to a threatening stimulus.

Fast start A sudden acceleration from resting or swimming. It can be an escape response or a predator strike, or a maneuver observed during social interactions.

Mauthner cells Two giant neurons located in the hindbrain of most fish species. These cells usually control escape responses. However, non-Mauthner-cell escapes also have been reported, and the Mauthner cells may be involved in other behaviors such as post-feeding turns.

Predator strike A sudden acceleration (i.e., a fast start), generally in line with the body axis, aimed at catching prey.

Single-bend response A C-start lacking stage 2.

S-start A fast start in which the body of the fish is bent into an S-shape at the end of stage 1. It mainly is used in a predatory strike, but a type of S-start also has been observed in escape response (e.g., in pike).

Stage 1 The first body bend occurring during a fast start, analogous to a half tail-beat in continuous swimming. Kinematically, it starts at the onset of the response, and it ends when the angular motion of the head reverses direction.

Stage 2 The second body bend occurring during a fast start, analogous to a half tail-beat in continuous swimming. Kinematically, its beginning corresponds to the end of stage 1 and it ends with the next reversal of the turning of the head.

Startle response Any reaction caused by a threatening stimulus. It may imply an escape response or other responses such as freezing and withdrawal.

Introduction

Fast starts are sudden accelerations performed by fish either from rest or while swimming. They are a fundamental component of a fish's locomotory repertoire, because they are used by most species for avoiding predation and by some species for capturing prey (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics). Fast-start maneuvers have been associated also with social interactions in a number of species. Fast starts are the result of the contraction of fast glycolitic (anaerobic) fibers and can be considered as a form of burst swimming that lasts only for a fraction of a second. Mechanically, fast starts are unsteady swimming behaviors that can be classified as body–caudal fin (BCF) transient motions.

Fast starts are divided into three stages: stage 1, the first body bend; stage 2, the second body bend; and stage 3, a variable stage, involving continuous swimming, coasting, or braking. Most experimental work has focused on the first two stages. Early work considered stage 1 and stage 2 as the preparatory and propulsive stages, respectively. However, recent work has demonstrated that stage 1 alone can provide thrust, which is consistent with the finding that fast-start escape responses lacking a stage 2 still result in forward motion.

Fast starts can be classified further based upon the type of curvature achieved by the fish at the end of the first muscular contraction (stage 1). The two main types of fast starts are the C-start and the S-start, in which the body curvature at the end of stage 1 assumes a C- or an S-shape, respectively. C- and S-starts often are associated with escape

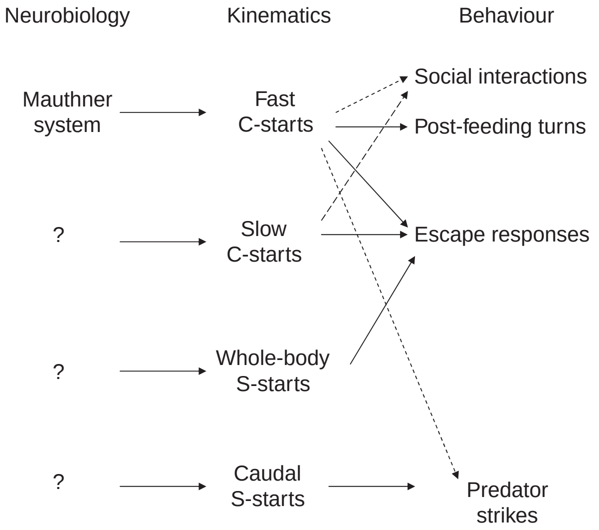


Figure 1 Diagram showing various types of fast starts and their relative neural control, kinematics and the behavioral context within which they are observed. Dotted arrows indicate uncertain links (i.e., the activation of the Mauthner system in fast C-starts has not been demonstrated in social interactions and predator strikes; the presence of distinct fast and slow C-start in social interactions has not been demonstrated). Whole-body and caudal S-starts are shown in **Figure 3**; fast and slow C-starts are shown in **Figure 6**. Based on Domenici P and Blake RW (1997) The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* 200: 1165–1178 and successive modifications by Schriefer JE and Hale ME (2004). Strikes and startles of northern pike (*Esox lucius*): A comparison of muscle activity and kinematics between S-start behaviors. *Journal of Experimental Biology* 207: 535–544. and Wohl S and Schuster S (2007) The predictive start of hunting archer fish: A flexible and precise motor pattern performed with the kinematics of an escape C-start. *Journal of Experimental Biology* 210: 311–324.

responses and strikes, respectively. However, recent work has demonstrated that C-starts can also be used as predator strikes, and that two kinematic types of S-starts exist in some species (e.g., pike *Esox lucius*) that can be used for escaping and for prey capture, respectively. In addition, while C-start escape responses usually are controlled by a pair of giant neurons (the Mauthner cells), experiments have shown that Mauthner cells can be used also in other contexts, such as in post-capture maneuvers. A diagram elucidating the kinematic classification of fast starts, the underlying neural control, and the behavioral contexts in which they are used is shown in **Figure 1**.

Neural Control

Most work on neural control has focused on escape responses. Escape responses usually are controlled by a pair of giant reticulospinal neurons located in the hindbrain of most fish species, the Mauthner cells (**see also Hearing and Lateral Line**: Auditory System Morphology and **Brain and Nervous System**: Physiology of the Mauthner Cell: Function), and in several other associated reticulospinal neurons. All of these neurons together make up the Mauthner system controlling the features of the initial phase of escape responses. In a typical, mechanically triggered C-start escape response, the generalized sequence of events is: fish perceive the threatening stimulus; the sensory neurons excite the Mauthner cell ipsilateral to the stimulus; the ipsilateral Mauthner cell inhibits the contralateral Mauthner cell and excites the contralateral axial musculature as well as other motor components (e.g., pectoral and dorsal fins and opercula); and the fish body bends into a C-shape, giving rise to the stage 1 of the response. Stage 2 often follows stage 1, although its execution is not directly activated by the Mauthner cells. The Mauthner cells receive stimulation from a number of ipsilateral sensory inputs, typically visual and/or mechano-acoustic, and escape responses can be triggered by a variety of stimuli such as visual (shadows or looming images), sudden noise, vibration, and chemical and tactile stimuli. An approaching predator is likely to provoke stimulation of more than one of these sensory modalities.

In light of the high variability in timing, performance, and kinematics (see below), escape responses cannot be considered highly stereotypic. Correspondingly, recent work has demonstrated the existence of many variants of the typical escape response described above. Escape responses may or may not include a stage 2 (double-bend and single-bend responses, respectively; **Figure 2**); in certain species, S-start escape responses can be observed in which contractions on both sides of the body occur during stage 1; and escape responses in which Mauthner cells are not activated have been observed in both intact fish and in fish with ablated Mauthner. Therefore, the Mauthner cells are not necessary for escape responses to occur, although Mauthner cell-mediated responses tend to show higher performance (e.g., shorter reaction times and higher turning rates) than non-Mauthner-cell responses.

Furthermore, while fast-start escape responses commonly are observed in fish when threatened, other startle responses that do not necessarily imply locomotion, such as withdrawal (head retraction) and freezing responses, can occur. Head retraction usually is observed as a response to head stimulation in some elongated species (e.g., *Anguilla rostrata* and *Petromyzon marinus*) and it does not involve a propulsive phase. These species avoid predators by retracting their head back into a burrow rather than by swimming away from the threat. Like escape responses, withdrawal responses are thought to be mediated by the Mauthner cells. The Mauthner cells of the lamprey, unlike those of teleosts, lack reciprocal inhibition – both cells fire at the same time.

Mauthner-mediated fast starts can be observed during feeding. When goldfish (*Carassius auratus*) capture a prey

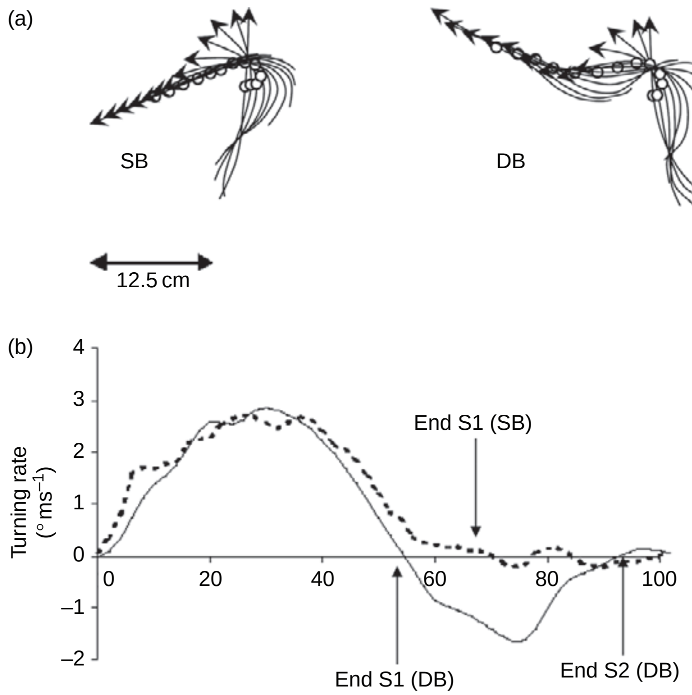


Figure 2 (a) Tracing of the mid-line of grey mullet (*Liza aurata*) in successive positions during a single-bend (SB) and a double-bend (DB) response. The arrows and the circles indicate the positions of the head and the CM, respectively. (b) Turning rate of the head for the responses shown in (a). While the DB response (continuous line) shows a reversal of turning rate in correspondence with stage 2 (S2), the SB response (dotted line) shows turning rate values around zero after the end of stage 1 (S1). Reproduced from Lefrançois C, Shingles A, and Domenici P (2005) The effect of hypoxia on locomotor performance and behaviour during escape in the golden grey mullet (*Liza aurata*). *Journal of Fish Biology* 67: 1–19, with permission from Wiley-Blackwell.

fallen on the water surface, prey capture often is followed by a quick Mauthner-mediated C-start. Hence, a startling stimulus is not a necessary event for triggering Mauthner cells. Little is known about the neural control of predatory strikes, although high-speed video observations suggest that S-start strikes may be controlled by different mechanisms than S-start escape responses because of the kinematic differences between these two fast-start types (**Figure 3**).

Muscle Activity

The muscle activity involved in C- and S-starts differs considerably. The C-bend commonly observed in C-type escape response is the result of the unilateral contraction of the anaerobic axial fibers. These fibers are designed for fast, high-power muscle contractions and typically constitute most of the muscle mass of fish. Electromyography shows that a unilateral contraction at the onset of an escape response is common among teleosts (due to the reciprocal inhibition of contralateral Mauthner cells), although primitive forms such as the bowfin (*Amia calva*) and bichirs (*Polypterus senegalus*) show bilateral muscle activity during stage 1. Muscle activation in stage 1 is simultaneous along the whole body length. However, the duration of muscle activity in stage 1 increases at posterior sites. Stage 1 usually is followed by stage 2 in double-bend responses, which corresponds to a contralateral wave of muscle activity propagating posteriorly.

Studies of muscle activity (based on electromyography) in predator and escape S-starts have revealed the muscular basis for differences in kinematics. In certain elongated species such as pike, posteriorly triggered escapes consist of whole-body S-starts, in which, during stage 1, the contralateral body bend of the S-shape occurs approximately halfway along the fish, with rostral and caudal muscle activity on opposite sides and almost simultaneous. A different type of S-start can be observed during predator strikes, in which the contralateral bend of the S-shape is restricted to the tail region (**Figure 3**), and the rostral muscle activity during stage 1 is delayed relative to the caudal muscle activity. Predatory S-starts may imply sudden S-bending in stage 1 followed by stage 2, or a relatively long-lasting S-posture during which the predator is relatively still, followed by successive muscle contractions.

The anaerobic fibers have different contractile properties depending on their location along the fish. Generally, rostral fibers show faster contractile properties than posterior ones. In addition, the anaerobic fibers, the main muscle fibers that fuel fast starts, are generally more massive anteriorly than posteriorly. As a result of these differences in contractile properties and muscle distributions, the forces developed

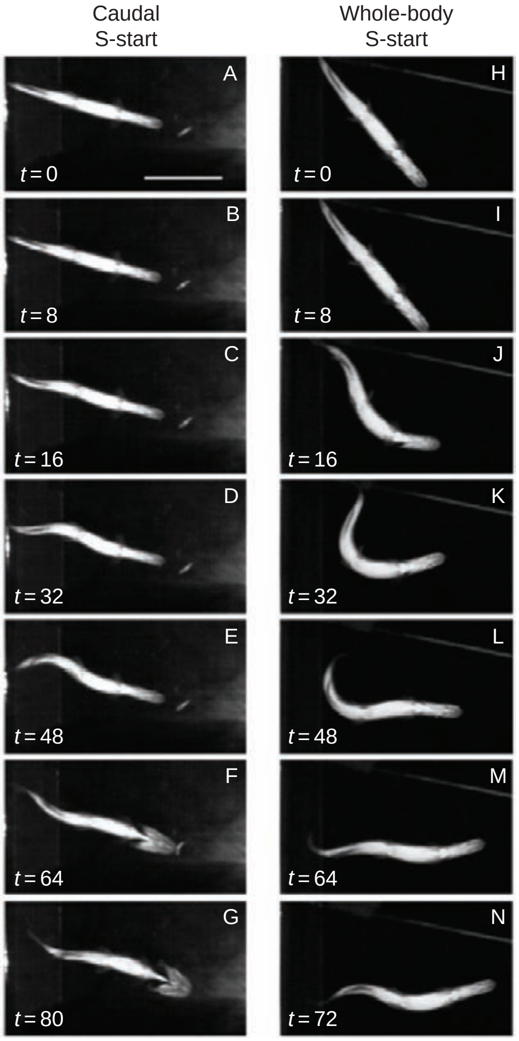


Figure 3 Predatory strike (caudal S-start; left panels) and escape response (whole-body S-start; right panels) S-starts in pike (*Esox lucius*) filmed from below using high-speed video at 500 Hz. The S-start of a predatory strike involves a contralateral bend restricted to the caudal region, while in escape responses the S-bend occurs along the whole length of the fish. *t* indicates time in millisecond. From Schriefer JE and Hale ME (2004). Strikes and startles of northern pike (*Esox lucius*): A comparison of muscle activity and kinematics between S-start behaviors. *Journal of Experimental Biology* 207: 535–544.

anteriorly are larger than those developed posteriorly. This large force development causes a delay in the peaks of bending in caudal sites relative to rostral sites during fast starts, resulting in a wave of body bending along the length of the fish, in line with kinematic observations.

Because most of the white (i.e., anaerobic) muscle mass in fish tends to be in the central region, this is where most of the mechanical power is produced during a fast start. However, most hydrodynamic forces are generated in the posterior region of the fish because muscle power is transmitted to the posterior region as a result of the stiffness of the muscle and the skeleton.

Hydrodynamics

Early work on fast start describes stage 1 as the preparatory phase and stage 2 as the propulsive phase. However, more recent kinematic work has shown that (1) the center of mass is accelerated even during stage 1 and (2) fast starts associated with considerable forward propulsion can occur even in the absence of a stage 2 in single-bend escape responses (**Figure 2**). This suggests that some thrust is produced during stage 1. Earlier work calculated the acceleration and lift forces during S- and C-start maneuvers. Acceleration forces were the dominant component during stage 1, while lift forces dominated during stage 2. Fast starts show high amplitude motions when compared with routine swimming, with large accelerations and decelerations, and forces produced not only in the direction of motion but also perpendicularly. As a result of these features, fast starts show relatively low efficiency of locomotion. However, fast starts are likely not designed for locomotor efficiency, but rather for high power and high acceleration, which maximize success in life-threatening situations.

Recently, digital particle image velocimetry (DPIV; **see also** (**Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics) was applied to C-type escape responses in order to estimate the flow pattern around the fish and the underlying hydrodynamics. DPIV is a video technique that allows visualizing and quantifying the flow around objects. Briefly, it consists of adding neutrally buoyant particles to the fluid. A given plane of interest is then illuminated by a laser sheet. Video recording allows quantifying the distance covered per unit time by the particles and therefore the motion of the fluid. Experimental work using DPIV suggests that both stages 1 and 2 produce thrust. Specifically, a C-start escape response produces three orthogonal jets (**Figure 4**). Jet 1 opposes the final swimming trajectory of the fish, in line with the low efficiency of fast starts and it may be an unavoidable property of such a high-amplitude maneuver. Jet 2 is the main propulsive jet, being in a direction opposite to the final swimming trajectory of the fish. This jet starts forming during stage 1, because bending the fish into a C-shape generates a region of suction within the C. This demonstrates that stage 1 is a propulsive phase of C-type fast starts, albeit most of the thrust is generated during stage 2. Jet 2 further develops during stage 2 as a result of the return flip of the tail. Flow generated by the dorsal and anal fins provides a relatively large proportion of the total momentum by adding to jet 2. Jet 3 develops as a result of stage 2 and is directed perpendicularly to the escape trajectory of the fish. This jet may contribute to steering, by opposing the angular momentum generated during stage 1. Further work using DPIV techniques is

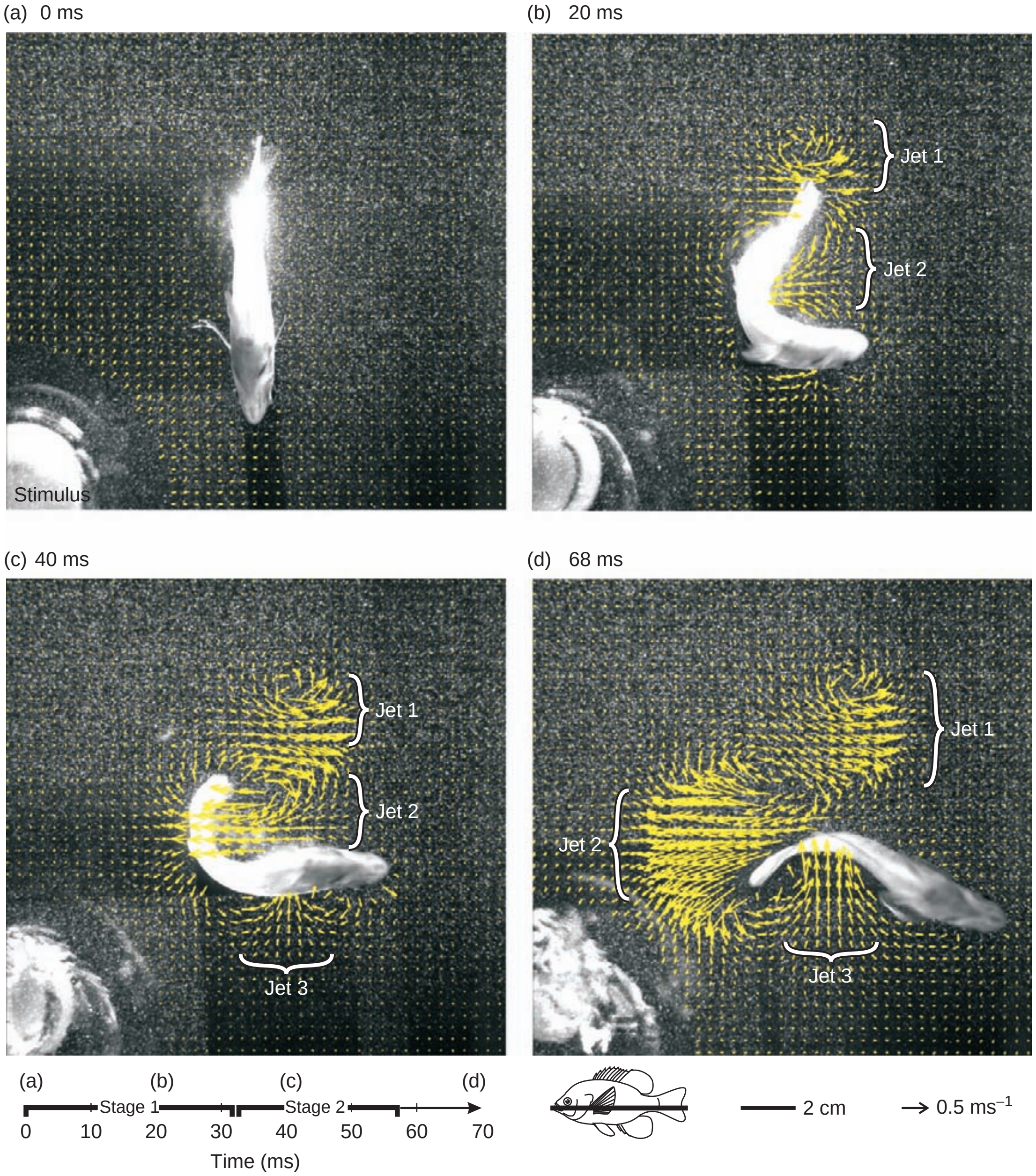


Figure 4 The jets created during an escape response in bluegill sunfish, *Lepomis macrochir*, based on digital particle image velocimetry. View from below. Stimulation is from the bottom left of each panel. Time is shown on the top left of each panel. Panels (a)–(d) show the position of the fish at different times, from the onset of the response (a, 0 ms) to 68 ms later (d). The timing of each panel relative to each stage of the escape response is shown in the bottom drawing. The bluegill drawn at the bottom shows the position of the laser light sheet on the vertical plane (black line). The arrow at the bottom right shows flow velocities of 0.5 m s^{-1}, for comparison with the flow vectors shown in the four panels. See text for details. From Tytell ED and Lauder GV (2008) Hydrodynamics of the escape responses in bluegill sunfish, *Lepomis macrochirus. Journal of Experimental Biology* 211: 3359–3369.

necessary to elucidate the flow fields and underlying hydrodynamics of other types of fast starts, including predatory strikes.

Performance and Kinematics

Fast-start performance can have a major effect on the outcome of predator–prey encounters, because fast starts are used by most species of prey and by many predators. When considering escape responses, there are a number of components that affect survival that can be related to both reactivity and swimming performance. The main components are described and defined below, together with the main factors affecting them. From a methodological point of view, high-speed video ($\geq$250 frames s^{-1}) is the most common tool used for

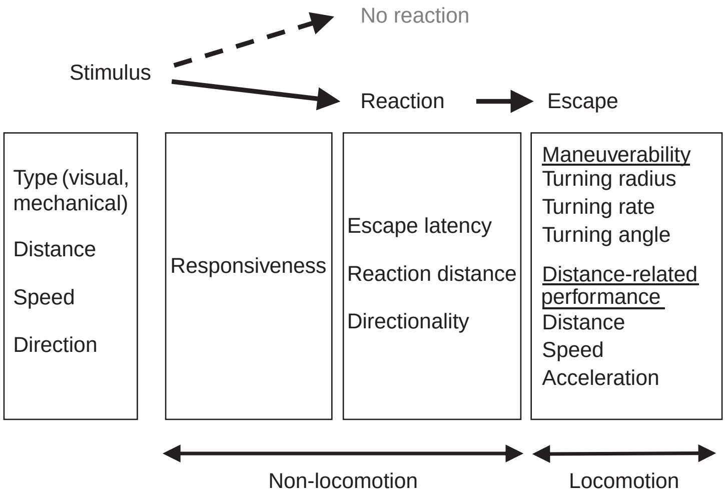


Figure 5 The components of an escape response, including stimulation, non-locomotion, and locomotion variables. Reproduced with permission from Domenici P, Lefrançois C, and Shingles A (2007) The effect of hypoxia on the antipredator behaviour of fish. *Philosophical Transactions of the Royal Society B* 362: 2105–2121.

assessing fast-start performance (**Video Clip 1**). The main components of fast-start performance are shown in **Figure 5**. For each component, a range of indicative performance levels is identified in **Table 1**. Once a predator attacks, the first step is to determine whether the prey executes an escape response or not. The proportion of responses over the total number of attacks for any given sample can be scored as responsiveness and was found to be a major determinant of prey vulnerability. Responsiveness is affected by the strength (or distance) of the stimulation, with supra-threshold stimuli yielding up to 100% of responses.

If a fish executes an escape response that is triggered by an approaching stimulus (e.g., a predator), its reaction distance can be measured as the distance between the prey and the snout of the predator at the onset of the response. Reaction distances tend to increase with the frontal size and speed of the approaching predator. This is because the rate of change of the angle of the predator's frontal profile as seen by the prey (the apparent

Table 1 Indicative performance levels for escape response variables of adult fishes

Variable type	*Variable*	*Size effect*	*Performance (indicative range)*
Escape behavior	Responsiveness	[a]	Up to 100%
	Latency	[a]	5–100 ms
	Prey reaction distance (apparent looming threshold)	↗[b]	0.1–1 rad s^{-1}
	Directionality	Not tested	50–100%
	Escape trajectories	[a]	90–180°
Kinematics (maneuverability)	Turning radius	↘[c]	0.05–0.2 L
	Turning angles	[a]	0-180°
	Stage 1 turning rate (average)	↘	500–5000° s^{-1}
Kinematics (distance–time variables)	Fast start duration	↗	30–200 ms
	Distance covered during fast start	↗	1–20 cm
	Distance covered within a fixed time[d]	[a]	1–20 cm
	Speed within fast start	↗	3–30 L s^{-1}
	Speed within a fixed time[d]	[a]	3–30 L s^{-1}
	Burst speed[e]	↗	5–30 L s^{-1}
	Acceleration	[a]	20–150 m s^{-2}

[a] Indicates that no size effect was found.
[b] Apparent looming threshold decreased with size in some species (i.e., reaction distance increases).
[c] Indicates a size-related increase in turning radius (turning radii measured in cm are larger in large fish, although when measured in Lengths (L), they are relatively constant)
[d] Fixed time taken as the average duration of stage 1 plus stage 2.
[e] For swimming performance beyond fast start stages.
Arrows (↗ and ↘) represent a size-related increase and a decrease in performance, respectively.
Indicative values are for a range of sizes spanning approximately 5–50 cm.

looming threshold) at the onset of the escape response is relatively constant for any given prey. In addition, reaction distance is affected by factors that affect prey risk and the cost of fleeing, such as the presence of refuges and the engagement in feeding activities, respectively. Sudden stimulation, as often used in laboratory settings, allows the measurement of escape latency, defined as the time between stimulus onset and the fish's reaction. Minimum latencies of the order of 5–10 ms are characteristic of Mauthner-cell-mediated responses, although longer latencies (as long as 50–100 ms) have been reported in responses possibly not mediated by the Mauthner cells. Latencies tend to decrease with stimulus strength and proximity. In C-type escape responses, fish tend to contract their body into a C-shape opposite to the threat as a result of the activation of the Mauthner cell ipsilateral to the stimulation. The proportion of this type of responses (also called away responses as opposed to the less common towards response, in which the C-bend is directed toward the threat) over the total number of responses for a given treatment can be scored as directionality. Directionality is largely affected by the orientation of the fish relative to the approaching stimulus, with values around 80–90% when the stimulation is perpendicular, decreasing to almost 50% for stimuli that are nearly head on or tail on. In addition, directionality is affected by the presence of nearby obstacles. Finally, the overall swimming direction (escape trajectory) shown by fish at the end of the escape response tends to be in the sector 90–180° away from the threat, with high variability related to multiple peaks in frequency.

A number of kinematic variables related to swimming performance affect escape success and consequently survival. These are mainly variables related to (1) maneuverability and (2) distance–time performance. In most cases, performance has been measured by considering the center of mass of the fish when stretched straight (CM_s). While this point is not the physical center of mass when the fish is moving (unless it is gliding in a straight position), this convention has been used because CM_s approximates the point upon which forces act and it is the point where many predators aim.

Maneuverability (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability) is often a determinant of predator–prey interactions. Generally speaking, small fish are more maneuverable than their larger predators, but are slower when swimming along a straight course (**Table 1**). Indeed, swerving is often used by fish under attack, allowing prey to benefit from their superior maneuverability. This behavior has the additional advantage of increasing the unpredictability of their movement. Maneuverability can be measured using a number of variables whose relative importance may depend on the type of interaction. Maneuverability has mainly been measured in escape responses, because predatory strikes tend to be in line with the stalking orientation of the predator and involve little turning during the strike.

The main maneuverability variables measured during escape fast starts are turning radius, turning angles, and turning rates. The turning radius is usually measured as the roughly circular path of CM_s during stage 1 and it provides an indication of how sharply a fish can turn. Turning radii in escape responses are independent of speed and proportional to fish length, such that turning radii measured in Length (L) tend to be relatively constant, around 0.05–0.2 L depending on the species. Fish with high maneuverability, living in structurally complex environments, tend to have small turning radii, while fish from pelagic environments tend to show relatively large turning radii. These differences are related mainly to differences in body flexibility. While fish from structurally complex habitats usually are quite flexible and can show high-amplitude maneuvers, fish from pelagic environments, such as tuna, usually are relatively rigid, which minimizes recoil and therefore drag. The ability of fish to accomplish a turn within one-half tail beat (i.e., stage 1 of an escape responses) can be assessed by simply measuring the angle between the head at the onset of the response and at the end of stage 1 (i.e., turning angle). Because fish tend to escape in a direction away from the stimulus, the average turning angle, when using various stimulus directions, tends to be around 90°. However, maneuverability should be assessed by measuring the full range of turning angles available to a fish using stimulation spanning 180° from both sides, rather than the mean turning angle. Relatively flexible fish such as angelfish (*Pterophyllum eimekei*) and goldfish show turning angles ranging from about 10 to 180°, while rigid fish such as tuna can achieve a 180° turn only if using 2–2.5 tail beats.

The agility of fish can be measured as the rate at which they can change their orientation (commonly measured as the turning rate or angular velocity of the line joining the tip of the head and CM_s). Turning rate can assess the ability for swerving and it is a fundamental variable for survival since it affects the outcome of predator–prey interactions. Small fish show a higher turning rate than large fish, because the minimum muscle contraction time increases with fish size. For example, a 90° turn is accomplished in about 20 ms in a 10-cm fish and in about 100 ms in a 30-cm fish. In addition, turning rate should be related to the amount of muscle power used in the contraction and inversely related to body rigidity and drag. Escape responses show fast and slow turning rates (possibly related to different neural control; **Figure 6**), although both are faster than the turning rates observed during routine swimming.

Propulsive performance in fast starts commonly is measured as distance traveled, speed, and acceleration of the CM_s. Fast starts are characterized by extremely high accelerations, of the order of 5–20 g. Locomotor performance shows relatively high variability and tends to be

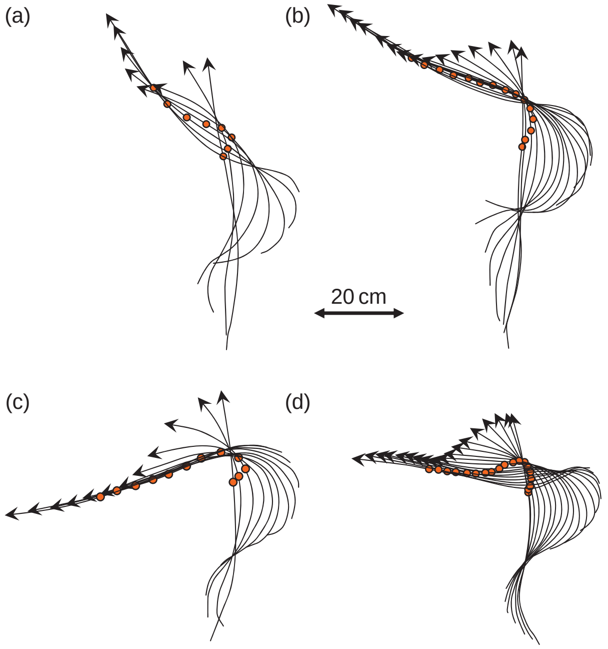


Figure 6 Escape responses in dogfish (*Squalus acanthias*) with slow (a, c) and fast (b, d) turning rates (corresponding to fast and slow responses in **Figure 1**). The arrows and the circles indicate the positions of the head and the CM, respectively. Time between positions is 40 ms. Fish in (b) and (d) take longer to achieve a similar stage 1 angle as (a) and (d), respectively. From Domenici P, Standen EM, and Levine RP (2004) Escape manoeuvres in the spiny dogfish (*Squalus acanthias*). *Journal of Experimental Biology* 207: 2339–2349.

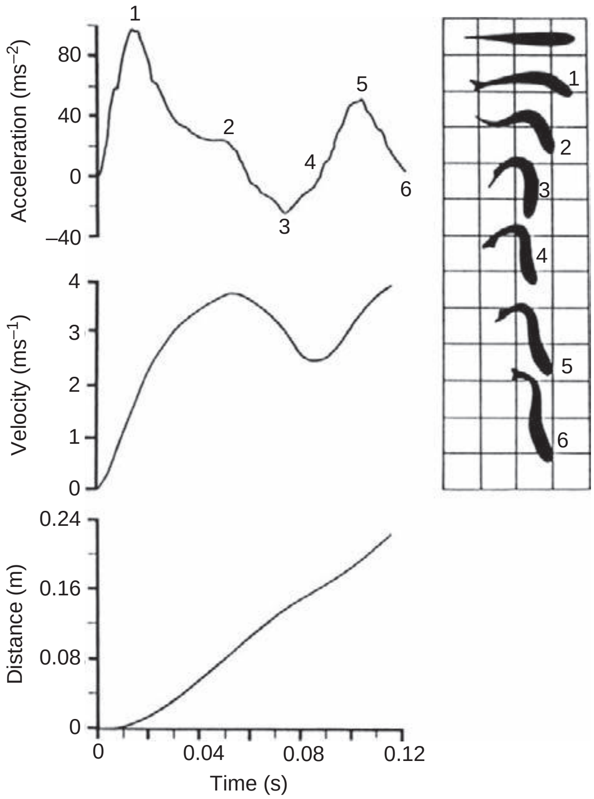


Figure 7 (Left panels) Locomotor performance in a fast-start escape response by rainbow trout (*Oncorhynchus mykiss*). Two main peaks occur in the acceleration trace, one per each kinematic stage. (Right panel) The silhouette of the trout during the escape response. Stage 1 (1–3) and stage 2 (4–6) are shown. Numbers correspond to those indicated on the acceleration plot. From Harper DG and Blake RW (1990) Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius* during escapes. *Journal of Experimental Biology* 150: 321–342.

higher in escape responses than in predator strikes, possibly because faster attacks cause larger reaction distances in the prey. In both escape and strike fast starts, a peak in acceleration is observed during stage 1 (followed by a peak in stage 2; **Figure 7**). Hence, the time course of the propulsive power is associated with the tail beats. Although, on theoretical grounds, acceleration is expected to decrease with size (because thrust, proportional to L^2, is used to move the inertia of the body and added mass, proportional to L^3), experimental data show no clear scaling trend. Because acceleration is obtained by a double differentiation of distance over time, it is usually noisy. Although a number of smoothing techniques have been developed, it is advisable to consider, in addition to acceleration, other less noisy measurements for the assessment of fast-start performance. In particular, speed and distance traveled are good predictors of the outcome of predator–prey encounters. The speed at the end of stage 1 is usually around $10\,\mathrm{L\,s^{-1}}$ (or even higher in small fish), and it increases in stage 2. In order to provide ecologically relevant measures, speed and distance traveled are commonly calculated within a fixed time. Experimental work using predator–prey encounters suggests that an ecologically relevant fixed time to use is the cumulative duration of stages 1 and 2. Nevertheless, the relevant time within which performance should be assessed may depend on the species of predators and prey involved, as well as on the habitat type in which they live. For example, structurally complex habitats providing shelters are likely to result in short-lived predator–prey encounters. Although burst speed increases with fish size, when measured within a fixed time, speed is independent of size, in line with data on the scaling of acceleration.

Body Design

Both shape and size can affect fast-start performance. A summary of the scaling issues mentioned in the above sections is listed in **Table 1**. Large fish are at an advantage when swimming in a straight line because of their higher burst swimming speed. However, predator–prey encounters often involve swerving and most maneuverability variables show higher performance in small fish.

Fish shape can affect a number of fast-start components. Theoretical considerations predict that fast-start performance should be higher in fish with a large posterior body section, a large tail for thrust production, a flexible body for high-amplitude motions, and a high proportion of white/red muscle mass. These characteristics trade off with those maximizing cruising, such as a drag – minimizing body shape with a fineness ratio (length/depth) of about 4.5, with the maximum depth positioned at about a third of the body length, a lunate tail for drag minimization, a rigid body for recoil (hence drag) minimization, and a high proportion of red/white muscle. Experimental work using intraspecific comparisons is in line with these predictions. However, body shape is likely to affect factors other than swimming performance that will in turn also affect the predator–prey encounters. For example, the fineness ratio of prey may affect their vulnerability to predation by gape-limited predators. Similarly, a narrow frontal section in predators allows them to minimize the reaction distance of the prey. In addition, body shape may affect other factors such as reproductive success. Body shape is therefore likely the result of a number of evolutionary forces that are not necessarily related only to swimming performance or to predator–prey relationships.

Environmental Factors

Fast-start performance is affected by a number of environmental factors. Temperature is one of the main factors affecting fast starts, because it affects nerve conduction speed and muscle power output (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). The effect of temperature on reactivity has been particularly studied in escape responses. Responsiveness increases with acclimation temperature, although it increases at low temperatures when acute exposure is used. As expected from the generalized effect of temperature on nerve conduction speed, escape latencies decrease with temperature. However, acute exposure to high temperatures can have a negative effect on reactivity of fish, measured as reaction distance. Similar to responsiveness, the effect of temperature on directionality is modulated by acclimation. Fish show low directionality when acutely exposed to low temperatures. On the other hand, low directionality was observed also in fish acclimated to high temperatures. These contrasting results are due to changes in the properties of the Mauthner cells in acute treatments, and to changes in the processing time of the neural circuitry in fish acclimated to high temperatures, respectively. For both escape responses and predator strikes, propulsive performance (distance, speed, and acceleration) tends to increase at high temperatures as a result of increased muscle performance. Acclimation can provide some degree of compensation. Maneuverability (turning rates and angles) also can be negatively affected at very low temperatures.

Fast starts are fueled anaerobically; therefore, it may be surprising to find that oxygen levels can affect fast-start performance. Recent work has found that exposure to hypoxia does have a negative effect on a number of escape components. Escape responsiveness decreases in hypoxia, possibly as a result of sensory malfunctioning or lower motivation or other physiological factors. While hypoxia did not have an effect on escape latency, directionality decreases in hypoxia, possibly as a result of impairment in the left–right discrimination of stimulus direction. In some species (e.g., Mugilidae), fish show a higher proportion of low-performance (i.e., single bend) responses than high-performance ones (double-bend responses), with negative consequences for propulsive performance. These observations may be explained by physiological exhaustion and negative effects on neural control. Therefore, while relatively acute hypoxia exposure may not directly affect the muscle performance of fish, it may have negative effects on their brain and sensory functions, with direct consequences on the execution of the escape response at all levels. Although specific studies on the effect of hypoxia on predatory strikes have not been carried out, it is likely that the main effect of hypoxia on predators might be a decrease in hunger level.

Other environmental factors such as salinity and turbidity affect reaction distance in escape responses. Osmotic stress in freshwater salmonid smolt exposed to seawater results in a decrease in reaction distance. As expected, turbidity decreases reaction distance, although it also decreases locomotor performance, possibly as a result of a lower amount of sensory information present in the visual stimulus.

A number of pollutants affect fast starts. Ammonia is toxic and it impairs nervous and muscle function in fish. Ammonia exposure increases escape latency and decreased propulsive performance in both escape responses and predator strikes. Other pollutants, such as metallic compounds originating from industrial discharges, can affect fast starts. Cadmium exposure decreases responsiveness, increases latency, and decreases speed in European seabass (*Dicentrarchus labrax*) as a result of impairment of the mechano-sensory system, and mercury-exposed zebrafish (*Danio rerio*) show a decrease in responsiveness and an increase in escape latencies, as a result of an alteration in the neuromuscular performance.

Future Directions

A number of unresolved issues related to the mechanistic basis of fast start remain to be elucidated. Among these, the neural control and the hydrodynamics of predatory strikes need to be investigated. In addition, more work is necessary on the hydrodynamics of different types of escape responses, as well as on the neurobiological basis of this diversity. More efforts taking into account trade-offs in functional morphology should be made to understand the evolutionary basis of the variety of body shapes found in fish. This should take into account the effect of body design on fast start and routine swimming as well as on other factors that are not necessarily related to locomotion. Fast starts have been studied from a number of perspectives, including biomechanics, physiology, functional morphology, neurobiology, behavior, ecology, and evolution. An approach that integrates these disciplines will be necessary in order to assess the fitness value of fast starts. For example, the relationship between fast-start performance and the outcome of predator–prey encounters should be studied both in the laboratory and in the field by combining biomechanics, physiology, and behavioral ecology approaches. Finally, environmental change can affect the fast-start performance of predators and prey to different extents, thereby potentially influencing their relative abundance and distribution. An integrated experimental and modeling approach that takes into account context-dependent variability in fast-start performance is necessary in order to generate predictive ability of environmental change at the ecological level. (**Video Clip 1**).

See also: **Brain and Nervous System**: Physiology of the Mauthner Cell: Function. **Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics; Feeding Mechanics; Functional Properties of Skeletal Muscle: Work Loops; Maneuverability. **Hearing and Lateral Line**: Auditory System Morphology.

Further Reading

Canfield JG and Rose GJ (1993) Activation of Mauthner neurons during prey capture. *Journal of Comparative Physiology Part A* 172: 611–618.

Domenici P (2009) Escape responses in fish: Kinematics, performance and behaviour. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp 123–170. Enfield, NH: Science Publishers (review).

Domenici P (2010) Context-dependent variability in the components of fish escape response: Integrating locomotor performance and behavior. *Journal of Experimental Zoology Part A* 313: 59–79 (review).

Domenici P and Blake RW (1997) The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* 200: 1165–1178 (review).

Domenici P, Lefrançois C, and Shingles A (2007) The effect of hypoxia on the antipredator behaviour of fish. *Philosophical Transactions of the Royal Society B* 362: 2105–2121 (review).

Domenici P, Turesson H, Brodersen J, and Brönmark C (2008) Predator-induced morphology enhances escape locomotion in crucian carp. *Proceedings of the Royal Society B* 275: 195–201.

Eaton RC, Lee RKK, and Foreman MB (2001) The Mauthner cell and other identified neurons of the brainstem escape network of fish. *Progress in Neurobiology* 63: 467–485 (review).

Hale ME (2002) S- and C-start escape responses of the muskellunge (*Esox masquinongy)* require alternative neuromotor mechanisms. *Journal of Experimental Biology* 205: 2005–2016.

Korn H and Faber D (2005) The Mauthner cell half a century later: A neurobiological model for decision-making? *Neuron,* 47(1): 13–28 (review).

Langerhans RB (2009) Morphology, performance, fitness: Functional insight into a post-Pleistocene radiation of mosquitofish. *Biology Letters* 5(4): 488–491.

Langerhans RB and Reznick DN (2009) Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective,* pp .200–248. Enfield: NH: Science Publishers. (review)

Rice AN and Hale ME (2009) Roles of locomotion in feeding. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp .171–199. Enfield, NH: Science Publishers (review).

Schriefer JE and Hale ME (2004) Strikes and startles of northern pike (*Esox lucius)*: A comparison of muscle activity and kinematics between S-start behaviors. *Journal of Experimental Biology* 207: 535–544.

Szabo TM, Brookings T, Preuss T, and Faber DS (2008) Effects of temperature acclimation on a central neural circuit and its behavioral output. *Journal of Neurophysiology* 100: 2997–3008.

Tytell ED and Lauder GV (2008) Hydrodynamics of the escape responses in bluegill sunfish, *Lepomis macrochirus*. *Journal of Experimental Biology* 211: 3359–3369.

Wakeling JA. (2006) Fast-start mechanics. In: Shadwick RE, Lauder GV (eds.) *Fish Biomechanics*, pp 333–368. San Diego, CA: Academic Press (review).

Walker JA, Ghalambor CK, Griset OL, McKenney D, and Reznick DN (2005) Do faster starts increase the probability of evading predators? *Functional Ecology* 19: 808–815.

Webb PW (1984) Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist* 24: 107–120 (review).

Webb PW and Skadsen JM (1980) Strike tactics of *Esox*. *Canadian Journal of Zoology* 58: 1462–1469.

Wilson R, Lefrançois C, Domenici P, and Johnston IA. (2009) Environmental influences on unsteady swimming behaviour: Consequences for predator–prey and mating encounters in teleosts. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp 269–295. Enfield, NH: Science Publishers (review).

Wohl S and Schuster S (2007) The predictive start of hunting archer fish: A flexible and precise motor pattern performed with the kinematics of an escape C-start. *Journal of Experimental Biology* 210: 311–324.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Feeding Mechanics

TE Higham, Clemson University, Clemson, SC, USA

© 2011 Elsevier Inc. All rights reserved.

How Do Fishes Capture Prey?
Feeding in Jawless Fishes
The Evolution of Suction Feeding
What Is Suction-Feeding Performance?
Hydrodynamics of Suction Feeding
Ecomorphology of Feeding
Jaw Protrusion
The Integration of Locomotion and Feeding
Future Directions
Further Reading

Glossary

Buccal cavity The mouth (oral) cavity at the anterior end of the fish in front of the gills.

Digital particle image velocimetry (DPIV) A modern computational technique used to visualize the movement of fluids. Neutrally buoyant reflective particles are placed in the water, illuminated using a laser light sheet and are used to measure water movement. The movements of the particles during feeding are captured using high-speed video.

Ecomorphology The relationship between morphology and ecology.

Exaptation A character, previously shaped by natural selection for a particular function, that later is co-opted for a new use.

Ram The forward movement of the fish.

How Do Fishes Capture Prey?

Suction (mouth expansion), ram (forward swimming), and manipulation are three mechanisms of prey capture in fishes. Despite the considerable diversity among the approximately 28 000 species of fishes, the most common mode of prey capture involves suction feeding. Although the general principles underlying suction feeding are relatively straightforward, several aspects of the feeding system can be modulated in order to alter feeding performance. For example, the rate and magnitude of mouth expansion, the distance between the fish's mouth and prey item, and body movements will influence suction-feeding performance. Jaw protrusion is an additional feature that can enhance suction-feeding performance. Based on recent evidence, intraoral processing (chewing) also appears to be a relatively common feature among fishes. For example, bowfin (*Amia calva*), salmonids (e.g., *Salmo salar*), and pike (e.g., *Esox niger*) all chew prey with their oral jaws. Modern experimental techniques, including sonomicrometry, digital particle image velocimetry (DPIV), *in vivo* pressure recordings, and computational fluid dynamics, all have propelled our understanding of the biomechanics of feeding in fishes. Some websites include videos that show examples of these features of fish feeding (**see** the Relevant Websites section).

The prevalence of suction feeding is the major focus of this article. The mechanisms underlying suction feeding in fishes are fairly complex. For example, the initial expansive phase is a result of hyoid depression, cranial rotation, jaw protrusion, and jaw opening. The musculature involved in feeding is discussed in other articles (**see also The Muscles**: Cartilaginous Fishes Cranial Muscles and Bony Fish Cranial Muscles).

Ram

Several species of fishes capture prey without generating suction or manipulating the prey. Ram feeding occurs when a fish swims over a prey item with its mouth open. Examples of fishes that employ ram include tunas and whale sharks.

Suction

As indicated above, suction feeding is the most common mode of prey capture. It involves the rapid expansion of the buccal cavity, which ultimately draws water into the mouth due to a pressure gradient formed (**Figure 1**). The majority of bony fishes (Osteichthyes) employs suction feeding in order to capture prey (see **Video Clip 1** for an example of a fish employing suction feeding).

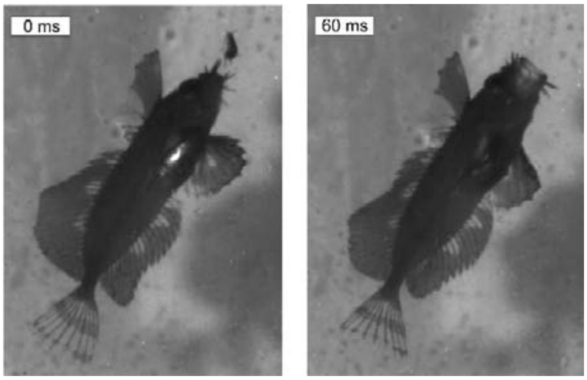


Figure 1 Suction feeding in the silverspotted sculpin, *Blepsias cirrhosus*. These images highlight the extent to which the jaw is protruded during suction feeding and the fact that ram (swimming) is not necessary for capturing prey with suction feeding.

Manipulation

In addition to suction and ram, fishes can obtain prey by physically contacting it and bringing it into the mouth, that is, biting. For example, biting is relatively common among shark and teleost fishes. Every species of heterodontid sharks is ecologically and functionally specialized for durophagy, which involves the consumption of hard prey such as mollusks or crabs. In many cases, the oral jaws of fishes are used to bite a prey item that is located on the substratum. Furthermore, some fishes will bite other animals (e.g., specialized for eating scales) and some will bite off small pieces of plants. As explained below, jawless fishes also use manipulation in order to obtain food.

Feeding in Jawless Fishes

Hagfishes and lampreys feed in a complex and very different way compared with other fishes (**see also Hagfishes and Lamprey**: Hagfishes, Lampreys: Energetics and Development, and Lampreys: Environmental Physiology). The feeding system includes posteriorly directed keratinous teeth that are attached to dental plates, a paired series of cartilages. The dental plates are moved in and out of the mouth during feeding via retractor and protractor muscles. The movement of the dental plate acts in a similar way to a fixed pulley. This system appears to have both advantages and disadvantages relative to fishes with jaws. The pulley system allows maximal force transmission, but does not permit speed amplification. Speed amplification is likely a key aspect of jaws that has facilitated the success of jawed fishes.

The Evolution of Suction Feeding

The invasion of new habitats by early fishes was likely facilitated by the evolution of jaws. The diversity of gnathostomes (jawed fishes) exploded in the Silurian and gnathostomes is currently represented by the chondricthyans and bony fishes. Later advances, such as jaw protrusion (discussed later) and pharyngeal jaws, then enhanced the basic feeding plan. However, it is important to discuss first the origin of jaws and ultimately suction feeding.

Jaws evolved in fishes over 460 million years ago. Recently, two hypotheses have been put forth explaining the origin of jaws. Several commonalities persist in both hypotheses. For example, both agree that jaws represent the first branchial arch, also termed the mandibular arch (**Figure 2**). The evidence supporting this includes the fact that most of the elements of the jaws (skeletal elements, muscles, etc.) resemble those of other branchial arches. Also, both agree that the lower jaw resides in the ancestral position of the mandibular arch, whereas the upper jaw

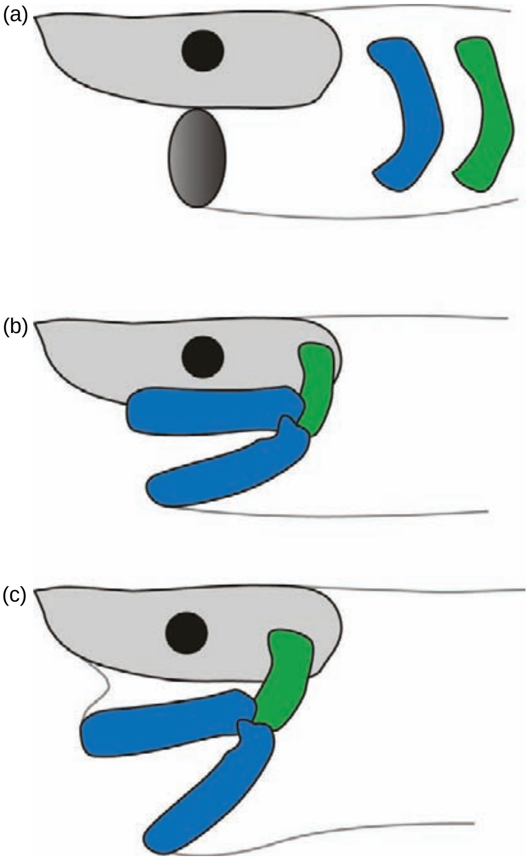


Figure 2 A hypothetical schematic the evolution of jaws for suction feeding. (a) Here the mandibular (blue) and hyoid (green) arches before being involved in the jaws. (b) Here the mandibular arch has become the jaws, which are suitable for respiration. In this amphistylic jaw suspension, the mandibular arch is directly connected tot the cranium. (c) Here the mandibular arch is not directly connected with the cranium, which is more suitable for feeding.

stems from the forward extension (in front of the mouth opening) of the maxillary part of the mandibular arch (**see also The Skeleton**: Bony Fish Skeleton).

The first hypothesis for the origins of jaws is the neoclassical hypothesis, which asserts that the jaw first evolved for stronger ventilation and then to grasp prey. As ventilation increases, prey would be sucked in and potentially consumed. Thus, what originally began with a ventilatory function eventually became utilized for suction feeding, indicating that the current use in feeding is an exaptation. Eventually, the ability to grasp prey was improved when dentition became associated with the mandibular arches. In the neoclassical hypothesis, few or no oral structures were lost during the jawless-to-jawed transition.

The second hypothesis for the evolution of the jaws is the heterotopic hypothesis, which is based mainly on modern developmental studies. This hypothesis asserts that the upper jaw did not evolve through a series of intermediate stages. Instead, certain genes are expressed farther back in the head of gnathostomes compared with lamprey embryos, which leads to different skeletal structures being generated by different parts of the neural crest between the two clades.

What Is Suction-Feeding Performance?

In general, generating high fluid speeds (due to negative pressure in the mouth cavity) during suction feeding is a good indication of suction-feeding performance. However, several other factors have been implicated in suction-feeding performance, including the ingested volume of water (IVW), the volumetric flow rate, strike accuracy, and fluid acceleration. The importance of these metrics depends on the ecology of the fish. For example, largemouth bass (*Micropterus salmoides*) depend strongly on a large IVW and high ram speeds in order to capture prey. For this species, maximum fluid speed is likely not as important for capturing prey. Thus, species-dependent metrics of suction-feeding performance are necessary to understand the feeding diversity of fishes.

Hydrodynamics of Suction Feeding

Modern techniques have greatly enhanced our understanding of feeding mechanics in fishes. One such area that has contributed substantially is hydrodynamics. Advances have emerged in both experimental and modeling techniques.

Visualization of Fluid Movement

The flow of water generated during suction feeding can be quantified using DPIV, which is a modern computational technique used to visualize the flow of fluids. This technique has recently been utilized to study the flow generated by suction in centrarchid fishes and sharks. The speed of the water being sucked into the fish's mouth is highest at the mouth aperture and decays rapidly moving away from the fish's mouth (**Figure 3**). Thus, suction feeding is most effective when the mouth of the predator is in very close proximity to the prey item.

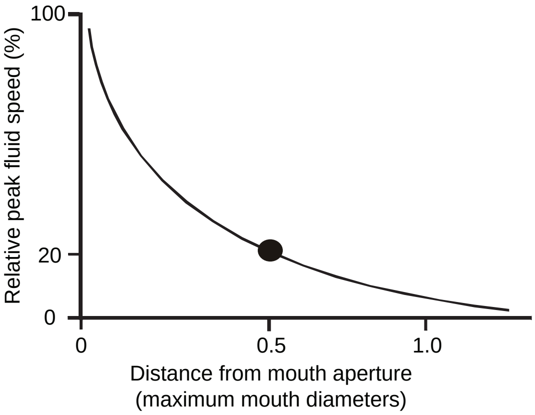


Figure 3 The general relationship between fluid speed and relative distance from the mouth opening (aperture). Fluid speed drops off exponentially going away from the mouth aperture, highlighting the importance of being close to the prey item for succesful suction feeding. The black circle indicates the location that fluid speed is typically measured using digital particle image velocimetry (DPIV).

Direct measurements of fluid flow permit the calculation of the ingested volume, which is strongly correlated with mouth size. Because the flow of water is unidirectional in fishes (i.e., flows in the mouth and out the opercular cavity), the amount of water ingested can exceed the size of the buccal cavity. In addition to the size, one can also calculate the shape of the IVW, which is strongly influenced by swimming speed.

Swimming during suction feeding can alter the hydrodynamics of suction feeding. Direct measurements from bluegill sunfish and largemouth bass indicate that, as swimming speed increases, the ingested volume becomes increasingly narrow and elongate (**Figure 4**). For further details, see section The Integration of Locomotion and Feeding.

Modeling

When experimental measurements are difficult (or impossible) to obtain, modeling the flow of water becomes increasingly informative. In addition, modeling allows one to predict the effects of manipulating a single variable while holding everything else constant. Modeling can ultimately provide insight into animal diversity and predator–prey interactions. Several attempts have been made to model the hydrodynamics of suction feeding in fishes. In the early 1980s, the expanding mouth cavity was

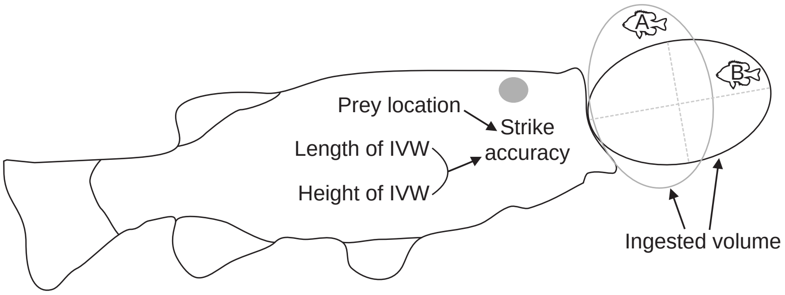


Figure 4 The influence of swimming (ram) speed on the shape of the ingested volume of water (IVW) and strick accuracy. In situation A, the forward swimming speed of the predator is negligible and prey item A is caught. However, prey B cannot be caught because the IVW is not long enough. In situation B, there is substantial ram speed and prey B is caught. However, prey A cannot be caught because the IVW is too narrow. Ultimately, the length of the IVW, and the location of the prey, will play a large role in capture success.

modeled as a single truncated cone with a gape (anterior end) and a posterior height. A number of input parameters define how the buccal cavity expands. This model yielded pressure and fluid speed for given dimensions and expansion profile of the feeding event. More than 20 years later, the model was modified to include three truncated cones connected in series.

Recently, the early model was tested by measuring both pressure and fluid speed simultaneously during suction feeding in largemouth bass and bluegill sunfish (*Lepomis macrochirus*). Pressure transducers were implanted through the dorsal aspect of the skull and they ultimately protruded into the buccal cavity. DPIV was used to quantify fluid speed. The dimensions of the buccal cavity, and the profile of expansion, were also used to predict fluid speed using the early model. Ultimately, the predicted values of fluid speed exceed those measured under *in vivo* conditions. Reasons that the model could not fully explain the actual fluid speeds may include complex patterns of expansion within the mouth cavity and/or unsteady flow normal to the long axis of the fish. However, more work is needed to understand the dynamics of water flow inside the mouth of a fish.

Another technique for modeling fluid flow during suction feeding is computational fluid dynamics - a branch of fluid dynamics that utilizes the incompressible Navier–Stokes equations, which govern the flow of water during suction feeding. An interesting conclusion from a recent study is that prey size is a very important factor when considering the timing and magnitude of peak forces exerted on prey. Based on modeling, it is apparent that peak force can be reached most quickly for prey items that are approximately 33% of mouth diameter. This has important implications for prey selection by fishes.

Ecomorphology of Feeding

The relationships between morphology and ecology, and ultimately performance, have been well studied in fishes. In particular, several studies have focused on the relationship between diet and structural aspects of the mouth. Recent work has provided a functional foundation for such studies, making the link between morphology and ecology more evident. For example, mouth size is strongly correlated with prey size such that species with larger mouths are able to ingest larger prey items.

Jaw lever mechanics are commonly studied in relation to ecology. One can measure the closing and opening in- and out-levers of the jaw, and then relate that to the diet or feeding style of the predator. In coral reef fishes of the Caribbean, species that use their oral jaws to grip, manipulate, bite, shred, or crush prey have very high jaw closing lever ratios. In contrast, those species that exhibit ram- or suction-based strike tactics exhibit much lower jaw closing ratios. Higher closing ratios indicate the ability to more efficiently transmit the force generated by the muscles to the tooth surface. Jaw opening ratios do not show an obvious pattern like the jaw closing ratios.

The ecomorphology of feeding in cottid fishes (sculpins) has also been examined in detail. The focus has primarily been on the species of the Northeastern Pacific. In these studies, a larger mouth was linked to feeding on more elusive prey (i.e., fishes, shrimp, mysids, and octopods). In addition to the general ecomorphological approach, functional studies have also been used to test whether the morphology exhibited by some species enhances their performance in an ecological context. Larger-mouthed species of sculpins exhibit significantly higher capture success on evasive shrimp than did smaller-mouthed species. This functional link is critical for providing a mechanism underlying the correlation between morphology and ecology.

Jaw Protrusion

As mentioned above, the flow of water generated by suction is limited to a narrow range outside of the fish's mouth. Thus, being able to get extremely close to a prey item is necessary for effective suction feeding. Jaw protrusion is one way in which the mouth can be extended closer to the prey without having to move the entire body (**Figure 1**). Taken another way, the predator could initiate a strike at a farther distance from the prey.

The ability of fishes to protrude their upper jaw has been cited as a key innovation that facilitated the trophic diversity among fishes. The premaxilla is rotated or pushed into an extended position, and multiple paths to jaw protrusion have evolved. The upper jaw movement can involve anterior, anterodorsal, or anteroventral aspects, and the lower jaw or mandible can pivot around a ball and socket articulation.

Recent work has revealed that jaw protrusion enhances forces exerted on a prey item. Although this had been suggested for quite some time, empirical data exploring the functional ramifications of jaw protrusion are limited. Utilizing a framework based on data from bluegill sunfish, a recent study utilized empirical data and simulations to calculate the contribution of jaw protrusion to the forces exerted on prey. The observed speed of jaw protrusion was positively correlated with the peak measured force on the prey. Ultimately, the independent source of acceleration provided by jaw protrusion can increase the total force on the prey by up to 35%.

The Integration of Locomotion and Feeding

For several reasons, locomotion is critical for most fishes to successfully capture prey. Locomotor performance (e.g., maximum swimming speed and maximum acceleration) will be important for overtaking an evasive or elusive prey item. In addition, controlling the locomotor system (e.g., stability and positioning) will play a significant role in the timing and accuracy of a strike, which will ultimately determine capture success.

Ram Speed

Although ram is often a component of a feeding event, the relative contribution can vary considerably. One ramification of increased ram speed is a decreased capture success. Among other things, swimming fast during prey capture decreases the time that the predator has to adjust its position relative to the prey. This can result in poor aiming and/or incorrect timing of mouth opening. One critical aspect of suction feeding that is altered with ram speed is the shape of the IVW (**Figure 4**). As a fish swims faster during feeding, the resulting IVW will be increasingly elongate and narrow. This is important with respect to positioning of the prey in front of the predator's mouth. If the prey item is located farther away from the predator, an elongated IVW will facilitate prey capture.

Pectoral Fins

As outlined earlier in this article, strike accuracy is necessary for successful prey capture in fishes, especially those species that rely heavily on suction. In acanthomorph fishes, the pectoral fins are well suited for braking without inducing any unsteady maneuvers because of their location relative to the fish's center of mass (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming). For these fishes, the reaction force generated by the pectoral fins during braking goes through the center of mass, thus maintaining the fish's position. Many fishes brake during prey capture, but why they do this is still in question. One explanation is that braking reduces the approach speed, which will allow the predator to adjust its position relative to the prey. Thus, braking may increase capture success. Another possible benefit to braking during prey capture is that it puts the predator in a position to quickly maneuver and follow the prey if it escapes. Finally, braking might prevent a collision with the substrate (if the prey is located close to structure), which will ultimately prevent injury.

Future Directions

Where is the field of feeding mechanics in fishes headed? With an increased understanding of the hydrodynamics and mechanics of suction feeding, we are now poised to explore the incredible diversity that is evident in the many thousands of extant species of fishes. In addition, few studies have attempted to understand the evolution of feeding mechanics, in part due to the incomplete understanding of the mechanics and also a lack of well-resolved phylogenies. This will be a fruitful area of research in the future. Finally, although we know much about the feeding and locomotor systems, our understanding of how multiple complex systems are integrated is poor. How do fishes move and rapidly expand their mouths with coordinated timing? What underlying neural control mechanisms permit the simultaneous actions of the systems?

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics; Maneuverability; Paired Fin Swimming. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **The Muscles**: Bony Fish Cranial Muscles; Cartilaginous Fishes Cranial Muscles. **The Skeleton**: Bony Fish Skeleton.

Further Reading

Carroll AM, Wainwright PC, Huskey SH, Collar DC, and Turingan RG (2004) Morphology predicts suction feeding performance in centrarchid fishes. *Journal of Experimental Biology* 207: 3873–3881.

Day SW, Higham TE, Cheer AY, and Wainwright PC (2005) Spatial and temporal patterns of water flow generated by suction feeding bluegill sunfish *Lepomis macrochirus* resolved by particle image velocimetry. *Journal of Experimental Biology* 208: 2661–2671.

Higham TE (2007) Feeding, fins and braking maneuvers: Locomotion during prey capture in centrarchid fishes. *Journal of Experimental Biology* 210: 107–117.

Higham TE, Day SW, and Wainwright PC (2005) Sucking while swimming: Evaluating the effects of ram speed on suction generation in bluegill sunfish *Lepomis macrochirus* using digital particle image velocimetry. *Journal of Experimental Biology* 208: 2653–2660.

Higham TE, Day SW, and Wainwright PC (2006) Multidimensional analysis of suction feeding performance in fishes: Fluid speed, acceleration, strike accuracy and the ingested volume of water. *Journal of Experimental Biology* 209: 2713–2725.

Holzman RA, Day SW, Mehta RS, and Wainwright PC (2008) Jaw protrusion enhances forces exerted on prey by suction feeding fishes. *Journal of the Royal Society Interface* 5: 1445–1457.

Lauder GV (1980) The suction feeding mechanism in sunfishes (*Lepomis)*: An experimental analysis. *Journal of Experimental Biology* 88: 49–72.

Mallatt J (2008) The origin of the vertebrate jaw: Neoclassical ideas versus newer, development-based ideas. *Zoological Science* 25: 990–998.

Motta PJ (1984) Mechanics and functions of jaw protrusion in teleost fishes: A review. *Copeia* 1984: 1–18.

Muller M, Osse JWM, and Verhagen JHG (1982) A quantitative hydrodynamical model of suction feeding in fish. *Journal of Theoretical Biology* 95: 49–79.

Nemeth DH (1997) Modulation of buccal pressure during prey capture in *Hexagrammos decagrammus* (Teleostei: Hexagrammidae). *Journal of Experimental Biology* 200: 2145–2154.

Norton SF (1995) A functional approach to ecomorphological patterns of feeding in cottid fishes. *Environmental Biology of Fishes* 44: 61–78.

Norton SF and Brainerd EL (1993) Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology* 176: 11–29.

Sanford CPJ and Wainwright PC (2002) Use of sonomicrometry demonstrates the link between prey capture kinematics and suction pressure in largemouth bass. *Journal of Experimental Biology* 205: 3445–3457.

Svanback R, Wainwright PC, and Ferry-Graham LA (2002) Linking cranial kinematics, buccal pressure, and suction feeding performance in largemouth bass. *Physiological and Biochemical Zoology* 75(6): 532–543.

Van Wassenbergh S and Aerts P (2009) Aquatic suction feeding dynamics: Insights from computational modelling. *Journal of the Royal Society Interface* 6: 149–158.

Wainwright PC, Carroll AM, Collar DC, *et al.* (2007) Suction feeding mechanics, performance, and diversity in fishes. *Integrative and Comparative Biology* 47: 96–106.

Wainwright PC, Ferry-Graham LA, Waltzek TB, *et al.* (2001) Evaluating the use of ram and suction during prey capture by cichlid fishes. *Journal of Experimental Biology* 204: 3039–3051.

Westneat MW (2004) Evolution of levers and linkages in the feeding mechanisms of fishes. *Integrative and Comparative Biology* 44: 378–389.

Wilga CD, Motta PJ, and Sanford CP (2007) Evolution and ecology of feeding in elasmobranchs. *Integrative and Comparative Biology* 47: 55–69.

Relevant Websites

http://www.youtube.com/watch?v=AWsbwQ-yxw4&feature=related – You Tube; Freshwater eel, *Anguilla* sp., feeding.

http://www.youtube.com/watch?v=MSzEGg-6wWo – You Tube; Particle image velocimetry video of suction feeding by a bluegill sunfish, *Lepomis macrochi.*

http://www.youtube.com/watch?v=OHivHHmYSDc – You Tube; Particle image velocimetry video of suction feeding by a largemouth bass, *Micropterus salmoides.*

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Biomimetics: Robotics Based on Fish Swimming

JH Long Jr., Vassar College, Poughkeepsie, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Fish and Robotic Fish
Biomimetics as an Approach to Fish Robotics
Classification of Fish Robots
Design of Fish Robots
Robotic Fish: The Next Generation
Further Reading

Glossary

Bioinspiration The analytical and synthetic process by which biologists and engineers extract functional principles from biological systems and instantiate those principles in engineered systems.

Biomimetics The analytical and synthetic process by which biologists and engineers duplicate biological structure, function, or both.

Instantiate To represent an abstract concept with a concrete example.

Robot A whole fish or part of a fish that is modeled digitally, using a computer-based simulation, or physically, using a mechanical device. The modeled whole or part moves and thus interacts mechanically with the environment.

Fish and Robotic Fish

Biologists classify fish as propulsive machines of two types: (1) body-caudal fin swimmers (BCF), who use body undulations and oscillations of the caudal fin (**see also Buoyancy, Locomotion, and Movement in Fishes:** Undulatory Swimming) and (2) median-paired fin swimmers (MPF), who use any combination of pectoral, pelvic, dorsal, and anal fins to produce thrust (**see also Buoyancy, Locomotion, and Movement in Fishes:** Paired Fin Swimming). Many species combine BCF and MPF mechanisms, using BCF swimming for cruising and escape and MPF swimming for station-holding and maneuvering in tight quarters (**see also Buoyancy, Locomotion, and Movement in Fishes:** Maneuverability and Stability and Turbulence).

Biological targets for BCF robotic fish have included lamprey (unspecifed species of the order Petromyzontiformes), eel (unspecifed species of the order Anguilliformes), sea bream (unspecified species of the family Sparidae), koi carp (*Cyprinus carpio*), coelacanth (*Latimeria chalumnae*), pike (unspecified species of the family Esocidae), trunkfish (unspecified species of the family Ostraciidae), and tuna (unspecified species of the family Scombridae). Biological targets for MPF robotic fish have included bluegill sunfish (*Lepomis macrochirus*), bird wrasse (*Gomphosus varius*), stingray (unspecified species of the family Dasyatidae), manta ray (*Manta birostris*), and knifefish (unspecified species of the order Gymnotiformes).

The first robotic fish is a subject of controversy. RoboTuna is often given credit as being the first. Working in the laboratory of Michael S. Triantafyllou at the Massachusetts Institute of Technology (United States), David Barrett began creating Charlie I, the first RoboTuna, in 1993. Charlie I undulated like a BCF fish (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming) in the water as it was towed from above on a gantry. Charlie I and then II provided the foundation that allowed Jamie Anderson of Draper Laboratories (United States) to build a self-propelled BCF descendent of the original RoboTunas, the Vorticity Control Unmanned Undersea Vehicle (VCUUV). In 1998, the VCUUV swam for the first time under its own power in a pool.

Some people consider the first robotic fish to be TwiddleFish. Invented by Charles Pell and Stephen Wainwright, the self-propelled BCF TwiddleFish was designed at Duke University (United States). Working with graduate students, the duo hit upon the simple idea that a fish-shaped piece of rubber would generate undulatory waves in water if the fish was actuated by an oscillating torque within the head. By 1995, Pell published a scientific paper using the TwiddleFish platform to study how changes in a fish's body stiffness control swimming performance. Descended from the TwiddleFish is the Nektor, a class of flapping flexible foils used as thrusters on underwater vehicles, instead of rotary propellers, when high-performance maneuverability is desired.

Working robotic fish are found in the entertainment industry as well as in academia. For example, autonomous, self-propelled BCF robotic fish swim with adaptive behavior in the London Aquarium. These entertainment robots were created by researchers at the Human Centered Robotics Group at Essex University (United Kingdom), under the leadership of Housheng Hu. The first robotic fish built for aquarium markets was the sea bream, a collaboration of Yuuzi Terada, Fukui University of Technology (Japan), and Ikuo Yamamoto, Mitsubishi Heavy Industries (Japan). By 2003, they and their collaborators had BCF robotic sea bream and coelacanth swimming in specially designed tanks that allowed the behavior of the robotic fish to be externally controlled.

In China, Wang Tainmiao, of Beijing University of Aeronautics and Astronautics, and Tan Min, of the Chinese Academy of Sciences, built a BCF robotic fish, SPC-03, for underwater archeology; it has operated successfully in exploration missions. Kristi Morgansen, University of Washington (United States), built a coordinated school of three self-propelled, autonomous, and communicating BCF/MPF robotic fish. In a multinational project, FILOSE, sponsored by the European Union (Seventh Framework Programme), Maarja Kruusma, Talinn University of Technology (Estonia), is coordinating biologists and engineers from the Italian Institute of Technology (Italy), Riga Technical University (Latvia), the University of Verona (Italy), and the University of Bath (England). They are designing a biomimetic lateral line and a BCF robotic fish to go with it; using the lateral line, the robotic fish will sense currents, detect objects, swim efficiently, and navigate.

Biomimetics as an Approach to Fish Robotics

Why make robotic fish? Researchers build robotic fish for three main purposes: (1) to test biological hypotheses about propulsion, perception, behavior, and evolution of fishes; (2) to engineer biologically inspired propulsion, perception, and behavior into underwater vehicles; and (3) to serve as objects of entertainment. Built for whichever purpose, robotic fish are, by definition, biomimetic.

The term 'biomimetics' refers to the process by which biologists and engineers duplicate biological structure, function, or both. To differentiate duplication *per se* from emulation, some researchers use the term 'bioinspiration', the process by which biologists and engineers extract functional principles from biological systems and instantiate those principles in engineered systems. The level (size, process, material, or element) of structural or functional duplication or inspiration is determined by the investigator's goals and knowledge of the biological source.

Among biologists, controversy exists about whether structure and function can be analyzed and duplicated independently or if structure and function occur only in one-to-one causal mappings. Functionalists argue that many different mappings exist: single structures may be capable of having multiple functions and multiple structures may be capable of having identical functions. This functionalist framework forms the premise for most work in biomimetics, since the biological target can, strictly speaking, only satisfy one-to-one mapping by duplication via reproduction. Duplication via engineering uses materials that are often nonbiological and are rarely identical to those of the biological source. Inspiration, by definition, assumes that many different kinds of structure can implement the same function.

Classification of Fish Robots

Within the fields of robotics, artificial intelligence, engineering, and biology, no single definition of 'robot' exists. For biologists interested in fish, 'robot' can mean a whole or part of a fish that is instantiated digitally, using a computer-based simulation, or physically, using a mechanical device. Robotic fish can be distinguished by the following dimensions:

1. proportion of fish (states: whole or part);
2. instantiation (states: mechanical, digital, or combination);
3. propulsive mode (states: self-propelled, towed, or tethered);
4. propulsive mechanism (states: body-caudal fin, median fin (s), paired fins, or combination);
5. control type (states: autonomous, remote-controlled, or open-loop);
6. control architecture (states: behavior-based, cognitive, or combination);
7. energy source (states: internal or external);
8. processor type (states: analog or digital);
9. processor location (states: internal or external);
10. actuator types (states: hydraulic, pneumatic, electric, polymeric, shape-memory alloy, piezoelectric, or combination);
11. actuator location (states: internal or external);
12. sensor types (states: single, paired, array, and/or various);
13. sensor locations (states: internal, body surface, and/or off-vehicle);
14. chassis type (states: rigid with hinges, flexible without hinges, or combination);
15. habitat (states: benthic, pelagic, surface, amphibious, or combination);
16. behavior types (states: navigation, escaping, exploring, schooling, other, or combination);

17. purpose (states: hypothesis-testing, commercial application, entertainment); and
18. biological target (states: structure, function, taxon, or combination).

The remainder of this article focuses on mechanical instantiations of robots.

Design of Fish Robots

The design process varies depending on the purpose of the robot (see item 17, above) as determined by the investigator or engineer. Options are constrained by practical considerations.

For hypothesis testing, the investigator must first generate or select a hypothesis that can be tested using robots. Hypotheses about the mechanics and control of swimming abound (**see also Buoyancy, Locomotion, and Movement in Fishes:** Maneuverability), Stability and Turbulence, Undulatory Swimming, and Paired Fin Swimming) and many of these hypotheses are testable with robots. Examples of robotically testable hypotheses include: (1) propulsion is mediated by vortex shedding at the trailing edges of fins; (2) gliding performance can be controlled by lifting surfaces on the body or fins; (3) lift-based flapping in fins produces more propulsive work than drag-based rowing; and (4) turning in yaw is enhanced by reducing the mass moment of inertia of the body.

For commercial application, the engineer must first identify one of the following: (1) a function of a fish or function within a fish that is novel to engineering and can be reduced to a mechanical or mathematical principle; (2) a behavior of a fish or system of fish that is novel to engineering and can be reduced to an algorithm; and (3) a structure of a fish that is novel to engineering and can be duplicated in other materials.

In practice, biologists and engineers often collaborate. Biologists bring expertise about fish, ecology, performance, and evolution. Engineers bring expertise about constructing the mechanical, electrical, and software systems. Also, cognitive scientists, computer scientists, and artists can be found on many design teams.

Following the hierarchical analytical approach of David Marr, the design team must answer the following questions within their purpose-specific framework:

1. What function(s) or behavior(s) will the robot perform? The answer to this question is informed by the hypothesis to be tested or the function or behavior to be targeted for application.

2. How, in terms of mechanical and algorithmic processes, will the robot accomplish the desired function(s) or behavior(s)? This level of design decomposes the overall design into information flow, interactions of physical subsystems, and the conceptual choices needed on each design dimension (see list of 18 dimensions in previous section). If the desired function(s) or behavior(s) require an autonomous, self-propelled robot, it is crucial at this stage of design to recognize that behavior depends on the robot's interaction with a specific environment. Because behavior cannot be fully predicted on the drawing table, it is likely that the team will iterate between this level and the next (number 3, below) during prototyping.

3. How will the robot be physically implemented? This is the level of engineering design, and the question can only be answered once the higher-level questions are addressed and specifications are created. Referring to the 18 dimensions of classification (see previous section), decisions must be made about specific hardware and software. The team must procure or create motors, sensors, power source, computer, and mechanical mechanisms that meet the specifications identified at the previous level.

Beyond these general guidelines, the specifics of the design process are different for every new robotic fish. To demonstrate different design processes and systems, two case studies are given in the next sections.

Case Study 1: Testing Biological Hypotheses Using Evolving Robots

One use of robotic fish is to reconstruct the ecological and evolutionary situation in which extinct species might have evolved structural and functional characters. Researchers at Vassar and Lafayette Colleges (United States) were interested in the evolution of vertebrae and caudal fins, macroevolutionary events that began in the earliest vertebrates, the jawless fishes of the Cambrian and Ordovician Periods that lived as long as 530 million years ago. The first vertebrates had no vertebrae and had caudal fins that tapered to a point. From these ancestral states, vertebrae and flared caudal fins evolved over the next 100 million years. The biological question was: what are the plausible selection pressures that might have increased the number of vertebrae (reducing the size of the intervertebral joints) and the span of the caudal fin?

Because predation is a powerful selection force in the evolution of living fish, such as the guppies of Trinidad, the researchers hypothesized that predation was likely to have been important in the fossil fish, too. Recognizing that prey, and not just the predator, must also eat, the team generated the following hypothesis:

selection for enhanced foraging and predator avoidance in a population of prey robots will evolve an increase in the number of vertebrae (=smaller intervertebral joints) and an increase in the span of the caudal fin (=larger flare). The connection between selection and the change of structures was detailed in two functional hypotheses: (1) increasing the number of vertebrae stiffens the axial skeleton, increasing elastic energy storage and (2) increasing the span of the caudal fin increases the thrust power.

For the design of a robotic system, the team decided that they needed an autonomous, self-propelled prey robot that could eat and avoid being eaten. This answered Marr's design question one (see previous section). To accomplish this task (Marr's question number two), the team decided to build a fish robot that would seek light as the equivalent of foraging; for predation avoidance, the robot would detect an approaching predator robot and then escape. Drawing from what is known about the neuro-motor patterns of swimming fish that perform an escape maneuver while cruising, the team wanted the prey robot to have its escape behavior override its foraging behavior. They also decided that they would build a predator fish robot that would not evolve; its only function would be to chase the prey robot and attempt to ram it. To swim, the prey robot needed to have a propulsive, undulating tail that could vary in number of vertebrae and span of the caudal fin. Finally, the characters needed to be genetically coded; genes needed to go through biological processes such as mutation, independent assortment, and gametogenesis. Random mating would also be conducted, with breeding parents determined by evolutionary fitness.

To address Marr's third design question, about physical implementation, the team decided to build the vertebral column and caudal fin as a biomimetic tail (**Figure 1**). The tail was built to be attached to a motorized shaft that input a sinusoidal bending couple that propagated down the tail as an undulatory wave, resulting in the caudal fin moving laterally and generating propulsion. The tail and caudal fin generated thrust and turning moments for steady cruising used during foraging; the tail and caudal fin also created the rotational forces used during an escape maneuver. The tail was driven by a servo motor attached to a round plastic bowl that acted as a hull for the surface-swimming robots (**Figure 2**). In the prey robot, two paired photoresistors acted as light-detecting eyes, and laterally placed infra-red proximity detectors acted as a predator-detecting lateral line. These sensors were monitored by an on-board microcontroller programmed to have the robot seek light in the absence of the predator and avoid the predator when it was detected. Power for the robots was also on-board in the form of batteries. The genetics were implemented on a separate computer using a genetic algorithm approach, with number of vertebrae, tail span, and predator detection threshold each coded as quantitative traits on separate chromosomes. Random mutation occurred during gametogenesis. A population size of 18 prey robots was used, with six different genotypes each cloned in triplicate to provide replication. A single predator robot was built. All experiments were conducted in an artificial ecosystem, a 3.1-m diameter aquaculture tank painted matte black to minimize reflections from the single over-hanging light source.

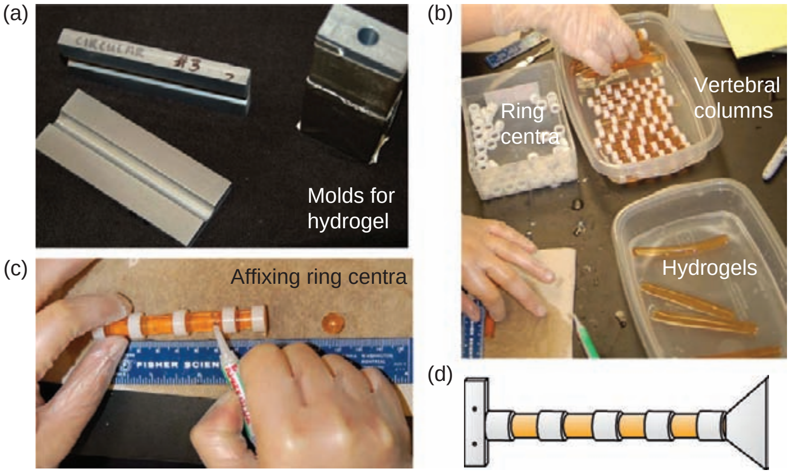


Figure 1 Building a biomimetic tail for a self-propelled fish robot: (a) Right, circular hydrogels are created from gelatin in molds. The hydrogels are cross-linked to achieve the desired Young's modulus. (b) Hydrogels are cut to size and then turned into vertebral columns by adding ring centra. (c) The ring centra are affixed using cyanoacrylate glue. (d) The final tail has a caudal fin (to right) and a grip (to left) by which to attach the tail to the robot's servo motor. Depending on a combination of random (mutation, drift, and reproduction) and deterministic (selection) processes, the number of vertebrae and caudal fin span for any individual varies from 2 to 13 and 0 to 50 mm, respectively. Photos by John Long; tail illustration by Hannah Rosenblum.

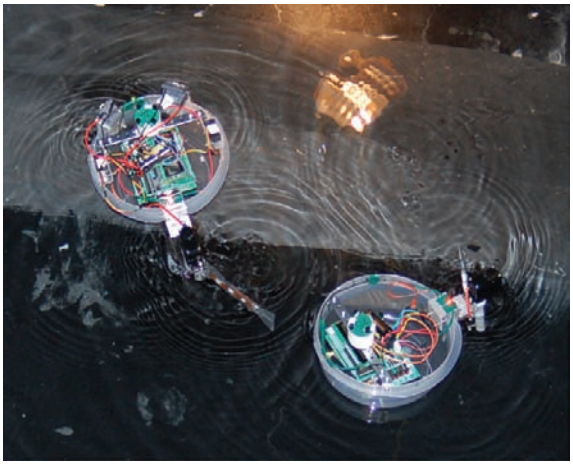


Figure 2 Biomimetic, self-propelled, and autonomous fish robots in action. A prey robot (upper left) and a predator robot (lower right), were built to test the hypothesis that the number of vertebrae and size of the caudal fin span increased in a population of prey as an evolutionary response to selection for improved foraging and predator avoidance. Note the biomimetic tail (see **Figure 1**) propelling the prey robot. In this selection experiment, the prey robot finds and orbits the light source (reflection in upper right) while being tracked by the predator (lower right). These biomimetic robots have control, energy, and actuators located on-board. Sensors include photocells, infrared proximity detectors, and TR-tracking Sensors. Photo by John Long.

For each generation, each of the 18 individual prey robots was tested in a 3-min trial with the predator. Overhead video and an on-board accelerometer were used to log information about the prey's speed, average distance to the light source, average distance from the predator, number of escape maneuvers initiated, and maximum acceleration during the escapes. These metrics were combined in a compound fitness function that rewarded individual genotypes, relative to other genotypes in that generation. The genotypes with the top three fitness values were permitted to breed. For the next generation, new offspring genotypes provided instructions for the new 18 prey robots.

Under these experimental conditions, the team found support for the hypothesis. Selection for enhanced foraging and predator avoidance resulted in an evolutionary response by the population: the mean number of vertebrae and the mean caudal fin span increased. This result suggests that the following scenario is plausible: selection for enhanced foraging and predatory avoidance in ancient vertebrates drove the evolution of vertebrae and caudal fins. Note that the researchers do not claim that this is what happened, only that they were unable to eliminate this particular selection scenario as possible.

Case Study 2: Engineering Fish-like Vehicles for Near-Shore and Benthic Operations

One use of robotic fish is to build a mobile instrument platform that combines low-power swimming, high maneuverability, and stable benthic station holding. Benthic interactions are particularly important in near-shore coastal environments that contain and combine high-energy tides, currents, surf, obstacles, and fixed and mobile marine structures. This zone, which includes busy harbors, rivers, and beaches, is the focus of terrestrial–marine interactions, and humans need mobile instruments that allow detection, tracking, and mapping of phenomena such as thermal gradients, salt isoclines, and seepage or spills. To build a mobile instrument platform for this environment, researchers and engineers at Vassar College and IntelliTech Microsystems (United States) collaborated to build a biomimetic fish robot designed to be a commercial-grade autonomous, underwater, and self-propelled vehicle.

To satisfy as many of the requirements for working in the nearshore zone as possible, the team sought a biological target. Skates (order Rajiformes) and rays (order Myliobatiformes) were a tentatively identified because they live in the zone, are stable on sandy bottoms by virtue of burrowing, and are maneuverable. Both groups were disqualified after considering implementation problems: members of those orders undulate or oscillate their body disk for propulsion, and this motion is either (1) difficult to emulate or (2) disruptive of payloads that might be housed in the body disk. Both of these implementation difficulties were obviated by the choice of electric rays (order Torpediniformes), because electric rays possess many of the desired engineering features: (1) propulsion by an undulatory tail that is independent of the nonpropulsive body disk; (2) the body disk houses a nonpropulsive payload, the electric organs; and (3) mid-water swimming is combined with high maneuverability in yaw and low-power station holding via benthic burrowing.

Duplication of an electric ray was not the goal for the team. In response to Marr's first design question (see section above) – what function(s) or behavior(s) will the robot perform? – the team decided to focus on the functions of propulsion, maneuverability, and simple gradient navigation for the first prototype, named RayBot. In terms of Marr's second design question – how will the robot accomplish the desired function or behavior? – the team decided to build an autonomous and self-propelled robot, to drive it with a biomimetic actuator connected to an undulatory tail, to house the actuator within the body of the robot, to place power and control on-board, to build the body of the robot as a single flexible unit shaped like an electric ray, to design the body disk to bend its trailing edges for

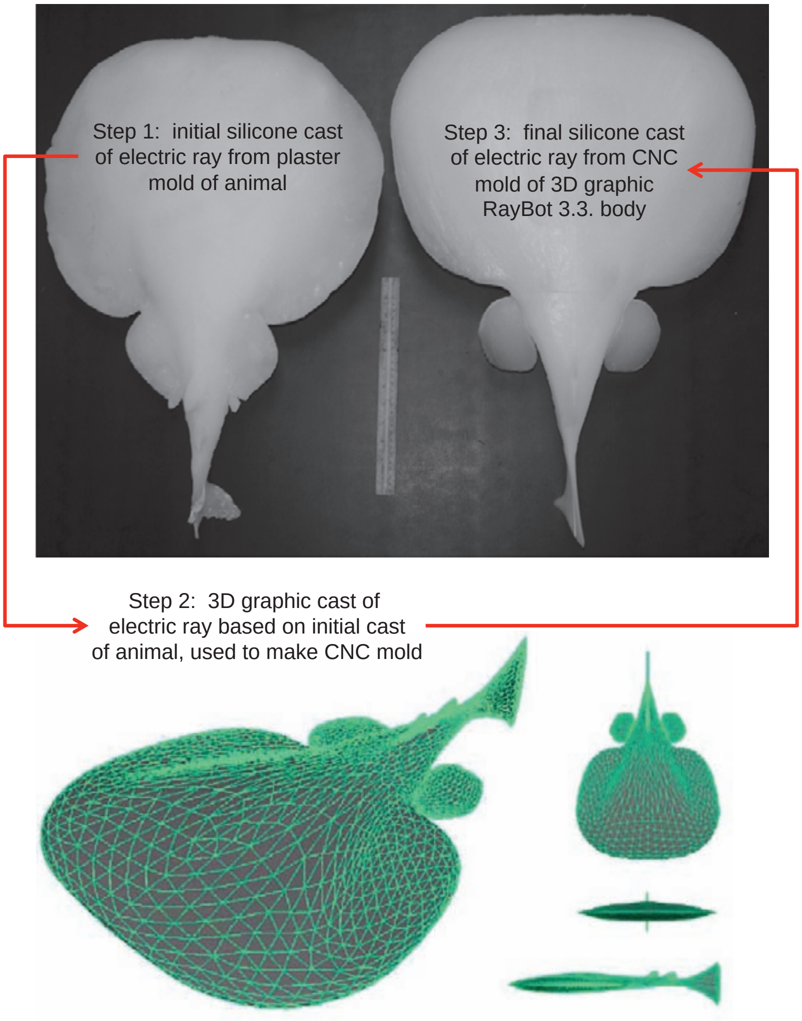

Step 1: initial silicone cast of electric ray from plaster mold of animal
Step 3: final silicone cast of electric ray from CNC mold of 3D graphic RayBot 3.3. body
Step 2: 3D graphic cast of electric ray based on initial cast of animal, used to make CNC mold

Figure 3 Creating a biomimetic commercial fish robot, RayBot. Step 1: a rubber cast made of a real electric ray (*Torpedo nobiliensis*) was the physical model for our 3D graphic. Step 2: From the electric ray cast, a 3D computer graphics cast was created. The 3D coordinates were then used by a CNC milling machine to cut a mold. Step 3: using the CNC mold (see **Figure 4**), the final body of RayBot was cast using a flexible silicone (Dragon Skin, Smooth-On Inc., Easton, PA). Since the initial cast contained asymmetries and transitions difficult to machine, the 3D graphic representation was a simplification. RayBot is collaboration between Vassar College and IntelliTech Microsystems. Figure created by John Long and Joshua de Leeuw.

control of maneuverability and body lift, to make the body negatively buoyant so that station holding on the bottom could occur passively, and to equip RayBot initially with simple paired sensors for navigation up or down light gradients.

Physical implementation of RayBot – the topic of Marr's third design question – centered around the design of the ray-like flexible body (**Figure 3**). The team acquired a large (50-cm-wide body disk) electric ray, *Torpedo nobiliensis*, from the Marine Biological Laboratory, Woods Hole, Massachusetts. A mold of this ray was made using plaster of Paris. From the mold, the team created a flexible silicone-based cast (Dragon Skin™, Smooth-On Inc., Easton, PA). The problem with the cast was its asymmetry; bulges and concavities on the body's surface were created by the displacement of viscera during mold making. To overcome these asymmetries, the team used the Dragon Skin™ cast as the model to create a three-dimensional (3D) rendering of the ray's surface (**Figure 3**) using Autodesk® Maya® (San Rafael, CA) 3D modeling software. This 3D rendering was a simplification of the real electric ray, with paired and median fins reshaped to allow the machining of a mold. The coordinates for the 3D rendering were transferred to a three-axis computer-numerically-controlled milling machine, which then cut the nine-piece mold. The nine-piece mold was assembled, including the internal housing and actuator system, and filled with Dragon Skin™. The resulting 50 cm × 75 cm, 5.5 kg cast was a single piece with the embedded payload, actuator, and microcontroller (**Figure 4**).

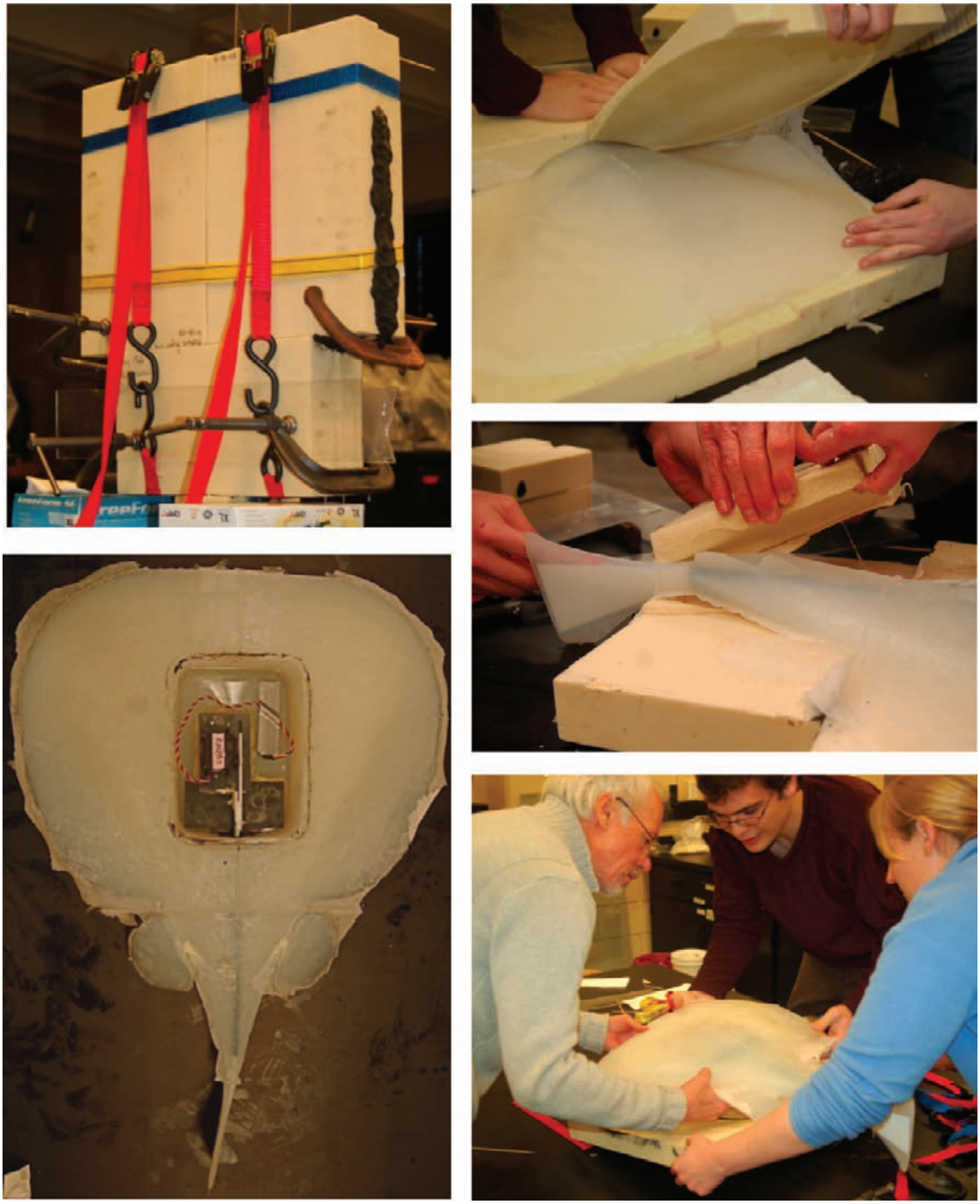


Figure 4 Casting the flexible body of RayBot. Clockwise from upper left: the mold machined to create the negative form of the 3D graphic model (see **Figure 3**) was assembled and filled with Dragon Skin (Smooth-On Inc., Easton, PA). Once set, the mold, in nine pieces, was removed and the body was freed. Lower left: ventral view of the cast body, untrimmed, showing the central payload for the servo motor and microcontroller. Also visible is the fin-shear actuation system that extends from the payload to the caudal fin, a system that permits a single, centrally located motor to drive bending distributed along the tail's axis. Photos by John Long.

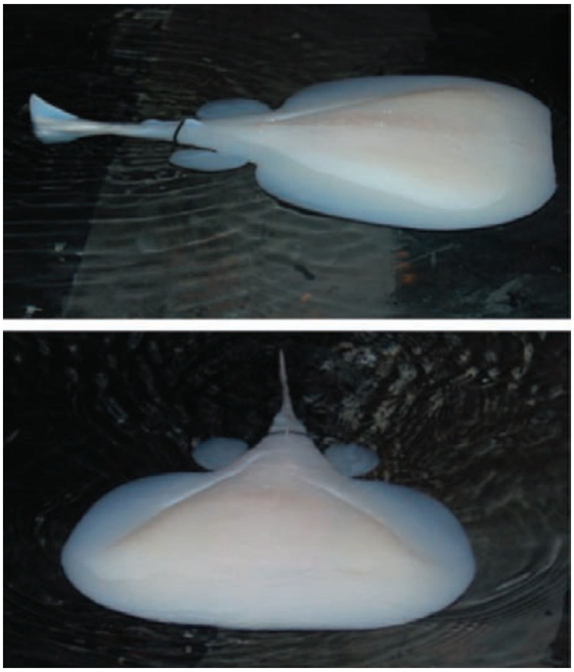


Figure 5 RayBot swimming. RayBot is self-propelled, autonomous, with control, energy, and actuators located onboard. Designed to be a submerged swimmer with benthic station-holding capabilities, RayBot is shown here swimming at the surface at the start of a mission. Sensors include photocells for navigation by light-gradient tracking. Photos by Joshua de Leeuw.

The actuator was a fin-based shear mechanism, inspired by the biomimetic work done in the laboratory of George Lauder at Harvard University. The RayBot team used a single servo motor to shear a movable shaft held parallel to a fixed shaft; with the parallel shafts attached distally and the shear of one causes the two to bend. With this implementation, a single, centrally located motor can do mechanical work on the flexible tail, creating undulations that propel and steer RayBot (**Figure 5**).

To measure RayBot's steady swimming performance, the team attempted to alter two kinematic variables that fish use to modulate steady swimming speed: tail-beat frequency (Hz) and tail-tip lateral amplitude (cm). Tail-beat frequency was altered by changing the drive signal to the servo motor. Top steady swimming speeds were obtained at an intermediate tail-beat frequency of 1 Hz (**Figure 6**(**a**)). The team attempted to alter tail-tip amplitude by driving the rotary servo pinion, attached to a rack that drove the fin-shear actuator, through high and low drive cycles of 22.5° and 45°; only at the performance peak of 1 Hz did the two drives yield different speeds (**Figure 6**(**a**)). Moreover, only when swimming with the low actuator drive did RayBot's speed increase linearly with increasing tail-tip amplitude (**Figure 6**(**b**)). The complexities in locomotor control are explained, in part, by the relation between tail-beat frequency and tail-tip amplitude (**Figure 6**(**c**)): as tail-beat frequency increased, tail-tip amplitude decreased, most likely as a result of increased hydrodynamic loading (added mass) with increasing accelerations. Programming RayBot to accelerate and then glide provided a method for estimating the robot's drag coefficient (**Figure 6**(**d**)). While able to swim, glide, and perform turning maneuvers with low power consumption, RayBot currently lacks the range of swimming behaviors and performance seen in real electric rays.

Robotic Fish: The Next Generation

The race has begun. Multidisciplinary teams of academic researchers and industrial engineers from around the world, fueled by funding from governments and industries, are competing to create the next generation of robotic fish. While the purposes of these fish vary from testing biological hypotheses, to engineering new exploratory vehicles, to creating fresh entertainment experiences, two themes are likely to emerge.

The first theme is that robotic fish will move into the wild. This migration from the controlled environment of the laboratory to the complex and variable world of rivers, lakes, and oceans is perhaps the biggest promise made by today's robot builders. Robotic fish will become the working tools of ecologists, fisheries managers, oceanographers, marine archeologists, ocean engineers, and navies. Highly maneuverable, stealthy, and energy efficient, these mission-capable robotic fish will carry a new generation of underwater sensors and be propelled by novel bioinspired and biomimetic actuators, tails, and fins.

The second theme is that robotic fish will become autonomous. Using the principles of artificial intelligence and embodied cognitive science that have been deployed effectively in robotic automobiles and the Mars Exploration Rovers, autonomous robotic fish, operating in the wild, will be programmed to make decisions on their own. In real time, robotic fish will use their on-board intelligence to decide how to navigate unfamiliar terrain and how to adjust to unexpected events. This autonomy will extend their mission time, increase their reliability, and, most importantly, increase the complexity of their activities.

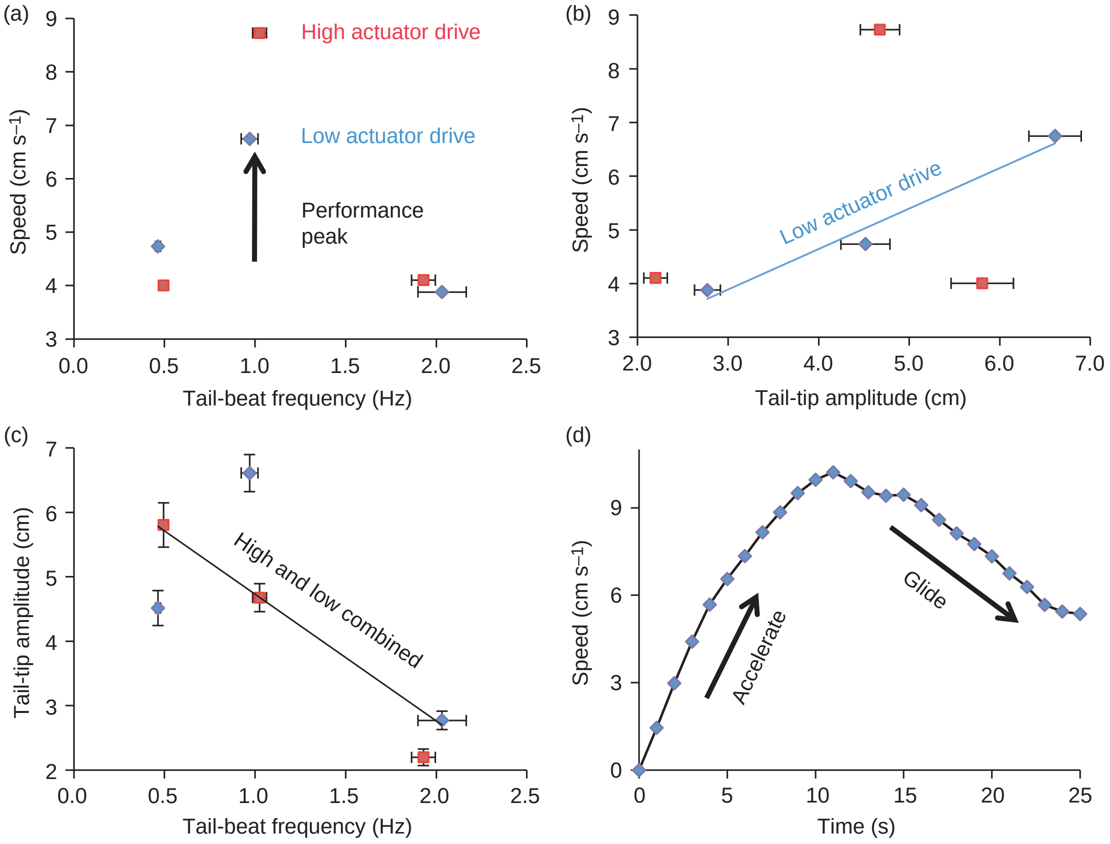


Figure 6 RayBot's performance as a self-propelled undulatory swimmer. (a) During steady swimming, RayBot swims fastest at the intermediate tail-beat frequency of 1.0 Hz. At peak performance, high actuator drive (±45° rotation of servo driving fin-shear mechanism) increases swimming speed relative to low actuator drive (±22.5°). (b) Tail-tip amplitude is proportional to swimming speed (slope of 0.75) in the low actuator drive. (c) In both high and low drives, tailtip amplitude decreases with increasing tail-beat frequency (combined slope of −1.97). (d) RayBot can be programmed to swim unsteadily, accelerating from rest and then gliding. Over the acceleration period, average acceleration is 0.93 cm s–2; average acceleration during the glide phase is –0.35 cm s^{-2}. Drag on the decelerating body was 0.02 N, which yielded a drag coefficient (based on projected area) of 0.032, in the range expected for streamlined bodies. Error bars (±1 standard error) represent within-trial variation; a total of six trials are shown for steady swimming. Figure created by John Long.

See also: **Buoyancy, Locomotion, and Movement in Fishes:** Maneuverability; Paired Fin Swimming; Stability and Turbulence; Undulatory Swimming.

Further Reading

Anderson JM and Chabra N (2002) Maneuvering and stability performance of a robotic tuna. *Integrative and Comparative Biology* 42: 118–126.

Bandyopadhyay PR (2005) Trends in biorobotics autonomous undersea vehicles. *IEEE Journal of Oceanic Engineering* 30(1): 109–139.

Bekey GA (2005) *Autonomous Robots: From Biological Inspiration to Implementation and Control*. Cambridge, MA: MIT Press.

Doorly N, Irving K, McArthur G, *et al.* (2009) Biomimetic evolutionary analysis: Robotically simulated vertebrates in a predator–prey ecology. *Proceedings of the 2009 IEEE Symposium on Artificial Life,* 147–154.

Epps BP, Valdivia y, Alvarado P, Youcef-Toumi K, and Techet AH (2009) Swimming performance of a biomimetic compliant fish-like robot. *Experiments in Fluids* 47(6): 927–939.

Ijspeert AJ, Crespi A, Ryczko D, and Cabelguen JM (2007) From swimming to walking with a salamander robot driven by a spinal cord model. *Science* 315: 1416–1420.

Jones JL (2003) *Robot Programming: A Practical Guide to Behavior-Based Robotics*. New York: McGraw-Hill.

Klein DJ, Bettale PK, Triplett BI, and Morgansen KA (2008) Autonomous underwater multivehicle control with limited communication: Theory and experiment. *Proceedings of the Second IFAC Workshop on Navigation, Guidance and Control of Underwater Vehicles* 2008.

Krishnamurthy P, Khorrami F, de Leeuw J, *et al.* (2009) A multi-body approach for 6 DOF modeling of biomimetic autonomous underwater vehicles with simulation and experimental results. *Control Applications (CCA) and Intelligent Control (ISIC) 2009 IEEE*, 1282–1287.

Lauder GV, Anderson EJ, Tangorra J, and Madden PGA (2007) Fish biorobotics: Kinematics and hydrodynamics of self-propulsion. *Journal of Experimental Biology* 210: 2767–2780.

Long JH, Jr. (2007) Biomimetic robotics: Building autonomous, physical models to test biological hypotheses. *Proceedings of the Institution of Mechanical Engineers, Part C. Journal of Mechanical Engineering Science* 221: 1193–1200.

Low K (2009) Modeling and parametric study of modular undulating fin rays for fish robots. *Mechanism and Machine Theory* 44: 615–632.

McHenry MJ, Pell CA, and Long JH, Jr. (1995) Mechanical control of swimming speed: Stiffness and axial wave form in an undulatory fish model. *Journal of Experimental Biology* 198: 2293–2305.

Nolfi S and Floreano D (2000) *Evolutionary Robotics: The Biology, Intelligence and Technology of Self-Organizing Machines*. Cambridge, MA: MIT Press.

Pell CA and Wainwright SA (2001) Swimming aquatic creature simulator. US Patent 6179683, issued 30 January 2001, assigned to Nekton Technologies, Inc. (now the marine division of iRobot, Inc.).

Tangorra JL, Davidson SN, Hunter IW, *et al.* (2007) The development of a biologically inspired propulsor for unmanned underwater vehicles. *IEEE Journal of Oceanic Engineering* 32(3): 533–550.

Terada Y and Yamamoto I (2004) An animatronic system including lifelike robotic fish. *Proceedings of the IEEE* 92(11): 1814–1820.

Yu JZ, Wang L, Zhao W, and Tan M (2008) Optimal design and motion control of biomimetic robotic fish. *Science in China Series F: Information Sciences* 51(5): 535–549.

Wang Z, Hang G, Li J, Wang Y, and Xiao K (2008) A micro-robot fish with embedded SMA wire actuated flexible biomimetic fin. *Sensors and Actuators A* 144: 354–360.

Webb B (2001) Can robots make good models of biological behavior? *Behavioral and Brain Sciences* 24: 1033–1050.

Relevant Websites

http://www.mitpressjournals.org – *Artificial Life* journal.

http://ausi.org – Autonomous Undersea Systems Institute.

http://www.iop.org – *Bioinspiration & Biomimetics* journal.

http://www.biorobotics.ttu.ee – FILOSE project on fish locomotion and sensing.

http://www.youtube.com – Gymnobot, the robotic knifefish from the University of Bath (UK).

http://www.essex.ac.uk – Human Centered Robotics Group, University of Essex (UK), and their entertainment robotic fish.

http://www.ieee-ras.org – IEEE Robotics and Automation Society.

http://www.isab.org – International Society for Adaptive Behavior.

http://www.irobot.com – iRobot, Maritime Division.

http://web.mit.edu – MIT Towing Tank.

http://www.youtube.com – Robotic fish for the London Aquarium from the University of Essex (UK).

http://www.youtube.com – Schooling robotic fish from the University of Washington (USA).

REPRODUCTION

The Diversity of Fish Reproduction: An Introduction

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Further Reading

Glossary

Embryogenesis Stages of development between fertilization and hatching.

Gonochorism Sexual pattern in which individuals mature as one sex and remain that sex.

Gynogenesis Formation of offspring possessing only maternal genetic information; a type of parthenogenesis where embryogenesis is triggered by the presence of sperm, although the sperm does not contribute any genetic material.

Hermaphroditism Sexual pattern characterized by individuals that function both as males and females either simultaneously or sequentially.

Iteroparous Capable of reproducing more than once (usually many times) in a lifetime.

Ovoviviparity Production of eggs that develop and hatch within the mother before being released as live young to the external environment.

Oviparity Production of eggs that develop and hatch after being released into the external environment.

Parthenogenesis The ability of an egg to develop without contribution from sperm.

Semelparous Capable of reproducing only once in a lifetime, usually just before dying.

Vitellogenins Precursors of the major egg yolk proteins comprised of up to five different domains, each of which corresponds to one of the stored yolk proteins (see also yolk proteins). Vitellogenins are large (> 350 kDa), homodimeric hospholipoglycoproteins. Common abbreviations for vitellogenin are Vg, VG, and Vtg.

Viviparity Production of live young that develop before being released into the external environment.

Given that there are well over 25 000 species of fish, it is perhaps not surprising that reproductive strategies in fishes are extremely diverse. In some species, mating pairs form between fish that matured as either male or female, a strategy that we perhaps perceive as a normal breeding strategy where a male and female form a lifelong breeding pair. In complete contrast, other species can change from being male to female in a matter of seconds. External fertilization through the release of unguarded eggs and sperm is a common reproductive strategy in fish, yet many species give birth to live young or care for their offspring until or beyond the time they reach sexual maturity. The diversity is simply amazing.

Consideration of the diversity of fish reproductive strategies can be traced back over centuries, from Aristotle's observations of fish hermaphroditism to Darwin's interest in the ability of male seahorses to raise offspring in brood pouches. Many articles within this encyclopedia review one or more aspects of fish reproduction and the aim of this article is to provide a very brief overview of fish reproduction with links to other articles in the encyclopedia that cover this fascinating topic in more detail.

Many fish species mature into either a male or female (gonochorism) and, at the point of reproduction, males and females simultaneously release gametes (sperm and eggs, respectively) into the water (known as broadcast spawning), with fertilization occurring externally. For this process to be successful, sexually mature males and females of the same species must arrive in the same place at the same time and be able to coordinate the release of gametes. Some fishes release chemical cues into the water to attract mates (**see also Hormones in Communication:** Hormonal Pheromones) while others use signals such as sound (**see also Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling) and electricity (**see also** Shocking Comments: Electrocommunication in Teleost Fish) to attract and secure potential mates. Visual courtship displays have also been documented in a myriad of fish species (**see also Social and Reproductive Behaviors:** Sexual Behavior in Fish).

The production and release of gametes are controlled by hormones (**see also Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction), and during egg formation, females of most fish species will invest energy into future offspring by providing the egg with nutrient stores. The main yolk protein present in fish eggs is vitellogenin (**see also The Reproductive Organs and Processes:** Vitellogenesis in Fishes), and for eggs released into drifting currents to fend for themselves from the point of fertilization, this nutrient supply is a vital lifeline, allowing the embryo to develop and grow while obtaining a size large enough to be able to catch its own food. This nutritional investment by the mother enhances the likelihood that her genes will be passed on through generations; if her offspring successfully feed and avoid predation for a long enough period of time, they will then mature into sexually reproductive adults, (**see also The Reproductive Organs and Processes:** Anatomy and Histology of Fish Testis, and **Fish Migrations:** Tracking Oceanic Fish) and have the potential to reproduce themselves. The process of sex differentiation (**see also Hormonal Control of Reproduction and Growth:** Endocrine Control of Sex Differentiation in Fish) and the functioning of the reproductive system are strongly influenced by the environment and may be negatively impacted by both natural and anthropogenic influences (**see also Behavioral Responses to the Environment:** Anthropogenic Influences on Fish Behavior).

In some fish species that show external fertilization and broadcast spawning, groups of fish congregate together with the potential for sperm from one male to fertilize the eggs of many females and the eggs from one female to be fertilized by many males. In other species, one male and one female form a breeding pair where males will vigorously defend the female from other males. It is not uncommon, under these circumstances, for males of the same species to adopt alternative reproductive tactics. Here, intrasexual variation in reproductive behavior and phenotype occurs, with bourgeois males that actively compete among themselves for access to females unlike parasitic males that sneak in to fertilize a female's eggs, thereby exploiting the courtship and resources of the bourgeois male.

Not all fish are gonochoristic, that is, male or female. The ability to change sex (hermaphroditism) is common among some families of fish, including wrasses, parrot fishes, damselfishes, and gobies (**see also Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes). Additionally, a few fish species are able to reproduce asexually without the egg having any contribution from sperm – this is known as parthenogenesis. In the Amazon molly, *Poecilia formosa*, females reproduce asexually; their eggs containing only female DNA. Interestingly, although there is no genetic contribution from male sperm during asexual reproduction of the Amazon molly, the presence of sperm from the males of closely related species is still required to trigger the development of the embryo (embryogenesis).

Not all oviparous fishes (those that release eggs) release their eggs unprotected into the external environment, leaving them to the mercy of prevailing water currents. Laying eggs on selected areas of substrate, or building nests to lay eggs in, provides offspring with additional protection (**see also Social and Reproductive Behaviors:** Sexual Behavior in Fish). Nest provision and design varies greatly from burying of eggs in gravel nests, as seen in salmon and trout, to the elaborate nests built by male sticklebacks, *Gasterosteus aculeatus*. Nests can also be used to remove offspring from detrimental water-quality conditions such as in the air-breathing catfish *Hoplosternum littorale* (**see also Behavioral Responses to the Environment:** Behavioral Responses to Hypoxia).

The location of offspring in one place, such as in a nest or more simply just attached to the substrate, allows parents to defend their young against predation during the early, more vulnerable, developmental period. Parental-care strategies in fishes are extremely diverse (**see also Social and Reproductive Behaviors:** Parental Care in Fishes) and may be carried out by the female, the male, or both parents. Types of care range from either guarding of eggs within a nest or fanning of eggs by parents to increase water flow, to complex cooperation behaviors among groups of fish where socially subordinate helpers work together to raise the young of more dominant breeding individuals.

Another way that parents can invest in offspring survival is through the additional provision of nutrients after hatch or through internal fertilization (**see also Social and Reproductive Behaviors:** Nutritional Provision During Parental Care). Particularly in sharks and rays, although eggs are produced, these are retained within the mother until after they have hatched. Following an internal developmental period where nutrients are provided by the mother, offspring are then released into the external environment as live young, a reproductive strategy known as ovoviviparity. True viviparity also occurs in fishes, with mothers developing placental relationships with offspring as seen in mammals.

The majority of fishes reproduce more than once in their life time (iteropary); although investment strategies in the future generation vary drastically from the release of hundreds of gametes for external fertilization on a regular basis with no parental care, to long gestation periods and low offspring numbers associated with viviparity. For a few fish species, the journey to reproductive success is extremely grueling (**see also Fish Migrations:** The Biology of Fish Migration). Several species of salmon migrate many hundreds of miles to spawning grounds undergoing complete osmoregulatory changes as they move through marine to freshwater environments and develop reproductively while no longer feeding (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle). These migrations can be so physiologically demanding that the journey to spawning grounds is made only once, leaving them only one opportunity to reproduce (semelparity) and pass their genes onto the next generation.

The collection of articles on aspects of fish reproduction within this encyclopedia provides an insight into the fascinating lives of fishes and the amazing variety of reproductive strategies that they employ. Yet, with fish displaying the widest diversity of reproductive strategies across all of the vertebrates, there is still so much that we have left to learn about fish reproduction.

See also: **Behavioral Responses to the Environment:** Anthropogenic Influences on Fish Behavior; Behavioral Responses to Hypoxia. **Fish Migrations:** Pacific Salmon Migration: Completing the Cycle; The Biology of Fish Migration; Tracking Oceanic Fish. **Hormonal Control of Reproduction and Growth:** Endocrine Control of Sex Differentiation in Fish; Endocrine Regulation of Fish Reproduction. **Hormones in Communication:** Hormonal Pheromones. **Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling; Shocking Comments: Electrocommunication in Teleost Fish. **Social and Reproductive Behaviors:** Nutritional Provision During Parental Care; Parental Care in Fishes; Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes. **The Reproductive Organs and Processes:** Anatomy and Histology of Fish Testis; Vitellogenesis in Fishes.

Further Reading

Oliveira RF (2006) Neuroendocrine mechanisms of alternative reproductive tactics in fish. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish,* 1st edn., vol. 24, pp. 151–196. San Diego, CA: Elsevier.

Pauly D (2004) *Darwin's Fishes. An Encyclopaedia of Ichthyology, Ecology and Evolution*. Cambridge: Cambridge University Press.

Schlupp I, Parzefall J, and Schartl M (2002) Biogeography of the Amazon molly, *Poecilia formosa. Journal of Biogeography* 29: 1–6.

REPRODUCTION

The Reproductive Organs and Processes

Contents
Anatomy and Histology of Fish Testis
Regulation of Spermatogenesis
Vitellogenesis in Fishes

Anatomy and Histology of Fish Testis

RW Schulz and RH Nóbrega, Utrecht University, Utrecht, The Netherlands

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Structure of the Teleost Testis
Anatomy of the Testis, Efferent Duct, and Seminal Vesicles
Germ-Cell Clones, Sertoli Cells, and Cystic Spermatogenesis
Distribution of Spermatogenic Cysts
Spermatogenesis – An Overview
Morphological Characteristics of Germ Cells during Spermatogenesis
Further Reading

Glossary

Crossing over An exchange of genetic material between homologous chromosomes.

Heterochromatin A tightly packed form of chromatin (DNA-protein complex) that is less accessible for the transcription of mRNA.

Self-renewal A process by which stem cells replicate themselves, maintaining, therefore, their pool in the tissue.

Spermatogenesis A cellular development during which spermatogonial stem cells give rise, via proliferation and differentiation steps, to a large number of sperm cells or spermatozoa. The germ-cell types formed successively during spermatogenesis are spermatogonia, spermatocytes, spermatids, and spermatozoa.

Spermatogonial stem cells The only stem cells in the body able to transmit genetic information to offspring. They are the foundation of spermatogenesis, being responsible for sustaining male fertility during the reproductive lifespan.

Stem cell Cell capable to both self-renew (in order to maintain their pool) and differentiate into a particular cell type. The balance between self-renewal and differentiation is crucial for tissue homeostasis.

Introduction

Sexual reproduction is the typical mode of reproduction in fish (**see also Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish); it requires eggs and sperm. Testes produce a large number of small gametes that evolved as motile genome vectors (spermatozoa), and ovaries produce a comparatively small number of large gametes (eggs) that are rich in reserves (**see also The Reproductive Organs and Processes**: Vitellogenesis in Fishes). This article describes the anatomy of teleost testis and sperm-duct systems, and then turns to the histological and morphological characteristics of male germ cells and to how these characteristics change during spermatogenesis. The crucial roles of a somatic cell type, the Sertoli cell, for germ-cell survival and development are also highlighted. Elsewhere (**see also The Reproductive Organs and Processes**: Regulation of Spermatogenesis), the testis is considered as an endocrine organ producing sex-steroid hormones and growth factors, and the roles of hormones and growth factors in the regulation of spermatogenesis are described. In both these articles, it is to be realized that fish are the most diverse and numerous group of vertebrates, and yet detailed knowledge on spermatogenesis and its regulation is limited to the few species used in basic and/or applied research. Hence, general statements often lean on information from only a handful of species from a few orders.

General Structure of the Teleost Testis

Following the general vertebrate scheme, the fish testis is composed of two compartments, the interstitial (or intertubular) tissue and the germinative (or spermatogenic) tissue (**Figure 1**). The interstitial compartment contains the steroid-producing Leydig cells, blood/lymphatic vessels, macrophages and mast cells, and neural and connective tissue elements, including the peritubular myoid cells (**Figure 1**); the connective tissue elements are continuous with the tunica albuginea, the testis organ wall. A part of the wall of the seminiferous tubules is a basement membrane that is formed by two cell types, the Sertoli cells in the seminiferous tubules, and the peritubular myoid cells in the interstitial compartment (**Figure 1**). The somatic Sertoli cells and germ cells are the only two cell types within the germinative compartment and they together form the germinal epithelium. In this epithelium, germ-cell survival and development depends on the constant and intimate contact with Sertoli cells (**Figure 1**). Depending on the age of the males, or the season, germ cells are found in different stages of spermatogenesis.

Central within, lateral to, and/or caudal of the testis, an efferent duct collects the spermatozoa formed by the spermatogenic tissue. The efferent duct also serves as a sperm storage site, participates in the control of the composition of the seminal fluid, and, thereby, determines the environment, in which capacitation (i.e., attaining the ability to fertilize eggs) of the spermatozoa occurs. Finally, the duct system transports sperm toward the genital pore for fertilization. In certain species, specialized tissue can differentiate along the efferent duct, which is then referred to as seminal vesicles.

Anatomy of the Testis, Efferent Duct, and Seminal Vesicles

Fish testes are usually elongated paired organs attached to the dorsal body wall by a fibrous sheath of connective tissue called mesorchium (**Figures 1** and **2**). In the

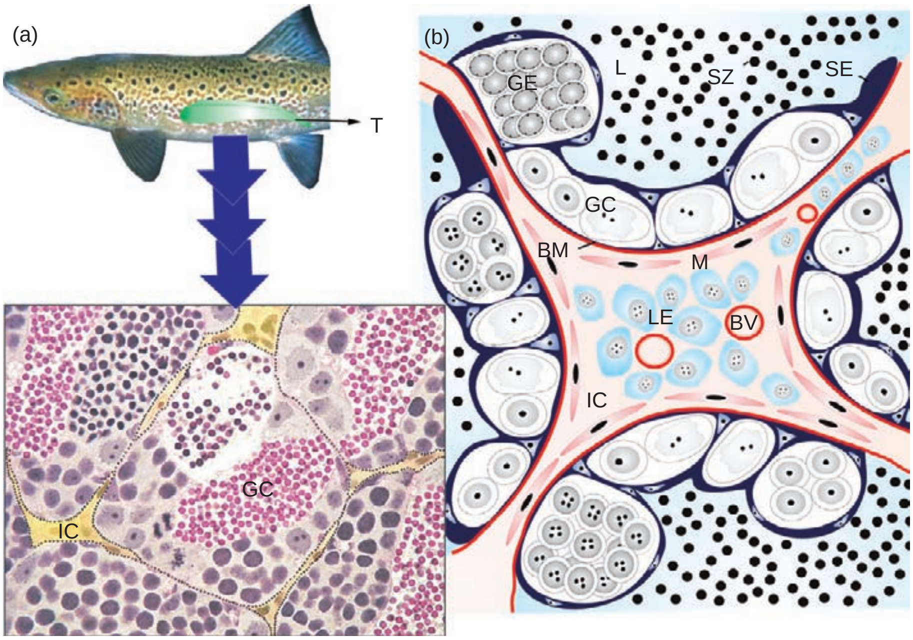


Figure 1 Schematic representation of the testicular structure in fish. (a) The fish testis (T) is divided into two compartments, the interstitial compartment (IC, yellow) and the germinative compartment (GC), separated from each other by a basement membrane (stippled line). (b) Schematic drawing of the two compartments. The interstitial compartment (IC) is composed of steroid-producing Leydig cells (LE, light-blue cytoplasm), blood/lymphatic vessels (BV), and connective tissue elements, including the peritubular myoid cells (M). The germinative compartment (GC) holds the germinal epithelium, formed by somatic Sertoli cells (SE, dark-blue cytoplasm) enveloping germ cells (GE) in different stages of development. Tubular lumen (L) and spermatozoa (SZ) are also shown. IC and GC are separated by a basement membrane (BM), which together with the layer of peritubular myoid cells, constitutes the wall of the spermatogenic tubules.

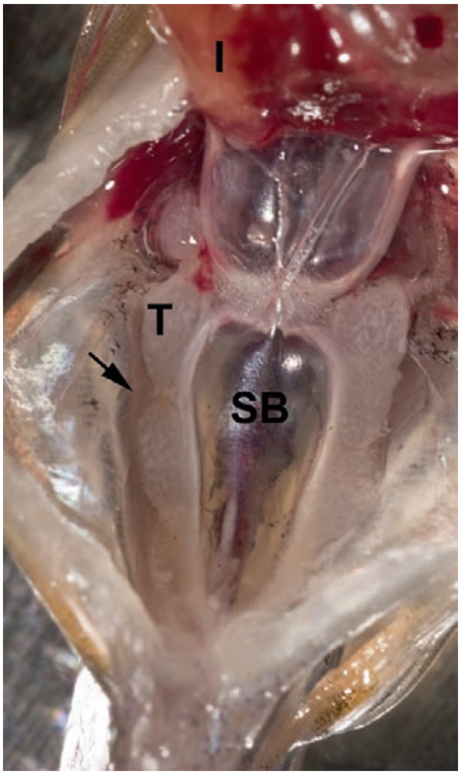


Figure 2 Zebrafish (*Danio rerio*) testis anatomy. Testes (T) are paired elongated organs (left and right) located ventral of the swimbladder (SB) and dorsal of the intestine (I), which was partially removed to expose the testes. The testes are attached to the dorsal body wall by the mesorchium (arrow).

coelomic cavity, the testes are located ventral of the swimbladder and dorsal of the intestine (**Figure 2**). The efferent duct arises from the meso/dorsal surface of each elongated testis, and continues into the spermatic duct that forms a short, unpaired segment before opening into the urogenital papilla (**Figures 3** and **4**). Together, the efferent and spermatic ducts form the testicular duct system, which transports sperm as well as is involved in storage, nutrition, and reabsorption of spermatozoa, secretion of seminal fluid, and induction of sperm motility by changing the ionic composition of the seminal fluid (**see also The Reproductive Organs and Processes**: Regulation of Spermatogenesis). Different variations of testes and testicular duct anatomy can be observed in different fish species. For example, in some species, for example, *poeciliids*, the two testes are fused to a single organ. In others, for example, in the order *Siluriformes*, it ranges from the basic type of elongated paired organs to testes composed of several fringes or lobes (**Figure 5**). The cranially located lobes contain germinal epithelium and the lumina are filled with sperm (**Figure 5**(**c**)) and the caudal lobes contain purely somatic, secretory epithelium producing seminal fluid. In some species of this order (e.g., the African catfish *Clarias gariepinus*), seminal vesicles differentiate under the influence of rising androgen levels during puberty from the caudal part of the immature testis where spermatogenic tissue merges into the spermatic duct system. Their columnar, single-layered epithelium is derived from Sertoli cells while the thin lamina propria, on which they reside, contains cells that show morphological characteristics of Leydig cells, which may be a source for pheromones.

Germ-Cell Clones, Sertoli Cells, and Cystic Spermatogenesis

Germ-cell development starts from spermatogonial stem cells (SSCs), the most undifferentiated type of spermatogonium, which usually occurs as single cells. When an SSC enters the differentiation pathway toward the spermatozoa, it shows a peculiar behavior upon dividing in all animals. At the end of mitosis, when the two new cells have formed, a cell division is incomplete in these differentiating cells since the daughter cells remain interconnected by a cytoplasmic bridge, instead of forming two separated cells. These bridges are also formed during all subsequent germ-cell divisions. In this way, the germ cells derived from a given SSC form a clone of interconnected cells. All members of the same clone are in the same stage of development, synchronized in their activities via the cytoplasmic bridges. These bridges are broken when spermatogenesis is completed and germ cells leave the germinal epithelium as spermatozoa. However, when dividing, an SCC can also form two independent cells. These daughter cells are two new SCCs and this mode of division is called stem-cell self-renewal.

Since germ-cell survival depends on its contact with Sertoli cells, the number of Sertoli cells determines the spermatogenic capacity of a testis. In birds and mammals, Sertoli cell numbers increase until puberty, when tight junctions are formed between neighboring Sertoli cells as a prerequisite to initiate spermatogenesis. However, once formed, these junctions are incompatible with further Sertoli-cell proliferation. Hence, the adult testis of higher vertebrates contains a fixed number of immortal Sertoli cells that support successive waves of spermatogenesis. During these waves, a given Sertoli cell supports at the same time different developmental stages of germ cells (i.e., cells belonging to different clones; **Figure 6**(**a**)).

In fish (like amphibians), on the other hand, we find the cystic type of spermatogenesis. There are two main differences when compared with higher vertebrates. First, cytoplasmic extensions of Sertoli cells contact each other to form a cyst that envelopes a single, synchronously developing germ-cell clone derived from a single spermatogonium (**Figures 1**(**b**) and **6**(**b**)); in this way, a Sertoli cell is usually in contact with only one germ-cell clone. Second, the cyst-forming Sertoli cells proliferate in both prepubertal and adult fish. Indeed, Sertoli-cell

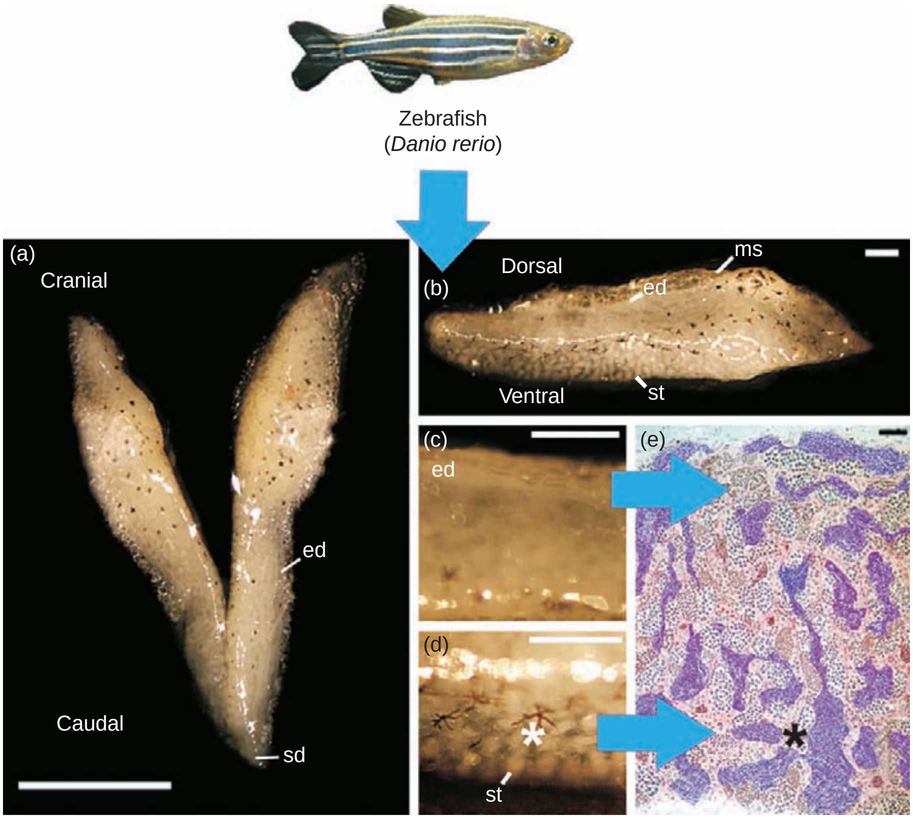


Figure 3 Zebrafish (*Danio rerio*) testis anatomy. (a) Testes are elongated paired organs (left and right) attached dorsally to the body wall by a connective tissue called mesorchium (ms). The efferent duct (ed) arises from the dorsal surface of each testis, and continues as a common, unpaired spermatic duct (sd). Spermatogenic tubules (st) are shown in (b). High magnification of efferent duct (c) and spermatogenic tubules (d), respectively. Tubular anastomoses are shown by an asterisk. (e) Histological section illustrating the areas demonstrated in (c) and (d). Tubular lumen is filled with sperm cells (dark blue). Sperm produced in the spermatogenic tubules is collected in the dorsal efferent duct. Scale = 1 mm (for (a) and (b); 0.5 mm for (c) and (d); and 50 μm for (e).

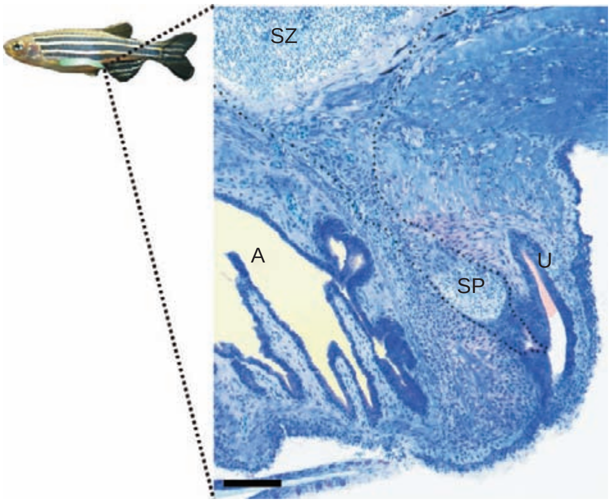


Figure 4 Zebrafish (*Danio rerio*) urogenital papilla. The urogenital papilla is an area between the anus (located cranially; A, yellow) and the ventral fin which gathers the openings of the spermatic duct (SP) and urethra (U, red). The spermatic duct (SP) is indicated by a stippled line and is filled with spermatozoa (SZ). Scale bar = 0.1 mm.

proliferation can be observed during the development of each germ-cell clone to exactly the number of Sertoli cells that is required to optimally support the increasing germ-cell number per cyst. The number of divisions a germ-cell clone undergoes is determined genetically (see below and **Figure** 7), so that the increases in both germ and Sertoli-cell numbers per cyst follow a predictable scheme. Moreover, we find a specific number of Sertoli cells per cyst for a given stage of development. The increases in germ-cell volume and number levels off toward the end of meiosis. Interestingly, this coincides with the formation of tight junctions between neighboring Sertoli cells in fish. Hence, as in higher vertebrates, the differentiation of tight junctions among Sertoli cells terminates their proliferation period.

When spermatogenesis is completed, the close contact between germ and Sertoli cells is broken and the cysts open such that the lumen of spermatogenic cysts becomes continuous with the lumen of the spermatogenic tubules. This process is called spermiation. The term spermiation is used in two ways in fish: in a strict sense to describe the

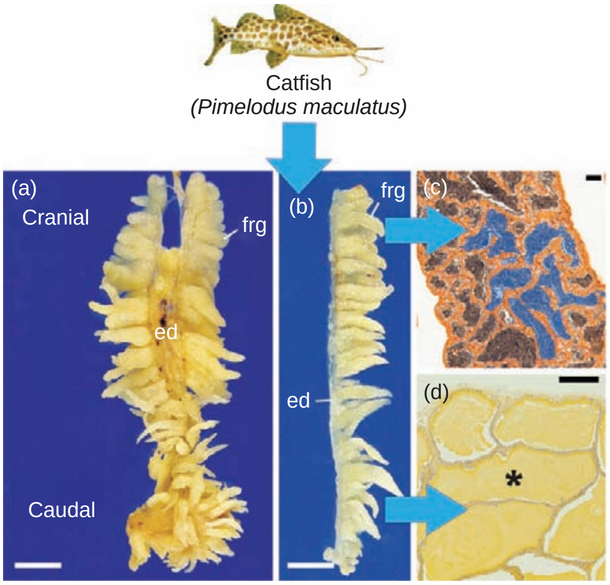


Figure 5 Catfish (*Pimelodus maculatus*) testis anatomy. (a, b) The testes are composed of fringes (frg) or lobes, which are connected centrally to the efferent duct (ed). (c, d) Histological sections of cranial (C) and caudal (D) lobes. (c) Spermatogenic tissue where the lumina are filled with sperm (blue colored). (d) Secretory tissue where the lumina are filled with acellular fluid (asterisk). Scale = 0.5 cm for (a) and (b); 100 μm for (c) and (d). Modified from Nóbrega RH and Quagio-Grassiotto I (2006) Morphological Changes of the Germinal Epithelium, Sertoli Cell Proliferation and 3βHSD Activity during the Reproductive Cycle of *Pimelodus maculates*. Master Thesis, UNICAMP.

termination of close Sertoli–germ cell contact and in a broader sense to indicate that strippable sperm (milt) is present in the spermatic duct system. Here, the term spermiation is used in the strict sense.

After spermiation, Sertoli cells can join the single-layered, flat epithelium that lines the inside of the spermatogenic tubules at places where no developing cysts are present. Thus, the basic functional unit of the spermatogenic epithelium in fish is a cyst formed by Sertoli cells that envelopes and supports one synchronously developing germ-cell clone. Different clones being at different stages of development generate the well-known histological picture of fish testes, where the seminiferous tubules contain groups of germ cells in different stages of spermatogenesis (**Figure 6**(**b**), inset).

Next to supporting germ-cell survival and development, Sertoli cells secrete the fluid present in and generating the tubular lumen. They are also the only phagocytotically active cell type in the seminiferous tubules. Material to be phagocytized is, for example, apoptotic germ cells, residual bodies (cellular material discarded by spermatids during spermiogenesis), or sperm that remained in the testis after the end of a spawning season. A certain loss of developing germ cells via apoptosis is normal during spermatogenesis. Cystic spermatogenesis in fish is more efficient in this regard than the noncystic spermatogenesis in rodents, for example, considering that only 30% of developing germ cells are lost to apoptosis in fish, while 60–70% is normal in laboratory rodents.

Distribution of Spermatogenic Cysts

Two types of spatial arrangements are found in the teleost testes with regard to the spatial distribution of cysts with early spermatogonia. In the restricted type, the cysts with early, undifferentiated spermatogonia, among which are probably the SSCs, are located close the tunica albuginea in the periphery of the testis. During differentiation, the developing cysts migrate toward the efferent ducts where spermiation occurs, that is, the cysts open to release spermatozoa. This type of arrangement is found in the higher teleosts, such as in the orders Atheriniformes, Beloniformes, and Cyprinodontiformes. In the second type, the unrestricted spermatogonial distribution is found, and early, undifferentiated spermatogonia are spread throughout the testis. The cysts do not migrate during their development. The unrestricted pattern is considered as the more primitive one and is found in orders such as the Characiformes, Cypriniformes, or Salmoniformes. Intermediate forms also exist, for example, in the orders Gadiformes, Perciformes, or Pleuronectiformes, where undifferentiated spermatogonia show a preferred, but not exclusive, location close to the tunica albuginea. In the restricted and intermediate types, the observed localization of undifferentiated spermatogonia results in a zonation of, or gradient of, development in the testis: early stages of germ-cell development are found distant from, and advanced stages close to, the efferent duct system.

Some tropical species do not display a seasonal variation of the spermatogenic activity. However, in many species with habitats at higher latitudes, reproduction is a seasonal or cyclic event related to environmental cues. Typically, cysts with rapidly proliferating spermatogonia are observed only at the beginning of testicular recrudescence, while after completion of spermatogenesis, tubules are filled with large numbers of spermatozoa and only some scattered, quiescent type A spermatogonia remain. In other cyclic species, germ cells remaining in the regressed testis may be spermatogonia and spermatocytes (e.g., tench, *Tinca tinca*), or even all germ cell types, such as in goldfish (*Carassius auratus*), where the seasonal regression is of a quantitative rather than of a qualitative nature.

Spermatogenesis – An Overview

During spermatogenesis, normal-looking diploid germ cells, the spermatogonia, transform during a suite of proliferation and differentiation steps, into flagellated

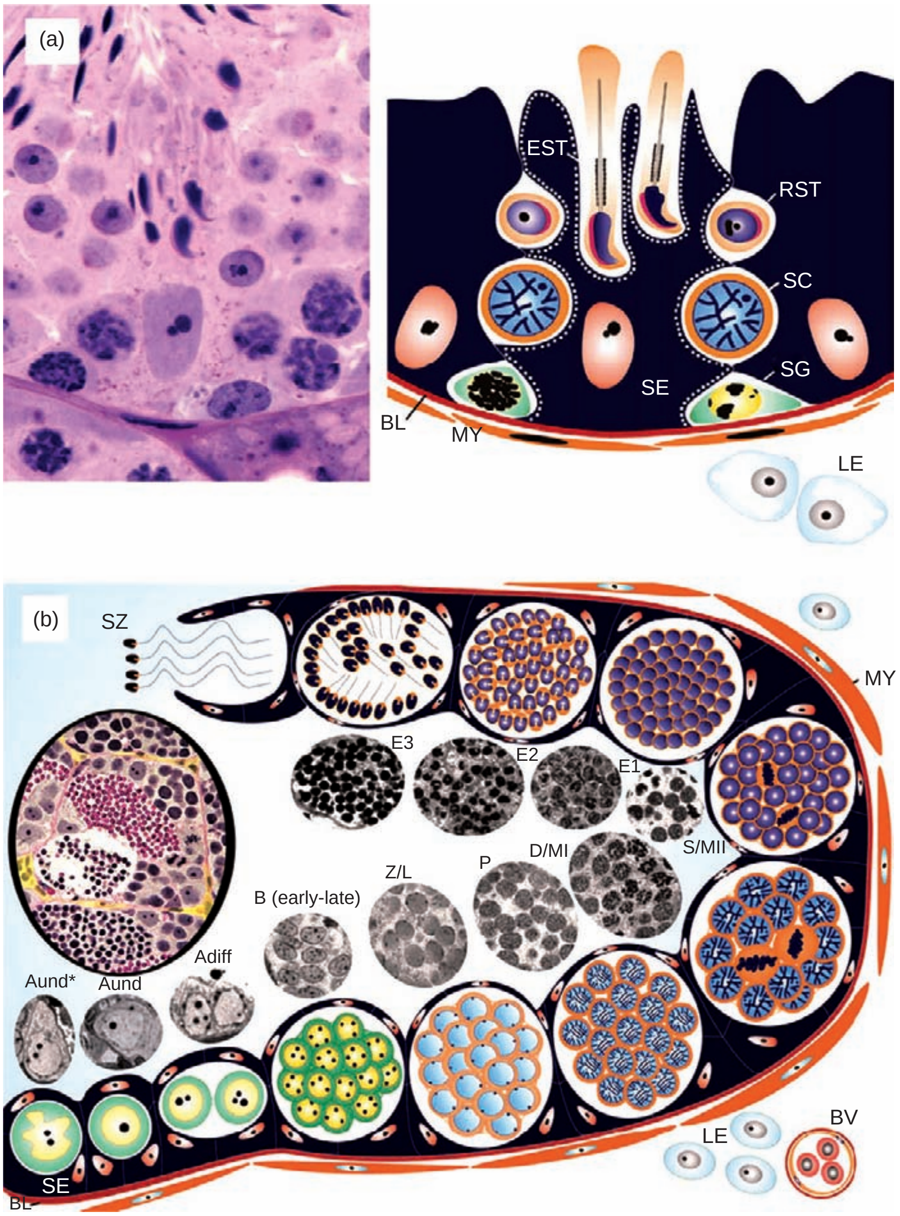


Figure 6 Comparison of mammalian ((a) mouse) and fish ((b) zebrafish) testis. Segments of spermatogenic tubules are shown to illustrate the differences in Sertoli/germ cell relation between cystic (b) and noncystic (a) spermatogenesis. The germinal epithelium contains Sertoli (SE) and germ cells, delineated by a basal lamina (BL) and peritubular myoid cells (MY). The interstitial Leydig cells (LE) and blood vessels (BV) are shown. (a) SG, spermatogonia; SC, spermatocyte; RST, round spermatid; EST, elongated spermatid. (b) $A_{und}*$ (*possible stem cell?), type A undifferentiated* spermatogonia; A_{und}, type A undifferentiated spermatogonia; A_{diff}, type A differentiated spermatogonia; B (early-late), spermatogonia type B; L/Z, leptotenic/zygotenic primary spermatocytes; P, pachytenic primary spermatocytes; D/MI, diplotenic spermatocytes/metaphase I; S/MII, secondary spermatocytes/metaphase II; E1, early; E2, intermediate; and E3, final spermatids; SZ, spermatozoa. From Schulz RW, de França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165(3): 390–411 (doi:10.1016/j.ygcen.2009.02.013).

spermatozoa. These motile cells carry a recombined, haploid genome to fertilize an egg. Spermatogenesis can be divided into three phases.

The initial one is the mitotic or spermatogonial phase. Here, the main increase in germ-cell number occurs during successive rounds of mitotic duplication of the spermatogonia. The number of spermatogonial generations (and hence the number of mitotic divisions they undergo before differentiating into spermatocytes) varies among species (**Figure 7**). For example, 14 generations of spermatogonia were found in the guppy (*Poecilia reticulata*), 10–12 in mosquito fish (*Gambusia affinis*), nine in zebrafish (*Danio rerio*), six in mudminnow (*Umbra limi*) and rainbow trout (*Oncorhynchus mykiss*).

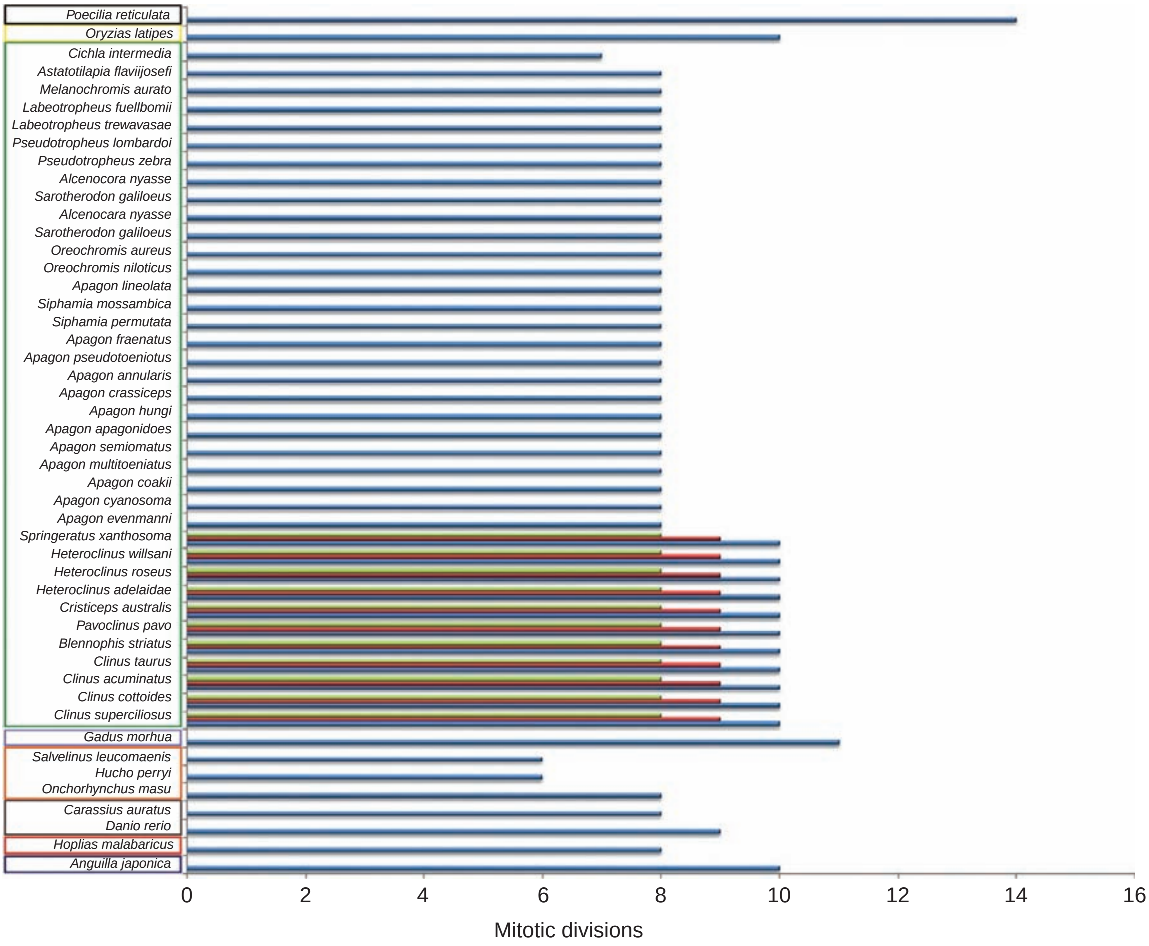


Figure 7 Number of mitotic divisions prior to meiosis. Note that the number of divisions seems to vary with the taxonomic position of the species. Colored squares delimitate orders which are phylogenetically arranged from the most derived (above) to the most primitive (below). The orders depicted follow the aforementioned sequence: Cyprinodontiformes, Beloniformes, Perciformes, Gadiformes, Salmoniformes, Cypriniformes, Characiformes, and Anguilliformes.

When entering the second phase, meiosis, the spermatogonia differentiate into spermatocytes that go through the two meiotic divisions. These are characterized by reshuffling of the paternal and maternal genetic information via crossing over during the first meiotic division and the reduction to a haploid genome during the second meiotic division.

Third, during the spermiogenic phase the haploid spermatids emerging from meiosis differentiate – without further proliferation – into flagellated spermatozoa. The morphological changes in germ cells taking place during spermiogenesis are quite similar among different species (e.g., reduction in cytoplasmic volume and organelles, maximum DNA condensation, and differentiation of a flagellum). However, the final shape of the spermatozoa can differ between species and sometimes provide taxanomic clues.

The duration of meiosis and spermiogenesis has been determined in a few fish species and varies between 1 and 3 weeks in four tropical species, but can take 1–3 months in species living in temperate or cold waters. No information is available yet in fish on the duration of the spermatogonial phase.

Morphological Characteristics of Germ Cells during Spermatogenesis

As all other germ cells, the SSCs in fish are in close contact with cytoplasmic extensions of Sertoli cells. Clear morphological or molecular markers have not been identified yet for SSCs in any vertebrate, but the general view is that SSCs are single cells and part of a population referred to as undifferentiated spermatogonia type A (A_{und}). There is a functional assay for SSCs though, which is based on their capacity to re-start, after transplantation, spermatogenesis in the testis of a recipient animal (in which endogenous spermatogenesis had been stopped experimentally). Transplantation assays exist for certain mammalian species and are being developed for fish now. Although a morphological characteristic of SSCs is that they are single germ cells, that is, not connected via cytoplasmic bridges to other germ cells, not all single type A_{und} spermatogonia seem to be stem cells. In fish, we find two types of single, undifferentiated spermatogonia, type A_{und*} and the type A_{und} spermatogonia (**Figures 8** and **9**). The type A_{und*} shows the most undifferentiated characteristics, such as a large nucleus with little heterochromatin, a high volume of the cytoplasm, a convoluted nuclear envelope and – particularly interesting – darkly staining material on the cytoplasmic side of the invaginations of the convoluted nuclear envelope (**Figure 8(a)**). Such patches are known as 'nuage' in the primordial germ cells, the earliest (embryonic) generation of germ cells (**Figure 8(b)**). The other type of single spermatogonia shows a smooth nuclear envelope and less nuage and these cells are referred to as type A_{und} spermatogonia (**Figures 8** and **9**). To date, it is not known if a mitotic division separates these two types of A spermatogonia.

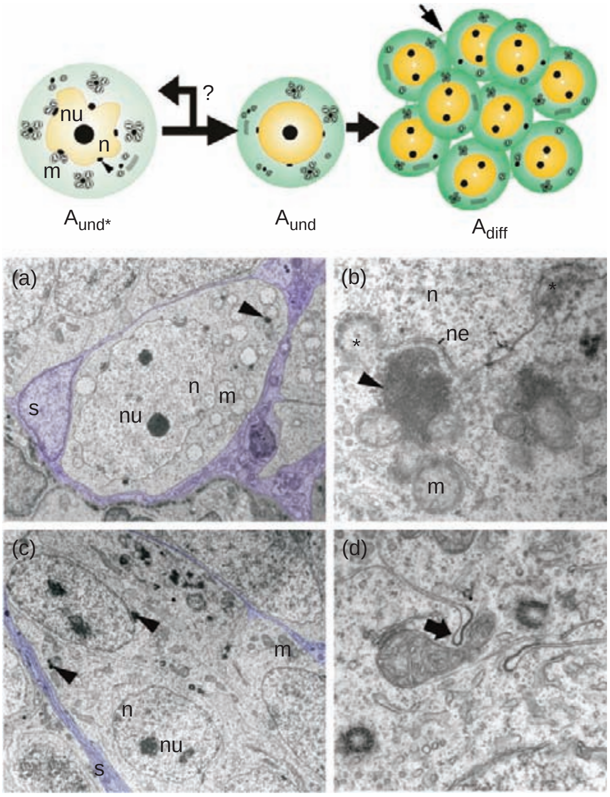


Figure 8 Morphological features of the most undifferentiated ($A_{und}*$ and A_{und}) and differentiated type A spermatogonia (A_{diff}). Schematic drawing (top) of the spermatogonial generations from $A_{und}*$ to A_{diff}. The question mark between $A_{und}*$ and Aund indicates that it is not known yet if they are separated by a mitosis. (a, b) Note that $A_{und}*$ is a single cell surrounded by cytoplasmic extensions (colored area) of Sertoli cells (S). $A_{und}*$ has a large and convoluted nucleus (n) with little heterochromatin and one or two nucleoli (nu). Close to the nuclear convolutions (asterisks) of the nuclear envelope (ne), or associated with mitochondria (m), their cytoplasm contains a large amount of electron-dense material, called nuage (arrowhead). (c, d) Differentiating type A spermatogonia are found in cysts with two, four, and eight cells that are enveloped by thin (colored) cytoplasmic extensions of Sertoli cells. These differentiating germ cells are connected by cytoplasmic bridges (arrow), shown at high magnification in D. A_{diff} have a round or oval nuclei, one or two nucleoli, denser chromatin, and little or no nuage.

A differentiating division produces a pair of spermatogonia A connected by a cytoplasmic bridge. Since this is the first step toward differentiation to spermatozoa, these cells therefore are called differentiating type A spermatogonia (A_{diff}) in fish (**Figures 8** and **9**). Type A_{diff} cells then undergo a species-specific number of mitotic divisions, during which the number of spermatogonia duplicates with each mitotic cell cycle. The last generation of spermatogonia that differentiates – without division – into primary spermatocytes, is always called type B spermatogonia. When several spermatogonial generations are present in a species, further subdivisons become evident, and we present the zebrafish (*Danio rerio*) as an example.

Type A_{diff} spermatogonia in zebrafish are found in cysts with two, four, and eight cells connected by cytoplasmic bridges (**Figures 8(c)**, **8(d)**, and **9**). Their nuclei are round to oval, contain no or very little nuage-like material, and show one or more nucleoli. They are smaller than the type A_{und} spermatogonia but share with them the low amount of heterochromatin in the nucleus, and a relatively large cytoplasmic volume (**Figures 8(c)** and **9**).

Further morphological changes are observed during development of the more rapidly dividing type B spermatogonia. In zebrafish, type B spermatogonia are found in cysts containing 16–512 cells. The successive generations of type B spermatogonia show a progressively decreasing cell and nuclear volume, with a small rim of cytoplasm surrounding an oval to round nucleus that gradually shows more heterochromatin (**Figure 9**).

After the final mitosis, type B spermatogonia differentiate into primary spermatocytes. Upon approaching the first meiotic division, the cells enter the very long prophase of meiosis I, which is subdivided into stages that are named according to the appearance of the meiotic chromosomes in the light microscope: preleptotene, leptotene (slender ribbon), zygotene (threads bound together), pachytene (thick threads), and diplotene (double threads). During this long prophase, crossing over can occur between the paternal and maternal chromosomes (usually one or two per pair of chromosomes), thereby reshuffling the genetic information; the crossing-over sites become visible as chiasmata (cross pieces) during diplotene. This is followed by the first meiotic division, during which the homologous (paternal and maternal) chromosomes segregate into the nuclei of the secondary spermatocytes. The latter quickly enter the second meiotic division, without a duplication of

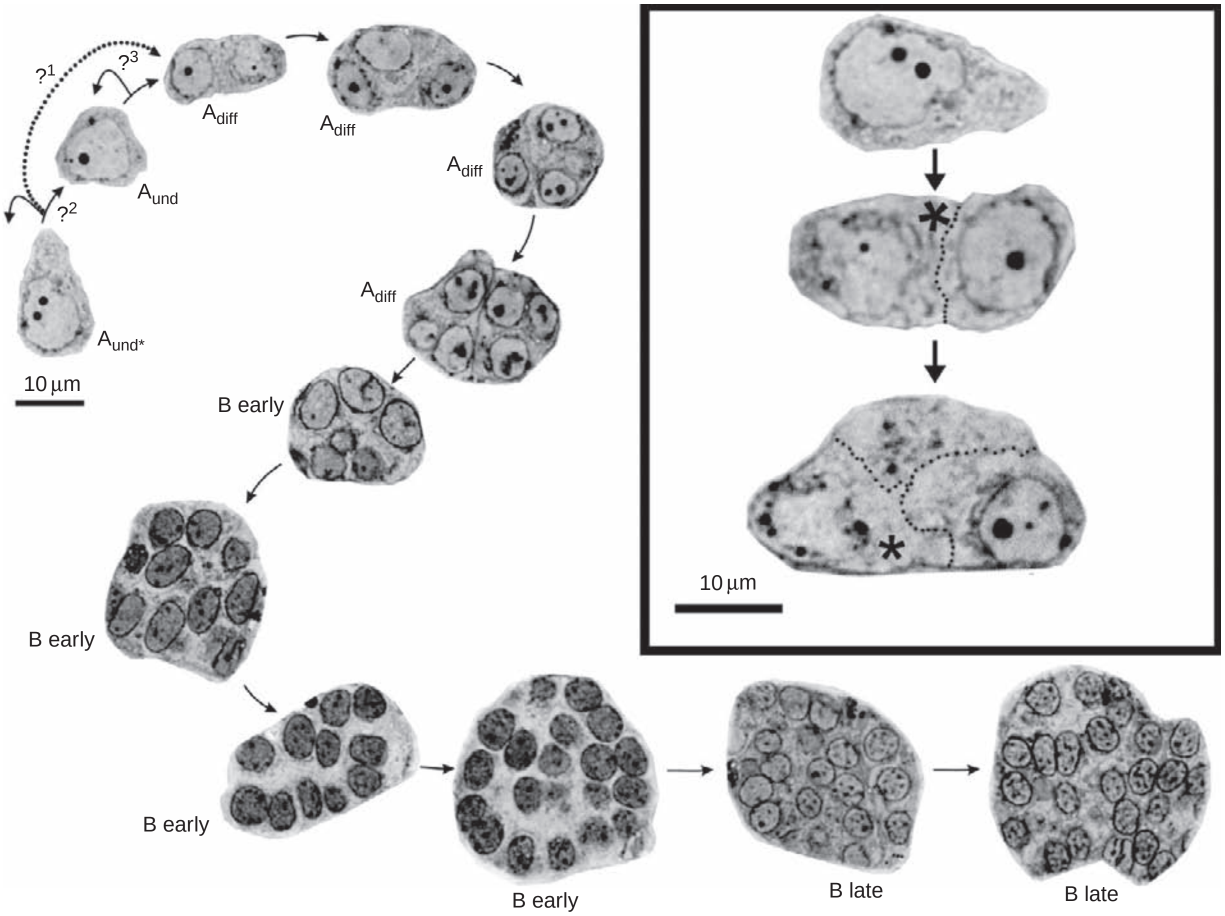


Figure 9 Spermatogonial generations in zebrafish. The terminology to address the spermatogonial generations is based on nuclear (heterochromatin amount) and nucleolar characteristics, and the number of germ cells inside the cyst. Using high-resolution light microscopy applied to araldite-embedded sections, five types of spermatogonia are described: type A undifferentiated* (A_{und*}), type A undifferentiated (A_{und}), type A differentiated (A_{diff}), type B early (B_{early}), and type B late (B_{late}). The curved arrows indicate self-renewal while the simple arrows indicate mitosis. The first question mark ($?^1$) indicates a doubt as regards a possible asymmetric division of A_{und*} (in the inset, note the stippled line toward a pair of spermatogonia). The second question mark ($?^2$) refers to the uncertainty if type A_{und*} is separated from A_{und} by mitosis or if they represent different stages of the same cell cycle. The third question mark ($?^3$) indicates a doubt as regards the stemness of A_{und}. Inset: The asterisks show undifferentiated spermatogonia belonging to cysts with other types of spermatogonia. This observation might indicate that asymmetrical divisions can occur but requires further studies in teleosts. From Schulz RW, de França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165(3): 390–411 (doi:10.1016/j.ygcen.2009.02.013).

the DNA, and the chromosomes go through an equational division, that is, the sister chromatids are segregated. This results in four haploid cells derived from each of the last type B spermatogonium, harboring one copy of each chromosome.

Using light microscopy, identification of meiotic cells is based mainly on the appearance of the germ-cell nuclei, such as size and shape, degree of chromosome condensation, and metaphasic figures (**Figures 6(b)** and **10**). In zebrafish, leptotene/zygotene spermatocytes have a larger nucleus (~ 5.5µm) and are more round than in the last type B spermatogonia; the chromatin is clear and shows small dots of heterochromatin outlining the nuclear membrane (**Figures 6(b)** and **10**). Pachytene spermatocytes are the most frequent meiotic cells due to the long duration of this stage. They have a large (~6.5 µm) and dense nucleus, with the chromosomes appearing as bold lines from the nuclear membrane to the center of the nucleus. Diplotenic primary spermatocytes are often found together with metaphasic figures (metaphase I); the chromosomes reach their maximum degree of condensation and are found close to the nuclear membrane that, however, starts to disappear at this stage. The secondary spermatocytes, resulting from meiosis I, are rare because they quickly enter meiosis II. They are small cells with a dense nucleus (~4 µm), usually found together with metaphasic figures from the second meiotic division (**Figures 6(b)** and **10**). A fine description of meiotic germ cells using light and electron microscopy has been reported for several species of fish.

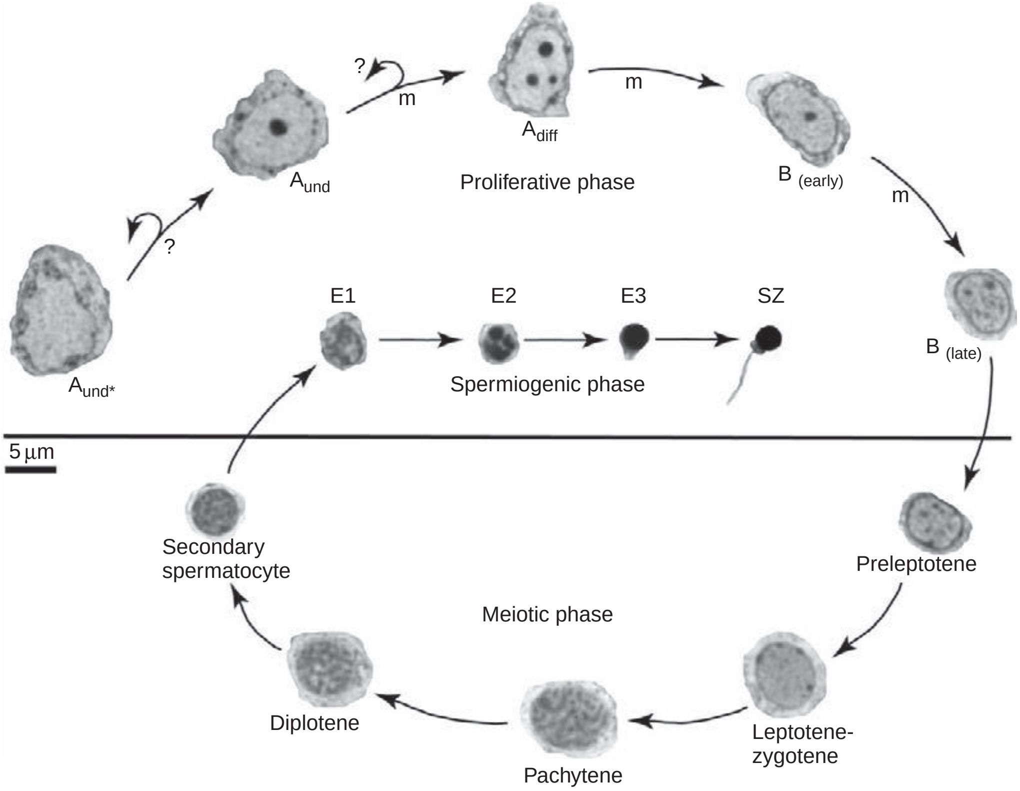


Figure 10 Step-by-step representation of zebrafish spermatogenesis from type A undifferentiated spermatogonia to spermatozoa, throughout the three phases of the spermatogenic process: proliferative or spermatogonial, meiotic or spermatocytary, and spermiogenic. The spermatogonial micrographs show the most characteristic features of each cell type, although there is a range of their morphology and size. A_{und}*(stem cell?), type A undifferentiated* spermatogonia; A_{und}, type A undifferentiated spermatogonia; A_{diff}, type A differentiated spermatogonia; B, type B spermatogonia; curved arrows, self-renewal; m, mitosis; E1, early spermatids; E2, intermediate spermatids; E3, final spermatids; SZ, spermatozoa. A_{und}* and A_{und} are single cells, whereas A_{diff}, B (early), and B (late) are grouped. The first question mark (?) indicates a doubt if A_{und}* and A_{und} are separated by a mitosis or represent different stages of the same cell cycle, while the second question mark indicates uncertainty as regards the stemness of A_{und}. From Schulz RW, de França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165(3): 390–411 (doi:10.1016/j.ygcen.2009.02.013).

Spermiogenesis, that is, the differentiation of spermatids into spermatozoa without further cell divisions, consists of series of morphological changes, such as condensation of the nucleus and its DNA, elimination of superfluous organelles and cytoplasm (discarded as residual bodies), flagellum formation, and the rearrangement of remaining organelles in the remaining cytoplasm. Three types of spermiogenesis (types I, II, and III) have been described based on the orientation of the flagellum to the nucleus, and on whether or not a nuclear rotation occurs. These patterns are reflected in the spermatozoa structure, are highly conserved within taxonomic units, and can be used for clarifying the phylogenetic position of fish species. The increasing nuclear condensation together with the decreasing nuclear size can be used to subdivide spermiogenesis. In zebrafish, three types of spermatids were described in this regard: early, intermediate, and final spermatids **Figures 6(b)** and **10**). Fish spermatozoa usually have no acrosome; a micropyle gives access to the membrane of the oocyte. Spermatozoa often show a spherical nucleus with homogenous, highly condensed chromatin, a nuclear fossa, a midpiece of variable size containing the mitochondria, and one or two long flagella.

At the end of spermiogenesis, the intercellular bridges between the spermatids are broken and the germ cells are individualized. The cyst-forming Sertoli cells lose contact with each other upon opening of the cyst to release spermatozoa into the tubular lumen (**Figure 6(b)**). In some species, the release of germ cells occurs at the spermatid (or even the spermatocyte) stage itself, and spermiogenesis is completed in the tubular lumen. This is called semicystic spermatogenesis and is found in different taxonomic groups (e.g., Bleniidae, Corydoradinae, Opheliidae, Scorpaenidae, and Soleidae). In semicystic spermatogenesis, Sertoli cells form, earlier than in other species, an epithelial lining of the spermatogenic tubules. There are cases, however, where spermatozoa are kept together during release, and sperm bundles are formed within the cyst when Sertoli cells secrete a capsule around a group of spermatids, thereby creating spermatophores.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish. **The Reproductive Organs and Processes**: Regulation of Spermatogenesis; Vitellogenesis in Fishes.

Further Reading

Billard R (1986) Spermatogenesis and spermatology of some teleost fish species. *Reproduction, Nutrition, Development* 26: 877–920.

Grier HJ (1992) Chordate testis: The extracellular matrix hypothesis. *Journal of Experimental Zoology* 261: 151–160.

Grier HJ (1993) Comparative organization of Sertoli cells including the Sertoli cell barrier. In: Russell LD and Griswold MD (eds.) *The Sertoli Cell*, pp. 703–739. Clearwater, FL: Cache River Press.

Leal MC, Cardoso ER, Nóbrega RH, *et al.* (2009) Histological and stereological evaluation of zebrafish (*Danio rerio*) spermatogenesis with an emphasis on spermatogonial generations. *Biology of Reproduction* 81: 177–187.

Matta SL, Vilela DA, Godinho HP, and Franca LR (2002). The goitrogen 6-n-propyl-2-thiouracil (PTU) given during testis development increases Sertoli and germ cell numbers per cyst in fish: The tilapia (*Oreochromis niloticus*) model *Endocrinology* 143: 970–978.

Nóbrega RH, Batlouni SR, and França LR (2009) An overview of functional and stereological evaluation of spermatogenesis and germ cell transplantation in fish. *Fish Physiology and Biochemistry* 35(1): 197–206.

Nóbrega RH and Quagio-Grassiotto I (2006) Morphological Changes of the Germinal Epithelium, Sertoli Cell Proliferation and 3βHSD Activity during the Reproductive Cycle of *Pimelodus maculates*. Master Thesis, UNICAMP.

Pudney J (1995) Spermatogenesis in nonmammalian vertebrates. *Microscopical Research Techniques* 32: 459–497.

Schulz RW, de França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165(3): 390–411 (doi:10.1016/j.ygcen.2009.02.013).

Regulation of Spermatogenesis

RW Schulz and RH Nóbrega, Utrecht University, Utrecht, The Netherlands

© 2011 Elsevier Inc. All rights reserved.

Introduction
Regulation of Testis Functions by Pituitary Gonadotropins
Sertoli Cells
Steroid Hormone Blood Levels
Estrogens
Androgens
Progesterones
Final Maturation of Male Gametes
Germ Cell Autonomous Regulation of Spermatogenesis
Synopsis and Outlook
Further Reading

Glossary

Paracrine signaling Refers to the observation that signaling molecules (e.g., hormones and growth factors) can exert effects in the (close) vicinity of their site of production.

Pituitary gonadotropins Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are pituitary glycoprotein hormones that regulate the two main functions of the gonads, germ cell and sex hormone production.

Sex steroids A group of lipophilic, small (~300 Da) signaling molecules that are produced from cholesterol in the gonads under the influence of gonadotropins. Androgens are male sex steroids, and progesterones; estrogens are female sex steroids.

Spermatogenesis A cellular development during which spermatogonial stem cells give rise, via proliferation and differentiation steps, to a large number of sperm cells or spermatozoa. The germ-cell types formed successively during spermatogenesis are spermatogonia, spermatocytes, spermatids, and spermatozoa.

Spermiation Describes the release of spermatozoa from the germ-cell-supporting Sertoli cells. In fish, this is associated with the opening of the spermatogenic cysts formed by cytoplasmic extensions of the cyst-forming Sertoli cells.

Introduction

Another article presents the testis in its function as a germ-cell-producing tissue (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis). In the present article, we focus on the testis as an endocrine organ. Both functions are intimately related, since hormones and growth factors produced by the testis are required for regulating germ-cell production (**Figure 1**). Testicular hormones also regulate the development of secondary sexual characteristics or reproductive behavior (**see also Hormones in Communication**: Hormonal Pheromones), and they feed back on the endocrine systems in the brain and the pituitary (**Figure 1**) that regulate pubertal maturation and adult reproductive cycles (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction).

The testis is composed of two main compartments, the interstitial and the tubular compartment. The interstitial compartment houses the steroidogenic (i.e., steroid-hormone producing) Leydig cells (**Figures 1** and **2**). The tubular compartment contains germ cells and Sertoli cells. The germ-cell-supporting Sertoli cells also produce signaling molecules, although they do not release steroids but growth factors, many of which work in a paracrine (short-range, cell-to-cell signaling) manner to regulate spermatogenesis (**Figure 1**).

Regulation of Testis Functions by Pituitary Gonadotropins

In many species living at higher latitudes, reproduction is a seasonal event related to environmental cues, such as changes in the photoperiod. However, some tropical species do not display apparent seasonal variations in reproductive activity and once pubertal maturation is completed, reproductive activity is seen throughout adult life. Pubertal or seasonal activation of spermatogenesis is closely associated with the activation of testicular hormone and growth-factor production. Surgical removal of the pituitary demonstrated that both production of hormones/growth factors and spermatogenesis require

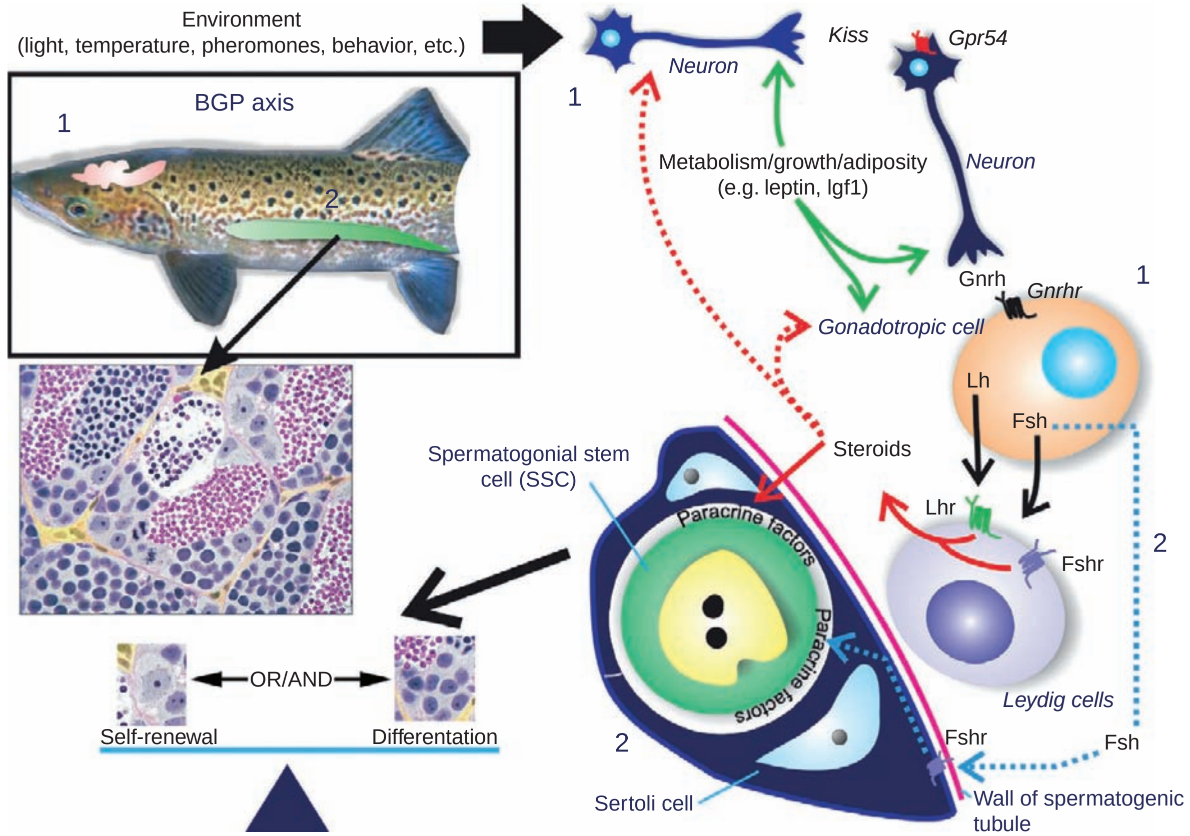


Figure 1 Schematic representation of the endocrine system regulating reproduction, the brain–pituitary–gonad (BPG) axis. In the brain–pituitary segment of the axis (1), Kiss producing neurons regulate via the Kiss receptor Gpr54 (red) the activity of the Gnrh-producing neurosecretory neurons. Gnrh interacts with its receptor Gnrhr (black) on the two gonadotropic cell types of the pituitary, which produce either LH or FSH. Both hormones reach the testis (2) via the blood stream and stimulate sex-steroid production by the interstitial Leydig cells by interacting with the LH receptor (green) or the FSH receptor (pink). FSH can also activate (blue stippled line) the FSH receptor expressing Sertoli cells, which moreover express steroid hormone receptors. Sertoli cells envelop developing germ cells and following steroid and/or FSH action, modulate the release of growth factors that influence the self-renewal, proliferation, and/or differentiation behavior of germ cells in a paracrine manner. Sex-steroid hormones feed back to the brain–pituitary system (red stippled line) and modulate, together with other internal messengers (e.g., reflecting the metabolic state, green arrows) and external signals (e.g., photoperiod, large black arrow), the activity of Kiss and Gnrh-producing neurons.

regulatory input from the pituitary. The pituitary, in turn, is controlled by neuro-endocrine centers in the brain (**Figure 1**).

The two most important pituitary hormones regulating testicular physiology are luteinizing hormone (LH) and follicle-stimulating hormone (FSH), collectively referred to as gonadotropins or gonadotropic hormones. In mammals, FSH and LH interact with their membrane-associated, G-protein-coupled receptors, FSHR and LHR, in a highly specific manner at physiological hormone concentrations. Leydig cells express the LHR gene (**Figure 1**). Accordingly, LH regulates Leydig-cell sex-steroid production. Sertoli cells express the FSHR gene and FSH regulates the structural, nutritional, and regulatory (paracrine) support that Sertoli cells provide to germ cells. In fish, the FSHR is less selective and binds, next to FSH, to LH as well. Moreover, the FSHR is not only expressed by Sertoli cells, but also by Leydig cells. These traits lead to the impression that the biological activities of fish gonadotropins are less clearly separated compared with those in higher vertebrates. However, cross-activation of the FSHR requires a rather high LH concentration that, if at all relevant physiologically, is restricted to the brief period with peak LH plasma levels during actual spawning events. FSHR expression by Leydig cells, on the other hand, explains the steroidogenic potency of FSH fishes, which is particularly relevant for understanding the regulation of sex-steroid production when LH plasma levels are very low or undetectable. Hence, in fish, Leydig-cell steroid production is directly regulated by LH and by FSH, while Sertoli cell functions are predominantly regulated by FSH.

Data on plasma levels of the gonadotropins during a complete reproductive cycle are available for a few salmonid species only. In rainbow trout (*Oncorhynchus mykiss*), LH plasma levels were low or undetectable during the start of

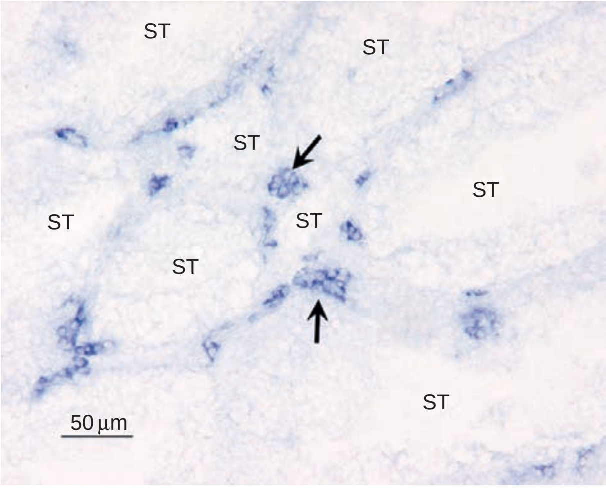


Figure 2 3β-Hydroxysteroid dehydrogenase (3βHsd) activity (blue staining) is demonstrated on a 5-μm thick cryosection from an adult African catfish (*Clarias gariepinus*) testis using an enzyme-histochemical technique. The enzyme is localized in the cytoplasm of Leydig cells that are arranged in cell groups (black arrow) in the interstitial compartment of the testis, between the seminiferous tubules (ST). Air-dried cryosections were treated with xylene for 2 min, and then incubated for 1 h at 37 °C in 3βHsd staining medium (containing 0.1 mg·ml^{-1} 5α-androstan-3β-ol-17one, 0.5 mg·ml^{-1} β-nicotinamide adenine dinucleotide, 0.2 mg·ml^{-1} nitroblue tetrazolium chloride, and 10% 7 dimethylformamide in 0.1 M phosphate buffer pH 7.4).

testis development (proliferation of spermatogonia), became detectable when meiosis started, and reached peak levels in the spawning season. FSH plasma levels, on the other hand, showed a transient increase at the beginning of spermatogonial proliferation, re-increased with the start of spermiation (opening of spermatogeneic cysts to release spermatozoa in the lumen of the tubules), and decreased before the spawning season was completed. In Chinook salmon (*Oncorhynchus tshawytscha*), FSH and androgen blood levels were elevated when spermatogonial proliferation started. In Japanese eel (*Anguilla japonica*), FSH-stimulated spermatogonial proliferation was prevented by blocking the steroidogenic activity of FSH. Hence, the present knowledge about fish indicates that FSH stimulates androgen production, which in turn initiates spermatogenesis. However, future work may uncover other aspects of FSH effects on spermatogenesis.

As in other vertebrates, fish Leydig cells express the LHR. When LH binds to its receptor, steroid production is upregulated by transporting cholesterol into the mitochondria, thereby fueling the steroidogenic pathway (**Figure 3**). Biochemically, all steroid hormones are derived from cholesterol, and are formed in a suite of reactions catalyzed by steroidogenic enzymes. The first step, the conversion of cholesterol to pregnenolone (**Figure 3**), is catalyzed by an enzyme located in the mitochondria. This is also the rate-limiting step, the bottleneck being the transport of cholesterol into the mitochondria. Cholesterol uptake by mitochondria is greatly enhanced by the steroidogenic acute regulatory protein, and its expression is stimulated by pituitary hormones inducing steroid production. The product of the first reaction is pregnenolone, which diffuses into the cytoplasm where most of the ensuing steps (production of progesterone, androgen, and estrogen) are catalyzed by enzymes associated with the endoplasmic reticulum. The type of steroid metabolites released by Leydig cells depends on the type and quantity of steroidogenic enzymes present. Expression of the respective enzyme genes changes during puberty or at different stages of the annual reproductive cycles. Enzyme expression is regulated by LH and FSH (mainly stimulatory), and by steroid hormones (mainly inhibitory). In mammals, Sertoli cell-derived growth factors can also participate in the regulations of Leydig-cell enzyme expression, but information is unavailable in fish.

The main Leydig-cell products in fish are androgens, in particular 11-ketotestosterone (11-KT) and testosterone (T). Apart from androgens, other sex steroids are produced in the fish testis, such as a progesterone (in many species 17α,20β-dihydroxy-progesterone; DHP), and also small amounts of the estrogen 17β-estradiol (E2). All three types of sex steroids have specific functions in the regulation of testis physiology, which are discussed later. First, however, a closer look at Sertoli cells is provided (**see also Hormonal Responses to Stress**: Hormone Response to Stress; and Impact of Stress in Health and Reproduction).

Sertoli Cells

Sertoli cells provide physical, nutritional, and regulatory (via growth factors) support for developing germ cells. Moreover, Sertoli cells are the only phagocytotically active cell type in the spermatogenic tubules, and they remove apoptotic germ cells and cellular remnants discarded by developing germ cells. Sertoli cells express receptors for sex steroid hormones as well as FSH receptors (**Figure 1**). One of the effects of steroids on Sertoli cells is to modulate growth-factor release (see below). Information on FSH effects on Sertoli-cell functions, such as growth-factor production, is unavailable in fish.

An aspect of great relevance for spermatogenesis is Sertoli-cell proliferation. In particular, during the period of mitotic proliferation of the spermatogonia, the cyst-forming Sertoli cells also proliferate to exactly the number required for supporting the growing germ-cell clone (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis). This tailored Sertoli-cell proliferation is very important for the quantity and efficiency of sperm production, since germ cells can neither survive nor develop without Sertoli-cell support. Hence, if

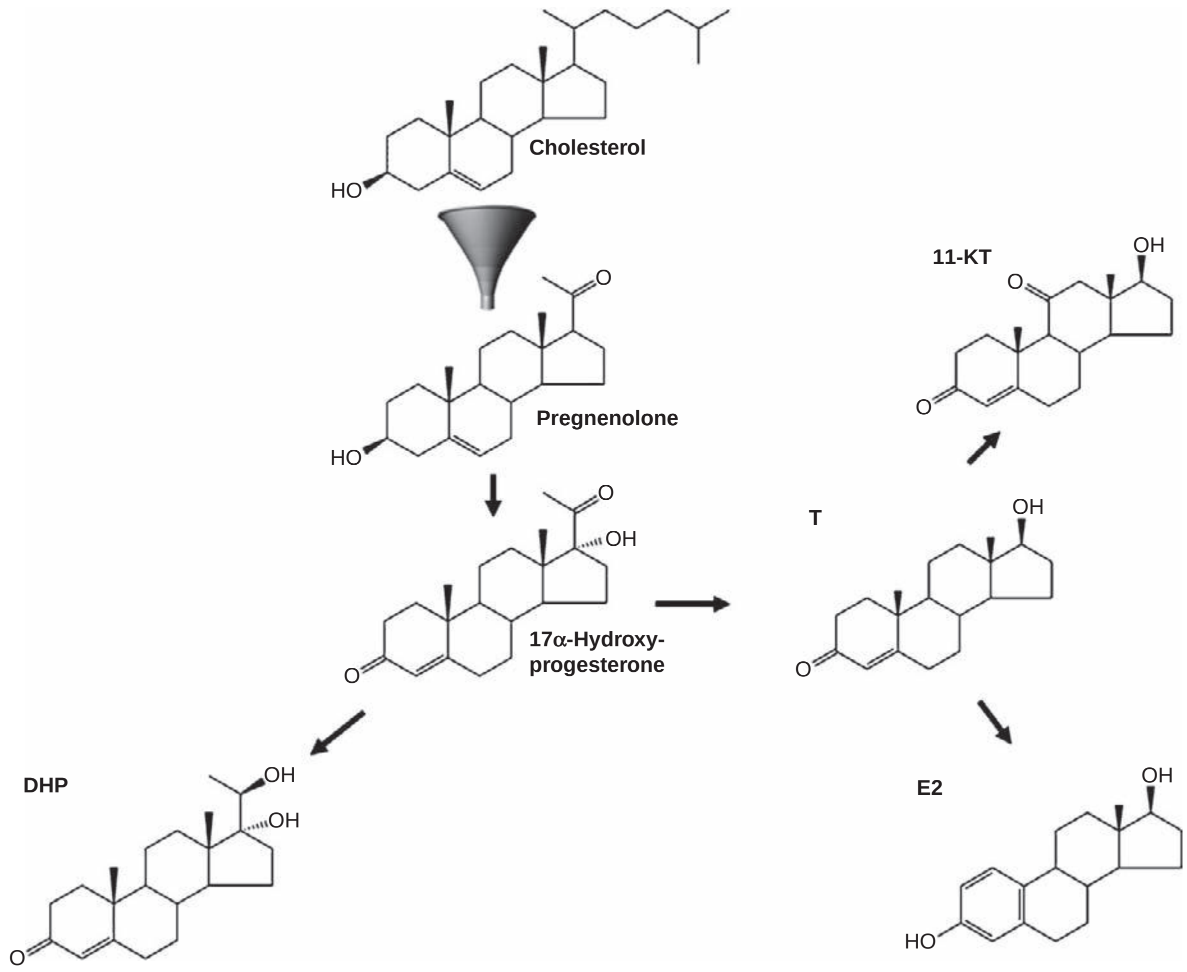


Figure 3 Schematic, simplified representation of the steroidogenic pathway in fish Leydig cells. Cholesterol is taken up from the blood or synthesized in Leydig cells. The first step in steroid production, the conversion of cholesterol to pregnenolone, is rate limiting (indicated by a funnel) and takes place in the mitochondria. Most subsequent steps take place in the cytoplasm, where pregnenolone is converted to 17α-hydroxyprogesterone. The latter is the substrate for androgen synthesis. The main product of the mature fish testis is 11-ketotestosterone (11-KT) that is produced in a number of steps from testosterone (T). T, in turn, is also the substrate for the production of 17β-estradiol (E2). Finally, in the spawning season, 17α-hydroxyprogesterone is also used as substrate for the production of 17α,20β-dihydroxyprogesterone (DHP).

Sertoli cells numbers are too low, increased germ-cell apoptosis and reduced sperm production will follow. Elevated FSH blood levels were observed during the start of seasonal testis growth, that is, when the numbers of spermatogonia and their accompanying Sertoli cells start expanding. In mammals, FSH is the most important stimulator of Sertoli-cell proliferation. The availability of recombinant fish FSH or synthetic FSH analogs allows more functional studies on FSH in fish, in the near future.

Steroid Hormone Blood Levels

Plasma levels of sex-steroid hormones change greatly during puberty or annual/seasonal reproductive cycles in adults. Estrogens are generally considered female hormones, but small amounts are formed in males as well. In rainbow trout, for example, E2 plasma levels increase transiently at the beginning of testis growth. The androgens 11–KT and T increase gradually as spermatogenesis proceeds, and reach peak levels shortly before or at the beginning of the spawning season. The progesterone DHP shows two peaks in salmonid fish, a small peak after the start of spermatogonial proliferation when the first meiotic germ cells are formed, and a prominent peak in the spawning season. These examples suggest that steroid hormones have distinct roles in controlling spermatogenesis. Indeed, as discussed later, estrogens modulate spermatogonial stem cell (SSC) self-renewal proliferation, androgens stimulate the rapid, differentiating proliferation of spermatogonia, and progesterone triggers the entry of spermatogonia into meiosis, that is, it promotes the development from spermatogonia to spermatocytes. Later on, DHP also supports final maturation of spermatozoa and sperm hydration.

Estrogens

Estrogens, like other steroid hormones, bind to nuclear receptors that function as hormone-activated transcription factors and modulate target-gene expression. Up to three estrogen-receptor (ER) subtypes can be found in fish. Testis tissue expresses the estrogen receptors in Sertoli cells and in spermatids. Estrogen treatment changed the expression of numerous genes in the testis of maturing sea bream (*Sparus auratus*). Some of the estrogen-regulated genes seem relevant for spermatogenesis since they are involved in lipid or protein metabolism, cellular communication, or proliferation.

Two types of germ-cell proliferations are observed at the beginning of spermatogenesis: on the one hand, the slow self-renewal proliferation of SSCs that produces new, individual SSCs; and on the other hand, the rapid, clonal proliferation of differentiating spermatogonia toward meiosis (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis). In immature Japanese eel testis, E2 induced in Sertoli cells the expression of platelet-derived endothelial cell growth factor (PD-ECGF) that in turn stimulated SSC self-renewal (**Figure 4**). In the fruit flie (*Drosophila melanogaster*), a number of different growth factors participate in the regulation of SSC proliferation, and future work will likely elucidate the roles of other factors regulating SSC proliferation in fish as well (**see** section Androgens). The stimulatory effect via PD–ECGF occurred at very low estrogen concentrations, while higher concentrations inhibited spermatogenesis via negative feedback effects on the brain and the pituitary, involving downregulation of the testicular androgen-production capacity.

Androgens

Androgens are required for spermatogenesis in all vertebrates. Depending on the fish species, one or two androgen receptor (AR) subtypes have been described, which are prominently expressed in the testis. AR expression is found in Sertoli cells (and other somatic cells), but not in germ cells. Therefore, androgens stimulate spermatogenesis in an indirect manner, for example, via the AR-expressing Sertoli cells. As can be expected in context with the molecular mechanism of action of steroid hormones, androgens strongly influence testicular gene-expression patterns. Detailed studies have been carried out in Japanese eel and in rainbow trout. With regard to androgen-sensitive Sertoli cell genes, for instance, the expression of the transforming growth factor β (TGFβ) family member anti-Müllerian hormone (AMH) is downregulated by androgens. AMH has two known biological activities in fish. It stimulates self-renewal proliferation of SSCs (demonstrated in medaka (*Oryzias latipes*)), and it prevents differentiation of SSCs into rapidly proliferating clones of spermatogonia (observed in Japanese eel (*Anguilla japonica*)) (**Figure 4**). Therefore, the observation that androgens stimulate the start of spermatogenesis is in line with the downregulatory effect of androgen on AMH gene expression, hence relieving the inhibition of SSC differentiation (**Figure 4**). Once the clones of differentiating spermatogonia have been formed, their further development toward meiosis continues to be stimulated by androgen-regulated, Sertoli-cell-derived growth factors, such as activin, another TGFβ family member (**Figure 4**).

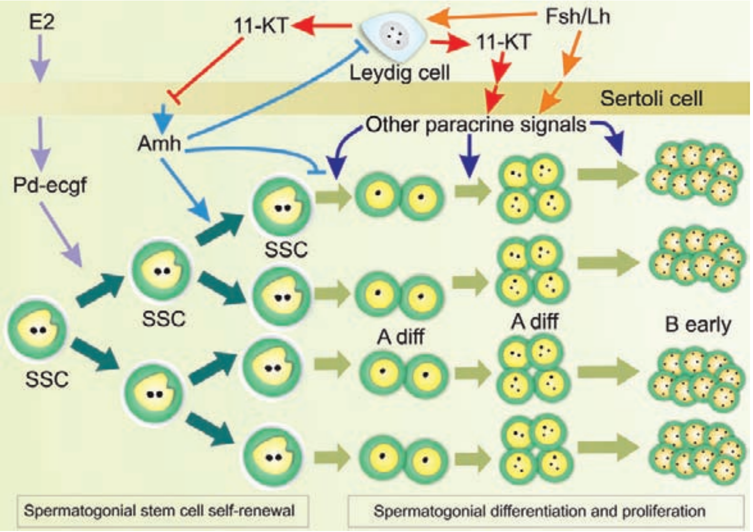


Figure 4 Schematic representation of the balance between spermatogonial stem cell self-renewal and differentiation. Spermatogonial stem cell (SSC), differentiating type A spermatogonia (Adiff), early type B spermatogonia (B early), 17β-estradiol (E2), platelet-derived endothelial cell growth factor (Pd–ecgf), 11–ketotestosterone (11–KT), anti-Müllerian hormone (Amh), follicle-stimulating hormone/luteinizing hormone (Fsh/Lh), stimulatory signal (→) and inhibitory signal (⊥).

In rainbow trout (*Oncorhynchus mykiss*), it was possible to compare changes in testicular gene expression after sex-steroid treatment with those occurring during natural testis maturation. For example, distinct subgroups of genes responsive to androgen treatment are highly expressed before the start of spermatogenesis, at the initiation of rapid spermatogonial proliferation, and during meiotic/post-meiotic stages, respectively. These gene groups therefore may play important roles during specific phases of spermatogenesis. Many of the maturational changes in gene expression are correlated with the changes in androgen levels in the blood. For instance, some genes are upregulated when androgen levels increased moderately at the beginning of spermatogenesis. Others are overexpressed in parallel to the prominent increase in androgen blood levels, in fully mature males. Interesting examples are genes coding for ion or water transporters, supporting the relevance of androgens for sperm hydration.

Progesterones

Testis tissue expresses nuclear and membrane-associated progestin receptor types in fish. Progesterone blood levels increase toward, and are high during, the spawning season. Progesterones are involved in the regulation of several testicular functions. For example, treatment with progesterone (DHP in freshwater species; 17α,20,21-trihydroxy-4-pregnen-3-one in marine species) induced spermiation, increased milt production, and stimulated spermatozoa motility in different fish species. Moreover, the involvement of DHP in triggering meiosis was recently reported.

Upon completion of the mitotic proliferation phase, type B spermatogonia differentiate into primary spermatocytes, thereby entering meiosis (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis). DHP plasma levels show a small peak during this period. Incubating immature testis tissue with DHP induced the expression of proteins typically associated with meiosis in eel. One of the proteins induced by DHP treatment was identified as trypsin that, secreted by Sertoli cells, mediated the DHP-induced entry of spermatogonia into meiosis. DHP also induced meiosis in oogonia in huchen (*Hucho hucho*) and carp (*Cyprinus carpio*) ovarian-organ-culture experiments. Together, these data show that DHP is an essential factor for the initiation of meiosis in both spermatogenesis and oogenesis.

Final Maturation of Male Gametes

During the actual spawning season, several plasma hormone levels show remarkable changes in male fish. LH secretion induces an increase in the testicular androgen and progesterone production. Androgen injections induced spermiation in goldfish (*Carassius auratus auratus*) and salmonids; progesterone injections had similar effects in salmonids and eels. While LH and these steroids are clearly involved in regulating spermiation in fish, the mechanism of action of these hormones on milt hydration, sperm migration from the spermatogenic tubules to the sperm duct, or sperm capacitation (see below), are still unclear.

After completion of spermatogenesis, Sertoli cells release the spermatozoa (spermiation) which are often not yet capable of fertilizing eggs, however. In some salmonid fish, for example, dilution with freshwater induces motility of spermatozoa collected from the sperm duct while testicular spermatozoa remain immotile. Thus, spermatozoa acquire the motility response to dilution during their passage through the sperm duct. Sperm maturation, the phase during which nonfunctional gametes develop into mature spermatozoa (fully capable of vigorous motility and fertilization), involves physiological but no morphological changes. In salmonids, sperm motility can also be induced by increasing the seminal plasma pH value to ~8, which in turn results in an elevation of cyclic adenosine monophosphate (cAMP) levels in spermatozoa. In Japanese eel, DHP triggered the expression of an enzyme in the efferent duct system that increases seminal plasma pH, in turn increasing the cAMP content, and hence sperm motility. Finally, progestins also may have a direct action on sperm motility. In Atlantic croaker (*Micropogonias undulatus*), progesterone bound to a membrane-bound receptor on the mid-piece (region rich in mitochondria) of the spermatozoa and induces vigorous motility.

The different phases of spermatogenesis and the role of steroid hormones are summarized schematically in **Figure 5**.

Germ Cell Autonomous Regulation of Spermatogenesis

While hormones exert the master control over spermatogenesis, regulation of spermatogenesis also operates on an intracellular level in germ cells, independent of external signals. This is elegantly demonstrated by transplanting germ cells between species that show differences in the amount of time required for spermatogenesis. For example, transplanting germ cells from rat (duration of one spermatogenic cycle is 14 days) to mice (10 days) showed that the timing of spermatogenesis was determined by the germ-cell genome, that is, it reflects (rat) germ-cell autonomous processes that are not influenced by the recipient's (mouse) endocrine system. We can conclude that endocrine signaling does not mediate a step-wise regulation of germ-cell development, but rather brings about the creation of different microenvironments that the germ cells require for proceeding through the

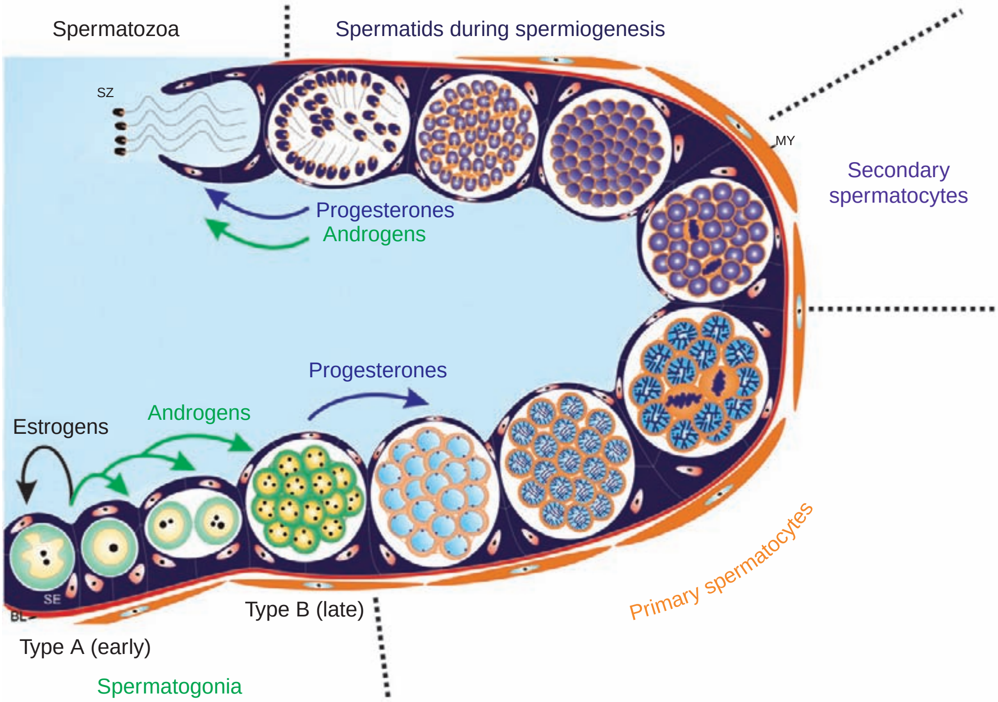


Figure 5 Schematic representation of the main phases of spermatogenesis starting from spermatogonia type A (early generations) through type B (later generations), primary and secondary spermatocytes, spermatids and spermatozoa). Estrogens stimulate self-renewal of spermatogonial stem cells (black curved arrow). Androgens (green arrows) stimulate differentiation and rapid proliferation of spermatogonia. Progesterones (blue arrows) trigger entry into meiosis and, together with androgens, spermiation (opening of the spermatogenic cysts). The germinal epithelium contains Sertoli (SE) and germ cells, delineated by a basal lamina (BL) and peritubular myoid cells (MY).

successive phases of the developmental program (stem cell, spermatogonial, meiotic, and spermiogenic phases) that constitute spermatogenesis.

The complexity of these germ-cell autonomous processes is exemplified in yeast. From the ~6200 genes in the yeast genome, ~1600 are expressed differentially during meiosis. When deleting a subset of these genes one by one, ~10% of them (~160 genes) were found to be required for just one process, the segregation of the meiotic chromosomes. In vertebrates, other spermatogenesis-specific genes function during the mitotic and spermiogenic phases. Indeed, in several cases, compromised or lost gene function was responsible for a failure of spermatogenesis, and many of these defects concern germ-cell autonomous processes, often based on germ-cell-specific (variants of) cell-cycle regulators, transcription factors, or enzymes. Some information in this regard is also available in fish.

The *mlh1*gene operates during meiosis. In spermatocytes, a large number of DNA double-strand breaks are generated in the chromosomes. One or two breaks per pair of chromosomes are repaired through homologous recombination with a non-sister chromatid of the homologous chromosome, resulting in crossing-over sites. The mutL protein complex consists of two proteins, Mlh1 and Mlh3. The complex is present at the sites of crossing over, and stabilizes the recombination sites until their repair. In zebrafish (*Danio rerio*) lacking a functional Mlh1 protein, males are sterile and lack fertile spermatozoa. Spermatogenesis stops at the metaphase of the first meiotic division, where completion of recombinatory DNA-break repair fails. Primary spermatocytes accumulate at this point, become apoptotic, and are finally phagocytized by Sertoli cells.

In addition, a new class of small, noncoding RNAs (so-called piRNAs) is involved in germ-cell autonomous differentiation processes. Loss-of-function mutations of the enzymes generating the piRNAs result in a loss of all germ cells before they can reach meiosis.

Synopsis and Outlook

Hormones of the brain–pituitary system control spermatogenesis. This endocrine master control of puberty and adult reproduction provides numerous evolutionary advantages, such as silencing of the reproductive system until somatic development and/or until seasonal conditions are sufficiently favorable to afford reproduction. Moreover, integrating activation of spermatogenesis, via its dependency on sex steroids, with reproductive behavior and secondary sexual characters, allows for sexual selection to have an impact. Seemingly wasteful (ornamental or behavioral) competitive traits, often in males, reflect the individual's competence to recruit resources from the environment, involve several genes spread over the genome, and therefore serve as genome-quality indicators. When used in mate choice, this efficiently removes deleterious alleles, while rapidly fixing advantageous ones in a population.

What are the most important steps in spermatogenesis under control of the endocrine system? Reproductive hormones seem to play a critical role at three points:

- the balance between self-renewal and differentiation of SSCs;
- the transition of type A spermatogonia to rapidly proliferating type B spermatogonia, and
- the entry into meiosis.

During later developmental stages, on the other hand, the endocrine system seems to assume a permissive rather than stimulatory role, enabling Sertoli cells to generate the environment germ cells needed to proceed through meiosis and spermiogenesis. After completion of spermatogenesis, the endocrine system is again important for spermiation and milt hydration in the context of spawning.

Knowledge about the mechanisms regulating SSC proliferation and differentiation are not only very interesting with regard to basic and comparative biology, but also with regard to applied aspects of science. For example, the early stages of spermatogenesis in fish are of great relevance for aquaculture biotechnology and ecotoxicology (**see also Hormonal Responses to Stress:** Impact of Stress in Health and Reproduction). Surface freshwater bodies contain compounds (referred to as endocrine-disrupting chemicals, EDCs) that function as agonists/antagonists of nuclear receptors involved in regulating the reproductive system. Detrimental effects of EDCs have been described in wild populations of fish as well as in experimental models, and are an important ecotoxicological concern. In aquaculture biotechnology, precocious sexual maturation, particularly in males, is a commonly observed problem, with adverse effects on growth, flesh composition, disease, and survival. Obtaining detailed knowledge about the underlying processes triggering pubertal testis maturation will be of great relevance not only for developing approaches to control male puberty, but also for estimating the impact of, and the requirement for, monitoring/regulating the presence of EDCs in surface freshwater bodies.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress**: Hormone Response to Stress; Impact of Stress in Health and Reproduction. **Hormones in Communication**: Hormonal Pheromones. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis.

Further Reading

Carrillo M, Zanuy S, Felip A, *et al.* (2009) Hormonal and environmental control of puberty in perciform fish: The case of sea bass. *Annals of the New York Academy of Sciences* 1163: 49–59.

Clutton-Brock T (2007) Sexual selection in males and females. *Science* 318: 1882–1885.

Davies EL and Fuller MT (2008) Regulation of self-renewal and differentiation in adult stem cell lineages: Lessons from the *Drosophila* male germ line. *Cold Spring Harbor Symposia on Quantitative Biology* 73: 137–145.

Feitsma H, Leal MC, Moens PB, Cuppen E, and Schulz RW (2007) Mlh1 deficiency in zebrafish results in male sterility and aneuploid as well as triploid progeny in females. *Genetics* 175: 1561–1569.

Houwing S, Kamminga LM, Berezikov E, *et al.* (2007) A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. *Cell* 129: 69–82.

Hutchinson TH, Ankley GT, Segner H, and Tyler CR (2006) Screening and testing for endocrine disruption in fish-biomarkers as "signposts," not "traffic lights," in risk assessment. *Environmental Health Perspectives* 114(supplement 1): 106–114.

Levavi-Sivan B, Bogerd J, Mañanós EL, Gómez A, and Lareyre JJ (2010) Perspectives on fish gonadotropins and their receptors. *General and Comparative Endocrinology* 165(3): 412–437 (doi:10.1016/j.ygcen.2009.07.019).

Miura T and Miura C (2001) Japanese eel: A model for analysis of spermatogenesis. *Zoological Science* 18: 1055–1063.

Nagahama Y (1994) Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology* 38: 217–229.

Rooij de DG and Boer de P (2003) Specific arrests of spermatogenesis in genetically modified and mutant mice. *Cytogenetic and Genome Research* 103: 267–276.

Schulz RW, de França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165(3): 390–411 (doi:10.1016/j.ygcen.2009.02.013).

Taranger GL, Carrillo M, Schulz RW, *et al.* (2010) Control of puberty in farmed fish. *General and Comparative Endocrinology* 165(3): 483–515 (doi:10.1016/j.ygcen.2009.05.004).

Zohar Y, Muñoz-Cueto JA, Elizur A, and Kah O (2010) Neuroendocrinology of reproduction in teleost fish. *General and Comparative Endocrinology* 165(3): 438–455 (doi:10.1016/j.ygcen.2009.04.017).

Vitellogenesis in Fishes

BJ Reading and CV Sullivan, North Carolina State University, Raleigh, NC, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Chemistry of Vitellogenins and Egg Yolk
Vitellogenin Structure and Product YPs
Nomenclature and Classification of Vitellogenins
Endocrine Control of Vitellogenesis
Vitellogenin Synthesis and Secretion
Vitellogenin Uptake and Processing into YPs
Vitellogenin Receptors
Maturational Proteolysis of YPs
Phosvitinless Vitellogenin: The Oddities of VtgC
Vitellogenesis in Livebearing Fishes
Alternative Functions of Vitellogenins
Applications and Perspectives of Vitellogenesis
Further Reading

Glossary

Egg The fully grown and ovulated female gamete, competent to undergo fertilization. After ovulation oocytes become eggs. Also called an ovum.

Follicular growth The period after onset of meiosis from the time when prefollicle cells envelop the oocyte to the end of vitellogenic oocyte growth.

Oocyte The female germ cell (gametocyte) from which an egg develops. After the onset of meiosis, oogonia become oocytes.

Oogenesis Process by which oogonia become mature eggs (or ova), the female gametes.

Ovarian follicle The basic unit of female gametogenesis, composed of a spheroid aggregation of cells found in the ovary. Follicles are comprised of an oocyte surrounded by a monolayer of granulosa cells overlain by a basement membrane (or basal lamina) and then a peripheral layer of theca cells and associated connective tissue and vasculature. During the reproductive cycle, these structures grow and develop, culminating in ovulation of the oocyte as a competent egg.

Ovarian maturation The series of events beginning with fully yolked (postvitellogenic) oocytes encapsulated in their follicles and culminating with the ovulation of fertilizable eggs.

Vitellogenesis The stage of oocyte growth characterized by the formation of egg yolk proteins and their accumulation in the ooplasm (Latin *vitellus* (yolk) and Greek *genesis* (production)). Vitellogenesis is characterized by five events: hepatic synthesis of the vitellogenin yolk precursors, which is induced by estrogen, delivery of vitellogenins to the growing oocyte via the bloodstream, selective uptake of vitellogenins by the oocyte through receptor-mediated endocytosis, processing of vitellogenins into their derivative yolk proteins, and storage of the yolk proteins in yolk granules, globules, or platelets.

Vitellogenins Precursors of the major egg yolk proteins comprised of up to five different domains, each of which corresponds to one of the stored yolk proteins (see also yolk proteins). Vitellogenins are large (>350 kDa), homodimeric phospholipoglycoproteins. Common abbreviations for vitellogenin are Vg, VG, and Vtg.

Yolk The content of the ooplasm of the egg, which serves as the nutrient source for the developing embryo. Yolk components of endogenous or maternal origin may include proteins, carbohydrates, lipids, vitamins, minerals, ions, and various other nutritive materials required to sustain embryogenesis, as well as nucleic acids encoding proteins essential for oogenesis and embryonic development.

Yolk proteins Proteolytic derivatives of the yolk precursors, vitellogenins, that are stored in the yolk granules, globules, or platelets, or fluid yolk of the ooplasm and comprise the major protein component of the egg yolk. There are five different yolk proteins, which may occur singly or as conjugates in pairs or groups: lipovitellin heavy chain, lipovitellin light chain, phosvitin, β'-component, and carboxy-terminal component.

Introduction

Ovarian follicle growth can be divided into previtellogenic (primary growth) and vitellogenic (secondary growth) stages, during which the major lipid and protein nutrients required for embryonic and larval development are stored within the oocyte (**Figure 1**). During previtellogenesis, oocytes begin accumulating neutral lipids, which are stored as lipid droplets in the ooplasm. During vitellogenic growth, oocytes also accumulate

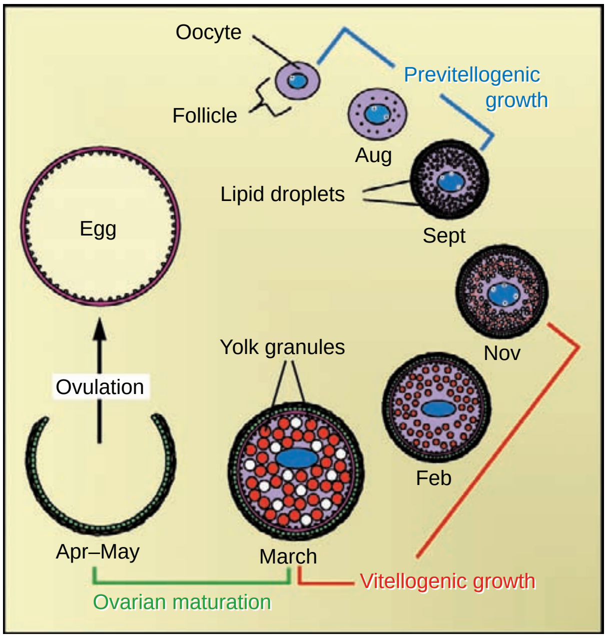


Figure 1 Model depicting oogenesis in a temperate perciform fish, the white perch (*Morone americana*). The oocyte along with its surrounding somatic tissues (the granulosa cells and theca layer) is called a follicle. The previtellogenic, primary growth oocyte accumulates neutral lipids that are stored in the ooplasm as lipid droplets. During vitellogenic growth, lipid deposition continues and the oocyte accumulates yolk proteins, which are stored in yolk granules. After vitellogenic growth is completed, the follicle undergoes maturation, which includes resumption of meiosis and cytoplasmic maturation by the oocyte, culminating in ovulation of an egg that is competent to undergo fertilization.

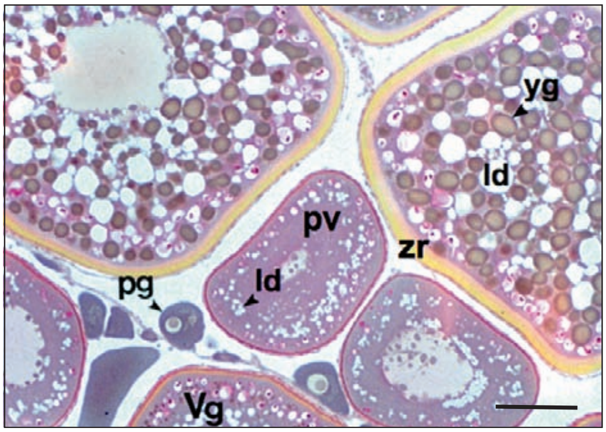


Figure 2 Light micrograph of a section through white perch ovarian follicles containing large, late vitellogenic oocytes (upper left and right) among small primary growth oocytes (pg), previtellogenic oocytes undergoing lipid deposition (pv), and early vitellogenic (Vg) oocytes. This section is typical of white perch ovaries collected just before the spawning season. The large vitellogenic oocytes contain abundant yolk granules (yg) and lipid droplets (ld) as inclusions in their ooplasm, and they have a well-developed egg envelope or zona radiata (zr). The previtellogenic oocytes contain only lipid droplets as ooplasm inclusions and their zona radiata is relatively undeveloped. Scale = 150 μm.

phospholipid-rich yolk protein (YP) precursors termed vitellogenins (Vtgs). Vtgs are synthesized by the liver and transported through the bloodstream to the ovary, where they are taken up by oocytes and processed into their derivative YPs. When vitellogenesis ends, the ovary is filled with fully yolked oocytes that subsequently undergo maturation and ovulation. The contribution to oocyte growth of Vtg-derived yolk can be substantial, comprising up to 80–90% of the dry mass of an ovulated egg in some species. The histological structure of vitellogenic ovarian follicles from white perch (*Morone americana*) is shown in **Figure 2**.

Chemistry of Vitellogenins and Egg Yolk

Vitellogenins are dimeric proteins, consisting of two identical subunits with phosphate, lipid, carbohydrate, and protein components. They are members of the large lipid transfer protein superfamily, which includes other serum lipoproteins such as low-density lipoprotein (LDL). The Vtgs are large lipoproteins (~350–600 kDa) and ~20% lipid by weight. They also are specialized carriers of important ions, such as calcium, magnesium, iron, zinc, copper, and various minerals and vitamins, such as retinoids and carotenoids. In addition, regulatory compounds found in fish egg yolk, including lipid soluble steroid and thyroid hormones, may be transported in part by Vtgs.

For fishes that spawn demersal (sinking) eggs that lack prominent oil droplets, Vtgs are major carriers of lipids into growing oocytes, most of which (~80%) are phospholipids, often phosphatidylcholines (**Figure 3**). In marine fishes that spawn pelagic (floating) eggs with no large oil droplet(s), phospholipids, triacylglycerides, and wax- or steryl-esters can account for >70%, ~8–12%, and ~4% of total egg lipids, respectively. Other teleosts produce pelagic eggs with large oil droplets comprised of neutral lipids (e.g., triacylglycerides and wax- or steryl-esters) that can occupy >50% of the ooplasm. As the deposition of neutral lipids is initiated before, and can occur independently from, accumulation of Vtg-derived YPs, Vtg is not a major source of neutral lipids in oocytes of these species. The neutral lipids must be delivered to the oocyte via a different mechanism, perhaps by LDL or related lipoproteins.

Alanine is the most abundant amino acid comprising Vtg polypeptides (~11–12% of total residues). In addition, Vtgs in some species (e.g., anguillid eels) contain polyalanine regions. It is suggested that the alanine serves as an important intermediary of carbohydrate metabolism, especially embryonic gluconeogenesis.

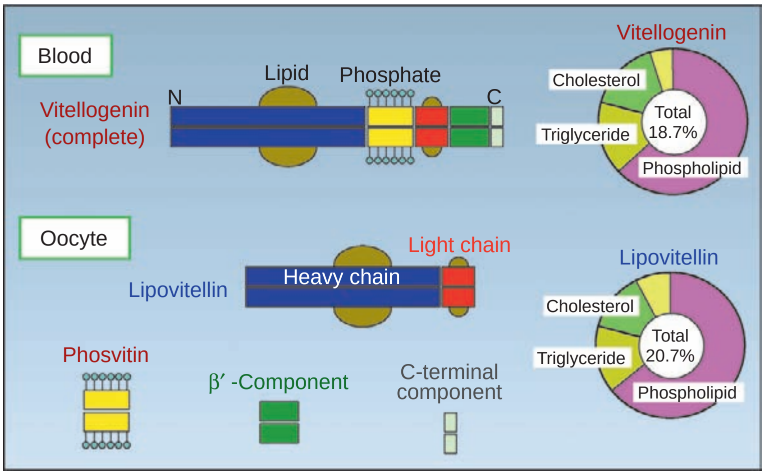


Figure 3 Model showing the pentapartite domain organization of native complete vitellogenin (dimer) in the blood and its corresponding dimeric yolk protein products in growing oocytes. The yolk protein products include: lipovitellin (Lv) heavy chain (LvH), Lv light chain (LvL), phosvitin (Pv), β'-component (β'-c), and C-terminal component (Ct). Phosphate groups attached to Pv are indicated by the aqua blue circles, and lipids bound to vitellogenin (Vtg) and Lv are indicated by the half-ovals shaded in brown. The amino (N)- and carboxy (C)-termini of Vtg also are indicated. The average percentage of total teleost Vtg and Lv lipid (by weight) is indicated within the white circles to the right (total %); the proportional composition of lipids by class (phospholipid, triglyceride, and cholesterol) is shown as colored wedges, with phospholipids being the dominant lipid class.

Vitellogenin Structure and Product YPs

Native Vtg monomers consist of covalently linked YP domains that are proteolytically processed into corresponding YPs after being taken up by the oocyte (**Figure 3**). Complete Vtg molecules consist of five linear YP domains from the amino-terminus: lipovitellin heavy chain (LvH), phosvitin (Pv), lipovitellin light chain (LvL), β'-component (β'-c), and C-terminal peptide (Ct).

The largest YP is the LvH lipoprotein that supplies offspring with amino acids and phospholipids, which can serve as catabolic energy substrates or in anabolic synthesis of membrane or protein structures, respectively. Teleost LvH has an average mass of ~114 kDa predicted from deduced amino acid sequences and resolves close to this position by acrylamide gel electrophoresis in most species. The LvH polypeptide consists of largely amphipathic secondary and tertiary structures that form a basket with a lumen of hydrophobic residues required to accommodate lipids. This characteristic structure is similar to vertebrate apolipoprotein B, the primary protein scaffold of other lipid-transporting particles such as LDL. Almost universally included in the LvH domain is a short sequence known to bind oocyte Vtg receptors. The LvL domain, which is smaller than LvH (~25 kDa), also forms part of the lipid-basket of Vtg and has chemical and structural characteristics similar to LvH. The LvH and LvL domains of Vtg usually contain one or more glycosylation sites to which carbohydrate moieties are attached and the LvH also contains a site that may bind zinc ion.

The Pv is a metalloprotein, consisting largely of serines (>50% of total residues), to which phosphates may be covalently attached prior to secretion by the liver. The negatively charged phosphates attract calcium, magnesium, zinc, and other multivalent metal cations (e.g., ferric iron) via ionic interactions occurring in the bloodstream or oocyte. In freshwater fishes, such as masu salmon (*Oncorhynchus masou*) and mosquitofish (*Gambusia affinis*), metal ions delivered by Pv are crucial for embryo survival, since they are not abundantly available for uptake from the environment. The egg yolk of marine fishes, such as barfin flounder (*Verasper moseri*), red seabream (*Pagrus major*), and Pacific herring (*Clupea pallasii*), contains less of the metal ions abundantly present in seawater (e.g., calcium and magnesium), suggesting that Pv plays a less significant role in maternal provision of these ions in marine fishes. The Pv also usually contains several potential glycosylation sites. Thus, Pv acts to transport important metabolic ions and carbohydrates into the yolk and, in doing so, helps maintain aqueous solubility of the largely hydrophobic Vtg particle. In addition, Pv may aid in stabilizing Vtg structure through interaction with the basket enclosing lipid cargo formed by LvH and LvL.

It is technically difficult to precisely resolve Pv by acrylamide gel electrophoresis, since it does not stain by traditional protein dyes and all species of Pv do not migrate to the same position in the gel (size range ~6–20 kDa) due to high and varying degrees of phosphorylation and to the presence of various Pv–Lv conjugates in the yolk. The size of Pv also is highly variable among fishes and the Pv domain may even be lacking in some incomplete forms of Vtg. The Pv-containing Vtgs from Ostariophysian and Protacanthopterygian fishes generally contain shorter Pvs with fewer serines (~24–34) than those from acanthomorph teleosts, which contain ~50 serines. In contrast, Japanese eel (*Anguilla japonica*) Vtgs have massive Pvs with 87–91 serines. Why this size variation occurs is unknown.

The remaining two small YPs (β'-c and Ct) are devoid of lipid and phosphate but together contain 14 highly conserved cysteine residues, which are known to be involved in disulfide linkages required for complex folding of the Vtg polypeptide and possibly for dimerization of native Vtg. This carboxy-terminal end of Vtg is highly similar in structure to cysteine-rich von Willebrand factor (vWF)-type D2 domain located in the short propeptide of vertebrate vWF. In addition, a conserved CGxC motif found in both vWF-type D2 domain and the carboxy-terminus of β'-c may be involved in facilitation of disulfide bond formation during peptide folding and/or adhesion when Vtg binds its oocyte receptor. The β'-c may have a glycosylation site and, like Pv, also has high aqueous solubility with many of its hydrophilic residues exposed on the surface of the Vtg particle. Although β'-c is known to be released from the carboxy-terminal end of Vtg as an ~16 kDa YP in a number of fish species, presence of Ct as a bona fide YP has only been verified in barfin flounder and deduced in Atlantic herring. The molecular weight of β'-c in the oocytes of several fishes indicates that Ct is cleaved from its carboxy-terminus; however, it may be degraded as opposed to stored as yolk, since a Ct YP typically cannot be detected (predicted size ~13 kDa).

Nomenclature and Classification of Vitellogenins

Fish Vtgs have a complex evolutionary history and they exhibit considerable variation in structure and function. The currently accepted Vtg nomenclature developed by R.N. Finn and associates is based on multiple types of Vtg having arisen via whole genome duplications (WGDs) during the evolutionary history of vertebrates. According to this scenario, the ancestral chordate Vtg (VtgABCD, present in silver lamprey, *Ichthyomyzon unicupsis*) gave rise to two forms of Vtg, VtgAB (chondrostean vitellogenin; present in chondrostean fishes and amphibian and avian species) and VtgCD. The VtgAB subsequently gave rise to VtgA (universally present in teleosts) and to VtgB, which is extinct. VtgCD gave rise to teleost vitellogenin type C (VtgC; also called Vtg3 or phosvitinless Vtg) (present in teleosts from diverse phylogenetic lineages) and to VtgD, which is extinct. Additional Vtg gene duplications, not always involving WGD, have occurred within different lineages of fishes. Dual or multiple A-type Vtgs are present in Protacanthopterygii (salmonids, VtgAsa, and VtgAsb), Ostariophysi (Ostariophysian vitellogenin type 1 (VtgAo1) and type 2 (VtgAo2)), and Elopomorpha (Elopomorph vitellogenin type 1 (VtgAe1), type 2 (VtgAe2), and type 3 (VtgAe3)). In a late round of WGD, the VtgA gave rise to two paralogous forms of Vtg (acanthomorph vitellogenin type Aa (VtgAa; also called Vtg1 or VtgI) and type Ab (VtgAb; also called Vtg2 or VtgII)), which are present in acanthomorph teleosts.

The various forms of fish Vtg may be either complete or incomplete and their phylogenetic distribution is shown in **Table 1**. Incomplete Vtgs are subdivided into those that contain Pv and those that lack or have severely reduced Pv domains (Pv-less). The VtgC is incomplete, being Pv-less and also lacking β'-c and Ct domains, and consists only of lipovitellin (LvH and LvL). Incomplete Pv-containing Vtgs occur only in Ostariophysian fishes (VtgAo1) and contain three YP domains: LvH, Pv, and LvL. A protein dendogram showing classification and domain structure of various types of Vtg present in diverse fish species for which full-length Vtg sequences are available is shown in **Figure 4**.

Endocrine Control of Vitellogenesis

Vitellogenesis is a seasonal or cyclic process depending on gonadotropins. The production of gonadotropin-releasing hormone (GnRH) by the brain (hypothalamus) is mediated

Table 1 Classification of multiple fish vitellogenins (Vtgs)

Taxon	*Complete Vtgs*	*Incomplete Pv-containing Vtgs*	*Incomplete Pv-less Vtgs*
Acanthopterygii	VtgAa and VtgAb	–	VtgC
Paracanthopterygii	VtgAa and VtgAb	–	VtgC
Protacanthopterygii	VtgAsa and VtgAsb	–	VtgC
Ostariophysi	VtgAo2	VtgAo1	VtgC
Elopomorpha	VtgAe1, VtgAe2, VtgAe3	–	?
Chondrostei	VtgAB	–	?
Primitive chordates	VtgABCD	–	?

A dash (–) indicates a Vtg type that is absent in a taxonomic group of fishes and a question mark (?) indicates not evaluated or described to date. The nomenclature is based on Finn and Kristoffersen, *PLoS ONE*, 2(1): e169.doi:10.1371/journal.pone.0000169, 2007.

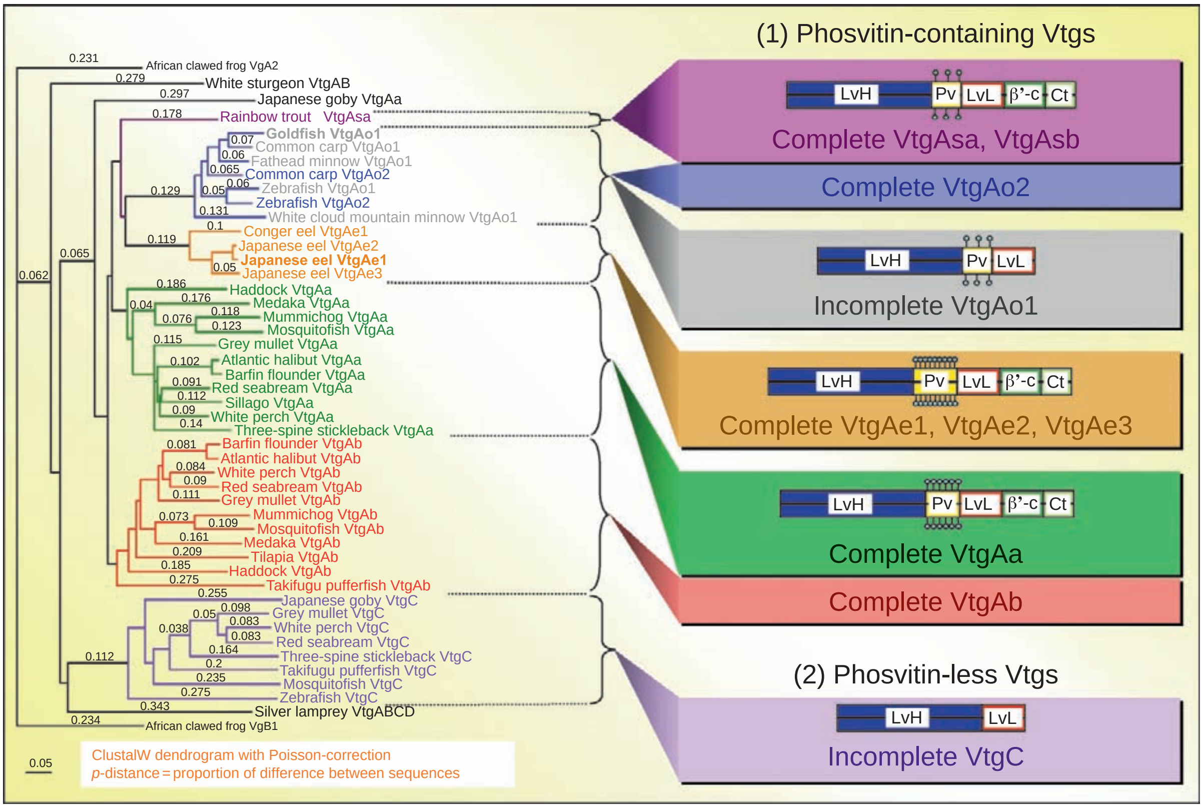


Figure 4 Protein dendrogram, nomenclature, and domain structure of vitellogenins (Vtgs) present in a diverse array of teleosts for which full-length Vtg cDNA and deduced polypeptide sequences are available. See text for details and abbreviations. The protein dendrogram was generated using the ClustalW (v 1.83) algorithm and MacVector™ (Oxford Molecular Ltd.) software with multiple alignment parameters: open gap penalty = 10.0; extended gap penalty = 0.2; delay divergent = 30%; gap distance = 4; and similarity matrix = gonnet. The Vtg polypeptide sequences were deduced from cDNA. Adapted from Reading BJ, Hiramatsu N, Sawaguchi S, *et al*. (2009) Conserved and variant molecular and functional features of multiple egg yolk precursor proteins (vitellogenins) in white perch (*Morone americana*) and other teleosts. *Marine Biotechnology* 11:169–187.

by a variety of endogenous and environmental factors, including innate biorhythms, nutritional status, and seasonal changes in day length and water temperature. In response to GnRH, follicle-stimulating hormone (FSH) is secreted by pituitary gonadotrophs and induces the theca and granulosa cells of the ovarian follicle to secrete estradiol-17β (E2), which instructs the liver to synthesize Vtgs and secrete them into the bloodstream (**Figure 5**). In some species, luteinizing hormone produced by the pituitary in response to GnRH also elicits follicular production of E2. Growth hormone, thyroid hormone, cortisol, androgens, and other estrogenic steroids, such as estrone, may also contribute to induction of hepatic vitellogenesis in some species (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction).

Vitellogenin Synthesis and Secretion

Circulating E2 enters liver cells and binds to one of three estrogen receptors (ERs), likely estrogen receptor alpha in tilapia, *Oreochromis mossambicus* (**Figure 5**). Once the E2 is bound, conformational changes occur, which allow the ER to bind protein factors involved in targeted transcriptional activation. The ER is translocated to the nucleus where it dimerizes and binds specific genomic DNA sequence motifs called estrogen response elements nearby basal *vtg* gene promoters and transcription is initiated. Ribosomes on the rough endoplasmic reticulum translate Vtg-encoding mRNAs and the polypeptides are translocated to the endomembrane system. The Vtgs undergo substantial posttranslational modification. They must be properly folded and dimerized, their Lv domains lipidated, Pv domains phosphorylated, 15–16 residue signal peptides cleaved, and carbohydrate moieties attached to their glycosylation sites. Mature Vtgs are exported to the *trans*-Golgi network where they are packaged into secretory vesicles and released into the circulatory system.

Rates of hepatic synthesis differ between Vtg types and some Vtgs are more sensitive to induction by E2. In Japanese goby (*Acanthogobius flavimanus*), VtgC has a lower sensitivity to induction by E2 and, in most species, is a minor form of Vtg based on transcription rate, plasma protein levels, and/or yolk composition. In contrast,

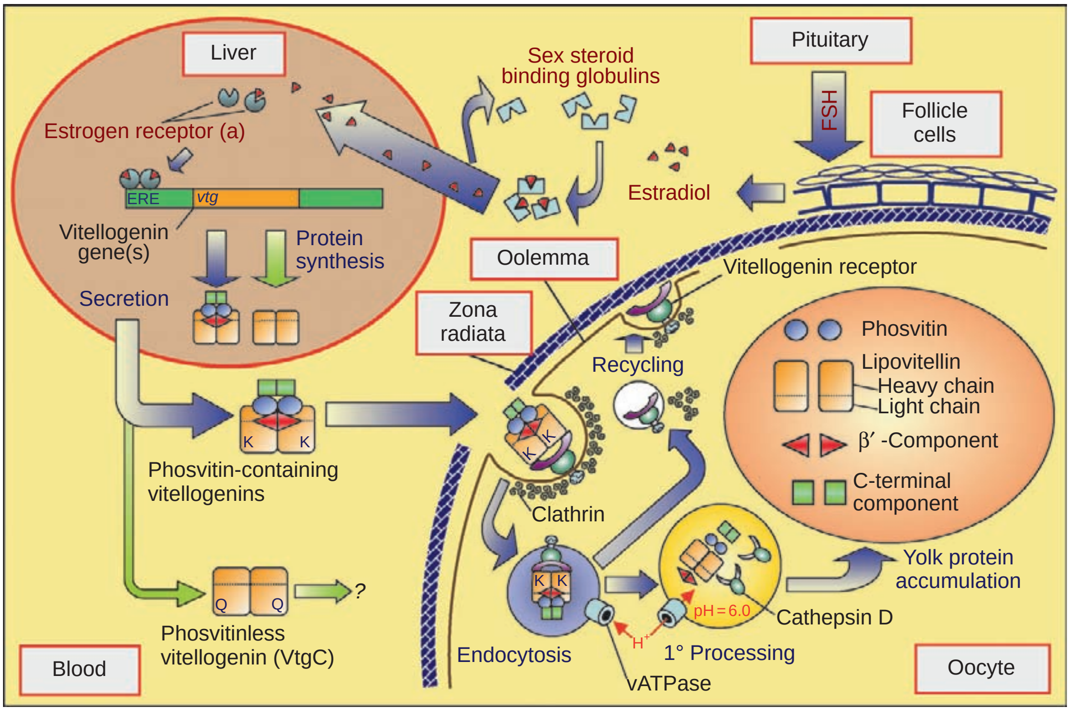


Figure 5 Model of vitellogenesis and oocyte growth. See text for details and abbreviations. Redrawn from Hiramatsu N, Cheek AO, Sullivan CV, Matsubara T, and Hara A (2005) Vitellogenesis and endocrine disruption. In: Mommsen TP and Moon TW (eds). *Biochemistry and Molecular Biology of Fishes*, vol. 6, pp. 431-471. Amsterdam: Elsevier.

VtgAb and VtgAa are the dominant types in barfin flounder and goldsinny wrasse (*Ctenolabrus rupestris*), respectively. Rates of hepatic secretion may also differ between Vtg types, as shown in barfin flounder. In this species, VtgAb is present at low levels in the liver compared to VtgAa and VtgC. However, VtgAb levels in the serum are relatively high, indicating that it is secreted at a higher rate than VtgAa and VtgC (see also **Figure 9**).

Vitellogenin Uptake and Processing into YPs

Circulating Vtgs travel through capillaries in the follicle theca and pass through the basement membrane and intercellular spaces among granulosa cells to enter the extracellular matrix of the granulosa epithelium where it contacts the oolemma. Here, Vtgs enter growing oocytes via selective endocytosis mediated by specific membrane receptors (vitellogenin receptors (Vtgrs)) (**Figure 5**). Once bound to Vtg, the Vtgrs cluster in clathrin-coated pits that invaginate and are endocytosed. In the peripheral ooplasm, the clathrin coat is stripped and Vtg dissociates from the Vtgr due to mild acidification of the vesicle. Vesicles are then sorted into a population of early endosomes that fuse with lysosome-like organelles to form multivesicular bodies. Inactive cathepsin D zymogens are co-localized with the imported Vtgs, and V-class vacuolar ATPases (i.e., proton pumps) further acidify the lumen of the multivesicular bodies. The resulting drop in pH (to ~6.0) activates cathepsin D, which cleaves Vtg into its product YPs, including LvH, LvL, Pv, β'-c, and Ct (**Figure 5**). Proteolytic variants of YPs have been demonstrated in some species, whereby the Pv domain either remains covalently linked to the carboxy-terminus of LvH or the amino-terminus of LvL, or whereby LvH–Pv–LvL conjugates remain largely intact. Smaller proteolytic fragments of LvH and β'-c have been reported in some fishes and may possibly result from incomplete or nonspecific cleavage of Vtgs.

This initial proteolytic event is termed 'primary YP processing'. Acidification may also strip calcium ions from Pv. The YPs are stored as noncovalently linked homodimers in yolk granules, globules or platelets, or as fluid yolk, in the ooplasm. In addition, noncovalent association of LvH with LvL to form lipovitellin (Lv), presumably as a result of the similar lipophilic characteristics of these YPs, has been demonstrated in many species.

Disparate accumulation of different types of Vtgs by the oocyte has also been reported. In grey mullet (*Mugil cephalus*), an acanthomorph species that spawns pelagic eggs, the accumulated ratio of VtgAa:VtgAb:VtgC in oocytes is 4:13.3:1 and in barfin flounder it is 9:15:1. Thus, a system for differential uptake of Vtgs exists and may be receptor mediated.

Vitellogenin Receptors

The teleost Vtgr is a member of the lipoprotein receptor superfamily characterized by eight ligand-binding repeats (LR8), a feature typical of other vertebrate Vtgrs and of the very low density lipoprotein receptor (VLDLR) (**Figure 6**). The LR8 receptors are ∼100–110 kDa and generally exist as splice variants either containing [LR8(+)] or lacking [LR8(–)] a serine- and threonine-rich O-linked sugar domain. The LR8(–) Vtgr is the dominant lipoprotein receptor in the ovary of fishes, whereas the LR8(+) may be a somatic VLDLR also expressed in the ovary of some species. Highest Vtgr mRNA expression occurs during previtellogenesis and expression is very low or absent in late-vitellogenic follicles or ovulated eggs. However, Vtgr-binding activity is not detected until transition to vitellogenic growth. The Vtgr transcripts are translated during vitellogenesis and Vtgrs are recycled back to the oocyte membrane throughout vitellogenic oocyte growth (**Figure 5**). The first three ligand-binding repeats of the Vtgr recognize a short motif rich in basic amino residues (HLTKTKDLN) in tilapia (*Oreochromis aureus*) VtgAb, to which it binds. Similar, but not identical, sequences are located in LvH of other fish Vtgs, usually between residues 172 and 191. The VtgC lacks this sequence.

Three distinct Vtgrs that specifically bind VtgAa and VtgAb have recently been discovered in the acanthopterygian white perch (**Figure 7**). One of these (>200 kDa) binds specifically to VtgAa (the vitellogenin Aa receptor (VtgAar)), whereas two other receptors (110 and 116 kDa) bind VtgAb (the vitellogenin Ab receptors (VtgAbrs)). It is hypothesized that these receptors may direct the different forms of Vtg into discrete populations of early endosomes resulting in disparate degrees of storage and proteolysis of their derivative YPs during ovarian maturation (see below). The disparate uptake of different Vtg types by the oocyte in barfin flounder and grey mullet may also relate to this discovery. Multiple ovarian receptors that bind Vtg have been detected in the rainbow trout (*Oncorhynchus mykiss*); however, disparate binding to the different Vtg ligands (Protacanthopterygian (salmonid) vitellogenin type a (VtgAsa) and type b (VtgAsb)) was not demonstrated.

Maturational Proteolysis of YPs

After vitellogenesis, oocytes undergo maturation, which, in addition to resumption of meiosis, includes lipid droplet coalescence, ooplasm clarification, and oocyte

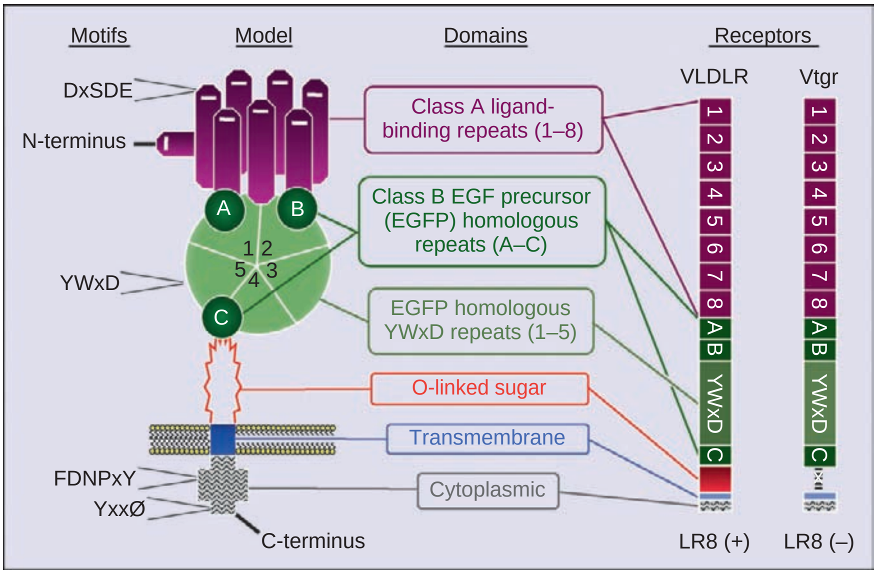

Motifs
Model
Domains
Receptors
VLDLR
Vtgr
DxSDE
N-terminus
YWxD
FDNPxY
YxxØ
C-terminus
A
B
C
1 2
5 4 3
Class A ligand-binding repeats (1–8)
Class B EGF precursor (EGFP) homologous repeats (A–C)
EGFP homologous YWxD repeats (1–5)
O-linked sugar
Transmembrane
Cytoplasmic
1 2 3 4 5 6 7 8 A B YWxD C
1 2 3 4 5 6 7 8 A B YWxD C
LR8 (+)
LR8 (–)

Figure 6 Model depicting typical structures of teleost oocyte lipoprotein receptors with eight ligand-binding repeats (LR8). Motifs present in each domain are indicated at the left and the linear organization of the defined receptor domains present in the receptor polypeptide sequences is shown on the right (**see** section Vitellogenin Receptors for details and abbreviations). The LR8 receptors contain four (Vtgr) or five (VLDLR) domains from the N-terminus: a ligand-binding domain, comprised of eight class A ligand-binding repeats; a domain that shares high homology with epidermal-growth factor precursor (EGFP) comprised of three class B repeats (A–C) and five YWxD repeats that form the β-propeller; an O-linked sugar domain which is present in the VLDLR [(LR8(+)], but is absent in the Vtgr [LR8(–)]; a transmembrane domain; and a short cytoplasmic domain that contains endocytosis signals (NPxY and YxxØ). Clusters of acidic residues (DxSDE) impart a negative charge at the surface of each ligand-binding repeat, indicated by the (–), which is required for their electrostatic interaction with specific positively charged residues in Vtg or other ligands.

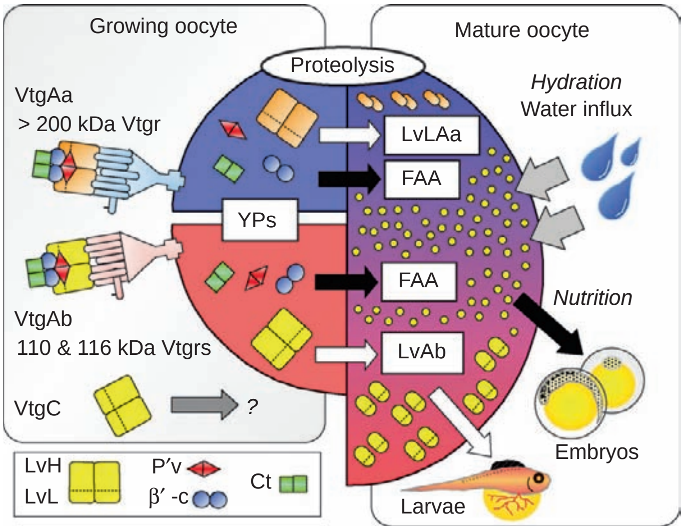


Figure 7 Hypothetical model of oocyte growth including recent findings on ovarian receptors for vitellogenins (Vtgrs) in white perch (**see** section Vitellogenin Receptors for details). One of these receptors specifically binds VtgAa (>200 kDa Vtgr), whereas the two other receptors bind VtgAb (110 and 116 kDa Vtgrs). During oocyte growth, these receptors may direct the different forms of complete Vtg into discrete populations of early endosomes (blue and red compartments) where they are cleaved into product yolk proteins (YPs): lipovitellin (Lv) heavy chain (LvH), Lv light chain (LvL), phosvitin (Pv), β'-component (β'-c), and C-terminal component (Ct). The YPs derived from the different forms of complete Vtg are later subjected to disparate degrees of maturational proteolysis yielding free amino acids (FAAs) and lipoprotein products that play different roles in oocyte hydration and embryonic and larval nutrition (**see** section Maturational Proteolysis of YPs for details). In white perch, VtgC does not bind any Vtgr; therefore, its method of entry into the oocyte is unknown and indicated with a question mark.

hydration. Collectively, these latter changes are termed ‘cytoplasmic maturation’. In acanthomorph teleosts that spawn pelagic eggs in marine or brackish water, a ‘secondary proteolysis’ occurs during this time, whereby most Vtg-derived YPs are degraded into free amino acids (FAAs). However, the major YP derived from VtgAb (LvHAb) persists largely intact (**Figure 8**), although it may dissociate from its native dimeric state into monomers (~170 kDa). In barfin flounder, the YPs derived from VtgAa (representing ~87% of the entire VtgAa polypeptide) are extensively cleaved into FAAs, whereas the proteolysis of YPs derived from VtgAb (representing only ~33% of the VtgAb polypeptide) is quite limited. A similar phenomenon has been described in haddock (*Melanogrammus agefinus*) and in Atlantic halibut (*Hippoglossus hippoglossus*). The VtgC has been shown to survive maturational proteolysis in grey mullet, mosquitofish, and white perch. The β'-c also survives this degradation in striped bass (*Morone saxatilis*), although it is degraded in barfin flounder, red seabream, and Atlantic halibut. In barfin flounder, LvL remnants, perhaps derived from Lv–Pv conjugates or from incomplete degradation of LvLAa, along with Vtg-derived yolk lipids, are reconstructed into novel, highly lipidated particles.

Maturational proteolysis of YPs requires acidification of yolk vesicles, which activates cathepsin proenzymes therein (**Figure 9**). Yolk vesicle pH decreases to ~5.0–5.5 due to the activation of bafilomycin-sensitive vacuolar H^+-ATPase in concert with chloride channels in the organelle membranes and of the chloride antiporter SLC26 (pendrin) in the oolemma (**Figure 9**). In mummichog (*Fundulus heteroclitus*), cathepsins B, F, and L have all been implicated in this process. However, cathepsin F is only found in yolk granules during vitellogenesis and, therefore, it may play a catalytic role in the activation of cathepsins B or L. The proteolytic activities of cathepsins B and L related to their roles in degradation of YPs may also be species specific. In barfin flounder, cathepsin B mediates this degradation; however, in seabream (*Sparus aurata*) cathepsin L does. Concomitant with the proteolysis of YPs, inorganic phosphates are liberated from Pv by phosphatase, which is activated by yolk vesicle acidification.

The small peptides and FAAs generated during secondary YP processing, along with inorganic intraoocytic ions, such as Pv-derived phosphate (and calcium) and potassium transported into the oocyte by oubain-sensitive Na^+, K^+-ATPase, act as osmotic effectors that drive the influx of water. Water diffuses through aquaporin channels transiently expressed in the oocyte membrane, resulting in oocyte hydration and in acquisition of proper egg buoyancy. Oocyte hydration also provides for dilution of embryonic wastes

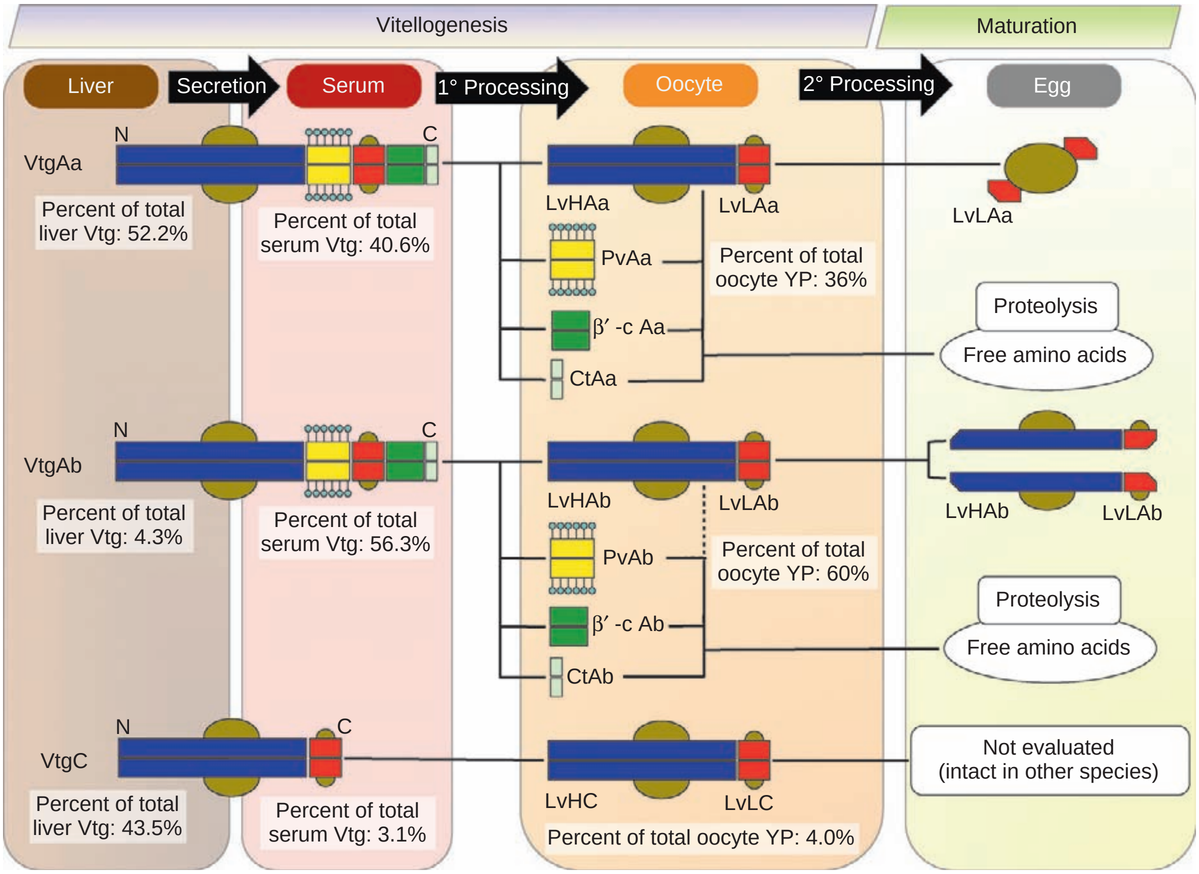

Vitellogenesis
Maturation
Liver
Secretion
Serum
1° Processing
Oocyte
2° Processing
Egg
N
C
VtgAa
Percent of total liver Vtg: 52.2%
Percent of total serum Vtg: 40.6%
LvHAa
LvLAa
PvAa
Percent of total oocyte YP: 36%
β′ -c Aa
CtAa
LvLAa
Proteolysis
Free amino acids
N
C
VtgAb
Percent of total liver Vtg: 4.3%
Percent of total serum Vtg: 56.3%
LvHAb
LvLAb
PvAb
Percent of total oocyte YP: 60%
β′ -c Ab
CtAb
LvHAb
LvLAb
Proteolysis
Free amino acids
N
C
VtgC
Percent of total liver Vtg: 43.5%
Percent of total serum Vtg: 3.1%
LvHC
LvLC
Percent of total oocyte YP: 4.0%
Not evaluated (intact in other species)

Figure 8 Model showing molecular alterations of the three types of vitellogenins (Vtgs) during vitellogenesis (1° processing) and ovarian maturation (2° processing) in barfin flounder. Structures of Vtgs and their product yolk proteins in serum of vitellogenic fish, vitellogenic oocytes, and ovulated eggs are shown (**see** sections Vitellogenin Uptake and Processing into YPs and Maturational Proteolysis of YPs for details and abbreviations). The yolk proteins (YPs), including LvH, LvL, Pv, β'-c, and Ct, are illustrated as in **Figure 1** with the exception that the Vtg-of-origin of each yolk protein (VtgAa, VtgAb, or VtgC) is indicated following the abbreviation for that YP (Aa, Ab, and C, respectively). The percentage of total Vtg comprised by VtgAa, VtgAb, and VtgC in liver and serum, and by the YPs products of each form of Vtg in fully yolked oocytes is also indicated (percent of total Vtg or YP). Although processing of LvHC during ovarian maturation was not evaluated in barfin flounder, in several other species this yolk protein remains largely intact. Compiled from Matsubara T and Swano K (1995) Proteolytic cleavage of vitellogenin and yolk proteins during vitellogenin uptake and oocyte maturation in barfin flounder (*Verasper moseri*). *Journal of Experimental Zoology* 272: 34–45; Matsubara T and Koya Y (1997) Course of proteolytic cleavage in three classes of yolk proteins during oocyte maturation in barfin flounder *Verasper moseri*, a marine teleost spawning pelagic eggs. *Journal of Experimental Zoology* 278: 189–200; Matsubara T, Ohkubo N, Andoh T, Sullivan CV, and Hara A (1999) Two forms of vitellogenin, yielding two distinct lipovitellins, play different roles during oocyte maturation and early development of barfin flounder, *Verasper moseri*, a marine teleost that spawns pelagic eggs. *Developmental Biology* 213: 18–32; and Sawaguchi S, Ohkubo N, Amano H, *et al*. (2008) Controlled accumulation of multiple vitellogenins into oocytes during vitellogenesis in the barfin flounder, *Varasper moseri*. *Cybium, the International Journal of Ichthyology* 32(2): 262.

(e.g., ammonia) and adjusts the spatial volume of the egg for embryonic development. The YP-derived FAA pool is later used as a source of diffusible nutrients by embryos at early developmental stages, while the remaining LvHs serve as lipoprotein nutrients for late-stage embryos and larvae, in which structures required to process large macromolecules (e.g., yolk syncytium) have formed.

In acanthopterygian teleosts spawning demersal eggs, such as mummichog, and those in less derived taxa, degradation of YPs into FAA is not as extensive and some major YPs (notably LvHAa) are only partially hydrolyzed or nicked to yield smaller peptides. For example, there is only a nearly threefold increase in absolute FAA content in maturing oocytes of the mummichog, which spawns demersal eggs, as compared to the barfin flounder, a pelagic egg spawner in which a ~12-fold increase in FAA content is observed. In addition, disparate hepatic production or oocytic accumulation of different types of Vtg (e.g., VtgAa:VtgAb ratios) may influence this phenomenon.

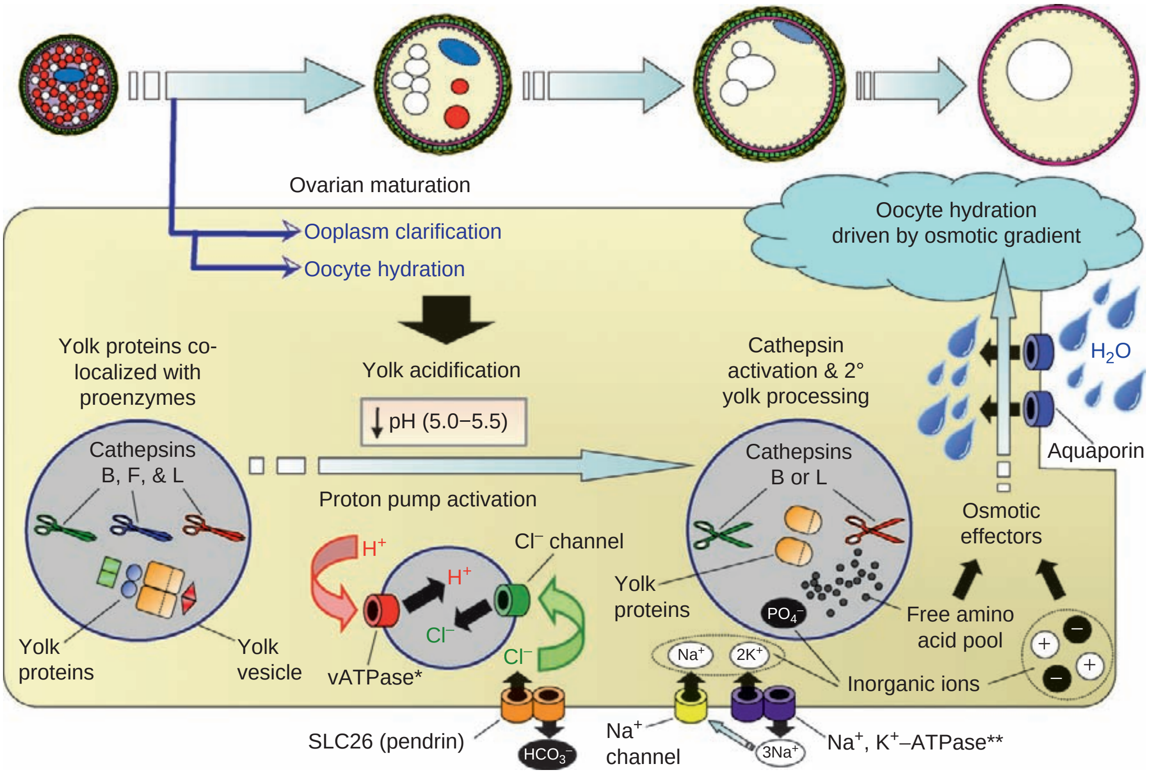


Figure 9 Model of ovarian maturation showing mechanisms of maturational proteolysis of yolk proteins (YPs) and ion transport during oocyte hydration (**see** the section Maturational Proteolysis of YPs for details and abbreviations). Ooplasm clarification and oocyte hydration are related to the proteolysis of vitellogenin (Vtg)-derived YPs, which requires acidification of the yolk vesicles to activate cathepsin proenzymes, yielding free amino acids (FAAs) that act as osmotic effectors influencing oocyte hydration. The single asterisk (*) and double asterisk (**) indicate bafilomycin- and oubain-sensitive ATPases, respectively.

Phosvitinless Vitellogenin: The Oddities of VtgC

The nearly universal presence of VtgC and its characteristic lack of some typical YP domains raise questions as to its physiological functions. The VtgC usually makes a minor contribution to the yolk. In grey mullet and barfin flounder, LvHC represents only 5.4% and 4.0% of the total LvH accumulated by oocytes, respectively. However, in mosquitofish a corresponding value as high as 25% has been reported. The VtgC has an aberrant Vtgr-binding site in its LvH domain and, in the white perch, it does not bind to any oocyte receptor, indicating a potentially different mechanism of oocyte entry, perhaps in the fluid endocytotic phase. This linkage between lack of receptor binding and the absence of oocytic proteolysis emphasizes receptor targeting of Vtgs to specific endocytotic compartments as an important mechanism regulating their proteolytic fates. In addition, the synthesis of VtgC appears to be less dependent on, and less sensitive to, E2 induction and it has been reported to begin accumulating in oocytes much earlier than the complete Vtgs in medaka (*Oryzias latipes*) and tilapia (*Oreochromis niloticus*). In mosquitofish, LvHC is consumed as a nutrient by late-stage larvae. Thus, VtgC may provide a mechanism for enriching the supply of large lipoprotein nutrients for late-stage larvae without altering the osmotic balance of oocytes or eggs.

Vitellogenesis in Livebearing Fishes

Livebearing fishes (**see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care) also produce Vtgs and utilize their derivative YPs as nutrients that support embryonic development. The ovoviviparous mosquitofish and the viviparous redtail splitfin (*Xenotoca eiseni*) produce VtgAa, VtgAb, and VtgC and deposit them in oocytes as YP nutrients. Although mosquitofish embryos develop in a closed system (egg), with the mother contributing no additional nutrients during gestation, *Xenotoca* embryos form vascular attachments with their mother called trophotenia, which function analogously to a placenta. This mode of embryonic development, termed matrotrophy, in which nutrients are supplied directly by the mother, has arisen independently in many fish lineages. However, the extent to which Vtg-derived YPs contribute to embryonic nutrition after formation of trophotenia remains to be verified, and direct transfer of Vtgs from mother to offspring via trophotenia also is unexplored.

Alternative Functions of Vitellogenins

In the rosy barb (*Puntius conchonius*), Vtg(s) have been shown to have hemagglutinating functions in blood clotting and males produce Vtgs in response to bacterial exposure. The basis for the hemagglutinating properties of Vtg is unclear at present; however, the vWF-like β'-c may be involved, since vWF is important in platelet and collagen adhesion during clotting. The Vtg(s) of greeling (*Hexagrammos otakii*) and carp (*Cyprinus carpio*) have been shown to exhibit multivalent pattern recognition, binding to various pathogen-associated structures including bacterial lipopolysaccharide, peptidoglycan, and lipoteichoic acid in a bacteriostatic fashion. Interaction of purified greeling Vtg(s) with lipopolysaccharide and lipoteichoic acid in the cell wall is actually bacteriocidal. Therefore, Vtgs may serve as antimicrobial agents in some species.

Vtgs have also been demonstrated to regulate their own synthesis in some fishes. Vitellogenesis in female walking catfish (*Clarias batrachus*) has been induced by administration of heterologous Vtg(s) purified from Indian mrigal carp (*Cirrhinus mrigala*). In rainbow trout, high concentrations of Vtg(s) inside the oocyte can alter Vtg synthesis in the liver, through suppression of E2 production by the ovary.

The specific types of Vtgs possessing these various alternative functions have not yet been identified.

Applications and Perspectives of Vitellogenesis

Circulating Vtgs have served as markers for onset of puberty and progression of gonad maturation in female fishes, especially in aquaculture. The presence of Vtg in blood, mucus, and muscle has also been used to identify the gender of fishes that do not exhibit sexual dimorphism (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish). Assessment of the proper maturational proteolysis of Vtg-derived YPs, or of expression of cathepsin transcripts and proteins, has also been used as a marker of egg quality. In addition, Vtgs are frequently used to assess exposure of animals in aquatic environments to endocrine-disrupting chemicals (EDCs), specifically to EDCs that mimic the action of estrogens (**see also Toxicology**: The Toxicology of Organics in Fishes). Since Vtgs are produced in response to endogenous E2, they are potentially ideal markers for assessment of estrogenic EDCs. However, as disparities exist between the different types of Vtgs (or *vtg* transcripts) with regard to their sensitivity to induction by estrogen(s), the specific type of Vtg being measured to detect EDCs should be taken into account.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Social and Reproductive Behaviors**: Nutritional Provision During Parental Care; Sexual Behavior in Fish. **Toxicology**: The Toxicology of Organics in Fishes.

Further Reading

Babin PJ, Carnevali O, Lubzens E, and Schneider WJ (2007) Molecular aspects of oocyte vitellogenesis in fish. In: Babin PJ, Cerdà J, and Lubzens E (eds.) *The Fish Oocyte: From Basic Studies to Biotechnological Applications*, pp. 39–76. New York, NY: Springer.

Cerdà J, Fabra M, and Raldúa D (2007) Physiological and molecular basis of fish oocyte hydration. In: Babin PJ, Cerdà J, and Lubzens E (eds.) *The Fish Oocyte: From Basic Studies to Biotechnological Applications*, pp. 349–396. New York, NY: Springer.

Davis LK, Hiramatsu N, Hiramatsu K, *et al.* (2007) Induction of three vitellogenins by 17beta-estradiol with concurrent inhibition of the growth hormone-insulin-like growth factor 1 axis in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). *Biology of Reproduction* 77: 614–625.

Finn RN (2007) Vertebrate yolk complexes and the functional implications of phosvitins and other subdomains in vitellogenins. *Biology of Reproduction* 76: 926–935.

Finn RN and Fyhn HJ (2009) Requirement for amino acids in ontogeny of fish. *Aquaculture Research* 41(5): 684–716 (doi:10.1111/j.1365-2109.2009.02220.x).

Finn RN and Kristoffersen BA (2007) Vertebrate vitellogenin gene duplication in relation to the "3R hypothesis": Correlation to the pelagic egg and the oceanic radiation of teleosts. *PLoS ONE* 2(1): e169 (doi:10.1371/journal.pone.0000169).

Grier HJ, Uribe Aranzábal MC, and Patiño R (2009) The ovary, folliculogenesis, and oogenesis in teleosts. In: Jamieson BGM (ed.) *Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes)*, pp. 26–84. Enfield, NH: Science Publishers.

Hiramatsu N, Chapman RW, Lindzey JK, Haynes MR, and Sullivan CV (2004) Molecular characterization and expression of vitellogenin receptor from white perch (*Morone americana*). *Biology of Reproduction* 70: 1720–1730.

Hiramatsu N, Cheek AO, Sullivan CV, Matsubara T, and Hara A (2005) Vitellogenesis and endocrine disruption. In: Mommsen TP and Moon TW (eds.) *Biochemistry and Molecular Biology of Fishes,* vol. 6, pp. 431–471. Amsterdam: Elsevier.

Hiramatsu N, Matsubara T, Fujita T, Sullivan CV, and Hara A (2006) Multiple piscine vitellogenins: Biomarkers of fish exposure to estrogenic endocrine disruptors in aquatic environments. *Marine Biology* 149: 35–47.

Li A, Sadasivam M, and Ding JL (2003) Receptor–ligand interaction between vitellogenin receptor (VtgR) and vitellogenin (Vtg), implications on low density lipoprotein receptor and apolipoprotein B/E. The first three ligand-binding repeats of VtgR interact with the amino-terminal region of Vtg. *Journal of Biological Chemistry* 278: 2799–2806.

Lubzens E, Young G, Bobe J, and Cerdà J (2010) Oogenesis in teleosts: How fish eggs are formed. *General and Comparative Endocrinology* 165(3): 367–389.

Matsubara T, Ohkubo N, Andoh T, Sullivan CV, and Hara A (1999) Two forms of vitellogenin, yielding two distinct lipovitellins, play different roles during oocyte maturation and early development of barfin

flounder, *Verasper moseri*, a marine teleost that spawns pelagic eggs. *Developmental Biology* 213: 18–32.
Mommsen TP and Korsgaard B (2008) Vitellogenesis. In: Rocha MJ, Arukwe A, and Kapoor BG (eds.) *Fish Reproduction*, pp. 113–169. Enfield, NH: Science Publishers.
Patiño R and Sullivan CV (2002) Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiology and Biochemistry* 26: 57–70.
Reading BJ, Hirmatsu N, Sullivan CV (in press) Disparate binding of three types of vitellogenin to multiple types of vitellogenin receptor in white perch. *Biology of Reproduction*.
Reading BJ, Hiramatsu N, Sawaguchi S, *et al.* (2009) Conserved and variant molecular and functional features of multiple egg yolk precursor proteins (vitellogenins) in white perch (*Morone americana*) and other teleosts. *Marine Biotechnology* 11: 169–187.
Selman K and Wallace RA (1989) Cellular aspects of oocyte growth in teleosts. *Zoological Science* 6: 211–231.
Sullivan CV, Hiramatsu N, Kennedy AM, *et al.* (2003) Induced maturation and spawning: Opportunities and applications for research on oogenesis. *Fish Physiology and Biochemistry* 28: 481–486.
Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries* 6: 259–286.

BEHAVIOR AND PHYSIOLOGY

Linking Fish Behavior and Physiology: An Introduction

KA Sloman, University of the West of Scotland, Paisley, Scotland UK

© 2011 Elsevier Inc. All rights reserved.

Sensory Systems, Perception, and Learning
Interactions with the Environment
Social and Reproductive Behaviors
Further Reading

Glossary

Animal behavior Any action or reaction of an animal in the field or in a laboratory setting.

Ethology The study of animal behavior derived from the Greek word *ethos* meaning character. Ethologists study animal behavior.

The award of the Nobel prize for physiology or medicine in 1973 to three ethologists, Karl von Frisch, Konrad Lorenz, and Nikolaas Tinbergen, marked a turning point in awareness of the fundamental links between organism behavior and physiology. Although physiologists have long recognized the importance of behavioral factors in understanding the way organisms respond to their environment, traditionally physiology and behavior have been studied as two separate fields. We now find ourselves in an era of multidisciplinary research supporting an increasing trend toward combining behavioral and physiological approaches. In particular, the vast diversity of fish species combined with the myriad of life histories that they display make fishes excellent models for comparative studies linking behavior and physiology.

This section of the encyclopedia is particularly diverse, reflecting the enormity of the interface between behavior and physiology in fish. In general, the section can be divided into three areas where current research is investigating behavior/physiology interrelations. These are sensory systems, perception, and learning; interactions with the environment; and social and reproductive behaviors.

Sensory Systems, Perception, and Learning

Fishes obtain information about their surrounding environment through their sensory systems. Some fish senses, such as hearing, vision, taste, and smell, are very similar to that in humans, while others, such as electrocommunication, are completely alien. These senses, sound (**see also Sensory Systems, Perception, and Learning**: How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling and **Hearing and Lateral Line**: Acoustic Behavior), vision (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals and **Vision**: Behavioral Assessment of the Visual Capabilities of Fish), taste, smell (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior), and electrocommunication (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish), among others, play vital roles in fish behaviors, from aggression toward predators, competition with conspecifics, to attracting and courting potential mates. Like humans, fish are also known to enter the behaviorally quiet state of sleep where sensory systems are not easily aroused by mild environmental stimuli (**see also Behavioral Responses to the Environment**: Sleep in Fish).

Central to the use of sensory information is the ability to interpret and process information received from the sensory systems. For example, if a fish can visually identify a competitor with which it has previously lost a social encounter, then, being able to remember this information may allow avoidance of any subsequent encounters with the same individual (**see also Sensory Systems, Perception, and Learning**: Fish Learning and Memory). Losing aggressive encounters can be very costly to a fish and while the ability of fish to perceive pain in the same way that we do remains a widely debated topic (**see also Sensory Systems, Perception, and Learning**: Nociception or Pain in Fish), remembering and consequently avoiding unpleasant experiences can aid survival. Integration of sensory information gained about the surrounding environment is also vital for formation of spatial maps that fish can use to orientate and navigate through their environment (**see also Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?).

Interactions with the Environment

Fishes have evolved many behavioral and physiological strategies ensuring that they acquire sufficient food from an environment, without becoming food themselves (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). Nevertheless, many fishes will experience periods of food deprivation with subsequent periods of compensatory growth having profound behavioral and physiological effects (**see also Behavioral Responses to the Environment**: Effects of Compensatory Growth on Fish Behavior). For those fish whose life-history requirements cannot be met by a single habitat, migration to alternative environments for reproduction or feeding may be essential (**see also Fish Migrations**: The Biology of Fish Migration).

Fish survival, like human survival, also depends on a supply of oxygen, which in many aquatic environments can become restricted on a regular basis. Fish that inhabit these environments display physiological and behavioral survival strategies (**see also Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia). Along with levels of dissolved oxygen, temperature can have profound effects on the way fish behave (**see also Behavioral Responses to the Environment**: Temperature Preference: Behavioral Responses to Temperature in Fishes). Parasites, such as trematodes and cestodes, have the ability to manipulate both the behavior and physiology of their fish hosts (**see also Behavioral Responses to the Environment**: Manipulation of Fish Phenotype by Parasites), and the fragile link that exists between fish behavior and physiology is vulnerable to anthropogenic disturbance as well (**see also Behavioral Responses to the Environment**: Anthropogenic Influences on Fish Behavior). For example, release of estrogenic endocrine disruptors into aquatic environments is now well known to cause alterations in the sexual behavior and physiology of male fish, having a negative impact on reproductive success and population health.

Social and Reproductive Behaviors

The majority of fishes reproduce sexually, where effective transmission of genes to the next generation requires finding a suitable mate. In many instances, there may not be a sufficient number of attractive mates within a habitat and competition over resources will lead to the formation of social structures (**see also Social and Reproductive Behaviors**: Dominance Behaviors). Many fishes use elaborate courtship displays to attract mates (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish) and/or release attractive smells (pheromones) into the water (**see also Hormones in Communication**: Hormonal Pheromones). Reproductive strategies among fishes are amazingly diverse (**see also Reproduction**: The Diversity of Fish Reproduction: An Introduction); some species forming mating pairs between individuals that matured as either a male or female, while in other species individuals have the capacity to change from being male to female in a matter of seconds (**see also Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes). Parental care is seen in a variety of fishes (**see also Social and Reproductive Behaviors**: Parental Care in Fishes) with some species providing their offspring with nutritional supplements during the pre- or post-hatch/birth period to enhance offspring survival (**see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care).

In conclusion, upon reading articles in this section of the encyclopedia on the links between fish behavior and physiology, it is hoped that the reader will be inspired to contribute to the ever-increasing body of literature that treats fish behavior and physiology as entities that are inextricably linked.

See also: **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food; Anthropogenic Influences on Fish Behavior; Behavioral Responses to Hypoxia; Effects of Compensatory Growth on Fish Behavior; Manipulation of Fish Phenotype by Parasites; Sleep in Fish; Temperature Preference: Behavioral Responses to Temperature in Fishes. **Fish Migrations**: The Biology of Fish Migration. **Hearing and Lateral Line**: Acoustic Behavior. **Hormones in Communication**: Hormonal Pheromones. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals; Fish Learning and Memory; How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling; Nociception or Pain in Fish; Shocking Comments: Electrocommunication in Teleost Fish; Spatial Orientation: How do Fish Find their Way Around?. **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior. **Social and Reproductive Behaviors**: Dominance Behaviors; Nutritional Provision During Parental Care; Parental Care in Fishes; Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes. **Vision**: Behavioral Assessment of the Visual Capabilities of Fish.

Further Reading

Gilmour KM, Wilson RW, and Sloman KA (2005) The integration of behavior into comparative physiology. *Physiological and Biochemical Zoology* 78: 669–678.

Reebs S (2001) *Fish Behavior in the Aquarium and in the Wild*. London: Cornell University Press.

Sloman KA, Wilson RW, and Balshine S (eds.) *Behavior and Physiology of Fish*, 1st edn., vol. 24, pp. 151–196. San Diego, CA: Elsevier.

BEHAVIOR AND PHYSIOLOGY

Social and Reproductive Behaviors

Contents

Dominance Behaviors
Sexual Behavior in Fish
Socially Controlled Sex Change in Fishes
Parental Care in Fishes
Nutritional Provision During Parental Care

Dominance Behaviors

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Dominance Hierarchies
How Is Dominance Measured?
Causes of Dominance
Physiological Consequences of Social Status
Why Study Dominance?
Further Reading

Glossary

Dominance The state of being dominant where dominant individuals obtain a high status within a social group.

Dominance hierarchy A social situation where one individual dominates all those below it, then the individual directly beneath it in social status dominates all those below it and so on (i.e., a pecking order). In a linear hierarchy, this relationship continues down to an individual dominated by all. Some social groups may have more complex dominance hierarchies that form networks rather than following a continuous linear relationship.

Hypothalamus Region at the floor of the forebrain (diencephalon) that controls several vegetative physiological processes, including food intake and secretion of hormones from the pituitary gland.

Interrenal cells These cells are found in the head kidney of teleost fish that synthesize and release cortisol.

Neuropeptide Any member of a class of protein-like molecules released by the nervous system. They consist of short chains of amino acids, functioning as neurotransmitters (direct synaptic effects) or hormones (indirect modulatory effects).

Neurotransmitters The signaling molecules released by nerves. This is a specialized form of paracrine signaling.

Pituitary An area of the brain involved with the release of certain hormones, including adrenocorticotropic hormone.

Preprohormone A precursor protein to a prohormone that is, in turn, a precursor of a peptide hormone.

Sclera The white of the eye.

Smoltification A series of physiological and morphological changes a fish may undergo when migrating from freshwater to seawater.

Social status Social standing or rank.

Dominance Hierarchies

To survive, an individual fish must, among other things, find food and avoid being eaten. To pass on its genes to future generations it must also find a mate and reproduce. However, in many instances, food supply may be limited, there may not be sufficient shelter within a habitat to support all individuals, and the number of available attractive mates may be restricted. Therefore, individuals may be left with no option but to compete among themselves for access to resources. Fighting can be costly to an individual. Minimally, it can be energetically expensive. It can result in serious or fatal injuries, and weakened individuals might be more prone to predators. Particularly, aggressive encounters also can distract an individual's attention away from any potential predators lurking nearby. Given the risks associated with fighting over resources, it is not surprising that many species form stable social structures, where once social status is established, direct competition is greatly reduced. In stable hierarchies, individual fish are likely to maintain their social position for long periods, although this will depend on stable environmental conditions and the absence of changes in population density.

The concept of dominance hierarchies was first developed through the observation of a 'pecking order' in chickens. Initial competition between pairs of individuals within a population results in winners and losers; winners of contests are termed 'dominant individuals' and losers as the 'subordinates'. A dominance hierarchy is then formed as a result of successive paired encounters within a group of animals. Dominance hierarchies have now been documented within many species of fish and described in particular detail for salmonids (e.g., salmon and trout). Juvenile salmonid fish often inhabit streams and rivers where water currents supply drifting food items and individuals will tend to orientate themselves in the water current accordingly. A section of stream may be inhabited by several individuals who will compete with each other for the most profitable position in the stream in terms of catching prey and providing access to shelter. In these instances, linear dominance hierarchies may form along the stream, with the most dominant fish upstream of its subordinate counterparts (**Figure 1**).

Many tropical fish form dominance hierarchies in which only the most dominant fish reproduces. For example, linear dominance hierarchies can be seen among individual clown fish (*Amphiprion percula*) co-inhabiting an anemone. Within an anemone, there will generally be one sexually mature male and female pair, which collectively dominates that anemone. Also living within the anemone will be a hierarchy of several more subordinate males that have no reproductive access to the female. When one of the breeding pair is eaten or dies, there is a progression up the hierarchy and the most dominant of the remaining males will take the breeding position. Of course, in the case of the clown fish, to replace the lost breeding female requires that one of the subordinate males within the group must change sex (**see also Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes). A similar situation is observed in the freshwater African cichlid, *Neolamprologus pulcher*, which live in groups consisting of a breeding pair and several subordinate helpers who assist the breeding pair in caring for their young. Once one of the breeding pair is lost, the most dominant helper will take its place.

Dominance hierarchies are not only seen among groups of fish in the natural environment but also seen in groups of many fish species confined together in a laboratory environment and under aquaculture conditions. Here, aggressive interactions and unequal distribution of food among individuals is disadvantageous, resulting in mortalities, injuries (such as fin damage), and a reduction in fish quality. Therefore, much research has focused on reducing the formation of social hierarchies within aquaculture conditions. Large stocking densities may reduce the likelihood of formation of strong linear hierarchies and if food is distributed randomly rather than from a single, defendable point source, then food consumption is more likely to be equally distributed. Water flow, allowing fish to orientate and swim within a water current, can also reduce aggression. Holding fish of similar size together additionally reduces the opportunity for larger fish to aggressively target smaller fish.

How Is Dominance Measured?

With the vast amount of research that has explored the formation and consequences of dominance hierarchies, it is important to understand how dominance is measured.

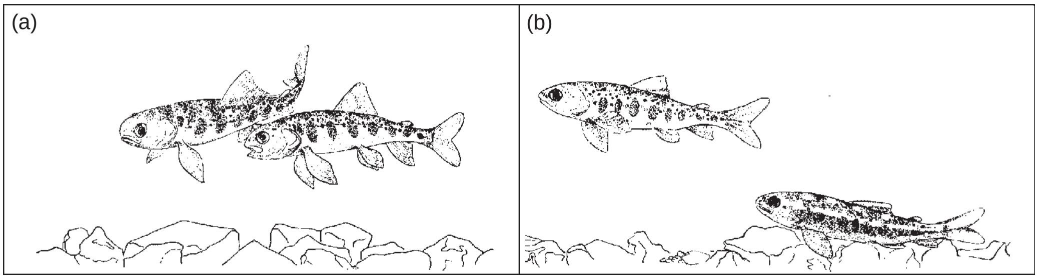


Figure 1 (a) Juvenile salmonid fish compete over limited resources and form dominance hierarchies as a result of pair-wise interactions. (b) Following hierarchy formation, dominant individuals will generally maintain position in the water column, while subordinate individuals take up a submissive posture on the bottom and may turn darker in coloration as a signal of defeat. Reproduced from figures 4 and 8 in Keenleyside MHA and Yamamoto FT (1962) *Territorial Behaviour of Juvenile Atlantic Salmon* (*Salmo salar* L.). The Netherlands: Brill, with permission.

Dominance is relative rather than absolute and in its simplest form can be tested by observing the interaction between two individuals when presented with a limited resource to compete over. The most dominant of the two individuals will be the one that wins the aggressive encounter and gains access to the resource. This test can be extended to investigate dominance hierarchies among groups of fish by allowing them to compete for a resource and by sequentially removing the most dominant individual. Each time the dominant fish is removed, the next most dominant individual will take its place and so on until all the individuals have been assigned a social ranking. This method of identifying the dominance hierarchy among a group of fish is known as the 'serial removal technique'. Dominance hierarchies can also be assessed by observing the interactions between individuals and by noting which individuals win aggressive encounters over others. However, as social hierarchies promote stability and reduce aggressive encounters, this is often easier to observe during the formation of hierarchies rather than after they have stabilized.

Causes of Dominance

The ability of one individual to win a competitive encounter and monopolize a resource at the expense of another individual relies inherently on individual variation in competitive ability. Therefore, what makes some individuals physiologically and behaviorally better at competing than others? Originally, it was believed that size was the main predictor of high dominance status with larger individuals consistently outcompeting smaller individuals. However, it appears that this only holds true when the size disparity is quite large. For example, in male green swordtails (*Xiphophorus helleri*) size uniquely determines dominance only when asymmetries in size greater than 20–30% occur. Thus, other factors may play more fundamental roles.

So far, the only physiological parameter that has reliably been demonstrated as a predictor of dominance status is metabolic rate. The success of salmonid fry at the outset of competitive interactions with conspecifics is determined based on a higher metabolic rate being correlated with greater competitive ability and dominance status. Whether the individual variation in metabolic rate that is responsible for varying competitive ability is genetically determined is unknown; however, approximately 70 genes have been identified so far that differ significantly between dominant and subordinate fish.

Prior residence of an area is also known to influence the outcome of competitive interactions. If an individual has prior experience of a habitat, then its chances of successfully outcompeting an individual unfamiliar to the area are increased. Under these conditions, an unfamiliar individual is likely to have to adjust to a new environment at the same time as competing with the resident individual, making success less likely. However, dominant individuals under certain environmental conditions may not necessarily prosper if the environment changes; fluctuations in water levels and flows have the potential to disrupt and alter existing social hierarchies. Thus, a physiology that suits one set of environmental conditions does not ensure dominance in another environment. Physiological condition may also affect the competitive ability of an individual. Elevated plasma concentrations of the stress hormone cortisol are known to be associated with a decrease in the competitive nature of individuals. In contrast, aggressive behavior of fish increases with administration of L-dopa, the immediate precursor of the neurotransmitter, dopamine.

A complication in predicting the outcome of dominance contests based on a combination of all the above is the additional ability of fish to learn and adapt their competitive tactics in successive aggressive encounters, that is, based on prior social experiences. In fact, there is positive feedback in this regard. Fish that have lost competitive encounters are more likely to adopt submissive behaviors in subsequent contests and become subordinate. Moreover, the simple observation of competitions between other individuals may influence the way that the observer will subsequently interact with either of these opponents. If the observer is subsequently required to compete with the observed loser, then the previous eavesdropping on its competitor's ability may bring confidence that adopting a dominant/aggressive tactic is likely to be successful in this instance. Alternatively, if the observer is required to compete with the observed winner, then it may decide that adopting a submissive role may be the least costly strategy. Thus, a whole myriad of innate and acquired physiological factors influence the competitive ability of an individual.

Physiological Consequences of Social Status

Growth

Access to premium resources is likely to result in advantages to dominant individuals. One of the most obvious advantages where a food source is monopolized is a higher growth rate compared to more subordinate animals. Many studies have documented lower growth rates in subordinate fish under culture or laboratory conditions. For example, among groups of green sunfish (*Lepomis cyanellus*), growth is directly correlated with social rank. Growth also correlates with dominance among groups of salmonids and among groups of *N. pulcher*. In field studies, however, investigations into the effects of social status on growth are equivocal. In groups of masu salmon

(*Oncorhynchus masu ishikawai*) living in a mountain stream, there was a clear correlation between a high dominant status and higher daily growth increments but in Atlantic salmon (*Salmo salar*) several studies have failed to find a clear relationship between dominance and growth in the natural environment. It is, therefore, likely that the effect of social status on growth is strongly influenced by environmental conditions.

Although short-term changes in growth rates have been documented in relation to position in a social hierarchy, we know very little about prolonged physiological effects of social status. Even so, there is evidence that among juvenile salmonids social status can have a profound effect on life history strategy as a consequence of initial short-term changes in growth. Within the freshwater stream environment, dominant Atlantic salmon grow faster than their subordinate counterparts and are more likely to undergo smoltification (**see also Fish Migrations:** The Biology of Fish Migration) and migrate to sea a year earlier. Subordinate individuals enter a 4–6-month period of anorexia and postpone seaward migration. Not only has this phenomenon been documented in laboratory studies, but there is strong evidence for bimodal size distribution seen in some natural salmon populations. A few male individuals may adopt an alternative reproductive strategy (**see also Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes) and mature in freshwater without migrating to sea. These fish, known as precocious parr, reach sexual maturity at a much smaller size, bypassing the usual increase in size associated with transition from a juvenile to adult stage.

Clown fish are another species in which subordinate individuals are believed to suppress their growth rates. As mentioned above, groups of clownfish living within an anemone form a queue for a breeding position. By maintaining a size smaller than a more dominant individual, subordinate individuals avoid the potential aggression they might attract if they were seen as a competitive threat. The exact mechanism of appetite suppression in fish awaits discovery, but is likely to be under the control of brain neurotransmitters. In the cichlid, *Haplochromis burtoni*, subordinate fish have larger neurons containing the neuropeptide somatostatin, which is known to inhibit the release of growth hormone.

Differences in growth rates among individuals of different social status, particularly under laboratory conditions, clearly do not appear to be solely explained by monopolization of a food resource by dominant individuals. In fact, in studies where fish of different social status are fed equal rations, subordinate fish will still exhibit lower growth rates and be less motivated to feed even when excess food is available. Furthermore, suppression of feeding in subordinate salmonids may be sustained for several days after removal of the dominant and appetite suppression can be linked to a stress-induced anorexia.

The Endocrine System, Brain Neurotransmitters, and Dominance

Stress in teleost fish results in an immediate activation of the sympathetic nervous system and release of the catecholamine hormones epinephrine and norepinephrine from chromaffin cells in the head kidney into the circulation (**see also Hormonal Responses to Stress:** Catecholamines). Catecholamines optimize cardiovascular and respiratory function and release stored energy to allow an individual to respond immediately to stress in the traditional fight-or-flight response. Because catecholamines are released so quickly into the circulation in response to stress, it makes accurate determination of plasma levels of catecholamines very difficult as the usual methods of obtaining a blood sample from a fish are stressful in themselves (e.g., capture, anesthetization, and withdrawal of blood by caudal venipuncture). Therefore, whether the levels of circulating catecholamines differ between dominants and subordinates is currently unknown.

Subsequent to the release of catecholamines under stressful situations is the activation of the hypothalamus–pituitary–interrenal (HPI) axis (**see also Hormonal Control of Metabolism and Ionic Regulation:** Corticosteroids). Here, the hypothalamus releases corticotrophin-releasing factor which causes the pituitary to release adrenocorticotropic hormone (ACTH), resulting in the synthesis and release of the stress hormone cortisol from the interrenal cells of the head kidney. Because of this hormone cascade, cortisol takes longer time to enter the circulation than the catecholamines.

Elevated cortisol concentrations elicit a myriad of physiological changes (**see also Hormonal Responses to Stress:** Stress Effect on Growth and Metabolism). In general, cortisol maintains the release of stored energy by breaking down carbohydrate, lipid, and protein stores to allow an individual to cope with a longer-term stress. If cortisol remains elevated for long periods of time (e.g., chronic elevation over days to weeks), it can often be associated with more deleterious effects, such as immunosuppression and deterioration in brain function.

A considerable number of studies have examined the effects of social status on activation of the HPI axis. During an aggressive encounter, both opponents will experience an increase in plasma cortisol and, for the eventual dominant individual, this increase in cortisol is transient. However, plasma cortisol concentrations are likely to remain chronically elevated for days to weeks in the subordinate individual, resulting in many physiological changes associated with chronic stress (**Figure 2**). Elevated concentrations of circulating cortisol have

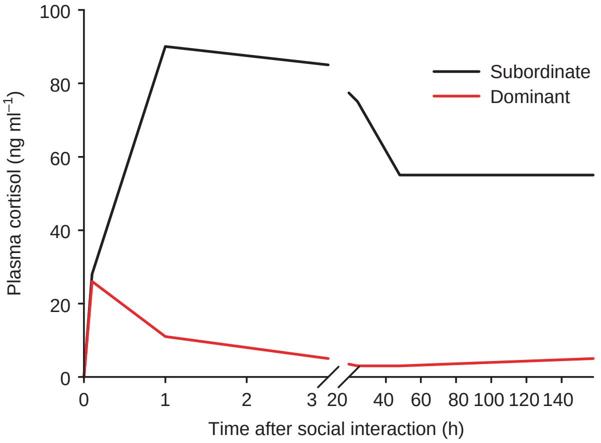


Figure 2 Typical plasma cortisol profile for dominant and subordinate rainbow trout held in pairs and allowed to establish a dominance hierarchy. Both individuals show an initial rise in plasma cortisol. While this elevation is transient in the dominant individual, concentrations of plasma cortisol remain chronically elevated in the subordinate individual.

been measured in the subordinates of many fish species including rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), coho salmon (*Oncorhynchus kisutch*), European eel (*Anguilla anguilla*), Arctic charr (*Salvelinus alpinus*), Gulf toadfish (*Opsanus beta*), and the cichlid (*H. burtoni*). The changes in interrenal-cell diameters that occur in conjunction with this chronic elevation of cortisol are likely to be associated with increased cortisol synthesis.

Elevated cortisol concentrations can also affect the functioning of the HPI axis through hormone feedback loops. Desensitization of interrenal cells to further stimulation by ACTH appears to occur in subordinate rainbow trout as they display a lower rate of cortisol secretion when stimulated *in situ* with ACTH than their dominant counterparts. This would suggest a decreased ability of subordinate animals to respond to further stressors as their endocrine system is already substantially challenged.

Cortisol is closely associated with the serotonergic system and levels of the brain neurotransmitter serotonin. Changes in brain monoamine neurotransmitters during social encounters have been documented in many fish species. These neurotransmitters include serotonin (5-hydroxytryptamine (5-HT)) and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of 5-HT). In particular, substantial activation of the serotonergic system has been associated with subordinate status. In dominant and subordinate salmonids, serotonergic activity (often measured as 5-HIAA/5-HT ratios) is observed to increase during aggressive encounters. As with cortisol, this increase is transient in dominants but persists in subordinates. In the bicolor damselfish (*Pomacentrus partitus*), higher serotonergic activity is seen in nonterritorial males. An antiaggressive effect of brain serotonin has been documented in fish along with a decrease in locomotor ability. Thus, long-term activation of the brain serotonergic system may perpetuate behavioral submission.

Social status may also influence levels of other circulating hormones. In juvenile Arctic charr, subordinate individuals show elevated levels of plasma α-melanocyte-stimulating hormone (α-MSH). The release of α-MSH is controlled by the HPI axis being derived from the same preprohormone, preopiomelanocortin, as ACTH. In fish, α-MSH is known to cause a darkening in skin coloration and a darker color is often observed in subordinate salmonids (**Figure 1**). In Atlantic salmon, overall body coloration and also sclera of the eye is seen to darken in subordinate individuals. It has been noted that immediately following this change in coloration, aggression is reduced between pairs of fish, suggesting that it may act as a signal of defeat.

Social dominance in fish has been associated with high levels of circulating androgens. In teleost fish, 11-ketotestosterone (11-KT) is the predominant androgen (**see also Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction); and in Arctic charr, elevated levels of 11-KT were found in dominant males. Similarly, in *Brienomyrus brachyistius*, a weakly electric fish, dominant males had high levels of 11-KT. In rainbow trout, dominant males have elevated levels of testosterone and another reproductive hormone 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) (**see also Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction), but in male redbelly tilapia (*Tilapia zilli*), elevated levels of androgens were absent in dominant males. In male swordtails, living in stable social communities, there was no relationship between aggressive behavior and androgens. However, when instability occurred, aggression was androgen related. Therefore, although there is the potential for dominance to be associated with elevated androgens, this is likely to be both context- and species-specific.

Metabolism and Disease

Chronic elevation of stress hormones will eventually become disadvantageous. During stressful encounters, energy stores are mobilized and energy is diverted toward fuelling the stress response and away from basic needs such as growth and maintenance. There is evidence that social position can compromise ionoregulatory ability. Subordinate freshwater salmonids leak sodium ions across both the kidney and gills at a faster rate than dominants and, as a result, require higher rates of sodium uptake from the water to maintain their osmotic balance. More energy may, therefore, be directed to ionoregulation in subordinate individuals.

Not surprisingly, chronic stress is also associated with a compromise in immune response and a decreased resistance to disease. Under captive conditions, competitive interactions among European eels result in increased levels of plasma cortisol, glucose, and lactate along with decreased hepatic glycogen stores and lower numbers of leukocytes in subordinate individuals. Subordinate salmonids are more susceptible to a wide range of diseases; subordinate rainbow trout exposed to the opportunistic ubiquitous bacterium, *Aeromonas hydrophila*, were 50% more susceptible to infection.

Susceptibility to Aquatic Toxicants

Because of the wide variety in physiological profiles of individuals of different social status, it is not surprising that social position has an effect on the susceptibility of individuals to aquatic toxicants. In bluegills (*Lepomis macrochirus*), subordinates were found to be more susceptible to a lethal concentration of zinc, dying significantly earlier than their dominant counterparts. In this study, addition of a shelter increased the survival of subordinates suggesting that refuge from the aggressive attention of a dominant reduces stress and susceptibility to a contaminant.

Differential accumulation of metals can occur in fish of different social status (**see also Behavioral Responses to the Environment:** Anthropogenic Influences on Fish Behavior). For example, when salmonid hierarchies are exposed to waterborne copper, the subordinates will take up more copper from water than the dominants. A similar pattern is observed when hierarchies are exposed to waterborne silver. Both copper and silver ions are taken up across the gills, and, in salmonid fish, it is believed that this process occurs through sodium channels. Therefore, it is possible that the higher uptake of sodium seen in subordinates as a result of their social status concomitantly drives a higher uptake of copper and silver. Similar patterns of uptake are not observed with metals such as cadmium and lead which do not cross the gills via sodium channels.

In contrast to the increased susceptibility of subordinates to some waterborne toxicants, dietary toxicants may be more problematic for dominant fish. Monopolization of a food resource can become deleterious if it is contaminated by a toxic substance. Among groups of fish fed a copper-contaminated diet, dominant fish are likely to experience toxic effects earlier than more subordinate fish and, consequently, lose their ability to monopolize a food source. The next most dominant fish will out-compete the dominant individual and gain greater access to the food source but will eventually suffer a similar fate. Dietary contaminants are, therefore, likely to destabilize hierarchies and the impacts of dietary toxicants on social hierarchies warrants further study.

Why Study Dominance?

Dominance hierarchies play an integral role in the structure of many fish populations and will also form under artificial conditions in the laboratory and in aquaculture. Understanding how behavior and physiology interact during social encounters is important if we are to understand how populations will respond to environmental change and to predict welfare implications of hierarchy formation in captivity. We still know very little about the potential long-term consequences of social stress and the majority of techniques used for quantifying social stress remains invasive. Developing noninvasive assays for stress and techniques that can be readily applied to field situations is a priority for furthering our understanding of dominance hierarchies in natural fish populations.

See also: **Behavioral Responses to the Environment:** Anthropogenic Influences on Fish Behavior. **Fish Migrations:** The Biology of Fish Migration. **Hormonal Control of Metabolism and Ionic Regulation:** Corticosteroids. **Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress:** Catecholamines; Stress Effect on Growth and Metabolism. **Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes.

Further Reading

Beaugrand JP, Payette D, and Goulet C (1996) Conflict outcome in male green swordtail fish dyads (*Xiphophorus helleri*): Interactions of body size, prior dominance/subordination experience, and prior residency. *Behaviour* 133: 303–319.

Buchner AS, Sloman KA, and Balshine S (2004) The physiological effects of social status in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Journal of Fish Biology* 65: 1080–1095.

Buston P (2004) Territory inheritance in clownfish. *Proceedings of the Royal Society of London B* 271(supplement 4): S252–S254.

Cardwell JR, Sorensen PW, Van der Kraak GJ, and Liley NR (1996) Effect of dominance status on sex hormone levels in laboratory and wild-spawning male trout. *General and Comparative Endocrinology* 101: 333–341.

Carlson BA, Hoplons CD, and Thomas P (2000) Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish. *Hormones and Behaviour* 38: 177–186.

Cutts CJ, Metcalfe NB, and Taylor AC (1998) Aggression and growth depression in juvenile Atlantic salmon: The consequences of individual variation in standard metabolic rate. *Journal of Fish Biology* 52: 1026–1037.

Ejike C and Schreck CB (1980) Stress and social hierarchy rank in coho salmon. *Transactions of the American Fisheries Society* 109: 423–426.

Heggenes J and Metcalfe NB (1991) Bimodel size distributions in wild juvenile Atlantic salmon populations and their relationship with age at smolt migration. *Journal of Fish Biology* 39: 905–907.

Johnsson JI, Winberg S, and Sloman KA (2006) Social interactions. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, 1st edn., vol. 24, pp. 151–196. San Diego, CA: Elsevier.

Metcalfe NB, Taylor AC, and Thorpe JE (1995) Metabolic rate, social status and life-history strategies in Atlantic salmon. *Proceedings of the Royal Society of London B* 236: 7–19.

Nakano S (1995) Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *Journal of Animal Ecology* 64: 75–84.

O'Connor KI, Metcalfe NB, and Taylor AC (1999) Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? *Animal Behaviour* 58: 1269–1276.

Oliveira RF, McGregor PK, and Latruffe C (1998) Know thine enemy: Fighting fish gather information from observing conspecific interactions. *Proceedings of the Royal Society of London B* 265: 1045–1049.

Øverli Ø, Harris CA, and Winberg S (1999) Short-term effects of fights for social dominance and the establishment of dominant–subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain, Behaviour and Evolution* 54: 263–275.

Peters G, Delventhal H, and Klinger H (1980) Physiological and morphological effects of social stress in the eel (*Anguilla anguilla* L.). *Archives Fischereiwissenschaft* 307: 157–180.

Sloman KA (2007) Effects of trace metals on salmonid fish: The role of social hierarchies. *Applied Animal Behaviour Science* 104: 326–345.

Sloman KA and Armstrong JD (2002) Physiological effects of dominance hierarchies: Laboratory artifacts or natural phenomena? *Journal of Fish Biology* 61: 1–23.

Sloman KA, Metcalfe NB, Taylor AC, and Gilmour KM (2001) Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. *Physiological and Biochemical Zoology* 74: 383–389.

Sloman KA, Montpetit CJ, and Gilmour KM (2002) Modulation of catecholamine release and cortisol secretion by social interactions in the rainbow trout *Oncorhynchus mykiss*. *General and Comparative Endocrinology* 127: 136–146.

Sloman KA, Morgan TP, McDonald DG, and Wood CM (2003) Socially induced changes in sodium regulation affect the uptake of waterborne copper and silver in the rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology C* 135: 393–403.

Winberg S and Nilsson GE (1992) Induction of social dominance by L-DOPA treatment in Arctic charr. *NeuroReport* 3: 243–246.

Sexual Behavior in Fish

I Katsiadaki and M Sebire, Cefas Weymouth Laboratory, Weymouth, UK

Crown Copyright © 2011 Published by Elsevier Inc. All rights reserved.

Overview of Sexual Behaviors in Fish
Hormonal Control of Sexual Behavior
Conclusions
Further Reading

Glossary

Agonist/antagonist An agonist produces an action. It is the opposite of an antagonist, which acts against and blocks an action.

Androgen Any natural or synthetic steroid hormone that is principally involved in growth, development, and maintenance of the male reproductive system and secondary sexual characteristics.

Bower A construction usually made by the male bowerbird for courtship display (i.e., not as a nest); the term may also be used to describe a sandcastle structure made by male cichlids that is used to attract females.

Estrogen Any natural or synthetic steroid hormone that controls female sexual development, secondary sexual characteristics, and stimulates egg production. Together with progesterone (another steroid hormone), it regulates the development and function of sex organs.

Hormone A chemical substance, often a peptide or steroid, released from endocrine glands that controls and regulates a range of physiological activities.

Neuropeptide Any member of a class of protein-like molecules released by the nervous system. They consist of short chains of amino acids, functioning as neurotransmitters (direct synaptic effects) or hormones (indirect modulatory effects).

Oviposition The act of laying eggs by oviparous animals (animals laying eggs that develop outside the female's body).

Prostaglandin Any member of a group of lipid compounds that are 20-carbon fatty acid derivatives containing a 5-carbon ring. They mediate a wide range of physiological functions, such as, in fish, ovulation (follicular rupture), and female sexual behavior, and also act as pheromones in some species.

Sex steroids (or gonadal steroid) Steroid hormones that are produced and secreted by the gonads (testes and ovaries). By interacting with androgen and estrogen receptors, they control reproductive development and functions and influence behavioral patterns (sexual and others).

Sympatric Organisms occupying the same or overlapping geographic areas without interbreeding.

Vitellogenesis Synthesis of the yolk protein vitellogenin, the precursor and main component of egg yolk protein, vitellin, which is synthesized in the liver, transported in the blood stream, and incorporated into oocytes providing energy reserves for the development of embryos.

Overview of Sexual Behaviors in Fish

A series of behaviors consisting of several specific acts are performed by teleost fish during the process of mating. A variable terminology is used for the description of these acts depending on the pattern of movement (e.g., courtship, chasing, clasping, mating, spawning, egg release, sperm release, oviposition, and ejaculation). Collectively, these acts are called 'sexual behavior' or 'reproductive behavior' while 'spawning behavior' is a commonly used term reserved for teleosts whose gametes are fertilized in the external environment. It is often quite difficult to clearly define sexual behavior in fish largely due to the diversity of behavior patterns observed (**see also Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes).

Some behaviors, usually performed after gamete fusion, can be classified as reproductive behaviors in a broader sense but are usually not referred to as a sexual behavior. For example, parental care is not generally classed as sexual behavior. In fish that demonstrate some sort of parental behavior (one or both sexes), two strategies can be discerned: building of a physical barrier for protecting the eggs (guarders) or morphological/physiological modifications to carry the eggs/young (bearers) (**see also Social and Reproductive Behaviors:** Parental Care in Fishes). Here, we consider pre-spawning nest-building and territorial behaviors as sexual behavior, important for successful spawning to take place. For some species, migration and preparation of a breeding site constitute the first phase of sexual behavior (e.g., salmonids; (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle)).

Construction (Nest-Building) Behaviors

The main function of a nest is to protect eggs from predators and adverse environmental conditions. Nest spawners show a great diversity in their building capacity, ranging from simple depressions or cavities in the sediment to hugely complex structures. Nest structures, however, may lead to secondary problems, such as low oxygen, that are overcome by further adaptations in behavior (e.g., fanning) or/and in the complexity of the structure. Some fish establish a territory around a nest or spawning site and defend it vigorously against other conspecifics such as the Mozambique tilapia, *Oreochromis mossambicus*, the bowfin, *Amia calva*, the three-spine stickleback, *Gasterosteus aculeatus*, and the Siamese fighting fish, *Betta splendens*. Fish that guard spawning sites tend to produce fewer numbers of eggs than those that do not (**see also Social and Reproductive Behaviors:** Parental Care in Fishes).

Most sunfish species, such as pumpkinseed *Lepomis gibbosus*, reproduce in shallow waters of slow rivers or lagoons and are not particularly choosy with respect to substrate type. Males construct a nest by sweeping the substrate, using their caudal fins much like brooms until they have made a clean, circular, slightly depressed, saucer-like area. Sunfish are usually colonial nesters, with nests in densely packed clusters. Many nests are so close that they touch each other, although males vigorously defend their individual nesting site. Another sweeping species is the bowfin, which prepares a nest in shallow, often poorly oxygenated water, by clearing a site from vegetation roots and creating a slight depression for the eggs to be deposited. The male also defends his nest very aggressively.

Some cyprinids, gobies, cichlids, tilefish, and wrasses are specialized in mound building. Some mounds can be quite impressive in comparison to the size of the fish. The male hornyhead chub, *Nocomis biguttatus,* achieves a total length of 90 mm and yet digs a depression up to 0.5 m in diameter and piles pebbles into the cavity to a height of 15 cm. After a female lays her eggs on the top of the mound, the male covers them with more pebbles.

Most anabantoids (labyrinth fish), for example, the gouramis (e.g., Siamese fighting fish) and species in the Callichthyiadae family (armored catfish), build characteristic bubble nests, an adaptation to an oxygen-depleted environment (**see also Behavioral Responses to the Environment:** Behavioral Responses to Hypoxia). The male blows air bubbles, coated with mucus through the gills, either at the water surface or under a leaf. The male incorporates the eggs, released by the females, into the mass of bubbles and guards them.

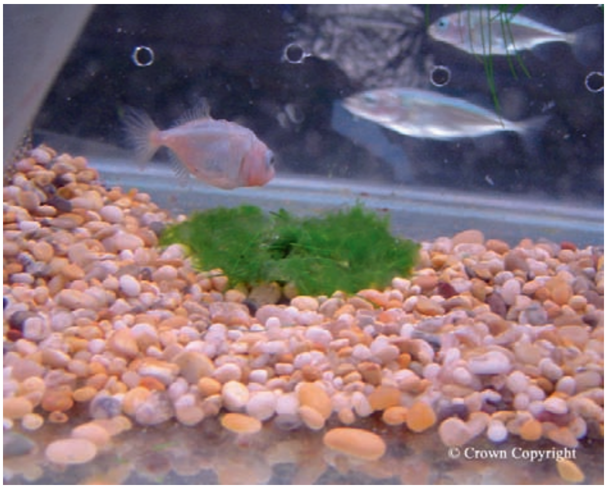


Figure 1 A male three-spine stickleback, *Gasterosteus aculeatus*, guarding his nest (an entrance is visible). Crown Copyright.

Finally, nests can be more elaborate in structure, such as an assemblage of pieces of vegetation. The most renowned example of such construction can be seen in the male three-spine stickleback (**Figure 1**). Once the male has established a territory, he tests several areas on the bottom by taking mouthfuls of sand and quickly ejecting them. Eventually he focuses on digging in one area, carrying the sand further away before spitting it out. This results in the formation of a pit where the nest will be built. The male collects nest material, for example, waterweed, and glues the pieces together with a mucus secretion of the kidney, a protein called spiggin from the Swedish name for stickleback. When a male performs the gluing behavior, he swims slowly over the nest with juddering movements of the pectoral and anal fins that result in both his head and his tail being raised while the cloacal region is pressed against the nest material to release spiggin (**Video Clip 1**). In the final stage, the last pieces of vegetation are glued together to form the nest entrance and the male performs a creeping behavior, swimming through the nest to form a tunnel and a more distinct entrance. After the female has spawned, the male takes care of the eggs alone by mainly fanning water currents through the nest for oxygenation.

Egg protection may not necessarily be the primary intention for nest construction. Some fish build houses predominantly for protecting themselves from predators; this is used secondarily for laying eggs, for example, mudskippers and the mouth-brooding jawfish (Opistognathidae). Mouth-brooding cichlids construct sand-scape bowers that they use mainly as a mating display and as egg-fertilization sites, but not for the protection of the young. Bower forms among different species range from huge craters 3 m in diameter (e.g., *Oreochromis* sp.) to small depressions of a few centimeters (e.g., *Copadichromis azureus*) and small sandcastles (e.g., *Lethrinops* sp.).

Fish that do not guard nests can also demonstrate spawning-site preparation. In these species, no parental care is provided and usually it is the female that displays site-preparation behavior. Female fish are generally considered nest diggers, preferring to dig down rather than build up a nest. After migration, female masu salmon, *Oncorhynchus masou*, use their tail to excavate gravel to form a nest or spawning bed, checking regularly the shape (depth and width) with their anal fins. Other female fish behave similarly; the smallmouth bass, *Micropterus dolomieui*, digs a nest up to 185 cm in diameter and both male and female lampreys excavate pits in streambeds using their mouths and tails. Shortly after high tide, on certain nights of the year, female California grunions, *Leuresthes tenuis*, swim as far as possible up onto a sandy beach using breaking waves and with their tails they drill their body into the sand up to their pectoral fins. They then wait for males to make their way up the beach and fertilize their eggs!

Courtship Behaviors

Courtship is, by definition, any communication behavior that occurs in order to initiate mating, and often includes elaborate displays. In fish, males usually display, while the role of females is mainly selective. In general, males prefer large females to small ones, a trait that is adaptive since brood size increases with female size. The state of arousal of a courting male is often (but not always) reflected in colored skin patches, the color intensity of which is under neural control. Together, female size (acting as the external stimulus) and internal arousal (signifying reproductive maturity) combine to determine the courtship acts of the male. Nuptial coloration patterns are very useful in the study of the motivation of courtship and can be examined in many species.

Expression of courtship behavior requires the occurrence of a number of signals in the following order: (1) visual recognition of the sexual status of the partner; (2) the expression of a state of sexual maturity by the male (male readiness); and (3) the willingness of the female to mate (female readiness).

Visual recognition (sexual dimorphism)

The more striking aspects of external appearance in fish are often associated with breeding. In most species, sexual characteristics are male specific and the male undergoes the most striking changes at sexual maturity. Alterations in female coloration are less common in fish. Some morphological changes are temporary, for example, nuptial coloration in the blenny and tubercles in the fathead minnow; others are permanent after sexual maturity is attained, for example, gonopodium in the guppy. The magnitude of sexual dimorphism is usually related to the mating system.

Sexual dimorphism in body size is known in a number of fish, for example, sunfish, blennies, sculpins, cyprinids, sticklebacks, gobies, labrids, guppies, salmonids, and an extreme example, anglerfish – for example, *Photocorynus spiniceps,* where the male measures less than a centimeter and lives as a parasite on a female, which can be up to five times bigger in size.

Sexual dimorphism in nuptial coloration can also be seen in a number of fish such as in cyprinids, cichlids, sticklebacks, greenlings, guppies, mollies, platies, and swordtails. Several males, such as the guppy, develop bright spots or bars on body and fins, as visual signals during the courtship display (**see also The Skin:** Coloration and Chromatophores in Fishes). There is a remarkable polymorphism of guppy color pattern with a great variation among individuals.

A special behavioral pattern occurs in the mouthbrooding cichlids *Haplochromis burtoni* and *Copadichromis virginalis*. The male develops egg-like spots on his anal fin. A receptive female lays her eggs in several batches within a site and collects them in her mouth. The male drags his egg-marked anal fin in front of her trying to tempt her to pick up the missed eggs; instead, she picks up his sperm and fertilizes the eggs in her mouth. Interestingly, the representation of the egg row on the male's fin is an exact one to the human eye.

Male readiness

A large range of courtship displays (from simple stimuli to a complex series of acts) exists among male teleost fish exhibiting both internal and external fertilization. Courtship behavior is commonly composed of a repetitive or rhythmic pattern in movement. These dance-like movements are often followed up by the male fish poking or butting the female in the flanks (Poeciliidae, Gasterosteidae, and genus *Phoxinus*), and in some species the male eventually curls himself around the body of the female (*Macropodus* sp., *Barbus* sp.).

The characteristic courtship behavior of the male guppy is the 'sigmoid' display, termed after the S-shape of the male's body when it is performed. The male guppy waits for a female to stop moving or slow down sufficiently for him to jump in front of her. When he performs the sigmoid display, he arches his body and quivers stiffly. Copulation may occur but the male could suddenly jump away from the female to test her responsiveness or/and to lead her away from a current position, especially away from a crowded male area.

The male three-spine stickleback is one of the best-studied examples of mating behavior. The courtship is composed of distinctive synchronized elements, which ensure that the male only mates with the females of his own species. The male first jumps away from and then toward the female with his mouth open and spines erect, performing the zigzag dance (**Video Clip 2**). If the female responds, the male interacts with her by a wide repertoire of moves including biting and dorsal pricking (where the male is below and across the female belly, pushing her with jerky movements with his erect spines toward the water surface). He then leads her to the entrance of the nest pushing his snout into and back from the nest to show the female. There are many variations in this courtship behavior both between individuals and populations. Pacific Ocean male three-spine sticklebacks perform the zigzag dance, while sympatric males of the Japan Sea perform a different dance, which is more like rolling. This different pattern of courting evolved over several thousand years of geographical isolation, and, while the two stickleback morphs now coexist, they do not interbreed. This difference in courtship behavior may have resulted in reproductive isolation; hence, courtship behavior is important for the ethological isolation of closely related species.

Sound (**see also Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling), olfaction (**see also Hormones in Communication:** Hormonal Pheromones), and electrocommunication (**see also Sensory Systems, Perception, and Learning:** Shocking Comments: Electrocommunication in Teleost Fish) may also be used during courtship behavior and are covered in more detail in other articles.

Female readiness

The female display of sexual receptivity is often less striking than that of the male, but maintains continuity through the female staying near the spawning ground or nesting site. It is important to emphasize that usually the females get to choose their mates; hence, there is not much point in developing attractive traits themselves. The logic behind this statement is simple. Males produce an enormous number of small cells (sperm) without investing too much energy, whereas females produce a few large and nutritious eggs that are very costly to them. If a male mates with several females, he increases the number of his progeny, but for females, it makes no difference. A female gains only a little from having a large number of egg fertilizations with different males. Therefore, males in general are promiscuous but females are choosy because they want their few progeny to be of highest possible quality.

The criteria for selecting a male mate vary enormously in the fish world and may include color, size, nest quality, aggressiveness, ability to win fights, relatedness (some species avoid inbreeding, others do not) and resistance to parasites. On choosing a mate, female readiness can manifest in a variety of ways. A sexually responsive female guppy displays a gliding motion, facilitating copulation. The displaying male moves in a circle around the female and attempts to inseminate the female with his gonopodium – this behavior may be accompanied by nips, that is, oral contacts with the female's gonopore. When both fish are successfully in contact, the pair rotates around each other rapidly. After mating, the male jerks his body up and forward several times; the significance of this behavior is not clear. Female guppies may mate with several mates and are able to store sperm so that sperm from different males may compete to fertilize their eggs. In this species, sneak copulation (a gonopodial thrust to an unwilling partner) occurs frequently, although most do not result in successful transfer of sperm.

In sticklebacks, receptive females adopt a characteristic head-up posture that serves as a stimulating clue to the male. When the female is interested in spawning, she will follow the leading male and inspect the nest before entering. If she is satisfied with the male courtship display and the nest quality, she will enter the nest. When inside the nest, the male stimulates her by giving a series of quick pushes on her flanks and caudal peduncle, a behavior that is called quivering (**Video Clip 3**). Finally, as soon as spawning is complete, she exits and moves away.

Hormonal Control of Sexual Behavior

As in other vertebrates, sexual behavior in fish is under hormonal control. For a number of years, hormones were seen as causal agents of behavior, acting directly on the expression of a given behavioral act; this view was supported by a number of studies that demonstrated that castration and hormone-replacement treatments could abolish and restore sexual behaviors, respectively. However, hormones are now considered major modulators of behavior, that is, they can modify the probability of the expression of a behavior but not necessarily the expression itself.

In fish, the actions of hormones in the occurrence of a particular sexual behavior have been classified into three categories. The first is a physiological trigger, which elicits sexual behavior in a relatively short time when other physiological and environmental conditions are appropriate. For example, in female goldfish the hormone prostaglandin (PG) F2α results in oviposition within several minutes of administration. Prostaglandin is produced in the ovary after ovulation (ovulated oocytes in the oviduct are the stimulus for its production) and acts on the brain of females, inducing this specific sexual behavior during natural spawning.

The second category of hormone action is as a requirement or primer; the hormone does not trigger sexual behavior *per se* but its presence is essential for the occurrence of the behavior. Testicular androgens often function in this priming manner. In many male teleosts, castrated males without circulating testicular androgens do not show any signs of an appropriate sexual behavior. Administration of androgens can restore male sexual behavior in castrated males, but merely administering the hormones does not trigger the behavior to occur. Likewise, a priming androgen injection in juvenile males allows male behavior to be triggered by external cues, such as pheromones or other behavioral signals from females.

The third category of hormone action is classed as 'potentiator'; it does not trigger the behavior and sometimes is not even essential for the actual occurrence of the behavior. However, the presence of this particular hormone enhances or increases the effect on the sexual behavior of the fish. Gonadotropin-releasing hormone (GnRH) is well known as a potentiator hormone in female goldfish. Without administration of GnRH, sexual behavior of female goldfish can still be triggered by PGF2α administration. However, PGF2α-induced spawning activity is enhanced by administration of a GnRH agonist and suppressed by a GnRH antagonist.

The equivalent hormone to progesterone in teleost fish is 17,20β-dihydro-progesterone (17,20β-P). Several functions for 17,20β-P have been proposed, including induction of oocyte final maturation and spermiation (milt production), enhancement of sperm motility (by alteration of the pH and fluidity of the seminal fluid), and acting as a pheromone.

Upstream migration in salmonids has been associated with a sharp increase in plasma cortisol levels, which is not associated with stressors such as long-distance migration and changes in salinity.

Regulation of the sexual behavior in fish has been extensively studied and is best understood in goldfish and salmonids. Some of these behavior regulators are produced by endocrine organs and are transported via the circulatory system where they act directly or indirectly on the brain, while others are produced in as well as act upon the brain. Although the neuroendocrine regulation of fish reproductive behavior has been the subject of many studies, our understanding of this modulation of sexual behavior remains incomplete. The relative importance of hormones and neuropeptides in modulating or controlling sexual behavior is different among species, reflecting the large variation observed in fish mating systems and is particularly complex in fish with alternate reproductive tactics (**see also Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes). For this reason, we attempt a brief account of the most typical examples of hormonal regulation of sexual behavior described in fish thus far; these include sex steroids, prostaglandins, and neuropeptides.

Male Fish

Androgens, particularly testicular androgens, generally control sexual behavior (both nest building and courtship) in male fish, although variation exists between species. For example, castration abolished nest-building and courtship behaviors in the three-spine stickleback and the Egyptian mouth-brooder, *Pseudocrenilabrus multicolor multicolor,* but had no effect on the nest-building behavior of the blackchin tilapia, *Sarotherodon melanotheron melanotheron.* In addition, administration of androgen to the castrated Egyptian mouth-brooder or the three-spine stickleback restored male courtship behavior but the same treatment was ineffective in castrated rainbow trout, *Oncorhynchus mykiss.*

The functional androgenic steroid varies between species, although the main physiologically relevant androgens in fish are 11-ketotestosterone (11-KT) and 11-ketoandrostenedione (11-KA). 11-Ketoandrogens are not present in gobies or sardines where dihydrotestosterone and testosterone appear to be the major androgens. In goldfish, 11-KT is important in males in the release of the luteinizing hormone (LH) – that stimulates milt production – and sexual behavior (weak chasing). Similarly, androgens play an important role in the upstream migratory behavior of male salmonids.

There are many examples of androgens being the potentiator rather than the requirement for the expression of sexual behaviors. For example, in some cyprinids, androgen treatment increases olfactory sensitivity to sex pheromones, having a facilitating role in the initiation of sexual behavior.

Brain aromatase, an enzyme that converts androgens to estrogens, is possibly involved in the expression of male sexual behavior. Both the neuropeptide arginine vasotocin (AVT) and the hormone GnRH appear to control certain acts of male sexual behavior in some species.

In many species, the initiation of spermiation is accompanied not just by a sharp rise in plasma 17,20β-P concentrations, but also by a sharp drop in plasma 11-KT concentrations. There is an abundance of data (mostly on salmonids) suggesting that plasma concentrations of 17,20β-P are highly regulated by social factors, including hierarchy, territoriality, and courting. It has also been established that 17,20β-P concentrations increased in males only when in chemical contact with nesting females. Pheromones that have the ability to raise 17,20β-P concentrations in males were subsequently shown to be present in the urine of female salmonids including PGF2α and L-kynurenine.

Female Fish

In females, neuroendocrine studies have focused on female receptivity and oviposition. Estrogens do not seem to be a requirement for the female sexual behavior in many externally fertilizing teleosts. In fact, plasma estradiol levels appear decreased during the spawning period, which coincides with completion of vitellogenesis. Therefore, estrogens are controlling processes associated with oogenesis and do not appear to be essential for the female sexual behavior. In contrast, in internal-fertilizing fish, such as the guppy, estradiol synthesis appears to be in synchrony with sex pheromone production and maintains receptivity in females just as it does in internal-fertilizing tetrapods.

LH release plays a role in the production of the maturation-inducing steroid, 17,20β-P, that also has pheromonal properties.

Although in female masu salmon, a surge in plasma levels of testosterone is required for the induction of nest-digging behavior, there are no other reports showing a stimulatory effect of testosterone on female sexual behavior in fish. T is involved in LH release but not in sexual behavior in goldfish.

It is also interesting to note that sex steroids may regulate rheotaxis (swimming in response to a current of water) of fish. Fish without sex steroids, sexually immature or with their gonads removed, swim with the downstream current or do not exhibit upstream swimming behavior. In contrast, fish with high levels of sex steroids swim against the current, resulting in reproduction in the river (resident and upstream migration).

Female sexual behavior can be induced by PG injection in a number of fish species such as goldfish, paradise fish (*Macropodus opercularis*) medaka (*Oryzias latipes*) and dwarf gourami, *Colisa lalia.* In addition, AVT induces the spawning reflex in female killifish (*Fundulus heteroclitus*) – the same effect is observed in males – medaka, bitterling (*Rhodeus sericeus*) and flagfish (*Jordanella floridae*) – where it also occurs in males. Finally, GnRH is implicated in female spawning behavior in goldfish and salmon but has not been extensively studied in other species.

Conclusions

Fish present a unique collection of mating strategies and reproductive behaviors that in turn offer a great field for research into not only fundamental aspects of reproductive biology but also evolution and speciation. Of particular interest, and as yet a field that is far from being exhausted in terms of research, is the endocrine control of sexual behavior and mate choice. Here, there is scope not only for a better understanding of mechanisms involved in sexual behavior but also for applying knowledge to management of species conservation and aquaculture practice. In addition, since many cues controlling sexual behavior are chemical, it is anticipated that anthropogenic pollution might have an adverse effect on fish reproductive fitness, and hence deserves further investigation.

See also: **Behavioral Responses to the Environment:** Behavioral Responses to Hypoxia. **Fish Migrations:** Pacific Salmon Migration: Completing the Cycle. **Hormones in Communication:** Hormonal Pheromones. **Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling; Shocking Comments: Electrocommunication in Teleost Fish. **Social and Reproductive Behaviors:** Parental Care in Fishes; Socially Controlled Sex Change in Fishes. **The Skin:** Coloration and Chromatophores in Fishes.

Further Reading

Adler HE (1975) *Fish Behavior: Why Fishes Do What They Do*. Neptune City: TFH Publications.

Balon EK (1975) Reproductive guilds of fishes: A proposal and definition. *Journal of the Fisheries Research Board of Canada* 32: 821–864.

Godin J-G (ed.) (1997) *Behavioural Ecology of Teleost Fishes*. Oxford: Oxford University Press.

Hofmann HA (2006) Gonadotropin-releasing hormone signaling in behavioral plasticity. *Current Opinion in Neurobiology* 16: 343–350.

Houde AE (1997) *Sex, Color, and Mate Choice in Guppies (Monographs in Behavior and Ecology)*. Princeton, NJ: Princeton University Press.

Keenleyside MHA (1979) *Diversity and Adaptation in Fish Behaviour*. Berlin: Springer.

Liley NR and Stacey NE (1983) Hormones, pheromones, and reproductive behaviour in fish. In: Hoar WS, Randall DJ, and Donaldson EM (eds.) *Fish Physiology*, vol. IX, pp. 1–63. London: Academic Press.

Munakata A and Kobayashi M (2010) Endocrine control of sexual behavior in teleost fish. *General and Comparative Endocrinology 165: 456–468* (doi:10.1016/j.ygcen.2009.04.011).

Oliveira RF (2004) Hormones, social context and animal communication. In: McGregor PK (ed.) *Animal Communication Networks*, pp. 481–520. Cambridge: Cambridge University Press.

Oliveira RF (2004) Social modulation of androgens in vertebrates: Mechanisms and function. In: Lehrman DS, Hinde RA, and Shaw E (eds.) *Advances in the Study of Behaviour*, vol. 34, pp. 165–239. New York: Academic Press.

Oliveira RF, Canário AVM, and Bshary R (1999) Hormones, behaviour and conservation of littoral fishes: Current status and prospects for future research. In: Almada VC, Oliveira RF, and Gonçalves EM (eds.) *Behaviour and Conservation of Littoral Fishes*, pp. 149–178. Lisbon: ISPA.

Oliveira RF and Gonçalves DM (2008) Hormones and social behaviour of teleost fish. In: Magnhagen C, Braithwaite VA, Forsgren E, and Kapoor BG (eds.) *Fish Behaviour*, pp. 61–150. Enfield, NH: Science Publishers.

Pitcher TJ (ed.) (1993) *Behaviour of Teleost Fishes*, 2nd edn. London: Chapman and Hall.

Reebs SG (2001) *Fish Behavior in the Aquarium and in the Wild*. Ithaca, NY: Cornell University Press.

Sloman KA, Wilson RW, and Balshine S (eds.) (2006) *Behaviour and Physiology of Fish*, Fish Physiology Series, vol. 24. San Diego, CA: Elsevier.

Wootton RJ (1976) *The Biology of the Sticklebacks*. London: Academic Press.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Socially Controlled Sex Change in Fishes

J Godwin, North Carolina State University, Raleigh, NC, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Sexual Patterns in Fishes
Steroid Hormone Patterns
Other Steroid Hormones and Sex Change
Neural Mechanisms of Sex Change
Neural Bases of Behavioral Sex Change
Summary
Further Reading

Glossary

11-Ketotesterone Potent androgenic steroid hormone in teleost fishes inducing the development of secondary sexual characters often associated with territoriality and courtship in large males.

Arginine vasotocin Neuropeptide homolog of arginine vasopressin found in mammals. These hormones are released at the posterior pituitary gland, and also widely in the brain where they act as neuromodulators.

Aromatase Enzyme belonging to the cytochrome P450 family of proteins and plays a key role in the biosynthesis of estrogens through metabolism of androgens such as testosterone.

Estradiol 17β The most important circulating estrogen in both teleost fishes and mammals, produced in the ovaries and also other tissues including brain.

Gonadotropin-releasing hormone Neuropeptide hormone produced in the hypothalamus, released at the anterior pituitary gland to regulate gonadotropin secretion and is a key regulator of reproduction.

Gonochorism Sexual pattern in which individuals mature as one sex and remain that sex.

Initial phase First sexual phenotype seen in many protogynous species, often characterized by relatively drab colors and relatively low displays of aggression and courtship behavior.

Monoamine neurotransmitter A group of neurotransmitters that contain one amino group in their chemical structure.

Protandry Sexual pattern in which individuals mature as males and can then later change functional sex to become female.

Protogyny Sexual pattern in which individuals can mature as females and then later change functional sex to become secondary males. In monandric (one male) protogyny, all secondary males first pass through a female stage. In diandric (two males) protogyny, individuals can mature as either males or females and both can change from the initial phase to become the larger and typically colorful and aggressive terminal phase males.

Simultaneous hermaphroditism Sexual pattern characterized by individuals possessing both mature ovarian and spermatogenic tissue within the same functional gonad.

Steroid receptors Proteins that bind steroid hormones and then function as ligand-activated transcription factors to regulate gene expression in target cells.

Testosterone Important androgenic steroid hormone in all classes of vertebrates; critically, this steroid often functions as a biosynthetic intermediate in estradiol or 11-ketotesterone production.

Introduction

This article focuses primarily on one of the more dramatic examples of reproductive adaptation to social environment seen in fishes and, indeed, any animal – socially controlled functional sex change. The adaptive significance (or why?) of sex change is generally better understood than the developmental and physiological mechanisms that underlie this process (i.e., how?). When individuals can reproduce more effectively as one sex when small or young and as the other sex when larger or older, then there is an obvious benefit in being able to change sex to maximize reproductive success for their body size. This is known as the 'size advantage model' of sex change and it has been quite successful in explaining patterns of sex change in many species.

While the size advantage model offers a compelling explanation for the presence of sex change in many species of fishes where this pattern is observed, it is equally striking that many species exhibit similar patterns of reproductive success across body size (especially mating advantages for large males) and should exhibit sex change according to the model, yet do not. This is true both within the fishes and especially for other vertebrate groups where sexual lability is both much rarer and restricted to early developmental periods if it occurs. What accounts for this variation?

In mammals, the sex of an individual is determined by sex chromosomes at fertilization and specifically the presence or absence of the gene SRY (for 'Sex determining Region of the Y' chromosome). The testis or ovaries then arise relatively early in development and their hormonal secretions (particularly testicular androgens) organize other tissues including the brain. By contrast, the gonads develop relatively late in fishes as compared with other tissues and often well after individuals have spent considerable time as independently living miniature adults. But why does this matter in terms of environmental control of sex determination?

When the gonads develop very early, the brain is neither capable nor has the opportunity to gather and interpret environmental information that might be used to adaptively guide sex determination. By contrast, fishes often have ample opportunity to collect such information prior to gonadal development and, in the case of social influences, possess a well-developed brain to interpret it.

Sexual Patterns in Fishes

Fishes show the broadest range of sexual patterns of any group of vertebrates. With respect to sex determination, this variation ranges from strict gonochorism, where sex is determined by sex chromosomes, to simultaneous hermaphroditism, where individuals can alternate male and female function on a second-to-second basis. Between these extremes are species where:

1. sex is determined by social or physical factors prior to maturation, but then fixed after maturation;
2. species that mature as one sex, either male or female, and then change to become the other sex following maturation either as part of an ontogenetic sequence dependent on age or body size or in response to changes in their social environment; and
3. species that can undergo serial adult sex changes in both directions in response to social environment.

The article primarily focuses on the latter two patterns where sex determination occurs in adults and is under social control. Many species that undergo sex change also exhibit alternate male phenotypes; these are fascinating reproductive adaptations, but a full treatment is beyond the scope of this article.

Socially controlled sex change was first described and is best characterized in tropical marine fishes associated with coral reefs. This is because sex change is relatively common in four families that are conspicuous and important parts of the reef fish community. These families are the wrasses (Labridae), parrotfishes (Scaridae), damselfishes (Pomacentridae including the anemonefishes, subfamily Amphiprioninae), and the gobies (Gobiidae). Socially controlled sex change is also found in some temperate species where studies have focused on wrasses and gobies.

Male-to-female adult sex change is termed protandry and is seen in several families of fishes. Socially controlled protandry is best described in the anemonefishes, an Indo-Pacific group of 26 species (25 in *Amphiprion* and one species of *Premnas*). The anemonefishes have been an object of fascination due to both the symbiotic relationship they show with large tropical sea anemones and their recognition as stars in the movie *Finding Nemo* that featured Nemo, a 'boy' anemonefish, being raised by his single father Marvin. However, had the movie been biologically accurate, Nemo's father would have likely changed sex to become female following the disappearance of Nemo's mother and Nemo would have been like other anemonefishes, developing as an immature female who would likely have matured into a functional male to form a breeding pair in the social group (**Figure 1**). This pattern of a breeding pair and up to several immature females forming social groups has now been described for several anemonefish species. The gonadal structure of males consists of peripheral active spermatogenic tissues surrounding immature ovarian tissue (oocytes in the previtellogenic stage of development, **Figure 2**). This pattern is interesting because it illustrates the fundamentally female-first pattern of development in sex changing fishes even when the adult sex change pattern is male-to-female (we can only speculate as to why the movie makers decided not to have Nemo's father change sex!).

The maturation of juveniles and sex change by breeding males appears to be prevented by aggressive dominance of large females in anemonefishes. In the cinnamon anemonefish, *Amphiprion melanopus*, dominant females rapidly and aggressively approach their male pair mates approximately 150 times a day. Disappearance or experimental removal of these females eliminates this inhibition and both the behavior and gonadal structure of the male begin to change rapidly. Male cinnamon anemonefish made experimentally dominant show significant increases in aggressive behavior within 1 day of the removal of their dominant female pair mates and extensive changes in gonadal structure with proliferation of oogonial-like cells by 10 days later. Replacement of spermatogenic tissue with ovarian tissue is complete by 20 days.

The most common form of socially mediated sexual plasticity seen in reef fishes is female-to-male sex change or protogyny. Protogyny can take two forms. In monandric (one male) protogyny, all individuals mature as females and only later potentially change to become males. In diandric protogyny, individuals develop initially as immature females, but may mature into the initial phase (IP) as either males or females and either of these

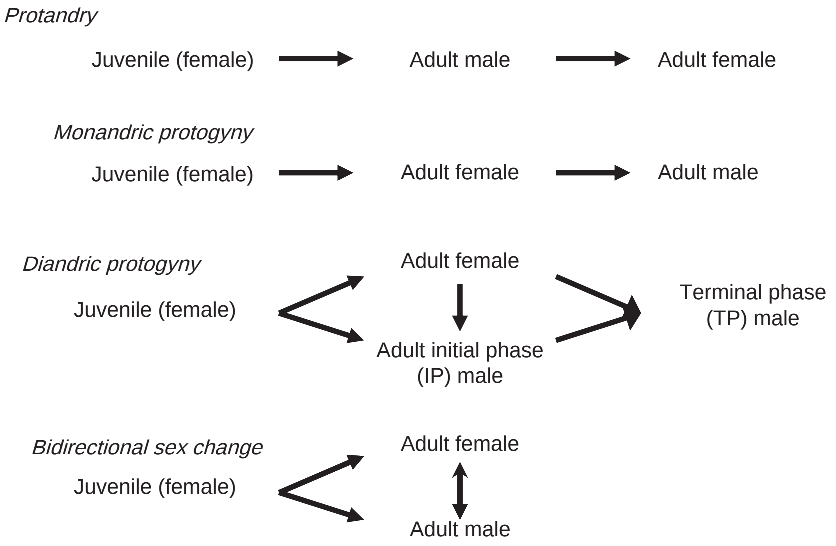


Figure 1 Sex change patterns in fishes. Modified from Godwin J (2010) Neuroendocrinology of sexual plasticity in teleost fishes. *Frontiers in Neuroendocrinology* 31(2), 203–216, with permission from Elsevier.

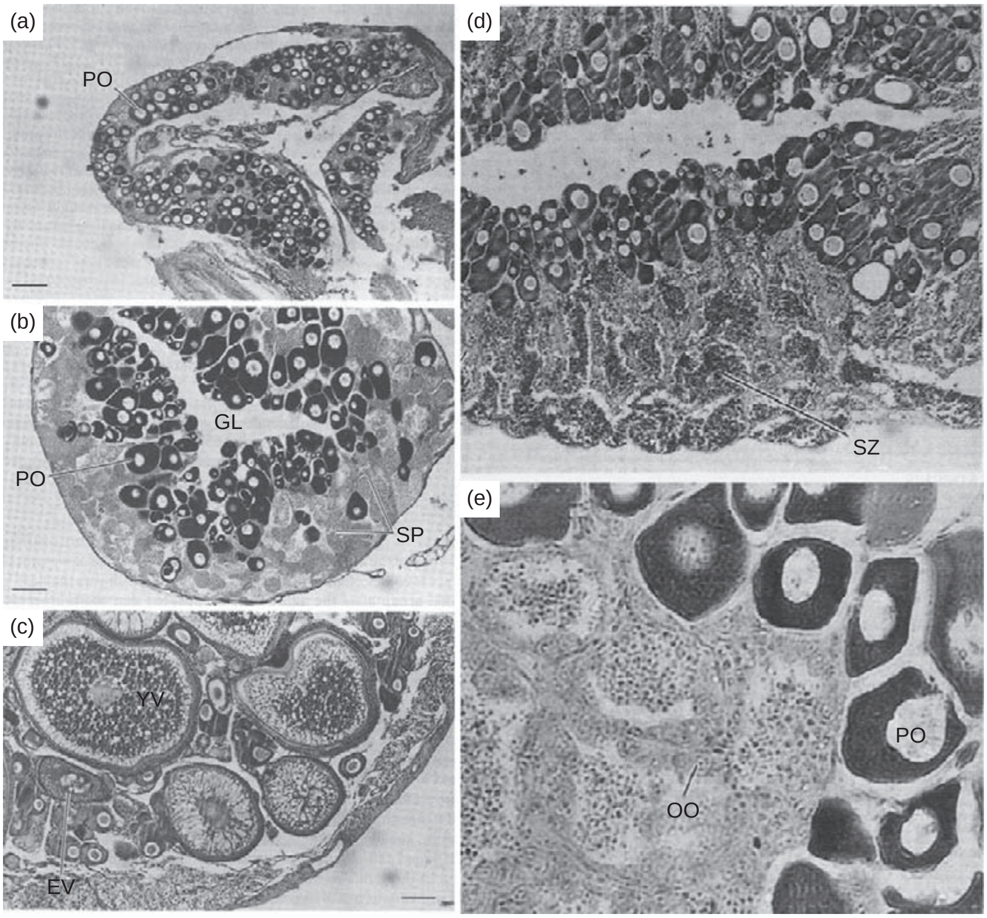


Figure 2 Gonad structure in the anemonefish *Amphiprion melanopus* for juvenile nonbreeders (a), breeding males (b), breeding females (c), and a sex changing fish 10 days after removal of the dominant female ((d) – low magnification, (e) – higher magnification). Abbreviations: EV, early vitellogenic oocyte; GL, gonadal lumen; PO, previtellogenic oocyte; SP, spermatogenic tissue (multiple stages); SZ, spermatozoa; OO, presumptive oogonia appearing as ovary develops during sex change. Scale bars = 100 mm. Reproduced from Godwin J (1994) Histological aspects of protandrous sex-change in the anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *Journal of Zoology* 232: 199–213.

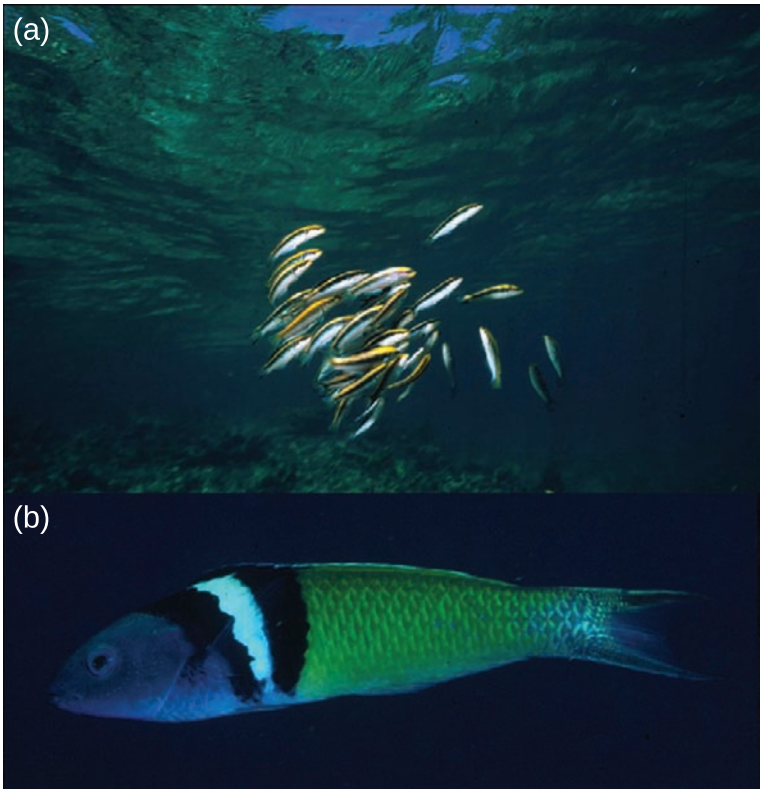


Figure 3 Initial phase and terminal phase males of the bluehead wrasse, *Thalassoma bifasciatum.*

phenotypes can then undergo change to the typically brightly colored terminal phase males (**Figures 1** and **3**). Monandric protogyny occurs in some damselfishes and is common in gobies and groupers with both monandric and diandric protogyny occurring in wrasses and parrotfishes. As predicted by the size advantage model discussed earlier, the adaptive significance of both monandric and diandric protogyny appears to be the high levels of reproductive success that accrue to large males who monopolize access to females or spawning territories.

Perhaps the most dramatic examples of sexual plasticity are seen in gobies and some serranid fishes. A number of serranid species are simultaneous hermaphrodites, maintaining both mature ovarian and testicular tissue in the same gonad. The hamlets (genus *Hypoplectrus*) are native to the Caribbean Sea and alternate female and male behavior and spawning on a second-to-second timescale in a behavior termed 'egg trading'. Other species of small serranid sea basses maintain gonads with mature tissue of both sexes and may lose the ovarian tissue when they become socially dominant members of social groups able to monopolize mating.

A number of goby species are protogynous. However, gobies also show an additional degree of flexibility in species that exhibit bidirectional sex change. Species of *Gobiodon* and *Paragobiodon* are obligate residents of branching corals in the west Pacific that can change sex in either direction. This flexibility allows any two individuals to form a mating pair on a coral, likely a strong advantage for a very small bodied species in which traveling large reef areas in search of mates could be very dangerous (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction; Endocrine Control of Sex Differentiation in Fish).

Steroid Hormone Patterns

The effects of androgens in a sex changing species were examined as early as 1962 in the wrasse *Coris julis*, but measurements of circulating levels of sex steroid hormones had to wait for the development of a technique termed 'radioimmunoassay' (RIA). Early studies using RIA showed that plasma levels of the primary estrogen in fishes (and mammals) 17-beta estradiol (E_2) were elevated in female saddleback wrasses (*Thalassoma duperrey*) and declined dramatically at the onset of sex change at the same time that oocytes in the gonad degenerated (**Figure 4**). Levels of E_2 remained very low across sex change and in terminal phase (TP) males. By contrast, a key androgen in fishes 11-ketotestosterone (11KT), was present at low levels in females and across sex change, only becoming elevated in TP males. Importantly, significant increases over female 11KT levels appeared to follow rather than precede or accompany the appearance of mature spermatogenic tissues.

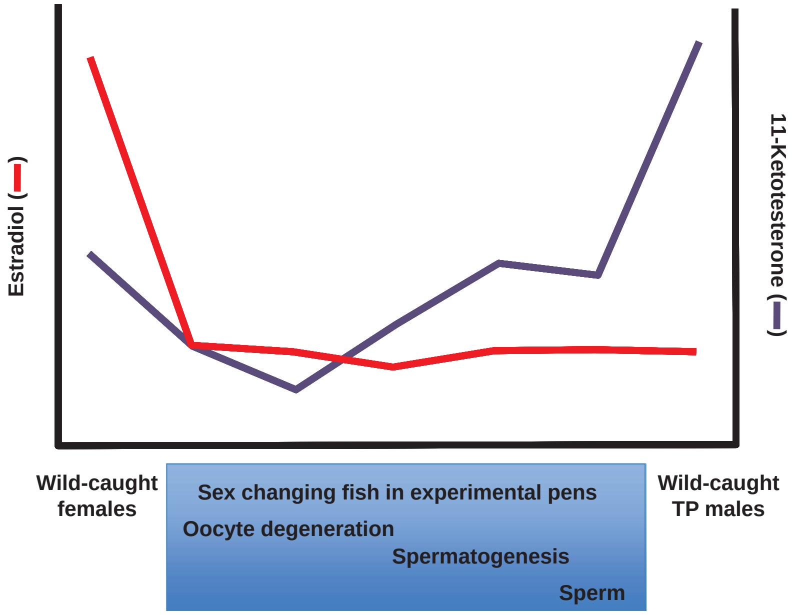


Figure 4 Relative steroid hormone levels in the plasma of saddleback wrasses shown for natural females and TP males as well as females undergoing socially induced sex change in experimental pens. Redrawn from Nakamura M, Kobayashi Y, Miura S, Alam MA, and Bhandari RK (2005) Sex change in coral reef fish. *Fish Physiology and Biochemistry* 31: 117–122.

Similar sex steroid hormone patterns are seen in the stoplight parrotfish (*Sparisoma viride*) and several other sex changing species that have been examined, including goby and grouper species. In an interesting twist, the anemonefish *A. melanopus* also shows higher plasma E_2 levels in females and higher 11KT levels in males despite the direction of sex change being male-to-female and females being the larger and aggressively dominant sex (**Figure 5**).

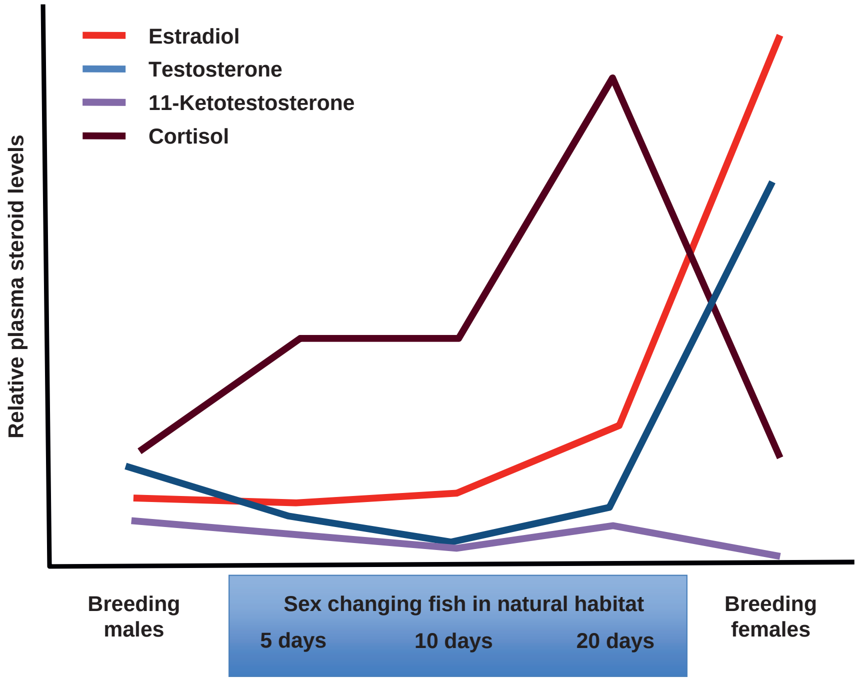


Figure 5 Relative steroid hormone levels in plasma of the anemonefish *Amphiprion melanopus* in natural males and females and as males underwent protandrous sex change following experimental female removals from social groups. Relative levels of different hormones are depicted approximately to scale except for cortisol, where levels were substantially higher. Redrawn from Godwin JR and Thomas P (1993) Sex change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *General and Comparative Endocrinology* 91(2): 144–157.

The role of steroid hormones in the control of sex change is supported by many experiments manipulating either steroid hormones directly or their synthesis. Androgen administration is effective for inducing male sex determination in a wide range of fishes and this approach is widely used in aquaculture to produce faster-growing males. Beginning in 1955 with bluehead wrasses, androgen administration has also been used to successfully induce sex change in a number of sex changing species. These include a number of wrasses, the stoplight parrotfish *S. viride,* the blackeye goby *Coryphopterus nicholsi,* and several species of groupers.

Blocking estrogen synthesis has similar effects to androgen administration in inducing female-to-male sex change. A key regulatory step in estrogen synthesis is the enzyme aromatase, a protein in the cytochrome P450 family of proteins. Fishes express two forms of this enzyme, known as gonadal and brain aromatase. Aromatase and estrogens generally appear to play key roles in sex determination in a range of species. Manipulations of estrogen signaling and aromatase activity are effective in manipulating the occurrence of sex change in several wrasse and grouper species as well as two goby species. For example, inhibiting aromatase action in the blackeye goby *C. nicholsi* induces sex change as effectively as 11KT implants. The bidirectionally sex changing gobies in the genus *Gobiodon* are especially interesting in this respect. As noted above, these gobies can change sex in either direction to form heterosexual pairs on the corals where they reside. Implants of the aromatase inhibitor fadrozole induce females to become males and males to remain male even when paired with a larger male. Conversely, E_2 implants induce sex change in males paired with other males while E_2-implanted females paired with other females do not change sex. Also consistent with a dominant role for estrogenic signaling in regulating sex change was a study where co-administering E_2 with androgens in three-spot wrasses blocked the female-to-male sex change that would otherwise occur. Taken together, these studies suggest that estrogenic inhibition may be the critical controlling factor for gonadal sex change.

The environmental cues for socially controlled sex change must clearly first be perceived in the brain. Consistent with this expectation, the more rapid decreases in brain aromatase activity seen in the brain compared to the ovaries of sex changing *Lythrypnus dalli* gobies suggest that a key early event in the sex change process is reduced estrogen signaling to neural structures.

Other Steroid Hormones and Sex Change

Because the social regulation of sex change is often closely related to aggressive behavioral dominance, there has been interest in the potential role of stress hormones. The key stress-related steroid in fishes is cortisol, as in humans, and is synthesized in the interrenal gland. Subordinate social status is quite often characterized by elevated cortisol levels in fishes and other species and elevated cortisol levels can inhibit reproductive function. These relationships led Perry and Grober to develop a model of the mediation of socially controlled sex change through the endocrine stress axis. This model was not supported in studies in anemonefish (*A. melanopus*) or the sandperch *Parapercis cylindrica*, where experimental increases in cortisol did not prevent sex change. However, stress effects can be exquisitely time sensitive and animals show physiological adaptation to chronic stressors in sometimes subtle ways, so more work remains to be done regarding the potential role of the endocrine stress axis in mediating sex change (**see also Social and Reproductive Behaviors**: Dominance Behaviors, **Hormonal Responses to Stress**: Impact of Stress in Health and Reproduction).

Neural Mechanisms of Sex Change

It has become clear that the often dramatic changes in behavior that accompany sex change are key parts of the change in sexual phenotype and may play an important role in stimulating the gonadal sex change process. This appreciation of the role of behavioral change as well as development of new techniques have shifted much of the focus in recent studies of sex change to neural and neuroendocrine mechanisms which may translate changes in social environment to changes in the gonads and other parts of the sexual phenotype.

The hypothalamus serves as a critical integrative center for both the reproductive axis and sexual behavior and is the site of gonadotropin-releasing hormone (GnRH) neurons which project to the pituitary gland. This brain region has, therefore, been a logical starting point for investigations of the neural mediation of sex change. Both correlative and experimental approaches support a role for the GnRH system in the sex change process. Working with bluehead wrasses in the early 1990s, Matthew Grober and colleagues described greater numbers of immunoreactive GnRH neurons in the hypothalamus of TP males than in that of females and that these numbers were increased in females by 11KT treatment. This pattern of greater numbers of GnRH neurons in males than females is also seen in the Ballan wrasse (*Labrus berggylta*) and in the protandrous anemonefish *Amphiprion bicinctus* (despite females being larger and aggressively dominant in anemonefishes). Expression of GnRH has not been closely monitored through the sex change process in any species, but findings from the cichlid *Astatotilapia burtoni* suggest that this would likely be informative. *A. burtoni* shows socially regulated sexual development that involves rapid activation of preoptic area GnRH neurons when changes in social conditions are perceived. This rapid activation of GnRH neurons underlies the rapid activation of the HPG axis when subordinate males ascend to social dominance.

What mechanisms might induce a change in the activity of GnRH neurons? Changes in neural estrogen signaling due to reductions in brain aromatase activity at the onset of sex change and a potential role for stress hormones were discussed above. Another intriguing possibility is the monoamine neurotransmitters – serotonin, noradrenaline, and dopamine which have been shown to vary in areas of the forebrain important for reproduction and sexual behavior, and their roles further confirmed by manipulation experiments.

Before leaving the discussion of neural mediation of gonadal sex change, this article discusses some recent work suggesting that the variation in target tissue responsiveness to gonadotropins is the critical change at the time of sex change. The bidirectionally sex changing goby *Trimma okinawae* expresses both ovarian and testicular tissues, but with only one type being mature and active at a given time. Kobayashi and colleagues found that the active portion of the gonad expressed gonadotropin receptors at much higher levels than the inactive portion and that this higher expression was associated with greater responsiveness to gonadotropin. Exposure to visual cues that induce a change to the opposite sex reversed the pattern of gonadotropin receptor expression within approximately 12 h.

Neural Bases of Behavioral Sex Change

Successful sex change requires coordinated changes in a suite of phenotypic features. Behavioral sex change in fishes is often very rapid, occurring within one to several days in anemonefishes and blue-banded gobies and within minutes of dominant male removal with cleaner and bluehead wrasses studied in nature. This short timescale suggests gonadal change does not drive behavioral change. Indeed, the reverse may be true. The role of the gonads, neural mediators of behavioral sex change, and potential connections between the behavioral and gonadal sex change are discussed in this section.

Surgically removing the gonads from female bluehead wrasses does not prevent behavioral sex change when those females are made socially dominant. While the gonads are not necessary for the behavioral components of sex change in this species, ovariectomized females do not develop TP male coloration on becoming dominant. This is presumably because they lack a source of 11KT, which can induce the development of these colors when administered even to subordinate, ovariectomized females. This lack of necessity for gonads in behavioral sex change is consistent with often very rapid time course of this process and highlights the fact that important changes in steroid hormone signaling do not need to occur in the gonad itself.

What neural mechanisms do mediate behavioral changes during the sex change process? Two areas of focus have been monoamine neurotransmitters and neuropeptide hormones. Changes in monoamines were mentioned above and in saddleback wrasses (*T. duperrey*) changes are especially pronounced during the first week of sex change when social dominance for sex changing females is being established. During the first week in experimental groups, dominance interactions develop accompanied by a number of alterations in monoaminergic signaling, although the patterns are complex across brain nuclei. In bluehead wrasses, serotonergic manipulations can decrease aggression in both laboratory and wild TP males, but no studies have closely examined the behavioral effects of manipulating monoamines during the sex change process in wrasses.

A second focus for studies of the neural mechanisms mediating behavioral sex change has been on neuropeptide hormones, especially arginine vasotocin (AVT). The first study to show differences in AVT neurons across sexual phenotypes in a sex changing fish was in the goby *T. okinawae*. In bluehead wrasse, TP males have significantly higher abundances of AVT mRNA in the preoptic area of the hypothalamus compared with females and these levels increase rapidly during experimentally induced sex change. Both the gonads and social interactions are changing as a female wrasse undergoes sex change; therefore, to tease these two factors apart, levels of AVT neurons in females changing sex with and without gonads and females that remain as subordinate females were compared. Social status affected AVT mRNA abundance, while gonadal status did not (**Figure 6**). Behavioral effects of AVT also appear to depend on sexual phenotype. Neither courtship nor territorial behavior could be induced by AVT injections in females or female-mimic IP males. However, injection of AVT increases courtship in TP males and induces nonterritorial TP males to establish territories.

This difference in responsiveness to AVT across sexual phenotypes is not surprising. In mammals, arginine vasopressin, the mammalian homolog of AVT, is more strongly implicated in male-typical socio-sexual behavior, while oxytocin, the homolog of the teleost isotocin, is implicated primarily in mediating female-typical socio-sexual behavior. The best data on the effects of isotocin in fishes come from an experiment by James Goodson and Andrew Bass, who examined neural responsiveness with a non-sex changing species, the plainfin midshipman *Porichthys notatus*. They found that the brains of large territorial males were strongly responsive to AVT while those of females and smaller nonterritorial type II males were not responsive at all. By contrast, the brains of females and type II males were strongly responsive to isotocin, while the large territorial type I males were completely unresponsive. The only data available

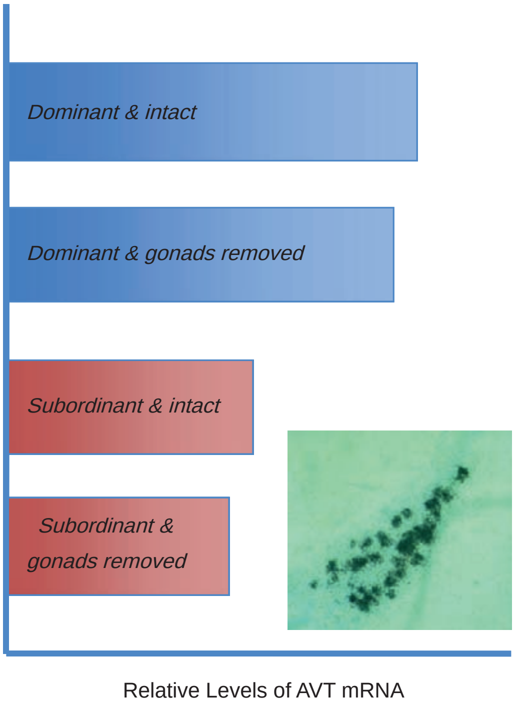


Figure 6 Social status influences AVT mRNA levels in the preoptic area of the hypothalamus in bluehead wrasses while gonadal status does not. Dominant individuals were the largest in their social groups while subordinants were not. Intact animals underwent sham operations to control for the surgical procedure. Inset shows AVT mRNA in the preoptic area visualized by the technique of *in situ* hybridization. Data from Semsar K and Godwin J (2003) Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. *Journal of Neuroscience* 23: 4386–4393.

regarding isotocin neuron patterns in sex-changing species come from blue-banded gobies, where more isotocin neurons were found in females than males. Further exploration of isotocin actions in sex changing fishes would be valuable.

Summary

Our understanding of the physiological basis of sex change has increased rapidly in recent years. This is due to both technical advances in endocrine techniques and especially molecular biology as well as the identification and exploitation of powerful model systems such as sex changing wrasses and bidirectionally sex changing gobies. Estrogen synthesis through the aromatase enzyme and the actions of the neuropeptides GnRH and AVT appear especially important in regulating sex change, but other physiological mediators are undoubtedly important contributors to the process. The discovery of these mechanisms and development of synthetic models of the mechanistic basis of sex change from the perception of changed social environment to gonadal change should be the next stage in study of this fascinating reproductive adaptation.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish; Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress**: Hormone Response to Stress; Impact of Stress in Health and Reproduction. **Social and Reproductive Behaviors**: Dominance Behaviors.

Further Reading

Black MP, Balthazart J, Baillien M, and Grober MS (2005) Socially induced and rapid increases in aggression are inversely related to brain aromatase activity in a sex-changing fish, *Lythrypnus dalli*. *Proceedings of the Royal Society B: Biological Sciences* 272: 2435–2440.

de Mitcheson YS and Liu M (2008) Functional hermaphroditism in teleosts. *Fish and Fisheries* 9(1): 1–43.

Devlin RH and Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208(3–4): 191–364.

Fishelson L (1970) Protogynous sex reversal in fish *Anthias squamipinnis* (Teleostei, Anthiidae) regulated by presence or absence of a male fish. *Nature* 227: 90–91.

Francis RC (1992) Sexual lability in teleosts – developmental factors. *Quarterly Review of Biology* 67(1): 1–18.

Ghiselin MT (1969) The evolution of hermaphroditism among animals. *Quarterly Review of Biology* 44: 189–208.

Godwin J (1994) Histological aspects of protandrous sex-change in the anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *Journal of Zoology* 232: 199–213.

Godwin J (2009) Social determination of sex in reef fishes. *Seminars in Cell and Developmental Biology* 20: 264–270.

Godwin JR and Thomas P (1993) Sex change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *General and Comparative Endocrinology* 91(2): 144–157.

Kroon FJ and Liley NR (2000) The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholsii* (Teleostei: Gobiidae). *General and Comparative Endocrinology* 118(2): 273–283.

Kroon FJ, Munday PL, Westcott DA, Hobbs JPA, and Liley NR (2005) Aromatase pathway mediates sex change in each direction. *Proceedings of the Royal Society B: Biological Sciences* 272(1570): 1399–1405.

Larson ET, Norris DO, Grau EG, and Summers CH (2003) Monoamines stimulate sex reversal in the saddleback wrasse. *General and Comparative Endocrinology* 130(3): 289–298.

Larson ET, Norris DO, and Summers CH (2003) Monoaminergic changes associated with socially induced sex reversal in the saddleback wrasse. *Neuroscience* 119(1): 251–263.

Nakamura M (2005) Sex change in coral reef fish. *Fish Physiology and Biochemistry* 31: 117–122.

Nakamura M, Hourigan TF, Yamauchi K, Nagahama Y, and Grau EG (1989) Histological and ultrastructural evidence for the role of gonadal steroid hormones in sex change in the protogynous wrasse *Thalassoma duperrey*. *Environmental Biology of Fishes* 24: 117–136.

Robertson DR (1972) Social control of sex reversal in a coral-reef fish. *Science* 177: 1007–1009.

Semsar K and Godwin J (2003) Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. *Journal of Neuroscience* 23: 4386–4393.

Stoll LM (1955) Hormonal control of the sexually dimorphic pigmentation of *Thalassoma bifasciatum*. *Zoologica* 40: 125–131.

Warner RR (1984) Mating behavior and hermaphroditism in coral-reef fishes. *American Scientist* 72: 128–136.

Parental Care in Fishes

S Balshine, McMaster University, Hamilton, ON, Canada

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Patterns and Diversity in Parental Care
Explaining the Patterns and Diversity of Fish Parental Care
Physiological Mechanisms Underlying Fish Parental Care
Future Research Directions and Conclusions
Further Reading

Glossary

Androgen Any natural or synthetic steroid hormone that is principally involved in growth, development, and maintenance of the male reproductive system and secondary sexual characteristics.

Arginine vasotocin A neuropeptide homolog of arginine vasopressin found in mammals. These hormones are released not only at the posterior pituitary gland, but also widely in the brain where they act as neuromodulators.

Isotocin Homolog of the mammalian hormone oxytocin, known to influence social behaviors in fish.

Oviposition An act of laying eggs by oviparous animals (animals laying eggs that develop outside the female's body).

Phylogeny Modification of the structure and function of a species or group of species throughout their evolutionary history.

Prolactin A protein hormone primarily produced in the pituitary acting on ion balance and reproduction.

Sneaker male A male phenotype that sneaks into the territory of a conventional male to mate with a female.

Sperm competition The competition between sperm from two or more males to fertilize the egg of a female.

Viviparity The production of live young that develop before being released into the external environment.

Zygote The initial cell of a new organism, usually formed by the joining of an egg and sperm at fertilization.

Introduction

Parental care, the investment in young after fertilization, may not be a behavior that immediately jumps to your mind when you think of fishes. However, many fishes provide care for their young, and they care for the young in different ways. Which parent provides the care also varies greatly from fish species to fish species. The impressive variation in parental-care tactics has made fishes an excellent group for testing our understanding of how parental care evolves. Indeed, studies with bony fishes have helped shape our theoretical understanding of how sexual selection operates.

In contrast, the physiological mechanisms underlying parental behavior in fishes remain poorly explored. Although to a limited degree prolactin, isotocin, and androgens have all been investigated in the context of parental care, the behavioral physiology of parental care in fishes is very much in its infancy. In this article, we examine the major forms of care and the common patterns of care found in fishes. We then provide an explanation for these patterns based on the costs and benefits of parental care. Finally, we review the current available information on physiological underpinnings of fish parental-care behavior, albeit limited, and suggest areas for much-needed future research.

Before embarking on a review of parental care in fishes, it is necessary to clarify the terms used when describing behavior of parents toward their young. The term 'parental care' is used to describe any behavior performed by parents that appears likely to improve the survival and reproduction of the young. Robert Trivers coined a more restrictive term 'parental investment' to refer to any action by parents that increases offspring fitness at a cost to parental fitness (because it curtails their ability to invest in future mating, fecundity, survival, or further parental care). The terms parental effort or parental expenditure refer specifically to the time and effort spent on parental care of offspring, again referring to actions that are thought to increase offspring fitness, for example, defending offspring against predators. This measure is related to parental investment but does not necessarily imply fitness costs for parents, as the costs of parental care may change with an individual's age, status, or condition.

The question of which behaviors to include or exclude when using the terms parental care or parental investment can also be challenging. For example, building and defending a nest may not only increase the chances of attracting a mate (reproductive effort), but also have a positive impact on offspring fitness (parental effort). Many fish species provide eggs with large quantities of yolk before releasing them to the external environment (**see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care); the largest known fish egg belongs to the coelocanth (*Latimeria chalumnae*) with a diameter between 7.5 and 9.0 cm and a mass of 325 g. Whether or not such provisioning should be considered as parental care or parental investment has been much debated. Females vary considerably (within and across species) in the extent to which they provision eggs and such provisioning has profound effects on development time, survival of young, and the ability for a female to invest in future young.

Patterns and Diversity in Parental Care

Form of Care

Unlike birds and mammals where offspring require some form of parental assistance, most fish species do not provide parental care. However, in 25% of fish species, care has evolved, and the way care is performed is highly variable (**Figure 1**). Fish care ranges from hiding of eggs and then abandoning the area, to guarding young in elaborately prepared structures for up to several months, and from carrying young in or on the parent's body cavity to the feeding of young.

The simplest form of fish parental care is hiding of the eggs. Female salmon and trout, for example, will excavate nests (redds) by digging simple depressions with their tails; the eggs that are laid in these redds are fertilized and then buried by the female. In contrast to the simple pit built by the female salmonids, some fish species are amazing construction workers. Lake Malawi's cichlid, *Cytocara eucinostomus,* creates crater-shaped nests that are more than 3 m in diameter, while the three-spined stickleback, *Gasterosteus aculeatus*, constructs elaborate nests woven of plant material carefully glued together with a special kidney glycoprotein secretion known as spiggin (**Figure 1**(**a**); **see also Social and Reproductive Behaviors**: Sexual Behavior in Fish).

Guarders are fish species that take parental care a step further than nest builders, by tending and defending eggs, embryos, or larvae until they hatch. Some guarding species build their own nests, while others choose an area (cavity, cave or natural burrow, underside of a rock, or log) to use as a spawning and guarding site. Typically, fish species that significantly modify the substrate to receive eggs (nest builders), also guard eggs against predation (**Figure 1**(**b**)). The amount of time spent protecting young varies from 1 day in the Sacramento perch, *Archoplites interruptus,* to over 4 months in the Antarctic plunderfish, *Harpagifer bipinis.* Many fish species go beyond simple guarding by aerating the eggs using their pelvic or pectoral fins. By fanning the eggs with their fins, parents ensure that fresh, aerated water is passed over the eggs on a regular basis. Many parent fish also clean the eggs, placing their mouths against the eggs and sucking away detritus or removing dead or fungus-ridden eggs.

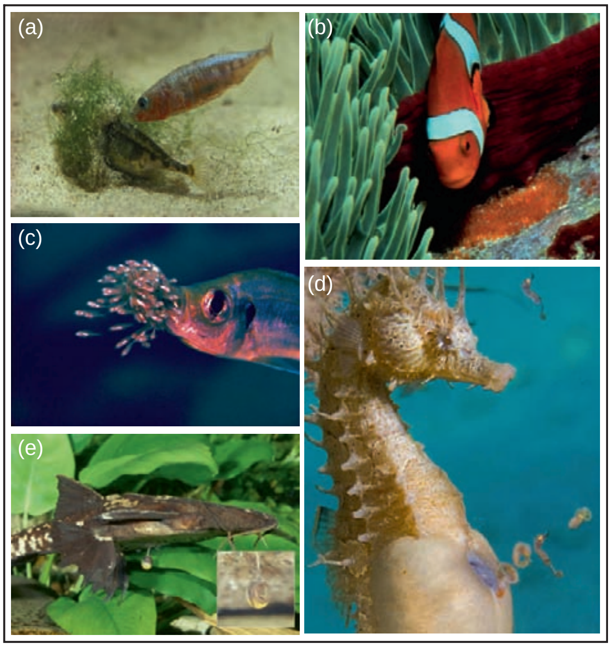


Figure 1 (a) A male three-spined stickleback (*Gasterosteus aculeatus*) observing a female entering his nest; (b) a male clownfish (*Amphiprion percula*), inspecting eggs in its nest; (c) a female mouth-brooding cichlid from Lake Tanganyika, *Haplotaxodon microlepis* collecting young in its mouth; (d) an unnamed seahorse, *Hippocampus* spp. giving birth to live young; and (e) A female eeltailed banjo catfish, *Platystacus cotylephorus,* carrying young embedded on the skin of her abdomen.
(a) Reproduced with permission from Photolibrary. (b) Reproduced from Buston PM and Balshine S (2007) Cooperating in the face of uncertainty: A consistent framework for understanding the evolution of cooperation. *Behavioural Processes* 76: 152–159, with permission from Elsevier. (c) Reproduced with permission from http://www.deeblestone.com (e) Reproduced with permission from Ingo Seidel.

Although the terms 'brooding' and 'incubation' are commonly used by fish biologists when describing fish parental care, fish do not truly incubate eggs, as heat is not transferred to the eggs by parents. However, many fish species protect their young internally and some even have live births (known as live-bearing or viviparous species; **see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care). Species of fish that protect or incubate their young internally may do so in their mouths (e.g., marine catfishes and cichlids, **Figure 1(c)**), in ventral brood pouches (e.g., sea horses and pipefishes, **Figure 1(d)**), on hooks (e.g., *Kurtus* spp.), embedded in skin (e.g., American banjo catfish, **Figure 1(e)**), or in gill chambers (e.g., cavefish). Internal brooding is an extremely effective method of protection because the only way a predator can capture or kill young fish is to injure or kill the parent or force the parent to eject its young. For example, male seahorses, *Hippocampus wheitei*, receive eggs from their female partners, which the males internally fertilize and then aerate and nourish for a few weeks in an enclosed pouch. The dads eventually give birth (via a series of forward and backward muscular contortions) to young, one at a time.

The most complex or elaborate parental-care behaviors found in fishes are undoubtedly the feeding of young post-hatch and caring for nutritionally independent young. *Symphysodon discus* young as well as the young of some other cichlid species ingest the epidermal mucus from their parents' body. Both male and female parents of the Central American convict cichlid, *Cichlasoma nigrofasciatum*, carefully lift up fallen leaves for their young providing them with benthic prey underneath the leaf litter. Many cichlid parents appear to be able to signal imminent danger to their young by using a specific jolt or calling behavior that consists of open-and-shut snapping of the pelvic fins while swimming backward with the head pitched downward. The young respond to these warning movements by settling down into the substrate or swarming and entering the parents' mouths where they can be kept safely until the danger has passed. Finally, in Tanganyikan cichlids, such as *Neolamprologus brichardi and Neolamprologus pulcher*, sexually mature young continue to be vigorously guarded.

Sex of Caregiver

In fishes, care can be provided by the female alone (maternal or female-only care), by the father alone (paternal or male-only care), or by both parents together or in sequence (biparental care, **Figure 2**). About 30% of the 500 known fish families show some form of parental care, and most often (78% of the time) care is provided by only one parent (usually the male). Male care (50%, **Figure 2(a)**) is much more common than female care (30%) with biparental care accounting for about 20%, although a more recent comparative analysis suggests that male care may be more common (84%; **Figure 2(b)**). In some species, such as Galilee St. Peter's fish (*Sarotherodon galileaus*) and the brown bullhead (*Ameiurus nebulosus*), parental care is variable with male-only, female-only, and biparental care occurring in the same population.

Explaining the Patterns and Diversity of Fish Parental Care

Many interesting questions arise from the patterns observed in fish parental behavior. For example, (1) why do so many more freshwater fishes provide care (57% of freshwater fish families) compared to marine species (only 16%); (2) why is female-only (maternal) care typically associated with internal bearing in fishes; and (3) why is male-only (paternal) care so common? This section addresses these related questions about who should care, and it reviews the costs and benefits associated with parental care in fishes:

1. *Why so much more care in freshwater species?* The open ocean provides a relatively stable, safe, and homogeneous environment for egg development; egg predators are relatively rare and water conditions do not fluctuate quickly. In contrast, freshwater biotas, especially shallow ones where fish eggs are typically released, vary tremendously in time and space. Hence, selection of particular spawning locations combined with parental care can have massive impacts on egg development and survival. This benefit has led to male defense of the best or favored female spawning sites and to female egg clumping. Under these conditions (male territoriality and multiple females being attracted to the same spawning site), guarding eggs in addition to a territory would not be particularly costly.
2. *Why is female-only or maternal care associated with internal bearing in fishes?* Internal gestation is rare in fishes (11% of families) and is strongly associated with female care. Once internal fertilization has evolved, the retention of egg requires little re-organization and would confer a substantial survival benefit to young. A protracted association between females and the zygote is considered to be the basis for selection of internal fertilization. In families with internal fertilization and care, in 86% of the cases it is the female that provides the care; in contrast, when external fertilization and parental care co-occur, it is usually the male that provides care (76% of cases).
3. *Why so much male-only (paternal) care in fishes?* A lot of theory has centered on why, in contrast to other animals, in fish, it is the males that usually provide care. Initially, it was thought that higher rates of paternity, associated with external fertilization in fishes predisposed males to care. However, external fertilization does not protect against paternity loss; sneaker tactics and strong sperm competition have evolved many times in fishes and paternity certainly does not cause male care. Later, it was argued that the order in which gametes are released predisposes male fish to care, because in external fertilizing fishes, females shed eggs before males shed sperm, effectively providing females with an opportunity to desert their partners. Hence, male externally fertilizing

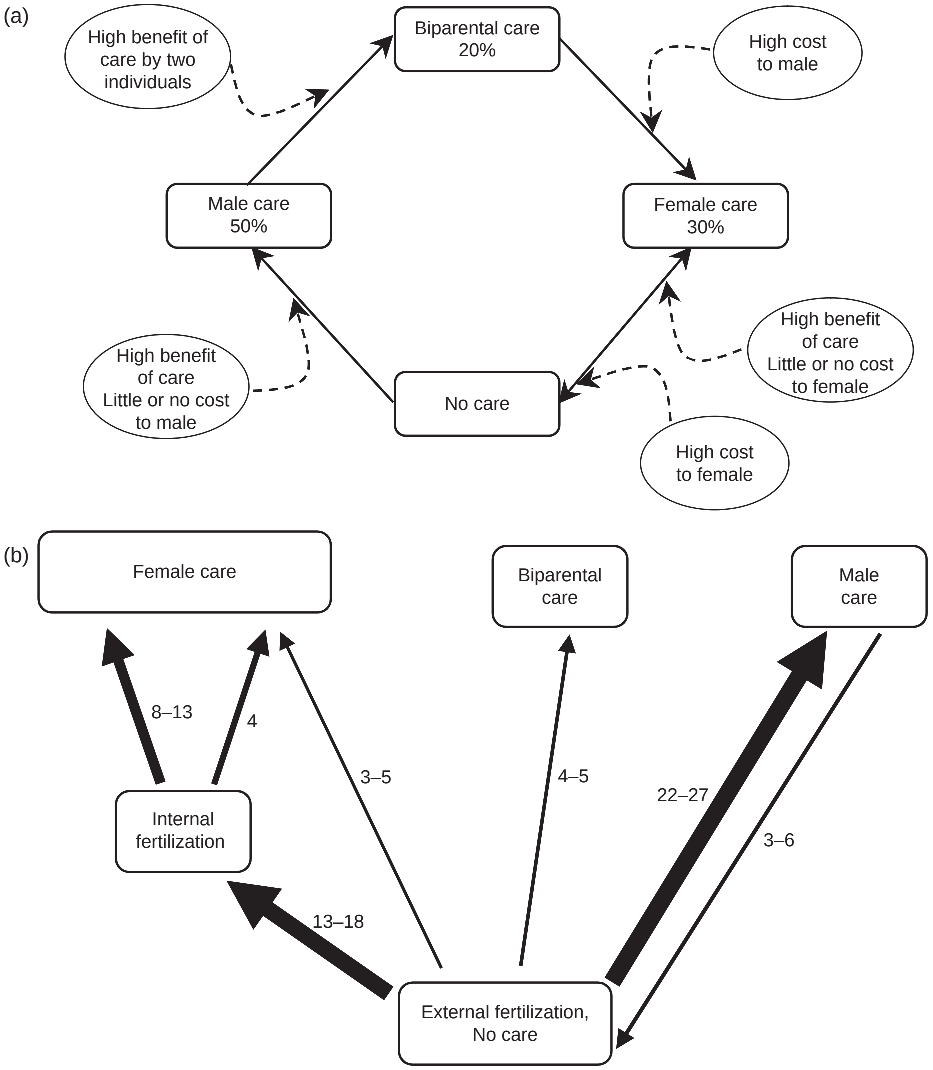


Figure 2 (a) The commonly accepted stepping-stone model of parental-care evolution in fishes with external fertilization (the data are based on 422 families of teleost fish families). There are four possible states of parental care: no care, male, female, or biparental care. The arrows show the likely direction of evolution among states. The dashed arrows refer to the selective factors promoting transitions in care. The percentage of teleost families in each state is shown, and families including species in more than one state are counted more than once. (b) An alternative evolutionary model for transitions among parental care states. Arrow size reflects the number of evolutionary transitions and the numbers by each arrow refer to the minimum and maximum number of possible transitions. The analysis is based on data for 228 families of ray-finned fishes (Actinopterygii). (a) Reproduced from Gross MR and Sargent RC (1985) The evolution of male and female parental care in fishes. *American Zoologist* 25: 807–822, by permission of Oxford University Press. (b) Reproduced from Mank JE, Promislow DEL, and Avise JC (2005) Phylogenetic perspectives in the evolution of parental care in ray-finned fishes. *Evolution* 59: 1570–1578, with permission of John Wiley and Sons.

fish are abandoned in the cruel bind of being left in charge of the babies. However, for the majority of externally fertilizing fishes, both males and females release gametes simultaneously. In some species (e.g., black gobies), caring males release sperm before females lay their eggs and in other species, females provide care even when males release gametes last.

A third hypothesis, known as the association hypothesis, is currently favored for explaining the preponderance of male care in fishes. The simple notion is that the sex with the lowest costs of care will provide care and that these costs of care are lower for the sex already associated with the young. This is the female for internal fertilizing fishes, while it is the male for external fertilizing species with male territory defense. Defending young does not represent a large energetic or time increase over and beyond the cost of defending a territory. Moreover, male reproductive rates are not necessarily curtailed by providing care, because multiple females may spawn with a single male. This is especially true when females are attracted to particular spawning areas because of reduced offspring mortality. In fact, in some fish species, females are most attracted to males that already have eggs in their nests. In contrast to males, the provisioning and protection of young can severely impact female growth and fecundity. Therefore, male care is more common in fishes because the costs of providing care are lower for males than for females.

Costs and Benefits of Parental Care

The answers to all of the above questions rely on understanding the costs and benefits of care. The benefit of parental care is that it improves survival and development of young. The three main costs of parental care are (1) decreased parental survival; (2) increased time until the next breeding attempt; and (3) reduced future fecundity (via suppressed feeding and growth). Experiments with various fish species have demonstrated these costs and benefits.

When parent sticklebacks and cichlids have been experimentally removed, eggs and young fail to survive. Experiments that have manipulated the benefits of parental care (relatedness and number of young) have shown that parents usually can dynamically adjust the level of care in relation to these changes in benefits.

Experimental manipulations have also quantified the costs of care. First, brooding male pipefish (*Syngnathus typhle*) have been shown to be 11 times less likely to survive than nonbrooding males. This may be because parents are more conspicuous or take bigger risks, or because caring is energetically costly while limiting foraging time, which in turn reduces body condition and makes parents more susceptible to predators, disease, and starvation. Second, in the cichlid fish, *Sarotherodon galileaus*, caring males and females have longer interspawn intervals (**Figure 3(a)**) and fewer opportunities to feed than noncaring fish. Caregivers also have a lower body mass, which reduces female fecundity as a result of smaller subsequent clutches compared to those that spawn eggs but do not provide care (**Figures 3(b)** and **3(c)**). Third, in another biparental cichlid, *Herotilapia multispinosa*, Miles Keenleyside showed by varying sex ratios that males desert their clutches far more frequently in female-biased areas, where their probability of mating again is high.

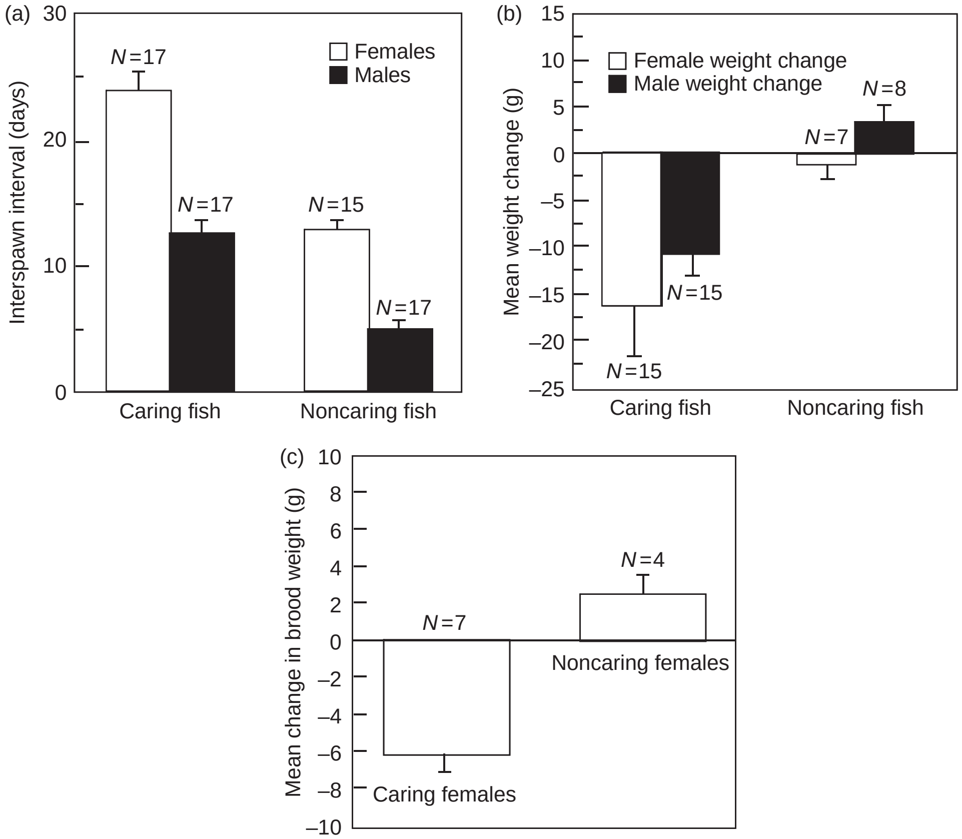


Figure 3 (a) The interspawn interval for caring vs. noncaring male (in black) and female (in white) Galilee St. Peter's fish. (b) Body mass change for caring vs. noncaring St. Peter's fish. (c) A decrease in fecundity was observed for caring but not for noncaring female St. Peter's fish. Reproduced from Balshine-Earn S (1995) The costs of parental care in Galilee St. Peter's fish *Sarotherodon galilaeus*. *Animal Behavior* 50: 1–7, with permission from Elsevier.

Evolutionary Trajectories of Parental Care among Fishes and Phylogenetic Comparisons

Parents and their young have often co-adapted to particular levels of care (constraining care tactics) so that sensible manipulative experiments to tease apart the causes and consequences of care are not always feasible. Fortunately, recent developments in fish phylogenies have allowed phylogenetic-based comparative studies to provide a complementary, powerful way to understand the evolution of parental care.

For 30 years, the most widely accepted hypothesis for the evolution of parental care in fishes has been a stepping-stone model in which care evolved from an ancestral state of no care to biparental care via two intermediate stages of female-only and male-only care (**Figure 2(a)**). While within-family phylogenetic-based comparative studies have largely supported this stepping-stone model, a recent cross-family comparison by Mank and co-workers, with representatives across 224 fish families, found no evidence for the stepping-stone model (**Figure 2(b)**). Instead, they showed that both female and male care have arisen from the precursor state of no care in fishes.

Transitions between care states are thought to have arisen via changes to the costs and benefits of parental care. For example, biparental care is thought to arise from male-only care when females lay very large eggs and the benefits of defense by two parents outweighs the female-fecundity costs of care. Biparental care is argued to lead to female-only care when male future mating opportunities increase. Female-only care is thought to have evolved from no care when the fecundity costs of providing care decrease and the benefits of care in terms of offspring survival surpass these costs. Comparative studies can test these ideas, examine what factors promote transitions in care, and explore the direction of change. For example, an analysis of 222 genera of cichlid fishes revealed 21–30 shifts from biparental to female-only care within the Cichlidae family. This removal of male care from the biparental state is related to a reduction in the need for biparental defense.

Physiological Mechanisms Underlying Fish Parental Care

There are still major gaps in our understanding of parental-care regulation in fishes. We know that a number of social, environmental, and physiological factors work together in a complex fashion to influence parental care. To date, few species have been physiologically studied and only a small handful of hormones and neuropeptides have been investigated in terms of how they mediate parental behavior.

Nest Building

Nest building can be considered a parental behavior because a well-placed, well-built nest can enhance the development of young. Nest building often occurs with territoriality and courtship in male fishes, and has been associated with high plasma androgen levels (0.85–400 ng ml^{-1}). For example, when male Siamese fighting fish, *Betta splendens*, build mucus-lined bubble nests and male three-spined sticklebacks weave vegetation nests together with glue from the special kidney protein spiggin, both of these actions are thought to be under the control of androgens (see **Figure 1(a)**; **see also Social and Reproductive Behaviors**: Sexual Behavior in Fish).

Defense

Male caring fishes, such as sticklebacks, damselfish, girabali, bluegill sunfish, plainfin midshipman, and the black-chinned tilapia, have high plasma androgen levels (testosterone and 11-ketotestosterone) during pre-spawning, when males compete for territories, construct nests, and court females. The androgen levels then gradually drop following spawning while males provide care. This was believed to indicate an androgen-mediated trade-off between aggression and parental care and a minimal role of androgens during parental care.

However, recent studies show that androgen levels often rise again to pre-spawning levels once eggs have hatched. Other studies even show that androgens remaining high in the early stages of care, correlated with the frequency of parental care/defense of young in the biparental cichlid *N. pulcher*. In addition, experimental elevation of androgen levels does not inhibit paternal behavior in a number of fish species, suggesting that an elevated androgen level is not necessarily incompatible with the expression of paternal behavior. This may be true of fish in general, but not other vertebrates. Male fish often continue to court and attract females even after they have begun to provide care, but in other taxa, the mating/courtship phase of reproduction is commonly temporally separated from the parental phase. Fish do not typically feed young, but instead defend young against predators (and sometimes fan the eggs); so, high androgen levels might, in fact, be beneficial and necessary for the aggression needed during parental care. More research is needed to clarify the role of androgens in mediating parental behavior with the importance of estrogen and mechanisms that modulate female care remaining particularly understudied.

Fanning and Brooding

The internal physiological mechanisms determining parental fanning behaviors remain largely unexplored.

Prolactin stimulates fanning behavior in the three-spined stickleback and in bluegills, where administration of a prolactin inhibitor reduces fanning and defense of offspring. In the mouth-brooding cichlid, *Oreochromis niloticus*, elevated levels of prolactin are seen in the brain and plasma during the brooding period, and in the cichlid, *Symphysodon discus*, injections of prolactin cause an increase in egg fanning and the production of mucus secretions on which newly hatched fry feed (**see also Social and Reproductive Behaviors**: Nutritional Provision During Parental Care). In *S. discus*, prolactin may, therefore, increase the production of mucus for young to feed from, in a manner similar to the role of prolactin in increasing milk production in mammals. Other hormones that have been associated with reproductive behaviors include arginine vasotocin (AVT) and isotocin (**see also Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes) and investigations into their role during parental care are warranted.

Future Research Directions and Conclusions

This article makes two main points. First, nowhere in the animal kingdom is the diversity of parental-care habits greater than among fishes. This diversity has meant that fishes will continue as extremely useful model organisms for clarifying our theoretical understanding of the evolutionary origins of parental care (i.e., who cares?) and revealing key factors influencing the adaptive amount of care (i.e., how much to care?). The idea of reproductive trade-offs (the costs and benefits of care) has provided a rich theoretical test bed for exploring the evolution of parental care in fishes. This approach has moved parental-care research from a descriptive qualitative science into a strongly quantitative predictive one.

Fishes also offer a wonderful opportunity to explore parental care's strong and intimate link with sexual selection. Parental care promotes the survival and vigor of young (so will clearly be influenced by natural selection), but in fishes can be often strongly influenced by sexual selection as well. The best parent, the one providing the most vigorous care, is often preferred as a mating partner. A fruitful avenue for future parental-care fish research will be to clarify the interactions between natural and sexual selection and more specifically to investigate the degree to which parental care (egg tending, defense, and nest building) can be viewed as a sexual ornament enhancing mate attraction.

Second, we know little about the physiology modulating care behavior in fishes. The scarce research in this area has largely focused on androgens and paternal care while the neuroendocrinological mechanisms and genetics modulating biparental and female care remain largely unexplored. There is an urgent need to expand physiological studies to encompass a larger range of hormones, more model fish species, and different care behaviors (e.g., fanning, offspring retrieval, and feeding). New brain-imaging techniques and the sequencing of entire genomes may provide powerful new insights on the neurophysiological systems and molecular changes that occur during parental care. An integration between ultimate and proximate analyses will undoubtedly shed light on the how and why of parental care in fishes. Understanding the physiological processes of fish parental behavior and their link to population biology and the health of fisheries will arm us with a valuable roadmap to navigate the possible impacts of all too frequent anthropogenic changes to environments and ecosystems.

See also: **Social and Reproductive Behaviors**: Nutritional Provision During Parental Care; Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes.

Further Reading

Balshine-Earn S (1995) The costs of parental care in Galilee St. Peter's fish *Sarotherodon galilaeus*. *Animal Behavior* 50: 1–7.

Baylis J (1981) The evolution of parental care in fishes, with reference to Darwin's rule of male sexual selection. *Environmental Biology of Fishes* 6: 223–251.

Blumer LS (1979) Male parental care in the bony fishes. *Quarterly Review of Biology* 54: 149–161.

Clutton-Brock TH (1991) *The Evolution of Parental Care*. Princeton, NJ: Princeton University Press.

Dawkins R and Carlisle TR (1976) Parental investment, mate desertion and a fallacy. *Nature* 262: 131–133.

Goodwin NB, Balshine-Earn S, and Reynolds JD (1998) Evolutionary transitions in parental care in cichlid fish. *Proceedings of the Royal Society of London Series B* 265: 2265–2272.

Gross MR (2005) The evolution of parental care. *Quarterly Review of Biology* 80: 37–45.

Gross MR and Sargent RC (1985) The evolution of male and female parental care in fishes. *American Zoologist* 25: 807–822.

Jones JC and Reynolds JD (1999) The influence of oxygen stress on female choice for male nest structure in the common goby. *Animal Behaviour* 57: 189–196.

Keenleyside MHA (1985) Bigamy and mate choice in the biparental cichlid fish, *Cichlasoma nigrofasciatum*. *Behavioural Ecology and Sociobiology* 17: 285–290.

Kindler PM, Philipp DP, Gross MR, and Bahr JM (1989) Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus*: Centrarchidae). *General and Comparative Endocrinology* 75: 446–453.

Knapp R, Wingfield JC, and Bass AH (1999) Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). *Hormones and Behaviour* 35: 81–89.

Lindstrom K and St. Mary C (2008) Parent care and sexual selection. In: Magnhagen C, Braithwaite VA, Forsgren E, and Kapoor BG (eds.) *Fish Behaviour*, pp. 377–410. Enfield, NH: Science Publishers.

Magee SE, Neff BD, and Knapp R (2006) Plasma levels of androgens and cortisol in relation to breeding behavior in parental male bluegill sunfish, *Lepomis macrochirus*. *Hormones and Behavior* 49: 598–609.

Mank JE, Promislow DEL, and Avise JC (2005) Phylogenetic perspectives in the evolution of parental care in ray-finned fishes. *Evolution* 59: 1570–1578.

Páll MK, Mayer I, and Borg B (2002) Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus* II. Castration and 11-ketoandrostenedione effects on courtship and parental care during the nesting cycle. *Hormones and Behavior* 42: 337–344.

Ros AFH, Bruintjes R, Santos RS, Canario AVM, and Oliveira RF (2004) The role of androgens in the trade-off between territorial and parental behavior in the Azorean rock-pool blenny, *Parablennius parvicornis*. *Hormones and Behavior* 46: 491–497.

Specker JL and Kishida M (2000) Mouthbrooding in the black-chinned tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae): The presence of eggs reduces androgen and estradiol levels during paternal and maternal parental behavior. *Hormones and Behavior* 38: 44–51.

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed.) *Sexual Selection and the Descent of Man 1871–1971*, pp. 136–179. Chicago, IL: Aldine.

Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist* 100: 687–690.

Nutritional Provision During Parental Care

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

J Buckley, University of Plymouth, Plymouth, UK

© 2011 Elsevier Inc. All rights reserved.

Why Provide Nutrients?
Future Directions
Further Reading

Glossary

Adelphophagy Where offspring feed on other embryos while still inside the mother.

Gestation The carrying of an embryo or fetus inside an ovoviviparous or viviparous parent.

Immunoglobulin An antibody playing a critical role in immunity.

Lecithotrophy Feeding on egg yolk or substances put into the egg by the mother.

Oophagy (egg eating) Where offspring feed on other eggs while still inside the mother.

Oviparity Production of eggs that develop and hatch after being released into the external environment.

Ovoviviparity Production of eggs that develop and hatch within the mother before being released as live young to the external environment.

Pseudoplacenta A structure which functions like a placenta, transferring nutrients from parent to offspring.

Trophotaeniae Vascular processes of an embryo that establish a placental relationship with the mother.

Vitellogenins Precursors of the major egg yolk proteins comprised of up to five different domains, each of which corresponds to one of the stored yolk proteins (see also yolk proteins). Vitellogenins are large (> 350 kDa), homodimeric phospholipoglycoproteins. Common abbreviations for vitellogenin are Vg, VG, and Vtg.

Viviparity Production of live young that develop before being released into the external environment.

Why Provide Nutrients?

A myriad of parental care strategies exist in fish (**see also Social and Reproductive Behaviors**: Parental Care in Fishes), ranging from the simple protection of eggs to more complex forms such as the provision of resources via a placental connection and offspring developing internally. In its broadest sense, parental care can involve any parental behavior that serves to maximize the survival of offspring. This can include behaviors whereby parents physically guard and protect both eggs and fry from predators through to the preparation of nests, and to mouth brooding. Besides providing physical protection to offspring, a number of strategies have also evolved to aid offspring survival through the parental provision of nutrients.

Fish hatch and begin development in what can be considered a hostile environment, frequently lacking an abundance of food and fraught with predators and pathogens. Newly hatched fish are often ill-equipped to deal with these problems due to a lack of development in immune organs and of locomotory systems that facilitate predation evasion and food capture; this period of early development is, therefore, considered one of the most delicate and dangerous periods of a fish's life where mortality rates are often highest.

One way parents can reduce the time offspring spend in this delicate period of development is to provide a form of nutrition to allow offspring to grow and develop quickly, thereby reducing their period of vulnerability. Parentally provided nutrition can be delivered to offspring during either the pre- or posthatch/birth period.

Prehatch/Birth-Provided Nutrition

The prehatch/birth provision of nutrients is the most common form of parental provision seen in fish. There are several variations including external development strategies such as the laying and provisioning of eggs with egg yolk, as seen in oviparous fish, to internal development strategies, such as the formation of a placental relationship between parent and offspring.

External development

Oviparous fish, those that lay eggs, provide their offspring with nutrients within the egg yolk, through a process known as lecithotrophy. Here, the majority of energy for development is derived from the yolk protein vitellogenin. Other non-nutritional factors such as hormones and immunoglobulins may also be present in the yolk, with recent work demonstrating the importance of maternally provided immunoglobulins for protection against pathogens. There are also trends, as seen in at least four families of oviparous fish (Cichlidae, Percidae, Salmonidae, and Centrarchidae), suggesting that egg size and the provision of egg yolk are positively correlated with the quality and duration of subsequent parental care – care which often involves egg guarding or mouth brooding of fry after hatch (**see also Social and Reproductive Behaviors**: Parental Care in Fishes).

Internal development

Besides providing offspring with a source of nutrition, internal development can be highly beneficial to the survival of offspring as it allows offspring to be released to the external environment at a more advanced stage of development; the vulnerability of very small embryos and larvae to predators is, therefore, reduced and parents can ensure that their offspring have an increased chance of survival at birth.

Fish that give birth to live young can be divided into ovoviviparous fish, where fertilized eggs develop and hatch inside the female, and true viviparous fish where embryos develop inside the mother (or father) without an egg case, most commonly with some kind of placental relationship. Nutrients may be provided either through direct parental contribution or through yolk stores (i.e., lecithotrophy) or a combination of both.

Oophagy and adelphophagy

Most elasmobranchs (sharks and rays) that display parental care are ovoviviparous. In this case, eggs hatch within the mother and are later released live into the environment. Developing embryos depend on their own yolk stores for energy but may also display a cannibalistic behavior where they feed upon sibling developing ova (oophagy) and embryos (adelphophagy). Oophagy is far more common than adelphophagy which has only conclusively been demonstrated in the sandtiger shark, *Carcharias taurus*.

In elasmobranchs where developing offspring supplement their energy reserves through oophagy, it is common for the female to continue ovulating during the gestation period to supply developing embryos with eggs. In the porbeagle shark, *Lamna nasus*, small developing embryos possess fangs designed for tearing open egg capsules (**Figure 1**). The embryos often have distended stomachs from the yolk they have ingested and in some cases the stomach may protrude between muscle layers and stretch the abdominal skin. Later in development, the stomach shrinks but leaves a characteristic scar on the abdomen, indicative of oophagy. The fangs possessed by the young embryos are present on both the upper and lower jaw and are progressively shed and replaced. The female continues to ovulate during the period when the embryos possess these fangs but appears to stop during the latter parts of gestation.

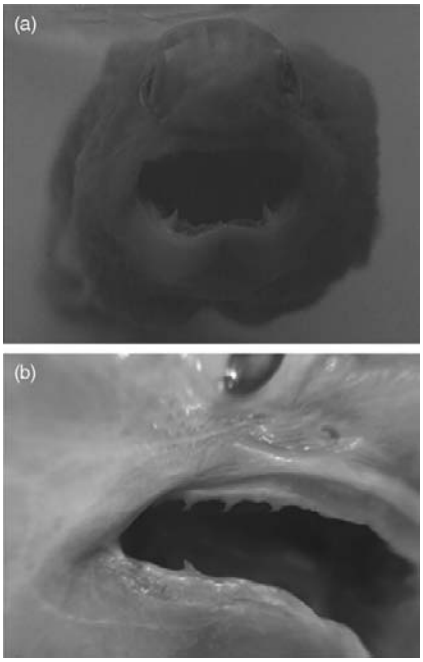


Figure 1 Anterior view of the heads of (a) 9.6-cm embryo and (b) 26.4-cm female *Lamna nasus* embryo showing the functional fangs. Photo (a) by G. Duhamel. Reproduction, embryonic development, and growth of the porbeagle shark, Lamna nasus, in the southwest pacific ocean, from Francis MP and Stevens JD (2000), Reproduced Fishery Bulletin 98: 41–63.

Oophagy has also been documented in the shortfin mako, *Isurus oxyrinchus*, the longfin mako, *I. paucus*, the great white shark, *Carcharodon carcharias*, and the thresher shark, *Alopias vulpinus*. The thresher shark has a gestation period of about 2 years with the young being released from the mother at about 60 cm in length and up to 10 kg in weight. Five or less individuals will survive until birth with many fertilized eggs being cannibalized during development. Searching behavior in the uterus when embryos are looking for eggs to eat is believed to occasionally result in injuries to other embryos within the uterus. This searching behavior may indicate the origins of adelphophagy.

Several ovoviviparous rays rely on histophagy as an additional source of nutrition for developing offspring. In this case, the uterus of the female develops vascularized appendages known as trophonemata which secrete a uterine milk-like substance rich in protein and lipid that can be ingested by the embryos. In some species of shark such as the smooth dogfish, *Mustelus canis*, nutrient provision to the developing embryo develops into a more direct placental relationship. Smooth dogfish embryos utilize yolk reserves for the first 3 months of gestation, at which point a placenta develops to provide energy until birth which occurs about 7–8 months later.

Placental relationships

Among the elasmobranchs, those species which show true placental relationships all belong to the family Carcharhinidae and tend to be tropical or subtropical. For example, in several species of hammerhead shark, *Sphyrna* spp., a placental cord develops between the mother and developing offspring with fine processes that aid in the absorption of maternal secretions.

There are also many teleosts where placental relationships develop between parent and embryo. For example, in the butterfly goodeid, *Ameca splendens*, embryos develop in the ovarian lumen of the female and develop a placental association with the mother. The placenta consists of an embryonic component known as the trophotaeniae and a maternal component derived from the internal ovarian epithelium. This joining of mother and embryo allows for nutritional provision to offspring during gestation.

Fish from the family Syngnathidae, the pipefish and seahorses, have a unique form of parental care that in some species involves a pseudoplacental relationship. Here, parental care is carried out by the male and during mating the female deposits her eggs on a brooding area or pouch where they are then fertilized by the male. The complexity of brooding structures in this family is very variable ranging from simple unprotected ventral areas to which the eggs are attached to well-defined pouches where the eggs are sealed from the external environment. In the pipefish *Solegnathus* and *Doryrhamphus* eggs are brooded in individual membranous compartments and in pipefish from the genus *Syngnathus*, a well-defined pouch partially or fully encloses the eggs. Seahorses, *Hippocampus* spp., display the most modified brood structures within this family consisting of sac-like fleshy pouches which are completely enclosed.

In seahorses, following intra-pouch fertilization, eggs implant into the brood pouch wall and form a pseudoplacental relationship with the father. The wall of the brood pouch undergoes morphological changes including a high level of vascularization (**Figure 2**), which is believed to aid gas exchange between the embryo and paternal blood supply. In sealing the brood pouch, the male is able to regulate the osmolality of the brood pouch environment to aid embryo osmoregulation. Gestation is variable in these species, lasting between 9 and 69 days depending on external temperatures. It has been noted that at birth the pseudoplacenta may be expelled along with juveniles.

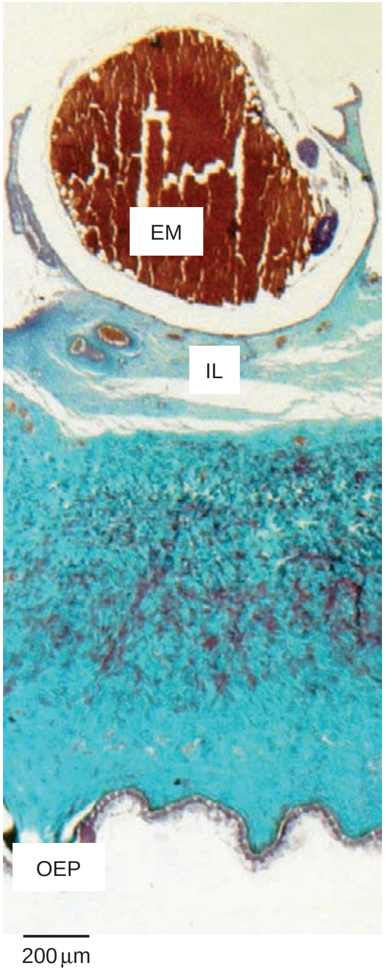


Figure 2 Cross section of an embryo-carrying stage brood pouch of the spotted seahorse, *Hippocampus kuda*, showing the embedded embryo (EM), distended inner epithelial and inner tissue layers (IL), and the folded outer epithelial layer (OEP). Reproduced with permission from Laksanawimol P, Damrongphol P, and Kruatrachue M (2006) Alteration of the brood pouch morphology during gestation of male seahorses, *Hippocampus kuda*. *Marine and Freshwater Research* 57: 497–502, copyright © CSIRO 2006.

There are several disadvantages of viviparity and placental relationships which include the reduced number of offspring that can be supported in this way and sometimes long gestation periods. Predation or disease in parents carrying offspring during gestation can potentially lead to the loss of both parents and offspring. However, placental relationships do allow a large amount of nutritional investment from the parent into their offspring, increasing the fitness and size of offspring at birth and, therefore, their survival chances. For example, the embryo dry mass of the butterfly goodeid increases remarkably by about 150-fold during the gestation period. This considerable energetic investment by the parent provides a remarkable fitness advantage to the offspring entering the competitive environment outside its parent.

Posthatch-Provided Nutrition

Oviparous parents may opt to supplement offspring nutrition after hatch. This can be extremely beneficial to the offspring during the first few weeks of development to ensure that nutritional requirements are met.

Mucus feeding

It is estimated that about 30 species of fish, predominantly from the family Cichlidae, provide nutrients to their offspring by allowing them to feed off their epidermal mucus secretions. The parent–offspring trophic transfer is associated with a parent-touching behavior. Examples of fish that display these parent-touching behaviors include the long-fin cichlid, *Cichlasoma longimanus*, the blue-eye cichlid, *C. spilurum*, the orange chromide, *Etroplus maculatus*, the midas cichlid, *C. citrinellum*, the red discus, *Symphysodon discus*, and the blue discus, *S. aequifasciatus*. It has also been suggested that some mouth-brooding cichlids may allow offspring to feed off mucosal secretions in their mouths. However, few studies have conclusively demonstrated a transfer of nutrients between parents and offspring. Parent-touching behavior of fry can be related to many other factors including a fear response and intraspecific communication, and it is also difficult to observe feeding in mouth-brooding cichlids. Here, we focus on the mucus feeding of the midas cichlid, discus fish, and some mouth-brooding *Tilapia* spp. where evidence exists for direct transfer of nutrients.

The midas cichlid is a substrate spawner where young begin free swimming about 1 week after the eggs are spawned. About 4 days posthatch, the young fry begin contacting their parents and micronip at their parents' bodies. This behavior continues until the young are about 5 weeks old. As the young grow, lesions on the parents may become apparent as mucus is continually removed. Survival of the offspring does not appear to be dependent on the provision of mucus and so this form of parental care may not necessarily occur under all circumstances. In the black-chinned tilapia, *Sarotherodon melanotheron*, the male picks up the eggs once they have been fertilized and after about 5 days of incubation in his mouth the eggs hatch and the hatched embryos remain in his mouth for just over a week. Brooding behavior lasts about 2 weeks in total and it has been hypothesized that due to the high levels of vitellogenin in the mucus of the male parent there is some provision of nutrients to the offspring during this brooding period.

The clearest example of mucus feeding by offspring is shown by the discus fish where mucus feeding appears to be obligate for offspring survival. Here, care is biparental with both parents providing epidermal mucus secretions. Eggs are laid and fertilized, attached to a hard surface, and both parents take turns in guarding and fanning the eggs (**Figure 3(a)**). Once hatched, the parents pick up the fry with their mouths and transfer them to a hard surface which the young adhere to with sticky cement glands on their head (**Figure 3(b)**). After approximately 4 days, fry become free swimming and promptly move to their parents' sides where they start to feed off mucus secretions (**Figure 3(c)**) (see **Video Clip 1**). Both parents are capable of feeding the fry and take turns by flicking all of the fry from one parent to another. In some pairs, parental care can be divided evenly between parents while in other pairs one parent may dominate offspring care. Fry will start consuming other food articles after about 1 week of feeding exclusively off their parents and by about 5 weeks posthatch are no longer consuming parental mucus.

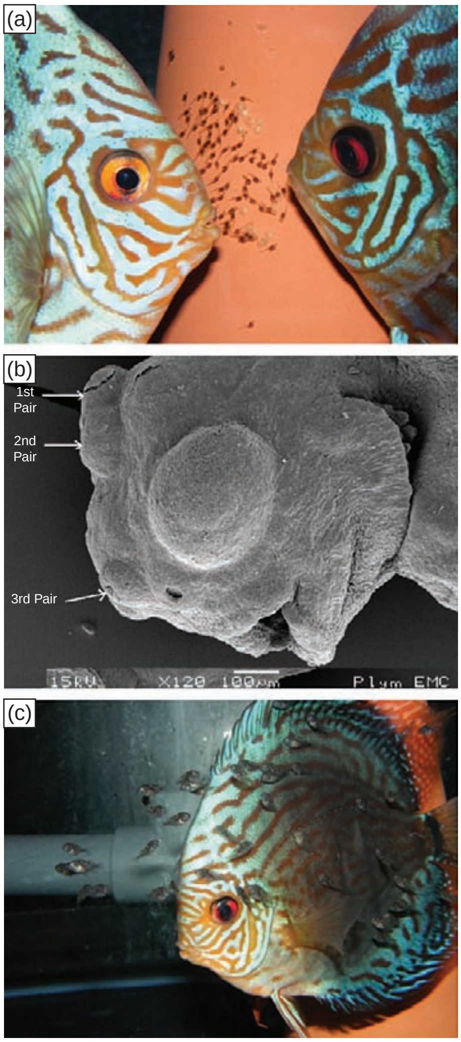


Figure 3 (a) Male and female discus parents guarding newly hatched fry. (b) Scanning electron microscope picture (120× magnification, scale bar represents 100 µm) of a discus fry showing the three pairs of cement glands used for attaching to substrates. (c) Discus fry, 1-week old, feeding from the mucus of a male discus fish. Photohraphs courtery of Dr. Richard Maunder and microscope image taken by Jonathan Buckley.

Fish mucus is known to contain many biologically active peptides and proteins and it is likely that mucus feeding may provide offspring with an array of nutritional and nonnutritional factors. Amino acids are a crucial part of the nutritional requirement of developing offspring. As many as 10 essential amino acids have been identified in the mucus of discus, including high levels of lysine and phenylalanine. It is, therefore, quite probable that the early needs of discus are met by the parental provision of basic peptides and amino acids in the mucus. Free amino acids are more easily digested by fry in comparision with complex proteins, which require energy-demanding catabolism for uptake. The early digestive system of discus larvae appears to be devoid of both an acidic environment and the digestive protease pepsin required to break down more complex proteins. Therefore, the behavior of fry feeding on parental mucus may give them access to a supply of free amino acids until their digestive system develops well enough to handle more complex proteins. By the time the fry have been feeding on parental secretions of mucus for 15–20 days, their digestive system has developed from an alkaline environment to an acidic environment containing several pepsin-like proteases. At this point, as the fry start to feed on alternative food sources, it appears that they are able to digest more complex nutritional items.

Beyond nutritional benefits

Several species of fish are known to provide some degree of passive immunity to their offspring via egg-yolk reserves and it is likely that the provision of mucus for young is an additional way of boosting offspring immunity. In the blue tilapia, *Oreochromis aureus*, it has been shown that mouth brooding, where offspring feed off mucus secretions in the mouth of the mother, increases the survival chances of the fry when the mother's immune system is enhanced through vaccination. Several studies have documented the presence of immunoglobulin (IgM) in cutaneous mucus of fish suggesting that a passive immunity could be passed on to the offspring via mucus. In mucus of the discus there is a c-type lectin uniquely expressed in parental mucus as opposed to nonbreeding adult mucus. The c-type lectins function as the preimmunity first line of defense by recognizing carbohydrate patterns found on the surface of a large number of pathogenic microorganisms. Potentially this c-type lectin could be transferred to offspring to provide them with a form of pathogenic protection.

As with immunity, there is also evidence in many fish species that transfer of maternal hormones to offspring occurs via egg reserves. It is also possible that mucus feeding may supply offspring with hormones. In the midas cichlid, prolactin, growth hormone, and thyroid hormones have been identified in parental mucus. Thyroid hormones are likely to enhance growth and survival in offspring and prolactin plays an important role in osmoregulation.

Provision of nutrients to developing offspring after hatch in these oviparous species can increase the survival chances of offspring. Some protection from predation may also be associated with these forms of nutrient provision. In particular, mouth-brooding parents can easily transport their fry away from predation threats and move the whole family to safer areas.

Parent–Offspring Conflict

There is no doubt that the provision of nutrients during parental care has an energetic cost. Extended periods of larval care in discus fish, for example, often negatively affect their subsequent reproductive performances. This energy drain can lead to conflicts between parents and offspring. For example, excessive parental investment in one brood of offspring may influence their ability to invest in future broods. An individual wishing to maximize their inclusive fitness would want to invest in current offspring up until the point where any further investment would only offer diminishing returns. Any investment past this point may squander energy that would have a greater return if invested into future offspring. Too much parental investment into current offspring will reduce the total number of an offspring that an individual could ultimately produce; this equates to a reduction in an individual's inclusive fitness. It is expected that parents should regulate the amount of care they provide to their current offspring to maximize their own fitness. Offspring, however, are also concerned with maximizing their own fitness and should seek to solicit more care than a parent wants to give.

Without a form of communication, it would be quite easy for a parent to either under- or over-provide parental care. It is possible in mucus-feeding fish that mechanical stimulation of the parental epidermis is itself a mechanism by which offspring communicate their need. Under aquarium conditions, it has been observed that adult discus fish show evidence of epidermal damage after a full term of parental care. This is due to an increase in both the size and feeding rate of discus offspring over the period of parental care, factors which serve to exacerbate the potential for mucosal feeding to cause noticeable epidermal damage. Supplementation of fry with live feed, however, reduces the impact of fry mucosal feeding. In viviparous vertebrates, there is evidence that fetal offspring can secrete compounds, most often hormones, to manipulate parental nutrient provision through placental connections. Thus, the apparently cooperative relationship between developing offspring and parent reveals itself to be more of a conflict between two different parties for survival.

Future Directions

The role of parental care in fish has been well researched (**see also Social and Reproductive Behaviors**: Parental Care in Fishes) and within mammalian systems the concepts of nutrient provision to offspring and parent–offspring conflict is well characterized. However, information on nutrient provision to offspring both before and after hatch/birth and its associated behaviors has received less attention in fish. There are inherent problems associated with studying these behaviors, for example, in observing the feeding behaviors of fry inside the mouths of their parents or developing offspring within parental reproductive organs. Many species showing oophagy or adelphophagy are large pelagic elasmobranchs where little is known about their reproductive biology in general. Research into the parent–offspring nutritional relationship in species that are more easily reared and observed in the laboratory environment, such as discus and seahorses, is now increasing with the potential to shed light on as-yet un-researched areas of parent–offspring conflict and the evolution of parental care in fish.

See also: **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food. **Social and Reproductive Behaviors**: Parental Care in Fishes.

Further Reading

Barnett C (1977) Chemical recognition of the mother by the young of the cichlid fish, *Cichlasoma citrinellum*. *Journal of Chemical Ecology* 3: 461–466.

Bone Q and Moore RH (2008) *Biology of Fishes*, 3rd edn. New York: Taylor and Francis.

Chong K, Ying TS, Foo J, Jin LT, and Chong A (2005) Characterisation of proteins in epidermal mucus of discus fish (*Symphysodon* spp.) during parental phase. *Aquaculture* 249: 469–476.

Clutton-Brock TH (1991) *The Evolution of Parental Care*. Princeton, NJ: Princeton University Press.

Crespi B and Semenuik C (2004) Parent–offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* 163: 635–653.

Francis MP and Stevens JD (2000) Reproduction, embryonic development, and growth of the porbeagle shark, *Lamna nasus*, in the southwest Pacific Ocean. *Fishery Bulletin* 98: 41–63.

Gross MR (2005) The evolution of parental care. *Quarterly Review of Biology* 80: 37–45.

Hildemann WH (1959) A cichlid fish, *Symphysodon discus*, with unique nurture habits. *American Naturalist* 93: 27–34.

Kishida M and Specker JL (2000) Paternal mouthbrooding in the black-chinned tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae): Changes in gonadal steroids and potential for vitellogenin transfer to larvae. *Hormones and Behaviour* 37: 40–48.

Kolliker M and Richner H (2001) Parent–offspring conflict and the genetics of offspring solicitation and parental response. *Animal Behaviour* 62: 395–407.

Masonjones HD (2001) The effect of social context and reproductive status on the metabolic rates of dwarf seahorses (*Hippocampus zosterae*). *Comparative Biochemistry and Physiology* 129: 541–555.

Laksanawimol P, Damrongphol P, and Kruatrachue M (2006) Alteration of the brood pouch morphology during gestation of male seahorses, *Hippocampus kuda*. *Marine and Freshwater Research* 57: 497–502.

Lombardi J and Wourms JP (2005) The trophotaenial placenta of a viviparous goodeid fish. II. Ultrastructure of trophotaeniae, the embryonic component. *Journal of Morphology* 184: 293–309.

Mor A and Avtalion RR (1988) Evidence of transfer of immunity from mother to eggs in tilapias. *Israeli Journal of Aquaculture – Bamidgeh* 40: 22–28.

Noakes DLG (1979) Parent-touching behaviour by young fishes: Incidence, function and causation. *Environmental Biology of Fishes* 4: 389–400.

Noakes DLG and Barlow GW (1973) Ontogeny of parent-contacting in young *Cichlasoma citrinellum* (Pisces, Cichlidae). *Behaviour* 46: 221–255.

Schütz M and Barlow GW (1997) Young of the midas cichlid get biologically active non-nutrients by eating mucus from the surface of their parents. *Fish Physiology and Biochemistry* 16: 11–18.

Takemura A and Takano K (1997) Transfer of maternally-derived immunoglobulin (IgM) to larvae in tilapia, *Oreochromis mossambicus*. *Fish and Shellfish Immunology* 7: 355–363.

Trivers RL (1974) Parent–offspring conflict. *American Zoologist* 14: 249–264.

Wilson AB, Vincent A, Ahnesjö I, and Meyer A (2001) Male pregnancy in seahorses and pipefish (family Syngnathidae): Rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. *Journal of Heredity* 92: 159–166.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

BEHAVIOR AND PHYSIOLOGY

Sensory Systems, Perception, and Learning

Contents

How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling
Communication Behavior: Visual Signals
Shocking Comments: Electrocommunication in Teleost Fish
Fish Learning and Memory
Nociception or Pain in Fish
Spatial Orientation: How do Fish Find their Way Around?
Magnetic Sense in Fishes
Circadian Rhythms in Fish

How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling

IM Kaatz, Stamford, CT, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction to Vocal Fishes: Quiet versus Loud Vocalizers
Discovery and Evolution of Vocal Fish Behavior
Fish Call Characteristics: Simple versus Complex
Chorusing Behavior: Schooling, Spawning, and Feeding
Reproductive Sound Signaling: Male Display and Female Mate Choice
Agonistic Sound Signaling within Species
Avoiding and Repelling Predators
Sound Signals Relative to Other Communication Modalities
Conclusions: What Fish Sounds Can and Cannot Do
Further Reading

Glossary

Agonistic Aggressive behavioral conflicts or threat displays that include fights between individuals, territory defense, attacks, and chases.

Bioacoustic parameter The amplitude (loudness), temporal (time or duration), or frequency (spectrographic) characteristics that describe a sound signal.

Call Sound signal associated with a particular posture, display, or context, for example, courtship call produced when a male displays to a potential female mate.

Communication Patterns of energy produced by a sender, perceived and decoded by the sensory apparatus of a receiver, and used to exchange information cooperatively or manipulate.

Communication modality Signals of a particular type often classified by their sensory perception category such as electric, chemical, tactile, visual, or vocal.

Display Stereotypical behavior patterns that may consist of one or several different postures and signals for communicating in both intra- and interspecific contexts.

Disturbance Behavioral context where physical restraint by a predator may cause sound production by prey or release of sounds similar to signals elicited by human handling.

Interception Communication signal perceived by an individual receiver for whom it was not intended and whose behavior changes in response to it.

Signal Energy pattern (sound, chemical, etc.) evaluated by a receiver that can convey false or honest information about physiological condition, social status, or motivational state of a sender.

Vocal Production of a sound signal for communication that may be alternatively designated as acoustic, sonic, or soniferous.

Introduction to Vocal Fishes: Quiet versus Loud Vocalizers

We are still in an age of discovering how many vocal fish taxa there are. Estimates of sound producing fish diversity identify over 1300 species. This number is likely to be much higher because many fishes have never been monitored with close proximity audio-visual recording techniques. While fish sounds are among the least complex in pattern and diversity among vertebrates, their importance in intraspecific communication is the same as for other vocal animals, functioning in social spacing, resource defense, mate attraction, species recognition, individual recognition, female mate choice, obstacle avoidance, and predator deterrence (**Table 1**; **see also Hearing and Lateral Line**: Acoustic Behavior).

Many fishes have impressive vocal communication abilities that are readily obvious to human observers and have been the focus of active research programs. For example, the Atlantic toadfish (*Opsanus tau*; **Figure 1**) generates sound using the fastest known contracting vertebrate muscle, which is attached to its swimbladder. The domino damselfish (*Dascyllus albisella*) male produces sounds that propagate up to 12 m in coral reef habitats. The plainfin midshipman (*Porichthys notatus*) can perform 1-h-long displays of sounds that can penetrate boat hulls and regularly alarm new boaters in California! The Atlantic cod (*Gadus morhua*) can produce startle clicks with peak call pressures as high as 153B at a 1-m distance.

Table 1 Behavioral contexts for fish sound signal production and the number of families they are currently known from

Behavioral context	*# Families*	*Sound signal function*
Schooling or chorusing	35	Mate assessment? Predator confusion?
Reproductive	35	Individual and species recognition Female mate assessment and preference Mate attraction and localization Male advertisement Male–female alert? Reproductive priming
Agonistic	57	Feeding competition? Fighting opponent assessment Nest defense Social dominance Territory defense
Disturbance or distress	107	Conspecific alarm? Distress noise? Other predator attraction to disrupt attack? Predator escape stotting, startle or warning? Release of social sounds
Feeding	22	Conspecific recruitment or competition?
Swimming	20	Obstacle avoidance? Predator alert?

Question marks denote hypothesized rather than confirmed functions.

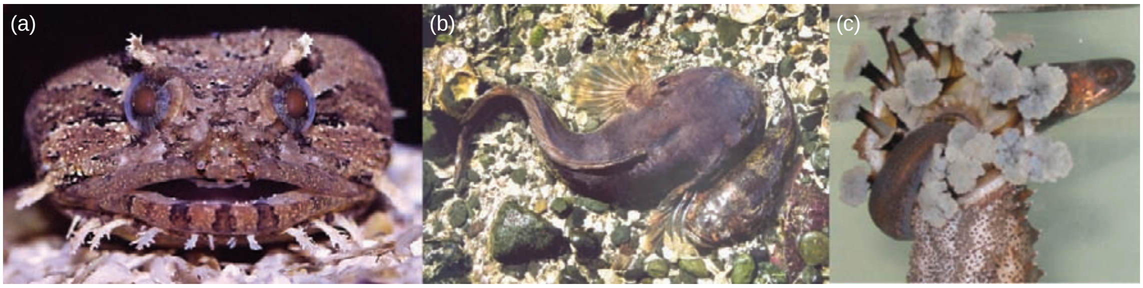


Figure 1 Examples of loud vocalizers (a) the Atlantic toadfish *Opsanus tau* and (b) the plainfin midshipman *Porichthys notatus* and a quiet vocalizer (c) the graceful pearlfish *Encheliophus gracilis*. Panel (b) shows a large type I singing male next to a smaller female fish. Courtesy of (a, b) M.A. Marchaterre, Cornell University, USA and (c) Eric Parmentier, University of Liege, Belgium.

Some fishes also produce a large repertoire of sounds, for example, the white weakfish (*Atractoscion nobilis*) has a repertoire of six statistically distinct sound types. Other fishes are considered less vocally active, and are only known to produce sounds when disturbed. However, it is possible that sounds may occur in these species in yet unmonitored behavioral contexts.

Recently discovered vocal species, once thought to be nonvocal, are simply less obvious sound communicators as they do not produce loud, year-round calls easily detected by a human observer. Such species would be better described as quiet vocalizing fishes (**Figure 2**). Quiet vocalizers include the fantail darter *Etheostoma flabellare*, the sheepshead minnow *Cyprinodon variegatus*, the pearlfish *Carapus acus*, the whitetail shiner *Cyprinella galactura*, and the cichlid *Tramitichromis intermedius*.

Quiet vocalizer calls require a hydrophone with amplifier at close proximity to a fish to be detected, usually within a few body lengths. Their sounds are characterized by sound amplitudes as low as 20 dB near the caller, short propagation distance (<1 m), occurring in lower abundance outside the breeding season or in discrete social contexts or narrow calling windows. Quiet fishes might still produce large repertoires and complex sounds and use them in the same behavioral contexts as loud vocalizing species. Many poorly studied vocal fishes are active at times or in places difficult for humans to study such as at night or in the deep sea, caves, seagrass beds, turbid waters, burrows, or buried in sand. Some fish even vocalize inside other animals. Five species of pearlfishes living inside sea cucumbers (**Figure 1**) have been discovered to produce quiet sounds either patrolling the territory around their host or once inside.

Discovery and Evolution of Vocal Fish Behavior

Vocal fishes have been known since the time of Aristotle. Even fishes producing quiet sounds were proposed as biologically significant communicators by researchers of the late 1800s, especially Leon Dufosse and William

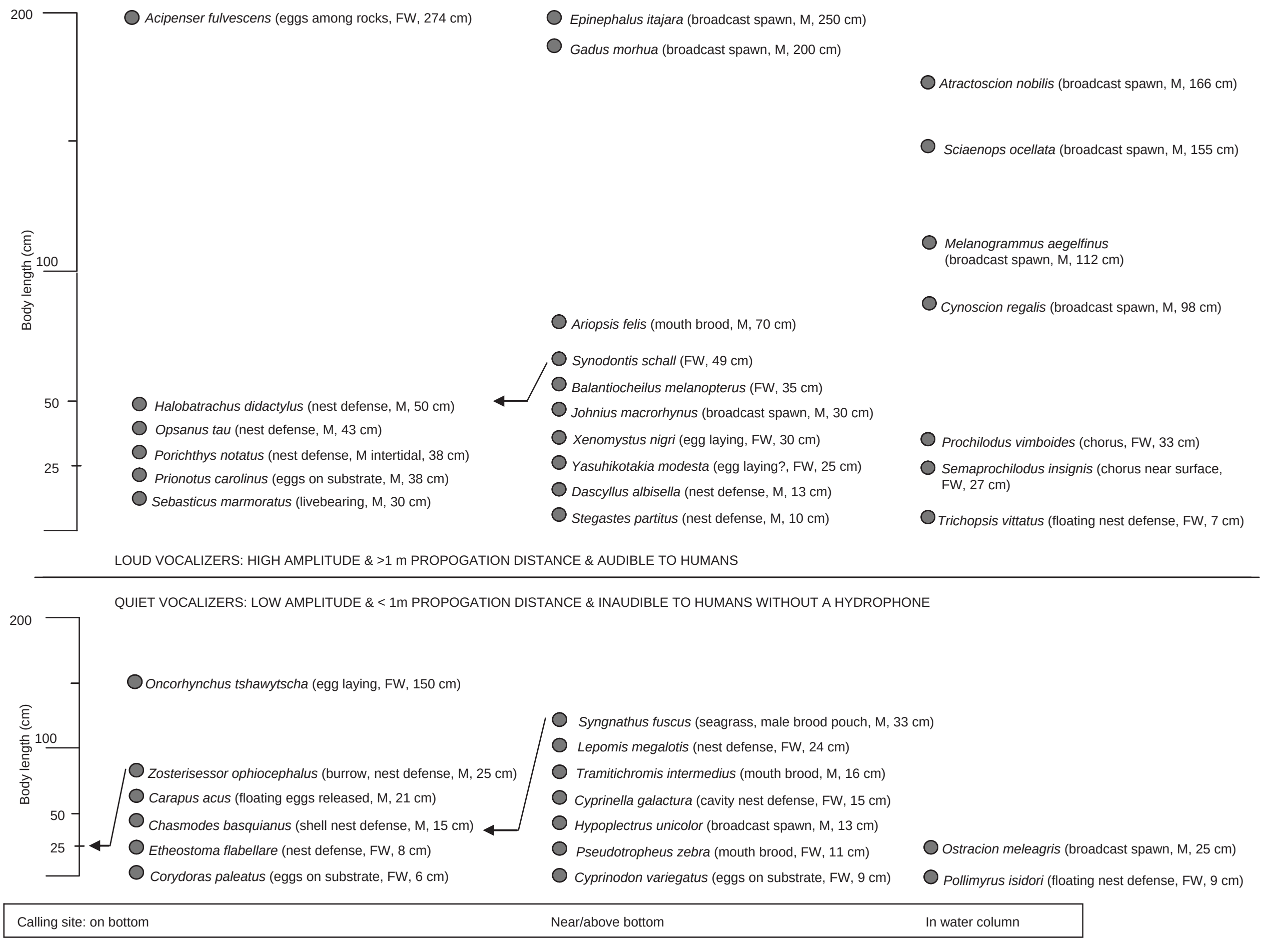


Figure 2 A sampling of vocal fish species (27 families) ranging from freshwater (FW) to marine (M) habitats, small to large body size, and varying in reproductive strategies (notes in parentheses when known). The upper panel is for loud and the bottom for quiet vocalizing fishes. Body length is for standard or total length as reported in Fishbase (http://fishbase.org/search.php).

Sorenson; both produced significant monographs on the subject. Few new fish families have been monitored with hydrophones since the extensive works of Marie Poland Fish especially in her book *Sounds of Western North Atlantic Fishes* with William Mowbray, a classic survey of marine fish sound behavior. The best-studied taxa include toadfishes, cichlids, croakers, damselfishes, cods, gobies, gouramis, elephantfishes, minnows, and catfishes. The earliest fishes evolved hearing to listen to the acoustic scene. Studies of the development of the neurological control of sound production in fishes that allows them to produce temporally patterned sounds, suggests an equally early evolutionary origin for sound communication. Modern teleost fishes have independently evolved different vocalizing mechanisms (**see also Hearing and Lateral Line**: Vocal Behavior of Fishes: Anatomy and Physiology). Absence of sound production in fishes can occur as a result of vocal mechanism loss suggesting the actions of some yet unknown evolutionary selection pressures. Several deep-sea groups have been examined for the presence of the most common vocal mechanisms. Pelagic species were found to lack the well-developed muscle and bony structures typically associated with sound production. If these groups do use sound in signaling, the mechanism involved would be novel compared to those currently known. This is not impossible; most gobies produce sound signals with a still as-yet unidentified vocal mechanism. Sharks and rays are considered nonvocal fishes. Like certain deep-sea fishes they lack key structures typically involved in sound production: bone, air-filled chambers, and flexible body parts for postural displays. However, there is some evidence for agonistic breaching behaviors that might produce hydrodynamic sounds and disturbance sounds in a few species.

Although no particular ecosystem (freshwater and marine), ecotype (benthic to pelagic), activity period (nocturnal or diurnal), life history strategy (egg laying on substrate vs. broadcast spawning into the water column), or social organization (solitary vs. closely aggregating or schooling) is exclusively associated with vocal behavior in fishes (**Figure 2**), some basic trends are emerging. Species whose social interactions are well developed have been identified as sound signalers; the greater the development of the social system, the more complex and abundant the sounds. Many vocal species are found in benthic communities where individuals defend territories and in pelagic chorusing aggregations where individuals form social dominance hierarchies. Another common theme among vocal fishes is male display while building and defending a nest with extended parental care.

Fish Call Characteristics: Simple versus Complex

The bioacoustic parameters of fish sounds are important for understanding their functions (**see also Hearing and Lateral Line**: Physiology of the Ear and Brain: How Fish Hear and Vocal Behavior of Fishes: Anatomy and Physiology). The basic sound unit or fish call (**Figure 3**) ranges in duration from 5 to 40 000 ms. An amazing exception is the plainfin midshipman whose hum call can last over 1 h. Among the simplest calls are single pulsed escape sounds with irregular temporal patterns that are produced when a fish moves away from a threatening source. Their duration ranges from <20 to 500 ms. Territorial or fighting sounds are highly variable but often of moderate duration, typically lasting from ~10 to 3000 ms. The most complex, longest (**Figure 4**), and most temporally stereotyped calls are produced during pre-reproductive activity. Prolonged courtship displays or songs may incorporate many basic sound units of different calls and can last for 55 s for the spawning chant of temperate white weakfish (>2000 purrs and 77 knocks), 143 min in a tropical marine big-snout croaker *Johnius macrorhynus* and up to several hours for the repeated male courtship boatwhistles of toadfish.

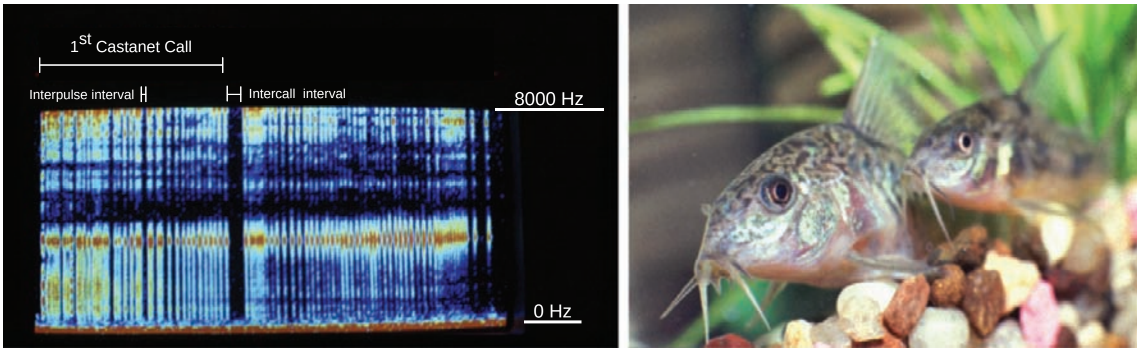
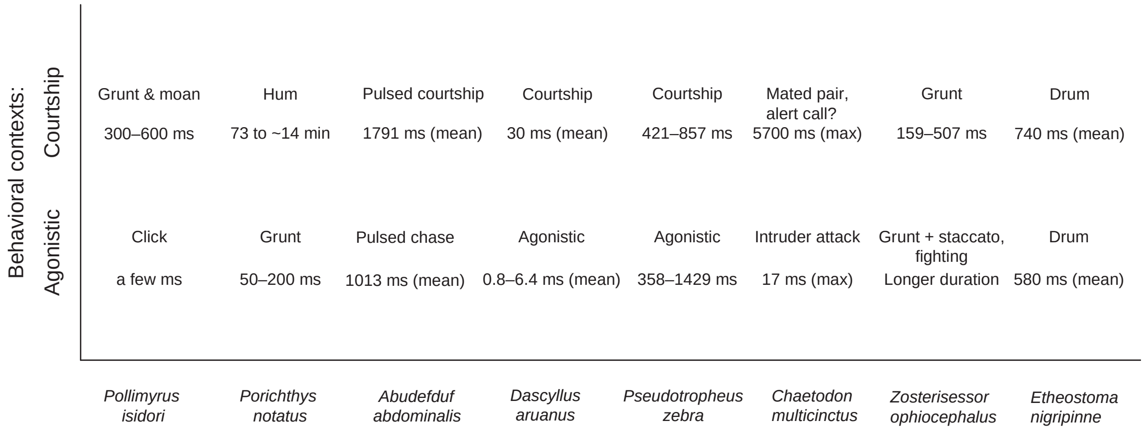


Figure 3 Spectrogram (frequency vs. time plot) of two male courtship associated sounds (basic sounds unit or call) of the diurnal quiet vocalizing miniature catfish *Corydoras paleatus*. The smaller male, at left, produces a courtship display to a silent female immediately prior to egg fertilization. These castanet calls (first call ~ 600 ms in duration) consist of a series of pulses with interpulses and cover a broad frequency range (~800–8000 Hz). Agonistic calls are simpler with fewer pulses and less regular interpulse interval durations.



Behavioral contexts:	*Pollimyrus isidori*	*Porichthys notatus*	*Abudefduf abdominalis*	*Dascyllus aruanus*	*Pseudotropheus zebra*	*Chaetodon multicinctus*	*Zosterisessor ophiocephalus*	*Etheostoma nigripinne*
Courtship	Grunt & moan 300–600 ms	Hum 73 to ~14 min	Pulsed courtship 1791 ms (mean)	Courtship 30 ms (mean)	Courtship 421–857 ms	Mated pair, alert call? 5700 ms (max)	Grunt 159–507 ms	Drum 740 ms (mean)
Agonistic	Click a few ms	Grunt 50–200 ms	Pulsed chase 1013 ms (mean)	Agonistic 0.8–6.4 ms (mean)	Agonistic 358–1429 ms	Intruder attack 17 ms (max)	Grunt + staccato, fighting Longer duration	Drum 580 ms (mean)

Figure 4 The duration of calls (range, unless otherwise indicated) representing reproductive vs. aggressive behavioral contexts as reported in the literature. Calls are identified by either their phonetic name or context description. The longest duration calls are from reproductive contexts, and when compared within species, most but not all have longer courtship calls.

While fish call patterns are diverse, there are two distinct types that have functionally different roles. Multipulsed calls have interpulse intervals or off-times within the basic sound unit (silence between regularly timed pulses, **Figure 3**) and are broad in their frequency range. In contrast, tonal sounds consist of pure tones (narrow frequency range) without any interpulse intervals. Pulse number and repetition rate may vary among species, suggesting the signaling potential for either species or individual recognition and discrimination. Indeed, playback studies have demonstrated that damselfish neighbor versus nonresident recognition and female preference for their own species calls is based on pulse number and interpulse interval. Likewise sympatric species differ predominantly in temporal characteristics, although for some congeners these differences are in amplitude, frequency, or waveform. The most complex fish sounds are male courtship sounds that combine multipulsed sounds with highly regular interpulse intervals and tonal components. Some goby males produce tonal sounds only as an advertisement call while a female is outside the nest and only multipulsed sounds during courtship of the female inside the nest. The croaking gourami (*Trichopsis vittata*) produces the same simple pulsed sounds in all contexts.

Amplitude range of species (and, therefore, the distance over which sound can be used to communicate) falls into two broad categories: those whose sounds propagate well over 1 m and those that utilize sound over much shorter distances (**Figure 2**). In sand gobies *Pomatoschistus minutus*, intraspecific male body size relates directly to courtship call loudness and shows higher stereotypy than other acoustic parameters. In gouramis, agonistic calls are twice as loud as pre-spawning calls indicating a more private channel for mate communication.

Frequency ranges of fish sounds vary widely from 10 to 8000 Hz. The most significant behavioral aspect of frequency is the highest amplitude component in a call referred to as the dominant frequency. This correlates with male size in numerous families and is hypothesized as an element in female mate choice and as a male fitness indicator. Female damselfishes prefer larger males that produce lower dominant frequency calls. Pure tone frequency (vs. broader band) sounds are almost exclusively associated with male reproductive display. Individual recognition of male toadfish is hypothesized based on frequency differences of mating calls.

With all of these different types of sounds, fish are very selective about when, where, and how long they call suggesting energetic or interception constraints. For example, calling activity level outside the breeding season in populations of diurnal freshwater miniature catfishes in the genus *Corydoras* equals one call per hour, while >100 sounds are produced during their several hour mating activities. The vocally active noctural toadfish call all year to maintain their benthic territories and more abundantly during the breeding season.

Chorusing Behavior: Schooling, Spawning, and Feeding

In relation to sound production, schooling fishes fall into two different groups. One is considered silent or only produces hydrodynamic noises from swimming movements, where the function of the noise, if any, is undetermined. The other is where many individuals produce patterned sounds unrelated to locomotor movements in a chorus, calling at a specific time and place (e.g., groupers and croakers). In the former, hydrodynamic noises and the prevalence of the tactile sense among tightly schooling and rapidly moving individuals may place some constraint on the use of sound signaling. In the latter, there may be many advantages to sound communication. For example, large schools of marine catfishes forage on the bottom in poor visibility coastal waters and produce nocturnal choruses of short pulse burst percolator sounds that may function in short-range orientation or act as contact calls. Schooling herring produce pulsed sounds in a predictable diel pattern perhaps promoting group cohesion. Several groups of fishes form schools associated with spawning. Chorusing activity for such aggregations typically begins close to sunset and peaks nocturnally. Fewer species have diurnal or crepuscular calling peaks. Many fishes produce noises as a by-product of feeding. The teeth grinding or plankton feeding popping sounds of freshwater and marine fishes are distinctive. In minnows and puffer fishes, these sounds are intercepted by conspecifics that are attracted to potentially more abundant feeding grounds.

Reproductive Sound Signaling: Male Display and Female Mate Choice

Sound production increases during the breeding season for many vocal fishes and the characteristics of these calls potentially convey information about an individual's age, sex, size, identity, and health or fitness. In cichlids, the physiological priming and conditioning of female gonads by male calls has been demonstrated. Female toadfish and plainfin midshipman are attracted to speakers broadcasting male calls. Male advertisement calls can be used for localizing conspecifics by gobies. Sound production during the breeding season is predominantly by males on territories, between males during conflicts over resources, by males displaying to females and less frequently between females and other individuals. Male calling is clearly multifunctional serving to attract mates and repel competitors. Sound displays that propagate greater than a few body lengths in distance are least likely when a mated pair is courting. This suggests a cost to loud sounds (interception by predators or conspecifics) and a priority for a close and intimate channel of communication between sender and receiver. In the plainfin midshipman and blennies, males exist in two different phenotypes. Smaller, sneaky males intercept sounds of larger displaying males to locate females and steal egg fertilization. Sneaky males vocally mimic the simple sounds of females while larger males defend nests and produce complex sounds (**see also Reproduction**: The Diversity of Fish Reproduction: An Introduction).

Female preference for a particular male call is likely a strong component of the selection forces that shape a male's vocal display. Playback demonstrates that female cichlids choose mates on the basis of male calls. Male fitness could be conveyed by signals with lower frequency and higher amplitude occurring as a consequence of increased size of vocal mechanisms (e.g., muscles). These are inherently honest signaling features that could indicate health, vigor, or energy reserves of individual males. Choice experiments have been conducted for midshipman where females showed preference for louder and longer duration male sounds and tonal over pulsed sounds. Females of many vocal species are presumed mostly silent during reproduction, but a recent discovery of very low amplitude pre-spawning calls in gouramis suggests that cooperative communication for spawning synchronization between the sexes may be intentionally less conspicuous. An alternation of male–female calling has been observed in a few species. Mating partners of the hermaphroditic butter hamlet (*Hypoplectrus unicolor*) alternately produce a spawning sound just before gamete release. The production of such sounds specific to spawning may ensure breeding synchrony during external fertilization.

Agonistic Sound Signaling within Species

Social dominance hierarchies among conspecifics are important for defending space, mates, offspring, and food (**See also Social and Reproductive Behaviors**: Dominance Behaviors). In defending resources, communicating the age, size, or strength of an individual is highly advantageous. Unlike during reproduction, when specially patterned or multipulsed sounds are essential for identifying species and distinguishing individuals, agonistic contexts may require less specificity. Increased call loudness may correlate with escalated agonistic interactions. Contest winning cichlid males produce calls with longer durations and lower peak frequencies. Winners also exhibit increased calling to females subsequent to contests. Calls during fights may serve an appeasement function by ameliorating aggression. Both females and males produce similar agonistic sounds in many species unlike in courtship contexts. Toadfish populations exhibit alternation of calling among neighboring males where social dominance is the proposed function. Keep-out signals are produced by damselfishes to repel intruders from resting crevice territories. Gobies and catfishes compete for and defend refuges while producing abundant sounds when intruders enter, often repelling them. Feeding competition is a context widely documented for sound production among fishes and may function to repel conspecific competitors from a contested food source.

Avoiding and Repelling Predators

Predators could place very strong selection pressures on whether or not fishes are vocal as well as how, where, and when sounds are produced. Predator and prey can vocally interact with each other in several ways: (1) predator interception of prey calls; (2) prey interception of predator calls; (3) prey production of distress, alarm, escape, startle, or threat sounds when seeing or attempting to flee from a predator before attack; and (4) disturbance, prey responding to a predator once physically restrained.

Predator interception of prey fish signals has been demonstrated. Dolphins can locate and are attracted to the vocal signals of sciaenids, making them unsuspecting prey fish. Simple noise production, the swimming sounds of the Bermuda anchovy, *Anchoa choerostoma*, may attract its predator, *Caranx latus*, the yellow jack. Eavesdropping by prey of predator calls has been demonstrated in toadfish. Calling is reduced when dolphins are present, a response that appears to be a direct result of ultrasonic dolphin calls. In fact, toadfish show increased levels of stress hormones in response to dolphin sounds, suggesting that they recognize the predation threat. The specialization of ultrasonic hearing in marine schooling fishes such as herring indicates that these fishes can detect echolocating predators. Sharks are attracted to pulsed but not tonal sounds, indicating a risk element to most fish calls.

Escape sounds are commonly produced by marine fishes and occur at the start of a prey individual's escape trajectory. They occur before any contact with the predator and are produced by the movement of body musculature. Escape sounds could be merely noise. Alternatively, they could function as 'stotting sounds', a vocal equivalent of visual jumping displays in African savannah gazelles. Escape sounds could indicate to a potential predator that it has been spotted and that the prey individual is in good physical condition to get away. A predator startling function is attributed to the loud clicks of the Atlantic cod produced only in the presence of seal-like body silhouettes. Mobbing squirrelfishes have been observed to approach and attack potential predators in small groups while vocalizing. Their calls in this context are proposed as alarm or threat signals and consist of rapid pulsed sounds. Several fish families produce disturbance sounds after a predator has physically attacked them in conjunction with weaponized defense mechanisms that include chemical secretions, pinching, stabbing, and envenomating devices (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food).

Sound Signals Relative to Other Communication Modalities

Sound may play an exclusive or supportive role in communication. Some gourami species lack social sounds and communicate visually while others are predominantly vocal. Calls are often directly associated with specific body postures. Cichlids vocalize quietly during body quivering, the common and often presumed nonvocal behavioral display of many fishes. In the frillfin goby *Bathygobius soporator*, females require visual stimuli in order to respond to a calling male; they will not respond to sound playback alone. Among catfishes the presence of chemical alarm signals is inversely related to the development of vocalization. Cichlid male vocal displays differ most among species where males are visually more similar indicating a communication modality trade-off. Some elephantfishes that produce a diversity of electric signals (**see also Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish) also produce sounds that males use to court females; however, sex discrimination depends solely on electric signals. The use of sound for male display is hypothesized as a better indicator of fitness because mechanical work is required to produce the signal. There is a significant overlap between the auditory and lateral line system in fishes (**see also Hearing and Lateral Line**: Auditory/Lateral Line CNS: Anatomy). Salmon produce sounds with inaudible and audible components during reproduction. Postural changes evaluated in butterfly fishes produce water currents that could be sensed via the lateral line while stronger movements could produce sounds detected by the inner ear. In cottids, substrate-transmitted drumming produces signals with lateral line and inner ear stimulating components. Such signal complimentarity is an exciting area for future research.

Conclusions: What Fish Sounds Can and Cannot Do

Like all other vocal animals, fishes use sounds to enhance their behavioral displays and convey information about sex, age, social status, species identity, reproductive condition, and spawning readiness. Tantalizing evidence exists for duets, call individuality and its discrimination, conspecific alarm and contact calls, and acoustic defense. Communication functions that fish sounds are not known to serve include parent–offspring signaling, sibling recognition, and mimicry. Many fish taxa remain unexplored in terms of bioacoustics. Because we now know that short propagation distance and low amplitude sounds occur more commonly than was once thought, we cannot be certain of the nonvocal status of any unmonitored fish. A developing pattern in fish bioacoustics is that simple and low-amplitude sounds can be sufficient for sound signaling. In addition, high-amplitude, interception-prone, complex calls function in species that can either avoid predation by calling from protected sites or avoid the risk of predation by other means to communicate over longer distances. New studies continue to reveal evidence of the importance of sound production in fish behavior. Current exciting studies include the influence of adult calls on juveniles indicating that sound production is innate, not learned, and the possible attraction of larval fish to nocturnal choruses on coral reefs that include fish sounds.

See also: **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food. **Hearing and Lateral Line**: Acoustic Behavior; Auditory/Lateral Line CNS: Anatomy; Physiology of the Ear and Brain: How Fish Hear; Vocal Behavior of Fishes: Anatomy and Physiology. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Sensory Systems, Perception, and Learning**: Shocking Comments: Electrocommunication in Teleost Fish. **Social and Reproductive Behaviors**: Dominance Behaviors.

Further Reading

Amorim MCP (2008) Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). *Journal of Fish Biology* 72: 1355–1368.

Bass AH and Ladich F (2008) Vocal–acoustic communication: From neurons to behavior. In: Webb JF, Fay RR, and Popper AN (eds.) *Fish Bioacoustics*, pp. 253–277. New York: Springer.

Fish MP and Mowbray WH (1970) *Sounds of the Western North Atlantic Fishes: A Reference File of Biological Underwater Sounds*. Baltimore, MD: Johns Hopkins Press.

Hawkins AD (1993) Underwater sound and fish behaviour. In: Pitcher TJ (ed.) *Behaviour of Teleost Fishes*, 1st edn., pp. 129–169. London: Chapman and Hall.

Kaatz IM (2002) Multiple sound-producing mechanisms in teleost fishes and hypotheses regarding their behavioral significance. *Bioacoustics* 12: 230–233.

Kasumyan AO (2008) Sounds and sound production in fishes. *Journal of Ichthyology* 48(11): 981–1030.

Ladich F, Collin SP, Moller P, and Kapoor BG (eds.) (2006) *Communication in Fishes*, 2 vols. Enfield, NH: Science Publishers.

Longrie N, Fine ML, and Parmentier E (2008) Innate sound production in the cichlid *Oreochromis niloticus*. *Journal of Zoology* 275: 413–417.

Malavasi S, Collatuzzo S, and Torricelli P (2008) Interspecific variation of acoustic signals in Mediterranean gobies (Perciformes, Gobiidae) comparative analysis and evolutionary outlook. *Biological Journal of the Linnean Society* 93: 763–778.

Myrberg AA, Jr. (1981) Sound communication and interception in fishes. In: Tavolga WN, Popper AN, and Fay RR (eds.) *Hearing and Sound Communication in Fishes*, pp. 395–426. New York: Springer.

Nordeide JT, Solberg C, Willumsen L, and Berge Amble S (2008) Seasonal variation and condition-dependence of the drumming muscles of cod, *Gadus morhua* L.?: An experimental approach. *Journal of Experimental Marine Biology and Ecology* 363: 66–74.

Parmentier E, Lecchini D, Frederich B, Brie C, and Mann D (2009) Sound production in four damselfish (*Dascyllus*) species: Phyletic relationships. *Biological Journal of the Linnean Society* 97: 928–940.

Phillips CT and Johnston CE (2008) Geographical divergence of acoustic signals in *Cyprinella galactura*, the whitetail shiner (Cyprinidae). *Animal Behaviour* 75: 617–626.

Remage-Healey L, Nowacek DP, and Bass AH (2005) Dolphin foraging suppress calling and elevate stress hormone levels in a prey species, the Gulf toadfish. *Journal of Experimental Biology* 209: 4444–4451.

Rice AN and Bass AH (2009) Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: Insights into the diversity of the Batrachoididae. *Journal of Experimental Biology* 212: 1377–1391.

Rollo A and Higgs D (2008) Differential acoustic response specificity and directionality in the round goby, *Neogobius melanostomus*. *Animal Behaviour* 75: 1903–1912.

Rosenthal GG and Lobel PS (2006) Communication. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behavior and Physiology of Fish. Fish Physiology* vol. 24 pp. 39–78. Amsterdam: Elsevier.

Sargent RC, Rush VN, Wisenden BD, and Yan HY (1998) Courtship and mate choice in fishes: Integrating behavioral and sensory ecology. *American Zoologist* 38: 82–96.

Tricas TC, Kajiura SM, and Kosaki RK (2006) Acoustic communication in territorial butterflyfish: Test of the sound production hypothesis. *Journal of Experimental Biology* 209: 4994–5004.

Verzijden MN, Heusden J, Bouton N, Witte F, Cate C, and Slabbekoorn H (2010) Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences. *Behavioral Ecology* 21: 548–555.

Zelick R, Mann DA, and Popper AN (1999) Acoustic communication in fishes and frogs. In: Fay RR and Popper AN (eds.) *Comparative Hearing: Fish and Amphibians*, pp. 363–411. New York: Springer.

Relevant Website

http://www.fishbase.org – FishBase.

Communication Behavior: Visual Signals

GG Rosenthal, Texas A&M University, College Station, TX, USA

NJ Marshall, The University of Queensland, Brisbane, QLD, Australia

© 2011 Elsevier Inc. All rights reserved.

Introduction
Bioluminescent Signaling
Signaling with Reflected Light
Evolution of Visual Signals
Further Reading

Glossary

Astaxanthin A naturally occurring red carotenoid pigment.

Collimating Aligning in parallel.

Cue A stimulus that may provide information to a receiver, but has not evolved in the context of communication.

Eavesdropper An individual, such as a predator or rival, other than the intended receiver, such as a potential mate, that modifies its behavior in response to a signal.

Monochromatic Having only one color.

Opsin A protein found in photoreceptor cells, covalently bound to a chromophore that changes conformation when it absorbs a photon of light.

Receiver In animal communication, an individual that modifies its behavior based on its perception, processing, or evaluation of a signal or a cue.

Signal In animal communication, a stimulus that has evolved in response to selection for a communicative function.

Signaler An individual producing a signal.

Tunaxanthin A naturally occurring red carotenoid pigment.

Introduction

Visual communication accounts for much of the popular fascination with fish. The profusion of pattern, color, and form in coral reef fish skin patterns is one of the most striking phenomena in the natural world; and many a kitchen table is graced with a male Siamese fighting fish, directing aggressive displays at passers-by. For researchers, visual signals in fishes present a powerful model for asking basic questions about the evolution of communication and about environmental effects on signaling. This is due to both the sheer diversity of communication systems, coupled with the fact that fish occupy the full gamut of possible visual environments: from lightless ocean depths and caves, to tea-stained lakes and streams, to sunny shallow reefs (**see also Vision**: Color Vision and Color Communication in Reef Fish and **Deep-Sea Fishes**).

At its most basic, communication is a two-way interaction between a signaler, who produces a sensory stimulus or signal, and a receiver, who perceives the signal and makes a behavioral decision. Signalers and receivers often exchange roles, and eavesdroppers, such as predators locating prey, often exploit communication interactions.

A basic challenge in animal communication is distinguishing between a signal – a stimulus that has evolved in an explicitly communicative context – and a cue – a stimulus whose communicative function is incidental. Signals are easy to identify if they involve postural changes, motor patterns, or skin-pattern changes performed only in the presence of an appropriate receiver, such as a ritualized courtship or aggressive display. Many of the most important visual signals in fish, however, are morphological structures such as fin extensions or constitutively expressed skin patterns that are always on. The situation is further complicated by the fact that some communication signals may have evolved in order to enhance transmission of a cue to a receiver. For example, close-range courtship motor displays may waft nonsignaling olfactory cues (e.g., metabolized steroid hormones) to the receiver (**see also Hormones in Communication**: Hormonal Pheromones).

Once a signal has been produced, it must be transmitted to the receiver, who then must detect it against background, evaluate it, and respond. An effective signal must therefore be both detectable in the environment in which it is produced and distinct from signals that would elicit a different response from the receiver.

From the receiver's point of view, a visual signal can be decomposed into spectral, spatial, and temporal components. The image that is projected onto each of a fish's two retinas consists of light varying in intensity and wavelength over space and time. Detection of visual signals is largely determined by the degree of spectral and spatiotemporal contrast between the signaler and the visual background, or among different elements of the signal.

Generating a visual signal involves multiple approaches. In rare cases, fish will directly generate light through bioluminescence (**see also The Skin**: Bioluminescence in Fishes). The vast majority of visual signals, however, involve modifying the spectral, spatial, and temporal distribution of light generated by the sun after it has been filtered through the atmosphere and the water column. This typically involves specializations in pigment cell distributions, posture, and motor patterns (**see also The Skin**: Coloration and Chromatophores in Fishes).

With the exception of a growing body of quantitative studies on the color component of communication, most work on visual communication in fishes (and in general) does not take a quantitative approach to characterizing the color, spatial, or temporal properties of stimuli, backgrounds, or ambient light conditions, whether characterizing signals themselves or evaluating receiver response. Nevertheless, the fact that visual stimuli can be readily characterized, as well as experimentally manipulated through dummies, models, videos, and computer animations, has given us substantial insight into this mode of communication in fishes.

Although relatively few studies have explicitly isolated visual components of communication signals, fishes use visual communication extensively in the context of mate choice and aggressive displays, as well as recognition of conspecifics, shoalmates, and individuals. While visual signaling has mostly been studied between conspecifics, it can play an important role in interspecific interactions. For example, the characid *Hemigrammus erythrozonus* has a visual antipredator display. Deep-sea anglerfishes use bioluminescent lures to snare prey and the monacanthid *Paraluteres prionurus* uses shape and color patterns to mimic a toxic pufferfish. Many fishes, notably the color-changing flatfishes (Pleuronectidae) use skin patterning and morphological structures for camouflage. Cooperative interactions between cleaner wrasses and their hosts involve the production of stereotypic postural and color signals by both parties.

Bioluminescent Signaling

Many marine fishes produce their own light energy, either endogenously or more commonly, by concentrating bacterial symbionts inside specialized light organs. Bioluminescence (**see also The Skin**: Bioluminescence in Fishes) is particularly useful at night and in the deep sea, where exogenous light is scarce. Bioluminescence has evolved independently many times in teleosts, exclusively in marine taxa. Bioluminescence serves a variety of functions, including prey capture and camouflage, and can play a role in shoaling and courtship.

Bioluminescence involves an enzymatic reaction whereby an oxygenase, luciferase, oxidizes a luciferin, producing oxyluciferin and emitting light. This is typically produced by symbiotic bacteria in specialized light organs, although in a few cases – midshipmen (Batrachoidae: *Porichthys* spp.), lanternfishes, and some apogonids – the luciferin–luciferase system is produced endogenously, with bioluminescence under neural control. Fishes have evolved a variety of structures to control the spectral, spatial, and temporal properties of emitted light. Both bacterial light organs and photophores can contain reflectors, filters, lenses, and light guides. Species with bacterial light organs use shutters controlled by specialized musculature to create flashes of light. Lampeyes (Anomalopidae) can use both musculature derived from the levator maxilla to draw a black pigmented curtain over the surface of the organ, or rotate the entire organ into a suborbital pocket. The fishes can cycle these movements up to 100 times per minute, creating a blinking effect that is used both for prey detection and intraspecific signaling. In the trachichthyid genus *Paratrachichthys*, bacterial organs are located around the anus, opening onto the rectum. The light is distributed anteriorly and posteriorly by translucent, collimating muscles piping light to various areas of the body in a manner analogous to fiber optics.

Three deep-sea genera of dragonfishes or loosejaws (Malacosteidae: *Malacosteus*, *Pachystomias*, and *Aristostomias*) couple a luciferin–luciferase system, emitting typical blue-green light, to red fluorescent protein and a series of filters to produce far-red light (maximum emission >700 nm) from suborbital photophores; in *Malacosteus*, the light is further modified using a superficial brown filter. Dragonfish detect these wavelengths not using a long-wavelength sensitive opsin, but with a novel, long-wavelength photosensitizer, derived from dietary chlorophyll. Accordingly, this light is invisible to most other deep-sea fishes, providing dragonfishes with a private channel with which to locate prey and signal to conspecifics. In all three of these genera, there is also an additional postorbital photophore that emits blue light, suggesting that these fish may be capable of sophisticated multicomponent signaling.

The difficulty of observing behavior in deep-sea fishes means that most inferences about bioluminescent signaling must be made from morphology. A rare exception is the leiognathids or ponyfishes, which occur in circumtropical shallow coastal waters of the Indo-Pacific. In some leiognathids, multiple shutters on the wall of the light organ mechanically control the duration and intensity of light emission. *Leiognathus elongatus* appears to be sexually dimorphic, with females lacking a light organ.

While bioluminescence allows the signaler to control the temporal characteristics of the signal, it has several limitations. Most bioluminescent signals are spatially simple and monochromatic; more important, they are fairly ineffective in brightly lit environments where vision plays a dominant role. Most visual signals, therefore, involve forms, patterns, colors, and movement that reflect available light.

Signaling with Reflected Light

Reflected-light signals are characterized by spectral reflectance as a function of space and time. Reflectance is defined as the light reflected back from a target illuminated by a uniform white light source, as a function of wavelength. Reflectance can change as a function of the angle of illumination, spatial position, and time. Many fishes express complex color patterns; over the course of a courtship or aggressive display, they vary the color and intensity of these patterns and engage in changes in position, orientation, and posture. The amount of information that can be conveyed in a display is thus considerable.

The color sensitivity of fishes ranges from around 340 nm (the near ultraviolet) to 750 nm (the near infrared), with considerable variation in visual sensitivity among species. Fishes produce colors across this spectrum. Spectral variation in signals is produced either by pigments, which absorb portions of the light visible to receivers, or by morphological structures that selectively scatter light as a function of wavelength. Pigments are typically used to generate long-wavelength colors (yellows, oranges, and reds), while morphological structures are used for shorter-wavelength colors (blues, indigos, violets, and ultraviolets) (**see also Vision**: Photoreceptors and Visual Pigments).

Pigment-Based Colors

The chemical nature of a pigment determines the wavelengths of light it can absorb (**see also The Skin**: Coloration and Chromatophores in Fishes). Many pigments in fishes are organic compounds containing long chains of conjugated double bonds, such as carotenoids and pterins. In general, small molecules absorb only high-energy (short) wavelengths, while larger molecules additionally absorb longer wavelengths of light. Carotenoids and pterins typically absorb shorter wavelengths, thereby reflecting yellows, oranges, and reds. Carotenoids bound to proteins can absorb middle wavelengths while reflecting both red and blue to produce purple. Many carotenoid-based color patches also exhibit a secondary peak in the ultraviolet, within the range of sensitivity of many fishes. Melanins are large proteins that absorb most visible and ultraviolet light to produce black. Pigments are housed in chromatophores, within small packets called chromatosomes.

Fishes cannot synthesize carotenoids; they must be obtained from dietary sources. In freshwater systems, notably threespine sticklebacks (*Gasterosteus aculeatus*) and guppies (*Poecilia reticulata*), males vary in carotenoid expression. Female sticklebacks and female guppies prefer more intense, purer carotenoid colors. The intensity of red color is determined by the concentration of astaxanthin carotenoids in the skin, while the chroma (color purity) of red is determined by the ratio of astaxanthins to tunaxanthin. Variation in astaxanthin deposition also appears to underlie the variation in the intensity and quality of orange coloration in guppies. The area of long-wavelength patches in guppies is highly heritable, while the intensity of color patches depends on carotenoid uptake from the diet and on parasite load. Intriguingly, while long-wavelength colors in freshwater fishes often appear to be limited by the availability of dietary carotenoids, this does not appear to be the case in marine fishes.

Structural Colors

Short-wavelength (green to ultraviolet) colors are often produced structurally, using small (<300 nm) particles embedded into a transparent layer backed by melanin pigment. Short wavelengths are scattered and reflected back to the receiver, while longer wavelengths are absorbed by the melanin. Adding filters to the transparent layer can further restrict the range of wavelengths being reflected.

Interference coloration is likely to account for the shimmering iridescent colors found in many freshwater fishes, notably poeciliids, cyprinodonts, and cichlids. This process involves a thin layer of transparent material, with a high index of refraction, coating the skin. Light hitting the surface at an angle is partially reflected to produce a primary reflection; much of the remaining light is refracted, reflected by the boundary at the bottom of the transparent material, then refracted again to produce a secondary reflection. The wavelengths for which these primary and secondary reflections are in phase depend on the viewing angle, the width of the transparent layer, and its refractive index. The apparent color can thus change substantially with viewing angle. Ultraviolet (UV) coloration in birds is produced by coupling multilayer interference with a scattering mechanism. Many fishes produce signals that reflect primarily in the UV (**Figure 1**).

White coloration often is produced by guanine forming microcrystalline platelets that reflect all wavelengths. If guanine platelets are packed in a dense array, they produce the specular (mirror-like) reflectance characteristic of silvery scales. These dense arrays can be concentrated in iridosomes, which intensify pigment

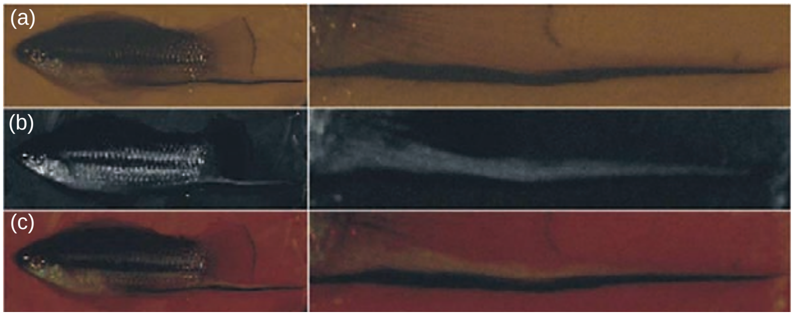


Figure 1 UV and visible spectrum imaging of male pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae). (a) Color photograph in human visible (400–700 nm) range. Enlarged sword sexual ornament is shown for detail. (b) Photograph taken with UV bandpass filter (300–400 nm). (c) Composite of the above two photographs showing black/UV contrast. From Cummings ME, Rosenthal GG, and Ryan MJ (2003) A private ultraviolet channel in visual communication. *Proceedings of the Royal Society B: Biological Sciences* 270: 897–904.

colors by reflecting wavelengths transmitted by chromatosomes.

The spectral curves produced by fish colors can be characterized as simple (reflecting strongly in one region of the spectrum) or complex (with separate reflectance peaks in distinct portions of the spectrum). Simple colors are either step-shaped, with high reflectance of all visible wavelengths above or below a cutoff point, or peak-shaped, reflecting within a particular range of wavelengths. Long-wavelength colors tend to be step-shaped, short-wavelength colors peak-shaped. Complex spectral curves contain multiple peaks and/or steps. The purple expressed by labroids, particularly tropical wrasses and parrotfishes, is one of the most complex animal colors, with distinct peaks around 400 and 580 nm and a step around 730 nm (**Figure 2**). These color spectra might be produced by combining several interference colors together.

Spatial Patterns

A reflectance spectrum only describes a single point in space and time. Visual perception, however, depends on processing of contrast relationships among elements of a visual scene. In teleosts, notably coral reef fishes, these patterns can be intricate and highly specified. The Picasso triggerfish, *Rhinecanthus aculeatus*, for example, exhibits a black-and-white polka-dot pattern just anterior to the caudal fin; four white stripes running diagonally from the insertion of the anal fin to the midline of the body; brown and gray bands on the dorsum; a filigree of electric blue lines originating parallel at the eye and curving to meet on the gill cover; and a blue mustache above the mouth contrasting with a broad russet band that slopes down to meet the white belly.

The specification of such complex patterns has been a focus of research in evolutionary developmental biology. A diversity of repeating patterns, or textures, can be produced by subtle changes to a relatively simple molecular mechanism. The noted mathematician Alan Turing proposed the reaction–diffusion system, whereby spot and stripe patterns arise as a result of instabilities in the diffusion of two or more morphogenetic chemicals (morphogens) in the skin during early development. Differences in boundary and initial conditions, and in the number of morphogens and diffusion properties of each morphogen, can interact to produce an array of stripe and spot patterns. Such a reaction–diffusion system is consistent with the development of the body pattern of juvenile angelfishes (*Pomacanthus* spp.), in which the body pattern is not fixed on the skin over ontogeny; rather, the size of the black melanophore coloration between yellow stripes is maintained constant, by continuous rearrangement of spatial patterns.

The developmental genetics of stripe formation have been extensively studied in *Danio* spp. (Cyprinidae). In the zebrafish, *D. rerio*, blue stripes consist of a mixture of xanthophores and melanophores, and white stripes consist of xanthophores and iridophores. While there is a fairly detailed picture of the mechanisms whereby pigment cells are recruited and aggregated, the forces underlying the spatial arrangement of stripes remain unclear. The lateral line system might be involved in recruiting stripes, as it is in salamander larvae; the zebrafish mutant puma exhibits defects in both lateral line and stripe development. The zebrafish leopard gene may be acting as a component of a reaction–diffusion system. Small changes in gene structure or regulation could thus induce qualitative changes in pattern elements (**Figure 3**(**a**)), producing dramatic differences in signal structure. Such a process could account for the striking differences in pattern sometimes seen among closely related species (**Figure 3**(**b**)).

In many cases, modifications of fin rays and axial vertebrae also appear to serve primarily in a signaling context. In many freshwater fishes, caudal and/or dorsalfins are prominently enlarged in adult males. In the Montezuma swordtail (Poeciliidae: *Xiphophorus montezumae*), the lower rays of the caudal fin are longer than the standard length in

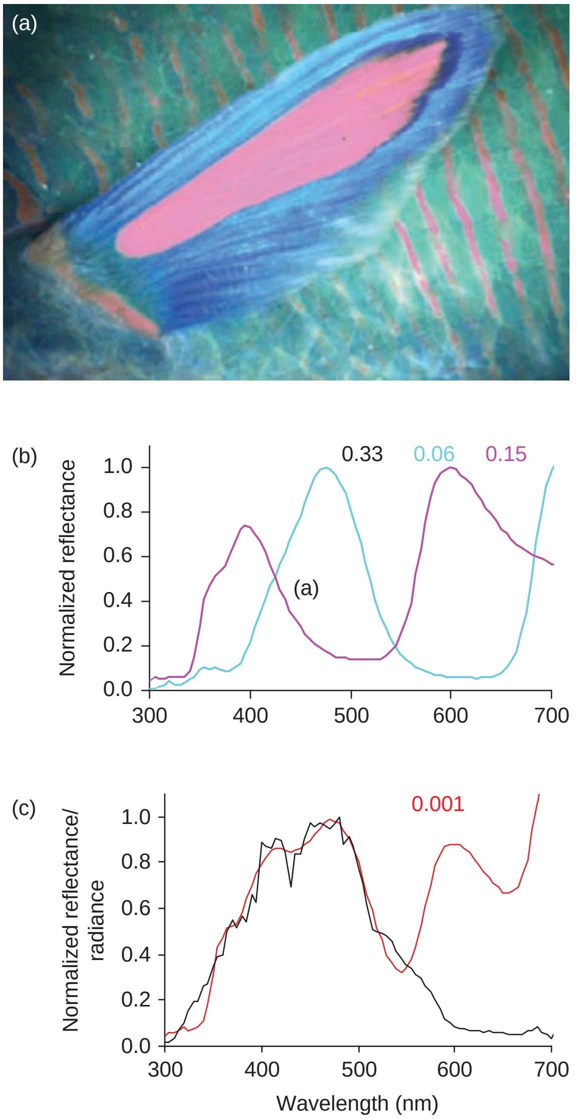


Figure 2 Communication and camouflage in an Indo-Pacific coral reef fish: (a) the purple-pink and blue pectoral fin of the wrasse *Thalassoma lunare* (Labridae) and (b) the colors of *T. lunare*'s pectoral fin. The black number 0.33 is the color distance between the two body colors. The blue number 0.06 is the color distance between blue color and background space light. The pink number 0.15 represents the color distance between purple-pink color and background space light. (c) The black curve shows background space light. The red curve indicates the purple-pink and blue reflectances of *T. lunare* added together. The red number 0.001 represents the color distance between the combined reflectances and background space light. Colors are highly conspicuous when viewed at close range, but smear into the background when viewed at a distance. Modified from Marshall NJ (2000) Communication and camouflage with the same 'bright' colors in reef fishes. *Philosophical Transactions of the Royal Society B* 355: 1243–1248.

some males. Females in several species prefer to mate with males bearing longer swords. By contrast, the highly dimorphic dorsal fin in the closely related sheepshead swordtail, *Xiphophorus birchmanni*, is unattractive to females but serves to deter rival males; accordingly, males modulate the elevation of the dorsal fin during courtship depending on whether rival males are nearby.

Temporal Properties of Visual Signals

In a signaling context, skin patterns and morphological structures are often coupled with stereotypical behaviors that maximize conspicuousness. Many fishes can change color patterns over a range of timescales, ranging from short-term flashes to ontogenetic changes over a lifetime. Male *Badis badis* (Badidae), for example, express nine distinct color patterns according to social context and dominance status. Simultaneously, hermaphroditic *Serranus subligarius* (Serranidae) alternate from a banded black and white male pattern to a solid black female pattern during a sex-changing courtship interaction. Males in several species of swordtails express conspicuous vertical bars on the flank, which they intensify over the course of courtship and agonistic interactions. Color change can operate over longer timescales as well. Male *Astatotilapia* (*Haplochromis*) *burtoni*, for example, express a drab, female-like pattern when subdominant and reproductively inactive, and a colorful, high-contrast sexually dimorphic pattern when dominant and reproductive. These shorter-term, reversible color changes are regulated via neural control of chromatophore cells, by aggregating or dispersing pigment granules within the cells.

Short-term temporal changes in reflected visual signals can also be effected by raising or lowering the fins, as in many poeciliids, or by postural changes which change the appearance of interference colors. Many signal interactions – courtship displays, aggressive displays, and cleaning solicitation displays – involve characteristic motor patterns which may also be providing information in nonvisual modalities, including olfaction and lateral-line detection of near-field vibration (**See also Social and Reproductive Behaviors**: Sexual Behavior in Fish). In some cases, fishes have evolved specialized motor control systems dedicated to communication. Male Siamese fighting fish *Betta splendens* (Osphronemidae) perform a dramatic frontal display to females and to other males, which involves a pronounced flaring of the gill covers. Both the musculoskeletal apparatus and motor control neurons appear to have been modified for this display. Female green swordtails *Xiphophorus helleri* attend specifically to the spatiotemporal visual characteristics of the male courtship display, and sticklebacks attend to variation in courtship tempo.

Color patterns also change over ontogeny (**See also Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes). Ontogenetic color changes are also quite common. Many sex-changing reef fishes have strikingly different color patterns according to

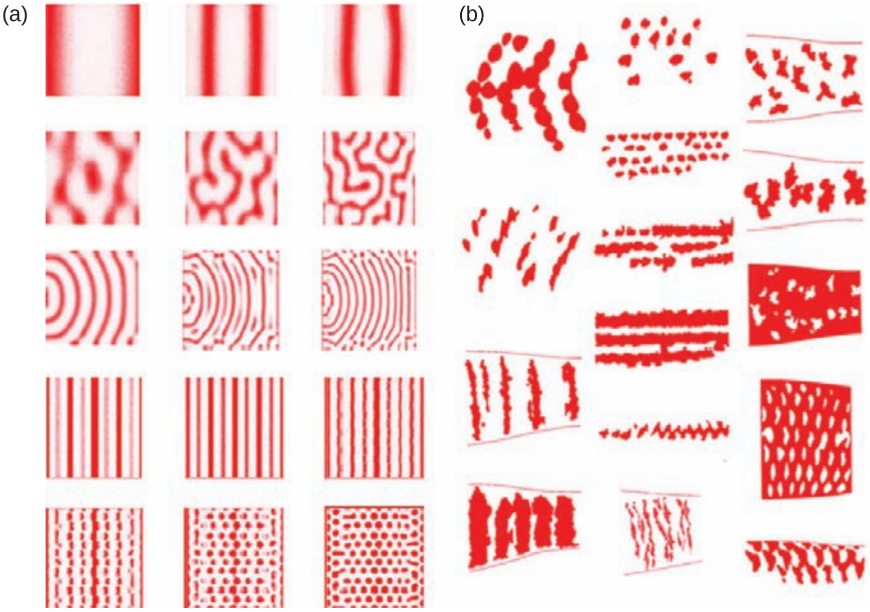


Figure 3 (a) Diversity of patterns generated by theoretical reaction–diffusion models of pattern formation. Small changes in gene structure or regulation can induce qualitative changes in pattern elements, producing dramatic differences in signal structure. (b) Such a process could account for the striking differences in pattern sometimes seen among closely related species. Distribution of red body pigmentation of 15 species of Cameroonian *Aphyosemion* killifish (Aplocheilidae). From Rosenthal GG (2007) Spatiotemporal aspects of visual signals in animal communication. *Annual Review of Ecology, Evolution, and Systematics* 38: 155–178.

sex and social status. In the bluehead wrasse *Thalassoma bifasciatum* (Labridae), both females and initial-phase males have yellow and black horizontal stripes; terminal males, which are derived from either females or initial-phase males, have completely different colors and spatial patterns: a blue head, black and white vertical bars, and a green posterior. Many pomacentrids and pomacanthids also have conspicuous juvenile color patterns strikingly different from the adult phase.

Evolution of Visual Signals

Visual signals in fishes are distinctive in the extent to which they depend on the underwater light environment. In aquatic environments, light quality and quantity varies with depth, water chemistry, and biotic factors such as plankton density. If a fish cannot make its own light, it must work with the available light environment to produce communication signals. Strong associations between transmission characteristics of the environment, visual sensitivity, and body color have been demonstrated in a variety of fish systems. Further, as on land, signal diversity is correlated with ecological complexity. The greatest profusion of fish visual signals is, accordingly, found in shallow waters on tropical coral reefs, where dozens of species coexist in a sensory environment that permits discrimination of fine spatial features and a broad range of colors (**See also Vision**: Color Vision and Color Communication in Reef Fish). In deeper or murkier water, fish tend to be drabber and spatially simpler.

Microhabitat differences can drive sensory systems and thereby speciation. Female sensitivity to red in sticklebacks correlates with the abundance of red light in the environment, and with female mating preference for red versus black males, producing divergent selection for red and black males in divergent environments. Human-induced disturbance can produce the reverse effect: in haplochromine cichlids, eutrophication of clear lake water masks the apparent color differences between two recently diverged species, promoting hybridization.

Sexual selection – both in the context of conspicuous advertisements to potential mates, and in the context of aggressive signals among sexual rivals – is a primary driver of visual exuberance in fishes. In swordtails, genus *Xiphophorus*, females exhibit an ancestral preference for the conspicuous sword elongation of the caudal fin. The preference appears to be a special case of a more general preference for large apparent size. Conspicuous signals are, however, open to exploitation by eavesdroppers. The Mexican tetra (*Astyanax mexicanus*, Characidae), a swordtail predator, shares the same broad visual biases as female swordtails, preferentially targeting males with swords. The visual spectrum is not universally shared among receivers, however. The UV (320–400 nm) is the primary area where visual sensitivity varies substantially across fish taxa, providing the opportunity for a degree of signal privacy. Only about 30% of reef-dwelling fish species are sensitive in the UV. Swordtails are sensitive to UV wavelengths, while *A. mexicanus* are not. Male swordtails in highly predated populations exhibit bright UV stripes, which females attend to in mate choice, but which are relatively inconspicuous to predators (**Figure 1**). Similarly, male guppies court at times of day and in microhabitats that minimize visual conspicuousness to long-wavelength (red) insensitive invertebrate predators, while retaining conspicuousness to females.

Sexual selection is insufficient to explain the flamboyant signals of many reef fishes, which are often highly stereotyped within a species, often identical in males and females, and in some cases, like beaugregory damselfish (*Stegastes leucostictus*) appear more conspicuous to us in juveniles than in adults. Konrad Lorenz, one of the founders of early ethology, proposed that reef-fish signals had evolved under pressure in a diverse, crowded community. By iconically encoding species identity, a distinctive pattern would minimize the incidence of aggression among heterospecifics not competing for resources. While Lorenz's idea of poster coloration remains to be thoroughly tested, some reef-fish signals are certainly targeting a broad array of receivers. For example, territorial males in many pomacentrid species express a distinct black-on-white pattern to warn off all intruders, and various species of cleaner fish have converged on a conspicuous blue-and-yellow cleaning pattern that serves to attract hosts to cleaning stations.

Spatial patterning may allow such patterns to be salient to receivers close by, while affording some degree of camouflage from larger midwater predators. High spatial frequency patterns (fine lines and small spots) provide high contrast at close range. When viewed at a distance, however, they smear together and blend into the background (**Figure 2**).

Fishes inhabit almost every conceivable visual environment, and the underwater environment poses considerable challenges for visual communication. Nevertheless, fishes have evolved a remarkable range of solutions to environmental constraints on signaling, and visual signals encode information that is critical to many fishes' social relationships, cooperative interactions, and reproductive biology. Recent advances in technology, including UV-sensitive cameras and hyperspectral imaging, will be instrumental in understanding how these sophisticated signals are detected and perceived in complex natural environments.

See also: **Deep-Sea Fishes**. **Hormones in Communication**: Hormonal Pheromones. **Social and Reproductive Behaviors**: Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes. **The Skin**: Bioluminescence in Fishes; Coloration and Chromatophores in Fishes. **Vision**: Color Vision and Color Communication in Reef Fish; Photoreceptors and Visual Pigments.

Further Reading

Bagnara JT and Hadley ME (1973) *Chromatophores and Color Change*. Englewood Cliffs, NJ: Prentice-Hall.

Bradbury JW and Vehrencamp SL (1998) *Principles of Animal Communication*. Sunderland, MA: Sinauer.

Cott HB (1940) *Adaptive Coloration in Animals,* 438pp. London: Methuen.

Cummings ME, Rosenthal GG, and Ryan MJ (2003) A private ultraviolet channel in visual communication. *Proceedings of the Royal Society B: Biological Sciences* 270: 897–904.

Fernald RD (1990) *Haplochromis burtoni*: A case study. In: Douglas RH and Djamgoz MBA (eds.) *The Visual System of Fish,* pp. 443–463. London: Chapman and Hall.

Lythgoe JN (1979) *The Ecology of Vision*. Oxford: Clarendon.

Marshall NJ (2000) Communication and camouflage with the same 'bright' colors in reef fishes. *Philosophical Transactions of the Royal Society B* 355: 1243–1248.

Marshall NJ and Vorobyev M (2003) The design of color signals and color vision in fishes. In: Collin SP and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments,* pp. 194–222. New York: Springer.

Rosenthal GG (2007) Spatiotemporal aspects of visual signals in animal communication. *Annual Review of Ecology, Evolution, and Systematics* 38: 155–178.

Rosenthal GG and Lobel P (2006) Communication. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish,* 1st edn., vol. 24, pp. 39–78. San Diego, CA: Elsevier.

Widder EA (2010) Origins of biological, chemical, and ecological diversity. *Science* 328: 704–708.

Shocking Comments: Electrocommunication in Teleost Fish

K Pappas and K Dunlap, Trinity College, Hartford, CT, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Discovery of Electrocommunication
Generating Electrocommunication Signals in Two forms: Wave versus Pulse EODs
Communication through the Continuous EOD: Function in Species and Sexual Identification
Communication through EOD Modulations: Function in Aggression and Courtship
Hormonal Influences on Electrocommunication Systems
Evolution of Electrocommunication
Electrocommunication as a Model in Neuroethology
Further Reading

Glossary

Chirps Brief frequency increases in the electric organ discharge of weakly electric fish that are used as communication signals in aggression and courtship.

Electric organ discharge (EOD) Electrical discharges resulting from the summed excitation of the cells (electrocytes) of the electric organ of electric fishes that function both in communication and in locating nearby objects. Short (100 microseconds to several tens of milliseconds) electrical pulses of various voltages (a few mV to several hundred V) are generated at various rates (a few to over 1000 times per second).

Electrocytes Effector cells or syncytium specialized for generating electric fields and located primarily in the tail of electric fish. They generate and control the waveform of the electric organ discharge.

Intentional jamming An electrocommunication behavior during agonistic interactions in which a fish will adjust its electric organ discharge frequency to match that of another fish, thereby causing interference in the EOD of the other fish.

Knollenorgans Tuberous electrosensory organs used for electrocommunication among mormyrid fishes. The sensory cells are equipped with numerous microvilli and are specialized for detecting the electric organ discharge of another conspecific fish.

Melanocortins A group of peptide hormones, including adrenocorticotropic hormone and melanocyte-stimulating hormone.

Pacemaker nucleus Region in the hindbrain of electric fish that is spontaneously active and sets the discharge frequency or discharge rate.

Prepacemaker nucleus Region in the midbrain of electric fish that regulates the production of electric organ discharge modulations used in communication.

Substance P A neuropeptide particularly associated with the brain and spinal cord.

Introduction

Hundreds of species of fish swim around in the freshwaters of tropical South America and Africa chattering in a unique language: electrocommunication. Unlike their more famous cousin, the electric eel (*Electrophorus electricus*), which can produce hundreds of volts of electricity to kill prey and predators, these so-called weakly electric fish generate signals in the millivolt range for communicating with members of their own species. Over evolutionary time, these fish have modified particular sets of muscle and nerve cells to form an electric organ that produces a communication signal termed the electric organ discharge (EOD). The EOD is also used for locating objects in the environment, but the focus of this article is its use in electrocommunication (**see also Detection and Generation of Electric Signals**: Active Electrolocation). They have also evolved specialized sensory organs, electroreceptors, that detect the signals produced by themselves and by other fish (**see also Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs). Fish emit the EOD continuously, and the EOD waveform and/or discharge rate conveys information about the species and gender identity of a fish. Fish can also briefly modulate this continuous discharge to send discrete signals that function in aggression and courtship. Electric signaling is a highly specialized form of communication, but, in a remarkable case of convergent evolution, it arose independently in two orders of the teleost fish: the Gymnotiformes in South America and the Mormyriformes in Africa.

Why communicate using electricity? Most of these fish are nocturnal and live in dark or muddy waters where visual communication would be impossible. Moreover, these fish often inhabit areas that are dense in aquatic vegetation, which would distort many acoustic communication signals. In such environments, electric signals are advantageous because they are neither influenced by light levels nor susceptible to environmental distortion. However, one disadvantage of electric signals is that they degrade in strength (amplitude) over a short distance. Consequently, electrocommunication is only effective for most species when fish are within about 1 m of each other.

Discovery of Electrocommunication

Ancient Egyptians and Greeks were well aware of the capacity of fish to generate electricity since they had experienced the powerful electric discharges of strong electric fish. However, it was not until the 1950s, largely through the work of zoologist Hans Lissmann, that scientists learned about the role of weak electric discharges in communication. Lissman collected weakly electric fish, mainly *Gymnarchus*, from the Upper Volta River in West Africa, and transported them to his laboratory at Cambridge University in England. First, he observed that whenever an electrical current was sent through a copper loop placed in the fish's tank, the fish would react aggressively. Then he recorded the continuous EOD of these fish and played it back through one of six sets of electrodes placed in the aquarium. The electric fish only attacked the electrodes that delivered the EOD, indicating that EOD was both detectable and behaviorally meaningful to the fish.

Generating Electrocommunication Signals in Two forms: Wave versus Pulse EODs

Most electric fish generate their electric field through modified muscle cells termed electrocytes. Like muscle cells, electrocytes fire action potentials, but unlike muscle cells, they fire nearly synchronously and have no contractile elements. The timing of electrocyte firing – the tempo and rhythm of electrocommunication – is controlled by a particular region of the hindbrain, the pacemaker nucleus. Neurons in the pacemaker travel down the spinal cord and synapse on to electromotor neurons that, in turn, synapse with electrocytes. The shape of the waveform – the timbre of the communication signal – is largely determined by the structure of the electrocyte membranes and the rate at which ions flow into the electrocyte (**see also Detection and Generation of Electric Signals**: Electric Organs and Generation of Electric Signals).

The timing and shape of EODs are extraordinarily diverse among species. Nevertheless, electric fish can be placed in one of two general categories based on their EOD form. In fish with a wave-type EOD, the EOD takes the shape of a continuous, quasi-sinusoidal wave that, when the fish is at rest, is remarkably constant in waveform and frequency (**Figure 1**). Indeed, the EODs of these fish are among the most precise biological oscillators

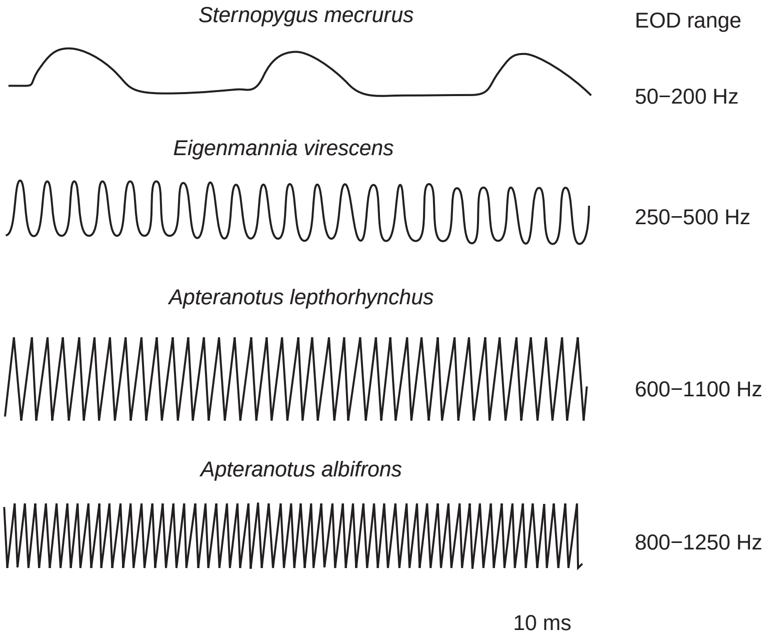


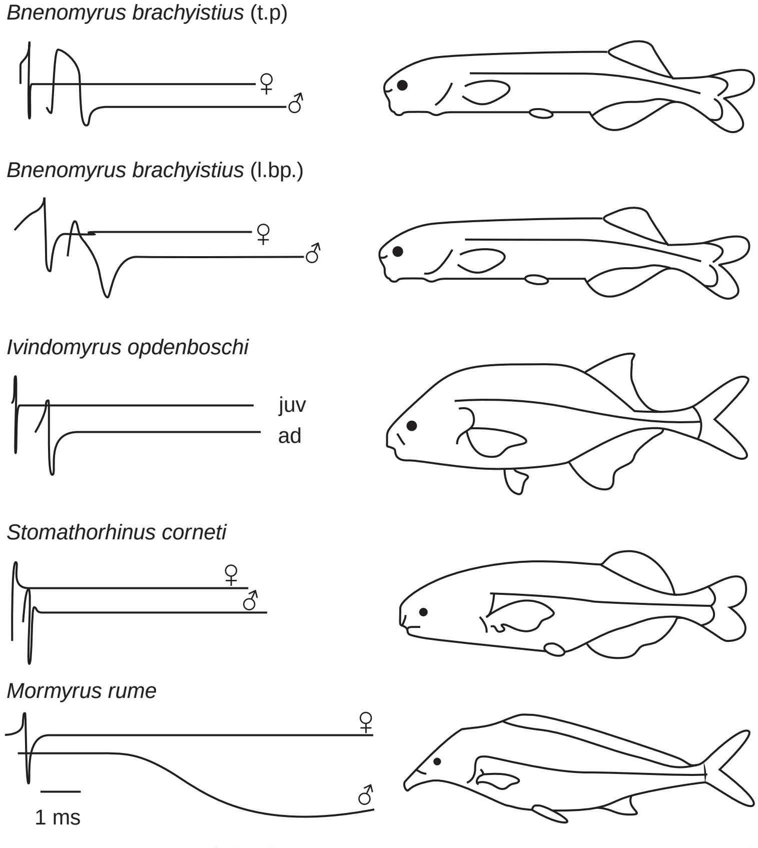
Figure 1 Wave-type electric organ discharges (EODs) of four Latin American weakly electric fish species. The EODs differ primarily in frequency, and these differences are used in species recognition. Reproduced from Smith GT (1999) Ionic currents that contribute to a sexually dimorphic communication signal in weakly electric fish. *Journal of Comparative Physiology A* 185: 379–387 and Zakon HH and Smith GT (2009) Weakly electric fish: Behavior, neurobiology, and neuroendocrinology. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, and Rubin R (eds.) *Hormones, Brain and Behavior*, vol. 2, ch. 17, pp. 611–638. New York: Academic Press, with permission from Elsevier.

known. When these signals are transduced into an audio signal, they sound like a constant ring at a single pitch that reflects the EOD frequency of the fish. Because EOD contains mostly a narrow range of frequencies, the electroreceptors are narrowly sensitive to this frequency range. Only one African species emits a wave-type discharge; about two-thirds of South American electric fish species have wave-type discharges.

Other fish generate pulse-type EODs, in which the time between pulses is longer than the duration of the pulse (**Figure 2**). The discharge rate within an individual is highly variable. When these signals are transduced into an audio signal, they sound like a rapid popping that frequently speeds up and slows down. Some species can even completely turn off their discharge for periods as long as 10 s. In pulse-type fish, the EOD contains a broad spectrum of frequencies, and their electroreceptors, compared to those of wave-type fish, are more broadly tuned. Mormyriform electric fish have a unique electroreceptor type, the Knollenorgan, that is specialized for electrocommunication. Through higher brain processing, the activity in Knollenorgan pathway is silenced when a fish produces its EOD, and thus the Knollenorgan activates only in response to the EOD of another fish. All but one African electric fish species and about one-third of gymnotiform electric fish in Latin America emit pulse-type EODs.

Communication through the Continuous EOD: Function in Species and Sexual Identification

Electric fish emit EODs continuously beginning from a few days after hatching (**see also Detection and Generation of Electric Signals**: Development of Electroreceptors and Electric Organs). The waveform and frequency of the continuous EOD conveys information to other fish about individual and/or species identity. At maturity, these parameters of the EOD commonly



Bnenomyrus brachyistius (t.p)
♀
♂
Bnenomyrus brachyistius (l.bp.)
♀
♂
Ivindomyrus opdenboschi
juv
ad
Stomathorhinus corneti
♀
♂
Mormyrus rume
♀
♂
1 ms

Figure 2 Pulse-type electric organ discharges (EODs) of five African weakly electric fish species. The EODs differ in both waveform and duration, and these features are used in species recognition. The figure also shows sexual and age-dependent variation in EOD waveform that is used in sexual identification. Reproduced from Zakon HH and Smith GT (2009) Weakly electric fish: Behavior, neurobiology, and neuroendocrinology. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, and Rubin R (eds.) *Hormones, Brain and Behavior*, vol. 2, ch. 17, pp. 611–638. New York: Academic Press and courtesy of C. Hopkins, with permission from Elsevier.

diverge between males and females and thus can be used to convey sexual identity as well.

Pulse-Type EODs

Among fish with pulse-type EODs, many species have their own characteristic waveform. For example, a few species have a single phase (monophasic); most have two (biphasic) or three phases while some have as many as four phases (**Figure 2**). Across species, the pulse duration ranges from less than 0.1 ms (e.g., *Polliyrus isidori*) to nearly 10 ms (e.g., *Paramormyrops gabonensis*). The ability of fish to discriminate variation in waveform was demonstrated by Heiligenberg and Altes, who measured the response of a pulse-type fish, *Hypopomus artedi*, to playback EODs that varied in waveform, but not in frequency content. Fish responded at highest rates to natural waveforms from their own species and considerably less to synthesized waveforms, in which the phases of the waveform were shifted. Other scientists demonstrated that, in another pulse-type fish *Gymnotus carapo*, each individual has its own stable and characteristic waveform. These fish are very territorial, and they can use these waveform signatures to distinguish familiar neighbors from potentially threatening intruders.

In the breeding season, the EOD waveform of pulse-type fish differentiates sexually (**Figure 2**). Most commonly, the pulse duration of males increases. In the mormyrid *Brienomyrus brachyistius*, mature males have an EOD pulse that is twice the duration of the female EOD. When males were presented with playbacks of another male, they tended to ignore the signal. But when presented with female EODs, males briefly accelerated their discharge rate to form a courtship signal termed a 'rasp'. Males also rasped to a synthetic square pulse, but only if it was the same duration as a natural female EOD, indicating that duration is the key component of the female EOD that males attend to.

In the gymnotiform pulse fish*, Brachyhypopomus gauderio*, males broaden their EOD pulses and increase their height (amplitude) during the night, and females are attracted to the males with the broadest, tallest discharge. However, broadening the pulse incurs at least two costs. First, males spend a great deal energy to generate this sex-typical signal. They expend over 20% of their daily energy use on electric signaling, compared to only 4% for females. Second, broadening the pulse adds low-frequency energy to their EOD and makes them more susceptible to predators that are sensitive only to such low frequency (see below).

Wave-Type EODs

Among wave-type fish, species identity is largely conferred through EOD frequency rather than waveform. Species vary in EOD frequency from 50 to 2000 waves per second or hertz (Hz), with each species commonly emitting their own characteristic pitch (**Figure 1**). When fish are presented with playbacks of different frequencies, they are much more likely to make attacks and electrical displays toward frequencies in their own species specific range than toward frequencies typical of other species.

In many wave-type species, EOD frequency is sexually dimorphic. The most common pattern is for males to have lower EOD frequency than females, though in some species, such as the well-studied *Apteronotus leptorhynchus*, that pattern is reversed. Male-typical frequencies usually provoke an aggressive response from males while female-typical frequencies commonly elicit a courtship response. Within a sex, EOD frequency also apparently signals social status. For example, in *A. leptorhynchus* living in a large social group, males with the highest (most masculine) EOD frequency tended to dominate the best retreat sites. Females with the highest EOD frequency outcompete low-frequency females for retreat sites as well.

Communication through EOD Modulations: Function in Aggression and Courtship

In addition to conveying information through the continuous EOD, fish communicate more context-specific information through transient modulations of the EOD. These modulations vary in duration from 10 ms to as long as 10 s, and fish produce them at different rates and proportions depending on whether they are engaged in aggression or courtship. For example, many pulse-type fish simply turn off their EOD for about 0.1 to 2 s during either same sex or opposite sex interactions. These signals, termed interruptions, are generated when neurons in the mid-brain send signals to the hindbrain pacemaker nucleus and inhibit its firing.

Some wave-type species that have particularly high EOD frequencies, such as those in the genus *Apteronotus*, cannot interrupt their EOD. Instead, they briefly accelerate their EOD frequency. These electrocommunication signals are termed chirps, based on the sound they make in an audio recording. Generation of chirps also originates in the mid-brain when a specific nucleus, the prepacemaker, stimulates cells in the pacemaker to transiently increase their firing frequency. During a chirp, the EOD frequency rises (50–400 Hz) for a brief period (10–200 ms) (**Figure 3**). Fish can distinguish between their own EOD chirps and those of other fish by comparing the timing or pattern of signals received by the electroreceptors on different parts of its body. When a fish chirps, it will experience the signal uniformly over its whole body. When a fish is detecting a chirp from another fish, it will feel the chirp

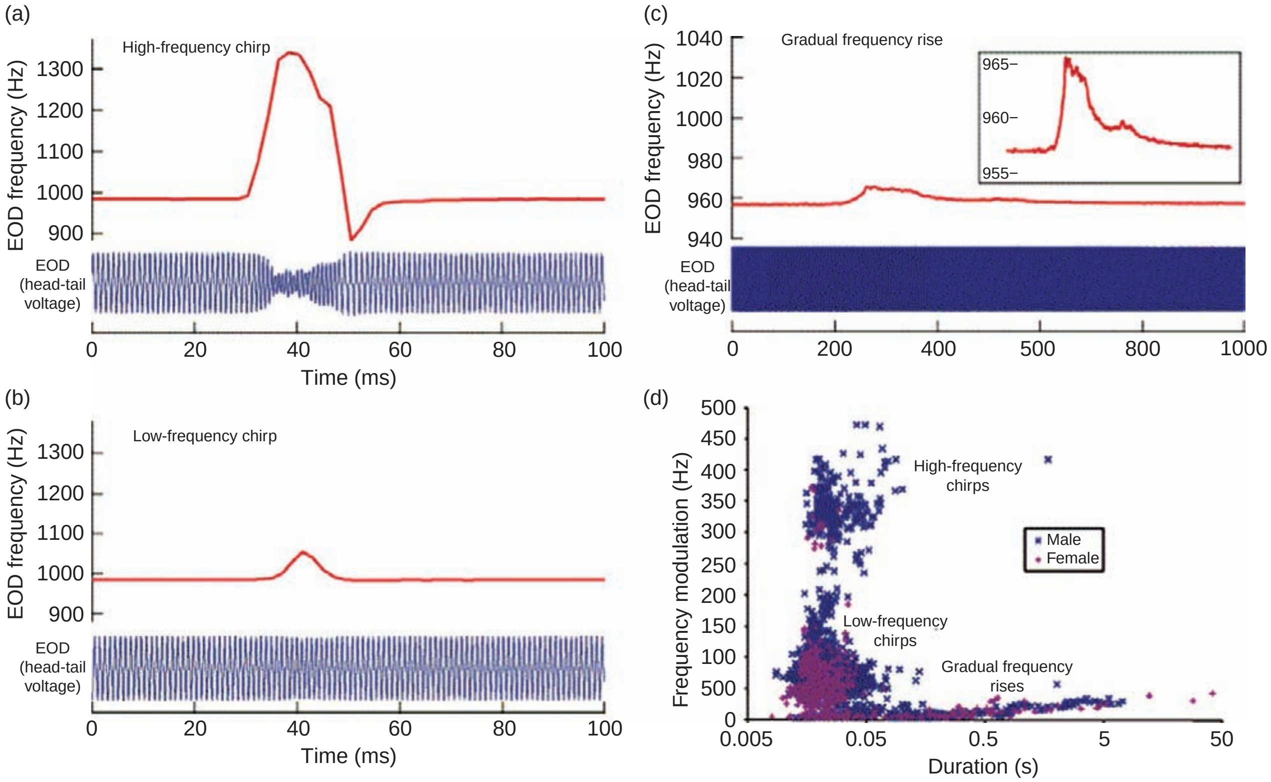


Figure 3 Electric organ discharge modulations – chirps and rises – used in electrocommunication in *Apteronotus leptorhynchus*. (a) High-frequency chirp type used primarily in courtship interactions. (b) Low-frequency chirp type used primarily in aggression. (c) Gradual frequency rise. (d) Scatter plot showing the three classes of EOD modulations and their differential production by sex. Compared to males, females rarely produce high-frequency chirps and are more apt to produce gradual rises. Reproduced from Zakon HH and Smith GT (2009) Weakly electric fish: Behavior, neurobiology, and neuroendocrinology. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, and Rubin R (eds.) *Hormones, Brain and Behavior*, vol. 2, ch. 17, pp. 611–638. New York: Academic Press, with permission from Elsevier.

asymmetrically on either side of its body, and its own EOD will be modified by the other fish's chirp.

Aggression

In nature, electric fish often live in rather dense aggregations and compete for food, mates, and retreat sites, so aggression between individuals of the same species is common. Fish chase and attack each other while modulating their EOD through frequency increases or interruptions. In the pulse-type mormyrid species *Gnathonemus petersii*, fish change their discharge rate at different phases of an aggressive encounter. Aggression often begins with a head butt, which is accompanied by an increased discharge rate. The aggressive fish frequently takes an antiparallel position next to the fish it is attacking, positioning its electric organ (located in the tail) next to the second fish's head, where the electroreceptors are at greatest density. In this position, fish increase their discharge rate from about 10 to 140 Hz. During a chase, the aggressor commonly signals through short (300–1000 ms) or long (<1 s) interruptions.

Some pulse-type gymnotiformes, such as *Brachyhypopomus* and *Gymnotus*, interrupt their EODs during aggression as a sign of submission. In fights between males, the defeated fish makes a sequence of interruptions once the outcome has been established. After these interruptions, the fighting subsides. In male–female pairings, the female often interrupts her discharge when she is persistently pursued by a male, as if she is electrically hiding.

Chirps are the most prominent aggressive electrocommunication signal in wave-type gymnotiforms such as *Apteronotus*. In *Apteronotus* agonistic chirps, EOD frequency rises about 50 Hz for about 20 ms (**Figure 3**). During male–male interaction, the largest males with the highest EOD frequency chirp at the highest rates, indicating that chirping signals dominance. Two males interacting at a close distance commonly trade chirps at the rate of about once per second in a regular back and forth rally – an echo response. Fish chirp most vigorously during the nonviolent period between attacks, suggesting that chirps may serve as a warning signal to deter aggression rather than a threat of impending attack.

Female *Apteronotus* are far less likely than males to produce chirps in agonistic interactions, but much more commonly produce longer duration EOD modulations termed rises. During a rise, the EOD frequency only increases by ~5–20 Hz, but the duration is much longer (1–200 s) than in chirps (**Figure 3**). When interacting with males, rises are usually long in duration (~30 s) and are commonly considered submissive or courtship signals. However, when females interact with each other, the meaning of rises appears to change. Females with a high EOD frequency make short-duration (~1 s) rises toward females with lower EOD frequencies. Because high-frequency females are usually dominant to low-frequency females, these rises appear to function in aggression, thereby establishing hierarchy among females.

Sometimes two interacting fish have very similar EOD frequencies, and their EODs interfere with each other. This phenomenon, referred to as jamming, disrupts a fish's ability to use its EOD frequency for electrolocation, and many fish adjust their EOD frequency up or down to avoid such interference. Despite the impairment caused by jamming, sometimes fish exhibit intentional jamming as an apparent aggressive signal (**Figure 4**). For example, when *Apteronotus* fight over a shelter, the fish with the lower EOD sometimes elevates its signal into the jamming range of the second fish, presumably as a means of threatening the second fish. In a study using EOD playbacks, fish elevated their EOD frequencies to match the frequency of the EOD playback while biting the electrodes immediately before and during the modulations, indicating that intentional jamming is an aggressive response.

Courtship

Electric signaling is equally important in courtship as it is in aggression. In the pulse-type mormyrid, *B. brachyistius*, males are very territorial and commonly lunge at and chase nearby females, while making quick bursts of ~100 pulses in a second. In this phase, females often make short interruptions of their discharge. During courtship, males circle around and follow the female while

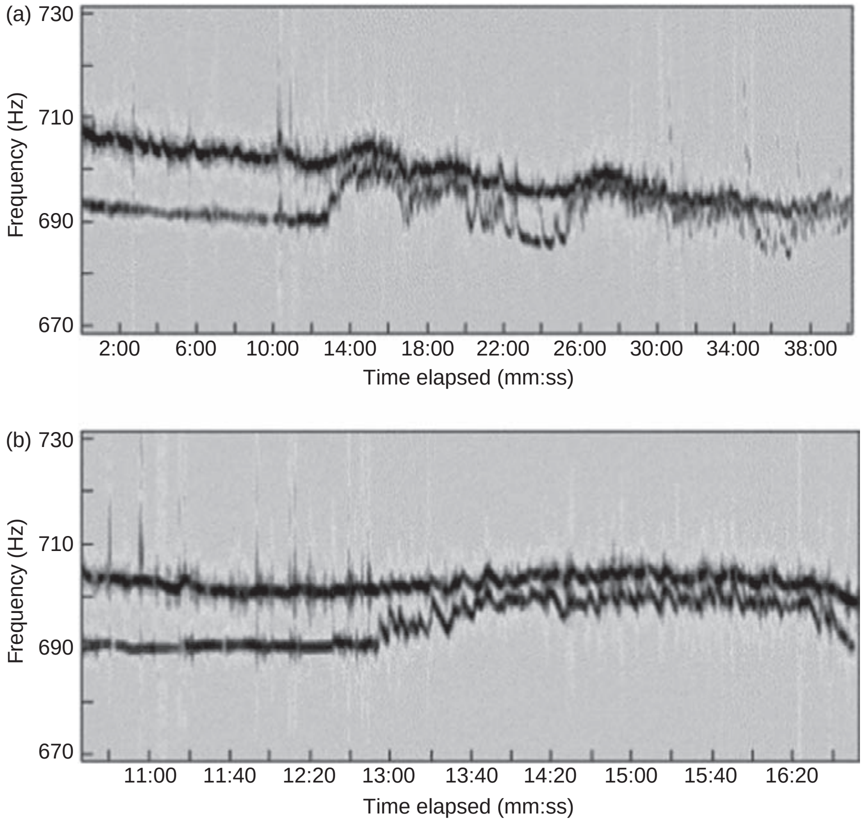


Figure 4 Intentional jamming during agonistic interactions between two female weakly electric fish, *Apteronotus leptorhynchus*. (a) Two fish began the interaction with electric organ discharge (EOD) frequencies at about 693 and 708 Hz. After 12 min, the fish discharging at 692 Hz increased its EOD frequency by about 10 Hz to match the EOD of the other fish and thereby causing interference. (b) Enlargment of (a) showing one detailed phase of intentional jamming. In both panels, chirps are visible as brief upward spikes emitted most commonly by the fish in the upper trace. Reproduced from Tallarovic SK and Zakon HH (2005) Electric organ discharge frequency jamming during social interactions in brown ghost knifefish, *Apteronotus leptorhynchus*. *Animal Behaviour* 70*:* 1355–1365, with permission from Elsevier.

producing rasps (a quick series of 4–10 pulses followed by a slow decrease in pulse rate). The female stops making interruptions and instead makes bursts of about 20 pulses in 1 s. They continue in a duet of rasps and bursts until spawning when the male begins to creak (a sequence of slow discharges and bursts, bracketed by interruptions) while the female continues to burst.

In wave-type gymnotiforms, courtship consists of a series of complex movements accompanied by bouts of chirping. These chirps are much different than those produced during aggression: EOD frequency increases are larger (200–300 Hz) and last longer (50–200 ms) than those of aggressive chirps (**Figure 3**). In *Eigenmannia,* the dominant male, who usually has the lowest EOD frequency among the males, increases his agonistic displays on the day before spawning, emitting regular chirps while patrolling a large area. After nightfall, the dominant female, who usually has the highest EOD frequency among the females, stops feeding and seeks shelter within floating plants. Meanwhile the male searches for the female while increasing aggression toward the lower-ranking males. After finding the female, the male swims back and forth underneath her while she remains in the plants. Right before spawning, the female raises her EOD frequency by tens of hertz, and the male responds with a series of courtship chirps. They then release their gametes and swim away from the plants.

Hormonal Influences on Electrocommunication Systems

Many sex differences in electrocommunication behaviors are exhibited only during the breeding period and, therefore, vary seasonally. Accordingly, the same physiological signals – sex steroid hormones such as androgens and estrogens – that control reproductive physiology also regulate electrocommunication behaviors. In many pulse fish, mature males have an EOD that is larger in amplitude and longer in pulse duration than that of females, and experimental treatment with androgens masculinizes the EOD. Androgens achieve this effect in mormyrids by acting on the morphology of the electrocytes; they increase the size of the electrocytes and the thickness of the electrocyte membranes. In gymnotiforms, androgens alter the physiology of electrocytes by changing the activity of ion channels in the electrocyte membrane. For example, in *Sternopygus,* androgen treatment causes a fish's electrocytes to produce longer, male-like discharges by slowing the rate at which sodium flows into the cell and potassium flows out of the cell. In some species, estrogens can also feminize the EOD. In *Sternopygus,* estrogen treatment speeds up the electrocyte discharge by causing changes in the sodium current that are opposite from those caused by androgens. In addition to acting on waveform and pulse duration, hormones influence EOD frequency and its modulations. Androgens decrease EOD frequency in species in which EOD frequency is lower in males than females. When sexual dimorphism in EOD frequency is reversed, such as in *A. leptorhynchus,* androgens have the opposite effect.

Steroids can directly affect the electrocommunication system by binding to receptors both in electrocytes and in neurons that control the EOD. However, steroids can also influence electrocommunication behavior indirectly by modifying other regulatory pathways. For example, androgens appear to promote chirping behavior in *Apteronotus* by acting through the serotonin and substance P systems in the brain. Androgens modify daily changes in male EOD waveform of *Brachyhypopomus* by altering the response of electrocytes to the peptide hormones in the melanocortin family.

An electric fish's electroreceptors are particularly sensitive to its own EOD, but their sensitivity also changes seasonally. If its electroreceptors did not adjust to seasonal changes in the EOD, they would decrease their ability to detect the fish's own discharge, compromising the fish's ability to electrolocate. Fortunately, electroreceptors are also steroid sensitive. When *Sternopygus* is implanted with androgens, masculinizing its EOD frequency, the electroreceptors simultaneously shift their tuning to keep them matched with their new EOD frequency.

Evolution of Electrocommunication

Electroreception, the ability to detect electric fields, arose in the earliest vertebrates over 500 million years ago and was retained in most cartilaginous fish. However, it was lost evolutionarily in the teleost fish, only to re-emerge independently twice during teleost evolution. In two orders (Gymnotiformes and Moryriformes), electroreception was coupled to the ability to generate EODs, and this opened the possibility for electrocommunication. The ancestral weakly electric fish likely had a monophasic, pulse-type EOD. However, these electric fish were likely detectable by predators, such as certain catfish, that could detect low-frequency electric components in this EOD. In subsequent evolution, species arose that had more complex multiphasic EODs, which eliminated the low-frequency components of the EOD, and thereby made them undetectable to electropredators. Although predation is commonly considered a force that simplifies communication signals, electric fish provide an example for which predation likely enhanced signal complexity.

In wave-type gymnotiforms, EOD frequency appears to have increased during evolution. The evolutionary relationships among electric fish are still the subject of debate, but many phylogenies show that the Apteronotidae, which discharge at the highest frequency, is the most recently evolved group of gymnotiforms. These fish abandoned their muscle-derived electric organ and instead evolved an electric organ that consists completely of neural tissue. Neurons in this system are connected only by electrical synapses, which enable them to discharge unusually rapidly (700–2000 Hz). This high-frequency EOD likely enhanced their ability to electrolocate in fast-moving streams and colonize waters that were unoccupied by other electric fish species. However, this full-throttle electrogenesis system is apparently unable to decrease firing rate or change EOD waveform, and this restricts the kinds of electrocommunication signals they can use. For example, apteronotids can only communicate through frequency elevations while pulse fish and low-frequency wave fish can communicate through changes in waveform, complex bursts, and decreases in pulse rate or complete interruptions.

Yet even within Apteronotidae, evolution has produced a spectacular diversity of EOD modulations in just the past ~5–10 million years. Across these closely related species, frequency elevations during a chirp can vary from 50 to almost 500 Hz, and the duration of chirps can range from 20 to 600 ms. Therefore, even though evolving a completely neural electric organ limited the production of certain classes of electrocommunication signals, these fish diversified their electrocommuncation signals through elaboration of one particular class of EOD modulations.

Electrocommunication as a Model in Neuroethology

Electric fish have become one of the most fruitful animal models in the field of neuroethology (the study for the neural basis of naturalistic behaviors). Indeed, certain electrocommunication behaviors, such as the jamming avoidance response, are understood at the cellular and circuit level more completely than almost any other vertebrate behavior. The utility of electric fish as a neuroethological model comes largely from the fact that the neural circuits underlying electrocommunication are relatively simple and electrocommunication signals are relatively easy to elicit and playback in the laboratory.

Electricity is the language of the nervous system in all animals. In most animals, the brain's electrical signals get translated into communication signals by activating a complex motor apparatus (such as the vocal apparatus). In electric fish, communication is far more direct; there is almost no translation between signal production by the nervous system of one fish and signal reception by another fish. The activity of specific cell types is very directly tied to the generation of the communication signal. Consequently, it is relatively easy to investigate how modification of cellular processes leads to changes in electrocommunication behavior. Moreover, because electric signaling varies so much among species, one can explore how electric circuits underlying behavior have evolved to generate diversity of communication systems.

See also: **Detection and Generation of Electric Signals**: Active Electrolocation; Development of Electroreceptors and Electric Organs; Electric Organs; Morphology of Electroreceptive Sensory Organs; Generation of Electric Signals.

Further Reading

Bass AH and Zakon HH (2005) Sonic and electric fish: At the crossroads of neuroethology and behavioral neuroendocrinology. *Hormones and Behavior* 48: 360–372.

Carlson BA (2002) Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *Journal of Physiology – Paris* 96: 405–419.

Curtis CC and Stoddard PK (2003) Mate preference in female electric fish, *Brachyhypopomus pinnicaudatus*. *Animal Behaviour* 66: 329–336.

Dunlap KD and Oliveri LM (2002) Retreat site selection and social organization in captive electric fish, *Apteronotus leptorhynchus*. *Journal of Comparative Physiology A* 188: 469–477.

Hagedorn M and Heiligenberg W (1985) Court and spark: Electric signals in the courtship and mating of gymnotoid fish. *Animal Behaviour* 33: 254–265.

Hopkins CD (1981) The neuroethology of electric communication. *Trends in Neurosciences* 4: 4–6.

Hupé GJ and Lewis JE (2008) Electrocommunication signals in free swimming brown ghost knifefish, *Apteronotus leptorhynchus*. *Journal of Experimental Biology* 211: 1657–1667.

McGregor PK and Westby GWM (1992) Discrimination of individually characteristic electric organ discharges by a weakly electric fish. *Animal Behaviour* 43: 977–986.

Möller P (1995) *Electric Fishes – History and Behaviour*. London: Chapman and Hall.

Silva A, Quintana L, Perrone R, and Sierra F (2008) Sexual and seasonal plasticity in the emission of social electric signals. Behavioral approach and neural bases. *Journal of Physiology – Paris* 102: 272–278.

Smith GT (1999) Ionic currents that contribute to a sexually dimorphic communication signal in weakly electric fish. *Journal of Comparative Physiology A* 185: 379–387.

Stoddard PK (2002) The evolutionary origins of electric signal complexity. *Journal of Physiology – Paris* 96: 485–491.

Tallarovic SK and Zakon HH (2005) Electric organ discharge frequency jamming during social interactions in brown ghost knifefish, *Apteronotus leptorhynchus*. *Animal Behaviour* 70: 1355–1365.

Tan EW, Nizar JM, Carrera GE, and Fortune ES (2005) Electrosensory interference in naturally occurring aggregates of a species of weakly electric fish, *Eigenmannia virescens*. *Behavior and Brain Research* 164: 83–92.

Turner CR, Derylo M, de Santana CD, Alves-Gomes JA, and Smith GT (2007) Phylogenetic comparative analysis of electric communication signals in ghost knifefishes (Gymnotiformes: Apteronotidae). *Journal of Experimental Biology* 210: 4104–4122.

Wong RY and Hopkins CD (2007) Electrical and behavioral courtship displays in the mormyrid fish *Brienomyrus brachyistius*. *Journal of Experimental Biology* 210: 2244–2252.

Zakon HH and Smith GT (2009) Weakly electric fish: Behavior, neurobiology, and neuroendocrinology. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, and Rubin R (eds.) *Hormones, Brain and Behavior,* vol. 2, ch.17, pp. 611–638. New York: Academic Press.

Zupanc GK, Sîrbulescu RF, Nichols A, and Ilies I (2006) Electric interactions through chirping behavior in the weakly electric fish, *Apteronotus leptorhynchus*. *Journal of Comparative Physiology A* 192: 159–173.

Fish Learning and Memory

VA Braithwaite, Penn State University, State College, PA, USA; University of Bergen, Norway

© 2011 Elsevier Inc. All rights reserved.

Introduction
Different Forms of Learning and Memory
Areas of the Fish Brain Involved in Learning and Memory
Factors That Affect Learning and Memory
Sensitive Phases for Learning or Imprinting
Learning and Memory in Applied Contexts
Summary
Further Reading

Glossary

Amygdala A small area of the mammalian brain that processes emotional information.

Cerebellum The small hindbrain found in vertebrates.

Cognition The process of sensing, learning, and remembering information.

Hippocampus A small discrete area of the mammalian brain that processes information connected with spatial learning or timing and also plays a role in consolidating memories.

Lateral pallium A defined area within the fish telencephalon that functions like a mammalian hippocampus.

Medial pallium A defined area within the fish telencephalon that functions like a mammalian amygdala.

Telencephalon The forebrain area in a vertebrate brain.

Introduction

Animals that can learn and remember information can alter their behavior in response to changes in their environment; such abilities help promote survival and success. Multiple experiments and observations across a broad range of fish species have shown that fish have impressive, sometimes even sophisticated, learning and memory abilities. Some species of fish are able to navigate long-distance migrations using memories of places they have previously been to guide their chosen routes. Other species that spend much of their time living within a territory learn and remember key facts about neighboring rivals. If a rival male invades your territory, knowing how strong or committed he is will enable you to modulate your own level of aggression and will help you to decide whether to escalate to a fight or not. Thus, learning and memory allow fish to vary and fine-tune their responses on a daily basis.

To integrate and consolidate information and to turn this into memories demands a certain degree of brain specialization. Fish have several distinct brain areas that are devoted to processing different forms of learning and memory. This article explains our current understanding of fish cognition, and it will consider the function of learning and memory and the kinds of complex behavior that it promotes. A few examples will explore the kinds of mechanism that underlie certain cognitive processes.

Different Forms of Learning and Memory

Even on a day-to-day basis, fish need to learn and remember different kinds of information. On a very basic level they can form simple associations. For example, they might learn that less aggressive opponents have lighter pigmentation in their skin and so lighter colored fish will be less of a threat. However, fish can also learn more complex associations. For instance, they can learn lists of landmarks to help them navigate to a specific place and they can learn elaborate escape routes when threatened. Some species learn to combine different kinds of information and then integrate these to make decisions about how to behave. For example, fish learn to integrate time information with spatial information – learning different events occur in certain parts of the environment at different time points in the day. Thus, fish show a range of learning abilities from simple to complex.

As fish learn new information, they build memories that can be recalled and used in the future. Studies with birds and mammals have found that they can store different types of memory – for instance, sometimes individuals recall single facts over a short period of time (e.g., short-term memory), but other forms of memory combine several pieces of information such as what kind of event occurred as well as where and when it happened (known as episodic memory). In contrast to the many studies in birds and mammals, we know less about memory processes in fish. Some work has shown that fish can remember and recognize the identity of specific individuals. Small groups of guppies, *Poecilia reticulata*, for example, can learn to recognize one another. Being able to recognize individuals also has been found to promote cooperation and even decreases competition in foraging situations in threespine sticklebacks, *Gasterosteus aculeatus*.

Areas of the Fish Brain Involved in Learning and Memory

The fish brain has clear similarities to other vertebrate brains – as we move from the front of the fish toward the back of the head, there is a distinctive forebrain, followed by a cluster of structures in the mid-brain and then to the hindbrain before reaching the spinal cord (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes). Many of the neural connections between different brain regions, including those seen in fish, are similar across different vertebrate taxa. Yet, as you look at a fish brain, it looks different to the brains of birds and mammals (**Figure 1**).

This is because fish lack a well-developed cortex; instead they have a much thinner pallial layer associated with the paired hemispheres that make up their forebrain – also known as the telencephalon. Our brains have a particularly thick cortex formed from six layers that make up the gray matter known as the neocortex. The convoluted neocortex is the material that gives our own brains their distinctive crinkled appearance.

Several distinct regions within the fish brain contribute to learning and memory processes. The small hindbrain, or cerebellum, helps to control various aspects of movement and motor coordination, but it is also involved in certain forms of simple learning. For instance, the cerebellum has been shown to affect some categories of associative learning such as classical conditioning – where fish learn about the predictive relationship between stimuli. Simple associative learning is the same process that made Pavlov's dogs salivate when they heard the sound of a bell. Over a series of trials Pavlov would ring a bell a few seconds before food was made available. Very quickly, a dog learns that the bell predicts food. Once the dog has learned the association, the sound of the bell is sufficient to start the dog salivating in anticipation of receiving food. For over 100 years (the earliest by Mobius in 1873), researchers have used classical conditioning experiments with fishes in thousands of experiments. Some have used appetitive conditioning (e.g., conditioning that a light or sound signals food and results in eating behavior) and others have used aversive conditioning (e.g., conditioning that a light or sound signals a shock and results in escape behavior).

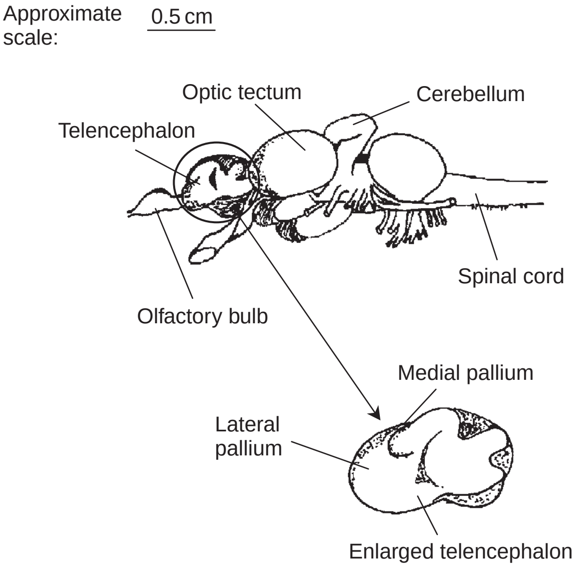


Figure 1 Schematic drawing of a goldfish brain. The telencephalon, or forebrain, has been enlarged to show more detail of this structure.

Many other kinds of stimuli and responses are used in associative learning. One commonly used technique in mammals pairs a light switching on followed by a puff of air blown at the eye resulting in a blink. Fish do not blink, but instead perform an eye retraction response, where their eyes briefly move a little way back into their sockets in response to a brief, mild electric shock close to the eye that induces a sensation similar to tingling in the skin. Goldfish (*Carassius auratus*) trained in this way quickly learn to retract their eye as soon the light switches on.

This kind of learning process is very widespread across the animal kingdom – even jellyfish and sea slugs learn to make connections if one stimulus predicts the onset of another. As learning associations between pairs of stimuli can be achieved by forming just a few connections within a small circuit of neurons, it is often considered to be a simple form of learning.

We know that the fish hindbrain, or cerebellum, plays a role in simple learning because damage to this area disrupts the fish's ability to learn the association between the light coming on and the delivery of the mild shock. Control experiments demonstrate that spontaneous delivery of the shock alone still generates the eye retraction response – so surgically lesioning the cerebellum does not prevent the fish from showing the eye retraction response. Rather, the lesioning affects the ability to learn the predictive link between the light coming on and the shock (**Figure 2**). More recently, experiments have shown that the metabolic activity of the cerebellum is increased when intact, normal goldfish are learning the association between the light followed by the delivery of the electric shock. Cytochrome oxidase (COX), a key component of metabolism, levels were significantly higher in fish trained with paired, predictive stimuli. This was not the case for

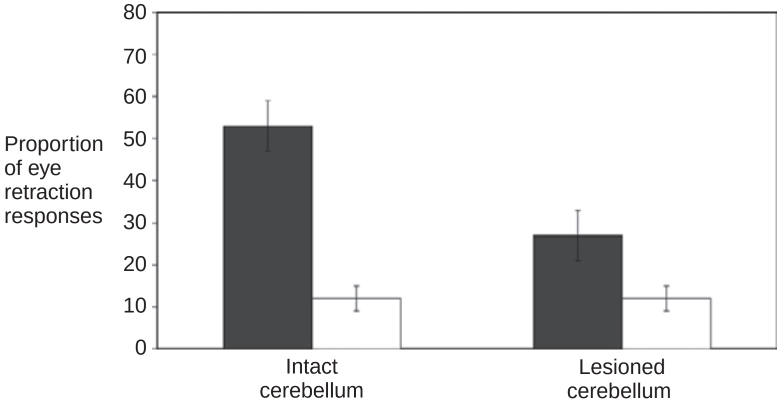


Figure 2 Mean proportion of eye retraction responses (±standard error) in fish with either an intact cerebellum or where it has been lesioned. Filled bars show the responses of fish that are given a light cue shortly before the onset of a mild electric shock. Fish with an intact cerebellum learn to respond to the light coming on, but learning this association is impaired in fish with a damaged cerebellum. The white bars are spontaneous responses to the shock delivered without a predictive light cue – both groups of fish responded in a similar manner to this kind of stimulation. Reproduced from Rodríguez F, Durán E, Gómez A, *et al.* (2005) Cognitive and emotional functions of the teleost fish cerebellum. *Brain Research Bulletin* 66: 365–370.

goldfish that were given the same two stimuli but where these were presented in a random fashion – so the light no longer reliably predicted when a shock would happen.

Toward the front of the fish brain the paired spheres of the telencephalon contain areas that are connected with different kinds of learning and memory. One area, known as the medial pallial region, has been linked with emotional learning. Emotions such as fear and aggression are an important component of cognition because they help the animal learn about potentially dangerous events to avoid similar situations in the future. The role played by different brain areas within the telencephalon has been studied using surgery to make very precise brain lesions to prevent defined areas of the brain from working. After fish recover from the surgery they are given a number of different tasks to perform and their solution is compared with control fish with no lesion.

Damage to the medial pallial area of the forebrain impairs tasks that involve some form of emotional learning. In one study, goldfish were trained to avoid a mild electric shock. How the fish learn to avoid electric shocks can be measured using a shuttle-box tank – this is a tank divided into two ends with a shallow channel connecting the ends (**Figure 3**). The fish learn that when a light is switched on, they must swim to the opposite end of the tank – if the fish fail to leave the side they receive an electric shock, but if they swim to the opposite end no shock is given. Normal goldfish have no difficulty in learning that the light cue means that they should quickly escape from the area they are currently in. After surgery, fish with a lesioned medial pallial area are slower in this shuttle-box task. It is believed that damage to the medial pallial region of the fish forebrain prevents the fish from recognizing the fear associated with the delivery of a shock and this impairs the formation of the memory.

Responses strikingly similar to those seen in the goldfish have been shown in mammals when a particular area called the amygdala is lesioned. Next to the amygdala in the mammalian brain is another important region called the hippocampus; this structure in both birds and mammals is known to play a vital role in two different kinds of learning and memory process. The first is associated with

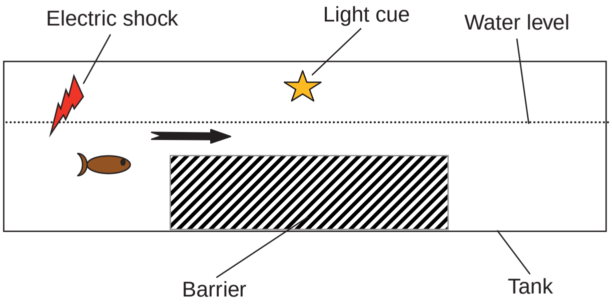


Figure 3 Schematic drawing of a shuttle-box tank. A light signal is shortly followed by the delivery of a mild electric shock. Fish learn to avoid the shock by swimming through a narrow area to the opposite end of the tank when the light cue is switched on.

spatial learning – being able to recognize where we are in an environment relative to key features such as a safe place to hide or a good place to find food. The second involves the timing or sequence of events or processes. To see if fish showed similar impairments, researchers began to look at the area next to the medial pallial region – the lateral pallial area. When this region was surgically lesioned, goldfish had deficits in learning and remembering spatial and timing tasks. Thus, the lateral pallial area functions in a very similar way to the hippocampus found in birds and mammals.

The results of these lesioning experiments are intriguing at several levels and clearly point toward a remarkable degree of anatomical conservation of brain function across the vertebrates from fish to birds and mammals. By carefully observing the early development of the fish brain and comparing this to the development of a mammalian brain, it has now been shown that the lateral and medial pallial regions of the fish brain have the same developmental origins as brain tissue that goes on to become the specialized hippocampus and amygdala (respectively) in the mammalian brain.

Factors That Affect Learning and Memory

The environment a fish lives in can influence many aspects of fish cognition. For fish that school or live in groups, the social environment can influence both what is learned and how it is learned. For example, social learning can be an effective way to learn new skills. Fish watching other fish perform a task can learn to copy the behavior. All kinds of information relevant to survival in nature can be learned by watching others, for example, the location of profitable feeding patches, the nest site of an attractive mate, a novel route that leads to a safe area with shelter, how to capture and handle a new kind of prey, or how to recognize species that are dangerous predators.

However, the process of learning about predators can be dangerous – if you make a mistake it may be your last (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). Detecting predator presence or categorizing different types of predator is possible in several fish species. In some species, such as guppies and minnows (*Phoxinus phoxinus*), pairs or small groups of fish will move closer to a predator to inspect it. This apparently intrepid behavior is used to determine how likely the predator is to seek out prey at that point in time. Once the group has gauged the predator's motivation to hunt, they return to their school.

Fish that lead a more solitary existence, or those that only school from time-to-time, typically rely on trial and error learning – learning new skills and recognizing what works or does not work for themselves. Even for these fish, the environment can shape the kinds of information that they learn and use. Different populations of threespine sticklebacks, for example, learn and use very different forms of spatial information. Fish sampled from ponds use visual landmarks to guide them to a particular location, whereas fish from river habitats use direction of water flow(rheotaxis). To explore these different preferences, researchers tested pond and river fish in a long channel down which there was a uniform flow of water and a series of visual landmarks of rocks or plants. At one end of the channel a small amount of food was hidden among gravel and the fish had to learn how to locate it. Sticklebacks from river environments almost always relied on the direction of water flow to help them locate the hidden food, whereas sticklebacks from pond environments relied on visual landmarks (**see also Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?; **Figure 4**).

A series of control trials proved that the sticklebacks did not rely on the smell of the food, but rather they learned how to find the food using concepts such as 'swim upstream', 'swim downstream' or 'the food is by the rock'.

In certain cases memory duration appears to be directly affected by how changeable different experiences are. For instance, fish from stable and unchanging environments develop longer-lasting memories than fish from places where things change on a regular basis. Examples of this can be seen in how different prey capture and handling skills are remembered. If fish consistently experience one or two forms of prey, they remember the specific techniques that enable them to effectively forage on those prey. However, fish that face changes in availability of different prey species appear to forget how to handle certain kinds of prey if they do not encounter them for a few days.

Sensitive Phases for Learning or Imprinting

Some forms of learning take place only during a particular phase in an animal's life. Learning that occurs during a particular sensitive time or phase is often referred to as imprinting. Not all fish undergo phases of imprinting, but one well-studied example is the Atlantic salmon, *Salmo salar*. During the first year or so of their life, juvenile Atlantic salmon live in freshwater streams and rivers. In late spring juvenile salmon start to change their behavior and physiology as they prepare for the migration that will take them out to sea (**see also Fish Migrations**: The Biology of Fish Migration and Pacific Salmon Migration: Completing the Cycle). As their bodies begin to prepare for life in saltwater, there are surges in hormones such as thyroxine that are believed to promote reorganization in specific parts of the brain associated with processing olfactory information. These areas change because the fish

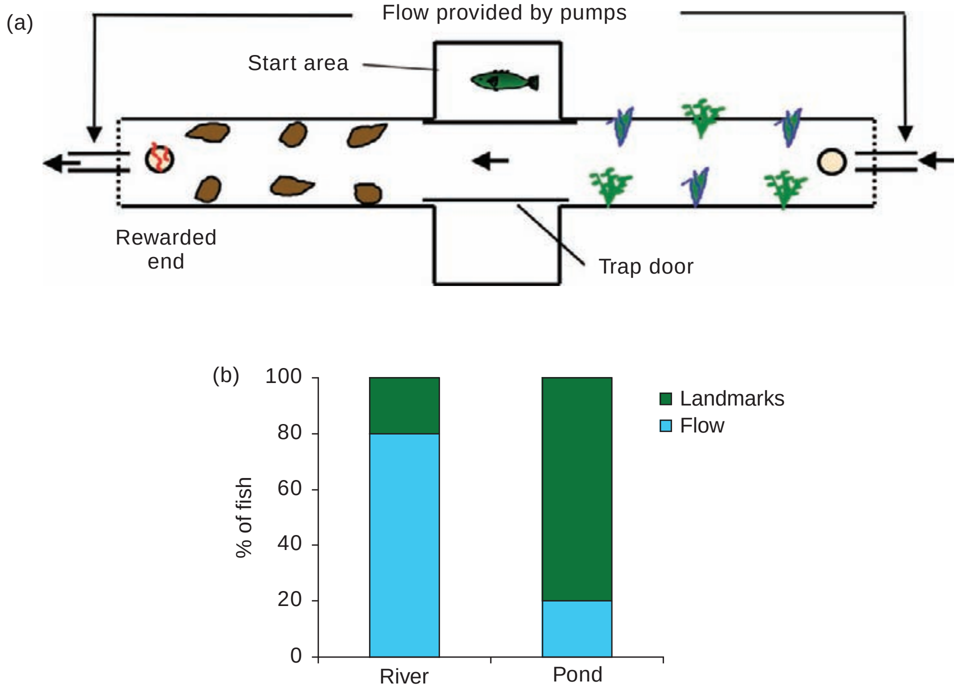


Figure 4 (a) Schematic diagram of the channel with two spatial cues: water flow direction or different visual landmarks. Threespine sticklebacks sampled from rivers and ponds are tested to determine which type of cue they prefer to use to help them locate the food reward (three worms) hidden at one end of the channel. The fish can be released from one of two possible start chambers and the order in which these are used is randomized to prevent the fish from learning a simple turn direction to solve the task. Using both start chambers during training prevents the fish from learning rules such as 'turn right on entering the channel'. Once fish have learned to locate the food reward, the spatial cue they use (water flow or visual landmark) can be tested by putting the two cues into conflict with each other. For example, the flow direction can be reversed within the channel, or the plant and rock landmarks could be switched between ends. (b) Results from a cue conflict trial. Fish sampled from rivers were shown to follow the flow direction to try and find the food reward. However, fish sampled from ponds preferred to remember which visual landmarks they should swim past.

need to learn and remember the signature odors of their river system. As the fish begin to swim downstream toward the sea they imprint, or learn, river-specific smells released by the plants and animals living within the river. These smells form a sequence, or list, of learned odor landmarks that can be remembered and reversed years later to help the fish find their way home when they are sexually mature.

Learning and Memory in Applied Contexts

Knowing that fish are capable of different forms of learning and memory and how this affects their behavior is used to help manage populations of fish. For species that are farmed, it is increasingly common for farmers to train the fish to feed themselves. Special feeders allow fish to obtain only a small meal of a few food pellets at a time as opposed to large numbers of pellets being sprayed across a sea cage or pen at random times of day. This method of self-feeding has a number of advantages; it means the fish are able to feed when they are hungry and motivated to feed; it results in better growth. Furthermore, this technique uses less food overall which leads to less waste which has both positive economic and environmental implications.

There are several different types of self-feeder now commercially available. Some require the fish to swim toward the feeder and this breaks a light beam signaling the feeder to release a small number of pellets. Other designs require the fish to push a paddle with their snout or pull on a string to release the food pellets (**Figure 5**).

In situations where populations of fish are threatened, various conservation programs try to restock captive-reared fish in attempts to increase the biomass of the fish population. Many reintroduction programs have failed because the majority of released fish perish shortly after release. The problem appears to be that once the fish are released into the wild, they need to learn how to forage on live prey and how to respond to predators. The hatchery environment does little to promote learning and memory in this regard – during early development all the fish experience is the constant, safe routine environment where pellet food is plentiful and predators are excluded. Various attempts have now been made to train the fish as to how to feed on live prey, how to recognize predators, and how to respond to change and novelty by learning and remembering previous experiences.

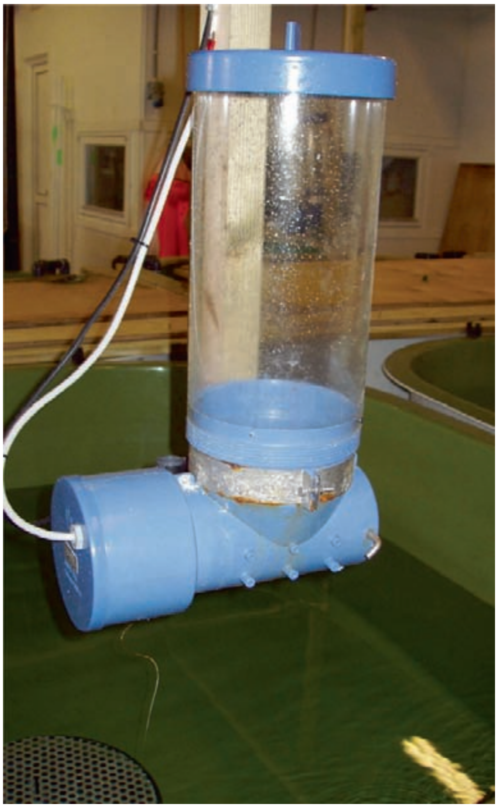


Figure 5 Photograph of an ArvoTec TD2000 self-feeder. The wire underneath the feeder can be pulled to deliver a small number of pellets into the water below the feeder. Fish learn to manipulate the wire using their mouths.

Summary

In this article the fundamental role played by learning and memory processes has been explored. These skills give fish the ability to adapt and shape behavior, allowing fish to out-smart predators and helping them find and relocate profitable feeding sites. Although fish were once portrayed as simple stimulus–response automata with limited memory capacity, we now recognize many fish as having impressive cognitive abilities, with some fish even rivaling other land-based vertebrates such as the birds and mammals.

See also: **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food. **Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes. **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle; The Biology of Fish Migration. **Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?.

Further Reading

Braithwaite VA (2006) Cognition in fish. *Behaviour and Physiology of Fish* 24: 1–37.

Braithwaite VA and Salvanes AGV (2008) Cognition: Learning and memory. In: Magnhagen C, Braithwaite VA, Forsgren E, and Kapoor BG (eds.) *Fish Behaviour*, pp. 33–60. Enfield, NH: Science Publishers.

Brown C, Laland K, and Krause J (eds.) (2006) *Fish Cognition and Behavior*. Oxford: Blackwell.

Fernö A, Huse G, Jakobsen PJ, and Kristiansen T (2006) The role of fish learning skills in fisheries and aquaculture. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behavior*, pp. 278–310. Oxford: Blackwell.

Griffiths SW and Watrd A (2006) Learned recognition of conspecifics. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behavior*, pp. 139–165. Oxford: Blackwell.

Odling-Smee L, Simpson SD, and Braithwaite VA (2006) The role of learning in fish orientation. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behavior*, pp. 119–138. Oxford: Blackwell.

Rodríguez F, Durán E, Gómez A, *et al.* (2005) Cognitive and emotional functions of the teleost fish cerebellum. *Brain Research Bulletin* 66: 365–370.

Rodríguez R, Broglio C, Durán E, Gómez A, and Salas C (2006) Neural mechanisms of learning in teleost fish. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behavior*, pp. 243–277. Oxford: Blackwell.

Shettleworth SJ (1998) *Cognition, Evolution and Behavior*. Oxford: Oxford University Press.

Nociception or Pain in Fish

LU Sneddon, University of Chester, Chester, UK

© 2011 Elsevier Inc. All rights reserved.

What Are Nociception and Pain?
Why Is Fish Pain Controversial?
Learning to Avoid Noxious Stimuli
Behavioral Responses to Pain or Nociception in Fish
Species-Specific Responses
How Important Would Pain Be to a Fish?
Future Research Perspectives
Further Reading

Glossary

Analgesia The absence of the sense of pain without loss of consciousness usually provided by an analgesic drug or endogenous opioids.

Analgesic A medication or drug (painkiller) that reduces or eliminates pain.

Classical conditioning A learning process in which an organism′s behavior becomes dependent on the occurrence of a stimulus in its environment by pairing a neutral stimulus with a stimulus that evokes a behavioral response; the stimulus that evokes the behavioral response is given whether or not the conditioned response occurs until eventually the neutral stimulus comes to evoke the behavioral response.

Habituation The gradual decline of a response to a stimulus resulting from repeated exposure to that stimulus.

Negative reinforcement A particular behavior is strengthened by the consequence of the stopping or avoiding (e.g., escape) of a negative condition (e.g., electric shock). Negative reinforcement strengthens a behavior because a negative condition is stopped or avoided as a consequence of the behavior.

Nociception The neural process of encoding and processing noxious stimuli that may damage tissue and be potentially painful. It is the afferent activity produced in the peripheral nociceptors that is transmitted to the central nervous system.

Nociceptors Sensory cells that preferentially detect mechanical, thermal, or chemical stimuli, above the noxious threshold. Nociception is usually accompanied by a reflex withdrawal response and may also result in the sensation of pain.

Noxious stimulus An agent that can potentially cause injury to tissue. Agents such as high mechanical pressure, extremes of temperature (usually above 40 °C for mammals and 30 °C for temperate fish or below 4 °C), and chemicals that excite the free nerve endings of nociceptors (e.g., acids or irritants), are noxious.

Pain An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

Stress-induced analgesia A physiological mechanism where increased stress reduces sensitivity to noxious stimulation or pain. Moderate stress provides an analgesia effect via the release of endogenous opioids from the central nervous system.

What Are Nociception and Pain?

The detection of a noxious stimulus that may produce or actually produces tissue trauma is termed ‘nociception’. This important sensory mechanism is typically followed by an instantaneous reflex withdrawal response away from the noxious stimuli. Nociception can be considered as an alarm system that alerts an animal to potential damage and enables it to take appropriate action to minimize or avoid injury. All vertebrates, including fish, and some invertebrates have nociceptors, receptors which preferentially detect noxious stimuli.

Tissue damage gives rise to the sensation of pain in humans but whether animals experience an internal emotional state and have feelings is subject to debate. Human pain is described by the International Association for the Study of Pain as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” For pain assessment to occur we rely on self-report between humans – we can tell each other about the pain we feel. However, this is impossible when trying to detect pain in other animals. Nociceptors, of course, do not detect pain *per se.* Instead, like all other sensory receptors, they simply detect an environmental change, which in this case is a noxious and potentially damaging stimulus (**see also Toxicology:** The Effects of Toxicants on Olfaction in Fishes). The sensation is produced by the nociceptive system and the brain.

To explore the possibility of pain perception in nonhumans we use indirect measures similar to those used for human infants who cannot convey whether they are in pain. We measure physiological responses (e.g., cardiovascular) and behavioral changes (e.g., withdrawal) to assess whether a tissue-damaging event is painful to an animal. If these responses are comparable to those of humans and are protracted negative changes, this may signify some degree of discomfort. In addition, if the administration of a painkiller or analgesic reduces any adverse reactions in animals, then it can be hypothesized that these responses are a direct result of a noxious, potentially painful, experience.

Definitions of animal pain are based upon behavioral and physiological changes rather than subjective states that cannot be measured. In addition to the detection of noxious stimuli and the accompaniment of an immediate reflex withdrawal, vegetative responses may also occur that signal pain (e.g., inflammation and fight-or-flight responses). Of the key behavioral changes, this article focuses on learning to avoid a noxious stimulus, nonreflexive changes in behavior, and suspension of normal behavior. Many behaviors shown by injured animals (including humans) aim to reduce additional damage, and also drive the individual to learn to avoid future damage and to allow healing. Other neurobiological criteria have been suggested to verify the capacity for pain in animals.

Why Is Fish Pain Controversial?

Fish have a nociceptive system, the simple detection and reflex withdrawal response to noxious stimuli – this is an accepted fact. The debate centers on the idea that fish may experience the suffering or discomfort necessary for pain perception. In part, the debate considers the lack of an enlarged neocortex identical to the human cortex (**see also Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes). Without an enlarged neocortex, it is suggested that fish are incapable of having an emotional state similar to human feelings and that any response to pain is reflexive and limited to the hindbrain and spinal cord. However, studies have now shown that the forebrain and midbrain of several fish species are activated in a specific way to tissue damage.

If the argument that fish do not possess the brain structures to experience pain is accepted, then only primates and humans can suffer from a painful experience; hence, fish, amphibians, and reptiles, including birds and nonprimate mammals, cannot suffer and are merely reflex in nature by making only automatic responses to external stimuli without any thought process. This opinion suggests that a function suddenly arises in evolution with no primitive ancestor and, as such, is contrary to the established knowledge of the mechanisms of evolution of other motivational drivers or emotions. Rather than suggesting that animal pain has to be indistinguishable from pain as experienced by humans, perhaps animal pain should be considered on a phylogenetic sliding scale with fish having a primitive, rudimentary type of pain and humans having an advanced pain sensation.

Recent research has demonstrated that fish fulfill the criteria for animal perception of pain as defined by authors such as Bateson, Molony, Sneddon, and Zimmerman. Fish possess a similar nociceptive apparatus compared with mammals; a potentially painful stimulus results in negative changes in physiological responses and detrimental changes in behavior (although some of these may aid recovery from pain); and administration of analgesia ameliorates these reactions.

The remainder of this article now leaves the issue of perception of pain and focuses on responses to noxious, potentially painful stimuli. Specific attention is given to the capacity of fish to learn to avoid noxious stimuli, behavioral responses to a potentially painful event, species-specific responses, and finally whether such stimuli are important to a fish.

Learning to Avoid Noxious Stimuli

Fish learn to avoid noxious stimuli and often exhibit learning after just one trial in experiments using classical conditioning with negative reinforcement. These experiments involve fish learning to associate a predictive or conditioned stimulus (CS) with an unconditioned stimulus (US) to perform a conditioned response or behavior (**see also Sensory Systems, Perception, and Learning:** Fish Learning and Memory). Negative reinforcement uses a punishment to train the fish and typically this is an electric shock that is painful to animals (including humans). Fish are, therefore, trained to associate an innocuous CS (e.g., a light or sound) with the US (e.g., an electric shock) which is given rapidly after the CS to produce an escape response. Eventually, the fish learns to perform the escape response to the CS even before the electric shock is given. In this way, the fish is successfully conditioned to exhibit the escape response prior to the shock in order to avoid what should follow the CS.

Goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) will avoid an area where they are given an electric shock for some time afterward. However, if this area is also the feeding area, starved goldfish will re-enter the area after 3 days to seek food (**Figure 1**) and trade off obtaining food with experiencing the electric

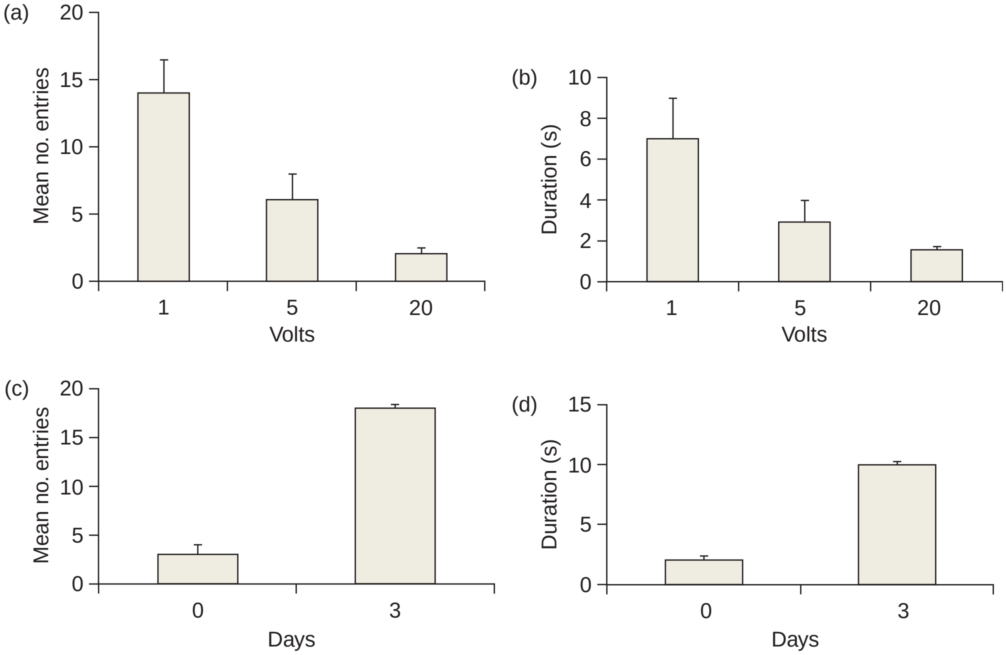


Figure 1 (a) Mean number (+SE) of entries and (b) duration (+SE) of time spent in a feeding/electric shock zone in goldfish after the application of an electric shock at 1, 5, and 20 V. (c) Mean number (+SE) of entries and (d) duration (+SE) of time spent in the feeding/electric zone in goldfish after 0 and 3 days food deprivation. Reproduced from Millsopp S and Laming P (2008) Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* 113: 247–254, with permission from Elsevier.

shock. However, as the intensity of the electric shock increases, the number of entries and time spent in this shock/food zone decrease remarkably (**Figures 1**(**a**) and **1**(**b**)). Food deprivation for 3 days results in goldfish increasing the number of entries and time spent in this shock area (**Figures 1**(**c**) and **1**(**d**)), but the fish perform more tail flips and escape responses when in this zone, possibly due to the previous experience of being shocked. This would suggest some form of fear or anticipation of the shock. Other experimental studies using classical conditioning with an electric shock show that when using morphine, a painkiller, goldfish do not respond to the shock. Therefore, teleost fish can learn to avoid a noxious, potentially painful stimulus.

Behavioral Responses to Pain or Nociception in Fish

Relatively few studies have explored the responses to a potentially painful event in fish. However, evidence is growing that fish exhibit detrimental changes in behavior while experiencing a potentially painful stimulus, although some of these changes may aid recovery. Noxiously stimulated rainbow trout, common carp (*Cyprinus carpio*), goldfish, and zebrafish (*Danio rerio*) display abnormal behaviors after subcutaneous injection of dilute acetic acid (0.1–5%) into the frontal lips. These include (1) rocking where the fish rocks from either pectoral fin on the substrate, which is a similar behavior to a fish struggling to maintain equilibrium and (2) rubbing the injection site typically against the side of the tank and/or into the substrate. These behaviors are mainly seen immediately after the injection of the noxious stimulus (**Figure 2**) when the stimulus is likely to be the greatest.

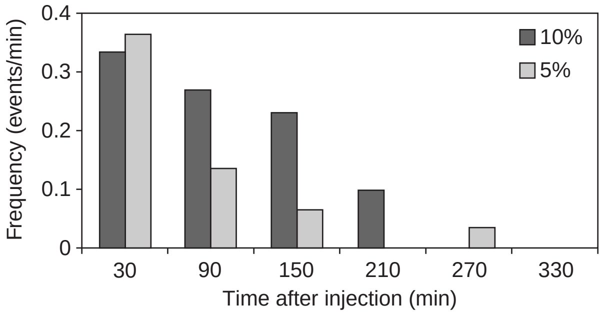


Figure 2 The frequency of anomalous behavioral events performed by common carp after subcutaneous injection of 5% or 10% acetic acid at 30-min intervals after the treatment. Reproduced from Reilly SC, Quinn JP, Cossins AR, and Sneddon LU (2008) Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114: 248–249, with permission from Elsevier

Rocking, as seen in primates and other animals, has been interpreted as an indicator of poor welfare, and the act of rubbing a painful area is seen in mammals (including humans). However, in fish these behaviors appear to be context specific. In barren conditions of rectangular or cylindrical tanks, the frequency of rubbing is reduced in rainbow trout relative to tanks that contain a gravel substrate. Increased stress also reduces the performance of these behaviors perhaps due to stress-induced analgesia. High stress results in the release of endorphins that can act as natural painkillers within the mammalian nervous system, thereby reducing the perception and performance of pain-related responses.

Decreased activity levels and frequency of swimming are also observed in zebrafish and rainbow trout (**Figure 3**), although this response appears to be species specific (see below). While the acute response to a noxious stimulus is a rapid escape response, it is typically followed by a reduction in frequency and duration of behaviors, which is believed to conserve energy for the healing process as well as having a protective role over further injury to the wounded area.

In addition to these behavioral changes, injection of acetic acid dramatically increases ventilation rate or opercular beat rate, suggesting increased metabolic rate. However, the ventilation rates observed during pain tests (90–100 beats/min) are much higher than those typically recorded for rainbow trout during other forms of stress such as handling (~69 beats/min) and are more comparable with rates recorded when trout are swimming at maximum speed. Given that activity is reduced in these fish, it would appear as if they are ventilating rapidly with no obvious extra energy demands except recovery from the painful event (**Figure 4**). This rise in ventilation rate subsides along with the reduced performance of anomalous behaviors and the return to normal activity.

One of the key factors for an animal to be considered experiencing pain is the suspension of normal behaviors. Acid-treated rainbow trout did not resume feeding for 180 min (**Figure 5**) unlike those injected with saline, which resumed feeding after 80 min. Acid-treated rainbow trout administered with morphine do not show this increased latency to feed and resume feeding at the same time as control fish. Indeed, morphine also ameliorated both the reduction in activity and the increase in ventilation rate, suggesting its efficacy as an analgesic in rainbow trout. However, another study conducted quite differently recorded rainbow trout feeding immediately after a similar noxious stimulus; the explanation for the differences between studies is unknown.

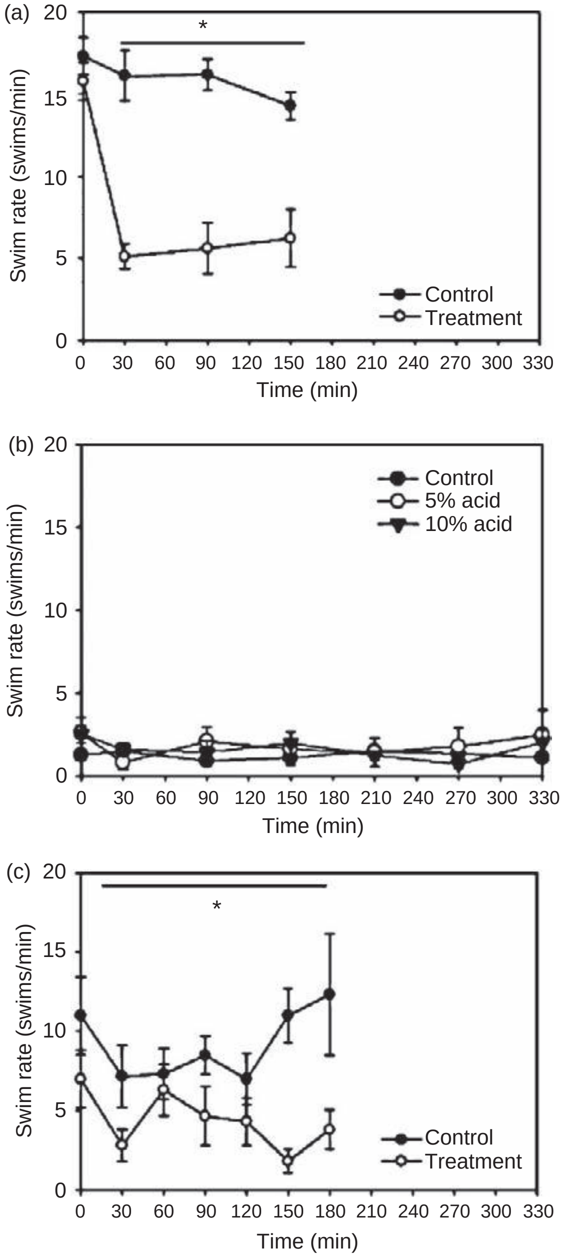


Figure 3 The mean frequency of swimming (±SE) before (0 min) and at subsequent intervals after the subcutaneous injection of saline as a control and acetic acid (treatment) in (a) zebrafish, (b) common carp, and (c) rainbow trout. Zebrafish and trout were injected with 1% acetic acid, whereas carp were injected with 5% and 10% ($^*P < 0.05$) (lines indicate significant difference between control and treatment, $^*P < 0.05$). Reproduced from Reilly SC, Quinn JP, Cossins AR, and Sneddon LU (2008) Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114: 248–249, with permission from Elsevier.

Species-Specific Responses

Pain-related responses differ between species. For example, rabbits are more likely to respond to pain by remaining motionless (freezing) for long periods unlike

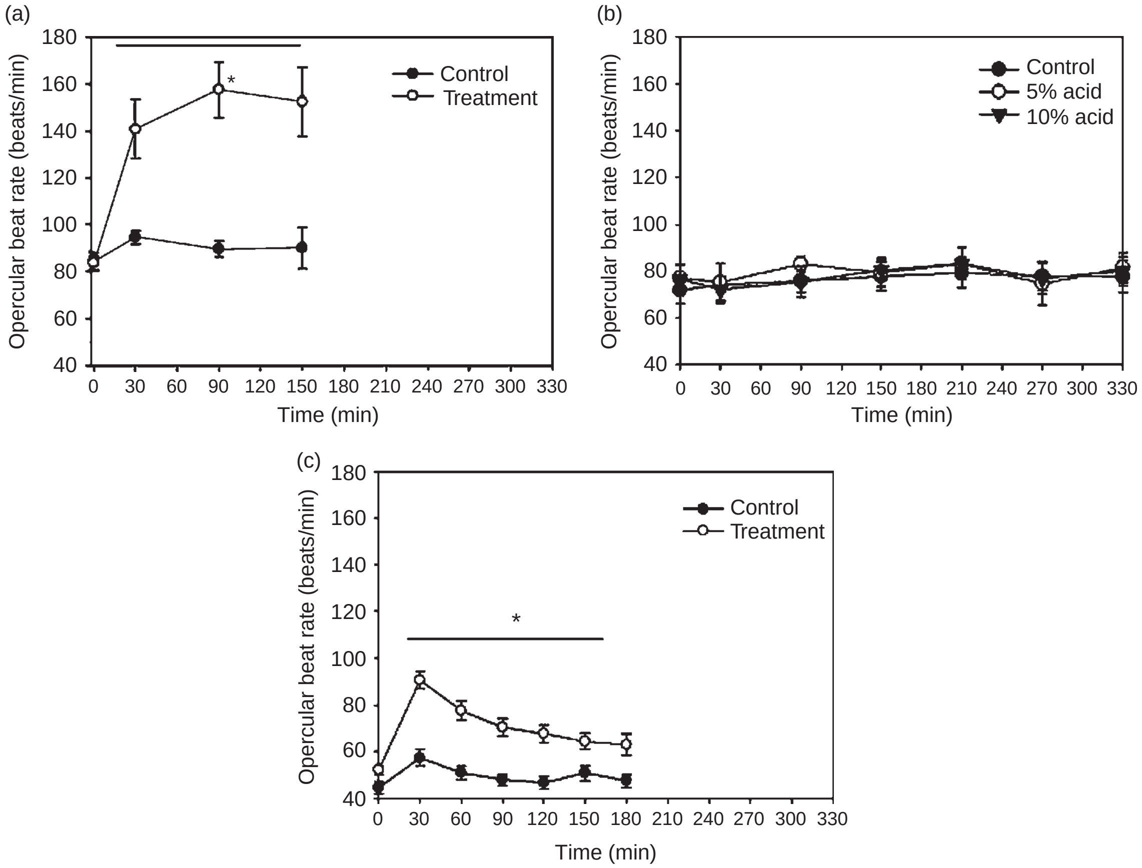


Figure 4 The mean frequency of opercular beats (±SE) before (0 min) and at subsequent intervals after the subcutaneous injection of saline as a control and acetic acid (treatment) in (a) zebrafish, (b) common carp, and (c) rainbow trout. Zebrafish and trout were injected with 1% acetic acid, whereas carp were injected with 5% and 10% (*$P < 0.05$) (lines indicate significant difference between control and treatment, *$P < 0.05$). Reproduced from Reilly SC, Quinn JP, Cossins AR, and Sneddon LU (2008) Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114: 248–249, with permission from Elsevier.

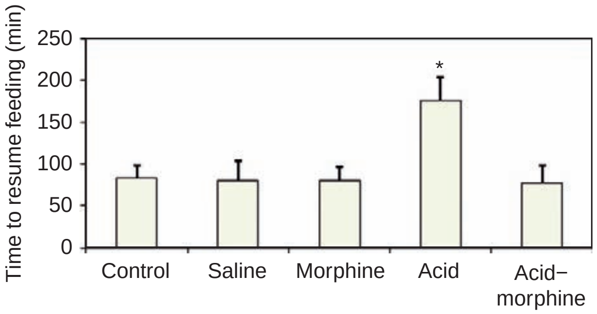


Figure 5 The mean time (+SE) rainbow trout took to resume feeding after being sham-handled but not injected (control), injected with only saline, or only morphine, or only acid, or acid plus morphine (*$P < 0.05$). Reproduced from Sneddon LU (2003) The evidence for pain perception in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science*, 83: 153–162, with permission from Elsevier.

rodents, which perform a variety of behaviors such as writhing and flinching. Common carp, rainbow trout, and zebrafish show different responses to noxious stimuli (**Figures 3** and **4**). Although all the three species express rocking behaviors, only rainbow trout and common carp rub the affected area. Zebrafish and rainbow trout decreased activity (**Figure 3**) and enhanced ventilation rate (**Figure 4**) but common carp did not (**Table 1**). This species variability has an impact on developing universal and robust indicators for assessing the perception of pain in fish. Putative pain indicators may be needed for each fish species since even within the cyprinid group, common carp and zebrafish display significant diversity in their response.

Intraspecific variation has also been recorded. Rainbow trout responses vary according to the degree of boldness (or stress coping style). Some rainbow trout are classified as bold due to their relatively high propensity for taking risks in the face of novel circumstances in contrast to shy fish that exhibit a cautious strategy and are less willing to take risks. Bold fish are much more active, aggressive, and perform high-risk behaviors when under predation risk compared with shy fish. These individual phenotypes influence many behaviors, including responses to a

Table 1 Behavioral changes seen in common carp, rainbow trout, and zebrafish after the administration of a noxious stimulus

Response to pain	*Common carp*	*Rainbow trout*	*Zebrafish*
Decrease in swimming	X	√	√
Increase in ventilation rate	X	√	√
Rubbing	√	√	X
Loss-of-balance rocking	√	√	√

√ indicates change occurs; X indicates change does not occur.
Reproduced from Reilly SC, Quinn JP, Cossins AR, and Sneddon LU (2008) Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114: 248–249, with permission from Elsevier.

potentially painful event. Under predation risk, noxiously stimulated bold rainbow trout actually reduce their use of a refuge area in response to a predator cue in contrast with both bold and shy controls which increase their refuge use. Bold trout are also more willing to perform rubbing behavior at a higher rate than shy fish. Dominance status also affects the response to noxious stimuli because subordinate trout exhibit no physiological or behavioral changes during noxious stimulation (see details below). Therefore, boldness and dominance status should be accounted for when assessing pain-related responses.

How Important Would Pain Be to a Fish?

Much of the controversy surrounding fish experiencing pain revolves around the question of whether or not the fish has a conscious awareness of pain. However, it is impossible to get into the mind of a fish and know what it truly experiences. Rather than tackling this impossible-to-measure concept, researchers have explored how significant the experience of noxious stimuli or pain is by providing competing stimuli. If pain is important, the animal should be unable or show impaired responses to other stimuli. However, if responses to competing stimuli take priority, then perhaps the perception of noxious stimuli is less significant to fish.

Rainbow trout normally avoid novel objects, but when given a noxious stimulus their fear response diminishes. Acid-injected fish spent approximately 33% of their time in close proximity to the object compared with <5% in control fish injected with saline. When given morphine, acid-injected fish avoid the object, demonstrating that analgesia resulted in a normal fear response. Similarly, noxiously treated rainbow trout do not perform anti-predator behaviors when a predator cue is introduced into their tank (**Figure 6**(**a**)). Control fish increase escape attempts and increase refuge use, whereas acid-injected fish do not and actually reduce cover use in response to a predator cue. These experiments demonstrate that pain may take priority in the context of fear and predation.

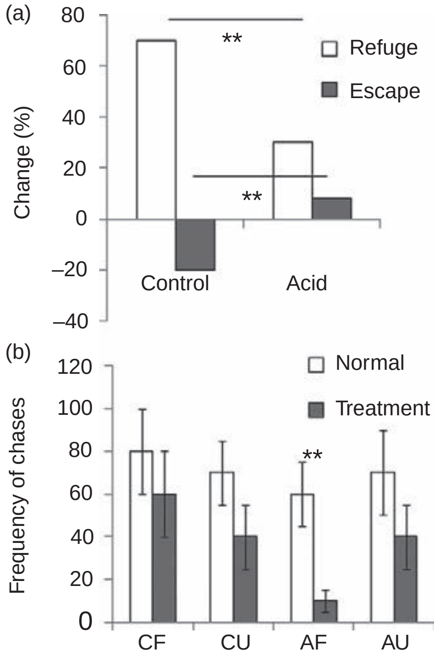


Figure 6 (a) The mean percentage change in refuse use and escape behavior in rainbow trout that were injected subcutaneously with saline as a control or acetic acid. (b) The mean frequency of aggressive chases performed by dominant rainbow trout before (normal) and after (treatment) injection with saline and placed into a familiar (CF) or unfamiliar (CU) social group or injected with acid ($^{**}P < 0.001$). AF, familiar; AU, unfamiliar (upper line indicates significant difference between control and acid fish in refuge use; lower line indicates significant difference between control and acid fish in escape behavior; $^{*}P < 0.05$). Reproduced from Ashley PJ, Ringrose S, Edwards KL, *et al*. (2009) Which is more important in fish: Pain, anti-predator responses or dominance status? *Animal Behaviour* 77: 403–410, with permission from Elsevier.

Most experiments have been conducted on individual fish and few have accounted for fish being held in social groups. Animals will readily exhibit pain-related changes in behavior in isolation, but not in the presence of conspecifics. This altered behavior may serve to protect the individual's social status. Alternatively, the external stimuli may produce opioids that would reduce pain-related changes.

Rainbow trout readily form dominance hierarchies (**see also Social and Reproductive Behaviors:** Dominance Behaviors). Acid-injected dominant trout do not exhibit any physiological changes seen in individually housed fish, such as increased plasma cortisol or elevated ventilation rate, but they do show a suspension in their normal rate of chasing the lower-ranked fish. However, when acid-injected dominants are placed in a tank with unfamiliar fish, the reduction in aggressive chases is not seen and it would appear that maintaining dominance status is the imperative in this context (**Figure 6**(**b**)). This behavior correlated with high plasma cortisol in the unfamiliar treatment, possibly resulting in stress-induced analgesia. Social status in rainbow trout and other salmonids is particularly crucial in obtaining the best resources with dominants monopolizing food and preferable habitats, whereas being subordinate comes at a high cost with increased stress levels. This may explain why status took priority over exhibiting signs of pain.

Future Research Perspectives

Research on nociception and its relationship to pain perception in fish is in its infancy. Even among the few fish species studied, profound species differences exist for the responses to noxious stimuli; therefore, many more species need to be tested to develop behavioral criteria for species-specific pain assessment. In addition, only electric shock, temperature, and subcutaneous injection of noxious stimuli to the lips have been used to evoke responses. Other tests such as application of noxious stimuli to fins may be more relevant given the prevalence of fin erosion, especially in spawning salmon in aquaculture. Confounding factors such as intraspecific variation in behavioral phenotype and environmental and/or social context also warrant further exploration. Fish may be less willing to perform pain-related behaviors in a certain context but this does not mean they are not experiencing the negative affective component of pain. Given the debate surrounding catch-and-release angling, there is little evidence to date that hooking a fish results in pain even though the tissue damage is likely to be nociceptive. Studies are needed to thoroughly examine this important area.

See also: **Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes. **Sensory Systems, Perception, and Learning:** Fish Learning and Memory. **Social and Reproductive Behaviors:** Dominance Behaviors. **Toxicology:** The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Ashley PJ, Ringrose S, Edwards KL, *et al.* (2009) Which is more important in fish: Pain, anti-predator responses or dominance status? *Animal Behaviour* 77: 403–410.

Ashley PJ and Sneddon LU (2008) Pain and fear in fish. In: Branson EJ (ed.) *Fish Welfare*, pp. 49–77. Oxford: Blackwell.

Cooke SJ and Sneddon LU (2007) Animal welfare perspectives on recreational angling. *Applied Animal Behaviour Science* 104: 176–198.

Dunlop R, Millsopp S, and Laming P (2006) Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception. *Applied Animal Behaviour Science* 97: 255–271.

ILAR Report (2009) Recognition and alleviation of pain in laboratory animals. http://www.nap.edu/catalog.php?record_id=12526 (accessed June 2010).

Millsopp S and Laming P (2008) Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* 113: 247–254.

Newby NC, Wilkie MP, and Stevens ED (2009) Morphine uptake, disposition, and analgesic efficacy in the common goldfish (*Carassius auratus*). *Canadian Journal of Zoology* 87: 388–399.

Reilly SC, Quinn JP, Cossins AR, and Sneddon LU (2008) Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114: 248–249.

Rodríguez F, Broglio C, Durán E, Gómez A, and Salas C (2006) Neural mechanisms of learning in teleost fishes. In: Brown C, Laland K, and Krause J (eds.) *Fish Cognition and Behaviour*, pp. 243–277. Oxford: Blackwell.

Rose JD (2002) The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science* 10: 1–38.

Sneddon LU (2003) The evidence for pain perception in fish: The use of morphine as an analgesic. *Applied Animal Behaviour Science* 83: 153–162.

Sneddon LU (2004) Evolution of nociception in vertebrates: Comparative analysis of lower vertebrates. *Brain Research Review* 46: 123–130.

Sneddon LU (2006) Ethics and welfare: Pain perception in fish. *Bulletin of the European Association of Fish Pathologists* 26: 7–11.

Sneddon LU (2007) Nociception. In: Hara T and Zielinski B (eds.) *Fish Physiology, Vol. 25: Sensory Systems Neuroscience*, pp. 153–179. London: Academic Press.

Sneddon LU (2009) Pain and distress in fish. *ILAR Journal* 50(4): 338–342.

Sneddon LU, Braithwaite VA, and Gentle MJ (2003) Do fish have nociceptors: Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society London B* 270: 1115–1122.

Sneddon LU, Braithwaite VA, and Gentle MJ (2003) Novel object test: Examining pain and fear in the rainbow trout. *Journal of Pain* 4: 431–440.

Yue S An HSUS Report: Fish and Pain Perception. http://www.hsus.org/web-files/PDF/farm/hsus-fish-and-pain-perception.pdf (accessed June 2010).

Relevant Websites

http://oslovet.veths.no – Norwegian School of Veterinary Science Fish Welfare Resource Site; Fish.

http://www.wellcome.ac.uk – The Wellcome Trust; Pain; Can animals feel pain?.

Spatial Orientation: How do Fish Find their Way Around?

T Burt de Perera, University of Oxford, Oxford, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Orientation Mechanisms
Sensory Systems Used to Obtain Spatial Information
Flexibility in Learning and Remembering Spatial Information
Conclusion
Further Reading

Glossary

Allocentric cues Features of the environment that are stored in an animal's memory and used to navigate.
Beacon A landmark that directly signals the position of a goal.
Compass A cue that imparts directional information.
Egocentric cues Body-centered movements related to direction traveled.
Ethmoid tissue Tissue around the nasal cavity.
Migration A regular directed movement of a group of animals.
Pilotage Orienting by chaining landmarks or locations together in sequence.
Rheotaxis A directional preference to flowing water.
Route integration An ability to gauge position in relation to a starting point, which would allow an animal to take a winding path outward and then travel directly back home. This is also known as dead reckoning.
Spatial map An internal representation of space based on surrounding objects.

Introduction

Fish are the most diverse vertebrate group, living in every aquatic niche from the open sea to small freshwater ponds, and differences in the behavior between species reflect this diversity. Some fish remain in a similar location for their entire lives and others migrate over vast distances to reach suitable feeding or breeding sites (**see also Fish Migrations:** The Biology of Fish Migration). These behaviors present a variety of navigational challenges to fish during their lifetimes. For example, Pacific salmon spawn in freshwater lakes or streams, and after a period of time, the offspring migrate to sea (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle). Once mature, the fish are able to home precisely to their natal stream to spawn, and so the cycle is repeated. These long-range migrations are one end of the navigational scale. At the other end, fish are challenged within their local home range to find mates, avoid predators, and find their way home. To achieve all of these fish must be able to orient efficiently, and in the aquatic environment this has to be in all three dimensions rather than the two with which we as terrestrial animals are familiar. Of course, fish need to obtain information about their surroundings to be able to navigate over both large and small distances. The sensory systems of fish have evolved to cope with the unique difficulties that they face in their aquatic environments. The different sensory systems that fish have also affect the way that they navigate.

This article deals with the way that fish navigate between locations. First, the mechanisms of long-range migration are discussed, before considering how fish orient over local areas using memory-based systems. Second, the senses that fish use to obtain information from their surroundings are examined. Together, this gives an overview of how this diverse group of vertebrates perceive and learn information about their spatial world, and how they act on this information to orient efficiently from place to place.

Orientation Mechanisms

Long-Range Migration

Some of the most famous examples of fish navigation are the incredible feats of salmon migration (Salmonidae). These fish spawn in freshwater systems where they reside for an amount of time that varies from days to years depending on the species, before migrating to the sea (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle). Migration is a costly process and many fish do not survive. There must, then, be overriding benefits to the fish for this behavior to have evolved. It is thought that migrating salmon take advantage of the high productivity of the marine environment. This increase in food quantity and/or quality allows the fish to grow fast, and to reach a larger overall size. Over evolutionary time, this would lead migrating fish to have a higher level of fitness (more surviving offspring), than fish that did not migrate. After a period of time in the open sea the salmon home back to their natal stream with an impressive level of spatial and temporal accuracy.

There are still many mysteries about the way that fish migrate in the open ocean. This is partly due to the difficulty of studying fish in marine environments and consequently, most of what we know about navigational mechanisms has been gleaned from controlled laboratory experiments. Salmon need two pieces of information to successfully return home. The first is the location of their current position with respect to home, and the second is the heading that they must take. In other words, they need a map and compass. It is not known how salmon ascertain their own position in the open ocean, but it is thought that they may use magnetic cues in a bicoordinate system, as some turtles do. More research has considered the cues that salmon may use as a compass, but the evidence is still scant.

A number of different sources of information could play a role in compass guidance including celestial and magnetic cues, both of which are used by terrestrial animals to navigate over large distances. By using polarizing filters, it was found that juvenile rainbow trout (*Oncorhynchus mykiss*) can use polarized light patterns in the sky to obtain directional information, but surprisingly they lose this ability as they reach adulthood, so polarized light cannot be used as a compass to guide adult salmon home. Other fish have been shown to use the sun as a time-compensated compass and this remains a candidate cue for migrating salmon. However, the problems with celestial cues are that they are unavailable when the sky is overcast, and their use is restricted to close to the water surface.

More recently, it has been suggested that salmon might orient to the Earth's magnetic field. Rainbow trout are able to detect changes in the intensity of an experimentally imposed magnetic field, and the mechanism behind detection appears to rest with the presence of biogenic magnetite particles in the snout of these fish (**see also Sensory Systems, Perception, and Learning:** Magnetic Sense in Fishes). In electrophysiological experiments, it was shown that the trigeminal nerve, near the snout of the trout, responded to changes in magnetic field intensity. Behavioral studies showed that the trout could discriminate between magnetic field intensities – the fish could be trained to swim to and strike a target to obtain food when only one of two intensities was presented. So together, the evidence shows that fish can respond to changes in the magnetic field in experimental situations. The challenge now is to determine whether they do use magnetic cues in their natural environment, when they are navigating across the open ocean.

Once they have orientated themselves in the direction of home, the salmon must locate their natal stream. Experiments have shown that they are able to do this by using site-specific olfactory cues, which the young salmon imprint on while swimming seaward. This sensitive phase in learning in the smolts coincides with an increase in the level of the hormone thyroxine, which is thought to trigger neural changes in the olfactory system. During their homing journey, the mature salmon use these learned olfactory signals to enable them to reach the precise location where their journey originated. The fish orient themselves against the direction that the signal is traveling (positive rheotaxis – i.e., facing into the current), allowing them to home to the olfactory source over long distances (**see also Fish Migrations:** Pacific Salmon Migration: Completing the Cycle and Eel Migrations).

Short-Range Orientation

Fish that do not migrate, but remain in the same area over their lifetimes, also face navigational challenges. Tracking current position in relation to important locations, such as a home site or shelter, would enable a fish to find a mate, escape predation, or find its way home. One way of achieving this is to remember body-centered movements about directions traveled (e.g., turn left, then right). This is termed egocentric orientation.

A second way to orient efficiently is to encode allocentric cues from the surrounding environment into memory. This requires an individual to learn and remember certain aspects of its surroundings, which are then used to orient. The process can be very simple, for example, a fish could learn to associate a single landmark with a food reward, but it can also be complex – some fish can even learn and remember information from different sources and integrate these together. The next section examines the experimental evidence for these processes in more detail.

Learning egocentric cues

Fish can be trained to swim through mazes to obtain a food reward. If they are not given any landmarks (either inside, or outside the maze) to help them solve the task, they can learn a sequence of body-centered movements. For example, turn left, then right, then right again. Fish could use this type of information in their natural environment – chaining a sequence of commands together to move from place to place.

So, fish can learn egocentric cues to find a goal, but what happens if the fish makes an incorrect turn in the sequence, would it then be lost? One possibility is that fish could route-integrate in a similar way that early transatlantic navigators navigated across sea. To do this they would need to gauge their position in relation to a starting point and would need information about both distance and direction. They could then take a winding path outward from a starting point, and could then travel directly back home by calculating the most direct route. However, we do not yet know whether fish are capable of storing distance and direction and route-integrating by this method.

Learning allocentric spatial cues

Another way of orienting would be to learn spatial information about the surrounding environment. One of the first experiments that tested whether fish can do this was carried out in the early 1970s. It had been noticed that tide-pool dwelling gobies (*Bathygobius soperator*) could jump from one rock pool to another when threatened by a predator. Impressively, they could do this even when they could not see the landing pool from takeoff. This suggested that the fish were remembering something about their surroundings, but how? A number of interconnecting artificial pools leading to a large pool simulating the sea were set up (**Figure 1**). Half of the fish in the experiment were allowed to experience simulated high tide; all of the pools were covered in water so that they could swim over the entire area. Half of the fish were denied this experience. The fish were prodded with a stick, and it was found that they could only jump accurately from pool to pool and eventually to the sea when they had previously experienced high tide. This suggested that the fish were learning their surroundings at high tide, and they were using this spatial knowledge at low tide when they could no longer see each pool. So fish do seem to have an ability to form a map of their surroundings, but what type of information is included?

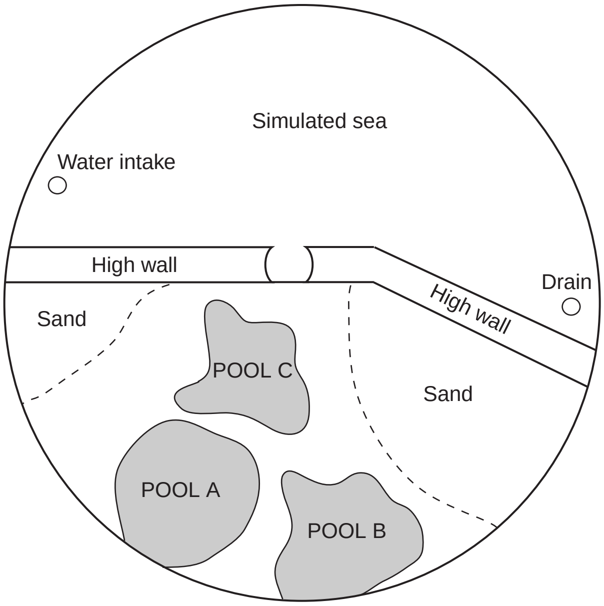


Figure 1 A plan view of the simulated tidepools used to test spatial memory in intertidal fish. At simulated high tide the fish were able to swim over the three pools and learn the general topography. At simulated low tide the fish were restricted to the pools, and could not swim over the area. Only the fish that had experienced high tide were able to jump from pool to pool and into the sea when threatened with a simulated predator. Modified from Aronson LR (1971) Further studies on orientation and jumping behavior in the Gobiid fish, *Bathygobius soperator*. *Annals of the New York Academy of Science* 188: 378–392.

Fish can use simple landmark cues to guide them. For example, goldfish (*Carrasius auratus*) were trained to use a plastic block as a beacon to label the location of a patch of food that was hidden under the substrate in a tank. However, what happens when the fish move out of range of such a landmark?

One possibility is that fish can learn a sequence of landmarks in order. We have already seen an example of this is salmon, which memorize a sequence of smells during their outward journey to the sea, and return by reversing this sequence. This ability to orient along sequences is also called pilotage and implies that fish can learn and remember the order of cues that they have previously encountered. Coral reef butterfly fish (*Chaetodon* spp.) swim backward and forward along a route to forage. When a coral reef head along this route was removed the fish got lost, suggesting that they were chaining features of the reef together in order in a similar way to the homing salmon.

This idea was tested further in blind Mexican cavefish (*Astyanax fasciatus*), which have no eyes and which orient using their mechanosensory lateral line organ. As the cavefish swim, they create a flow field in the water around them. When the fish pass an object, this field is distorted; cells in the lateral line detect these distortions and relay the information to the brain where an image of the fish's immediate surroundings is built (**see also Bony Fishes:** Blind Cavefish, **Hearing and Lateral Line:** Lateral Line Neuroethology, and Lateral Line Structure). Usefully from an experimental point of view, the cavefish swim faster when they encounter something that they do not recognize. This behavior enables a test of what the fish can learn and remember. By allowing the fish to learn an environment, changing a feature, and testing whether they speed up, one can test whether they have recognized the manipulated change, and therefore see whether they encoded that information during training. This was done in an experiment where the fish first learned a sequence of landmarks (which was shown by their speed decreasing). The landmark order was then switched. As the fish swam faster, it was concluded that they had recognized the change, and that they could learn and remember order. The perceptual range of the cavefish lateral line is less than two body lengths, but in the wild they would range across much larger areas. The experiments showed that they might be able to orient outside of their perceptual range by chaining landmarks together in order.

So, fish can use single landmarks to orient and can pilot along sequences in order. But what happens if the fish loses its way along a sequence, or if one of the landmarks moves? Fish may be able to learn and remember a spatial map that includes distances and directions between a number of landmarks. One of the first indications that fish can encode geometry came from work on goodeids. These fish appear to be able to use the angles and distances between the walls of an arena to find a reward. If placed in a rectangular arena (where the corners diagonal from one another are indistinguishable geometrically), the fish made errors as they could not tell the opposite corners apart. However, if the experimenters painted each wall a different color, the fish were able to find the reward with very few mistakes. It was concluded that they were able to learn and remember information about features and could combine this with the information about the geometry of the area to solve the task. However, recent work has revealed that instead of fish learning the complete geometry of an arena, they might just learn a snapshot-like view of one corner. This may have been enough to learn the task. Further work is being undertaken to work out how fish, and other animals, encode these kinds of spaces.

A further complication that fish face is that their environment is intrinsically three-dimensional. Aquatic systems, unlike terrestrial, are volumes through which fish can swim in all directions – up and down, side to side, and back to front. This potentially increases the difficulty of the orientation task. Aquatic environments have a unique extra cue that may aid this process, hydrostatic pressure, which fish might use to give them information about movement in the vertical axis. There has been very little work on three-dimensional orientation, and we are only just beginning to work out how fish cope with this problem.

Sensory Systems Used to Obtain Spatial Information

Fish have a number of different ways of sensing their surroundings and, not surprisingly given the difference in environments that fish and humans inhabit, these are very different from our own. Humans are highly visual animals – we tend to rely on our eyes to find our way around our local surroundings. Fish can also use visual cues, but their eye structures are different to terrestrial animals to cope with the demands of life underwater (**see also Vision:** Behavioral Assessment of the Visual Capabilities of Fish). There are two main problems for an animal that senses information using its eyes in aquatic systems. The first is the high refractive index of water, and the second is the high level of light absorption. The first is dealt with by the shape of the cornea, which is spherical with a high refractive index. The second problem is more difficult to solve. Light is absorbed by water, and scattered by suspended particles. In extremely clear water, objects cannot be seen for more than a few hundred meters, but in most cases, a fish's visual perceptive range is far shorter than this, sometimes only a few centimeters. Hence, vision may not always be the most appropriate sensory system to rely on.

Some senses impart general information about direction, or objects. For example, as seen above, fish have sensitive olfactory organs, which can be used to orient along chains of smells. Recently, sound cues have also been revealed to play an important role in orientation of coral reef larval fish. These fish return to reefs following a pelagic larval phase, and it appears that they may be attracted to reef sounds at settlement. Similar course-scale information is gained from the magnetic sense, which gives salmon long-range directional information during migration. Aquatic systems have a unique cue that is not present on terrestrial environments – hydrostatic pressure. Fish can use their gas-filled swimbladder to sense changes in pressure, and they can use this to ascertain their depth. Dogfish (*Scyliorhinus canicula*), which do not have a swimbladder, can use vestibular hair cells in their inner ear to sense changes in hydrostatic pressure. This allows fish to determine whether they are swimming up or down, which is essential in the three-dimensional aquatic environment.

Other senses impart fine-scale information. Vision is one, but as seen above, fish can sometimes be precluded from obtaining information in this way. The mechanosensory lateral line is a sensory system that is unique to aquatic animals, and that has specifically adapted to cope with the demands of a watery existence. Using this sense, fish can get extremely fine-scale information on their immediate surroundings. Some fish have also evolved an electrosense, which can operate passively, allowing a fish to respond to electric cues emanating from prey, or actively. The weakly electric elephantnose fish (*Gnathonemus petersii*) is in the latter category; they emit electric signals using a specialized organ in the tail. When these signals hit an object, they are bounced back and projected onto receptors on the surface of the skin. This is analogous to vision where images are projected onto the retina of the eye. Surprisingly, electric fish can make out object depth using electrolocation, which means that they can even perceive landmarks in three dimensions (**see also Detection and Generation of Electric Signals:** Active Electrolocation).

A fish's sensory systems can sometimes be integrated together during navigation, or they can be used in parallel systems, where one sense takes priority over another at certain times. For example, weakly electric fish use electrolocation to locate prey, but can switch to other sensory cues if the electric sense is impeded. Interestingly however, while schooling, these fish integrate both vision and electrolocation together – they need both for the behavior to persist.

Flexibility in Learning and Remembering Spatial Information

As seen above, there are a large number of ways that fish can orient efficiently from place to place. Recent work has aimed at working out when fish will use particular mechanisms. Two important factors influence what and when spatial information is learned: the first is the environment that the fish live in, and the second is the sensory system that the fish use to gain information.

Experiments have shown that fish that originate in ponds and rivers differ in whether they are likely to use landmarks or egocentric cues. Three-spined sticklebacks (*Gasterosteus aculeatus*) were trained to find food at the end of an arm of a maze-shaped like a 'T'. They could either learn to turn in one direction (e.g., left), or they could use a landmark, that was placed at the decision point, to guide them. Fish that were from ponds tended to use landmarks to orient through the maze, but fish from rivers were more likely to use body-centered cues. This seems to be due to the increased stability of ponds compared to rivers, where landmarks are more likely to be displaced by currents (**see also Sensory Systems, Perception, and Learning:** Fish Learning and Memory).

The sensory system that a fish has is also likely to influence the spatial mechanisms that it uses to orient. As described above, blind Mexican cavefish orient using their lateral line organs. These blind fish evolved from sighted ancestors that invaded caves in Northeastern Mexico about 100 000 years ago. Some of the eyed ancestors remained above ground; hence, there are two clear morphs of the same species – an eyed, surface and a blind, cave morph (**Figure 2**). The eyed fish are highly visual and can use their eyes to orient, whereas the blind fish must reply on their lateral line to gain fine-scale information about their surroundings.

Recent work has suggested that these different sensory systems affect the way that the fish orient. The eyed fish use landmarks, whereas the blind are less likely to. Presumably, this is because the perceptual range of the lateral line is smaller, so they cannot perceive landmarks from as far away as a visual animal is able.

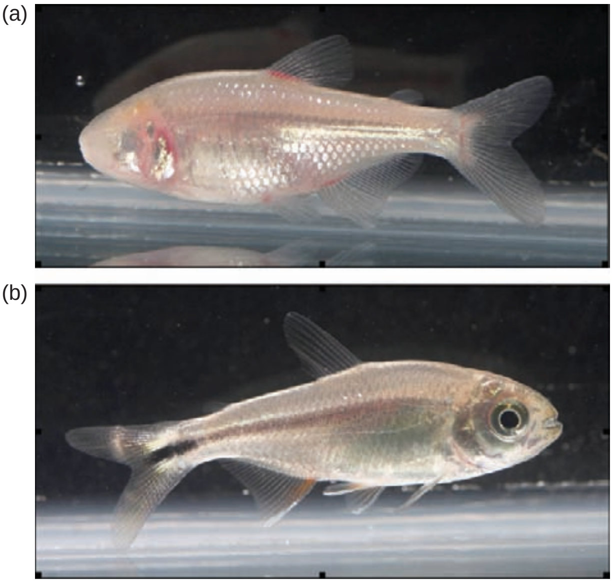


Figure 2 The two morphs of the species *Astyanax fasciatus*. (a) Blind cave morph, which relies on its lateral line to obtain fine-scale information of its surroundings. (b) Highly visual surface morph.

Conclusion

Fish are able to orient through their environments in a myriad of ways. They can migrate vast distances through relatively featureless oceans, and they can orient precisely within a short range. They use a huge variety of different senses to obtain information about their environment, ranging from senses that we are familiar with, such as vision, olfaction, and audition, to senses that are completely beyond our perceptual abilities. These include a magnetic sense which allow salmon to determine compass direction, and electrolocation, which enable weakly electric fish to perceive objects in three dimensions. The lateral line is a sense that is only found in aquatic animals, and has been exquisitely shaped to an aquatic existence. These sensory systems themselves appear to shape the way that fish orient through their surroundings, as does the environment that the fish live in. Fish can learn and remember an array of sophisticated information, which matches most terrestrial animals. They can learn simple pieces of body-centered information about whether to turn left or right to reach a goal. They can also encode spatial information from the surroundings into their memory. This process might be simple, telling a fish which landmark signals a goal, or it could be complex, including a memory of distances and directions between landmarks. In addition, fish must cope with the complexity of their volumetric world. Our challenge now lies in discovering how fish manage this, given that it is difficult for artificial intelligence systems and also for us humans.

See also: **Bony Fishes:** Blind Cavefish. **Detection and Generation of Electric Signals:** Active Electrolocation. **Fish Migrations:** Eel Migrations; Pacific Salmon Migration: Completing the Cycle; The Biology of Fish Migration. **Hearing and Lateral Line:** Lateral Line Neuroethology; Lateral Line Structure. **Sensory Systems, Perception, and Learning:** Fish Learning and Memory; Magnetic Sense in Fishes. **Vision:** Behavioral Assessment of the Visual Capabilities of Fish.

Further Reading

Aronson LR (1971) Further studies on orientation and jumping behavior in the Gobiid fish, *Bathygobius soperator*. *Annals of the New York Academy of Science* 188: 378–392.

Braithwaite VA (1998) Spatial memory, landmark use and orientation in fish. In: Healy S (ed.) *Spatial Representations in Animals*, pp. 86–102. Oxford: Oxford University Press.

Braithwaite VA and Burt de Perera T (2006) Short-range orientation in fish: How fish map space. *Marine and Freshwater Behavior and Physiology* 39: 37–47.

Burt de Perera T (2004) Fish can encode order in their spatial map. *Proceedings of the Royal Society B* 271: 2131–2134.

Cheng K (2008) Whither geometry? Troubles of the geometric module. *Trends in Cognitive Sciences* 12: 355–361.

Dittman AH and Quinn TP (1996) Homing in pacific salmon: Mechanisms and ecological basis. *Journal of Experimental Biology* 199: 83–91.

Quinn TP (2005) *The Behavior and Ecology of Pacific Salmon and Trout*. Seattle: University of Washington Press.

Holbrook RI and Burt de Perera T (2009) Separate encoding of vertical and horizontal components of space during orientation in fish. *Animal Behaviour* 78(2): 241–245.

Odling-Smee L and Braithwaite VA (2003) The role of learning in fish orientation. *Fish and Fisheries* 4: 235–246.

Sovrano VA, Bisazza A, and Vallortigara G (2003) Modularity as a fish (*Xenotoca eiseni*) views it: Conjoining geometric and nongeometric information for spatial reorientation. *Journal of Experimental Psychology: Animal Behavior Processes* 29: 199–210.

Walker MP, Diebel CE, Haugh CV, *et al.* (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390: 371–376.

Magnetic Sense in Fishes

MM Walker, University of Auckland, Auckland, New Zealand

© 2011 Elsevier Inc. All rights reserved.

Introduction
Magnetic Fields in Nature and the Laboratory
Behavioral Responses to Magnetic Fields
Neural Responses
Magnetite-Based Magnetoreceptor Cells?
Neuroanatomy
Do Fish Use the Earth's Magnetic Field to Navigate?
Outlook
Further Reading

Glossary

Magnetic moment The torque exerted on a magnet or dipole placed in a magnetic field.

Vector A quantity, such as a magnetic field, that has a magnitude (intensity) and a direction.

Introduction

Both teleost and elasmobranch fishes have been shown in behavioral experiments to possess a magnetic sense. Like birds, fish respond to both the direction and the intensity (the strength of the field or the force it exerts on, e.g., a compass needle) of the Earth's magnetic field. In compass reactions, fish orient their bodies or swim in particular directions in response to the direction of the Earth's magnetic field. Behavioral responses to the intensity of the Earth's magnetic field may be involved in position determination during navigation, in a manner similarly used by humans to determine position from geographic latitude and longitude. Taken together, these responses to magnetic intensity and direction, respectively, are consistent with the hypothesis that fish can navigate using a map to determine their current location and a compass to set a course toward a goal such as a feeding site, a migratory destination, or a home range.

How fish detect magnetic fields, however, remains unresolved. It seems likely that teleost fishes use chains of small crystals of magnetite found in the nose that act like very small compass needles in response to the external magnetic field. In contrast, the elasmobranch fishes might use either magnetite or the highly sensitive electroreceptors in the ampullae of Lorenzini, which are exposed to induced electric fields as the fish, the saltwater medium, or both the fish and medium move through the Earth's magnetic field. Neural responses to magnetic field stimuli have been demonstrated in the trigeminal and octavolateralis nerves, respectively, of teleost and elasmobranch fishes. These results are consistent with the use of both these mechanisms, but the issue of just how magnetic fields are detected in the fishes is still being debated by researchers.

Our knowledge of and ability to study the magnetic sense have grown in recent decades, but whether or not fish can use the Earth's magnetic field for navigation remains unclear. We now have reliable techniques to investigate the behavioral and neurophysiological properties of the magnetic sense. New techniques are also being developed to detect and characterize cells that contain magnetite, which may be the cells that detect magnetic fields in teleosts and perhaps also in the elasmobranch fishes. Although experimental designs for investigation of the potential use of the magnetic sense in navigation are yet to be developed, recent developments in equipment for tracking experiments suggest that studies of navigation by fishes will advance rapidly in the near future.

Magnetic Fields in Nature and the Laboratory

The Earth's magnetic field is like the field that would be produced by a very large bar magnet embedded in the core of the Earth (**Figure 1**). The north and south magnetic poles constitute a magnetic dipole that dominates (generally >90%) the field observed at the surface of the Earth. The magnetic field observed at a point on the Earth is a vector that has an intensity and a direction as indicated by a compass needle that is free to rotate in three dimensions. The declination of the Earth's magnetic field (the angle between the directions of geographic and magnetic north) arises from the displacement of the magnetic

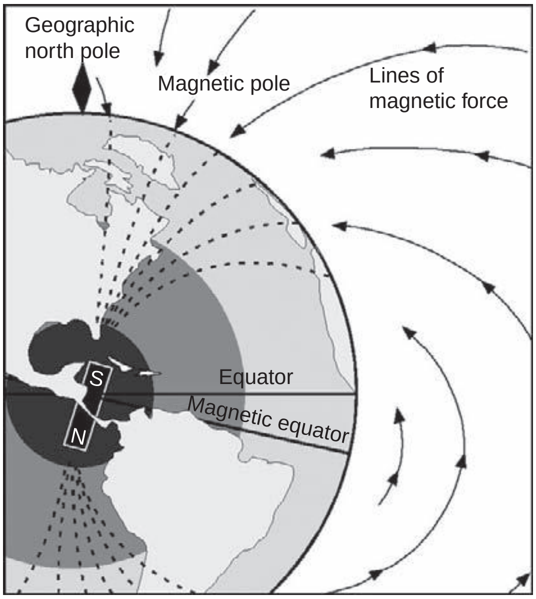


Figure 1 The main magnetic field of the Earth. The main magnetic field is a dipole (represented by the bar magnet in the core of the Earth) but contains some non-dipole components (not shown). The field due to the magnetic dipole in the core is represented by field lines, which show how the intensity (through increasing concentration of field lines) and inclination (through increasing angles of intersection of field lines with the surface of the Earth) of the field increase systematically between the magnetic equator and the magnetic poles. Adapted from Walker MM, Dennis TE, and Kirschvink JL (2002) The magnetic sense and its use in long-distance navigation by animals. *Current Opinion in Neurobiology* 12: 735–744.

poles relative to the geographic poles, and means that a magnetic compass needle seldom points directly toward geographic north.

The dipole field causes the intensity and inclination (the angle at which magnetic field lines intersect the surface of the Earth) of the vector to vary systematically from 25 to $65-70\,\mu$T and from 0° to 90° between the magnetic equator and poles, or 2–5 nanotesla per kilometer (nT km^{-1}) and about 1° per 100 km between the magnetic equator and poles. Local magnetic anomalies cause nonsystematic variations in intensity that can range from tens to thousands of nT km^{-1}, whereas the solar wind (a flow of charged particles from the sun that causes electric currents to flow in the upper atmosphere) can cause rapid variations in intensity over short periods (seconds to hours). These nonsystematic variations appear to overwhelm the systematic variation in the field and make it difficult to understand how animals such as fish might navigate using the Earth's magnetic field.

Laboratory study of the magnetic sense requires careful consideration of the magnetic fields present in the laboratory together with the use of magnetic fields produced by either magnets or, more usually, electromagnetic coils. As magnetic fields from different sources interact, an experimental situation, such as an orientation arena, should ideally be located in a purpose-built structure that is free of magnetic materials and, failing that, as close as possible to the center (in three dimensions) of a room. Electromagnetic coils provide greater control over stimulation because they permit experiments in which only magnetic field direction or intensity changes, and permit easy variation in the size of changes in field parameters.

Behavioral Responses to Magnetic Fields

Fishes from at least two teleost orders have been shown to respond to magnetic field direction in orientation experiments, whereas both teleost and elasmobranch fishes have been successfully conditioned to magnetic fields. The following sections examine key results from these experiments.

Orientation to Magnetic Direction

The critical assumption of orientation experiments is that the spontaneous directional choices made by animals placed in featureless orientation arenas match the directions they would choose in their normal environment. The directions chosen by the fry and smolts of sockeye salmon (*Oncorhynchus nerka*) in orientation arenas were consistent with the hypothesis that they were orienting to the axis of the lake in which they would live until they reached the smolt stage and began their migration to the sea. Similarly, when placed in a featureless arena where there was a radial current flow after experience with a current flowing from west to east in their home tank, juvenile chinook salmon (*O. tshawytscha*) oriented preferentially in the east–west axis despite being able to choose any orientation direction. The orientation directions of the fish in the arenas used in both sets of experiments could be changed by varying the direction of the magnetic field in the tanks using electromagnetic coils.

Laboratory Conditioning

Freely moving fish, including yellowfin tuna (*Thunnus albacares*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), sharks, and short-tailed stingrays (*Dasyatis brevicaudata*), have been trained to discriminate the presence and absence of a magnetic intensity anomaly in experimental tanks. Responses produced by the fish in different experiments included swimming through a hoop, pressing a lever, and swimming through a defined area in anticipation of food reward given at the end of experimental trials depending on whether the anomaly was present or absent. Although the tuna learned to discriminate the presence and absence of the anomaly field

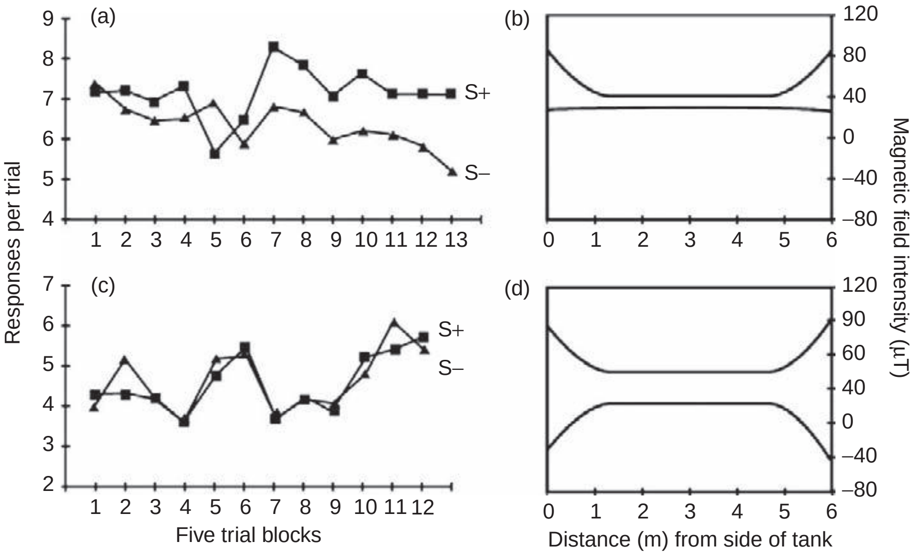


Figure 2 Behavioral responses to magnetic fields by teleost fish. (a, b) Magnetic discrimination learning in individually trained yellowfin tuna. In the training procedure, individual fish swam through a hoop during 30-s trials. Depending on the magnetic field presented, response by the fish was reinforced with food (S+, squares) given at the end of each trial or unreinforced (S–; triangles) no matter how often the fish responded. Each point is the mean of five S+ (squares) or S– (triangles) trials. Fish (*n* = 7) were required to discriminate the presence and absence (indicated by varying and constant intensity values across the tank in (b) of a localized magnetic-field anomaly projected into the experimental tank by a coil through which direct current could be passed. To control for possible generalized effects of the experimental field on behavior, some of the fish were trained with the magnetic field of the Earth as S+ and the localized anomaly as S–, whereas for the remaining fish the anomaly was S+ and the Earth's field was S–. (c) and (d) Fish (*n* = 2) were required to discriminate between two magnetic anomalies (indicated by the mirror-image curves of varying intensity values across the tank in (d) produced by reversing the polarity of the current passed through the coil. Adapted from Walker MM (1984) Learned magnetic field discrimination in the yellown tuna, *Thunnus albacares*. *Journal of Comparative Physiology A* 155: 673–679.

(**Figures 2**(**a**) and **2**(**b**)), they could not discriminate between the fields (**Figures 2**(**c**) and **2**(**d**)) that resulted from the addition and subtraction of the anomaly from the Earth's magnetic field in the tank. That is, the fish failed to distinguish between the mirror-image patterns of variation in intensity that occurred when the anomaly was added to or subtracted from the uniform Earth's field as the fish swam in the tank during trials. Finally, round stingrays (*Urobatus halleri*) have also been trained to respond to magnetic field direction by entering one of two enclosures at opposite ends of their tank depending on the direction of the magnetic field in the tank.

In cardiac conditioning, an animal that is not moving is presented with a stimulus that is followed by a brief electric shock. If the animal can detect the stimulus, the presentation of the stimulus alone will come to be followed by a decrease in heart rate. Nishi and his colleagues reported cardiac conditioning of Japanese eels (*Anguilla japonica*) to magnetic fields that differed in both magnetic field direction and intensity. Stimuli presented included changes in magnetic field intensity and direction ranging from 12 663 to 192 473 nT and from 21° to 80° easterly shift in the direction of the horizontal component of the magnetic field in the experimental tank (**Figure 3**(**a**)). Significant reductions in heartbeat rates (**Figure 3**(**b**)) occurred in 17 of 19 eels in both the marine and freshwater phases of the life cycle. Further experiments showed that these responses did not occur when the eels had been made anosmic and argued that magnetoreception takes place in or around the nares of the eel. Taken together, these experimental results demonstrate that the magnetic sense can be analyzed using conditioning approaches in the same manner as better-known sensory systems.

Neural Responses

Behavioral responses depend on information about an external stimulus, such as a magnetic field, that must be collected by specialized sensory cells and converted into streams of action potentials moving along a sensory nerve. As they travel along the sensory nerve, these action potentials can be detected by electrodes, permitting researchers to record and analyze the information transmitted to the brain from the sensory cell.

Responses in the Trigeminal Nerve of Teleost Fish

Neural recordings in response to changes in magnetic field intensity and direction, separately and together, have been made from individual nerve fibers (single

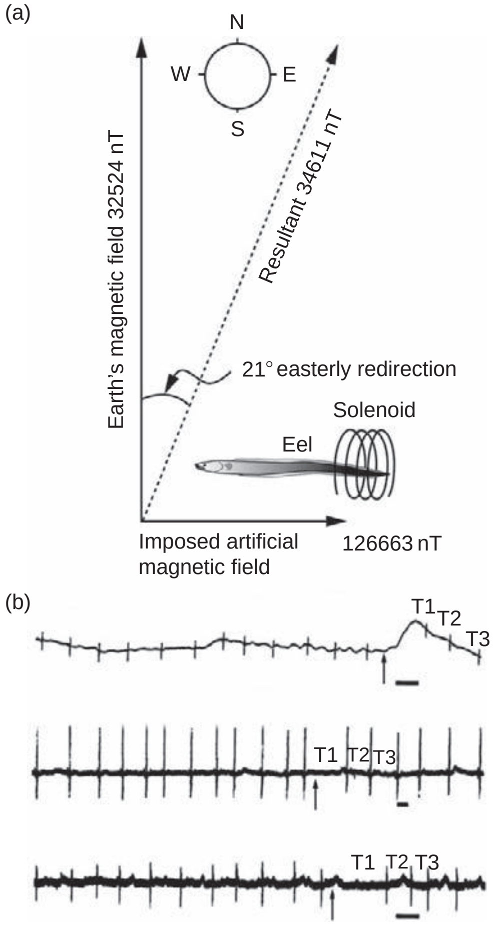


Figure 3 Cardiac conditioning responses to magnetic fields in eels. (a) The eel's head lay at the center of a solenoid (35-cm diameter) wrapped around a polyvinyl chloride (PVC) aquarium and oriented in the east–west axis. The solenoid in the apparatus produced a field that added to the geomagnetic field. The imposed field ranged between 12 663 and 192 473 nT with the resultant intensity ranging from 35 611 to 187 298 nT. These intensity changes produced resultant fields whose directions ranged from 21° to 80° to the east of magnetic north. (b) Electrocardiograms showing changes in heartbeat rate in response to magnetic-field changes in Japanese eels (scale = ~1 s). Arrows indicate the onset of the conditioning stimulus (magnetic field); T1–T3 indicate beats used to compare response to the magnetic field change with heartbeat rate during control tests in the absence of the experimental magnetic field. Adapted from Nishi T, Kawamura G, and Matsumoto K (2004) Magnetic sense in the Japanese eel, *Anguilla japonica*, as determined by conditioning and electrocardiography. *Journal of Experimental Biology* 207: 2965–2970, with permission from the Company of Biologists.

units) in the superficial ophthalmic (SO) branch of the trigeminal nerve (TN) of rainbow trout. The responsive units in the trout showed regular firing patterns except during phasic (transient) responses to a trebling of magnetic intensity presented as square waves at frequencies of 0.5 and 1 Hz (**Figures 4**(**a**)–**4**(**c**)). Both excitatory and inhibitory responses were observed, but each unit responded only to either the onset or the offset of a stimulus and responded more or less strongly to the stimulus when it was presented at different frequencies. For the units shown in **Figure 4**(**b**), post-stimulus time histograms (PSTHs) showed that the latency (10–15 ms; the first point after the stimulus step and the period during which the firing rate was more than two standard deviations above the mean for each unit) and time course (~100 ms) of the responses by the two units exposed to both stimulation frequencies were similar. The peak amplitudes of the responses in the units in the upper and lower panels of **Figure 4**(**b**) decreased and increased, respectively, when the presentation rate of the step change in intensity increased from 0.5 (left panels) to 1 Hz (right panels). **Figure 4**(**c**) (the same unit as in the upper panels of **Figure 4**(**b**)) shows the response to the onset but not to the offset of a change in magnetic intensity (upper panels) but shows no response to either the onset or the offset of a reversal in magnetic field direction. As with the unit shown, no unit responded when magnetic field direction was reversed without a simultaneous change in intensity. This finding could result from there being only a small proportion of units that respond to magnetic direction, by detection of magnetic field direction through another detection mechanism, or by integration in the brain of the signals from many different detector cells with different orientations and that each detect the field in a primary axis.

Induced Electrical Signals in Ampullary Electroreceptors

Evidence consistent with the hypothesis that elasmobranch fishes could use their ampullary electroreceptors to detect electric-current flows, induced by their own or the water's movement through the Earth's magnetic field, has been obtained by recording responses to electrical and magnetic field stimuli in the Black Sea skate (*Raja clavata*). Afferent nerves from ampullary electroreceptors in the wings, and primary area acoustico-lateralis in the dorsolateral region of the medulla oblongata of the brain, responded to both electrical and magnetic field stimuli in the skate. Excitatory and inhibitory responses were recorded in the afferent nerves so long as the magnetic field was varying continuously when the fish was not moving or when either the fish or the water were moving but the field was static. That is, responses occurred when electrical fields were likely to be induced. These results clearly confirmed that the electroreceptors do detect the electric current flows induced by magnetic field variations and by the movement of either the fish or the saltwater through a static magnetic field. Subsequent demonstration that elasmobranch fishes detect magnetic fields, in behavioral experiments, now present an

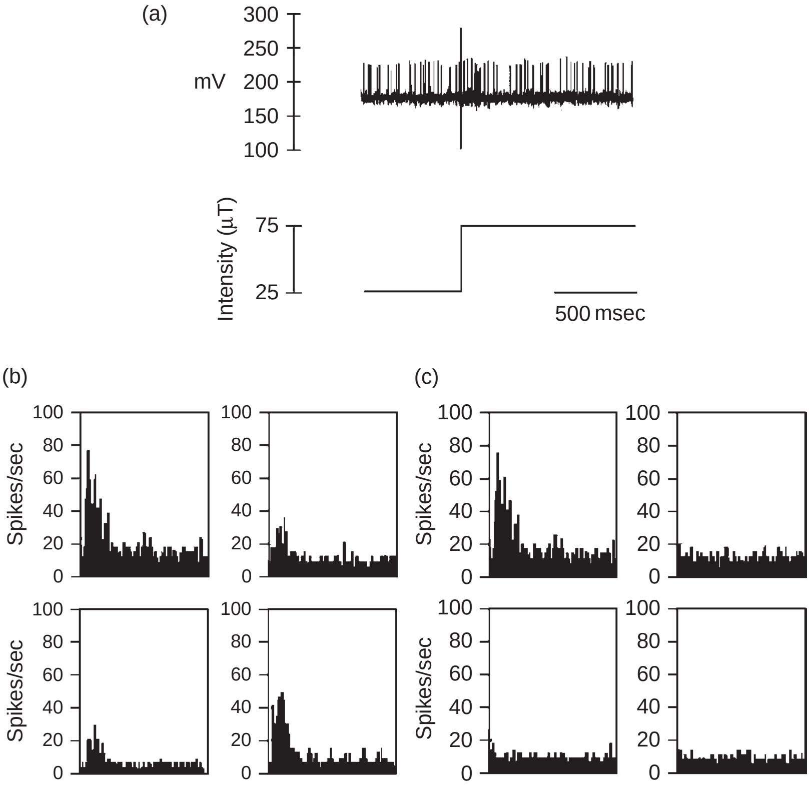


Figure 4 Neural responses to magnetic-field stimuli. (a) Spontaneous activity of a single unit (upper trace) in the TN of the rainbow trout in the background magnetic field followed by the activity for 1 s following the onset of a stimulus (lower trace) that produced a step change in magnetic field intensity from 25 to 75 μT within the experimental tank. (b) Post-stimulus time histograms (PSTHs) of responses by two spontaneously active units (unit 1 in top panels, unit 2 in bottom panels) to the same stimulus presented 128 times each at 0.5 (left panels) and 1 Hz (right panels). The magnetic stimulus trebled the magnetic field intensity but did not change field polarity. Each plot begins at stimulus onset and is of 500-ms duration with the magnetic field remaining constant throughout the period shown in each panel. Sampling bin width is 2 ms. Note that the top left panel in (b) is the same record as the top left panel in (c). (c) PSTHs of responses by one spontaneously active unit to the onset (left panels) and offset (right panels) of a trebling of magnetic field intensity (upper two panels) or a reversal of magnetic-field polarity in the experimental tank but with no change in field intensity (lower two panels). The presentation rate together with the number and duration of stimulations were as for the left-hand side panels in (b). Adapted from Walker MM, Diebel CE, Haugh CV, *et al*. (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390: 371–376.

opportunity to explore further the potential role of ampullary electroreceptors in magnetic field detection by elasmobranchs.

Magnetite-Based Magnetoreceptor Cells?

Following the discovery of magnetotactic bacteria and their use of chains of magnetite crystals for orientation, a search for magnetic material suitable for use in magnetoreception in animals identified magnetite in a variety of animals. Crystals of magnetite are divided into three categories based on their size, shape, and magnetic properties. Superparamagnetic (SPM) particles are typically less than about 35 nm in size with a magnetic moment that will rotate without movement of the crystal. Single-domain (SD) crystals are typically between 35 and 100 nm in size, are permanently magnetized, and have the maximum magnetic moments for magnetite; they will physically rotate in response to an external field if they are free to move. Multidomain (MD) particles of magnetite are typically >100 nm in size and only weakly magnetized. Of these, single domains have the strongest possible interaction with the relatively weak magnetic field of the Earth.

The simplest hypothesis for transduction of the magnetic field of the Earth into a signal that can be detected by the nervous system involves the use of chains of SD magnetite such as those that are observed in the magnetotactic bacteria and fish. The motion of these chains in response to the external magnetic field will then convert the magnetic field stimulus into a mechanical stimulus that could readily be detected using mechanically gated ion channels linked to the magnetite chain.

The crystal and magnetic properties of SD magnetite have been used to identify chains of SD crystals (**Figure 5**(**a**)) within cells in the nose of the rainbow

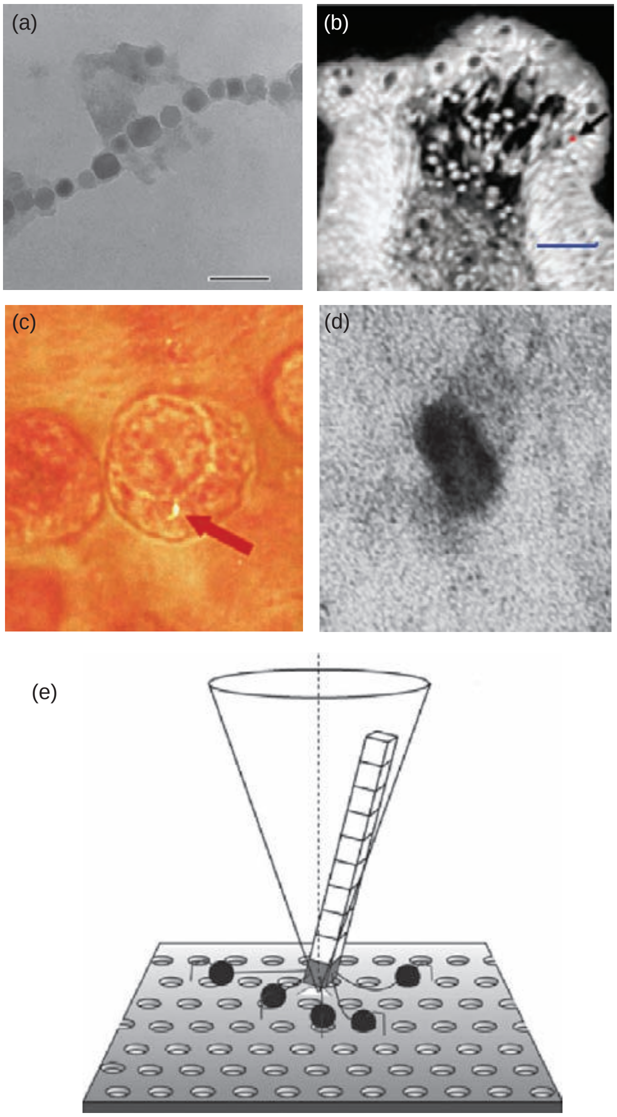


Figure 5 Detection of intracellular magnetite. (a) A chain of single-domain magnetite crystals extracted from sockeye salmon (*Oncorhynchus nerka*). Scale = 100 nm. (b) Autofluorescence image of a magnetite-containing cell within an olfactory lamella of rainbow trout viewed using a confocal laser scanning microscope (CLSM) in transmission mode. The red spot indicates where the reflection of the laser light by the magnetite was detected. Scale = 25 μm. (c) CLSM 30 − μm wide overlay in reflected and transmitted light modes of a candidate magnetoreceptor cell isolated from a trypsin digest of an olfactory lamella. The arrow indicates a chain of reflecting crystals. (d) Transmission electron micrograph of a crystal associated with a reflectance in the trout olfactory lamellae. The crystal is 50 nm long, iron rich, and was distinguished as a crystal from other dark particles in the thin section by its bright reflection in dark field. (e) Conceptual model showing how a magnetosome chain might be connected at one end to the cell membrane (surface) and linked by cytoskeletal filaments (lines attached to spheres) to an array of mechanically gated ion channels (holes) located in the cell membrane and centered on the end of the chain. The filaments will restrict the range of movement of the chain to a cone aligned perpendicular to the membrane. Movement of the chain to one side of the array under thermal agitation will cause the cytoskeletal filaments to pull open channels on the opposite side of the array (illustrated by the spheres that normally block the channels being pulled out of the channels as the chain rotates away from them). When the chain moves back toward the axis of cone, relaxation of the tension on the filaments allows gating springs (short lines connecting the spheres to the membrane) to close the channels. Within the cone, the variance of motion of the chain will depend on the intensity of the external field.
(a) From Mann S, Sparks NHC, Walker MM, and Kirschvink JL (1988) Ultrastructure, morphology and organization of biogenic magnetite from sockeye salmon, *Oncorhynchus nerka*: Implications for magnetoreception. *Journal of Experimental Biology* 140: 35–49. (b) Adapted from Walker MM, Diebel CE, and Green CR (2000) Structure, function and use of the magnetic sense in vertebrates. *Journal of Applied Physics* 87: 4653–4658. (c) From Diebel CE, Proksch R, Green CR, Neilson P, and Walker MM (2000) Magnetite defines a magnetoreceptor. *Nature* 406: 299–302. (d) From Walker MM, Diebel CE, Haugh CV, *et al*. (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390: 371–376.

trout despite the small size (~50 nm) and extreme rarity (<5 ppb by volume) of the crystals. Reflections of laser light off crystal surfaces permitted detection of the chains of magnetite crystals in reflection mode confocal laser scanning microscopy (CLSM) (**Figures** 5(**b**) and 5(**c**)). Mapping the reflections in three dimensions, then permitted imaging of single crystals in thin sections in the transmission electron microscope (**Figure** 5(**d**)) and unique identification of the crystals as magnetite using atomic and magnetic force microscopy.

The cells containing the magnetite particles are $10 - 12\,\mu\text{m}$ in length, have a distinctive multilobed shape, and are consistently located near the basal lamina of the olfactory epithelium (**Figures** 5(**b**), (**c**)). The cells are relatively rare and were found only near the tips of the olfactory lamellae (distal to the cells of the olfactory sensory epithelium). The chain of magnetite crystals in each cell is about $1\,\mu\text{m}$ long and the chains interact strongly enough with the Earth's magnetic field to permit detection of the field by the cell. The location of the chain of magnetite crystals within each cell suggests that a mechanical linkage of the chain to the cell could transduce the movement of the chain, in response to the external magnetic field, into changes in the membrane potential of the cell. **Figure** 5(**e**) illustrates how a chain of magnetite particles might be linked by filaments to mechanically gated ion channels to convert the signal from the magnetic field of the Earth into a mechanical signal that changes the receptor potential of the detector cell to cause release of neurotransmitter to a sensory neuron. If, indeed, the cells work in the manner suggested, the magnetoreceptor system might be able to measure both the intensity and the direction of the Earth's magnetic field.

Neuroanatomy

Although the mechanism of magnetic field detection in the teleost fishes may be different from the elasmobranchs, this section focuses on the teleost fishes, as the elasmobranch electroreceptor system is already well described. Detection of neural responses to magnetic stimuli in the trout led to use of a fluorescent dye (Di-I) to trace the nerve from the recording site in the SO branch of the TN to both the endings of the individual nerve cells and to the brain (**Figure 6**). Posterior to the orbit (the eye socket), the SO branch joined other branches of the TN and ended in cell bodies that make up part of the anterior ganglion (**Figure** 6(**c**)). Anterior to the orbit, the SO branch has branches that innervate the skin, surround the olfactory nerve and olfactory capsule (processes 1–3 in **Figure 6** (**c**)), and also penetrate the olfactory lamellae within the olfactory capsule itself (**Figure** 6(**c**)). Fine branches of the SO penetrate the olfactory lamellae from both the top and the base before terminating in finer processes within the olfactory lamellae, where the magnetite-containing cells are most often found (**Figures** 6(**a**), 6(**d**), and 6(**e**)). The proximity of the fine branches of the TN to the cells that contain the magnetite in the trout invites the hypothesis that the magnetite-containing cells transduce magnetic field information for transmission through the TN to the brain. Testing this hypothesis will require the ability to demonstrate that the magnetite-containing cells make synaptic contacts that permit encoding and transmission of magnetic field information by the TN.

Do Fish Use the Earth's Magnetic Field to Navigate?

Navigation can be defined as the processes by which an animal first estimates its current position relative to some goal that it cannot detect directly, and then sets a course to reach that goal (e.g., using a map and a compass). Evidence that fish use the Earth's magnetic field for navigation is currently limited to one study in which scalloped hammerhead sharks were tracked around seamounts in Baja, California. The tracked fish made repeated round-trip journeys each night, traveling away from El Bajo Espiritu Santu seamount to spend the night in deep water and returning each morning. Monte Carlo simulations demonstrated that the paths of the fish were associated with magnetic field features but not with bathymetric features associated with the seamount. Consistent with the above definition, **Figure** 7(**a**) shows a broadbill swordfish that clearly traveled independently of the flow directions of either local or major currents, including the Gulf Stream, and maintained the same course for some days.

The discovery that juvenile lemon sharks will home rapidly after being experimentally displaced (**Figure** 7(**b**)) opens the way for more detailed studies of the role of the magnetic sense in navigation. The likely motivation for the lemon sharks to home as rapidly as they did was to escape predation because numerous large sharks that feed on smaller sharks inhabit the water around the Bimini Islands. There are very likely to be other fish species in which juvenile animals inhabit nurseries to which they return after experimental displacement. Thus, the opportunity exists to begin experimental study of the navigation behavior of different fish species.

Outlook

Fishes have played a central role in studies of the structure and function of the magnetic sense. More is known about the crystal properties of biogenic magnetite, the candidate magnetite-based magnetoreceptor cells, and the magnetic-field detection pathway in the fishes than in any other metazoan group. We are, however, only just beginning the systematic study of the magnetic sense in

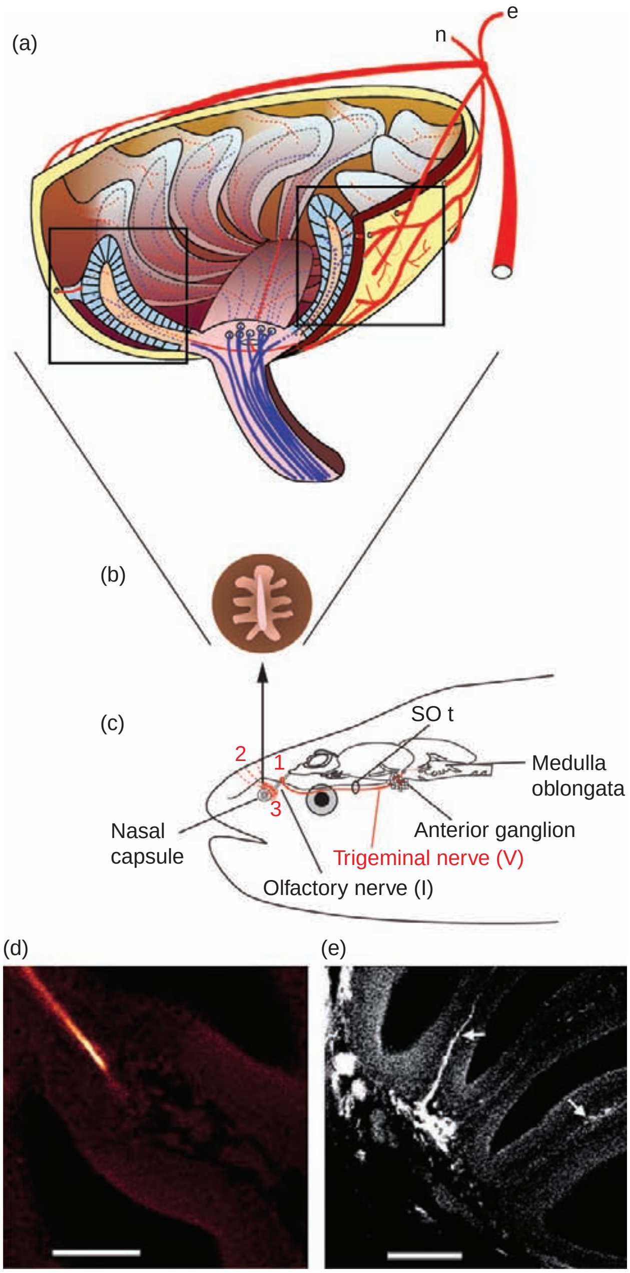


Figure 6 Relationship between the superficial ophthalmic branch of the trigeminal nerve and magnetite-containing cells in rainbow trout. (a) Schematic diagram of the innervation of the head region and nasal capsule of the trout by the superficial ophthalmic (SO; shown in red in (a)–(c)) branch of the trigeminal nerve (TN). One process innervates the nasal membrane and flap (n), and the other (right (e)) is process 2 in (c). Others form a network of nerves that surround the nasal capsule (right box). Within this network, the smaller branches have fine processes that pass through the nasal membrane, lining the nasal capsule, and innervate, both at the top and the base, individual olfactory lamellae that form the olfactory rosette. The olfactory nerve (blue) is the combination of all axons of the olfactory sensory cells which are situated in the mucosa and send their axons to the olfactory bulb. The network of nerves surrounding the capsule generally lies in a fatty layer (light shading on outside surface of capsule; right box in (a)), which is typically found between the neurocranium (not shown) and the outer membrane (brown) that lines the nasal capsule. The pale area in the front two lamellae represents the folded layers of the olfactory epithelium that are separated internally by the lamina propria. New lamellae are formed in the area of the nasal capsule (not shown). The left box outlines the area shown in (d). (b) Olfactory rosette within the trout nasal capsule (top view). The nasal flap that lies over the top of the olfactory rosette has been removed for clarity. (c) Innervation of the SO in the head region of the trout. The label SOt identifies the SO trunk where the SO branches of the trigeminal (cranial nerve V) and anterior lateral line (cranial nerve VIII) nerves pass together across the top of the eye before diverging to innervate the front of the head. (d) Optical slice through a single olfactory lamella (Scale = 100 μm). A labeled fine process from a branch of the SO branch can be seen entering the lamella through the top. (e) Fine processes can also be seen entering the lamina propria of several lamellae (arrows) from their bases (Scale = 100 μm). These processes originate from a different branch of the SO than the one that innervates the area in (d). Adapted from Walker MM, Diebel CE, Haugh CV, *et al*. (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390: 371–376.

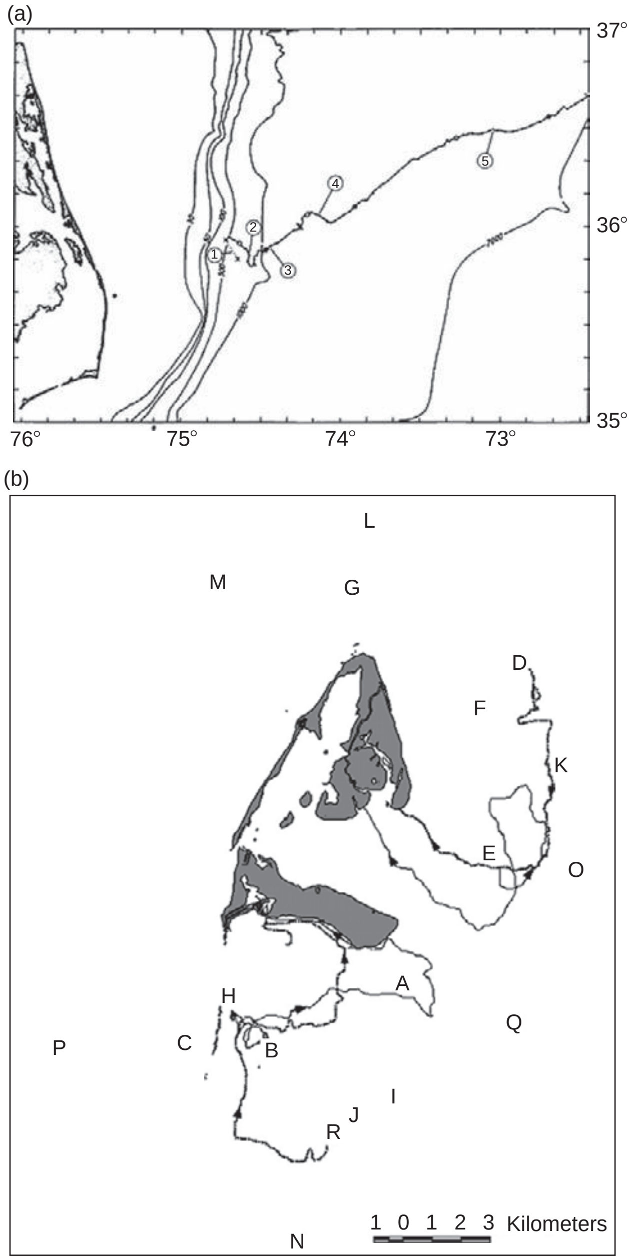


Figure 7 Orientation behavior of fish in the sea. (a) Sustained directional swimming in a swordfish. Path of a swordfish tracked continuously for several days in the Atlantic Ocean near Cape Hatteras, North Carolina. Numbers at points along the track represent the following events: (1) The fish was caught on long-line gear. (2) The fish moved beneath a layer of cold surface water. (3) The fish emerged from beneath the cold surface layer 10 h later. (4) Nine hours later, the fish was in the Gulf Stream. (5) By mid-afternoon on the following day, the fish had left the Gulf Stream and entered the Sargasso Sea. Filled circles represent sunset; open circles represent sunrise. (b) Homing behavior of juvenile lemon sharks. Tracks from 4 (of 41) juvenile lemon sharks, *Negaprion brevirostris*, displaced to release points (A–R) located 4–15 km offshore from Bimini Islands, Bahamas. On release (at points B, D, E, and R), the sharks swam to the bottom and moved in a pattern of oscillating directions for 2–10 min before setting off on sustained, relatively straight courses that quickly brought them back to the islands. (a) Adapted from Carey FG and Robison BH (1981) Daily patterns in the activities of swordfish, *Xiphias gladius*, observed by acoustic telemetry. *Fishery Bulletin* 79: 277–292. (b) Adapted from Sundström LF, Gruber SH, Clermont SM, *et al.* (2001) Review of elasmobranch behavioural studies using ultrasonic telemetry with special reference to the lemon shark, *Negaprion brevirostris*, around Bimini Islands, Bahamas. *Environmental Biology of Fishes* 60: 225–250, with kind permission of Springer Science and Business Media.

fish and other animals, and there is far more yet to be discovered than has been learned to date. Thus, for a magnetite-based magnetoreceptor system, descriptions of the internal structure of candidate magnetoreceptor cells, their synaptic links to afferent nerves and central projections in the brain, are priorities for structural study of the magnetic sense in all animals, including the fishes, which are well suited for such work. Similarly, functional studies of these magnetoreceptor cells, the coding of magnetic field information for transmission to the brain, analysis of the psychophysical properties, and demonstration of dependence of sensory capacity on the structure of the sense are all required if we are to understand the magnetic sense in detail. Finally, the opportunity exists to begin comparative study of the navigation behavior of fish and homing pigeons, in which navigation has been studied intensively for many decades. Although powerful experimental evidence for the use of the Earth's magnetic field in navigation exists only for homing pigeons, there will be much to learn from such comparative studies just as there will be from comparative studies of the magnetic sense itself in fish and birds. We look forward to exciting advances in our understanding of the properties and use of this most enigmatic of animal senses in the years to come.

See also: **Detection and Generation of Electric Signals**: Morphology of Electroreceptive Sensory Organs; Physiology of Ampullary Electrosensory Systems. **Fish Migrations**: Eel Migrations; Pacific Salmon Migration: Completing the Cycle; The Biology of Fish Migration; Tracking Oceanic Fish. **Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?.

Further Reading

Carey FG and Robison BH (1981) Daily patterns in the activities of sword fish, *Xiphias gladius*, observed by acoustic telemetry. *Fishery Bulletin* 79: 277–292.

Diebel CE, Proksch R, Green CR, Neilson P, and Walker MM (2000) Magnetite defines a magnetoreceptor. *Nature* 406: 299–302.

Hodgson ES and Mathewson RF (eds.) (1978) *Sensory Biology of Sharks, Skates, and Rays* Arlington, VA: US Govt. Printing Office.

Kirschvink JL (1992) Uniform magnetic fields and double-wrapped coil systems: Improved techniques for the design of bioelectromagnetic experiments. *Bioelectromagnetics* 13: 401–412.

Kirschvink JL, Jones DS, and MacFadden BJ (eds.) (1985) Magnetite biomineralization and magnetoreception by living organisms: A new biomagnetism. In: *Topics in Geobiology*, vol. 5, New York: Plenum.

Kramer G (1953) Wird die Sonnenhöhe bei der Heimfindeorientierung verwertet? *Journal für Ornithologie* 94: 201–219.

Mann S, Sparks NHC, Walker MM, and Kirschvink JL (1988) Ultrastructure, morphology and organization of biogenic magnetite from sockeye salmon, *Oncorhynchus nerka*: Implications for magnetoreception. *Journal of Experimental Biology* 140: 35–49.

Nishi T, Kawamura G, and Matsumoto K (2004) Magnetic sense in the Japanese eel, *Anguilla japonica*, as determined by conditioning and electrocardiography. *Journal of Experimental Biology* 207: 2965–2970.

Skiles DD (1985) The geomagnetic field: Its nature, history and biological relevance. In: Kirschvink JL, Jones DS, and MacFadden BJ (eds.) *Magnetite Biomineralization and Magnetoreception by Living Organisms: A New Biomagnetism*. New York: Plenum Publishing Corporation.

Sundström LF, Gruber SH, Clermont SM, *et al.* (2001) Review of elasmobranch behavioural studies using ultrasonic telemetry with special reference to the lemon shark, *Negaprion brevirostris*, around Bimini Islands, Bahamas. *Environmental Biology of Fishes* 60: 225–250.

Walker MM (1984) Learned magnetic field discrimination in the yellown tuna. *Thunnus albacares*. *Journal of Comparative Physiology A* 155: 673–679.

Walker MM (2008) A model for encoding of magnetic field intensity by magnetite-based magnetoreceptor cells. *Journal of Theoretical Biology* 250: 85–91.

Walker MM, Dennis TE, and Kirschvink JL (2002) The magnetic sense and its use in long-distance navigation by animals. *Current Opinion in Neurobiology* 12: 735–744.

Walker MM, Diebel CE, and Green CR (2000) Structure, function and use of the magnetic sense in vertebrates. *Journal of Applied Physics* 87: 4653–4658.

Walker MM, Diebel CE, Haugh CV, *et al.* (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390: 371–376.

Wiltschko R and Wiltschko W (eds.) (1995) Magnetic orientation in animals. In: *Zoophysiology*, vol. 33. Berlin, New York: Springer.

Circadian Rhythms in Fish

SG Reebs, Université de Moncton, Moncton, NB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Expressions of Circadian Rhythmicity
Entrainment of the Circadian Clock
Diurnality and Nocturnality
Location of the Circadian Clock
Clock Genes
Circatidal Rhythms
Other Rhythms
Further Reading

Glossary

Circadian Internally generated with a periodicity of about 24 h. From the Latin *circa* (about) and *dies* (day).

Circadian clock Cellular mechanism or multicellular structure that spontaneously expresses a rhythm with a periodicity of about 24 h. Also called circadian oscillator or, in the case of a master clock that drives others, circadian pacemaker.

Circatidal Internally generated with a periodicity of about one cycle of the tide, that is, about 12.4 h.

Daily With a periodicity of exactly 24 h. Synonymous with 'diel' or 'nycthemeral'. Used for environmental cycles. Should also be used for physiological and behavioral rhythms whose endogenous nature is not certain and whose observed periodicity is 24 h.

Entrainment Forced synchronization of an endogenous rhythm to the periodicity of an external factor, called a zeitgeber. Opposite of freerun.

Freerun State of an endogenous rhythm that follows its own natural periodicity, called the 'freerunning period'. Usually expressed under constant conditions. Opposite of entrainment.

Hourglass A mechanism that triggers an output after a certain amount of time has elapsed from a resetting event (which can be the output itself). It is a conceptual alternative to circadian clocks in some phenomena, although there is little evidence for it.

Masking Effect of an external factor on a circadian variable, resulting in the variable expressing a different state than that prescribed by the internal clock. For example, light can mask the wake–sleep cycle by rendering an animal active at a time when it would normally be asleep.

Phase A particular reference point or segment during an oscillation.

Phase shift A resetting of the internal clock or its zeitgeber. In a phase advance, the rhythm is temporarily shortened and its expression occurs earlier than expected. In a phase delay, the rhythm is temporarily lengthened and its expression occurs later than expected. Advance values (usually in hours) are assigned a positive sign and delay values are assigned a negative sign.

Subjective day or night Under constant light or constant darkness, the time segment during which the internal clock would expect to see day or night.

Transients Cycles that are temporarily shortened or lengthened during a phase shift. They reflect a gradual readjustment of the clock to a new steady state.

Zeitgeber An environmental factor that varies cyclically (e.g., light intensity) and that synchronizes (entrains) an endogenous clock to its own periodicity.

Introduction

The rotation of the Earth on its axis imposes a daily cycle of light and darkness on almost all organisms. Depending on the habitat, temperature and humidity can also vary on a 24-h basis. Such environmental cycles have led to the evolution of internal clocks in almost all living things. These clocks are cellular or multicellular structures that generate an output with a periodicity of about 24 h. The daily approximation defines the clock and its output rhythm as being circadian (from the Latin *circa* (about) and *dies* (day)). Other biological clocks exist with different periodicities, such as the duration of the tide cycle (12.4 h, circatidal clock) or the year (circannual clock), but the circadian variety is by far the most common and the most studied.

Circadian clocks have adaptive value for animals. With them, animals can keep track of time and anticipate significant events, such as dawn, dusk, and food arrival. Physiological systems can thus be revved up in advance of activity and food intake. Circadian clocks can also be involved in daylength measurement and thus in photoperiodic responses. Finally, circadian clocks can synchronize sleep with that portion of the day to which the animal's sensory abilities are less well adapted. If slumber were only a blind response to darkness, for example, any animal venturing into a permanently dark place would be transformed into a sleeping beauty, a serene but not very profitable state of affairs.

The basic procedure to demonstrate the existence of a circadian clock is to continuously measure a physiological or behavioral variable under constant laboratory conditions (constant light (LL) or darkness, stable temperature and oxygen content, and food available *ad libitum* or given at random intervals), and observe cycles that persist for at least a few days. The rhythm always exhibits a periodicity (called the 'freerunning period') slightly different from 24 h. This deviation from 24 h is a fundamental feature of circadian rhythms. It illustrates the fact that biological clocks (like most manufactured watches) need not keep exact time. Some clock drift is allowed as long as there is a mechanism that resets the clock every day to the environmental cycle being tracked. This synchronization of the biological clock by an external cycle is called 'entrainment'.

The deviation from 24 h is also providential, for it proves the endogenous nature of the rhythm. If the freerunning period was exactly 24 h, it could be argued that the animals were responding directly to an environmental cycle which the laboratory conditions failed to control (e.g., noise in the building, or some hard-to-detect geophysical phenomenon). However, no such environmental cycles could explain circadian rhythms of, say, 23 h. Moreover, there is often considerable variation between individuals in the exact value of their freerunning periods, another indication that the rhythms are generated internally and not driven by an exogenous factor.

Circadian physiology has been extensively studied in mammals, most notably in humans, rats, mice, and hamsters. Comparatively little work has been done with fishes. This is due, in part, to the fact that we are yet to find a fish species that is easy to keep, obtainable in great numbers, and that gives precise rhythms which last for a long time in constant conditions. As compared to laboratory rodents (the workhorses of circadian rhythms research), whose clocks remain self-sustained for months, fishes in constant conditions exhibit circadian rhythms that usually damp out within several days, or several weeks at most. We do not know the reason why these endogenous cycles seem to persist longer in mammals than they do in fishes.

Expressions of Circadian Rhythmicity

As is the case for other animal groups, locomotion is the most commonly measured circadian variable in fishes, probably because it is relatively easy to automate the continuous recording of swimming movements. Usually, an infrared light beam is set up across the aquarium and beam interruptions are recorded by computer. Other less-common methods include automated video-image analysis and recorded disruptions of a standing ultrasound wave. Freerunning rhythms of locomotion have been observed in more than 40 different fish species, including hagfish, lamprey, sharks, cyprinids, ictalurids, gymnotids, salmonids, and labrids. Freerunning periods generally vary between 20 and 29 h. The activity rhythms are often represented in the form of an actogram, as in **Figure 1**.

Another variable that can freerun is 'demand feeding'. Fishes can be taught to push an object in order to obtain

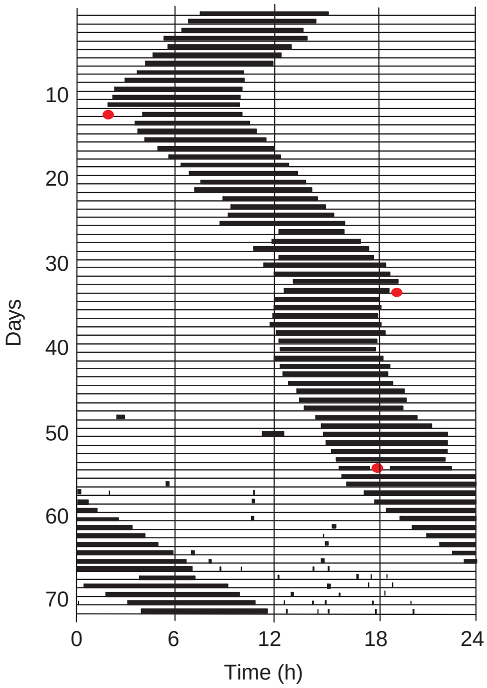


Figure 1 Actogram of the freerunning activity rhythm of an inshore hagfish in constant darkness. Lines represent successive days, with the thick parts showing the times when the fish was active. The fish was active for about 7.5 h each day. On the first day, the fish was active from 7.22 a.m. to 3.05 p.m.; these times gradually changed so that on day 10 the fish was active from 2.14 a.m. to 9.46 a.m. Thus, the biological clock in this fish, using activity onsets as reference points, had a freerunning period of 23.43 h during the first 10 days. The three circles represent 15-min pulses of light shown to the fish to phase-shift its rhythm. Adapted from figure 3 in Ooka-Souda S and Kabasawa H (1995) Circadian rhythms in locomotor activity of the hagfish, *Eptatretus burgeri* V. The effect of light pulses on the freerunning rhythm. *Zoological Science* 12: 337–342.

food. The hits are recorded by a computer. Freerunning rhythms of demand feeding with periodicities of 20–30 h have been obtained from the European sea bass (*Dicentrarchus labrax*), the rainbow trout (*Oncorhynchus mykiss*), the wels catfish (*Silurus glanis*), and the goldfish (*Carassius auratus*).

Freerunning rhythms have also been reported for the electric discharges of gymnotid fishes, air gulping of the river loach *Indoreonectes evezardi*, body color change in mummichogs (*Fundulus heteroclitus*), as well as oxygen consumption, retinal cell proliferation, and hormone levels in several species.

The freerunning rhythms of fishes are usually noisy (there is a fair amount of activity during the supposed rest phase, or inactivity during the supposed activity phase). Moreover, as already mentioned, seldom are the rhythms self-sustained for more than several weeks. The most precise and long-lasting fish rhythms to date have come from the electric discharges of *Gymnorhamphichthys hypostomus*, and the locomotor activity of the inshore hagfish (*Eptatretus burgeri*), the white sucker (*Catostomus commersoni*), the lake chub (*Couesius plumbeus*), the burbot (*Lota lota*), and various wrasses, with the inshore hagfish leading the pack with maximum reported durations of 75 days (**Figure 1**). It is noteworthy that *G. hypostomus*, the inshore hagfish, the burbot, and the wrasses prefer to sleep hidden, either buried in sand or underneath rocks. The search for a model subject in fish circadian research might benefit from a focus on species that sleep deeply and out of sight.

The value of the freerunning period (symbolized by τ, the Greek letter tau) is calculated by fitting a regression line through predefined reference points (e.g., daily activity onset), or, more commonly in the case of the noisy rhythms of fishes, with the help of a special technique called 'periodogram analysis'. This mathematical tool assesses the most likely period of a rhythm and assigns a statistical probability value to it.

The freerunning period of fishes can be influenced by the seasonal timing of the animals' capture. Lake chubs express longer freerunning periods of activity immediately after a winter capture than after a summer capture. Burbot and longnose dace (*Rhinichthys cataractae*) also show a summer–winter difference, but reversed: their freerunning period is longer after a summer capture. The causes of such seasonal variation and interspecific differences remain unknown.

Freerunning periods can also vary depending on whether the fish are single or in shoals. In both the white sucker and the mummichog, groups of 25 fish have yielded longer freerunning periods than fish kept separately. The reasons for this are not understood.

Finally, freerunning periods depend on whether the rhythm is measured in LL or in constant darkness (DD). Early work on the circadian physiology of animals led to the formulation of what is known as Aschoff's rule: increased light intensity lengthens the period in nocturnal animals but shortens it in diurnal ones. It is difficult to say whether fishes follow this rule; first because many species are hard to categorize as diurnal or nocturnal (see below), and second because fish rhythms are not always expressed in LL. However, a few studies have managed to document some influence of light intensity on the freerunning period of fishes. Zebrafish (*Danio rerio*) and mummichogs are diurnal in the laboratory and show longer freerunning periods in DD than in LL, as predicted by Aschoff's rule. Conversely, goldfish go against Aschoff's rule: they are mostly diurnal, and their period is longer in LL than in DD. In the nocturnal gymnotids, the period is lengthened as light intensity is increased from 0 to 100 lux, congruent with Aschoff's rule. However, in the nocturnal Amur catfish (*Silurus asotus*), the period decreases as light intensity increases from 0 to 10 lux. It has been suggested that Aschoff's rule should be altered to state that the periods are lengthened with increasing light intensity in animals whose freerunning period in DD is less than 24 h, with the opposite being true for animals with a freerunning period in DD over 24 h. The validity of this new formulation remains to be established for fishes. Whatever the most correct formulation turns out to be, the physiology behind these effects of light intensity on freerunning periodicity is still unknown.

Some fishes have failed to show freerunning rhythms in the laboratory, be it under LL or under DD. Most interesting among them are cave populations. The Mexican tetra (*Astyanax mexicanus*) includes both a blind cave form and a sighted surface form; the cave form is arrhythmic in the laboratory, while the sighted one freeruns in both LL and DD. Lack of rhythmicity in cave dwellers is a likely case of regressive evolution in an environment devoid of daily cycles. However, not all cavernicolous fishes are arrhythmic. There is need for further research to determine how widespread the loss of internal clocks is in cavefishes.

Another expression of circadian rhythmicity, besides freeruns, is the anticipation of daily recurring events. When fishes are fed at the same time every day, they commonly become more active and pay more visits to the feeding site a few hours before mealtime. This so-called food-anticipatory activity is not an expression of hunger because activity dips down to its normal level past the usual mealtime even when food is withheld. Even though the fish are still hungry, their internal time sense tells them that foraging past the regular mealtime would be unprofitable. This time sense could be based on an hourglass mechanism reset by dawn or dusk rather than on a circadian clock; however, this hypothesis has been refuted by a few observations of freeruns of food-anticipatory activity when scheduled feeding was interrupted and constant conditions were put in place, and also by cases of anticipation that began before dawn or dusk when the food arrived soon after dawn or dusk (an hourglass set by a light signal would trigger anticipation after the light signal, and not before).

More than one feeding time can be anticipated. Shoals of golden shiners (*Notemigonus crysoleucas*) and individual rainbow trout are capable of anticipating two daily feeding times, even when one is at night and the other during the day. In the same vein, golden shiners and inangas (*Galaxias maculatus*, ex-*Galaxias attenuatus*) can anticipate the arrival of food in one part of their aquarium in the morning and in another part in the afternoon, a phenomenon called 'time–place learning', which is commonly thought to be based on a circadian mechanism. Golden shiners can also learn to feed in one-half of their aquarium in the morning, the other half at midday, and back to the first half in the afternoon.

Fish can anticipate events unrelated to food, such as dawn and dusk. Activity often increases or decreases a few hours before dawn or dusk even in the absence of changes in sunlight intensity. For example, nocturnal American eels (*Anguilla rostrata*) in the wild have been seen moving up toward the entrance of their dark cave shelter several hours before dusk. In addition, parental convict cichlids (*Argocentrus nigrofasciatus*) can retrieve their young a few minutes before nighttime, even in a laboratory setting where no light attenuation announces the end of the day.

A somewhat indirect way to demonstrate the existence of a circadian clock is to phase-shift the light–dark cycle, advancing or delaying it by a few hours, and to observe transient cycles of activity in the next few days. Rather than start right away at the new time of lights-on or lights-off (depending on whether the fish is diurnal or nocturnal) following the phase shift, activity onset occurs at first at the old time of lights-on or lights-off and slowly drifts across clock times from day to day until it is resynchronized with the new phase of the light–dark cycle. This reflects the gradual readjustment of the clock. As a rule, fishes seldom provide such evidence, as they usually resynchronize very quickly to phase shifts of the light–dark cycle. They show no, or at most one, transient cycle. However, two cases of prolonged transits have been documented: in one, golden shiners exhibited 2–3 days of transients after a 6-h shift; in the other, inshore hagfish showed 7–13 days of transients after a 12-h shift (**Figure 2**). It is unclear whether cases with no or very few transients reflect an easily shifted clock or a masking effect of light and darkness on activity levels.

Circadian clocks are also likely to be involved in sun-compass orientation, the ability to use time of day and the position of the sun to find cardinal points. At least a dozen fish species are known to orient by sun compass. For example, when bluegill sunfish (*Lepomis macrochirus*) are displaced from shore to open waters, they swim in a fairly consistent direction (the one that would take them to shore) on sunny days, but they set off in random directions on overcast days; moreover, their orientation is shifted by 90° when sunrise is artificially made to appear 6 h ahead of time (6 h is a quarter of the full day, just as 90° is a quarter of a full circle).

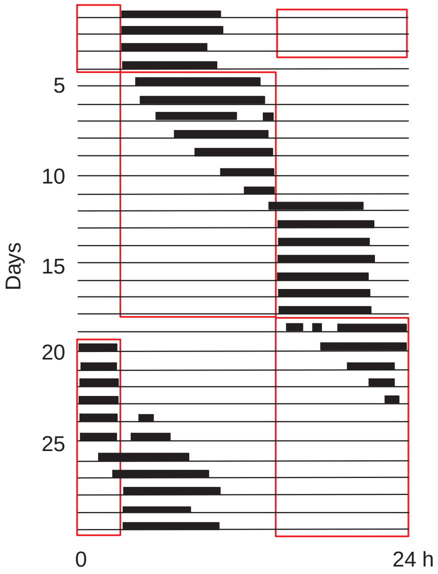


Figure 2 Transient cycles in the activity rhythm of an inshore hagfish following 12-h phase shifts of the light–dark cycle. Boxes show light times. Adapted from figure 6 in Ooka-Souda S, Kabasawa H, and Kinoshita S (1985) Circadian rhythms in locomotor activity in the hagfish *Eptatretus burgeri*, and the effect of reversal of light–dark cycle. *Zoological Science* 2: 749–754.

Entrainment of the Circadian Clock

As a rule, circadian clocks do not freerun in nature. (There are a few exceptions, such as beavers in their winter lodge.) Instead, they are synchronized by daily environmental cycles. The entraining cycle is called a 'zeitgeber', a German word that translates literally as time giver. By far the most important zeitgeber in nature is the light–dark cycle.

Two models explain how an exogenous cycle could entrain an endogenous one. The first one is parametric entrainment. This is the notion that an environmental factor can speed up or slow down the internal clock in a manner proportional to the intensity of the factor, so as to synchronize the clock to 24 h. The second one is nonparametric entrainment. According to this model, the clock is synchronized by daily jumps, or phase shifts, caused by the environmental factor being perceived by the animal at specific times. For example, to be entrained to a period of 24 h, a 26-h freerunning rhythm would need to jump backward (to be phase advanced, in the jargon of the trade) by 2 h every day. In a 26-h rhythm, the end of the subjective night (the end of the activity phase in a nocturnal animal, or the end of the sleep phase in a diurnal one) soon runs into dawn. If light at the end of the subjective night causes a 2-h phase advance in the clock, then the rhythm would become synchronized to 24 h.

Almost all research to date supports the theory of nonparametric entrainment. One key evidence is the phase-response curve, a graph depicting phase-shift size as a function of the circadian time at which a stimulus is given to the animal. Nonparametric entrainment theory predicts that pulses of light shown at the end of the subjective night should yield phase advances that become larger as the light pulse is administered earlier into the subjective night. The theory also predicts phase delays at the beginning of the subjective night, delays that become larger as the light pulse is given later into the subjective night. Somewhere around the middle of the subjective night, phase delays should quickly give way to phase advances.

Phase-response curves, with the correct shape, have been obtained in almost all organisms tested to date with light. Unfortunately, very few of these organisms have been fishes. This is because the study of phase shifts requires long-lasting rhythms with precise reference points (e.g., activity onsets), and fishes seldom show such rhythms. However, at least three phase-response curves have been obtained with fishes, all with roughly the expected shape. The first two came from the locomotor rhythms of the inshore hagfish and the canary wrasse (*Halichoeres chrysus*), two species that bury themselves in sand to sleep and that show exceptionally clean separation between their activity and rest phases. The third phase-response curve came from the rhythm of melatonin release from the pineal organ of the ayu (*Plecoglossus altivelis*) in flow-through culture.

Besides light, two other possible zeitgebers in fishes are time-restricted food availability and temperature cycles. Food delivery at the same time every day can entrain rhythms of activity in otherwise constant conditions, as has been shown in mummichog, goldfish, wels catfish, European seabass, and oriental weatherfish (*Misgurnus anguillicaudatus*). Entrainment of locomotor activity to cycles of 12 h of 20 °C and 12 h of 26 °C has also been reported for zebrafish. The mechanism of entrainment in these cases has not been identified. In the case of entrainment by food, the possibility exists that the circadian clocks being entrained are anatomically distinct from the ones normally entrained by light. This has led to the notion of separate light-entrained oscillators (LEOs) and food-entrained oscillators (FEOs). There is some evidence for the existence of FEOs in fishes, but work remains to be done to distinguish them from LEOs that could have a memory of mealtime being stamped onto a particular phase of their oscillation.

In mammals, there are known examples of entrainment by daily social interactions, induced physical exercise, and sleep interruptions. These factors appear not to have been tested in fishes. Another potential zeitgeber apparently untested in fishes, despite its presence in many freshwater habitats, is a daily cycle of oxygen concentration in water.

Diurnality and Nocturnality

The circadian clock of mammals and birds is responsible for maintaining the integrity of the sleep–wake cycle and its synchronization to the light–dark cycle. Conceivably, the same could be said of fishes. However, many fishes (mostly freshwater ones) do not sleep very deeply and do not appear too restricted by light or darkness in the timing of their activity. For example, goldfish can be nocturnal, diurnal, or constantly active, even in the same laboratory. There have even been reports of individual goldfish starting a study as diurnal and then switching to a nocturnal mode, or vice versa. Lake chub tend to be diurnal in the laboratory, but they have been seen feeding at night in the wild, and during their spawning season they migrate only at night. The spawning season itself tends to disrupt daily activity cycles in many species. Sticklebacks and cichlids are normally diurnal but during their parental phase they fan their eggs day and night. Some species switch from a nocturnal or crepuscular pattern of activity to a diurnal one when daylight is dimmed (e.g., in turbid waters). Many more species change the timing of their activity phase between summer and winter. Salmonids also can be nocturnal, diurnal, or aphasic; however, they consistently suppress daytime activity either in winter or simply when water turns colder. In captivity, European seabass and white suckers are mostly diurnal when kept in groups, but nocturnal when housed individually. Many cyprinids can be either diurnal or nocturnal, depending on when food is made available. Interestingly, all these flexible species (with the exception of the lake chub) exhibit circadian rhythms of activity that are neither particularly clear-cut nor long-lasting in the laboratory.

However, there are examples of fish species that do sleep soundly, marine ones in particular. Such species tend to keep to the same time of day for their activity phase, and those that have been tested for circadian rhythmicity have yielded rhythms that are better than most. It is interesting to note that the two species, which had activity rhythms strong enough to yield phase-response curves (see above), were both sound sleepers and marine as well. At this stage, the relationship between habitat, sleep soundness, and the self-sustainability of circadian rhythms remains tentative, but it may become better studied in the future. A worthy goal would be to identify the mechanism by which the circadian clock determines the exact phasing of activity relative to the light–dark cycle, something that remains a mystery at present in fishes as well as in other animals.

Location of the Circadian Clock

Circadian clocks can be located anywhere in the body of an animal, and there may be many present. In mammals, one clock drives the others. This so-called pacemaker, or master clock, is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. It receives input from the retina allowing entrainment to the light–dark cycle. In birds, both the hypothalamus and the pineal gland can act as circadian pacemakers, independently or in tandem depending on the species. Light can reach these structures directly through the thin skull or via messages from the eyes.

In fishes, no master clock has been identified yet; no structure analogous to the mammalian SCN has been documented in them. Nevertheless, the pineal is present, it is directly photosensitive, and it contains an intrinsic circadian clock. The fish pineal exhibits a circadian rhythm of melatonin production, even when isolated in culture. This rhythm can be entrained by light–dark cycles and phase-shifted by light pulses, both *in vivo* and *in vitro*. Melatonin is produced only during the subjective night, and, being a hormone, it could act as a body-wide signal to synchronize other, more peripheral clocks. Thus, the fish pineal has all the necessary characteristics of a master circadian clock.

However, pinealectomy usually does not eliminate circadian rhythms of activity. The ablation often alters freerunning periods (**Figure 3**), and has been reported to split rhythms in white suckers (**Figure 4**), but the rhythms usually persist. Moreover, in some species, such as salmonids, the pineal is photosensitive but does not express any freerunning rhythms of melatonin production; melatonin is just secreted whenever there is darkness. Thus, for most fish species, the pineal cannot be the master clock in and of itself, although it may be an important cog in the circadian machinery.

The fish retina is capable of several circadian phenomena. Rod and cone migrations anticipate daily changes in lighting and they freerun in DD. Dopamine release also follows a circadian pattern in constant conditions, even in isolated retina, with production being high during the subjective day. However, ophthalmectomy does not abolish freerunning rhythms of activity, nor does it prevent entrainment of these rhythms to light.

Clock genes are transcripted on a circadian basis under constant conditions in isolated heart and kidney tissue from zebrafish. Moreover, these tissues are responsive to light. Hearts and kidneys must therefore have circadian clocks. We must seriously envision the possibility of a complex network of central (brain) and peripheral clocks in fishes, either linked or responding independently to the same zeitgebers to maintain physiological integrity within the whole body, rather than a single pacemaker driving all other clocks as in mammals.

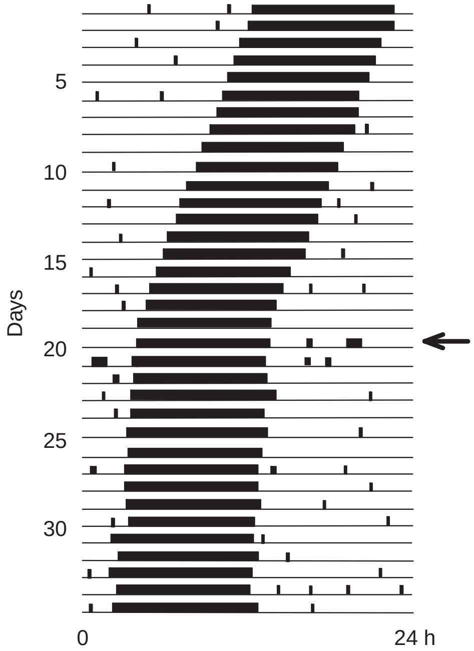


Figure 3 Freerunning activity rhythm of a burbot in constant darkness. The arrow on the right indicates the day when the fish was pinealectomized. The endogenous rhythm persisted after the pinealectomy, but the periodicity increased slightly from 23.61 to 23.89 h. Adapted from figure 1A in Kavaliers M (1980) Circadian locomotor activity rhythms of the burbot, *Lota lota*: Seasonal differences in period length and the effect of pinealectomy. *Journal of Comparative Physiology A* 136: 215–218.

Clock Genes

The molecular basis of circadian rhythmicity has been described in fruit flies and mice. Four main genes are involved. The *Clock* and *Bmal* genes encode proteins that are transcription factors. These activate the expression of the genes *Period* (*Per*) and *Cryptochrome* (*Cry*). The PER and CRY proteins, in turn, repress the transcription of their own genes by the CLOCK and BMAL proteins. This system is a feedback loop in which the expression of the genes is suppressed by their protein products with a periodicity of about 24 h.

Homologs of these genes have been found in zebrafish. All have multiple copies in the genome. Three *Clock* genes (*zfClock1*, *2*, and *3*), three *Bmal* genes (*Bmal1*, *2*, and *3*), three *Per* genes (*Per1*, *2*, and *3*), and six *Cry* genes (*zCry1a*, *1b*, *2a*, *2b*, *3*, and *4*) have been identified in zebrafish. All of the *Clock* and *Bmal* genes are expressed rhythmically in various tissues in DD. The first four *Cry* genes are also expressed rhythmically in the retina, pineal organ, and other parts of the brain.

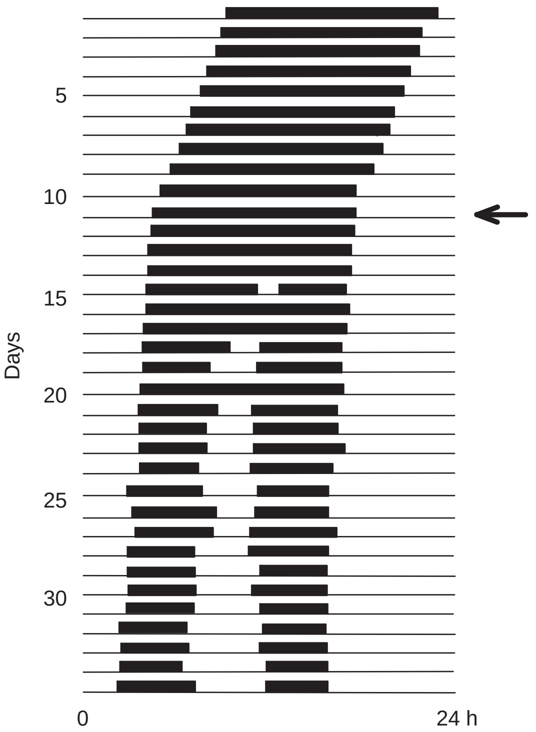


Figure 4 Freerunning activity rhythm of a white sucker in constant light. The arrow on the right indicates the day when the fish was pinealectomized. The rhythm persisted but split after the pinealectomy. Adapted from figure 2 in Kavaliers M (1980) Circadian organization in white suckers *Catostomus commersoni*: The role of the pineal organ. *Comparative Biochemistry and Physiology* 68A: 127–129.

Circatidal Rhythms

Tides along coasts and tidal currents offshore represent another type of environmental cycle that can impact the life of fishes. Many species use intertidal zones as feeding and spawning grounds. These fishes should benefit from being able to anticipate the flooding and ebbing tides. They should therefore have a circatidal clock, one that runs with a periodicity of about 12.4 h, which is the duration of the tidal cycle in most locations (sometimes, geographical features can greatly dampen one of the tides, resulting in a 24.8-h cycle). Indeed, some intertidal species do exhibit self-sustained rhythms of activity with a period of about 12.4 h under constant laboratory conditions. Such species include the rock goby (*Gobius paganellus*), the shanny (*Lipophrys pholis*), the Montagu's blenny (*Coryphoblennius galerita*), the Molly miller (*Scartella cristata*, ex-*Blennius cristatus*), and the mudskippers *Scartelaos histophorus* and *Periophthlalmus modestus.*

Unfortunately, circatidal rhythms in fishes under constant conditions are even noisier and shorter-lived than circadian rhythms. In this regard, fishes are bested by invertebrates. Various species of intertidal crabs and mollusks have proved to be better subjects of study. Despite this, some entrainment and phase-shift studies have been conducted with fishes and they suggest that cyclic changes in hydrostatic pressure and periodic wave action are the most likely zeitgebers of fish circatidal rhythms in nature. However, nothing is known of the anatomical location of the circatidal clock, nor of its molecular mechanism.

Other Rhythms

Lunar rhythms have a periodicity of 29.53 days, as exemplified by the recurrence of the full moon every month. Semilunar rhythms have a periodicity of 14.76 days, such as the recurrence of spring tides (tides of maximum amplitude) on the days of new moon and full moon. Many fishes exhibit lunar or semilunar rhythms, but we do not know if these rhythms are the product of an endogenous clock or simply a direct response to external stimuli, such as tide amplitude, moonlight intensity, and gravitational pull by the moon. The problem is threefold: first, it is essentially impossible to place the animal in truly constant conditions (the gravitational pull of the moon is always present); second, it is difficult to reproduce the complex variables of a lunar or semilunar environment in the laboratory; and, third, these rhythms are slow and require a relatively long time to express many cycles. With the possible exception of ovarian activity in mummichogs, freerunning lunar or semilunar rhythms have not been convincingly demonstrated in fishes (or in other animals, for that matter).

Many intertidal species spawn only during the high tides that surround the days of new moon and full moon. Examples include the California grunion (*Leuresthes tenuis*), the Gulf grunion (*Leuresthes sardina*), the Atlantic silverside (*Menidia menidia*), the mummichog, the inanga, the surf smelt (*Hypomesus pretiosus*), and the foureye (*Anableps microlepis*). The eggs are deposited high on the shore on spring tides, and, during the following 2 weeks, they remain in wet sand, uncovered by water and thus unexposed to marine predators. They hatch only during the next series of spring tides, which will take the larvae away. Semilunar patterns of spawning have also been observed in some blennies and damselfishes that live near but not on the intertidal zone, and, in such cases, the rhythm might help synchronize the mating activity of the population and facilitate dispersal of the larvae by tidal currents. Semilunar rhythms also exist in a few freshwater species, especially in the growth rate and food intake of various salmonids.

Examples of lunar rhythms include the seaward migration of adult eels (more fish move during the waning moon), the downstream migration of juvenile Atlantic salmon (*Salmo salar*, more movement around the time of new moon), and commercial catches of various clupeids (more captures around the full moon).

A final type of clock is circannual; it runs with a periodicity of about 1 year. Many examples are known from birds and mammals. Under conditions of constant temperature and stable photoperiod in the laboratory, starlings keep on molting, ground squirrels still fatten up and hibernate, and deer continue to grow and shed their antlers at a periodicity that is often short of the full year. In fishes, however, no endogenous circannual rhythms have been found. Annual cycles obviously exist in wild fish, in growth for example, but they may not be endogenous.

See also: **Behavioral Responses to the Environment**: Sleep in Fish. **Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?.

Further Reading

Ali MA (ed.) (1992) *Rhythms in Fishes*. New York: Plenum.

Dunlap JC, Loros JJ, and DeCoursey PJ (2004) *Chronobiology: Biological Timekeeping*. Sunderland: Sinauer Associates.

Gerkema MP, Videler JJ, de Wiljes J, *et al.* (2000) Photic entrainment of circadian activity patterns in the tropical labrid fish *Haliochoeres chrysus*. *Chronobiology International* 17: 613–622.

Iigo M, Mizusawa K, Yokosuka M, *et al.* (2003) *In vitro* photic entrainment of the circadian rhythm in melatonin release from the pineal organ of a teleost, ayu (*Plecoglossus altivelis*) in flow-through culture. *Brain Research* 982: 131–135.

Kavaliers M (1980) Circadian locomotor activity rhythms of the burbot, *Lota lota*: Seasonal differences in period length and the effect of pinealectomy. *Journal of Comparative Physiology A* 136: 215–218.

Kavaliers M (1980) Circadian organization in white suckers *Catostomus commersoni*: The role of the pineal organ. *Comparative Biochemistry and Physiology* 68A: 127–129.

Northcott SJ, Gibson RN, and Morgan E (1991) Phase responsiveness of the activity rhythm of *Lipophrys pholis* (L.) (Teleostei) to a hydrostatic pressure pulse. *Journal of Experimental Marine Biology and Ecology* 148: 47–57.

Ooka-Souda S and Kabasawa H (1995) Circadian rhythms in locomotor activity of the hagfish, *Eptatretus burgeri* V. The effect of light pulses on the free-running rhythm. *Zoological Science* 12: 337–342.

Ooka-Souda S, Kabasawa H, and Kinoshita S (1985) Circadian rhythms in locomotor activity in the hagfish *Eptatretus burgeri*, and the effect of reversal of light–dark cycle. *Zoological Science* 2: 749–754.

Palmer JD (2002) *The Living Clock: The Orchestrator of Biological Rhythms*. Oxford: Oxford University Press.

Reebs SG (2002) Plasticity of daily and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries* 12: 349–371.

Refinetti R (2006) *Circadian Physiology*, 2nd edn. Boca Raton, FL: CRC Press.

Sánchez-Vázquez FJ and Madrid JA (2001) Feeding anticipatory activity. In: Houlihan D, Boujard T, and Jobling M (eds.) *Food Intake in Fish*, pp. 216–232. London: Blackwell Science.

Schwassmann HO (1971) Biological rhythms. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 6, pp. 371–428. New York: Academic Press.

Tabata M, Minh-Nyo M, and Oguri M (1991) The role of the eyes and the pineal organ in the circadian rhythmicity in the catfish *Silurus asotus*. *Nippon Suisan Gakkaishi* 57: 607–612.

Thorpe JE (ed.) (1978) *Rhythmic Activity of Fishes*. New York: Academic Press.

Zhdanova I and Reebs SG (2006) Circadian rhythms in fish. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Fish Physiology, Vol. 24: Behaviour and Physiology of Fishes*, pp. 197–238. New York: Elsevier.

BEHAVIOR AND PHYSIOLOGY

Behavioral Responses to the Environment

Contents

A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food
Effects of Compensatory Growth on Fish Behavior
Temperature Preference: Behavioral Responses to Temperature in Fishes
Behavioral Responses to Hypoxia
Sleep in Fish
Manipulation of Fish Phenotype by Parasites
Anthropogenic Influences on Fish Behavior

A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food

MV Abrahams, Memorial University of Newfoundland, St. John's, NL, Canada

© 2011 Elsevier Inc. All rights reserved.

The Need for Food and Demands Placed upon Its Acquisition
Foraging Models – What Should You Try to be Doing?
What Should You Eat?
Where Should You Go to Get Food?
How Should You Capture and Consume Your Food?
Dealing with Competition
The Role of Predation Risk and How It Changes the Rules
Tactics for Avoiding Predators
Are There Overall Consequences?
Further Reading

Glossary

Benthic Lowest level of a body of water.
Chemotrophic Ability to obtain energy from the oxidation of organic or inorganic compounds.
Crypsis Blending into the background, most usually visual crypsis, although crypsis can refer to other senses.
Foraging theory Identifying the rules that govern how animals acquire resources.
Limnetic Open surface waters of a lake away from the shore line.
Morph Shape or form.
Ontogenetic The development of an organism.
Zero–one rule Always rejecting or including specific food items.

The Need for Food and Demands Placed upon Its Acquisition

A fundamental feature of ecosystems, indeed perhaps their defining feature, is the flow of energy from some initial source. Typically, this begins with photosynthetic plants that are capable of using solar energy that then creates the food for herbivores. Ecosystems whose bases are chemotrophic bacteria are less well known. No fish is capable of producing its own energy and so all fish must obtain their food from other organisms, either living or dead. This food provides the raw materials necessary for growth and reproduction, and the energy for all activities.

Ecologists have long known that animals need to be very good at obtaining food. As a consequence, a number of research approaches have been developed to understand foraging theory that are united by an evolutionary approach: those that are the most successful at obtaining food will then have the resources necessary to find mates and produce offspring. Consequently, food is considered a surrogate for evolutionary fitness, and individuals are assumed to be subject to evolutionary pressure to maximize their net rate of energy acquisition.

Here, the focus is on the net rate of energy acquisition. Simply obtaining food at a very high rate is of little use if this is an extremely expensive strategy. What is of benefit to any animal are the resources that are made available by food, less the cost of obtaining them.

Foraging Models – What Should You Try to be Doing?

The simplest foraging models require that animals maximize their net rate of energy intake in an environment that contains only a reliable distribution of food. While overly simplistic, these models form the foundation of more sophisticated and realistic models. One complication is variation in the availability of food. When deciding how to feed, fish have to consider both the net rate of benefit and the variation around this benefit (=reliability of finding food). Normally, high rates of food availability are good, while variation about that rate is bad. The question is how to integrate these two parameters, and whether all individuals are affected equally. In general, reliability can trump a higher but more variable rate of return.

Another complication is access to the information that allows fish to make good decisions about how to obtain food. The information may be acquired directly, requiring that the fish travel to different locations, assess their value, and then retain that information long enough and in an accurate enough form with which to make a decision. Alternatively, they may acquire their information by copying others (i.e., public information). While the former mechanism is expensive in time, energy, and somatic resources, it is also more precise than the latter. The question again is do the benefits justify the expense.

What Should You Eat?

One of the earliest and most successfully applied aspects of foraging models is the diet model. It considers whether a food item should or should not be consumed by a foraging fish and argues that food items can be ranked from highest to lowest based upon their net energetic value. Thus, the energy obtained from a type of food should be divided by the time spent searching for, capturing, and consuming it. The model then determines which food items will maximize the net rate of energy intake and predicts that strict adherence to this diet will mean that some food items are always ignored, while others are always consumed – the zero–one rule.

The logic behind the model is simple. Should an animal choose to devote time to consuming items of lower net energetic value, it will come at the expense of time that could be spent consuming more profitable food. This model has also attracted considerable attention for fish in part because it is relatively easy to obtain the important information. What is required is some estimate of the food that is available in the natural habitat, and then examining the contents of their stomachs to determine whether they select food randomly, or in a fashion more consistent with the diet model. The diet model performs well but seldom satisfies the zero–one rule. This usually means that the underlying assumptions of the model are an oversimplification of the task confronting animals in the wild.

One of the key omissions in the diet model is it just treats energy as the currency and fails to include access to other resources. For fish, and juvenile fish in particular, this can be a large error. Nonenergetic resources are crucial for the survival of juvenile fish populations. Perhaps the most critical decision is first-feed – the point at which larval fish must now obtain their food from the environment, having consumed all of their energetic reserves from their yolk. In many species, not all food has equal value and often there are times when these juvenile fish must consume prey that contains required nutrients. If critical prey are unavailable at the right time, all the juvenile fish will perish independent of the energetic value and accessibility of other prey.

Where Should You Go to Get Food?

Two modeling approaches where a fish should go to feed are discussed in the following.

The Patch Model

The patch model assumes that food is discretely distributed, fish know the average net rate of energy acquisition within their environment, and the net rate of energy gain declines at a nonlinear rate as they consume and deplete food within a patch. As with the diet model, the economics of foraging then argue that if energy is the only consideration, the food within any location should be

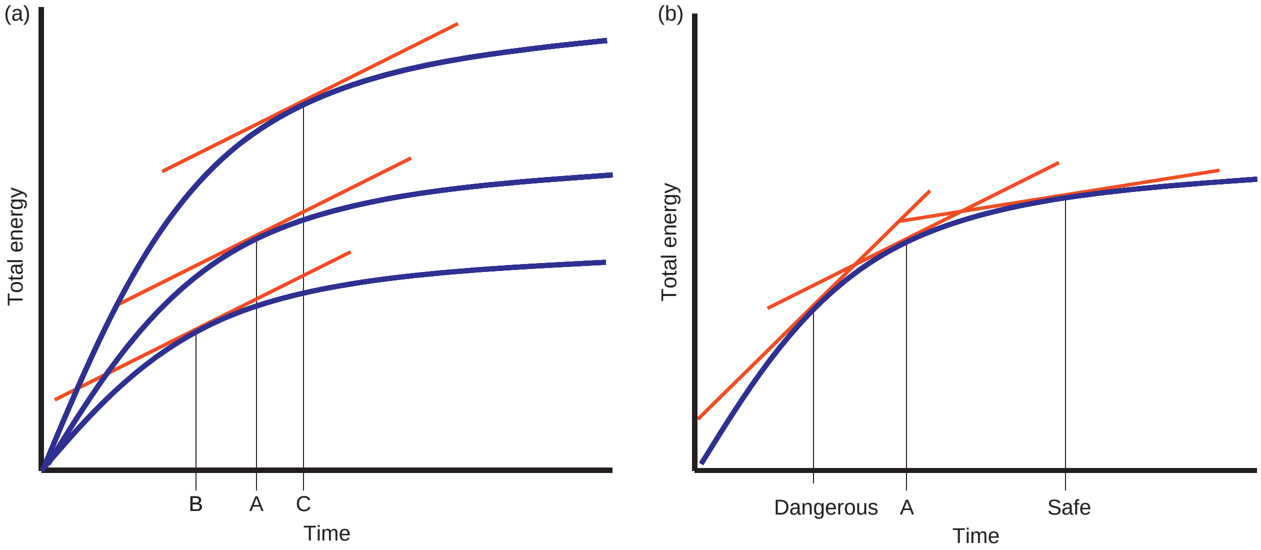


Figure 1 When an animal enters a feeding a location, it will accumulate food through time. Since the density of food will decline as the animal consumes it, so too will the rate of accumulation meaning that these functions will be nonlinear. Foraging is defined as the rate of energy gain, and therefore can be determined at any time as the tangent to the curve. (a) The point at which an animal should stop foraging in a patch occurs when its feeding rate has declined to the average for the environment, as identified by a tangent with a known slope. For a feeding patch of intermediate value (the middle curve), this occurs at point A. The lower curve identifies a location that contains a lower density of food, and as a consequence a feeding animal will drive the feeding rate down to the environmental average in less time (B). Conversely, the upper curve illustrates a more profitable feeding location that provides a feeding animal with more time before its foraging rate declines to the environmental average (C). (b) This technique can be used to assess environmental quality when more than energy affects quality. If energy were the only consideration, a foraging animal would feed in that location until the feeding rate equaled the environmental average, leaving behind a density of food equal to A. But if that location is dangerous, the animal will leave earlier when its feeding rate exceeds the environmental average. If the location is safer, the animal will remain longer until its feeding rate exceeds the environmental average. Measuring these densities makes it possible to assess habitat quality from a feeding animal's perspective.

depleted to the point where the net rate of energy intake equals that for the environment (**Figure 1(a)**).

What if safety from predators is an important consideration? If this is the case, a fish could leave a patch with food remaining if threatened by a predator. This important consideration can be assessed as habitat quality by measuring the density of food remaining within an artificial feeding patch when fish choose to move on to other areas (a GUD – giving-up density, **Figure 1(b)**). This approach is being used to assess habitat quality for foraging fish in the wild after being used for some time to assess habitat quality for terrestrial rodents.

A feature of most fish species is that as they grow, so too do the potential food items that they can consume. A result is that locations that were very profitable for fish when they were small may no longer be so as they grow larger. This phenomenon is known as the ontogenetic niche and recognizes that fish occupy different ecological niches as they move through different size stages. While the decision-making process may be similar to that described above, the solution tends to be size specific.

Another important consideration of where a fish should go to feed is dependent upon where others have selected to feed. If feeding rate is determined positively by the amount of food, but negatively by the number of competitors, then both factors will determine the distribution of feeding animals. If these animals conform to two assumptions, 'ideal' in that they have perfect information about the distribution of their resources, and 'free' because all have equal competitive ability, then the distribution of fish should conform to the ideal free distribution (IFD). A consequence of the IFD is that independent of the amount of food available within any patch, all fish will have the same intake rate across all feeding patches (see **Figure 2(a)**). Furthermore, this distribution is stable (known in economics as a Nash equilibrium) since any individual that moves to a different feeding location will have a lower feeding rate.

IFD Model

As with the patch model above, the IFD model requires that food be distributed discretely in space and time. However, these different feeding locations may provide food either continuously (such as fish that hold a feeding territory within a river or stream) or discretely, as above. A distinction is important since this will determine the relation between foragers and their food. The simplest case is when food is provided continuously. Under the assumptions of the IFD,

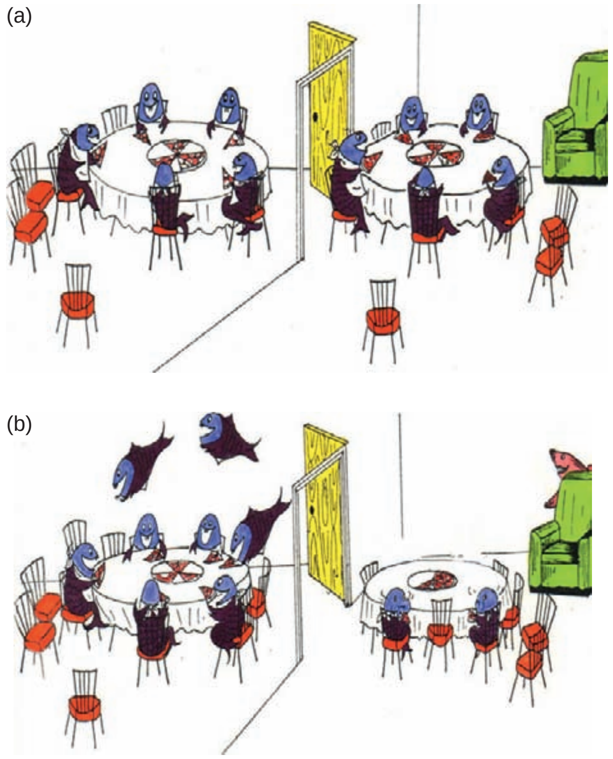


Figure 2 A cartoon illustrating habitat matching associated with the IFD. (a) With two equal-size pizzas distributed between two rooms, 10 fish will distribute themselves equally between these two locations. Note that this distribution is stable since any fish that deviates from this distribution will get less food (e.g., more fish to share the same amount of food). (b) In the same environment as in (a) but with a predator on the right. As foragers move from the dangerous (right) to safe locations (left), they change individual feeding rates to the point where the increased rate of energy acquisition of those feeding in the presence of the predator will compensate them for the risk they incur.

the result is a phenomenon known as habitat matching – the spatial distribution of predators will be the same as the spatial distribution of their prey (**Figure 2(a)**). This occurs because individual feeding rates are assumed to be equal to the total rate of food availability divided by the total number of foragers competing for this food. If 60% of the rate of food is available at a particular location, then this location should account for 60% of the foragers.

How Should You Capture and Consume Your Food?

Deciding where a fish obtains food is in part determined by how the food is consumed. Sit-and-wait feeders, by definition, select feeding locations that vary in quality by the rate at which food becomes available (**Figure 3**). This contrasts with a pursuit strategy where predators move through their environment to hunt and capture prey (**Figure 4**). Some species adopt both strategies, depending upon life stage and circumstances; other species are morphologically adapted to one or other tactic. The sit-and-wait tactic has the obvious advantage of a very low cost. Profitability hinges upon the rate at which prey can be successfully captured

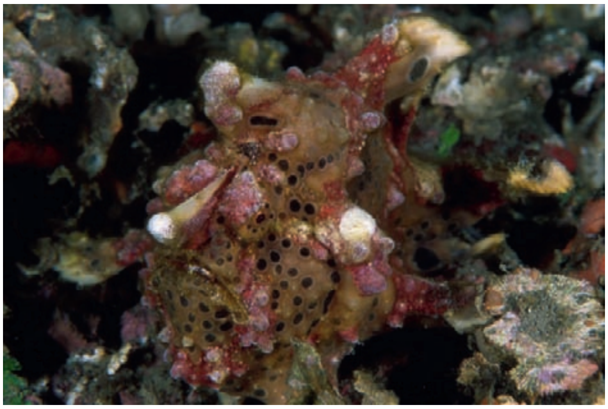


Figure 3 Image of giant frogfish (*Antennarius commersonii*) as an example of a sit-and-wait predator. Adaptations for this foraging strategy include a large mouth to increase the range of potential prey, and a visually cryptic morphology to reduce the probability of detection. Courtesy of Sergey Parinov.

Figure 4 A barracuda as representative of a pursuit predator. Success in this strategy is determined by swimming efficiency, both sustained for contacting prey and burst for prey capture. This image is from a painting by Robert W. Hines and is provided courtesy of the US Fish and Wildlife Service.

and consumed. How to go about doing this is dependent upon the type of prey being consumed. If the prey has no defense, then no special modifications are required. But if they do, success hinges upon defeating the prey. First, sit-and-wait predators need an antidetection mechanism. We observe such tactics primarily using vision and notice tactics that employ visual crypsis. For example, species such as the giant frogfish (*Antennarius commersonii*) that have an irregular, rock-like shape (**Figure 3**). Other species may burrow in sand. Regardless of mechanism, the goal is to allow prey to get close enough to increase the probability of capture success, the next critical adaptation to this mode of foraging.

Maximizing capture success depends upon the type of prey being sought. Animals that are mobile and capable of detecting predators have an antipredator strategy to avoid capture. Predators counter this by capturing their prey very rapidly. Key components of this strategy include the ability to very rapidly draw large amounts of water (plus prey) into their mouth, teeth that are specifically designed to grab and hold prey, and a gape size that makes it possible to consume a wide range of size classes (**Figure 3**). This is a particularly important strategy where prey exist at very low density and therefore the cost of search may be prohibitive (e.g., some deep ocean locations). Deep-sea fishes provide excellent examples of adaptations to avoid detection and the capacity to consume prey larger than themselves. They also have enhanced their sit-and-wait strategy by having evolved morphological and behavioral means to actively attract potential prey: by providing something they may be seeking, such as food as bait.

By their very nature, pursuit predators tend to be more commonly observed since they actively move through their environment to feed, which requires they be efficient at sustained swimming and have a well-developed visual system that enables them to detect prey (**Figure 4**). Unlike sit-and-wait predators, the key to success is to postpone detection to a point where they can initiate an attack with a high probability of success rather than avoid detection.

As noted above, whether to pursue or wait for prey is mainly driven by economics. If your chance of encountering prey is not greatly increased by moving, then it makes no energetic sense to pursue prey. However, moving to a different feeding patch may provide a significant benefit to moving through your environment. Often, other factors at play will influence these decisions. A relevant example is a juvenile salmon feeding in a stream. The preferred strategy is to establish a feeding territory and let the food come to them. Because there are more fish than territories, those not capable of competitively obtaining and retaining a territory are forced to use less profitable tactics.

Dealing with Competition

IFD is a consequence of competition among foragers. Animals may share the resource equally as assumed by the IFD, which is known as scramble competition. Alternatively, the outcome of competition may be determined by interactions between individuals and is appropriately termed contest competition.

If animals do conform to the assumptions of the IFD, then the distribution of foragers will match the distribution of their food (**Figure 2(a)**). However, the assumptions are unlikely to be met in reality. In particular, it is unlikely that all animals will be equal in their competitive ability (**see also Social and Reproductive Behaviors**: Dominance Behaviors). When there is variation in competitive abilities, a different type of spatial distribution will result with those of superior competitive ability being in locations that provide the greatest amount of food, and vice versa. Under this scenario, rather than the number of animals matching the spatial distribution of food, the prediction is that the number of competitive units (i.e., assume all animals scaled in units of the poorest competitor in the population) will match the spatial distribution of food. While such a distribution will involve considerable individual interactions, it is intermediate between an IFD and territoriality, in which an individual defends and monopolizes food within a certain territory. For territoriality to exist, the distribution of food must be similar to that necessary for the IFD. In addition, there must be sufficient difference in competitive ability that will make the cost of defending the location profitable.

The Role of Predation Risk and How It Changes the Rules

The vast majority of fishes seek food while simultaneously avoiding becoming food. While many of the strategies described above are very effective at obtaining food, they typically make fish vulnerable to predators, which can significantly change the foraging rules.

The most obvious influence of a predator is the trade-off between access to safety and energy (**Figure 2(b)**). Examples include reducing the number of animals that may choose to feed in dangerous but profitable locations, choosing to feed at times when predators are not present, and even occupying habitats that generate physiological stress but safety from predators.

Understanding the relative importance of safety and food requires a common currency for both resources. Laboratory techniques employ approaches such as IFDs that provide animals with only energy and safety, making it possible to measure their value in energetic units. In field locations GUDs can be used (as described above and in **Figure 1(b)**). Here, fish are provided with artificial feeding patches designed such that food density determines feeding rate. The initial amount of food provides a high feeding rate, but the more they consume, the lower their feeding rate. In safe locations animals drive the food density of these patches down to a much lower level than in dangerous areas, demonstrating how much energy they are prepared to forego in order avoid predators. The application of dynamic state variables as a modeling technique has also made it possible to integrate internal physiological characters, as well as other environmental factors, into better understanding so as to how animals make decisions that trade off food access against safety.

General outcomes from these three approaches do support the conclusion that animals do forfeit considerable foraging opportunities to gain access to safety. The significant range in this forfeiture has been experimentally determined. Fishes whose reproductive success is tightly linked to feeding rate are not as affected by predation risk when making foraging decisions, and vice versa. Likewise, fishes that are energetically stressed are also less affected by the presence of predators. Fishes also appear to take their vulnerability to predators into account when making such decisions, meaning that larger individuals, and those possessing anti-predator morphology (armor, spines, toxins, etc.) are less affected by predators than are those that are smaller or lacking in such morphology.

Tactics for Avoiding Predators

The probability of being killed by a predator (e.g., risk) is determined by the time spent being vulnerable to predators, the probability of encountering a predator while being vulnerable, and, if a predator is encountered, the probability that such an encounter will be fatal. In the presence of predators, foraging tactics will be modified with respect to their impact upon these three parameters.

Approaches used by fish to reduce the time spent being vulnerable to predators hinge upon spatial and temporal foraging decisions. The spatial option requires that there must be locations in the prey's environment that are not accessible to their predators. These include burrows, rocky crevices, weed beds, or other complex environments that provide locations small enough to permit the prey to gain entry, but not their predator. If there is also food within these locations, then it will also contain the majority of the fish competing for access to this food. The net effect is that an energetic price must be paid for safety.

Locations that vary in key environment variables, such as temperature, turbidity, dissolved oxygen, or natural or anthropogenic contaminants, provide an opportunity for fishes to take advantage of differences in tolerance. For example, if prey tolerate hypoxic environments better than their predators (e.g., warm, shallow regions that contain decaying vegetation), then these locations will provide prey with a spatial refuge (**see also Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia). Ironically, the very areas that may appear to be the least desirable fish habitats may be critical for the survival of some species (e.g., the marshes of Lake Victoria for haplochromine cichlids).

Many predators visually detect small fish and must hunt during daytime. An obvious tactic of small fish then is to use locations that contain the majority of the food at times when their predators are not active. Such behavior results in vertical migration of some fish that move to the upper, more productive regions of lakes and oceans in the evening to feed. It also accounts for the migration off reefs during twilight periods, allowing these resident species to feed under conditions that reduce their risk (**see also Fish Migrations**: The Biology of Fish Migration).

Fish in the superorder Ostariophysi also have specialized cells in their epidermis that contain alarm substance (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior). This chemical is released when a fish is injured or killed. The location of this chemical in the environment identifies high-risk areas that are usually avoided by small fish. Some predators also secrete this chemical after consuming a prey, thereby directly identifying their location.

While reducing the time spent being vulnerable is likely a preferred option to minimizing the risk of death from predators, the energetic cost may be too great to avoid incurring some risk. The options available to fish include avoiding detection if encountered, communicating with the predator in some way that convinces it not to attack, possessing specialized morphology that renders itself less susceptible to attack, or specialized behaviors that assist in escape.

The best way for prey to avoid detection is to blend in with their background. When this is done visually, this also requires that the prey must remain still in order for this approach to be effective.

If prey have detected a predator first and are capable of doing something to avoid capture, it is mutually beneficial to both parties that this information be communicated. It is also in the predator's interest that if it receives this information from the prey, it has some certainty that it is accurate. Examples do exist in terrestrial mammals, but they are not well known in fish.

If predators cannot be discouraged through dialog, then communicating the presence of toxins that are contained by prey may also deter attack. Species that are toxic are, in general, brightly colored in order to make clear the presence of these chemicals. Predators will learn very quickly to associate the bright colors with toxins and hence should avoid such encounters in the future. The

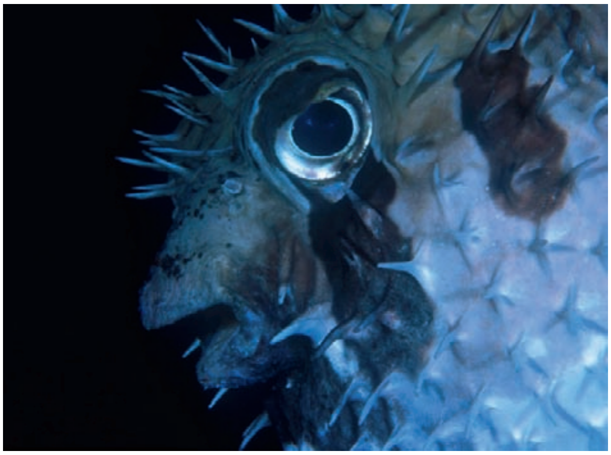


Figure 5 The antipredator morphology of the black-blotched porcupinefish (*Diodon liturosus*). This fish possesses spines over the entire body and, when disturbed, is capable of inflating with water to dramatically increase their size, and erect spines that make them a poor fit for most predator's mouths. Courtesy of Sergey Parinov.

caveat however is that other species will mimic the colors of toxic species without containing the toxins (a model mimic complex). The relative abundance of the mimics will impact the effectiveness of this approach.

Some species have unique morphologies, such as spines and armor plating, that make them very difficult for predators to consume (**Figure 5**). Perhaps the best example is the porcupine fish, which considerably swell their body and spines when disturbed, making them almost impossible to consume for all but the largest predators. Tuna, however, may attack these fish so rapidly that an effective defense cannot be mounted.

Are There Overall Consequences?

At the outset, we had noted the role of evolution in shaping fish design and behavior. Finding food and avoiding predators are fundamental, and the genetic characteristics of individuals that do better than their peers will shape the traits of successive generations. What is important here is that the presence of predators may also be considered to be an important resource. Typically, most individuals avoid locations that are dangerous resulting in greater feeding opportunities in more dangerous locations. Those individuals that are prepared to incur greater risk or that possess specialized morphologies to mitigate the risk can only take advantage of these traits if there is a healthy predator population within their environment. Furthermore, if there is a cost to possessing such traits, reduction in the density of the predator population will mean that for these individuals there will be no compensating benefit to the possession of these traits that will in turn result in their ability to survive in their environment which too is now being compromised.

It is from this perspective that the importance of predators in stabilizing ecosystems can be appreciated. While predators consume individuals at lower trophic levels, they also serve as an important resource for some species and therefore play an important role in competition. The converse is also true. If ecosystems are perturbed resulting in a significant decline or loss of the top predators, then they become destabilized. Those prey species that are adapted to obtaining food under such conditions will no longer be designed for thriving under these new conditions. As a consequence, new, invasive species will better be able to establish themselves and may displace the native species.

This perspective may also be important in understanding how to re-introduce species. Simply adding more individuals through hatchery programs or otherwise artificially increasing the population may not necessarily work if their environment has also changed. Properly understanding how that species is adapted to make foraging decisions in the presence of predators and what species currently occupy the environment into which this species will be introduced should provide insight as to how well this animal is designed for the environment it will now confront.

See also: **Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia. **Fish Migrations**: The Biology of Fish Migration. **Sensory Systems, Perception, and Learning**: Fish Learning and Memory. **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior. **Social and Reproductive Behaviors**: Dominance Behaviors.

Further Reading

Abrahams MV (2006) The physiology of anti-predator behaviour: What you do with what you've got. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, 1st edn., vol. 24, pp. 79–108. San Diego, CA: Elsevier.

Abrahams MV, Pink M, and Klassen C (2008) Predator avoidance. In: *Encyclopedia of Life Sciences*. Chichester: Wiley.

Arnegard ME and Carlson BA (2005) Electric organ discharge patterns during group hunting by a mormyrid fish. *Proceedings of the Royal Society* 272: 1305–1314.

Bestley S, Patterson TA, Hindell MA, and Gunn JS (2008) Feeding ecology of wild migratory tunas revealed by archival tag records of visceral warming. *Journal of Animal Ecology* 77: 1223–1233.

Brown J (1988) Patch use as an indicator of habitat preference, predation risk, and competition. *Behavioural Ecology and Sociobiology* 22: 37–47.

Ferrari M, Sih A, and Chivers DP (2009) The paradox of risk allocation: A review and prospectus. *Animal Behaviour* 78: 579-585.

Killen SS, Brown JA, and Gamperl AK (2007) The effect of prey density on foraging mode selection in juvenile lumpfish: Balancing food intake with the metabolic cost of foraging. *Journal of Animal Ecology* 76: 814–825.

Kingsley DM and Peichel CL (2007) The molecular genetics of evolutionary change in sticklebacks. In: Östlund-Nilsson S, Mayer I, and Huntingford FA (eds.) *Biology of the Three-Spined Stickleback*, pp. 41–82. Boca Raton, FL: CRC Press.

Mclaughlin RL (2001) Behavioural diversification in brook charr: Adaptive responses to local conditions. *Journal of Animal Ecology* 70: 325–337.

Mittelbach GG (2002) Fish foraging and habitat choice: A theoretical perspective. In: Hart PJB and Reynolds JD (eds.) *Handbook of Fish Biology and Fisheries,* vol. 1, pp. 251–266. Malden: Blackwell.

Peckarsky BL, Abrams PA, Bolnick DI, *et al.* (2008) Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator–prey interactions. *Ecology* 89: 2416–2425.

Preisser EL, Bolnick DI, and Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology* 86: 501–509.

Reitan KI, Rainuzzo JR, Gunvor Ø, and Olsen Y (1997) A review of the nutritional effects of marine fish larvae. *Aquaculture* 155: 207–221.

Robb TL and Abrahams MV (2004) Variation in tolerance to hypoxia in a predator and prey species: An ecological advantage to being small? *Journal of Fish Biology* 62: 1067–1081.

Stephens DW, Brown JS, and Ydenberg RC (2007) *Foraging: Behaviour and Ecology*. Chicago, IL: University of Chicago Press.

Effects of Compensatory Growth on Fish Behavior

D Álvarez, Universidad de Oviedo, Oviedo, Spain

© 2011 Elsevier Inc. All rights reserved.

Introduction
Behavioral Changes during Compensation
Deferred Costs of Compensatory Growth
Summary
Further Reading

Glossary

Compensatory growth A faster-than-normal growth that some species exhibit after a period of resource deprivation.

Dominance The state of being dominant where dominant individuals obtain a high status within a social group.

Hormone A chemical substance, often a peptide or steroid, released from endocrine glands that controls and regulates a range of physiological activities.

Hyperphagia Rate of food consumption significantly higher than that shown by animals that have been feeding continuously on an *ad libitum* ration.

Smolting Behavioral and physiological changes crucial to the successful rapid entry of fish that were born in freshwater and are migrating to the marine environment.

Introduction

Fish are capable of reacting after a period of resource deprivation by increasing their growth rates to higher-than-normal levels. This implies that animals under normal conditions are growing at a rate below their physiological potential and therefore they are able, to some extent, to adjust their growth. The main question regarding compensatory growth is why fish do not usually grow as fast as they can, since it is well known that the risk of predation reduces with increased body size (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). There is some evidence that indicates that there is a trade-off between growth and fitness such that rapid growth can produce deleterious effects compromising further survival. Growth compensation will be favored if the benefits of catching up in size outweigh these costs. Many consequences of accelerated growth rates are related to behavioral changes that animals show during and after compensation, particularly associated with changes in feeding habits and the trade-off between accelerated growth and the increased risks than the fish must take foraging for the extra food. These changes in behavior can be grouped into two different classes depending on whether these changes occur during the period of compensation or are a consequence of increased growth rates (**Figure 1**).

The main mechanism involved in the compensatory growth response is hyperphagia, an increased rate of food consumption, although increased conversion efficiencies or behavioral adjustments can play a role. Hyperphagia implies various behavioral changes, mainly an increase in the boldness and reduction in the use of shelters. During the period of compensation, fish increase their feeding activity and spend more time searching for food in open waters where the chances to be preyed upon are higher than in normal circumstances. The need to grow at a higher rate also affects intra-specific behavior, increasing aggressiveness and competition between fish. However, in the majority of studies on compensatory growth, study animals are held individually and therefore the behavioral changes that they experience during compensation are very difficult to evaluate due to the absence of social interactions (**Figure 2**).

After full growth compensation, fish can show deferred physiological and behavioral effects as a consequence of the rapid growth experienced. There is evidence in a variety of taxa that rapid growth can affect locomotor performance, possibly because of its effects on muscle cellularity and development, and therefore the benefits of growth acceleration should be matched against the locomotor costs. Swimming performance is strongly related to many fish behaviors, such as anti-predator behavior or breeding performance via sexual displays or offspring care.

Behavioral Changes during Compensation

Hyperphagia can result from increasing meal frequency, food consumption per session, or the combination of both. There is significant inter-specific variation in the

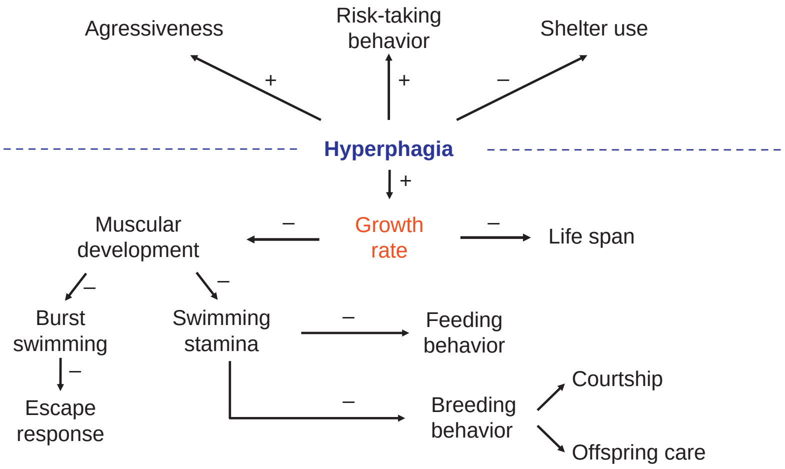


Figure 1 Schematic representation of the direct and indirect effects of compensatory growth on fish behavior where hyperphagia indicates the elevated rate of food consumption associated with compensatory growth. Above the blue line: behavioral changes during compensation; under the blue line: deferred effects of compensatory growth on fish behavior. +, positive relationship; –, negative relationship.

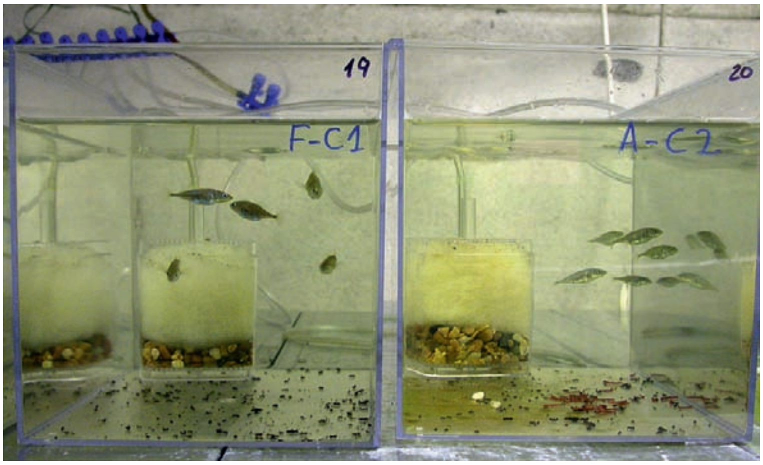


Figure 2 Three-spined sticklebacks (*Gasterosteus aculeatus*) held in groups of five fish during the phase of growth compensation. Many fish species live in shoals as juveniles, and isolation of individuals during laboratory experiments could affect growth performance. The majority of studies investigating compensatory growth separate fish into individual tanks making it impossible to study additional effects on social behavior. Photo: David Álvarez.

temporal pattern of hyperphagia. However, fish, like other vertebrates, can regulate their hyperphagic response according to the quality of the food and the energy needed. Animals that are compensating in growth have a greater food intake than fish that have continuous access to food but which have not undergone a growth depression. This suggests that the maximum daily rates observed during hyperphagia represent a rate close to the maximum possible rate at which the gut can process food. Although the mechanisms that allow compensatory growth are physiological, behavioral adjustments must be involved as high growth rates require an increase in food intake.

Hyperphagia imposes several behavioral costs. Increased foraging to obtain the extra ration of food needed to increase their growth rate may expose the animals to a greater risk of predation. Compensating fish are bolder in the presence of a potential predator than the fish growing at normal rates, and fish exhibiting hyperphagic behavior can increase the number of feeding attempts by more than 50% compared to fish of the same age growing at normal rates.

Risk-Taking Behavior

Feeding activity can increase the risk of being eaten, since foraging often decreases vigilance. Moreover, after a period of growth depression, a hungry fish may have difficulties avoiding an attack if it is weakened by its poor body condition. For the fish, this is a behavioral dilemma: on the one hand, a high growth rate will reduce the time that a fish spends in smaller, more vulnerable stages, but on the other hand to have a high growth rate, a fish has to take more risks and obtain more food. The search for the food necessary to increase their intake rates implies that fish have to use open waters more frequently than fish growing at normal rates and, therefore, they will have a greater chance of being captured. Studies carried out with coho salmon (*Oncorhynchus kisutch*) showed unequivocally that catch-up growth after a period of growth depression involves a change in the trade-off between food consumption and risk of mortality due to predation. In a study carried out with reef fish ambon damsel (*Pomacentrus amboinensis*), Monica Gagliano and her collaborators demonstrated that young fish with an intense selective pressure to grow at a faster rate during their benthic life had a high feeding motivation, and were willing to take a greater predation risk as a cost of gaining more resources.

Shelters are commonly used by inactive fish to give security against predators but usually imply a cost of reduced feeding opportunities. Fish experiencing compensatory growth after a period of growth depression change their habits and are more likely to spend increased time out of refuges than fish growing at normal rates. Studies carried out with three-spined sticklebacks demonstrate that the recovery time taken for a fish to emerge from a refuge after a predator attack is less in fish that are compensating than in normal growing fish. In Atlantic salmon (*Salmo salar*), after a simulated threat of predation, juvenile fish growing at normal rates are reluctant to move away from refuges to intercept prey items drifting past in the current. In contrast, fish that are displaying hyperphagic behavior after a period of food shortage spend less time in refuges after a simulated predation attack and assume more risks. This behavior increases the time spent for feeding but at the same time increases the risks of being preyed upon since they are more exposed to predatory attacks.

Treatment of fish with growth hormone (GH) can be used to evaluate the behavioral consequences of rapid growth. GH stimulates tissue growth by increasing deoxyribose nucleic acid (DNA) synthesis and the rate of cell multiplication and differentiation, increasing the metabolic demands of the animal and the hunger level it experiences. In brown trout (*Salmo trutta*), GH-treated fish increased their growth rate and also reduced their anti-predator responses. Fish treated with GH spent more time swimming and in the upper part of the water column where they were more accessible to predators.

Various studies suggest that GH increases feeding motivation. Similar results are obtained with transgenic Atlantic salmon bred to contain and transmit a GH transgene (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish): they grow faster than control fish but spend more time foraging in risky environments. Although it has not been exhaustively investigated, some authors suggest that fish that experience a phase of compensatory growth produce more GH which could increase growth rates above normal levels.

Aggressiveness and Risk of Aggression

Hyperphagia may induce higher levels of intra-specific aggression as individuals compete for food, with associated costs in terms of higher rates of energy expenditure and increased risk of mortality. Intra-specific aggression can result in the formation of dominance hierarchies (**see also Social and Reproductive Behaviors**: Dominance Behaviors) and lead to a trade-off between increased resource acquisition and energy losses associated with agonistic encounters. Growth rates of subordinate fish can be depressed as a consequence of dominant aggression but aggression also involves energetic costs and loss of feeding opportunities during time spent in territory defense.

Studies carried out with Atlantic salmon have demonstrated links between growth bimodality, aggressiveness, and compensatory growth with food-deprived Atlantic salmon juveniles showing increased levels of aggression during the compensatory growth phase. In salmonids, life-history pathways are not fixed and life-history decisions depend on growth rates or size during specific decision windows. For example, several months prior to smolting and migration to sea, juveniles must decide whether to migrate to sea or whether to postpone seaward migration until the subsequent year. Slow-developing fish from the initially poorer competitive fraction of the population are likely to postpone migration, whereas fast-developing fish from the more competitive fraction of the population are likely to migrate to sea at least 1 year before slow-growing individuals. Fish that adopt the faster development pathway show compensatory growth and may experience higher aggression than slower-growing fish. Aggression arises over competition for food and fish that are compensating for growth are more likely to receive attacks from other competing fish. Fish that postpone migration initiate less aggressive actions needing only to maintain their energy reserves above a critical level with a further year in the river to grow; competition for food with the faster-growing fish is not necessary. These different growth strategies in Atlantic salmon result in the fast-growing juvenile Atlantic salmon that are more aggressive than slow-growing fish, but are also more vulnerable to receiving attacks from other conspecifics.

Activity Patterns

Some fish species become nocturnal to reduce the risk from diurnal predators, and shelters may be used by inactive animals to give security against predation risk. However, fish that compensate in growth need to eat more than normal and changes in foraging habits can affect diel activity patterns. Individuals that are nocturnal in normal circumstances can increase their diurnal activity and hence the risk of predation. In contrast, in species such as Atlantic salmon where nocturnal fish tend to be subordinates that could not find a secure foraging place during the daylight, it may be more risky to feed at night. If energy demand increases as a consequence of compensatory growth, fish will be forced to eat during the hours when they would normally reduce their activity, therefore succumbing to a greater risk of predation.

Time–Place Learning

Animals organize their daily activities, particularly foraging, by associating different places with food availability at different times of the day; this is called time–place learning (TPL) (**see also Sensory Systems, Perception, and Learning**: Fish Learning and Memory). In rats it has been observed that animals that were fed *ad libitum* lost their ability to learn time-of-day discriminating tasks, whereas food-restricted rats were able to acquire associations between two different places and two different times of day. Experiments carried out with Nile tilapia (*Oreochromis niloticus*) demonstrated that fish that were fed *ad libitum* failed to learn the time and place synchronization task, but when the experiment was repeated with fish that had experienced a period of 17 days of starvation, those fish were able to switch places at the correct period of the day to get food, suggesting that food restriction facilitates TPL discrimination.

Deferred Costs of Compensatory Growth

Effects on Predator Avoidance

Rapid growth can affect locomotor performance, probably through effects on muscle cellularity and development. In some species of fish, fast-growing individuals have a higher percentage of small-diameter white muscle fibers and greater numbers of similar-diameter red muscle fibers than the slow-growing individuals, which could detrimentally influence fish swimming ability. In the case of compensatory growth, the benefits of growth acceleration can be matched against the locomotor costs. Many fish species use burst swimming as a response to evade predators, and the speed of this response is directly related with the probability of surviving predation attempts. In a study on Atlantic silversides (*Menidia menidia*) faster-growing fish had poorer prolonged and burst swimming performances, suggesting a trade-off between growth rate and swimming performance.

In three-spined sticklebacks, fish that had undergone compensatory growth after a period of food deprivation had a reduced burst swimming speed compared to fish growing at normal rates. Interestingly, there was a strong interhabitat difference in the impact of compensatory growth on swimming performance. Following compensatory growth, fish from stream populations showed impaired escape speeds, but this was not seen in the pond populations. The explanation for this habitat difference appears to lie in the nonlinear nature of the trade-off curve. Small but sustained increases in growth rate (as observed in the pond populations of sticklebacks) had relatively little effect on burst swimming, whereas the greater growth acceleration seen in the stream populations of sticklebacks incurred a much greater cost. Lakes and ponds are structured environments in which fish need to be able to dart quickly into a refuge and then remain still; burst swimming is a key factor in determining likelihood of escape and may be too important a trait to be traded off against growth. In contrast, in rivers and streams the importance of burst swimming in evading predators is less important and fish can assume the cost of impaired swimming ability to achieve a larger size in a shorter period of time.

Many fish species possess a defensive morphology that protects them from predators (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). For example, in three-spined sticklebacks there is an interesting potential trade-off in resource allocation between investment in body growth and investment in defensive morphology. In this species, there is marked intra- and interpopulation variation in the average extent of their anti-predator defensive structures (the pelvic and dorsal spines, pelvic girdle and lateral plates), which is linked to the intensity of predation and the willingness of the fish to take risks when foraging. Fish from populations without predatory fish tend to have fewer lateral plates and spines than the fish from populations with intense predation. This reduction of spines and plates has been interpreted as an evolutionary regression of defensive structures in populations from small lakes and streams that lack predatory fish. Due to the phenotypic plasticity of these structures, individual sticklebacks that experience compensatory growth after a period of growth depression may compromise on allocation of resources to defensive armor in order to increase their skeletal growth rate. Hence, these fish will be less protected than the fish that have grown at normal rates.

Effects on Breeding Behavior

Fish and other animals have to reach a minimum size to have the opportunity of finding mates and breeding. A further dimension is the fact that the cost of being small during the breeding season differs between the sexes. In three-spined sticklebacks, an increase in body length has a positive effect on reproductive success in both the sexes. However, the cost of a small size is greater for males, because females can breed when as small as 32 mm, whereas even a 40-mm male will likely never breed. Moreover, while an increase in body size from 40 to 50 mm doubles the clutch size of a female, the same increase in body size leads to an approximately 15-fold increase in reproductive success in males. This necessity to achieve a minimum size is complicated by the need to allocate resources to other external characteristics that affect the sexual attractiveness of an individual. As with predator avoidance, a large body size at the time of breeding is clearly a fitness advantage, but the rapid growth needed to achieve it may be costly.

Effects on Breeding Coloration and Sexual Attractiveness

Numerous male fish develop secondary sexual traits when they reach maturity. These attributes could be hyper-developed fins or conspicuous appendices that males exhibit during sexual displays to attract females. As these attributes would be expected to impair swimming performance, they are considered honest signals of the quality of the male because there is a trade-off between anti-predator escape responses and sexual attractiveness. This trade-off can be affected by a previous diet restriction and further resource allocation during compensatory growth. Green swordtails (*Xiphophorus helleri*) are small, tropical, live-bearing fish whose males have developed a long sword which is an extension of the caudal fin that develops at sexual maturity. In an experiment carried out to study the effects of compensatory growth on female selection, females did not visually discriminate between males that had experienced poor early nutrition and a subsequent compensatory growth strategy and similar control males. However, physical aspect is not the only trait that female swordtails search for in a potential mate. Courtship displays are also very important in female selection, and male swordtails that have undergone a period of growth compensation have poorer swimming performance than control fish. Therefore, growth compensation following poor initial growth can indeed reduce the mating success of male swordtails much later in life.

Other fish species swap the dull coloration that they exhibit during the majority of the year for a bright and colorful coloration during the breeding season (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals). To achieve this, fish must quickly mobilize a large amount of resources to the skin. Some of these resources, such as the carotenoids, cannot be synthesized and must be obtained from the diet. Thus, fast growth may result in more resources being allocated to growth at the expense of sexual ornaments, which could negatively affect female preference. Some authors suggest that males that have experienced compensatory growth early in life after a period of food restriction could be unable to develop phenotypic traits associated with mate attraction when older. They predict that such males may suffer a more severe decline in phenotypic traits than males that have not undergone growth compensation. This would result in females choosing males with normal growth trajectories over the growth-compensating males because the general appearance of the latter could be interpreted as an indicator of lower genetic quality.

Effects on Clutch and Offspring Care

As shown previously in this article, compensatory growth may impair muscle development and swimming performance. While this effect can be similar in males and females, there may be greater implications for males in terms of reproductive success as in many species females are the choosy sex and are likely to avoid males in poor condition or with reduced sexual attributes. However, in females compensatory growth can have a significant impact on reproductive investment (i.e., a reduced quality and number of eggs). Fast-growing males that show parental care after egg laying may be poorer fathers due their reduced ability to swim, which will adversely affect their ability to fan the eggs and defend the nest against intruders, but to demonstrate this potential effect, further experimental research is needed. Many salmonid fish spawn in rivers and streams where the female digs a nest in the gravel by lying on her side against the bottom and swimming forward energetically (**Figure 3**). These important movements might be impaired by the poor

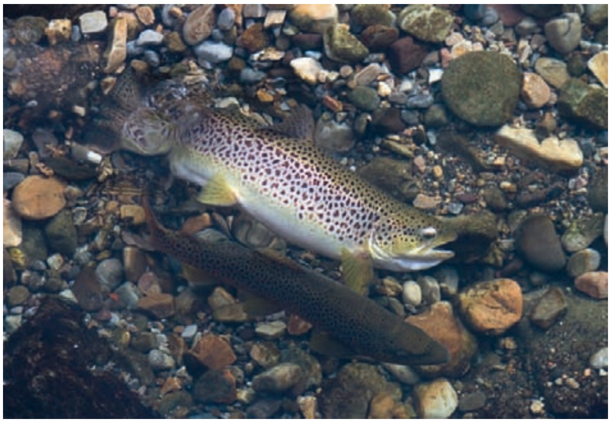


Figure 3 A pair of brown trout spawning. The female uses her tail to dig out a hollow in the gravel using a fanning motion. This behavior could be impaired if muscle development is hindered after a period of growth compensation. Photo: David Álvarez.

development of her muscles after a period of growth compensation, and could affect nest structure and further egg development.

Summary

The study of compensatory growth in fishes has received much recent attention, especially in relation to aquaculture, but many behavioral consequences of this accelerated growth remain unexplored. The main behavioral consequence of growth compensation is that fish have to take more risks to obtain the extra ration of food necessary to increase their growth rates. However, compensatory growth also affects intra-specific relationships between individuals and may have consequences for breeding behavior and parental care through impaired muscle development after a period of rapid growth. Increased research efforts are now needed in the study of long-term effects of compensatory growth on fish behavior and the consequences of these behavioral changes for natural populations.

See also: **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish. **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals; Fish Learning and Memory. **Social and Reproductive Behaviors**: Dominance Behaviors.

Further Reading

Ali M, Nicieza A, and Wootton RJ (2003) Compensatory growth in fishes: A response to growth depression. *Fish and Fisheries* 4: 147–190.

Álvarez D and Metcalfe NB (2005) Catch-up growth and swimming performance in sticklebacks (*Gasterosteous aculeatus*): Seasonal changes in the cost of compensation. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2169–2173.

Álvarez D and Metcalfe NB (2007) The tradeoff between catch-up growth and escape speed: Variation between habitats in the cost of compensation. *Oikos* 116: 1144–1151.

Billerbeck JM, Lankford TE, and Conover DO (2001) Evolution of intrinsic growth and energy acquisition rates. I. Tradeoffs with swimming performance in *Menidia menidia*. *Evolution* 55: 1863–1872.

Damsgård B and Dill LM (1998) Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. *Behavioral Ecology* 9: 26–32.

Gagliano M, McCormick MI, and Meekan MG (2007) Survival against the odds: Ontogenetic changes in selective pressure mediate growth–mortality trade-offs in a marine fish. *Proceedings of the Royal Society B* 274: 1575–1582.

Gotceitas V and Godin J-GJ (1991) Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.) – effects of social status and hunger. *Behavioral Ecology and Sociobiology* 29: 255–261.

Jonsson E, Johnssonand JI, and Bjornsson BT (1996) Growth hormone increases predation exposure of rainbow trout. *Proceedings of the Royal Society of London B* 263: 647–651.

Lima SL and Dill LM (1990) Behavioural decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology* 68: 619–640.

Metcalfe NB and Monaghan P (2001) Compensation for a bad start: Grow now, pay later? *Trends in Ecology and Evolution* 16: 254–260.

Nicieza AG and Metcalfe NB (1999) Costs of rapid growth: The risk of aggression is higher for fast-growing salmon. *Functional Ecology* 13: 793–800.

Royle NJ, Lindström J, and Metcalfe NB (2006) Sexual selection, growth compensation and fast-start swimming performance in green swordtails *Xiphophorus helleri*. *Functional Ecology* 20: 662–669.

Walling CA, Royle NJ, Metcalfe NB, and Lindström J (2007) Early nutritional conditions, growth trajectories and mate choice: Does compensatory growth lead to a reduction in adult sexual attractiveness? *Behavioral Ecology and Sociobiology* 61: 1007–1014.

Temperature Preference: Behavioral Responses to Temperature in Fishes

LI Crawshaw and JE Podrabsky, Portland State University, Portland, OR, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Species Preferences
Environmental Observations
Further Reading

Glossary

Behavioral thermoregulation Regulation of body temperature by utilizing behavioral movements to control body temperature. It is potentially utilized by all fish.

Final thermal preferendum The temperature preference obtained when a species remains in a temperature gradient long enough that a stable preferred temperature is observed.

Physiological (or autonomic) thermoregulation Utilization of autonomic responses to control body temperature; utilized in rare cases by fish.

Temperature preference The temperature that a particular species selects, under a particular set of conditions, in a situation where a range of temperatures are available.

Thermal acclimation The responses of a fish to maintain in a particular thermal regimen in the laboratory under a specified set of conditions.

Thermal acclimatization Organismal adjustments to temperature that occur in nature, and are thus affected by other factors such as season and day length.

Introduction

Temperature can be considered a master factor for fish as it affects all physiological processes and ecological systems. Thermal extremes can prove detrimental or even lethal (**see also Temperature**: Measures of Thermal Tolerance). Within the range of temperatures that a fish normally encounters, temperature directly speeds up and slows down biochemical reactions (**see also Temperature**: Effects of Temperature: An Introduction), thus impacting all aspects of a fish's behavior and physiology.

While all motile animals have evolved behaviors that deal with the thermal variation present in their environment, fish and other vertebrates possess a particularly sophisticated system to maintain body temperature within narrow limits. The properties of this regulatory system are very similar to those found in other vertebrates, although in fish such responses are largely limited to behavioral adjustments and, with only a few exceptions (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks), body temperature quickly equilibrates to within a few tenths of a °C ambient water temperature. When a range of temperatures is available, fish are able to maintain body temperature at or near the preferred level by positioning themselves in water of that temperature, that is, behavioral temperature regulation.

However, despite the power and sophistication of the nervous elements involved with behavioral temperature regulation, a particular species of fish at a given time is not always found at their preferred temperature. During some seasons, the preferred temperature is simply not part of the prevailing environment, while at other times predator avoidance, food acquisition, territorial defense, mating requirements, and gradients of light, oxygen, or salinity may displace a fish from its normally preferred temperature.

Nevertheless, temperature is a predominant factor in determining habitat selection by fish, and is a major influence on their daily and seasonal movements (**see also The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis) as well as in their overall distribution and their physiological and biochemical capabilities (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and **Temperature**: Membranes and Temperature: Homeoviscous Adaptation). Examples of these effects can be seen in the behavior of the chimaera (*Hydrolagus colliei*) and the Atlantic mackerel (*Scomber scombrus*). In its southerly extremes, the chimaera is a deep-water fish, usually being found at depths that exceed 90 m. However, in the cold North Pacific, the chimaera is typically found in shallow water. In both the southern and northern environments, this species is found at the same temperature. Thus, the temperature preference of this species may explain its depth distribution. In contrast to the chimaera, the Atlantic mackerel is a rapidly moving fish and can quickly shift its location in order to maintain a particular body temperature. For the Atlantic mackerel, a 1 °C increase in ocean temperature causes the fish to swim north about 110 km to maintain its preferred temperature.

Species Preferences

The preferred temperature can vary under different states, and for each species, the conditions under which the preferred temperature is determined should be specified. Thermal preference can be most clearly demonstrated in laboratory temperature gradient or choice devices, which provide fish with the ability to choose specific temperatures from a broad range of available temperatures. If nonthermal influences are minimized, and unstressed fish are placed in a thermal gradient, results similar to those shown in **Figure 1** are typically obtained. In **Table 1**, the temperature preferences of various species of fish are shown. The thermal preference is seen to relate to the general habitat of the fish, and is one of many responses that serve to maintain a species within its recognized habitat.

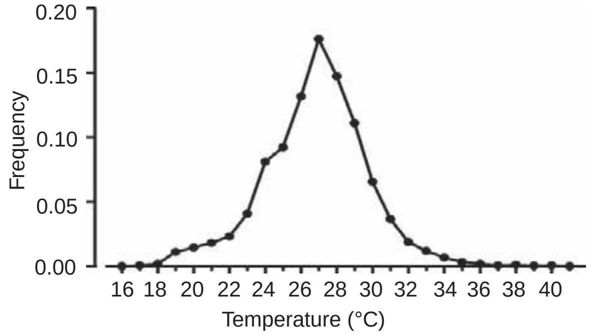


Figure 1 Fish prefer a narrow range of temperatures when presented with a continuous gradient of temperature options. This figure represents the frequency of temperatures selected by seven female *Austrofundulus limnaeus* over a period of 24 h in a linear thermal gradient ranging from 12 to 42 °C. The data are calculated from 10 321 one-min averages calculated from data collected every 6 s. Fish were acclimated to a cycling temperature regime ranging from 20–37 °C daily with a photoperiod of 14L:10D for several weeks prior to determination of temperature preference.

Small Alterations in the Preferred Temperature

In addition to examples of the preferred temperature for various species, **Table 1** also illustrates some of the conditions where the preferred temperature may be altered within a species. These shifts in preferred temperature are typically on the order of 2 or 3 °C, and are often made by a given species in order to better utilize available temperatures under different conditions. Such effects include time of day (**see also Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish), season, sex, reproductive stage, presence of a bacterial infection (fever), nutritional status, and geographical location of a subspecies. The degree and presence of such shifts in preferred temperature are specific to each fish species.

Major Alterations in the Preferred Temperature

In certain cases, there can be major alterations in the preferred temperature within a species. One case involves developmental state. Typically, younger animals prefer warmer temperatures, which can be considerably higher than the adult preferences. Thus, young salmonids often select temperatures in the upper teens, whereas larger adults typically exhibit temperature preferences in the lower teens. Larger individuals may also exhibit a lower tolerance to high temperatures than juveniles. These differences likely reflect both environmental and physiological differences between the developmental stages. Smaller fish can avoid predation more effectively in shallow, covered areas and are also more likely to find appropriate nourishment in these areas. Higher temperatures, up to a point, can

Table 1 The laboratory temperature preference of a variety of fish species

Common name	*Species*	*Laboratory temperature preference (°C)*	*Conditions*
Desert pupfish	*Cyprinodon macularius*	38–40	Summer
Common killifish	*Fundulus heteroclitus*	28–30	Northern population
		24–26	Southern population
Common goldfish	*Carassius auratus*	28–30	Small
		24–25	Adult
Large mouth bass	*Micropterus salmoides*	27–29	Adult
Rainbow trout	*Oncorhynchus mykiss*	21–22	Fed fingerlings
		17–18	Starved fingerlings
		13–17	Adults
Coho salmon	*Oncorhynchus kisutch*	11–12	Adult
Polar cod	*Boreogadus saida*	2–3	Early morning
		4–5	Afternoon – early evening

From Richards FP, Reynolds WW, and McCauley RW (1977) Temperature preference studies in environmental impact assessments: An overview with procedural recommendations. *Journal of the Fisheries Research Board of Canada* 34: 728–761.

also support faster growth. Larger fish, on the other hand, are more vulnerable to predation in shallow, warm areas.

A second case involves adjustment to extreme temperatures. While fish can adjust body processes to operate effectively over a range of different environmental temperatures (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation), this process takes time and varies for different species, with individuals from areas of high thermal variability showing greater capabilities for such thermal acclimatization. With acclimatization to extreme temperatures, metabolic and other physiological processes are greatly altered, and the normally preferred temperature can become harmful or lethal. Not surprisingly, thermal acclimatization alters the preferred temperature, and to a greater degree for temperatures farther from the normally preferred temperature. Thus, the brown bullhead (*Ictalurus nebulosus*), which normally prefer water temperatures of about 30 °C, prefer a temperature of 16 °C after being maintained in the laboratory at 7 °C for several weeks. When left undisturbed in a temperature gradient, the preferred temperature gradually increases, and returns to 30 °C in about 1 day. The thermal preference that fish finally stabilize at after a long period in a thermal gradient is termed the 'final thermal preferendum'.

A third case involves survival under physiological stress. Many species of fish have the ability to survive severe hypoxia (**see also Hypoxia**: The Expanding Hypoxic Environment) by selecting very cold temperatures. While broad variations in levels of dissolved oxygen in the water normally do not affect the temperature preference, when oxygen levels fall to near-lethal levels, thermal preference decreases drastically. Adult goldfish, for example, normally prefer 25 °C. If the oxygen partial pressure is lowered from a normal saturated value of 160 torr (21.3 kPa) to 31 torr (4.1 kPa), there is little change in the preferred temperature. However, if the oxygen level is decreased further, to 10 torr (1.3 kPa), the preferred temperature drops by about 10 °C. While this low temperature is stressful, it enables the fish to survive by increasing the affinity of hemoglobin for oxygen (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin), augmenting gill extraction efficiency, and by reducing the metabolic demands for maintaining minimal function.

Accuracy and Constancy of the Preferred Temperature

Given the general ability of fishes to adapt to a broad range of temperatures (just below 0 °C to just above 40 °C), the stability of their preferred temperature, as seen in **Figure 1**, is somewhat enigmatic. A closer look at several factors may elucidate the reason for such persistent maintenance of a particular temperature.

First, as mentioned earlier, adjustments to a new temperature take both time and energy. When a fish moves to water of a different temperature, all aspects of its physiological and behavioral capabilities are somewhat compromised. The rapidity with which a change in ambient water temperature affects overall body temperature is often underestimated. Typically, body temperature is measured at a deep site and the temperature at that site is found to change relatively slowly, even if a fish quickly moves to water of a radically different temperature. The net thermal effect on a fish is better estimated by evaluating the change in mean body temperature, which can be done by placing a fish in a calorimeter containing water of a different temperature. The results of such a maneuver for a 0.56 kg carp (*Cyprinus carpio*) are seen in **Figure 2**. While 50% of the change in mean body temperature occurs within 1 min, 50% of the change in brain and deep dorsal muscle temperature requires 4 and 5 min, respectively. The magnitude and rapidity of heat transfer

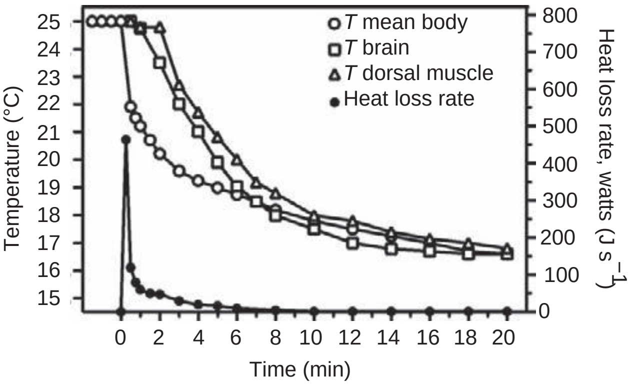


Figure 2 Changes in temperature and heat loss rates in a 0.56-kg carp transferred from 25 °C into a calorimeter at 15 °C. About half of the heat loss occurs within the first minute after transfer. Temperatures asymptote above 15 °C due to an increase in calorimeter temperature. Adapted with permission from Figure 2 in Crawshaw LI (1976) Effect of rapid temperature change on mean body temperature and gill ventilation in carp. *American Journal of Physiology* 231: 837–841.

are emphasized by the heat loss rate illustrated in **Figure 2**. Note that the metabolic heat produced by the carp in this situation would be less than 1 W. For such a carp, when equilibrium is finally reached, about half of the heat exchange occurs at the body surface and about half is due to conductance across the gill surface. The thermal exchange times exhibited for this 0.56-kg carp would be faster for smaller fish and slower for larger fish due to the differences in surface-area-to-volume ratios (**see also Temperature**: Effects of Temperature: An Introduction).

Another reason for the persistent selection of a particular temperature relates to the overall optimization of organismal function. While most fish can make adjustments to function well over a range of temperatures, there is a much narrower temperature range that, after thermal acclimatization, provides an optimal behavioral and physiological set of responses for functioning in the fish's particular habitat. Such responses include optimization of growth (**see also Energy Utilization in Growth**: Growth: Environmental Effects), metabolic scope, cardiac scope, power output of aerobic muscles, and overall performance. In the cases investigated, the preferred temperature fell within the narrow range of temperatures for overall optimal function.

Characteristics of the Thermoregulatory System

The regulatory system which is utilized by fish to maintain a particular body temperature is anatomically and functionally similar in Chondrichthyes and Osteichthyes, and is closely related to the system utilized by other vertebrates (see **Figure 3** and **Brain and Nervous System**: Autonomic Nervous System of Fishes). To function correctly, a thermoregulatory system must sense the controlled variable, make a comparison with an ideal value, and determine an appropriate behavioral response. In fish and other vertebrates, temperature sensing occurs both peripherally and centrally. Sensitivity occurs over the entire fish surface, and is likely subserved by highly responsive, rate-sensitive, free nerve endings. In conditioning experiments, fish have proved capable of responding to a surface temperature change of less than 0.05 °C. While, in most conditions, fish would probably not respond to such small temperature differences, they clearly have a capability for detecting extremely small thermal variation. This capability is undoubtedly useful for specific situations that could involve locating critical thermal refuges or guiding large-scale migrations. An important area for central thermal sensing and processing occurs in the anterior brainstem regions in vertebrates, as shown in **Figure 3**. In fish, an area of particular importance is the nucleus preopticus periventricularis. Lesions within the anterior brainstem area destroy thermoregulatory behavior in fish, and heating and cooling this area (including adjacent tissue) leads to the selection of cooler and warmer water, respectively.

Environmental Observations

Field observations that relate fish location (and sometimes actual body temperature) to local water temperature are particularly valuable. Such data clarify

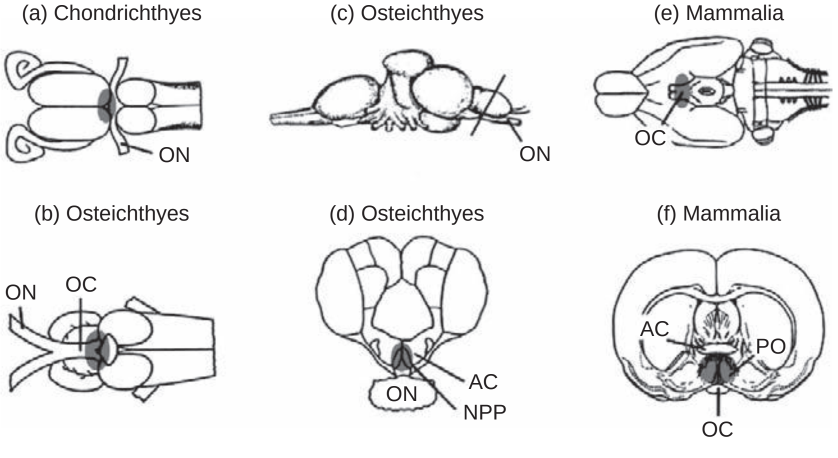


Figure 3 Similar anatomical locations are responsible for body temperature regulation in various groups of vertebrates. Ventral brain surfaces are represented in (a), (b), and (e). Shaded areas represent surfaces that overlie interior regions of the brain that are critical for thermoregulatory control and integration. (c) A side view of the brain with a line depicting the plane of the cross section depicted in (d). A cross section of the shaded region of (e) is depicted in (f). Major thermoregulatory effects are induced by the application of bioactive compounds to the shaded areas of the cross sections in (d) and (f). AC, anterior commissure; OC, optic chiasma; ON, optic nerve; NPP, nucleus preopticus periventricularis; PO, preoptic nucleus. Adapted from Fig. 1 in Crawshaw LI and O'Connor CS (1997) Behavioral compensation for long-term thermal change. In: Wood CM and McDonald DG (eds.) *Society for Experimental Biology Seminar Series 61: Global Warming: Implications for Freshwater and Marine Fish*, pp. 351–376. Cambridge: Cambridge University Press.

the operation of temperature selection in a natural environment, illustrate its use in the maintenance of an optimal body temperature, and show how fish avoid deleterious environmental temperatures in a local environment and during migrations. The value and limitations of temperature selection for fish attempting to survive during an era of global climate change will also be touched upon.

Avoidance of Dangerous Temperatures

While the lethal limit for brook trout (*Salvelinus fontinalis*) is just below 25 °C, they prefer to remain in temperatures below 17 °C. In spite of this, during the summer period of low flow and warm water, brook trout are often found in habitats where mainstream temperatures exceed lethal limits. **Figure 4** illustrates an example of how brook trout are able to survive such dangerously warm conditions. In this instance, the trout congregated near the confluence of the main river and a much cooler tributary. Although their thermal preference is considerably lower than the area of the river they occupied, they likely avoided moving into even cooler areas nearer the tributary due to the dangers of predation involved with occupying very shallow water. The location of these fish indicates a compromise between the preferred temperature and a combination of water depth plus river surface characteristics. Over the course of the summer, the brook trout maintained a body temperature of about 3 °C lower than that of the main river temperature. In addition to utilizing confluences with cooler streams, fish in overly warm rivers have also been observed to make use of cold groundwater discharges and thermally stratified deep pools to avoid thermally induced injury or death. Similar examples exist for migrating Pacific salmon (*Oncorhynchus* sp.) that, during their migration through large rivers, can take advantage of cooler water from tributaries and sometimes lakes. However, in this case, this preference behavior can only act as a temporary respite because the river migration must be completed in relatively short order (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle).

Fish utilize similar strategies in reservoirs and lakes. **Figure 5** depicts the body temperature of striped bass (*Morone saxatilis*) that were introduced into a reservoir in the southeastern USA. This area has warmer temperatures than those present in the normal habitat of this species. The larger adults prefer to remain at temperatures below 22 °C, but this becomes very difficult during the months of summer. The cooler, lower depths become progressively deoxygenated, while the surface waters become untenably warm. Nevertheless, as seen in **Figure 5**, the large adults were usually able to maintain body temperatures and (presumably) dissolved oxygen within acceptable levels by utilizing discrete areas near cold groundwater discharge or cool tributary entrances.

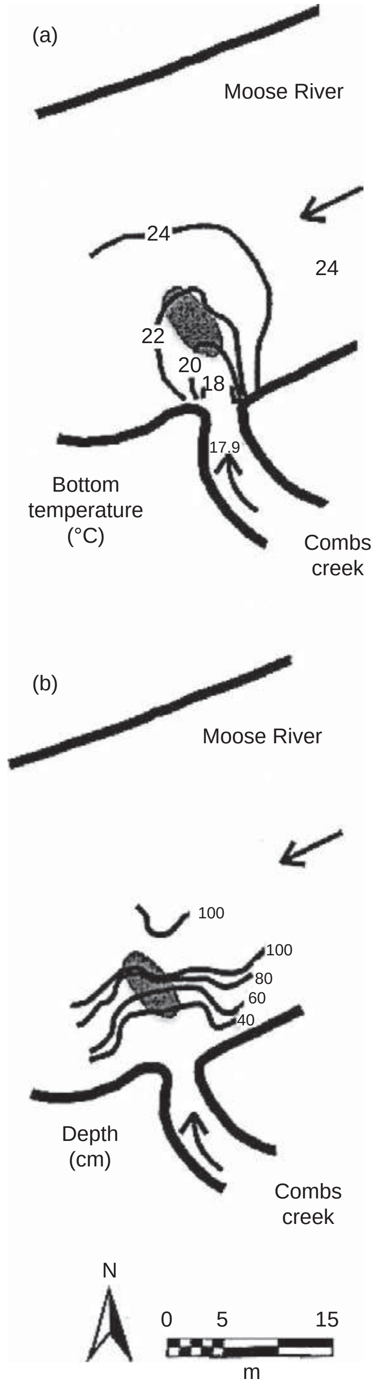


Figure 4 Aggregation of brook trout (shaded areas) depicted in relationship to surface water temperature (a) and depth (b) in the Moose River at the confluence with the cooler Combs Creek. Adapted from Fig. 5 in Baird OE and Krueger CC (2003) Behavioral thermoregulation of brook and rainbow trout: Comparison of summer habitat use in an Adirondack river, New York. *Transactions of the American Fisheries Society* 132: 1194–1206.

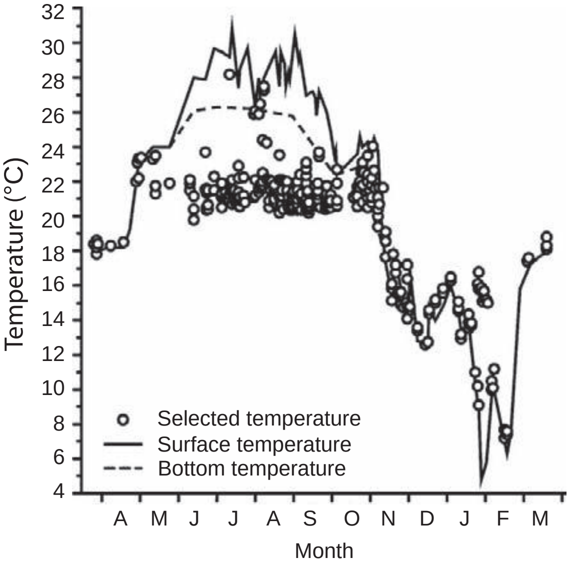


Figure 5 Temperatures selected by free-living striped bass (open circles) in Lake Seminole for a 1-year period starting in March/April of 1984. Surface and bottom water temperatures are plotted to indicate that the fish utilize a unique subset of their thermal habitat for large portions of the year. Redrawn from Fig. 3 in van den Avyle MJ and Evans JW (1990) Temperature selection by striped bass in a Gulf of Mexico coastal river system. *North American Journal of Fisheries Management* 10: 58–66.

Global Climate Change, Environmental Degradation, and Temperature Preference

Although the above strategies will, to some extent, ameliorate gradual increases in ambient water temperature due to climate change (**see also Temperature**: Effects of Climate Change) and other anthropomorphic effects (**see also Behavioral Responses to the Environment**: Anthropogenic Influences on Fish Behavior), disadvantages also accrue. In scenarios where preferred temperatures become limiting in a fish's natural environment, the usable habitat may also become restricted due to behavioral limitations. For example, fish may be forced to occupy small tributaries with cooler temperatures and be excluded from using or migrating through large mainstem rivers where temperatures can reach lethal heights or at least be many degrees above the fish's preferred temperature. As large numbers of fish are forced to aggregate into small areas, crowding can increase stress, parasitism, disease, as well as decrease food availability and perhaps the oxygen contained in the water. For species which are linked to a particular geographic area, environmental warming brings added hazards.

Migratory salmonids are a prime example, since nonthermal (largely olfactory) factors provide the primary cues that tie them to their natal streams. Adult salmon require relatively low body temperatures during migration to maintain energetic stores necessary for the arduous events required for swimming upstream and spawning. In many cases, migrants have already become dependent upon judicious behavioral thermoregulatory strategies in order to survive.

Chum salmon (*Oncorhynchus keta*), migrating along the coast in Japan, encounter high surface temperatures in early fall as they search for their spawning grounds. When the surface waters are warm, they achieve a compromise by mainly remaining in cool, deep water, but with occasional movements to the surface, presumably to obtain directional cues. Later in the year when the surface water is cooler, these migrating salmon spend most of the time in shallow water. There are also examples of sockeye salmon (*Oncorhynchus nerka*) being unable to complete their spawning migration because the river water temperature was simply too hot for them to swim.

Upstream migrating Chinook salmon (*Oncorhynchus tshawytscha*) also routinely encounter temperatures well above the optimal level. The importance of both behavioral thermoregulation and thermal refuges was highlighted by studies measuring deep body temperature as the salmon moved up the Columbia River. For all measurements obtained, body temperature was below the mean river temperature. The average measurement was 2.5 °C below mid-river temperature. This difference between the river temperature and fish body temperature extended down to river temperatures as low as 9 °C. This indicates that, during upstream migration, the preferred temperature of these large fish is likely below 9 °C.

Problems for juvenile salmon migrating into the ocean can also accrue due to ocean warming. As increased numbers of large and fast predatory fish migrate northward to attain their preferred temperature, the young salmon face the dual challenge of unduly warm temperatures and novel highly effective predators that they did not historically encounter.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Energy Utilization in Growth**: Growth: Environmental Effects. **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle. **Hypoxia**: The Expanding Hypoxic Environment. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish. **Temperature**: Effects of Temperature: An Introduction; Measures of Thermal Tolerance; Membranes and Temperature: Homeoviscous Adaptation. **The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin.

Further Reading

Berman CH and Quinn TP (1991) Behavioral thermoregulation and homing by spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. *Journal of Fish Biology* 39: 301–312.

Bicego KC, Barros RCH, and Branco LGS (2007) Physiology of temperature regulation: Comparative aspects. *Comparative Biochemistry and Physiology, Part A* 147: 616–639.

Coutant CC (1987) Thermal preference: When does an asset become a liability? *Environmental Biology of Fishes* 18: 161–172.

Crawshaw LI (1975) Attainment of the final thermal preferendum in brown bullheads acclimated to different temperatures. *Comparative Biochemistry and Physiology* 52A: 171–173.

Crawshaw LI (1976) Effect of rapid temperature change on mean body temperature and gill ventilation in carp. *American Journal of Physiology* 231: 837–841.

Crawshaw LI and O'Connor CS (1996) Behavioral compensation for long-term thermal change. In: Wood CM and McDonald DG (eds.) *Society for Experimental Biology Seminar Series 61: Global Warming: Implications for Freshwater and Marine Fish.* pp. 351–376. Cambridge, UK: Cambridge University Press.

Crawshaw LI, Wollmuth LP, O'Connor CS, Rausch RN, and Simpson L (1990) Body temperature regulation in vertebrates: Comparative aspects and neuronal elements. In: Schonbaum E and Lomax P (eds.) *Thermoregulation: Physiology and Biochemistry*, pp. 209–220. New York, NY: Pergamon Press, Inc.

Farrell AP (2009) Environment, antecedents and climate change: Lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology* 212: 3771–3780.

Farrell AP, Hinch SG, Cooke SJ, *et al.* (2008) Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology* 81: 697–708.

Hazel JR (1993) Thermal biology. In: Evans DH (ed.) *The Physiology of Fishes*, ch. 14, pp. 427–467. Boca Raton, FL: CRC Press.

High B, Peery CA, and Bennett DH (2006) temporary staging of Columbia River summer steelhead in coolwater areas and its effect on migration routes. *Transactions of the American Fisheries Society* 135: 519–528.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP (2006) Behavioral physiology of fish migrations: Salmon as a model approach. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish. Fish Physiology* 24. San Diego, CA: Elsevier Academic Press.

Imsland AK, Foss A, Folkvord A, Stefansson SO, and Jonassen TM (2005) The interrelation between temperature regimes and fish size in juvenile Atlantic cod (*Gadus morhua*): Effects on growth and feed conversion efficiency. *Fish Physiology and Biochemistry* 31: 347–361.

Jobling M (1996) Temperature and growth: modulation of growth rate via temperature change. In: Wood CM and McDonald DG (eds). *Society for Experimental Biology Seminar Series 61: Global Warming: Implications for Freshwater and Marine Fish* pp. 351–376. Cambridge, UK: Cambridge University Press.

Norris KS (1963) The functions of temperature in the ecology of the percoid fish *Girella nigricans* (Ayres). *Ecological Monographs* 33: 23–62.

Podrabsky JE, Clelen D, and Crawshaw LI (2008) Temperature preference and reproductive fitness of the annual killifish *Austrofundulus limnaeus* exposed to constant and fluctuating temperatures. *Journal of Comparative Physiology A* 194: 385–393.

Rausch RN, Crawshaw LI, and Wallace HL (2000) Effects of hypoxia, anoxia, and endogenous ethanol on thermoregulation in goldfish, *Carassius auratus*. *American Journal of Physiology. Regulatory, Integrative, and Comparative Physiology* 278: R545–R555.

Richards FP, Reynolds WW, and McCauley RW (1977) Temperature preference studies in environmental impact assessments: An overview with procedural recommendations. *Journal of the Fisheries Research Board of Canada* 34: 728–761.

Schurmann H and Christiansen JS (1994) Behavioral thermoregulation and swimming activity of two arctic teleosts (subfamily Gadinae) – the polar cod (*Boreogadus saida)* and the navaga (*Eleginus navaga)*. *Journal of Thermal Biology* 19: 207–212.

Tanaka H, Takagi Y, and Naito Y (2000) Behavioral thermoregulation of chum salmon during homing migration in coastal waters. *Journal of Experimental Biology* 203: 1825–1833.

Behavioral Responses to Hypoxia

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Overview
Altered Activity Patterns
Hypoxia as a Refuge
Surfacing Behaviors
Trade-Off with Predation Risk
Surfacing Behaviors and Parental Care
Terrestrial Behaviors
Future Studies
Further Reading

Glossary

Anoxia Complete absence of dissolved oxygen in water.

Aquatic surface respiration Behavioral response of ventilating gills using the surface layer of water, which tends to be fully or close to fully saturated with air even in hypoxic environments.

Buccal cavity Mouth or oral cavity.

Chemoreceptors Receptors that detect chemical stimuli and convert them into signals within the nervous system.

Estivation A state of dormancy usually to protect animals against heat and/or desiccation.

Friagem A weather phenomenon occurring in South America associated with rain, wind, and a sharp fall in temperature.

Hypoxia Reduced, below that of fully air-saturated, dissolved oxygen content in water.

Kilopascal SI unit for measuring pressure (pascal) often used as a unit of measurement for the partial pressure of oxygen in water.

Normoxia Water with fully air-saturated levels of dissolved oxygen.

Supra-terminal mouth Mouth that tends to open upward.

Overview

Hypoxia is a common phenomenon in many aquatic environments (**see also Hypoxia**: The Expanding Hypoxic Environment). Aquatic habitats can experience diurnal or seasonal fluctuations in oxygen availability and thus it is vital that the fish that inhabit these environments have biochemical, physiological (**see also Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations), and behavioral survival strategies.

There are a variety of ways in which fish behaviorally respond to hypoxia. Perhaps the easiest is simply to avoid it and to move away from areas of low oxygen. However, often fish do not have this option and, therefore, need strategies to survive in hypoxia. One immediate response can be to alter activity patterns in order to better balance the demand for energy with that available from aerobic respiration (**see also Gas Exchange**: Respiration: An Introduction). For example, reducing activity lowers energy demand perhaps more in line with the decreased availability of oxygen in the water. However, if this strategy does not provide sufficient savings, a fish may need to alter its physiology (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes) or behavior further in order to survive.

One of the most conspicuous and common behaviors is that some fish species move to the water surface to ventilate their gills using surface waters (aquatic surface respiration) or to breathe air in response to aquatic hypoxia. This behavior is a last resort for many species, especially since it additionally exposes them to aerial and terrestrial predators. Nevertheless, some fish species have evolved to inhabit environments that frequently experience hypoxia, and for them the journey to the water surface is a regular and controlled occurrence, allowing efficient acquisition of oxygen at the air/water boundary.

Altered Activity Patterns

Changes in activity patterns in response to hypoxia involve both increases and decreases in swimming activity. Benthic and demersal fish species are most likely to reduce swimming activity and minimize energy expenditure under hypoxic conditions; resting on the bottom and hiding from predators can effectively conserve energy. For example, the common sole, *Solea solea* will reduce its activity level under moderate hypoxia, as will the small spotted catshark, *Scyliorhinus canicula.* Reduced activity also may be accompanied by metabolic rate suppression (**see also Hypoxia**: Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia), a common physiological response to hypoxia.

Alternatively, some pelagic fish species may increase activity when they encounter hypoxia, presumably with the aim of escaping hypoxic areas as quickly as possible. For all species, the effects of hypoxia on activity patterns are likely to be context specific. When schools of Atlantic herring, *Clupea harengus*, are exposed to gradually falling oxygen tensions, they initially show an increase in swimming speed followed by a decrease in swimming speed if they are unable to escape hypoxia. This suggests that the herring first attempt to swim away from hypoxia but, if this behavioral response fails to locate normoxic waters, they are forced to reduce energy expenditure.

Hypoxia as a Refuge

Inevitably, fish of different shapes and sizes will differ in their ability to tolerate hypoxia and the mechanisms they employ for survival. Such differences in hypoxia tolerance allow some species to use hypoxia to their advantage, choosing to live in hypoxic environments and thereby minimize predation risk from hypoxia-intolerant species. An excellent example of this strategy came about with the introduction of the Nile perch, *Lates niloticus*, as a top predator in Lake Victoria. The Nile perch was such a successful and voracious predator that many native haplochromine fish species disappeared from the main lake. However, it was later discovered that several species, initially believed to have become locally extinct, were successfully using wetlands adjacent to Lake Victoria as a refuge from the Nile perch; the waters were too hypoxic for the Nile perch to tolerate.

Within a species, body size affects hypoxia tolerance, although the precise relationship varies greatly among species. In the oscar, *Astronotus ocellatus*, larger individuals appear to be able to tolerate hypoxia better than smaller individuals, but the reverse is true of the yellow perch, *Perca flavescens*. In species such as the yellow perch, where larger fish will cannibalize smaller individuals if the opportunity arises, the greater hypoxia tolerance of small fish may allow them to utilize hypoxic refuges (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food).

Surfacing Behaviors

Aquatic Surface Respiration

The surface layer of water tends to have the highest oxygen saturation within the water column due to diffusion from the air. Aquatic surface respiration (ASR) takes full advantage of this situation when the remainder of the water column is hypoxic. ASR is a behavior, which is performed by a variety of fish species, involving an individual journeying to the water surface to ventilate its gills with this relatively oxygen-rich surface water. The behavioral response itself is a reflex driven by oxygen-sensitive chemoreceptors that detect oxygen levels both in the blood and the external water. These chemoreceptors are believed to be located mainly in the gills (**see also Control of Respiration**: Oxygen Sensing in Fish).

Many intertidal species perform ASR. This behavior is necessitated because the tide pools they inhabit often become cut off from the sea at low tide, preventing an escape behavior. For tide pools near the low-tide mark, the duration of isolation may be infrequent and for only a short portion of the tidal cycle. However for tide pools near the high tide mark, the isolation may be much greater. Furthermore, there are diurnal considerations. When the tide goes out during the day, oxygen levels in the tide pool typically remain high (**Figure 1**(**a**)), because photosynthesis by algae continuously replenishes any oxygen removed from the water by animals. In fact, the water can easily become super-saturated with oxygen. However, photosynthesis ceases during the night, and all organisms within the tide pool, including the algae, continue to respire. Therefore, oxygen levels fall overnight (**Figure 1**(**b**)), and can reach almost anoxic (no oxygen) conditions in a crowded tide pool (**Figure 1**(**c**)). Consequently, many intertidal fish that have become stranded in tide pools start to perform ASR during the night (**Video Clip 1**). A good example of such a fish is the tidepool sculpin, *Oligocottus maculosus*, which inhabits rocky intertidal shores on the Pacific coast of North America.

Of the variety of intertidal fish that perform ASR, many have head morphologies that appear specially adapted to optimize access to surface water and minimize head exposure above the water. Supra-terminal mouths and dorsoventrally flattened heads enable these fish to get right up to the water surface to ventilate their gills without their heads breaking the water surface. Breaking the water surface would create a downward force, necessitating fish to swim harder to maintain position at the surface, and would also act as a clear signal for predators.

Fish species that perform ASR during hypoxia are not limited to tide pools. In fact, the Amazonian tambaqui, *Colossoma macropomum*, is an example of a fish that not only performs ASR in response to hypoxia but undergoes a morphological transformation to enhance the efficiency of ASR. When the tambaqui encounters hypoxia, its lower lip becomes highly vascularized and is remodeled into a flat skin within just 2 h; this rapid morphological transformation greatly enhances the ability of tambaqui to skim the oxygen-rich surface layers of water (**Figure 2**). Another unusual way of increasing ASR efficiency is seen in the upside-down catfish, *Syndontis nigriventris*, which as the name suggests

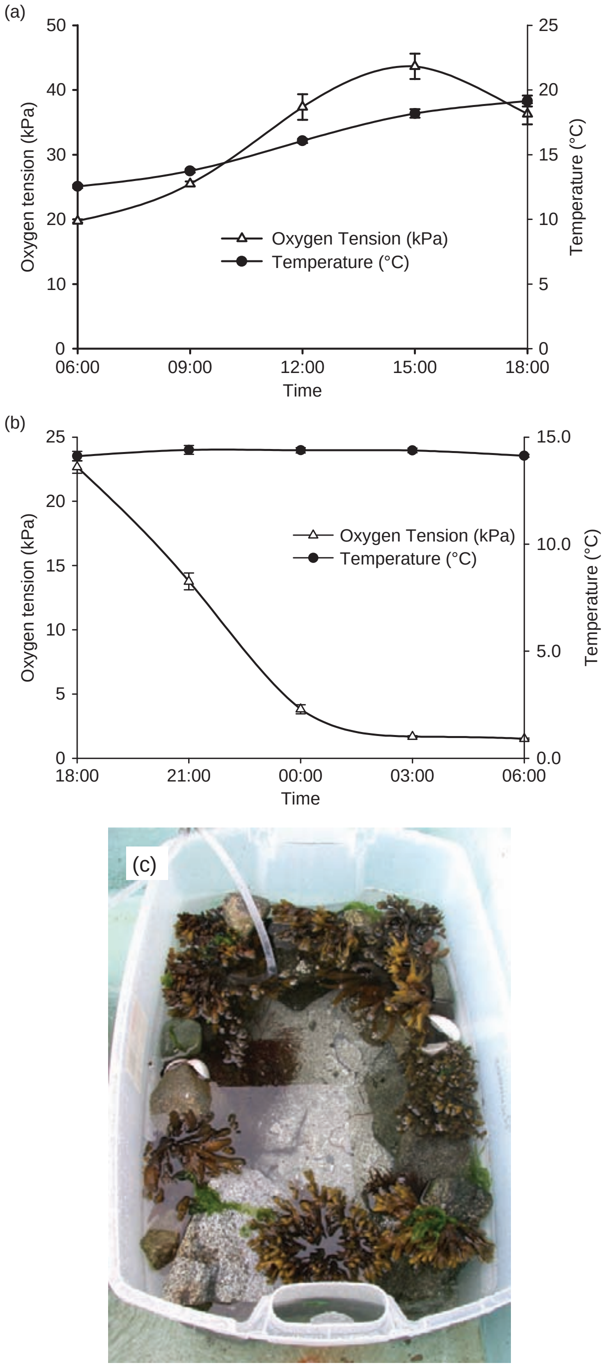


Figure 1 (a) Oxygen and temperature profiles of mesocosms (illustrated in (c)) isolated from water flow for 12 h during the day and (b) overnight. Measurements of physical parameters were taken every 3 h ($n = 16$ for each time point). Data are presented as means ± SEM. (a, b) Reproduced from Sloman KA, Mandic MM, Todgham AE, *et al*. (2008) The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments. *Comparative Biochemistry and Physiology A* 149: 284–292, with permission from Elsevier.

improves ASR by swimming upside down. During ASR, *S. nigriventris* is able to keep its body fully submerged and reduce surface activity levels by adopting this upside-down posture.

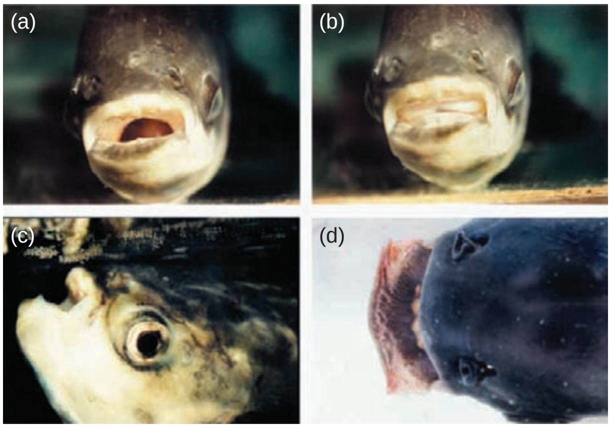


Figure 2 Morphological changes in the lips of the tambaqui, *Colossoma macropomum*, occurring during exposure to hypoxia. (a) A frontal view with the mouth open during lip expansion. (b) Shows a frontal view with the mouth closed. (c) A lateral view of the head approaching the water surface with the initial swelling of the lower lip. (d) A dorsal view of the fish illustrating the forward expansion of the lip near full development. Reproduced from Sundin L, Reid SG, Rantin FT, and Milson WK (2000) Branchial receptors and cardiorespiratory reflexes in a neotropical fish, the tambaqui (*Colossoma macropomum*). *Journal of Experimental Biology* 203: 1225–1239, with permission from the Company of Biologists.

There are costs to ASR. For example, for mid-water species of fish that would normally spend the majority of their time free-swimming, the additional energetic burden of maintaining buoyancy and their position at the water surface during ASR may not be that great. However, for benthic species, which are often negatively buoyant, maintaining position at the top of the water column can be energetically expensive even if the water is shallow. Consequently, many intertidal fish perform ASR on rocks or structures that provide support at the water surface. An alternative, or additional aid, to this strategy is seen in intertidal gobies such as *Cryptocentroides cristatus* and *Mugilogobius paludis*. These fish hold a gas bubble in their mouths while performing ASR, providing neutral or positive buoyancy to the head and allowing their mouth to easily rest at the water surface. The bubble could additionally provide oxygenation of water passing through the mouth and over the gills.

Emergence Behaviors

ASR enables fish to utilize the oxygen-rich upper layers of the water column when the main water body becomes hypoxic. However, if aquatic hypoxia persists, oxygen levels may fall so low that ASR is no longer sufficient. At this point, many fish species are able to extract oxygen from the air (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). In the majority of marine air-breathing fish, this process involves part or total emergence from the water (**Figure 3**) and the utilization of skin, gills, and buccal cavities as gas-exchange surfaces.

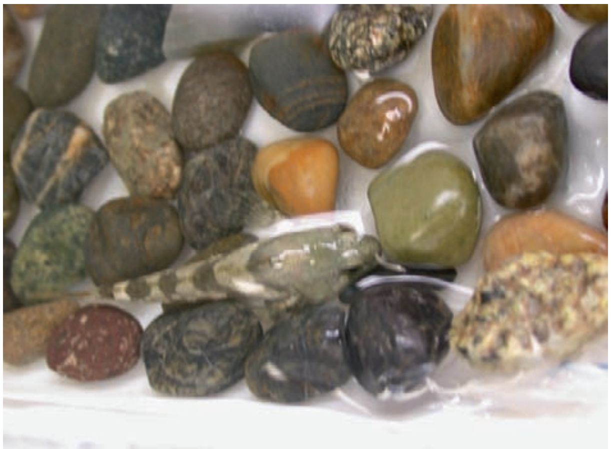


Figure 3 A tidepool sculpin, *Oligocottus maculosus*, emerging from the water during aquatic hypoxia.

In freshwater air-breathing fish, air is usually taken in at the water surface using surfacing behaviors similar to ASR and oxygen is extracted across air-breathing organs (ABOs) including areas of the intestine, modified gas/swimbladders, and lungs (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). Air breathing is typically a facultative process allowing fish to supplement their oxygen requirement when levels of dissolved oxygen fall. However, for some fish like the piraracu, *Arapaima gigas*, air breathing, and therefore surfacing behavior, is an obligate process; these fish will not survive when denied access to the water surface (**see also Behavioral Responses to the Environment**: Anthropogenic Influences on Fish Behavior).

Trade-Off with Predation Risk

Fish that make frequent journeys to the water surface or that are continuously breathing at the surface face a trade-off between the need to obtain oxygen and the increased risk of predation that may be associated with surfacing. Being at the water surface may make individuals easier prey items for aerial and terrestrial predators and any other larger aquatic predators that have also migrated to the water surface. To address this trade-off, fish have evolved a variety of behavioral and physiological strategies to allow them to reach the water surface but minimize their chances of being eaten (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food).

Use of Cover

If a fish needs to come to the water surface for ASR or to breathe air, then one way to minimize predation risk is to surface under shelter. This is clearly seen in intertidal fish such as the tidepool sculpin that will surface around the edges of tide pools and, when emerging from the water, is often found hiding under algae; algal cover has the additional advantage of reducing desiccation while the sculpin is exposed to air. The flathead grey mullet, *Mugil cephalus*, threatened by avian predators, will modify ASR behavior to surface under shelter or near the edges of aquaria; when the water is turbid, they may be unable to visually detect predators and will respond in a similar manner. For smaller juvenile fish, access to shelter may be particularly important. Juvenile oscar, *Astronotus ocellatus*, given a choice between staying in a hypoxic environment that had overhead floating plant shelter and a normoxic environment with no shelter, chose to remain in hypoxia for as long as they were physiologically able, rather than give up the protective overhead cover.

Postponement of Surfacing Behaviors

Some fish may choose to remain deeper in the water column in hypoxic waters rather than coming to the water surface when predation risk is perceived to be high. Therefore, avoiding predation may be associated with a physiological cost of remaining in hypoxia (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes). In the tidepool sculpin, adults will start to perform ASR when the oxygen tension in the water falls to about 2.5 kPa. However, if an alarm substance, a chemical released from the skin of some fish species when they are injured (**see also Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food), is added to the water, sculpins will delay the performance of ASR until oxygen tensions fall to about 1.5 kPa. As mentioned above, tidepool sculpins display ASR until oxygen tensions fall too low, at which point they emerge from the water. Although these sculpins appear to be able to delay the performance of ASR in response to predation, they do not appear to be able to delay emergence behavior, which occurs as a last resort response to hypoxia. Once oxygen tensions fall too low, the requirement for oxygen is too great and the fish must accept the increased predation risk and emerge from the water in order to stay alive.

Group Surfacing Behaviors

An alternative to surfacing under shelter or delaying surfacing behaviors is to surface in social groups. In a similar manner to the dilution effect associated with fish shoaling where an individual in a large group effectively minimizes its chances of being eaten, surfacing in social groups provides safety in numbers. Many catfish perform these synchronized surfacing behaviors, including *Hoplosternum littorale*. *Hoplosternum* is a facultative air

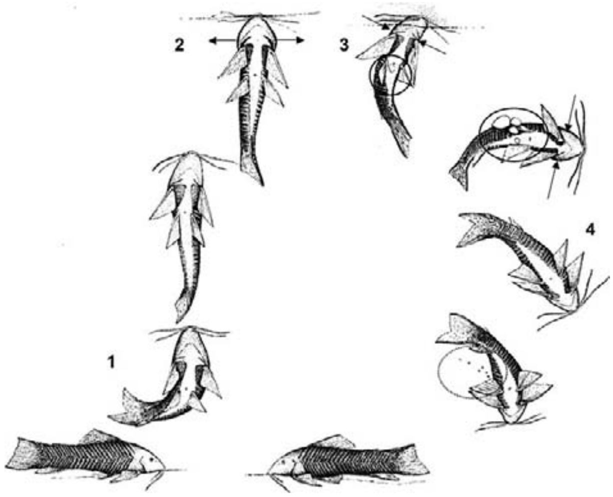


Figure 4 Air-breathing cycle of *Hoplosternum littorale*: (1) ascent to the water surface; (2) in contact with the surface the fish expands its oral cavity, inspiring air; (3) turning head down, with compression of the oral cavity pushing air into the intestine and causing the expiration of old air from the anus (circle, continuous line); (4) return to bottom and release of small air bubbles through the opercula (circle, dotted line). Arrows indicate the buccal pump. Reproduced from Jucá-Chagas R and Boccardo L (2006) The air-breathing cycle of *Hoplosternum littorale* (Hancock, 1828) (Siluriformes: Callichthyidae). *Neotropical Ichthyology* 4: 371–373 with permission from Sociedade Brasileira de Ictiologia.

breather that inhabits the Amazon basin and can supplement aquatic oxygen with oxygen from the air when necessary. Unlike many other teleosts of the Amazon, it can survive a seasonal phenomenon known as a ‘friagem’ where water becomes hypoxic, acidic, and rich in hydrogen sulfide. *Hoplosternum* utilizes its posterior intestine as an ABO and gulps in air at the surface, which is then passed into this highly vascularized area of its digestive tract. As the fish take new air into their intestines at the water surface, old oxygen-depleted air is then released through the anus and as the fish descends away from the water surface, any excess air is also expelled through the operculum (**Figure 4**). Oxygen can then be extracted from the air in the intestine; this air can also be used for buoyancy and to aid passage of food through the digestive tract.

When *H. littorale* are held individually in tanks and exposed to hypoxia, smaller individuals will generally travel to the water surface earlier, whereas larger individuals will postpone air breathing until oxygen tensions have fallen further. This would suggest that smaller individuals are physiologically unable to remain in hypoxic waters for as long as larger individuals and, therefore, benefit from the synchronous air breathing performed by these fish when held in groups. Within a group of *Hoplosternum*, the most active fish tend to surface first which may be related to the higher oxygen consumption associated with activity. Once one fish has moved toward the surface, all members of the group generally follow closely behind.

Surfacing Behaviors and Parental Care

A wide variety of fish species exhibit some form of parental care, which can range from guarding of externally developing eggs to the gestation of live young (**see also Social and Reproductive Behaviors**: Parental Care in Fishes and **Social and Reproductive Behaviors**: Nutritional Provision During Parental Care). Parental care is energetically expensive. For example, parents may provide energy to their offspring by supplying them with nutritional secretions or they may actively defend offspring against predators. For fish inhabiting hypoxic waters, this extra energy demand can be a potential problem.

Gestating female live-bearing fish need to supplement oxygen supply by journeying to the air–water interface. The sailfin molly, *Poecilia latipinni*, is a small live-bearing species found from South Carolina south and west through Florida and the Gulf Coast into Mexico. Its natural environment has the potential to become hypoxic and it is known to perform ASR. Gestating females have a higher metabolic rate compared to nongestating females and as a consequence, when experiencing low levels of dissolved oxygen, nongestating females spend approximately 15% of their time performing ASR as compared to gestating females spending 50% of their time performing ASR.

Mouth-brooding fish maintain embryos and young fry in their mouths for protection during development. For example, in the mouth-brooding African cichlid, *Pseudocrenilabrus multicolour victoriae*, brooding females will start to perform ASR at higher oxygen concentrations compared with nonbrooding females. There are several potential reasons for this. First, there is the increased energy demand associated with the transportation of young. In addition, young have to be provided with a supply of highly oxygenated water or air without being accidentally swallowed; most mouth brooders do not actually eat while they are incubating young. Lastly, holding developing broods within the mouth cavity has the potential to interfere with the pumping of water across the gills and to compromise both normal ventilation and ASR.

Several air-breathing fish species that inhabit oxygen-deplete areas care for their offspring by building floating bubble nests for their eggs that lift the eggs out of oxygen-poor water into the oxygen-rich surface water without creating desiccation. In *H. littorale*, this is achieved by producing a foam-like substance by swimming upside down at the water surface, pumping water over their gills and using their pelvic fins to mix water with mucus. Many species of gourami, for example, the flame-red dwarf gourami, *Colisa lalia*, also build floating bubble nests (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish).

Terrestrial Behaviors

While many intertidal fish species emerge from the water during severe hypoxia, they typically remain inactive during emergence and seek cover to reduce risk of predation and desiccation. However, a few intertidal fish have evolved a more amphibious lifestyle; they emerge fully from the water and locomote great distances on mud flats at low tide. Mudskippers locomote on land using their pectoral fins and are probably the most well known of amphibious fish, with the genus *Periophthalmus* being the most widely distributed. These fish can utilize their skin and the lining of their mouth cavity to extract oxygen from the air (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction).

Many mudskipper species inhabit burrows. For example, *Scartelaos histophorus* builds J-shaped intertidal burrows where they store air in an upturned area that is not connected to the water surface. To do this, they take air in their mouths from the external environment at low tide and transport it into their burrows. The air pocket within the burrow allows the mudskipper to breathe air if it depletes its aquatic oxygen supplies during the period that it is confined in its burrow at high tide. Once the oxygen in the air has been depleted during a high tide cycle, it needs to be replaced at the next available opportunity. Renewal of the air store occurs in a concentrated period of time, involving 6–15 air-deposition trips per minute. In complete contrast to the amphibious mudskippers that are able to move around easily in a terrestrial environment, some lungfish species can enter a period of estivation during dry periods when they have no access to water. There are three genera of lungfish, *Neoceratodus*, *Lepidosiren* and *Protopterus*, all of which journey to the water surface to breathe air when there is water to inhabit. *L. paradoxa* and the four species of *Protopterus* are obligate air-breathers and cannot survive without access to air, whereas *Neoceratodus* is a facultative air breather. *Protopterus* spp. are known to estivate during dry periods where they enter a period of dormancy or inactivity and build a cocoon around themselves until the dry season is over. Some species such as *P. aethiopicus* and *P. annectens* estivate in mud cocoons underground, whereas *P. dolloi* can estivate on land within a layer of dried mucus (**Figure 5**). During estivation, metabolic rate is depressed and the lungfish rely entirely on their lung to obtain oxygen from the air (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction).

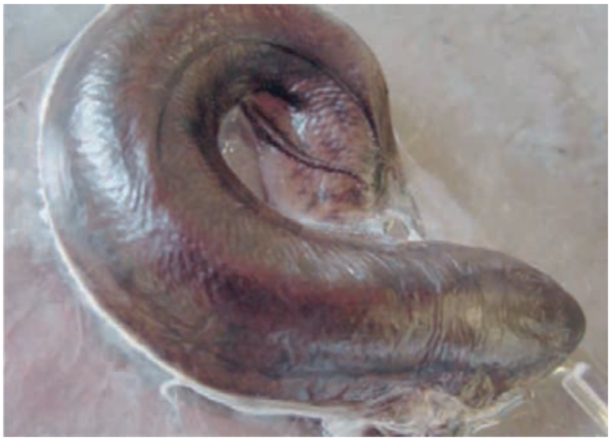


Figure 5 *Protopterus dolloi* estivating in a dried mucus cocoon on land in the laboratory. Reproduced from Chew SF, Chan NKY, Loong AM, *et al*. (2004) Nitrogen metabolism in the African lungfish (*Protopterus dolloi*) aestivating in a mucus cocoon on land. *Journal of Experimental Biology* 207: 777–786, with permission from the Company of Biologists.

Future Studies

The physiological and biochemical responses of fish to hypoxia have been well researched (**see also Hypoxia**: Anaerobic Metabolism in Fish, Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia, Respiratory Responses to Hypoxia in Fishes, **Bony Fishes**: Crucian Carp, and The Expanding Hypoxic Environment). Less research has focussed on the behaviors that allow fish to behaviorally avoid the metabolic consequences of hypoxia by avoiding or by supplementing their oxygen supply from the air or upper surface waters. Many studies on these behavioral responses have been laboratory based where complex interactions with predation risk and social structures are removed. There is now a need to consider these behaviors in ecologically relevant contexts and to understand how the ways in which fish can utilize behavior in response to hypoxia interact with other essential behaviors.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Behavioral Responses to the Environment**: A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food; Anthropogenic Influences on Fish Behavior. **Bony Fishes**: Crucian Carp. **Control of Respiration**: Oxygen Sensing in Fish. **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: Anaerobic Metabolism in Fish; Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia; Respiratory Responses to Hypoxia in Fishes. The Expanding Hypoxic Environment. **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations. **Social and Reproductive Behaviors**: Nutritional Provision During Parental Care; Parental Care in Fishes; Sexual Behavior in Fish.

Further Reading

Abrahams M (2006) The physiology of antipredator behaviour: What you do with what you've got. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, 1st edn., vol. 24, pp. 79–108. San Diego, CA: Elsevier.

Andrade DV and Abe AS (1997) Foam nest production in the armoured catfish. *Journal of Fish Biology* 50: 665–667.

Chapman LJ, Kaufman L, and Chapman CA (1994) Why swim upside down? A comparative study of two mochokid catfishes. *Copeia* 1994: 130–135.

Chapman LJ and McKenzie DJ (2009) Behavioural responses and ecological consequences. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Hypoxia*, pp. 26–77. San Diego, CA: Elsevier.

Chew SF, Chan NKY, Loong AM, *et al.* (2004) Nitrogen metabolism in the African lungfish (*Protopterus dolloi*) aestivating in a mucus cocoon on land. *Journal of Experimental Biology* 207: 777–786.

Domenici P, Steffensen JF, and Batty RS (2000) The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. *Journal of Fish Biology* 57: 1526–1538.

Gee JH and Gee PA (1995) Aquatic surface respiration, buoyancy control and the evolution of air-breathing in gobies (Gobiidae: Pisces). *Journal of Experimental Biology* 198: 79–89.

Jucă-Chagas R and Boccardo L (2006) The air-breathing cycle of *Hoplosternum littorale* (Hancock, 1828) (Siluriformes: Callichthyidae). *Neotropical Ichthyology* 4: 371–373.

Lee HJ, Martinez CA, Hertzberg KJ, Hamilton AL, and Graham JB (2005) Burrow air phase maintenance and respiration by the mudskipper *Scartelaos histophorus* (Gobiidae: Oxudercinae). *Journal of Experimental Biology* 208: 169–177.

Perry SF, Gilmour KM, Swenson ER, *et al.* (2005) An investigation of the role of carbonic anhydrase in aquatic and aerial gas transfer in the African lungfish *Protopterus dolloi. Journal of Experimental Biology* 208: 3805–3815.

Robb T and Abrahams MV (2003) Variation in tolerance to hypoxia in a predator and prey species: An ecological advantage to being small? *Journal of Fish Biology* 62: 1067–1081.

Shingles A, McKenzie DJ, Claireaux G, and Domenici P (2005) Reflex cardioventilatory responses to hypoxia in the flathead gray mullet (*Mugil cephalus)* and their behavioural modulation by perceived threat of predation and water turbidity. *Physiological and Biochemical Zoology* 78: 744–755.

Sloman KA, Mandic MM, Todgham AE, *et al.* (2008) The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments. *Comparative Biochemistry and Physiology A* 149: 284–292.

Sloman KA, Sloman RD, De Boeck G, *et al.* (2009) The role of size in synchronous air-breathing of *Hoplosternum littorale*. *Physiology and Biochemical Zoology* 82: 625–634.

Sundin L, Reid SG, Rantin FT, and Milson WK (2000) Branchial receptors and cardiorespiratory reflexes in a neotropical fish, the tambaqui (*Colossoma macropomum*). *Journal of Experimental Biology* 203: 1225–1239.

Timmerman CM and Chapman LJ (2003) The effect of gestational state on oxygen consumption and response to hypoxia in the sailfin molly, *Poecilia latipinna. Environmental Biology of Fishes* 68: 293–299.

Wen-Chi Corrie L, Chapman LJ, and Reardon E (2008) Brood protection at a cost: Mouthbrooding under hypoxia in an African cichlid. *Environmental Biology of Fishes* 82: 41–49.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Sleep in Fish

IV Zhdanova, Boston University School of Medicine, Boston, MA, USA

© 2011 Elsevier Inc. All rights reserved.

What Is Sleep?
Sleeping Fish
Further Reading

Glossary

Chronotype Individual pattern of circadian phase preference, defining preferred time of day for certain activities, such as sleep or work: early-morning chronotype in humans reflects preference for early-morning activity.

Circadian Internally generated rhythm with a periodicity of about 24 h. From the Latin *circa* (about) and *dies* (day).

Crepuscular Daily pattern with two peaks of activity centered around dusk and dawn, although not excluding some activity in-between these periods. From the Latin *crepusculum* (twilight).

Histamine A biological amine, with important role in multiple physiological functions (e.g., immune reactions and smooth muscle regulation), also serving as a neurotransmitter.

Hypocretin (or orexin) Excitatory peptides produced in the hypothalamus.

Isodendritic core A diffuse but morphologically similar group of cells in the brainstem. From the Latin *isos* (similar, uniform).

Melatonin A biological amine, hormone produced by the pineal gland (epiphysis cerebri).

Narcolepsy A chronic sleep disorder characterized by excessive daytime sleepiness, sudden attacks of muscle weakness, and unusual hallucination-like experiences at the onset of sleep (hypnagogic hallucinations). Most cases result from hypocretin deficiency.

Suprachiasmatic nucleus Small paired nuclei of the hypothalamus, serving a master clock function in mammals.

Zeitgeber A time cue sufficient to entrain circadian rhythms. From the German for time giver.

> Now, blessings light on him that first invented sleep! It covers a man all over, thoughts and all, like a cloak; it is meat for the hungry, drink for the thirsty, heat for the cold, and cold for the hot. It is the current coin that purchases all the pleasures of the world cheap, and the balance that sets the king and the shepherd, the fool and the wise man, even.
>
> Miguel de Cervantes, Don Quixote, 1605.

What Is Sleep?

None of us would doubt that sleep is an essential need. Lack of it makes us tired and irritable, clouding our optimism and increasing anxiety. Without a proper amount of sleep every day, we lose mental sharpness, as though our brain were working at half speed. Considering that, on average, we spend about one-third of our life in sleep, the question of what is happening with us during this time and why we need sleep has bothered scientists and laymen alike for centuries. Many ideas, hypotheses, and theories have been put forward to explain the ubiquitous phenomenon of sleep. In spite of this, as of now, no hypothesis has convincingly explained the principal physiological function of sleep. Perhaps you, our young and curious readers, will be the ones to explain the purpose of sleep.

Although the function of sleep remains an enigma, the in-depth scientific research into the problem of sleep that started in the nineteenth century and flourished in the twentieth, taught us a great deal about the sleep process and what happens with different physiological systems during sleep. Before we approach our main topic of sleep in fish, let us go over the principal facts about sleep in other species, including humans. This might help to explain why we are interested in the sleep of these cold-blooded and water-bound vertebrates from both a basic science point of view and in the selfish hope that understanding sleep in fish will help us to clarify the enigma of the human sleep process.

How do we know that somebody sleeps? First of all, their behavior is changed. A sleeping human or animal is behaviorally quiet, but unlike during quiet wakefulness, is not easily aroused by mild environmental stimuli. That said, sleep, unlike coma or hibernation, is rapidly reversed into wakefulness by substantially strong stimuli. Other notable behavioral characteristics of sleep are muscle relaxation and, often, a typical sleep posture. Look at an old gentleman sleeping in an arm chair, in front of a still-working television set, with his head sunk on his chest and his relaxed hand letting the remote control fall onto the rug. Watch your sleeping cat, lying curled up, with his head between his paws. Think of yourself getting comfortable in your bed before falling asleep at night.

There is another small sign of sleep that you might have thought of if you ever tried to pretend to be sleeping. It is lack of eye movement and blinking. However, sleep is not a homogenous state and, during one of its distinct stages, the eyes actively move. For this reason, this sleep stage is called rapid eye movement (REM) sleep. It was the last sleep stage to be discovered but it is so remarkable that the rest of sleep stages, combined, are now called non-REM sleep. They are numbered from 1 to 4, with stage 1 being a transition from wakefulness to sleep, and 4 being the deepest non-REM sleep stage.

You might wonder how we know which stage of sleep a person is in. This brings us to another way of studying sleep, one based on the recording of brain activity. In 1875, the British neurophysiologist Richard Caton recorded brain electrical activity using electrodes implanted directly in the animal brain; and in the 1920s, a German psychiatrist Hans Berger recorded human brain waves through electrodes attached to the scalp and reported that they are distinctly different depending on whether a person is awake or asleep. During wakefulness, the brain waves are fast (i.e., have high frequency) and have low amplitude. This is because the millions of neurons of our cerebral cortex that produce these waves fire rapidly and at different times. In other words, their activity is high but desynchronized. When we start falling asleep, these neurons slow down their activity but, more importantly, they synchronize with other neighboring neurons and, as a result, the recorded brain waves become slow (i.e., of low frequency) and their amplitude increases substantially. During stage 4 sleep, brain waves have the lowest frequency and the highest amplitude.

What is happening with us during these sleep stages? With the onset of non-REM sleep, starting with stage 1, our eyes close, and either stay still or have occasional slow movements. We start losing our regular perception of the environment, our muscles relax, our breathing and heart rate gradually slow down, and we progress into deeper stages of quiet sleep. Occasionally, we might move our limbs in sleep or turn to the other side but, overall, our body and brain are in a relaxed state. However, after about an hour or an hour and a half, an amazing change happens with both our body and brain activity. The body virtually loses all muscle tone, while the brain starts working almost as actively as when we are awake. Not only do our brain waves become fast and desynchronized, but also our still closed eyes start moving rapidly, as if we have opened them and are watching something. This remarkable state is called REM sleep, the time when we watch dreams, while our body remains virtually paralyzed.

Another interesting fact about sleep is that, throughout the night, the periods of non-REM and REM sleep alternate. Hence, every night we have four to six sleep cycles, each composed of a period of non-REM and REM sleep. Non-REM periods are longer and deeper at the beginning of the night, during the first two to three sleep cycles, while REM sleep is more prevalent in the early-morning hours. Although it is more difficult to wake up somebody from REM than from non-REM sleep, spontaneous awakening in the morning happens more often in REM. That is why, in the morning, you tend to remember that last dream that you saw in REM sleep right before your awakening.

Other important questions that researchers are working on include what makes us feel sleepy and for how long do we need to sleep. Depending on which time of day a species is active, animals are divided into those with diurnal and those with nocturnal chronotypes. For example, humans are typically alert at daytime, in contrast to mice, which are active at night. There are also species with a so-called crepuscular chronotype, such as cats, which have several, approximately similar sleep episodes during the day and at night. In both nocturnal and diurnal species, the timing of sleep is controlled by the circadian clock. Once this intrinsic biological clock strikes the habitual time of sleep, an individual becomes sleepy.

What is this biological clock and where does it reside? Every cell of our body has such a clock, which consists of a complex molecular mechanism of periodic gene transcription and protein synthesis. The period of activity of these numerous clocks is about 24 h; hence, they are called 'circadian' (from the Latin *circa* – around and *dies* – a day; **see also Sensory Systems, Perception, and Learning:** Circadian Rhythms in Fish). However, each of these clocks runs at a slightly different speed. Even though the difference might be only a few minutes per day, over weeks and months this would lead to complete desynchronization between the cells. Thus, as any orchestra needs a conductor, an organism needs a master circadian clock that can synchronize these individual cellular clocks.

In mammals, including humans, the master clock is located in a small paired brain structure, the suprachiasmatic nucleus (SCN) of the hypothalamus. The neurons of this structure can have their own distinct rhythms as well, but they are very cooperative in nature and, under normal circumstances, manage to effectively synchronize their rhythmicity within the SCN. As a result, this well-coordinated chorus of SCN cells, loudly singing the same time signal through their neuronal and hormonal outputs, dominates all other mini-clocks in the body, synchronizing them within the entire organism.

Every evening, the circadian clock signals to our organism that it is time to fall asleep and every morning helps it to wake up on time. How it does this, however, is not entirely clear. Such signals could be conveyed via the clock mechanisms within the brain structures responsible for sleep regulation, leading to their activation or inhibition at certain hours of the day. The principal hormone of the circadian system, melatonin, can also promote sleep. It is produced by the pineal gland and the retina of the eyes every night and, when taken as a drug, can promote sleep initiation. If light hits the eye in the middle of the night, melatonin production is acutely inhibited. This is one of the reasons why light is such a powerful zeitgeber (from the German for time giver).

Although the timing of the habitual sleep period, controlled by the circadian clock system, is very important, there is another critical mechanism of sleep regulation, the homeostatic one, which makes sure that we spend enough time sleeping every day. Under the pressure of some important task, we can overcome this need for sleep, but later, we have to compensate for sleep loss by sleeping more than usual. Perhaps, if we knew what exactly this homeostatic mechanism is balancing out, we would know the mysterious physiological function of sleep, why we fall asleep, and how we wake up after the sleep function is fulfilled.

Humans tend to sleep 7–8 h a day. Other mammals can have substantially different daily sleep durations. For example, elephants sleep only 3–4 h, while opossums sleep an astounding 20 h per day. The reasons for such interspecies differences in sleep duration remain unknown. Studying them might help to solve the enigma of sleep function but would also, most certainly, uncover new adaptive strategies that involve sleep physiology. Perhaps one of the most remarkable strategies was discovered by Lev Mukhametov and his colleagues when they studied sleep in dolphins. These marine mammals, which need to get to the water surface in order to breathe, have unihemispheric sleep. When one of their brain hemispheres is sleeping, the other one is awake, helping the animal to maintain its posture and stay close to the surface. Perhaps, for the same reason, these animals lack REM sleep, at least in the form that other mammals have. Later, a similar sleep strategy was found in other marine mammals, for example, seals, while they are at sea. Remarkably, when seals sleep on land, they can have normal bilateral sleep, that is, both brain hemispheres sleep simultaneously.

Sleeping Fish

Unlike marine mammals, fish do not need to get to the water surface to breath. With the exception of some fish, such as sharks, that have to move constantly so that their gills can be exposed to enough water and thus oxygen, most of the fish species show typical behavioral features of sleep. They have prolonged behavioral quietness, species-specific sleep postures, elevated arousal threshold, and rapid reversibility from quietness to activity in response to moderately intense stimulation. Since fish lack eyelids, their eyes stay always open but eye movements are largely inhibited during sleep.

The behavioral adaptations of fish species during sleep are as diverse as their daytime activity and reflect their specific adaptive ways of foraging or hiding from predators. Some fish sleep predominantly at night (e.g., herring, *Clupea* spp.; tuna, *Thunnus* spp.; spotties, *Pseudolabrus celidotus*), while others (e.g., bass or perch, family percichthyidae) rest mostly during the day or have many daytime naps. The environmental light–dark cycle powerfully affects sleep physiology in fish. They also have pronounced intrinsic periodicity, modulating their behavior and physiology. It remains to be seen, however, whether the SCN plays the master clock role in fish, as it does in mammals. So far, it appears that other clock structures, such as the pineal gland and the retina of the eye, can be sufficient to orchestrate the circadian system in teleost fish and to entrain their daily sleep–wake cycles. Moreover, in some fish, especially those that are semi-transparent, light sensitivity is not limited to the retina and pineal gland but is present in the skin and even deeper structures, for example, in the heart. These tissues thus can entrain to the light–dark cycle directly.

The sleep postures in fish vary from floating in a horizontal or vertical position, head up or down, to lying on their side or belly. The level of inactivity during sleep also widely varies, with some fish completely ceasing their locomotion, and others only slowing it down. Moreover, the increase in arousal threshold and disengagement from environmental stimuli associated with the sleep state can be so substantial that blueheads, *Thalassoma bifasciatum*, Spanish hogfish, *Bodianus rufus*, and several species of wrasses, family Labridae, can be lifted by hand to the surface at night before waking up.

The typical daytime hiding places also serve as bedrooms for some fish. Those that hide in the mud or sand of the water bed while hunting or escaping from predators, such as sole, use the same adaptive behavior during their sleep period. This provides camouflage that is critically important for the state of reduced alertness that prevents adaptive escape behavior when danger threatens. The coral fish, for example, damsels or clownfish, family Pomacentridae, typically active during the day, rest within the reef structure at night, protected by its crevices. Freshwater fish often hide under a rock or log at night. Some fish species, however, design elaborate, truly remarkable hiding strategies. For example, parrotfish family Scaridae, can envelop themselves in a protective sleeping bag of secreted mucus, which misguides predators, by hiding a clever sleeper.

Many fish species are highly social, forming large schools or shoals, synchronizing complex locomotor activities in the middle of the ocean or freshwater stream. These species can rest together as well, forming a slowly moving or drifting colony. In such cases, individual guards, floating on the periphery of the colony, can warn the others of impending danger. Other schooling fish, however, develop individual sleep strategies. They prefer to spread throughout rather large territories, sleeping far from each other, hidden by rocks or floating in seaweed. How do they know that it is the right time to wake up and join the rest of their shoal? So far, nobody really knows the answer to this question.

Over the years, sleep studies have been conducted in a variety of fish. However, lately, special attention has been paid to sleep in zebrafish, *Danio rerio*, largely due to the extensive knowledge of zebrafish genetics and development that has been accumulated over the years through the effort of many research groups (**see also Bony Fishes:** Zebrafish).

Research into sleep in zebrafish has been particularly explored from a biomedical perspective. Because of this, when considering the zebrafish as a model for studying sleep, the first steps had to address the extent to which those processes that are known to affect human sleep can also modulate the sleep state in zebrafish. Like humans, they have a distinct diurnal sleep–wake cycle, demonstrating a high arousal threshold and reduced breathing rate during the sleep state. While sleeping, zebrafish slow down their activity, sometimes sluggishly moving around the fish tank, often staying suspended in the water (**Video Clips 1(a) and 1(b)**). Interestingly, when zebrafish are very young (and called larvae), they tend to sleep with their head down. Adult zebrafish, on the contrary, tend to sleep with their head slightly upward. This might reflect some age-dependent regulation of the swimbladder sphincter or other mechanisms of posture control that change during maturation.

Although the circadian clock system synchronizes numerous physiological processes equally well in both nocturnal and diurnal organisms, its most core gears are virtually identical in both types of animals: the master clock, the SCN, has high electrical and metabolic activity during the day, the core clock genes are expressed at similar times, and the principal hormone of the circadian system, melatonin, is secreted only at night. Thus, the rest of the organism should know how to read the clock and keep a specific adaptive schedule of activity and physiological functions in order to maintain its adaptive diurnal or nocturnal lifestyle. We have yet to discover where this important interpreter resides, allowing for a diurnal organism to translate a specific clock time into action, while a nocturnal organism reads it as a command to fall asleep. Thus, so far, studying circadian regulation of sleep in a diurnal vertebrate, such as zebrafish, has clear advantages for biomedical research.

Once it was documented that zebrafish sleep has a circadian pattern, it was important to determine whether melatonin, which is produced by the pineal gland and retina exclusively at night, can modulate sleep in zebrafish. This is because melatonin, being the principal neurohumoral output of the clock, is also known to have acute sleep-promoting effects in humans and diurnal nonhuman primates, whether administered at daytime or at night, thus directly linking the circadian system and sleep. Indeed, administered at daytime, tiny amounts of melatonin that increase melatonin levels in fish brain to approximately those that it normally has at night, make zebrafish sleepy. After such treatment, both larval and adult zebrafish start moving slowly and their arousal threshold increases to the level typical of the nighttime sleep period. However, if there is an urgent need for an active response to some environmental perturbations, zebrafish treated with melatonin, even in relatively high doses, can easily become fully alert. Such remarkable reversibility of the sleep-promoting effect of melatonin was reported earlier in humans. Together, this suggests distinct similarities in the melatonin-related sleep mechanisms in zebrafish and humans.

The second piece of evidence that sleep in zebrafish has features in common with sleep in mammals came from experiments targeting the most widespread inhibitory neurotransmitter, gamma-aminobuytric acid (GABA). GABA affects primarily chloride-permeable $\mathrm{GABA_A}$ receptor channels, hyperpolarizing neurons and inhibiting their action potentials. The majority of currently prescribed hypnotic drugs utilizes this effect and targets the GABA-ergic system. Zebrafish respond to such hypnotic substances, for example, barbiturates or benzodiazepines, by reducing activity and increasing arousal threshold. As in mammals, an increase in the dose of GABA-ergic compounds leads to loss of consciousness in fish.

Another neurochemical system that modulates sleep in humans and other mammals is that of histamine. Although histamine is an important part of the immune response and is produced by several cell types in the periphery, it is also an important neurotransmitter. It is synthesized in the tuberomammilary nuclei of the hypothalamus, with neuronal projections reaching many cortical areas. Loss of these neurons reduces alertness, while antagonists of H1 receptors, blocking their ability to convey histamine-based messages, induce sleepiness. This is one of the reasons for the widespread and often excessive use of over-the-counter antihistamine medications. What if zebrafish had a chance to use those over-the-counter antihistamines? Would they help fish to sleep? It turns out that the answer is: yes. When antihistamines are administered to a fish tank, both juvenile and adult zebrafish take a nap.

These findings in zebrafish strongly suggested that there are principal similarities between sleep processes in mammals and fish, making it even more interesting to study sleep-related mechanisms in diverse fish species. Then, however, a number of surprising observations proved once again that sleep regulation might be more complex than expected and that keeping in mind species- and class-specific differences is a must for any biomedical researcher.

Although there is a range of sleep disorders, their nature is still poorly understood. That is why finding a cause of one of the most mysterious sleep disorder, narcolepsy, was an exciting event. The underlying reason for most of the clinical cases of narcolepsy, typically associated with increased sleepiness, altered dreaming, and sudden attacks of muscle paralysis, turned out to be a deficiency in the production of a hypothalamic peptide hypocretin, also known as orexin. Hypocretin is an evolutionarily well-conserved molecule and is present in the fish brain. However, when mutant zebrafish lacking receptors for hypocretin were generated, the expected increase in sleepiness was not observed. On the contrary, these fish seemed to sleep less, not more, than their normal counterparts did. That said, other experiments addressing the role of hypocretin in fish sleep suggested that increasing production of hypocretin could promote and consolidate wakefulness, and inhibit sleep behavior in zebrafish, as would be expected from the mammalian-based studies. Currently, several research groups are trying to untangle this seemingly complex relationship between hypocretin signaling and sleep in zebrafish.

The characterization of sleep-related behaviors in zebrafish larvae and in adult zebrafish demonstrated that, similar to mammals or birds, sleep deprivation can lead to rebound of sleep in this species. Young, larval zebrafish compensate for sleep deprivation by increasing sleep time. Following several hours of forced wakefulness at nighttime, adult zebrafish also show somewhat reduced activity levels and tend not to perform cognitive tasks as efficiently as control animals that were not sleep deprived. However, if sleep deprivation lasts longer, for many hours or even days, alterations in fish locomotor activity appear to become less pronounced. This is in striking contrast to the response of mammalian species to prolonged lack of sleep, with a characteristic increase in sleepiness, behavioral alterations, and physiological abnormalities resulting from prolonged wakefulness. It is plausible that fish, like marine mammals, have some specific adaptive mechanisms for coping with the need for prolonged periods of continuous alertness, or at least a partial one. Learning about the neurochemical mechanisms involved in such adaptations might help us not only to better understand the overall phenomenon of sleep but also, potentially, to design some ways for temporarily increasing periods of human vigilance with minimal or no side effects.

The lower vertebrates have obvious anatomical limitations for the expression of some of the electrographic patterns commonly found in mammalian sleep. For example, it is unlikely that animals, such as fish, with only a rudimentary neocortex, if any, would generate the brain waves characteristic of mammalian slow-wave sleep. Thus, some of the sleep-related processes found in mammals cannot be studied in fish. However, the neurons central to sleep regulation are contained in structures within the isodendritic core of the brain, extending from the medulla through the brainstem, hypothalamus and up into the basal forebrain. All of those structures are well represented in fish (**see also Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes). Thus, fish, with their limbic brain, may be ideal for studying the principal, core sleep mechanisms manifesting in the absence of overpowering modulation by the cerebral hemispheres. Perhaps, it might take a sleepy swim to uncover the mystery of sleep.

See also: **Bony Fishes:** Zebrafish. **Brain and Nervous System:** Functional Morphology of the Brains of Ray-Finned Fishes. **Sensory Systems, Perception, and Learning:** Circadian Rhythms in Fish.

Further Reading

Cahill GM, Hurd MW, and Batchelor MM (1998) Circadian rhythmicity in the locomotor activity of larval zebrafish. *NeuroReport* 9(15): 3445–3449.

Campbell SS and Tobler I (1984) Animal sleep: A review of sleep duration across phylogeny. *Neuroscience and Biobehavioral Reviews* 8(3): 269–300.

Mukhametov LM (1987) Unihemispheric slow-wave sleep in the Amazonian dolphin, *Inia geoffrensis*. *Neuroscience Letters* 79(1–2): 128–132.

Prober DA, Rihel J, Onah AA, Sung RJ, and Schier AF (2006) Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. *Journal of Neuroscience* 26(51): 13400–13410.

Renier C, Faraco JH, Bourgin P, *et al.* (2007) Genomic and functional conservation of sedative-hypnotic targets in the zebrafish. *Pharmacogenetics and Genomics* 17(4): 237–253.

Shapiro CM and Hepburn HR (1976) Sleep in a schooling fish, *Tilapia mossambica*. *Physiology and Behavior* 16(5): 613–615.

Yokogawa T, Marin W, Faraco J, *et al.* (2007) Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biology* 5(10): 2379–2397.

Yu L, Tucci V, Kishi S, and Zhdanova IV (2006) Cognitive aging in zebrafish. *PLoS* 1(1): E14.

Zhdanova IV (2006) Sleep in zebrafish. *Zebrafish* 3(2): 215–226.

Zhdanova IV, Wang SY, Leclair OU, and Danilova NP (2001) Melatonin promotes sleep-like state in zebrafish. *Brain Research* 903(1–2): 263–268.

Zhdanova IV, Yu L, Lopez-Patino M, *et al.* (2008) Aging of the circadian system in zebrafish and the effects of melatonin on sleep and cognitive performance. *Brain Research Bulletin* 75(2–4): 433–441.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Manipulation of Fish Phenotype by Parasites

O Seppälä, EAWAG, Swiss Federal Institute of Aquatic Science and Technology, and ETH-Zürich, Institute of Integrative Biology (IBZ), Dübendorf, Switzerland

© 2011 Elsevier Inc. All rights reserved.

The Parasitic Way of Life
Trophic Transmission and Host Manipulation
Future Directions
Further Reading

Glossary

Adaptation A characteristic which increases an individual's fitness and has evolved through natural selection.

Cestoda A class of parasitic worms called tapeworms (within the phylum Platyhelminthes). Most cestodes have complex life cycles in which they use invertebrates as intermediate hosts and vertebrates as definitive hosts.

Definitive host A host in which the parasite reaches maturity and reproduces sexually.

Fitness Contribution of an individual to the next generation relative to other individuals, i.e., the proportion of the individual's genes in all the genes of the next generation. Traits affecting an organism's survival and reproductive success are tightly connected to fitness i.e., fitness = survivorship • fecundity.

Host manipulation Ability of a parasite to induce alterations in host phenotype which enhance parasite transmission.

Intermediate host A host that is used by the parasite, for example, for development, asexual reproduction, and transmission between definitive hosts.

Parasite An organism living in or on another organism – the host – and obtaining its resources by utilizing the host.

Parasite life cycle A period involving the development of a parasite to maturity and reproduction. Parasite life cycles can involve the exploitation of one (direct life cycles) or more host species (complex life cycles).

Phenotype Observable characteristics of an organism including different traits. Phenotype results from the expression of an organism's genes and the influence of environmental factors.

Target host The next host in the complex life cycle of a parasite to which it should be transmitted from its current host.

Trematoda A class of parasitic worms called flukes (within the phylum Platyhelminthes). Most trematodes have complex life cycles in which they use mollusks as intermediate hosts and vertebrates as definitive hosts.

Trophic transmission Form of parasite transmission from one host to another in which an infected host needs to be ingested by the target host. Trophically transmitted parasites utilize the predator–prey interactions existing in natural food webs.

The Parasitic Way of Life

Parasites spend at least a part of their lives in or on other organisms – the hosts – and obtain their resources by utilizing host individuals. This way of life offers several benefits. For example, hosts generally are resource-rich and fairly predictable living habitats, which protect parasites from the adversity of the environment outside their hosts. Moreover, a vast number of potential host species and sites of infection (different organs) offer a wide variety of resources for parasites. These factors have led to the independent evolution of parasitism in several taxa, and to a high rate of parasite speciation. Therefore, parasitism is perhaps the commonest way of life on Earth.

Despite the advantages of the parasitic way of life, parasites also face several challenges in their life histories. For instance, since parasites usually cause harm to their hosts (i.e., virulence), hosts tend to evolve resistance to parasite infections and/or tolerance of their harmful effects. Especially in vertebrates, highly developed immune responses replenished by immunological memory form the main physiological barrier against parasite infections. Furthermore, other defense mechanisms such as avoidance of those circumstances under which infections take place and group formation to dilute parasite exposure have been described in several host species.

However, probably the most severe challenge parasites need to overcome is that hosts are relatively short-lived habitats compared to those utilized by many free-living organisms. Therefore, because parasites are dependent on their hosts, transmission of parasite individuals between hosts is an essential process. This, however, is a very uncertain stage in parasite life histories, because hosts are usually patchily distributed in the environment. Especially in parasites with complex life cycles, including several different host species, an individual parasite only has a small probability of surviving and completing its life cycle because of high parasite mortality during transmission between different hosts. Thus, natural selection can favor parasite genotypes that can reduce these losses by being better at infecting the target hosts (the next host in the life cycle) relative to other genotypes. In fact, a wide variety of adaptations to enhance parasite transmission have been described in parasite–host relationships. These include, for example, release of parasite eggs or infective stages at the time when successful transmission is most likely to occur, and production of phenotypically dissimilar offspring to reduce the risk of transmission failure in unpredictable environments (i.e., 'bet hedging').

Trophic Transmission and Host Manipulation

Several complex parasite life cycles include at least one stage at which the infected host has to be consumed by the target host for successful transmission. Thus, parasites utilize the predator–prey interactions existing in natural food webs. Since the probability of an individual parasite completing its life cycle is typically very small, transmission success is a major determinant of overall parasite fitness. Therefore, to maximize transmission probability and thus fitness in trophic transmission, it would be beneficial for the parasites to alter host behavior or other phenotypic traits to make infected hosts easier prey for target hosts. In fact, phenotypic alterations in infected hosts have been described in several parasite–host interactions, and the ability of parasites to cause such effects has usually been considered as an evolutionary adaptation to increase parasite transmission efficiency. This might be the case in many systems, but not all parasite-induced alterations in host phenotype necessarily enhance parasite transmission. For example, in addition to increased host susceptibility to predation by target hosts, manipulation can also predispose infected hosts to capture by predator species which are unsuitable next hosts for the parasites (i.e., nonhost predators). Nonhost predation always leads to death of the parasites and thus phenotypic alterations in infected hosts can also induce severe costs to parasites. Therefore, it is difficult to distinguish parasite–host interactions in which parasite-induced alterations in host phenotype actually increase parasite transmission efficiency and thus could be considered as adaptive host manipulation. Thus, the effect of parasites on the susceptibility of hosts to different predator species should be understood.

Host Manipulation by Fish Parasites

In general, aquatic habitats are ideal for the maintenance of several different parasite life cycles. First, water provides a physiologically stable and viscous environment which enables the survival and effective dispersal of parasite eggs and free-living larvae. Second, aquatic food webs are often relatively long and complex, which facilitates the evolution of complex parasite life cycles. Furthermore, due to their commonness, high species diversity, and central role in aquatic food webs (both as consumers and prey), fish serve as hosts to a wide range of parasite species that exhibit different life cycles and life history strategies (note that some fish species are parasitic but this article considers fish only as hosts). Fish have also been widely used as study systems to examine the effects of parasites on their hosts. This is not only because of the importance of fish in natural ecosystems but also because of the high economical impact of fish parasites in fisheries and aquaculture. Most of the conducted studies have examined if and how parasites can alter fish phenotype (behavior and appearance) so that infected fish would become more susceptible to predation, and if the ability of parasites to predispose fish to predators could be an evolutionary adaptation by the parasites to enhance their transmission. This article briefly reviews these aspects of host manipulation in two relatively well-studied fish–parasite (*Diplostomum spathaceum* eye fluke and *Schistocephalus solidus* tapeworm) interactions.

Diplostomum spathaceum

The trematode *D. spathaceum* has a three-host life cycle with a bird definitive host, and snail and fish intermediate hosts (**Figure 1**). The parasite matures in the intestine of fish-eating birds, where it reproduces sexually. Several bird species are suitable hosts for the parasite, but gulls and terns are probably most common. After sexual reproduction, eggs of the parasite are released into water along with the bird's feces, where they hatch into free-swimming miracidia. Miracidia then infect aquatic snails (first intermediate host) mainly of the genus *Lymnaea.* Infection in a snail gives rise to sporocysts in which cercariae larvae are produced through asexual reproduction. Parasite development in snails takes 4–10 weeks depending on the water temperature, after which an individual snail can produce thousands of cercariae per day for several weeks. Cercariae infect a wide variety of freshwater and brackish water fish species (second intermediate hosts) by penetrating the gills and skin. In fish, these parasites migrate to eye lenses where they develop into metacercariae. For successful transmission to the avian definitive host, an infected fish has to be eaten by a fish-eating bird. In the lenses of fish, metacercarial stages of the parasite reduce host vision by inducing cataract formation (**Figure 2**) and disrupting lens structure. Therefore, by

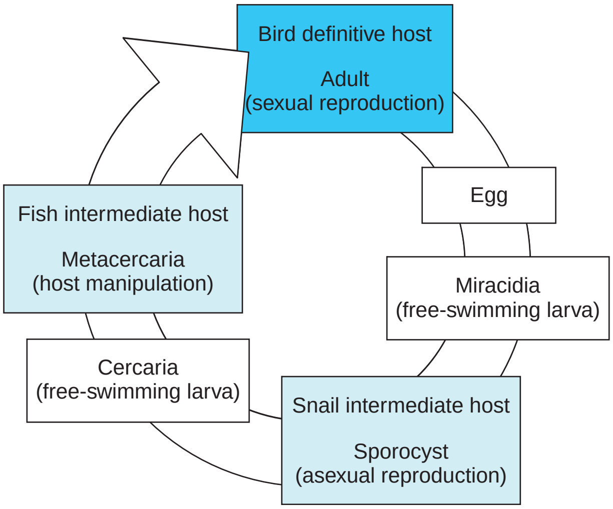


Figure 1 Life cycle of *Diplostomum spathaceum* (Trematoda). Colored boxes refer to the stages in which exploitation of hosts is involved (dark blue: definitive host; light blue: intermediate host).

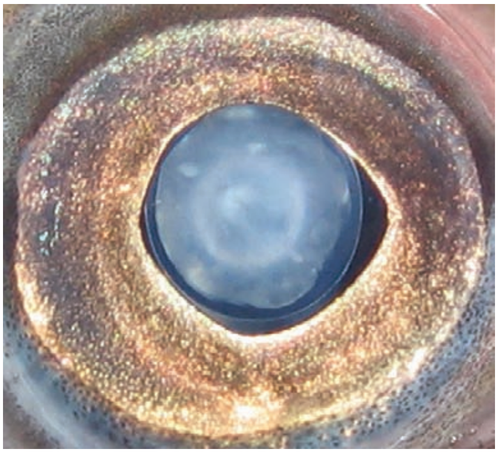


Figure 2 Parasite-induced cataracts in an eye lens of a juvenile rainbow trout infected with *Diplostomum spathaceum* eye flukes. Photo by Anssi Karvonen and Otto Seppälä.

injuring an important sensory organ, *D. spathaceum* has the potential to alter fish behavior and other phenotypic traits so that their vulnerability to predation could be increased.

D. spathaceum is one of the parasite species that is suggested to manipulate its fish hosts to increase transmission efficiency. By locating in the eye lenses and inducing cataract formation (**Figure 2**), it impairs fish vision and thus has the potential to predispose fish to predation. For example, dace (*Leuciscus leuciscus*) infected with *D. spathaceum* eye flukes spend more time near the water surface compared to uninfected fish. This can predispose fish to predation by gulls and terns, thus enhancing parasite transmission. However, the same effect has not been observed in rainbow trout (*Oncorhynchus mykiss*), with infected and uninfected fish preferring the same water depths. Instead, in rainbow trout, eye fluke infection impairs fish antipredator behavior. First, infection reduces shoaling of fish (infected fish are less likely to be part of a shoal and shoals of infected fish are smaller when compared to uninfected fish), which reduces the protection fish could get against predators from their conspecifics (**see also Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). Furthermore, these effects become more pronounced when the risk of predation is increased because only uninfected fish react to the presence of a predator by forming more cohesive shoals. Second, *D. spathaceum* infection reduces the intensity of fish escape reaction to an approaching avian predator by reducing the escape of fish to deeper water layers. These effects are likely mechanisms to predispose infected fish to predation and thus to enhance parasite transmission.

In addition to the behavioral responses discussed above, fish can also use other defense mechanisms, such as crypsis to avoid predators (**see also Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). In *D. spathaceum*–fish interaction, the ability of an eye fluke-infected fish to adjust itself to the environment using cryptic coloration and cryptic behavior is reduced when compared to an uninfected fish. In environments with a dark bottom coloration, both infected and uninfected rainbow trout are able to match their color to a substrate when observed from above. However, when maintained against lighter backgrounds, the contrast between fish color and background increases. Interestingly, this increase is higher in infected fish that remain darker than control fish. Furthermore, when fish can select between dark and light bottom colorations, the preference of infected fish for the dark background is reduced. This indicates that the camouflage of fish is reduced by the eye fluke infection. Therefore, infected fish should not only be easier to capture by predators but they can also be more conspicuous and thus more easily observed by avian predators.

To enhance parasite transmission, phenotypic alterations should predispose infected hosts to predation by target hosts. In laboratory experiments, eye fluke-infected rainbow trout are more prone to artificial predation, which simulates attacks by surface-feeding birds such as gulls and terns. When fish were caught with a dip net from shoals containing both heavily infected fish and uninfected fish, infected fish were caught more often as compared to controls. Similarly, when predation vulnerability of fish was determined by counting the number of attempts required to catch individual fish from the tanks, the number of attempts needed was lower in infected fish. These results suggest that infected fish are easier prey for bird hosts. However, these results have not been successfully verified in the field through real predation by gulls and terns, and thus the overall effect of manipulation on parasite transmission efficiency in the wild cannot be evaluated.

In many natural ecosystems, organisms are concurrently exposed to several predator species. Therefore, host manipulation may not only predispose infected individuals to predation by target hosts, but also to predator species which are unsuitable next hosts for the parasites. Nonhost predation always leads to death of the parasites, and thus parasite transmission may not be enhanced through manipulation. Since phenotypic changes induced by *D. spathaceum* are probably caused by impaired vision of fish (see below), manipulation could predispose fish not only to predation by avian hosts but also to nonhost predator species such as piscivorous fish. However, eye fluke infection has not been observed to predispose fish to predation by pike (*Esox lucius*), which represents a dead-end predator for the parasite. Reasons for the difference in the susceptibility of fish to different types of predators are unclear, but possibly differences in predator behavior are involved. Fish-eating birds typically actively search for prey, contrary to pike which ambush prey by staying motionless. Therefore, the effect of phenotypic alterations on the susceptibility of fish to predation may differ between predator species. If, for instance, activity of infected fish is reduced, it may lead to a lower contact probability with pike. Furthermore, when avoiding underwater attacks, fish are able to use not only their vision but also their lateral line and olfaction to detect predators. Hence, the role of vision may be particularly important when avoiding aerial avian predators (**see also Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food.

Findings presented above suggest that eye fluke-induced alterations in fish phenotype can increase parasite transmission success and thus they could be considered as adaptive host manipulation. But how did the ability of the parasite to manipulate fish phenotype evolve? In *D. spathaceum*–fish interaction, the parasites lodge themselves in the lens of fish eyes, and induce cataract formation (**Figure 2**) through their movements and metabolic wastes. In predation experiments discussed above, the susceptibility of fish to predation (capture by dip net) increased with the coverage of parasite-induced cataracts, indicating that impaired vision of fish is likely to be the underlying physiological mechanism leading to manipulation in this system. Therefore, it is possible that the eye as the site of infection has been favored by natural selection if it increases parasite transmission efficiency. On the other hand, natural selection may have also favored parasites escaping the host immune defense by locating in the lens, which has been suggested to be immunologically naïve because of the lack of blood vessels. Moreover, since cataract formation is probably tightly connected to parasite metabolic rate, increased susceptibility of fish to predation could be just a side effect of selection favoring faster parasite growth and development in fish. Whatever the original reason for locating in the eyes and inducing cataract formation is, natural selection may still act on all parasite features that influence transmission efficiency. For example, in *D. spathaceum*–fish interaction, cataract formation is slow when metacercariae are growing, but fast after they have completed development. This leads to an increase in the susceptibility of fish to predation only after parasites are infective to bird hosts. Delayed timing of manipulation has also been observed in several other parasite–host interactions, and it may be highly advantageous under selection favoring higher parasite transmission efficiency, because predation of uninfective larvae always leads to death of the parasites. These results suggest that precise timing of cataract induction, after metacercariae have reached infectivity to birds, leads to increased vulnerability of fish to predation, and thus may be a parasite strategy evolved to increase transmission efficiency in this system.

Schistocephalus solidus

Similar to *D. spathaceum*, the cestode *S. solidus* has a complex life cycle in which it exploits three different host species (**Figure 3**). The parasite matures in the intestine of fish-eating birds, where its reproduction takes place. After reproduction (*S. solidus* is a hermaphrodite and capable of self-fertilization), eggs of the parasite are released into water with bird feces, where they develop into the first larval stage called coracidia. The free-swimming coracidia that hatch from the eggs are ingested by freshwater copepods (first intermediate

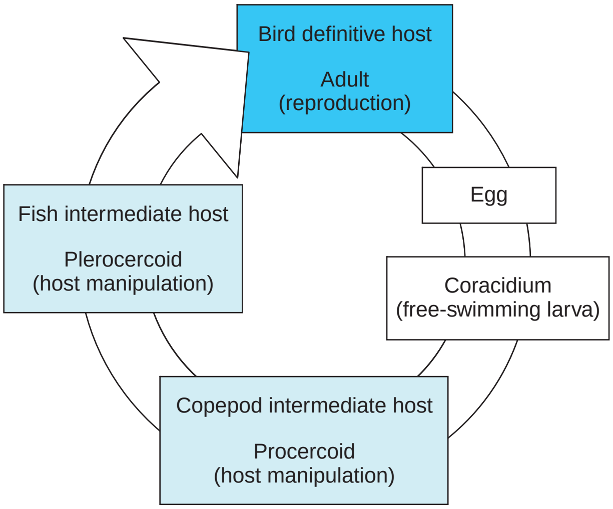


Figure 3 Life cycle of *Schistocephalus solidus* (Cestoda). Colored boxes refer to the stages in which exploitation of hosts is involved (dark blue: definitive host; light blue: intermediate host).

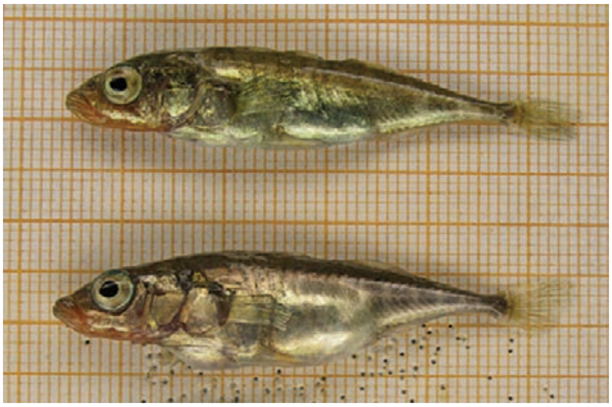


Figure 4 A healthy (top) and a *Schistocephalus solidus*-infected three-spined stickleback (bottom). The abdomen of the infected fish is swollen due to parasite infection. The fish are shown on a graph paper in which the distance between fine lines is 1 mm. Photo by Katja Räsänen.

host) where the parasites penetrate through the intestine to the body cavity and develop into procercoids. A copepod infected with a *S. solidus* procercoid needs to be ingested by a three-spined stickleback (*Gasterosteus aculeatus*; second intermediate host) for successful onward transmission. The parasite is known to manipulate the behavior of infected copepods by increasing their activity, which predisposes them to fish predation. In sticklebacks, parasites reach the last larval stage called plerocercoid, and grow in the abdominal cavity of the fish. For successful completion of the life cycle, an infected fish has to be consumed by a fish-eating bird. In sticklebacks, *S. solidus* plerocercoids grow for several weeks before they are able to infect a bird. During that period, parasites reach a very large size (they can equal the biomass of the fish), and the abdomen of an infected fish swells (**Figure 4**). Therefore, by inducing a strong pathological effect, *S. solidus* has the potential to alter fish phenotype in ways that could increase their vulnerability to predation.

Phenotypic alterations in sticklebacks infected with *S. solidus* plerocercoids show several similarities to those observed in fish infected with *D. spathaceum* eye flukes. For example, *S. solidus*-infected fish spend more time close to the water surface, and their escape responses are weaker when compared to uninfected individuals. Furthermore, in some stickleback populations in North America, *S. solidus* alters fish coloration, but contrary to eye flukes, the parasite induces demelanization of fish. This results in fish with a light body and dark eyes, which together with a swollen body are likely to make infected fish very easy to observe by predators. Furthermore, in addition to the phenotypic alterations mentioned above, *S. solidus* induces an interesting behavioral alteration in fish – increased risk taking. Contrary to uninfected fish, infected sticklebacks do not adjust their foraging areas according to predation risk but they forage close to predators. Infected fish also recover more quickly from predatory attacks as compared to uninfected individuals, returning closer to the water surface and starting to forage sooner after a disturbance. Although solid experimental evidence demonstrating the increase in predation vulnerability of infected fish is still lacking, the risky behavior of infected sticklebacks is very likely to increase parasite transmission success to piscivorous birds. As with *D. spathaceum*, the behavior of those fish, containing parasites ready to infect target hosts, is more strongly affected than those where the parasites are still developing. However, risk taking could also predispose sticklebacks to nonhost predators which can counterbalance its benefits (see above).

But why do *S. solidus*-infected sticklebacks take risks? Altered behavior of fish in relation to avoidance of predators could probably be explained by the energetic requirements of the parasite. In infected fish, *S. solidus* reaches a very large size so that the biomass of the parasite can equal that of the fish host. Thus, the worm can take so much energy from its host that infected sticklebacks simply need to forage despite the risk of predation. Furthermore, infected individuals are weaker competitors than uninfected fish. Thus, to avoid competition with their conspecifics, infected sticklebacks may be forced to forage close to predators. Increased energy requirements of infected fish are likely to explain the observed alterations in the behavior of infected sticklebacks especially because the effect of the parasite increases with plerocercoid size. However, also other mechanisms might play a role in host manipulation in this system. For example, monoamine neurotransmitters are involved in the control of animal behavior, and the monoaminergic systems are highly sensitive to several stressors, including parasitism. In *S. solidus*-infected sticklebacks, the concentrations of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) are reduced in the telencephalon, and the ratio between 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) is changed in both the hypothalamus and brainstem. Such effects are consistent with chronic stress in fish and indicate enhanced release of neurotransmitters. These effects could also lead to the observed behavioral alterations in infected sticklebacks, and may even be caused by direct manipulation of the fish neuroendocrine system by the parasite. However, further studies are needed to determine their role in host manipulation.

Future Directions

Similar to the studies presented above, most of the earlier work on host manipulation has mainly concentrated on its adaptive value to parasite transmission. Both the underlying physiological mechanisms leading to manipulation and the ecological importance of host manipulation are still poorly understood in most systems. Such knowledge is important as it could have wider implications for parasitological and ecological research. First, although many previous studies suggest that trophically transmitted parasites can increase their transmission success by manipulating the phenotype of their intermediate hosts, we do not know much about the evolutionary processes that have led to manipulation. If we understand how parasites alter host phenotype in detail, we can propose how natural selection could have led to the evolution of host manipulation, and also examine parasite evolution as a whole, additionally considering other parasite traits that are tightly connected to the fitness (e.g., host exploitation and growth).

Second, the ecological importance of manipulating parasites in natural ecosystems has received only recent interest. The potential role of host manipulation in determining, for example, parasite/host population dynamics, interactions between host species, food web structure, and ecosystem functioning is little understood. However, such effects can be important because manipulated hosts can be seen as complex organisms, not only retaining some of the properties of uninfected individuals but also displaying new parasite-induced characteristics. This can lead to novel interactions with other species and/or conspecifics. Furthermore, because parasites are extremely common, such alterations can potentially influence biodiversity and ecosystem functioning. Most of the research on host manipulation has been conducted under laboratory conditions. Although such experiments are extremely important when investigating the mechanisms and adaptive value of manipulation, they cannot address the extent of manipulation in natural host populations. Furthermore, most studies on host manipulation have investigated the effect of parasites only on one host species although many parasites infect several different hosts. Thus, to estimate the importance of manipulation at ecosystem level it would be necessary to examine the occurrence and effects of manipulation at a host community level in the field. Such studies could help to resolve how strong host manipulation is in nature, which host species are most strongly manipulated, and whether parasites can affect ecosystem processes by altering food web structure and energy flow.

See also: **Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food. **Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes.

Further Reading

Barber I and Rushbrook BJ (2008) Parasites and fish behaviour. In: Magnhagen C, Braithwaite V, Forsgren E, and Kapoor BG (eds.) *Fish Behaviour*, pp. 525–561. Enfield, NH: Science Publishers.

Barber I and Ruxton GD (1998) Temporal prey distribution affects the competitive ability of parasitized sticklebacks. *Animal Behaviour* 56: 1477–1483.

Lefèvre T, Lebarbenchon C, Gauthier-Clerc M, *et al.* (2009) The ecological significance of manipulative parasites. *Trends in Ecology and Evolution* 24: 41–48.

Lobue CP and Bell MA (1993) Phenotypic manipulation by the cestode parasite *Schistocephalus solidus* of its intermediate host, *Gasterosteus aculeatus*, the three-spine stickleback. *American Naturalist* 142: 725–735.

Milinski M (1990) Parasites and host decision-making. In: Barnard CJ and Behnke JM (eds.) *Parasitism and Host Behaviour*, pp. 95–116. London: Taylor and Francis.

Moore J (2002) *Parasites and the Behaviour of Animals*. New York: Oxford University Press.

Ness JH and Foster SA (1999) Parasite-associated phenotype modifications in three-spine stickleback. *Oikos* 85: 127–134.

Øverli Ø, Páll M, Borg B, Jobling M, and Winberg S (2001) Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. *Proceedings of the Royal Society of London B* 268: 1411–1415.

Seppälä O, Karvonen A, and Valtonen ET (2004) Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behaviour* 68: 257–263.

Seppala O, Karvonen A, and Valtonen ET (2005) Impaired crypsis of fish infected with a trophically transmitted parasite. *Animal Behaviour* 70: 895–900.

Seppälä O, Karvonen A, and Valtonen ET (2005) Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. *Animal Behaviour* 70: 889–894.

Seppälä O, Karvonen A, and Valtonen ET (2006) Host manipulation by parasites and risk of non-host predation: Is manipulation costly in an eye fluke–fish interaction? *Evolutionary Ecology Research* 8: 871–879.

Seppälä O, Karvonen A, and Valtonen ET (2008) Shoaling behaviour of fish under parasitism and predation risk. *Animal Behaviour* 75: 145–150.

Anthropogenic Influences on Fish Behavior

KA Sloman, University of the West of Scotland, Paisley, Scotland, UK

© 2011 Elsevier Inc. All rights reserved.

Interactions between Physiology and Behavior
Disruption of Sensory Information
Neurotoxins and Interference with Brain Function
Toxicants that Interfere with Respiration
Reproductive Impairment
Future Studies
Further Reading

Glossary

Acetylcholine A neurotransmitter particularly associated with motor control.

Acetylcholinesterase An enzyme that degrades the neurotransmitter acetylcholine.

Alarm substance A chemical or group of chemicals released in some species of fish from the skin of injured individuals.

Cholinesterase An enzyme (technically a group of enzymes) that degrades acetylcholine and butyrylcholine.

Endocrine disruptor An exogenous substance that causes adverse health effects in an organism or its progeny, subsequent to changes in endocrine function.

Monoamine neurotransmitter A group of neurotransmitters that contain one amino group in their chemical structure.

Neurotransmitters A group of chemicals that are released from nerve endings that transmit information to neighboring cells.

Ototoxin A chemical that causes damage to hair cells of the ear or lateral line.

Rheotaxis Orientation in a water current; most fish orientate to face into the water current.

Interactions between Physiology and Behavior

The physiology and behavior of fish are closely related and as such represent a fragile link vulnerable to anthropogenic disturbances. There are a myriad of ways in which humans have altered the aquatic environment, including chemical pollution, acoustic disturbances, physical disturbances, such as habitat destruction, and the release of invasive or transgenic species. The effects of human disturbance on fish behavior and physiology have been most studied in relation to chemical contaminants, including organic pollutants (e.g., some pesticides; **see also Toxicology:** The Toxicology of Organics in Fishes), pharmaceuticals, inorganic pollutants (e.g., metals; **see also Toxicology:** The Toxicology of Metals in Fishes), and radionuclides. Many types of anthropogenic disturbance target specific physiological systems and may exert their effects on fish behavior via physiological pathways. The performance of behaviors by individual fish generally follows specific physiological sequences acting as a link between physiological and ecological processes. Indeed, the use of fish behavior as a sensitive and ecologically relevant tool for monitoring anthropogenic effects in the aquatic environment is widely accepted. This article considers the main ways in which human activities are known to affect the fragile link that exists between behavior and physiology, focusing particularly on the effects of chemical contaminants.

Disruption of Sensory Information

Olfaction

The sense of smell is a key factor in allowing a fish to gain information about its surrounding environment and to behaviorally respond in an appropriate way. Olfaction is important in a plethora of behaviors. For example, salmonids rely on their sense of smell to find their way back to natal streams during migration (**see also Fish Migrations:** The Biology of Fish Migration). Many fish species, when injured by a predator, release a chemical known as ‘alarm substance’ into the water, sending olfactory warning signals to neighboring individuals that there is a predator present (**see also Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food). Reproductive pheromones play a vital role in courtship behaviors of many fish species (**see also Social and Reproductive Behaviors:** Sexual Behavior in Fish and **Hormones in Communication:** Hormonal Pheromones). Therefore, any chemicals that damage or interfere with the olfactory system in fish could disrupt a vast array of behavioral processes.

The olfactory system of fish consists of an olfactory rosette in direct contact with the external environment. Receptors in the rosette send information about olfactory stimuli via the olfactory nerve to the olfactory bulb located in the brain (**see also Smell, Taste, and Chemical Sensing:** Chemoreception (Smell and Taste): An Introduction and Morphology of the Olfactory (Smell) System in Fishes). Direct contact between the olfactory rosette and the environment of the fish can allow damage of the olfactory rosette by waterborne contaminants and some may actually be transported along the olfactory nerve to the brain. In pike, *Esox lucius*, mercury is transported along olfactory nerves, and, in salmonids (e.g., salmon and trout), cadmium has been found to accumulate in the olfactory rosette, olfactory nerve, and the anterior part of the olfactory bulb of the brain of fish held in cadmium-contaminated water, as illustrated in **Figure 1**. **Figure 1(a)** shows a cross section through a rainbow trout, *Oncorhynchus mykiss*, that has been exposed to waterborne radiolabeled cadmium. The olfactory system can be clearly seen. **Figure 1(b)** shows exactly the same section of fish but viewed using whole body phosphor screen autoradiography, which highlights the areas where radiolabeled cadmium has accumulated. From this, it is possible to see that cadmium has accumulated in the olfactory rosette, olfactory nerve, and olfactory bulb. Accumulation of toxicants in the olfactory rosette may also lead to a decrease in the number of functional receptors.

Contaminant accumulation in the olfactory system and the knock-on effects they have on cellular and

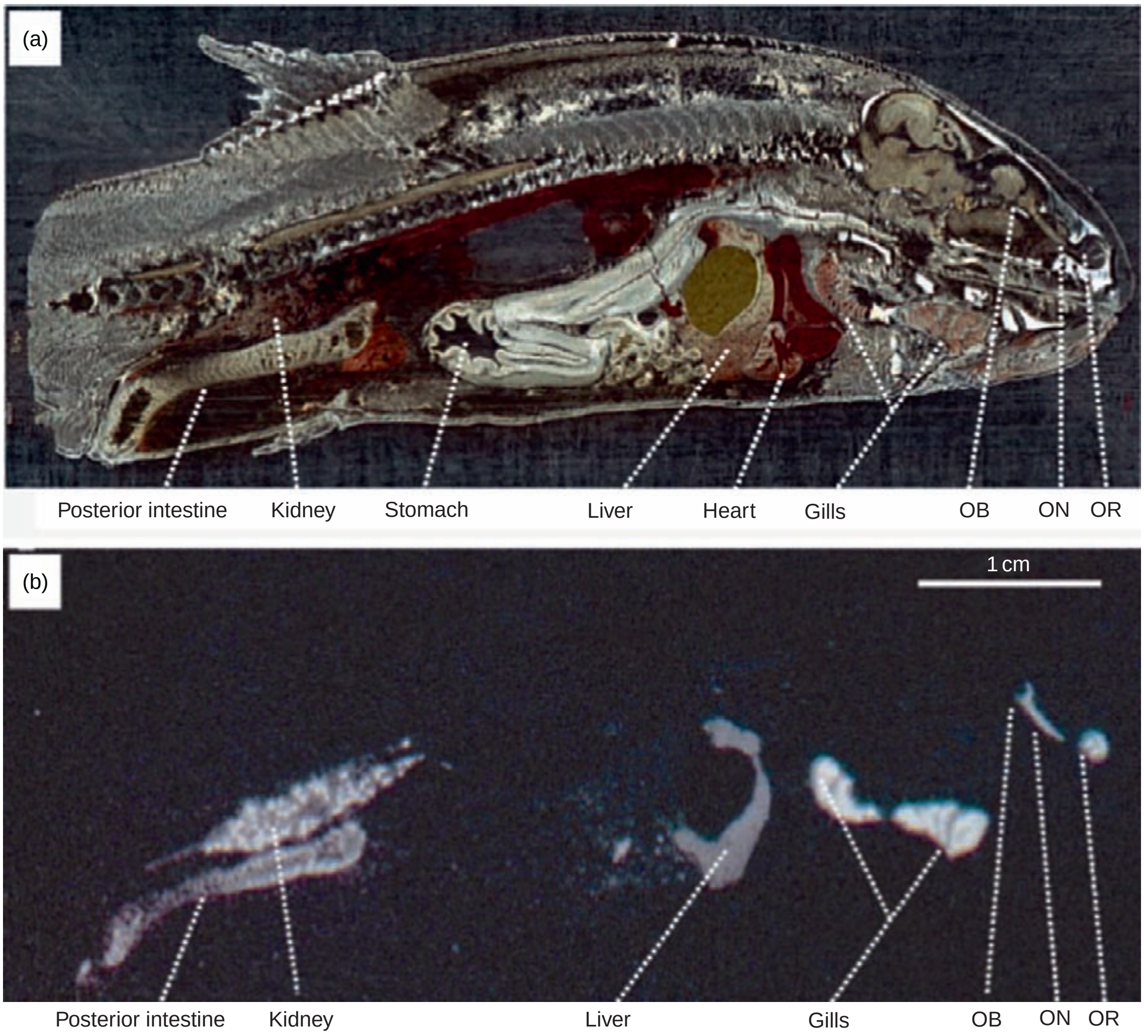


Figure 1 (a) Cross section through a rainbow trout showing a variety of tissues, including the olfactory bulb (OB), the olfactory nerve (ON), and the olfactory rosette (OR). (b) The same cross section of a rainbow trout viewed using phosphor screen autoradiography showing accumulation of radiolabeled cadmium following a waterborne exposure. Reproduced from Scott GR, Sloman KA, Rouleau C, and Wood CM (2003) Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 206: 1779–1790, with permission from The Company of Biologists.

physiological processes can ultimately affect the behavior of the fish. Alarm substance is a chemical or group of chemicals that is released when a fish is injured and its skin cells are ruptured. For example, in rainbow trout, individuals will demonstrate freezing behaviors and an overall decrease in activity when alarm substance is added to the water – characteristic behaviors seen in response to a potential predation threat. However, in rainbow trout that have been exposed to waterborne cadmium prior to the addition of alarm substance, no changes in behavior are seen. In contrast, exposure to dietary cadmium does not affect the behavioral response of fish to alarm substance. The effects of waterborne cadmium on the ability of fish to respond to alarm substance can be seen at relatively low concentrations that do not cause mortality or major physiological disturbances. However, the inability to detect and respond to alarm substance when a predator is present is a potentially lethal situation for the fish. Toxicants that interfere with detection of olfactory stimuli can also disrupt other behaviors, including courtship, prey capture, migration, and social interaction.

Mechanoreception

The lateral line functions to detect vibrations and water movement and allows fish to orientate themselves in a water current (rheotaxis), gain information about their spatial environment, and also plays a vital role in schooling (**see also Hearing and Lateral Line:** Lateral Line Structure). The sensory cells within the lateral line are known as hair cells and are also present in the ear. In the lateral line, hair cells are contained in sensory units known as neuromasts. Toxicants that interfere with hair-cell function, therefore, have the potential to disrupt behaviors reliant on hearing and the lateral line. Ototoxins are contaminants known to specifically affect hair-cell function and include the pharmaceuticals such as gentamicin sulfate, streptomycin, and amiloride.

Trace metals may also interfere with lateral line function. For example, banded kokopu (*Galaxius fasciatus*) exposed to waterborne cadmium show a reduced ability to orientate in a water current. Waterborne copper exposure in zebrafish larvae reduces the number of functional neuromasts in the lateral line. In control zebrafish larvae, functional neuromasts can be visualized by staining with the fluorescent dye, 2-(4-dimethylaminostyryl)-*N*-ethylpyridinium iodide) (DASPEI). **Figure 2(a)** shows control zebrafish larvae with functional neuromasts running along either side of the body in the lateral line stained with DASPEI. **Figure 2(b)** shows zebrafish larvae exposed to waterborne copper before staining with DASPEI; a clear reduction in functional neuromasts can be seen. In consequence, zebrafish larvae exposed to waterborne copper during development have a reduced ability to orientate and maintain their position within a water current.

Sound

Sound plays a major role in many fish behaviors, including social communication and mating (**see also Sensory Systems, Perception, and Learning:** How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling). In species with planktonic dispersal, for example many coral reef fish, sound also plays a vital role in locating a suitable habitat. Studies using artificial reefs with audio recordings of reef sounds have demonstrated how important sound is for settlement and habitat choice. In addition to ototoxins that can damage the hair cells of the ear, anthropogenic noise pollution can be problematic for fish although not all noise pollution has been found to have an effect on fish behavior (**see also Hearing and Lateral Line:** Effects of Human-Generated Sound on Fish). Marine fish from an inshore reef in Loch Ewe, Scotland, displayed a startle response to deployment of seismic air guns; however, there was little effect on day-to-day behavior of the fish. In the laboratory, initial exposure of Chinook salmon, *Oncorhynchus tshawytscha*, and rainbow trout to

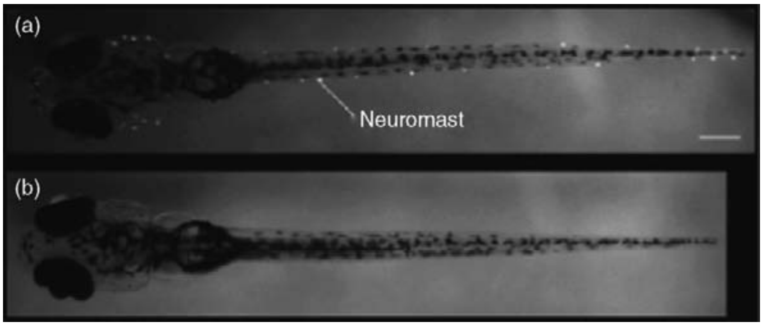


Figure 2 (a) Control and (b) copper-exposed zebrafish larvae stained with DASPEI illustrating the presence (in (a)) and absence (in (b)) of DASPEI-stained neuromasts. Scale = 0.25 mm. Reproduced from Johnson A, Carew E, and Sloman KA (2007) The effects of copper on the morphological and functional development of zebrafish embryos. *Aquatic Toxicology* 84: 431–438, with permission from Elsevier.

10-Hz-frequency sound causes a flight response away from the source; however, upon repeated exposure, this behavior changes to a less-dramatic avoidance response. This kind of sound avoidance by fish is well documented. Indeed, sound is often used as a fish repellent. For example, an acoustic deterrent system producing 20–600 Hz sound resulted in a 60% avoidance of a power station cooling water inlet by estuarine fishes. The clupeiod species, Atlantic herring, *Clupea harengus*, and European sprat, *Sprattus sprattus*, were most affected; a characteristic feature of the Clupeidae family is their advanced hearing capacity (**see Hearing and Lateral Line:** Physiology of the Ear and Brain: How Fish Hear).

Taste

Feeding behavior of fish depends on taste with many fish being able to distinguish between different food sources and display food preferences. Fish may also rely on taste to alter their diet in response to environmental change; for example, there may be potential for them to select a high-sodium diet, which is known to counteract the toxicity of some trace metals. In yellow bullheads, *Ictalurus natali*, detergents cause disintegration of taste buds and reduce the ability of individuals to chemically detect food. In catfish, such as yellow bullheads, taste buds, known as barbels, are located on whisker-like projections from the mouth, which are used for probing the sediment and searching for food. Damage to these sensory receptors may reduce the ability of individuals to detect potential prey.

Vision

The major anthropogenic influence affecting the vision of fish is increased turbidity through increased suspended material in the water column. Dredging, soil erosion, and sewage output are just a few examples of activities that can increase turbidity. The variety of bright colors utilized by fish indicates the importance of vision in fish communication for a myriad of reasons, including individual recognition and mate attraction (**see also Sensory Systems, Perception, and Learning:** Communication Behavior: Visual Signals). A reduction in visibility in the surrounding environment can disrupt fish visual communication and, in extreme cases, can lead to constraints in color vision.

Some toxicants, including methylmercury, may also interfere directly with the eye. Optic nerve function can be affected by the drug ethambutol, which is used to treat tuberculosis, and signal processing in the eye may be disrupted by the herbicide thiobencarb and the insecticide dichlorodiphenyltrichloroethane (DDT).

Neurotoxins and Interference with Brain Function

Neurotransmitters are chemicals that transmit signals both within the nervous system and from the nervous system to other cells. These chemicals play a vital role in many physiological and behavioral processes and, in order to function as reliable signals, they must be effectively degraded to give the signal a temporal property. Production and breakdown of the neurotransmitter allow transmission of a signal from one cell to another. A vast array of different neurotransmitters exists and several have been closely associated with behavioral patterns. Acetylcholine (ACh) is a neurotransmitter particularly involved with motor coordination and is broken down after release by the enzyme acetylcholinesterase (AChE). Total brain cholinesterase (ChE) activity is often measured as an indicator of AChE activity.

Many studies have considered the impact of toxicants on fish brain ChE activity. The insecticides malathion and diazinon are known to inhibit ChE activity, resulting in a buildup of ACh and subsequent effects on motor function. Studies on rainbow trout found that exposure to both of these insecticides resulted in fish swimming shorter distances at slower speeds. Other chemicals known to affect ChE activity include the organophosphate insecticides chloropyrifos and fenitrothion and several carbamate pesticides and herbicides, including thiobencarb and diuron.

A group of monoamine chemicals, which includes serotonin (5-hydroxytryptamine, 5-HT) and dopamine, also function as neurotransmitters. These monoamines are associated with the stress response and social behaviors. Elevated concentrations of serotonin and dopamine have been linked to submissive and aggressive behaviors, respectively. Methylmercury, the organochlorine pesticide lindane, polychlorinated biphenyls (PCBs), and copper can disrupt levels of these monoamines and, therefore, have the potential to impact behaviors associated with these neurotransmitters. Toxicants may also interfere with brain function through direct damage to brain tissue, including the formation of vacuoles within cell body layers and decreases in antioxidant enzymes, which function to break down free radicals and protect tissues from DNA damage.

Disruption of neurotransmitters and damage to brain tissue have many implications for behavior. The vital role of ChE activity in motor control means that chemicals, such as organophosphate pesticides that inhibit ChE activity, have profound effects on the myriad of behaviors that are dependent on locomotion. In particular, the decrease in swimming speeds associated with impaired ChE activity has implications for predator avoidance and prey capture. The escape response in most fish in response to a predatory threat occurs via the Mauthner system, consisting of two large nerve fibers located on either side of the brainstem with connections to hair cells of the lateral line (**see also** Brain and **Nervous System:** Physiology of the Mauthner Cell: Discovery and Properties). Activation of these nerve fibers in response to detecting pressure waves on one side of the body results in a classic reflex response away from the potential predator. This response occurs within milliseconds and is an extremely effective predator-avoidance mechanism. Exposure to the broad-spectrum insecticide, carbaryl or the chemical phenol, affected Mauthner cell function in medaka, *Oryzias latipes*. Sublethal concentrations of ammonia also affect the fast-start escape response in fish by interfering with the function of the Mauthner cells, and, additionally, by exerting toxic effects on white muscle. Exposures to sublethal levels of contaminants that impair swimming ability and reduce the effectiveness of the escape response can become lethal situations for the fish if they are unable to escape predation. Likewise, if fish are unable to catch their prey, then sublethal exposure to a toxicant can soon lead to starvation.

Neurotoxins are also known to interfere with fish social behavior. Exposure of rainbow trout to the neurotoxic metal, cadmium, decreased their ability to form social hierarchies and socially compete with nonexposed fish. Social hierarchies are known to form among groups of juvenile salmonid fish over resources such as food and shelter (**see also Social and Reproductive Behaviors:** Dominance Behaviors). The ability to win dominance contests can, therefore, translate into increased food acquisition and better territories. Initial competitions between pairs of juvenile trout often involve chases and bites by each fish, and occasionally mouth fighting until dominance is ascertained. In cadmium-exposed fish, it has been noted that individuals have difficulty making accurate judgments of the exact location of their competitor, resulting in aggressive chases that fail to make physical contact with their opponent. Consequently, hierarchy formation is either delayed where both competitors have been exposed to this neurotoxin, or cadmium-exposed fish are at a disadvantage when paired with a nonexposed fish.

Toxicants that Interfere with Respiration

There are two major ways in which fish gills or respiratory organs come into direct contact with contaminants. Some toxicants dissolve directly in the water column and thus are passed over the gills during ventilation. Other immiscible toxicants, such as oil, form layers on top of the water surface and can be inhaled by fish coming to the water surface to breathe air (**Behavioral Responses to the Environment:** Behavioral Responses to Hypoxia). Fish coughing has been observed as a general behavioral response to many waterborne toxicants. Aluminum is a trace metal that has been particularly associated with respiratory toxicity in fish, causing changes in gill morphology and impaired respiratory function. Fish exposed to waterborne aluminum reduce metabolically costly fast swimming behavior, suggesting a decrease in respiratory efficiency and, therefore, available energy. Other trace metals that can negatively impact on the energetic capacity of fish include copper and nickel.

Many fish species show adaptations to low oxygen levels in the water which involve moving to the water surface either to ventilate their gills in the thin layer of water in immediate contact with the air (aquatic surface respiration) or to actually breathe air (**see Behavioral Responses to the Environment:** Behavioral Responses to Hypoxia). When a contaminant forms a thin layer on the water surface, such as in the case of oil spills, there can be severe consequences for fish that rely on access to the water surface. Some fish, such as the piraracu, *Arapaima gigas*, are obligate air breathers and will die when denied access to air, regardless of levels of dissolved oxygen in the water. These fish are particularly susceptible to pollutants, such as oil, which can enter their air-breathing organs causing respiratory distress and suffocation.

Reproductive Impairment

Research into the effects of contaminants on fish reproduction has focused in recent years on compounds that can act as endocrine disruptors. An endocrine disruptor is an exogenous substance that causes adverse health effects in an organism or its progeny subsequent to alterations in endocrine function. Many endocrine disruptors mimic endogenous hormones, while others may interfere with the production of endogenous hormones or block their effects. In particular, a vast array of estrogenic endocrine disruptors have been identified, chemicals that mimic the natural hormone estrogen with potentially more potent effects. Androgenic endocrine disruptors also exist along with known anti-androgens and anti-estrogens that interfere with the functioning or production of male or female hormones, respectively.

Estrogenic chemicals, such as nonylphenol, used as a surfactant in detergents, bisphenol A, an important component of many plastics and the synthetic estrogen found in the contraceptive pill, ethinyl estradiol, are known to have feminizing effects in many fish species. Natural estrogenic chemicals, known as phytoestrogens, are found in some plant species and may be released into the environment through pulp and paper-mill processing. Male fish exposed to these compounds in the water may develop intersex reproductive organs that demonstrate the characteristics of both testes and ovaries. A commonly used indicator of estrogenic exposure in male fish is the production of vitellogenin, an egg-yolk precursor protein. Vitellogenin is normally only produced in female fish where high concentrations of the female hormone estrogen cause the liver to produce vitellogenin, which is then transported in the blood stream to the ovaries and packaged into eggs. Synthetic estrogens have the capacity to induce a similar effect in male fish, resulting in uncharacteristically high levels of plasma vitellogenin.

Exposure to these feminizing chemicals also has the potential to alter male reproductive behavior. Effects of endocrine disruptors on reproductive behavior have been particularly studied in fathead minnows, *Pimephales promelas*. Reproductively mature male fathead minnows develop secondary sexual characteristics, such as a fat pad on their head and tubercles on their snout, which aid in attracting a mate. Following courtship behaviors, these fish form pairs, and the female lays eggs, which the male then fertilizes. Exposure to estrogenic chemicals, such as nonylphenol, has been shown to reduce fat-pad size and the number of face tubercles in male fathead minnows, reducing their chances of attracting a mate and successfully reproducing. In three-spined sticklebacks, *Gasterosteus aculeatus*, males build a nest to attract a female to lay eggs in and use elaborate courtship behaviors to attract females to their nest (**see Social and Reproductive Behaviors:** Sexual Behavior in Fish). Exposure to estrogenic endocrine-disrupting chemicals has been shown to reduce courtship success and nest building behavior in male sticklebacks.

Other pollutants may interfere with reproductive behavior without necessarily disrupting endocrine function. Many fish use sexual pheromones to attract mates (**see Hormones in Communication:** Hormonal Pheromones). As mentioned previously, there are many chemicals that can interfere with olfactory function and thus disrupt courtship behaviors that depend on olfactory cues. For example, female Atlantic salmon, *Salmo salar*, release prostaglandin $F_{2\alpha}$ as an olfactory cue that has a priming influence on males, resulting in elevations of male hormones and sperm availability. Exposure of male Atlantic salmon to many insecticides and pesticides, including carbofuran, atrazine, cypermethrin, and diazinon, reduces the ability of males to detect prostaglandin $F_{2\alpha}$ and, therefore, their ability to behaviorally and physiologically respond to female cues.

Future Studies

The ways in which human activity can and has disrupted the fragile link that exists between fish behavior and physiology are vast and, here, I have only scraped the surface of the detrimental effects that we know we have exerted on fish biology. However, there are many areas where we are still lacking in knowledge. For example, a plethora of research has focused on chemicals that interfere with the functioning of reproductive hormones but little considered are chemicals that might interfere with other hormonal systems, such as stress hormones and thyroid hormones. Many fish species undergo sex change during their life history (**see Social and Reproductive Behaviors:** Socially Controlled Sex Change in Fishes), what impacts do estrogenic or androgenic chemicals have on this process? Also lacking is our understanding of how laboratory toxicity studies translate into field exposures. For example, laboratory exposure to toxicants can affect the ability to both detect predators and forage for food. In the field, these behaviors do not occur in isolation and it is likely that both prey and predator will be exposed to toxicants at the same time. It is imperative that we continue to strive to understand the anthropogenic influences on fish behavior and physiology and to add ecological relevance to our understanding of these issues.

See also: **Behavioral Responses to the Environment:** A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food; Behavioral Responses to Hypoxia. **Brain and Nervous System:** Physiology of the Mauthner Cell: Discovery and Properties. **Fish Migrations:** The Biology of Fish Migration. **Hearing and Lateral Line:** Effects of Human-Generated Sound on Fish; Lateral Line Structure; Physiology of the Ear and Brain: How Fish Hear. **Hormones in Communication:** Hormonal Pheromones. **Sensory Systems, Perception, and Learning:** Communication Behavior: Visual Signals; How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling. **Smell, Taste, and Chemical Sensing:** Chemoreception (Smell and Taste): An Introduction; Morphology of the Olfactory (Smell) System in Fishes. **Social and Reproductive Behaviors:** Dominance Behaviors; Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes. **Toxicology:** The Toxicology of Metals in Fishes; The Toxicology of Organics in Fishes.

Further Reading

Abrahams M (2006) The physiology of antipredator behavior: What you do with what you've got. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, 1st edn., vol. 24, pp. 79–108. San Diego, CA: Elsevier.

Allin CJ and Wilson RW (1999) Behavioural and metabolic effects of chronic exposure to sublethal aluminium in acidic soft water in juvenile rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 56: 670–678.

Baker CF and Montgomery JC (2001) Sensory deficits induced by cadmium in banded kokopu, *Galaxias fasciatus*, juveniles. *Environmental Biology of Fishes* 62: 455–464.

Bardach JE, Fujiya M, and Holl A (1965) Detergents: Effects on the chemical senses of the fish *Ictalurus natalis* (le Sueur). *Science* 148: 1605–1607.

Borg-Neczak K and Tjalve H (1996) Uptake of $^{203}Hg^{2+}$ in the olfactory system in pike. *Toxicology Letters* 84: 107–112.

Brewer SK, Little EE, De Lonay AJ, *et al.* (2001) Behavioural dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykiss)* exposed to cholinesterase-inhibiting chemicals. *Archives of Environmental Contamination and Toxicology* 40: 70–76.

De Boeck G, Nilsson GE, Elofsson U, Vlaeminck A, and Blust R (1995) Brain monoamine levels and energy status in common carp (*Cyprinus carpio)* after exposure to sublethal levels of copper. *Aquatic Toxicology* 33: 265–277.

Harries JE, Runnalls T, Hill E, *et al.* (2000) Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas)*. *Environmental Science and Technology* 34: 3003–3011.

Johnson A, Carew E, and Sloman KA (2007) The effects of copper on the morphological and functional development of zebrafish embryos. *Aquatic Toxicology* 84: 431–438.

Knudsen FR, Schreck CB, Knapp SM, Enger PS, and Sand O (1997) Infrasound produces flight and avoidance responses in Pacific juvenile salmonids. *Journal of Fish Biology* 51: 824–829.

Maes J, Turnpenny AWH, Lambert DR, *et al.* (2004) Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet. *Journal of Fish Biology* 64: 938–946.

McKenzie DJ, Shingles A, Claireaux G, and Domenici P (2009) Sublethal concentrations of ammonia impair performance of the teleost fast-start escape response. *Physiological and Biochemical Zoology* 82: 353–362.

Moore A and Waring CP (1996) Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon parr. *Journal of Fish Biology* 48: 758–775.

Niyogi S, Kamunde CN, and Wood CM (2006) Food selection, growth and physiology in relation to dietary sodium chloride content in rainbow trout (*Oncorhynchus mykiss)* under chronic waterborne Cu exposure. *Aquatic Toxicology* 77: 210–221.

Scott GR and Sloman KA (2004) The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology* 68: 369–392.

Scott GR, Sloman KA, Rouleau C, and Wood CM (2003) Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (*Oncorhynchus mykiss)*. *Journal of Experimental Biology* 206: 1779–1790.

Sloman KA (2007) Effects of trace metals on salmonid fish: The role of social hierarchies. *Applied Animal Behaviour Science* 104: 326–345.

Sloman KA, Scott GR, Diao Z, *et al.* (2003) Cadmium affects the social behaviour of rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology* 65: 171–185.

Sloman KA and Wilson RW (2006) Anthropogenic impacts upon behaviour and physiology. In: Sloman KA, Wilson RW, and Balshine S (eds.) 1st edn., *Behaviour and Physiology of Fish,* vol. 24, pp. 413–468. San Diego, CA: Elsevier.

Wardle CS, Carter TJ, Urquhart GG, *et al.* (2001) Effects of seismic air guns on marine fish. *Continental Shelf Research* 21: 1005–1027.

This page intentionally left blank

ENCYCLOPEDIA OF
FISH PHYSIOLOGY:
FROM GENOME TO
ENVIRONMENT

This page intentionally left blank

ENCYCLOPEDIA OF
FISH PHYSIOLOGY: FROM GENOME TO ENVIRONMENT

Volume 2

GAS EXCHANGE, INTERNAL HOMEOSTATIS, AND FOOD UPTAKE

Editor-in-Chief
ANTHONY P. FARRELL
Department of Zoology,
University of British Columbia,
Vancouver, BC, Canada

ELSEVIER
AMSTERDAM BOSTON HEIDELBERG LONDON NEW YORK OXFORD
PARIS SAN DIEGO SAN FRANCISCO SINGAPORE SYDNEY TOKYO
Academic Press is an imprint of Elsevier
ACADEMIC PRESS

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NWI 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

Copyright © 2011 Elsevier Inc. All rights reserved

The following articles are US government works in the public domain and are not subject to copyright:
Energetic Models: Bioenergetics in Ecosystems
Hormonal Control of Metabolism and Ionic Regulation: The Hormonal Control of Osmoregulation in Teleost Fish
Swimming and Other Activities: Applied Aspects of Fish Swimming Performance
The Skin: Functional Morphology of the Integumentary System in Fishes; The Many Functions of Fish Integument

The following articles are Crown Copyright:
Fish Migrations: Eel Migrations
Social and Reproductive Behaviors: Sexual Behavior in Fish

No part of this publication may be reproduced, stored in a retrieval system
or transmitted in any form or by any means electronic, mechanical, photocopying,
recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at (http://elsevier.com/locate/permissions), and selecting *Obtaining permission to use Elsevier material*

Notice
No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein, Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Catalog Number: 2011923567

ISBN: 978-0-12-374545-3

For information on all Elsevier publications
visit our website at books.elsevier.com

Printed and bound in Italy

11 12 13 14 15 10 9 8 7 6 5 4 3 2 1

For supplementary material please visit the companion site:
http://www.elsevierdirect.com/companions/9780123745453

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER BOOK AID International Sabre Foundation

Editorial: Kate Mittell
Production: Nicky Carter

EDITORS

EDITOR-IN-CHIEF

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ASSOCIATE EDITORS

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island,
Charlottetown, PE, Canada

This page intentionally left blank

EDITORS' BIOGRAPHIES

Editor-in-Chief

Anthony P. Farrell, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Tony Farrell is a graduate of Bath University, where he was fortunate to study with Peter Lutz. His fortunes grew further when he moved in 1974 to Canada and the Zoology Department at the University of British Columbia (UBC) to complete his PhD degree under the excellent tutelage of Dave Randall. In 2004, Tony returned to UBC when he accepted an endowed research chair in sustainable aquaculture. In between these positions at UBC, Tony was employed at the University of Southern California (PDF), the University of New Brunswick (sessional lecturer), Mount Allison University (first real job), and Simon Fraser University (moving through the ranks to a full professor). In addition to highly controlled laboratory experiments on fish cardiorespiratory physiology, Tony is committed to working on animals in their own environment. Therefore, his research on fish physiology has taken him on an *Alpha Helix* expedition to the Amazon, the University of Gothenburg and the Kristineberg Marine Research Station in Sweden, the Portobello Marine Biological Station in New Zealand, the University of Christchurch and Massey University in New Zealand, the Bamfield Marine Science Station and the Huntsman Marine Station in Canada, the University of Aarhus in Denmark, the University of Adelaide Charles and Darwin University in Australia, and to the Danish Arctic Marine Station on Disco Island in Greenland. These travels have allowed him to work with many superb collaborators wordwide, as well as study the physiology of over 70 different species of fish. Tony has received a number of awards for his scientific contributions: an honorary degree from the University of Gothenburg in Sweden; Awards of Excellence from the American Fisheries Society for Fish Physiology, Conservation and Management; the Fry Medal from the Canadian Society of Zoologists; and the Beverton Medal from the Fisheries Society of the British Isles.

Associate Editor

Joseph J. Cech, Jr., Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

Joe Cech grew up in northern Illinois <1 km from the Salt Creek Preserve, where he spent many hours as a boy. Summer trips to northern Wisconsin, northern Minnesota, and southern Ontario increased his interest in aquatic habitats and their fishes. He graduated from the University of Wisconsin (UW, Madison), where he was fortunate to study oceanography under Robert Ragotzkie and fish biology under Art Hasler and Fran Henderson in the UW Laboratory of Limnology. Before completing his BS degree in zoology, he was fortunate to serve as the resident zoologist aboard the R/V *Dante Deo*, for a year, as it crossed the Caribbean Sea and the South Pacific Ocean. Completing his degree upon his return, he was hired as a research assistant at the University of Texas' Marine Science Institute (UTMSI), where he earned his MA and PhD degrees in zoology, under the supervision of Curly Wohschlag. During his graduate education in Austin (Texas), he studied fish biology under Clark Hubbs and physiology under Jack Myers and Jim Larimer. After a 2-year postdoctoral position in Maine, he was hired as a fish physiological ecologist faculty member at the University of California (UC), Davis, in the Department of Wildlife, Fish, and Conservation Biology.

During his 32 years as an active faculty member at UC Davis, his research on the physiological ecology of fishes took him to Canada (University of British Columbia, Bamfield Marine Station), Finland (University of Helsinki), and to other laboratories in the USA (University of Tennessee, Scripps Institution of Oceanography, R/V *David Starr Jordan* in the North Pacific Ocean, UTMSI, Oregon Institute of Marine Biology, and the UC Bodega Marine Laboratory) where he collaborated with many excellent scientists. During this period, he taught courses in physiology of fishes, physiological ecology, and field studies in fish biology; he supervised 25 graduate students (10 MS and

15 PhD) in the disciplinary areas of ecology, physiology, zoology, and pharmacology/toxicology at UC Davis. He is a fellow of the AIFRB and AAAS, and he received Awards of Excellence from the American Fisheries Society (Physiology Section, and California-Nevada Chapter) and the UC Davis Prize for Undergraduate Teaching and Scholarly Achievement. Finally, he co-authored a popular ichthyology textbook, with Peter Moyle, and he published more than 160 peer-reviewed publications on the physiological ecology of fishes. He is currently Professor Emeritus of Fish Biology at the University of California Davis.

Associate Editor

Jeffrey G. Richards, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Jeffrey Richards is an associate professor in the Department of Zoology at the University of British Columbia, Canada. He started his academic career in the Department of Biology at Wilfrid Laurier University obtaining a BSc in 1995. He then went on to do an MSc at the University of Waterloo under the guidance of the late Prof. Richard Playle. With Prof. Playle, he studied environmental physiology and toxicology in fish. Following his MSc, he entered the laboratory of Prof. Chris Wood at McMaster University and focused his research on energy metabolism in swimming fish. He completed his PhD in 2002 and took up a postdoctoral fellowship at the University of British Columbia with Prof. Patricia Schulte. After a 2-year postdoctoral work, he started at the University of British Columbia as an assistant professor in 2004. His lab studies the relationship between plasticity and adaptive variation in the mechanisms that initiate, maintain, and coordinate metabolic energy balance in animals during exposure to environmental stress. He is particularly interested in the adaptations to low oxygen (hypoxia) in fish and other lower vertebrates. He prefers, whenever possible, to study animals in their natural habitat; thus, he travels extensively to unique and challenging environments, including the Amazon and the Tibetan plateau.

Associate Editor

E. Don Stevens, Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

Don Stevens did an MSc with the late Edgar Black on the effects of severe exercise on metabolites in trout in the Physiology Department at UBC after an undergraduate degree in mathematics and zoology at the University of Victoria. He then moved across the street to do a PhD on effects of moderate exercise on respiration and circulation in trout with Dave Randall, who just had been hired in the Zoology Department at UBC (summarized in a JEB classics article, *Journal of Experimental Biology* 211: 1521). His first position was at the Zoology Department at the University of Hawaii where he discovered that there was more to the fish world than trout, and that there was no such thing as the physiology of fish, rather there was a physiology of fishes. In Hawaii, he worked mostly on the biology of tropical tunas with a focus on their warm-bodiedness, in particular how they achieved higher than ambient body temperatures and the adaptive advantage of them. He then joined the Zoology Department at the University of Guelph working in the general area of comparative physiology with a focus on exercise effects until he retired in 2004. He now has an adjunct position at the Atlantic Veterinary College and continues to do research in fish physiology.

SECTION EDITORS

C Brauner
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

AB Butler
Department Molecular Neuroscience, George Mason University, Fairfax, VA, USA

BA Carlson
Department of Biology, Washington University, St. Louis, MO, USA

BM Casper
Department of Biology, University of Maryland, College Park, MD, USA

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

G Goss
Biological Sciences, University of Alberta, Edmonton, AB, Canada

JB Graham
Department of Economics, University of California at San Diego, La Jolla, CA, USA

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

S Holmgren
Zoophysiology, University of Goteberg, Goteberg, Sweden

N Maclean
Biological Sciences, University of Southampton, Hampshire, UK

JA Nelson
Department of Biological Sciences, Towson University, Towson, MD, USA

KR Olson
Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA

C Olsson
Zoophysiology, University of Goteberg, Goteberg, Sweden

AN Popper
Department of Biology, University of Maryland, College Park, MD, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

PM Schulte
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

RE Shadwick
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

K Sloman
University of the West of Scotland, Paisley, Scotland, UK

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

AP Summers
Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, USA

M Vijayan
Department of Biology, University of Waterloo, Waterloo, ON, Canada

H-J Wagner
Universitaet Tuebingen, Tuebingen, Germany

This page intentionally left blank

CONTRIBUTORS

MV Abrahams
Memorial University of Newfoundland, St. John’s, NL, Canada

RMcN Alexander
University of Leeds, Leeds, UK

D Alsop
McMaster University, Hamilton, ON, Canada

N Aluru
Woods Hole Oceanographic Institution, Woods Hole, MA, USA

D Álvarez
Universidad de Oviedo, Oviedo, Spain

B Austin
University of Stirling, Stirling, UK

M Axelsson
University of Gothenburg, Gothenburg, Sweden

R Baker
New York University Medical Center, New York, NY, USA

AM Bakke
Norwegian School of Veterinary Science, Oslo, Norway

JS Ballantyne
University of Guelph, Guelph, ON, Canada

S Balshine
McMaster University, Hamilton, ON, Canada

AH Bass
Cornell University, Ithaca, NY, USA

TL Beitinger
University of North Texas, Denton, TX, USA

TJ Benfey
University of New Brunswick, Fredericton, NB, Canada

M Berenbrink
University of Liverpool, Liverpool, UK

D Bernal
University of Massachusetts, Dartmouth, MA, USA

TR Binder
Hammond Bay Biological Station, Millersburg, MI, USA

BA Block
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

A Böhne
Université de Lyon, Lyon, France

NC Bols
University of Waterloo, Waterloo, ON, Canada

JK Bowmaker
University College London, London, UK

I Braasch
University of Oregon, Eugene, OR, USA

VA Braithwaite
Penn State University, State College, PA, USA; University of Bergen, Norway

MH Braun
University of Calgary, Calgary, AB, Canada

CJ Brauner
University of British Columbia, Vancouver, BC, Canada

DL Breitburg
Smithsonian Environmental Research Center, Edgewater, MD, USA

F Brunet
Université de Lyon, Lyon, France

J Buckley
University of Plymouth, Plymouth, UK

T Burt de Perera
University of Oxford, Oxford, UK

D Burton
Memorial University of Newfoundland, St. John's, NL, Canada

AB Butler
George Mason University, Fairfax, VA, USA

DC de Caprona
University of Iowa, Iowa City, IA, USA

AA Caputi
Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

BA Carlson
Washington University in St. Louis, St. Louis, MO, USA

MA Caruso
North Dakota State University, Fargo, ND, USA

MR de Carvalho
Universidade de São Paulo, São Paulo, Brazil

BM Casper
University of Maryland, College Park, MD, USA

T Castro-Santos
USGS Biological Resources Discipline, Turners Falls, MA, USA

JJ Cech Jr.
University of California, Davis, CA, USA

JN Cech
Howard Hughes Medical Institute, Boston, MA, USA

D Chabot
Institut Maurice-Lamontagne, Mont-Joli, QC, Canada

MJ Chacron
McGill University, Montreal, QC, Canada

K Cheney
The University of Queensland, Brisbane, QLD, Australia

KM Claeson
University of Texas at Austin, Austin, TX, USA

SJ Cooke
Carleton University, Ottawa, ON, Canada

SL Coombs
Bowling Green State University, Bowling Green, OH, USA

AJ Cotel
University of Michigan, Ann Arbor, MI, USA

LI Crawshaw
Portland State University, Portland, OR, USA

GT Crossin
Simon Fraser University, Burnaby, BC, Canada

S Currie
Mount Allison University, Sackville, NB, Canada

CP Cutler
Georgia Southern University, Statesboro, GA, USA

MN Dean
Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

J-P Denizot
CNRS, Gif sur Yvette, France

RH Devlin
Fisheries and Oceans Canada, West Vancouver, BC, Canada

RJ Díaz
Virginia Institute of Marine Science, Gloucester Point, VA, USA

KA Dickson
California State University Fullerton, Fullerton, CA, USA

P Domenici
CNR – IAMC, Torregrande (Or), Italy

SI Doroshov
University of California, Davis, CA, USA

RH Douglas
City University London, London, UK

WW Dowd
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

K Dunlap
Trinity College, Hartford, CT, USA

S Egginton
University of Birmingham, Birmingham, UK

DJ Ellerby
Wellesley College, Wellesley, MA, USA

DG Elliott
Western Fisheries Research Center, US Geological Survey, Seattle, WA, USA

J Engelmann
University of Bielefeld, Bielefeld, Germany

DH Evans
University of Florida, Gainesville, FL, USA

DS Faber
Albert Einstein College of Medicine, Bronx, NY, USA

AP Farrell
University of British Columbia, Vancouver, BC, Canada

RR Fay
Loyola University Chicago, Chicago, IL, USA

LA Ferry
Arizona State University, Phoenix, AZ, USA

PA Fields
Franklin & Marshall College, Lancaster, PA, USA

IP Forster
Fisheries and Oceans Canada, West Vancouver, BC, Canada

ME Forster
University of Canterbury, Christchurch, New Zealand

ES Fortune
Johns Hopkins University, Baltimore, MD, USA

B Fritzsch
University of Iowa, Iowa City, IA, USA

DS Fudge
University of Guelph, Guelph, ON, Canada

D Galiana-Arnoux
Université de Lyon, Lyon, France

GLJ Galli
University of Manchester, Manchester, UK

AK Gamperl
Memorial University of Newfoundland, St. John's, NL, Canada

DP German
University of California, Irvine, CA, USA

TE Gillis
University of Guelph, Guelph, ON, Canada

KM Gilmour
University of Ottawa, Ottawa, ON, Canada

D Giordano
National Research Council (CNR), Naples, Italy

J Godwin
North Carolina State University, Raleigh, NC, USA

A González
University Complutense of Madrid, Madrid, Spain

RJ Gonzalez
University of San Diego, San Diego, CA, USA

A Gräns
University of Gothenburg, Gothenburg, Sweden

AY Gracey
University of Southern California, Los Angeles, CA, USA

JB Graham
University of California at San Diego, La Jolla, CA, USA

S Grillner
Karolinska Institutet, Stockholm, Sweden

H Guderley
Université Laval, Montreal, QC, Canada

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

MC Hastings
Georgia Institute of Technology, Atlanta, GA, USA

AD Hawkins
Loughine Ltd., Aberdeen, Scotland, UK

LP Hernandez
George Washington University, Washington, DC, USA

JE Herr
University of Guelph, Guelph, ON, Canada

MJ Herrero-Turrión
Institute of Neurosciences of Castilla y León, Salamanca, Spain

TE Higham
Clemson University, Clemson, SC, USA

SD Hillyard
UNLV School of Dental Medicine, Las Vegas, NV, USA

EJ Hilton
Virginia Institute of Marine Science, Gloucester Point, VA, USA

SG Hinch
University of British Columbia, Vancouver, BC, Canada

MH Hofmann
University of Bonn, Bonn, Germany

S Holmgren
University of Gothenburg, Gothenburg, Sweden

DR Huber
University of Tampa, Tampa, FL, USA

P-P Hwang
Academia Sinica, Taipei, Taiwan, China

JM Jørgensen
University of Aarhus, Aarhus, Denmark

WR Jeffery
University of Maryland, College Park, MD, USA

E Jönsson
University of Gothenburg, Gothenburg, Sweden

M Jobling
University of Tromsø, Tromsø, Norway

DR Jones
University of British Columbia, Vancouver, BC, Canada

MG Jonz
University of Ottawa, Ottawa, ON, Canada

F Jutfelt
University of Gothenburg, Gothenburg, Sweden

IM Kaatz
Stamford, CT, USA

I Katsiadaki
Cefas Weymouth Laboratory, Weymouth, UK

A Kawano
University of Waterloo, Waterloo, ON, Canada

M Kawasaki
University of Virginia, Charlottesville, VA, USA

CJ Kennedy
Simon Fraser University, Burnaby, BC, Canada

P Kiilerich
Institut National de la Recherche Agronomique (INRA), Rennes, France

SS Killen
University of Glasgow, Glasgow, Scotland, UK

M Kinoshita
Kyoto University, Kyoto, Japan

F Kirschbaum
Humboldt-Universität zu Berlin, Berlin, Germany

CS Krahforst
East Carolina University, Greenville, NC, USA

Å Krogdahl
Norwegian School of Veterinary Science, Oslo, Norway

RHH Kröger
Lund University, Lund, Sweden

D Kültz
University of California, Davis, CA, USA

F Ladich
University of Vienna, Vienna, Austria

LEJ Lee
Wilfrid Laurier University, Waterloo, ON, Canada

B Levavi-Sivan
Hebrew University of Jerusalem, Rehovot, Israel

MW Levine
University of Illinois at Chicago, Chicago, IL, USA

D Liedtke
University of Würzburg, Würzburg, Germany

JH Long Jr.
Vassar College, Poughkeepsie, NY, USA

Z Lu
University of Miami, Coral Gables, FL, USA

JJ Luczkovich
East Carolina University, Greenville, NC, USA

WI Lutterschmidt
Sam Houston State University, Huntsville, TX, USA

P Machnik
University of Bayreuth, Bayreuth, Germany

N Maclean
University of Southampton, Southampton, UK

CP Madenjian
Great Lakes Science Center, Ann Arbor, MI, USA

H Malte
Aarhus University, Aarhus, Denmark

F Marino
University of Messina, Messina, Italy

NJ Marshall
The University of Queensland, Brisbane, QLD, Australia

GB McClelland
McMaster University, Hamilton, ON, Canada

CA McCormick
Oberlin College, Oberlin, OH, USA

SD McCormick
USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, USA

DJ McKenzie
Université Montpellier 2, Montpellier, France

DB McMillan
University of Western Ontario, London, ON, Canada

M Megías
University of Vigo, Vigo, Spain

AF Mensinger
University of Minnesota Duluth, Duluth, MN, USA

JD Metcalfe
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

WK Milsom
University of British Columbia, Vancouver, BC, Canada

CMR Le Moine
University of Ottawa, Ottawa, ON, Canada

JC Montgomery
University of Auckland, Auckland, New Zealand

TW Moon
University of Ottawa, Ottawa, ON, Canada

PC Morris
Heriot-Watt University, Edinburgh, UK

CD Moyes
Queen’s University, Kingston, ON, Canada

CA Myrick
Colorado State University, Fort Collins, CO, USA

JA Nelson
Towson University, Towson, MD, USA

M Nikinmaa
University of Turku, Turku, Finland

GE Nilsson
University of Oslo, Oslo, Norway

S Nilsson
University of Gothenburg, Gothenburg, Sweden

RH Nóbrega
Utrecht University, Utrecht, The Netherlands

RG Northcutt
University of California, San Diego, La Jolla, CA, USA

D Northmore
University of Delaware, Newark, DE, USA

KR Olson
Indiana University School of Medicine – South Bend, South Bend, IN, USA

C Olsson
University of Gothenburg, Gothenburg, Sweden

K Pappas
Trinity College, Hartford, CT, USA

JC Partridge
University of Bristol, Bristol, UK

MA Peck
University of Hamburg, Hamburg, Germany

B Pelster
University of Innsbruck, Innsbruck, Austria

AE Pereda
Albert Einstein College of Medicine, Bronx, NY, USA

SF Perry
University of Ottawa, Ottawa, ON, Canada

S Pieperhoff
University of British Columbia, Vancouver, BC, Canada

F Piferrer
Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain

JE Podrabsky
Portland State University, Portland, OR, USA

MA Pombal
University of Vigo, Vigo, Spain

H-O Pörtner
Alfred-Wegener-Institute, Bremerhaven, Germany

AN Popper
University of Maryland, College Park, MD, USA

G di Prisco
National Research Council (CNR), Naples, Italy

P Prunet
Institut National de la Recherche Agronomique (INRA), Rennes, France

DJ Randall
City University of Hong Kong, Hong Kong, China

BJ Reading
North Carolina State University, Raleigh, NC, USA

SG Reebs
Université de Moncton, Moncton, NB, Canada

SG Reid
University of Toronto Scarborough, Toronto, ON, Canada

JG Richards
University of British Columbia, Vancouver, BC, Canada

DA Righton
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

JW Robinson
University of Guelph, Guelph, ON, Canada

RE Rodríguez
Institute of Neurosciences of Castilla y León, Salamanca, Spain

I Rodríguez-Moldes
Universidad de Santiago de Compostela, Santiago de Compostela, Spain

PJ Rombough
Brandon University, Brandon, MB, Canada

I Rønnestad
University of Bergen, Bergen, Norway

GG Rosenthal
Texas A&M University, College Station, TX, USA

JL Rummer
University of British Columbia, Vancouver, BC, Canada

R Russo
National Research Council (CNR), Naples, Italy

FM Sánchez-Simón
Institute of Neurosciences of Castilla y León, Salamanca, Spain

PM Schulte
University of British Columbia, Vancouver, BC, Canada

RW Schulz
Utrecht University, Utrecht, The Netherlands

W Schulze
University of Bayreuth, Bayreuth, Germany

S Schuster
University of Bayreuth, Bayreuth, Germany

CJW Scott
Heriot-Watt University, Edinburgh, UK

M Sebire
Cefas Weymouth Laboratory, Weymouth, UK

SM Secor
University of Alabama, Tuscaloosa, AL, USA

O Seppälä
EAWAG, Swiss Federal Institute of Aquatic Science and Technology, and ETH-Zürich, Institute of Integrative Biology (IBZ), Dübendorf, Switzerland

H Seth
University of Gothenburg, Gothenburg, Sweden

RS Seymour
University of Adelaide, Adelaide, SA, Australia

MA Sheridan
North Dakota State University, Fargo, ND, USA

HA Shiels
University of Manchester, Manchester, UK

KR Siegfried
Massachusetts General Hospital, Boston, MA, USA

JA Sisneros
University of Washington, Seattle, WA, USA

KA Sloman
University of the West of Scotland, Paisley, Scotland, UK

LU Sneddon
University of Chester, Chester, UK

MC Soares
Universidade de São Paulo, São Paulo, Brazil

GN Somero
Stanford University, Pacific Grove, CA, USA

PW Sorensen
University of Minnesota, St. Paul, MN, USA

MW Sprague
East Carolina University, Greenville, NC, USA

N Stacey
University of Alberta, Edmonton, AB, Canada

EM Standen
McGill University, Montreal, QC, Canada

ED Stevens
University of Prince Edward Island, Charlottetown, PE, Canada

H Straka
Ludwig-Maximilians-Universität, Martinsried-Planegg, Germany

CV Sullivan
North Carolina State University, Raleigh, NC, USA

A Sundby
Norwegian School of Veterinary Science, Oslo, Norway

KS Sundell
University of Gothenburg, Gothenburg, Sweden

F Syeda
University of Birmingham, Birmingham, UK

DA Syme
University of Calgary, Calgary, AB, Canada

EW Taylor
University of Birmingham, Birmingham, UK

H Thorarensen
Holar University College, Saudarkrokur, Iceland

KB Tierney
University of Alberta, Edmonton, AB, Canada

L Tort
Universitat Autonoma de Barcelona, Cerdanyola, Spain

ED Tytell
Johns Hopkins University, Baltimore, MD, USA

C Verde
National Research Council (CNR), Naples, Italy

J-N Volff
Université de Lyon, Lyon, France

H Volkoff
Memorial University of Newfoundland, St. John's, NL, Canada

G von der Emde
University of Bonn, Bonn, Germany

M Vornanen
University of Eastern Finland, Joensuu, Finland

H-J Wagner
Universität Tübingen, Tübingen, Germany

MM Walker
University of Auckland, Auckland, New Zealand

KN Wallace
Clarkson University, Potsdam, NY, USA

T Wang
Aarhus University, Aarhus, Denmark

Y Wang
Queen's University, Kingston, ON, Canada

W Waser
University of Turku, Turku, Finland

JF Webb
University of Rhode Island, Kingston, RI, USA

PW Webb
University of Michigan, Ann Arbor, MI, USA

NC Wegner
Scripps Institution of Oceanography, La Jolla, CA, USA

SE Wendelaar Bonga
Radboud University Heiweg 8, EB Malden,
The Netherlands

CR White
The University of Queensland, St. Lucia, QLD, Australia

MP Wilkie
Wilfrid Laurier University, Waterloo, ON, Canada

TM Winegard
University of Guelph, Guelph, ON, Canada

JM Wilson
Centre for Interdisciplinary Marine and Environmental Research (CIIMAR), Porto, Portugal

RW Wilson
University of Exeter, Exeter, UK

RJ Wootton
Aberystwyth University, Aberystwyth, Wales, UK

PA Wright
University of Guelph, Guelph, ON, Canada

Y Yamamoto
University College London, London, UK

Z Yaron
Tel-Aviv University, Tel Aviv, Israel

D Zaccone
University of Messina, Messina, Italy

G Zaccone
University of Messina, Messina, Italy

DG Zeddies
Marine Acoustics, Inc., Arlington, VA, USA

IV Zhdanova
Boston University School of Medicine,
Boston, MA, USA

LI Zon
Howard Hughes Medical Institute, Boston, MA, USA

This page intentionally left blank

HOW TO USE THE ENCYCLOPEDIA

STRUCTURE OF THE ENCYCLOPEDIA

The material in the Encyclopedia is arranged as a series of articles within a certain topic.

There are three features to help you easily find the topic you're interested in: a contents list, cross-references to other relevant articles in the Encyclopedia, and a full subject index.

1. CONTENTS LIST

The contents list, which appears at the front of all volume, lists the entries in the order that they appear in the Encyclopedia. It includes both the volume number and the page number of each entry.

2. CROSS-REFERENCES

All of the entries in the Encyclopedia have been extensively cross-referenced. The cross-references which appear at the end of an entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere
ii. To draw the readers attention to parallel discussions in other entries
iii. To indicate material that broadens the discussion

Example

The following list of cross-references may appear in text and at the end of the entry
The Muscles | Cartilaginous Fishes Cranial Muscles

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **The Muscles**: Bony Fish Cranial Muscles. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

3. INDEX

The index includes page numbers for quick reference to the information you're looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4. CONTRIBUTORS

At the start of each volume there is list of the authors who contributed to the Encyclopedia.

This page intentionally left blank

CONTENTS

VOLUME 1 – THE SENSES, SUPPORTING TISSUES, REPRODUCTION, AND BEHAVIOR

SENSING THE ENVIRONMENT

BRAIN AND NERVOUS SYSTEM

Functional Morphology of the Nervous System: An Introduction *AB Butler* 1

Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern *B Fritzsch and DC de Caprona* 6

Functional Morphology of the Brains of Agnathans *MA Pombal and M Megías* 16

Functional Morphology of the Brains of Cartilaginous Fishes *I Rodríguez-Moldes* 26

Functional Morphology of the Brains of Ray-Finned Fishes *AB Butler* 37

Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and *Latimeria* *A González and RG Northcutt* 46

Motor Control Systems of Fish *S Grillner* 56

Physiology of the Mauthner Cell: Discovery and Properties *AE Pereda and DS Faber* 66

Physiology of the Mauthner Cell: Function *DS Faber and AE Pereda* 73

Autonomic Nervous System of Fishes *S Nilsson* 80

Opioids and Opioid Receptors in Fishes *MJ Herrero-Turrión, FM Sánchez-Simón, and RE Rodríguez* 89

VISION

Vision in Fishes: An Introduction *H-J Wagner* 98

Physiological Optics in Fishes *RHH Kröger* 102

Photoreceptors and Visual Pigments *JK Bowmaker* 110

Adaptations of Photoreceptors and Visual Pigments *JK Bowmaker* 116

Inner Retina and Ganglion Cells *MW Levine* 123

Optic Tectum *D Northmore* 131

Behavioral Assessment of the Visual Capabilities of Fish *S Schuster, P Machnik, and W Schulze* 143

Color Vision and Color Communication in Reef Fish *J Marshall and K Cheney* 150

Extraretinal Photoreception *H-J Wagner* 159

Visual Adaptations to the Deep Sea *RH Douglas and JC Partridge* 166

see Communication Behavior: Visual Signals, Volume 1, p. 692
see Coloration and Chromatophores in Fishes, Volume 1, p. 489
see Bioluminescence in Fishes, Volume 1, p. 497

SMELL, TASTE, AND CHEMICAL SENSING

Chemoreception (Smell and Taste): An Introduction *TJ Hara* 183
Morphology of the Gustatory (Taste) System in Fishes *TJ Hara* 187
Morphology of the Olfactory (Smell) System in Fishes *TJ Hara* 194
Neurophysiology of Olfaction *TJ Hara* 208
Neurophysiology of Gustation *TJ Hara* 218
Chemosensory Behavior *TJ Hara* 227
see The Effects of Toxicants on Olfaction in Fishes, Volume 3, p. 2078
see Oxygen Sensing in Fish, Volume 2, p. 871

HEARING AND LATERAL LINE

Fish Bioacoustics: An Introduction *AN Popper and BM Casper* 236
Vestibular System Anatomy and Physiology *H Straka and R Baker* 244
Auditory System Morphology *AN Popper* 252
The Ear and Hearing in Sharks, Skates, and Rays *BM Casper* 262
Biomechanics of the Inner Ear in Fishes *MC Hastings* 270
Psychoacoustics: What Fish Hear *RR Fay* 276
Auditory/Lateral Line CNS: Anatomy *CA McCormick* 283
Physiology of the Ear and Brain: How Fish Hear *Z Lu* 292
Sound Source Localization and Directional Hearing in Fishes *DG Zeddies, RR Fay, and JA Sisneros* 298
Effects of Human-Generated Sound on Fish *AD Hawkins* 304
Acoustic Behavior *J Luczkovich, MW Sprague, and CS Krahforst* 311
Vocal Behavior of Fishes: Anatomy and Physiology *F Ladich and AH Bass* 321
Lateral Line Neuroethology *JC Montgomery and S Coombs* 329
Lateral Line Structure *J Webb* 336
see How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling, Volume 1, p. 684

DETECTION AND GENERATION OF ELECTRIC SIGNALS

Detection and Generation of Electric Signals in Fishes: An Introduction *BA Carlson* 347
Morphology of Electroreceptive Sensory Organs *JM Jørgensen* 350
Physiology of Ampullary Electrosensory Systems *MH Hofmann* 359
Physiology of Tuberous Electrosensory Systems *ES Fortune and MJ Chacron* 366
Active Electrolocation *G von der Emde and J Engelmann* 375
Electric Organs *AA Caputi* 387
Generation of Electric Signals *M Kawasaki* 398

Development of Electroreceptors and Electric Organs *F Kirschbaum and J-P Denizot* 409
see Shocking Comments: Electrocommunication in Teleost Fish, Volume 1, p. 699

MUSCLES, SKELETON, SKIN, AND MOVEMENT

Functional Anatomy of Bones, Muscle, and Skin: An Introduction *ED Stevens* 416

THE SKELETON

Cartilaginous Fish Skeletal Anatomy *KM Claeson and MN Dean* 419
Cartilaginous Fish Skeletal Tissues *MN Dean* 428
Bony Fish Skeleton *EJ Hilton* 434

THE MUSCLES

Cartilaginous Fishes Cranial Muscles *DR Huber, MC Soares, and MR de Carvalho* 449
Bony Fish Cranial Muscles *LA Ferry and L Hernandez* 463

THE SKIN

The Many Functions of Fish Integument *DG Elliott* 471
Functional Morphology of the Integumentary System in Fishes *DG Elliott* 476
Coloration and Chromatophores in Fishes *D Burton* 489
Bioluminescence in Fishes *AF Mensinger* 497
Hagfish Slime *DS Fudge, JE Herr, and TM Winegard* 504

BUOYANCY, LOCOMOTION, AND MOVEMENT IN FISHES

Buoyancy, Locomotion, and Movement in Fishes: An Introduction *ED Stevens* 516
Buoyancy in Fishes *RMcN Alexander* 520
Swimbladder Function and Buoyancy Control in Fishes *B Pelster* 526
Experimental Hydrodynamics *ED Tytell* 535
Undulatory Swimming *DJ Ellerby* 547
Functional Properties of Skeletal Muscle: Work Loops *DA Syme* 555
Paired Fin Swimming *EM Standen* 564
Maneuverability *PW Webb* 575
Stability and Turbulence *PW Webb and AJ Cotel* 581
Fast Start *P Domenici* 587
Feeding Mechanics *TE Higham* 597
Biomimetics: Robotics Based on Fish Swimming *JH Long Jr.* 603
see Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems, Volume 2, p. 935
see Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder, Volume 2, p. 929
see The Retia, Volume 2, p. 1119
see Deep-Sea Fishes, Volume 3, p. 1953

see Applied Aspects of Fish Swimming Performance, Volume 3, p. 1652
see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215
see The Effect of Exercise on Respiration, Volume 2, p. 812

REPRODUCTION

The Diversity of Fish Reproduction: An Introduction *KA Sloman* 613

THE REPRODUCTIVE ORGANS AND PROCESSES

Anatomy and Histology of Fish Testis *RW Schulz and RH Nóbrega* 616
Regulation of Spermatogenesis *RW Schulz and RH Nóbrega* 627
Vitellogenesis in Fishes *BJ Reading and CV Sullivan* 635
see Parental Care in Fishes, Volume 1, p. 670
see Endocrine Regulation of Fish Reproduction, Volume 2, p. 1500
see Socially Controlled Sex Change in Fishes, Volume 1, p. 662
see Nutritional Provision During Parental Care, Volume 1, p. 678
see Sexual Behavior in Fish, Volume 1, p. 656

BEHAVIOR AND PHYSIOLOGY

Linking Fish Behavior and Physiology: An Introduction *KA Sloman* 647

SOCIAL AND REPRODUCTIVE BEHAVIORS

Dominance Behaviors *KA Sloman* 649
Sexual Behavior in Fish *I Katsiadaki and M Sebire* 656
Socially Controlled Sex Change in Fishes *J Godwin* 662
Parental Care in Fishes *S Balshine and KA Sloman* 670
Nutritional Provision During Parental Care *KA Sloman and J Buckley* 678
see Hormonal Pheromones, Volume 2, p. 1553
see The Diversity of Fish Reproduction: An Introduction, Volume 1, p. 613

SENSORY SYSTEMS, PERCEPTION, AND LEARNING

How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling *IM Kaatz* 684
Communication Behavior: Visual Signals *GG Rosenthal and NJ Marshall* 692
Shocking Comments: Electrocommunication in Teleost Fish *K Pappas and K Dunlap* 699
Fish Learning and Memory *VA Braithwaite* 707
Nociception or Pain in Fish *LU Sneddon* 713
Spatial Orientation: How do Fish Find their Way Around? *T Burt de Perera* 720
Magnetic Sense in Fishes *MM Walker* 726
Circadian Rhythms in Fish *SG Reebs* 736
see Bioluminescence in Fishes, Volume 1, p. 497

see Acoustic Behavior, Volume 1, p. 311
see Behavioral Assessment of the Visual Capabilities of Fish, Volume 1, p. 143
see Chemosensory Behavior, Volume 1, p. 227

BEHAVIORAL RESPONSES TO THE ENVIRONMENT

A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food *MV Abrahams* 744
Effects of Compensatory Growth on Fish Behavior *D Álvarez* 752
Temperature Preference: Behavioral Responses to Temperature in Fishes *LI Crawshaw and JE Podrabsky* 758
Behavioral Responses to Hypoxia *KA Sloman* 765
Sleep in Fish *IV Zhdanova* 772
Manipulation of Fish Phenotype by Parasites *O Seppälä* 777
Anthropogenic Influences on Fish Behavior *KA Sloman* 783
see The Biology of Fish Migration, Volume 3, p. 1921

VOLUME 2 – GAS EXCHANGE, INTERNAL HOMEOSTATIS, AND FOOD UPTAKE

GAS EXCHANGE

Respiration: An Introduction *JJ Cech Jr. and CJ Brauner* 791

VENTILATION AND ANIMAL RESPIRATION

Plasticity in Gill Morphology *GE Nilsson* 796
Gill Respiratory Morphometrics *NC Wegner* 803
The Effect of Exercise on Respiration *H Thorarensen* 812
Efficiency of Gas Exchange Organs *H Malte* 820
Respiratory Gas Exchange During Development: Models and Mechanisms *PJ Rombough* 829
Respiratory Gas Exchange During Development: Respiratory Transitions *PJ Rombough* 838
Techniques in Whole Animal Respiratory Physiology *JJ Cech Jr. and CJ Brauner* 846
see Branchial Anatomy, Volume 2, p. 1095

CONTROL OF RESPIRATION

Generation of the Respiratory Rhythm in Fish *EW Taylor* 854
The Ventilatory Response to CO_2/H^+ *WK Milsom* 865
Oxygen Sensing in Fish *MG Jonz* 871

TRANSPORT AND EXCHANGE OF RESPIRATORY GASES IN THE BLOOD

Red Blood Cell Function *M Nikinmaa* 879
Hemoglobin *M Nikinmaa* 887
O_2 Uptake and Transport: The Optimal P_{50} *T Wang and H Malte* 893
Carbonic Anhydrase in Gas Transport and Exchange *KM Gilmour* 899

Carbon Dioxide Transport and Excretion *D Randall* 909
Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange *CJ Brauner and JL Rummer* 916
Evolution of the Bohr Effect *M Berenbrink* 921
Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder *W Waser* 929
Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems *M Berenbrink* 935
Hemoglobin Differentiation in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 944
see Cellular Composition of the Blood, Volume 2, p. 984

TISSUE RESPIRATION

Cellular Respiration *GB McClelland* 951
Mitochondrial Respiration *CD Moyes and CMR Le Moine* 959
Specializations in Mitochondrial Respiration of Fish *CD Moyes and CMR Le Moine* 966

CIRCULATION

The Circulatory System: An Introduction *AP Farrell* 973
Circulatory System Design: Roles and Principles *KR Olson* 977

BLOOD

Cellular Composition of the Blood *AP Farrell* 984
Erythropoiesis in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 992
see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Red Blood Cell Function, Volume 2, p. 879
see Hemoglobin, Volume 2, p. 887

DESIGN AND PHYSIOLOGY OF THE HEART

Cardiac Anatomy in Fishes *AP Farrell and S Pieperhoff* 998
Cellular Ultrastructure of Cardiac Cells in Fishes *GLJ Galli* 1006
The Outflow Tract from the Heart *DR Jones and MH Braun* 1015
Physiology of Cardiac Pumping *AP Farrell* 1030
Action Potential of the Fish Heart *M Vornanen* 1038
Cardiac Excitation–Contraction Coupling: Routes of Cellular Calcium Flux *HA Shiels* 1045
Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element *TE Gillis* 1054
Cardiac Cellular Length–Tension Relationship *HA Shiels* 1060
Intracardiac Neurons and Neurotransmitters in Fish *G Zaccone, A Mauceri, and D Zaccone* 1067
Accessory Hearts in Fishes *AP Farrell* 1073
The Coronary Circulation *AP Farrell* 1077

DESIGN AND PHYSIOLOGY OF ARTERIES AND VEINS

Anatomical Pathways and Patterns *KR Olson* 1085
Branchial Anatomy *KR Olson* 1095
Physiology of Resistance Vessels *KR Olson* 1104

Physiology of Capacitance Vessels *KR Olson* 1111

The Retia *ED Stevens* 1119

The Gastrointestinal Circulation *M Axelsson and H Seth* 1132

see Secondary Circulation and Lymphatic Anatomy, Volume 2, p. 1161

see The Coronary Circulation, Volume 2, p. 1077

DESIGN AND PHYSIOLOGY OF CAPILLARIES AND SECONDARY CIRCULATION

Capillaries, Capillarity, and Angiogenesis *S Egginton and F Syeda* 1142

Circulatory Fluid Balance and Transcapillary Exchange *KR Olson* 1154

Secondary Circulation and Lymphatic Anatomy *KR Olson and AP Farrell* 1161

INTEGRATED CONTROL AND RESPONSE OF THE CIRCULATORY SYSTEM

Integrated Control of the Circulatory System *KR Olson* 1169

Central Control of Cardiorespiratory Interactions in Fish *EW Taylor* 1178

Hormone Metabolism in the Circulation *KR Olson* 1190

Integrated Responses of the Circulatory System to Temperature *AK Gamperl* 1197

Integrated Responses of the Circulatory System to Digestion *H Seth and M Axelsson* 1206

Integrated Cardiovascular Responses of Fish to Swimming *AP Farrell* 1215

Integrated Responses of the Circulatory System to Hypoxia *AK Gamperl* 1221

see Hormone Metabolism in the Circulation, Volume 2, p. 1190

see Accessory Hearts in Fishes, Volume 2, p. 1073

see Respiratory Adaptations for Air-Breathing Fishes, Volume 3, p. 1861

see The Osmorespiratory Compromise, Volume 2, p. 1389

THE GUT

The Gastrointestinal Tract: An Introduction *C Olsson and S Holmgren* 1229

Appendix A 1232

GUT ANATOMY AND MORPHOLOGY

Gut Anatomy *C Olsson* 1268

Pancreas *MA Caruso and MA Sheridan* 1276

Development of Fish Gut *KN Wallace* 1284

see The Gastrointestinal Circulation, Volume 2, p. 1132

INTEGRATED FUNCTION AND CONTROL OF THE GUT

Gut Motility *A Gräns and C Olsson* 1292

Gut Secretion and Digestion *Å Krogdahl, A Sundby, and AM Bakke* 1301

Intestinal Absorption *KS Sundell I Rønnestad* 1311

Barrier Function of the Gut *F Jutfelt* 1322

Nervous System of the Gut *S Holmgren and C Olsson* 1332
Endocrine Systems of the Gut *E Jönsson and S Holmgren* 1341
see Integrated Responses of the Circulatory System to Digestion, Volume 2, p. 1206

OSMOTIC, IONIC AND NITROGENOUS-WASTE BALANCE

Osmoregulation in Fishes: An Introduction *DH Evans* 1348
Mechanisms of Gill Salt Secretion in Marine Teleosts *DH Evans* 1354
Mechanisms of Ion Transport in Freshwater Fishes *P-P Hwang* 1359
Water Balance and Aquaporin *CP Cutler* 1366
Osmosensing *D Kültz* 1373

ROLE OF THE GILLS

Morphology of Branchial Ionocytes *JM Wilson* 1381
The Osmorespiratory Compromise *RJ Gonzalez* 1389

ROLE OF THE KIDNEYS

Histology of the Kidney *DB McMillan* 1395
The Kidney *SF Perry* 1411

ROLE OF THE GUT

Gut Ion, Osmotic and Acid-Base Regulation *RW Wilson* 1419
Dogfish Rectal Gland *WW Dowd* 1429
see The Hormonal Control of Osmoregulation in Teleost Fish, Volume 2, p. 1466
see Barrier Function of the Gut, Volume 2, p. 1322
see Endocrine Systems of the Gut, Volume 2, p. 1341
see Intestinal Absorption, Volume 2, p. 1311

NITROGENOUS-WASTE BALANCE

Excretion of Ammonia *D Randall* 1437
Ureotelism *PA Wright* 1444

HORMONAL CONTROLS

THE PITUITARY

Development of the Hypothalamus-Pituitary-Interrenal Axis *D Alsop and N Aluru* 1450
Pituitary Gland or Hypophysis *DB McMillan* 1457

HORMONAL CONTROL OF METABOLISM AND IONIC REGULATION

The Hormonal Control of Osmoregulation in Teleost Fish *SD McCormick* 1466
Corticosteroids *P Kiilerich and P Prunet* 1474

HORMONAL CONTROL OF REPRODUCTION AND GROWTH

Endocrinology of Fish Growth *MA Sheridan* 1483

Endocrine Control of Sex Differentiation in Fish *F Piferrer* 1490

Endocrine Regulation of Fish Reproduction *Z Yaron and B Levavi-Sivan* 1500

Control of Appetite in Fish *H Volkoff* 1509

HORMONAL RESPONSES TO STRESS

Hormone Response to Stress *SE Wendelaar Bonga* 1515

Catecholamines *SG Reid* 1524

Stress Effect on Growth and Metabolism *TW Moon* 1534

Impact of Stress in Health and Reproduction *L Tort* 1541

HORMONES IN COMMUNICATION

Hormonal Pheromones *N Stacey and P Sorensen* 1553

see Hormone Metabolism in the Circulation, Volume 2, p. 1190

see Integrated Control of the Circulatory System, Volume 2, p. 1169

VOLUME 3 – ENERGETICS, INTERACTIONS WITH THE ENVIRONMENT, LIFESTYLES, AND APPLICATIONS

ENERGETICS

Energetics: An Introduction *JA Nelson* 1563

General Energy Metabolism *JA Nelson and D Chabot* 1566

Physiological Functions that Scale to Body Mass in Fish *CR White and RS Seymour* 1573

FOOD ACQUISITION AND DIGESTION

Energetics of Prey Capture: From Foraging Theory to Functional Morphology *LA Ferry* 1583

Energetics of Foraging Decisions and Prey Handling *S Killen* 1588

Digestive Efficiency *DP German* 1596

Cost of Digestion and Assimilation *SM Secor* 1608

Dietary Requirements of Fish Under Culture Conditions *IP Forster* 1617

see A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food, Volume 1, p. 744

ENERGY UTILIZATION IN GROWTH

Energetics of Growth *RJ Wootton* 1623

Growth: Environmental Effects *RJ Wootton* 1629

see Effects of Compensatory Growth on Fish Behavior, Volume 1, p. 752

SWIMMING AND OTHER ACTIVITIES

Energetics of Fish Swimming *DJ McKenzie* 1636
Cellular Energy Utilization: Environmental Influences on Metabolism *H-O Pörtner* 1645
Applied Aspects of Fish Swimming Performance *T Castro-Santos* 1652
see Dominance Behaviors, Volume 1, p. 649
see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215
see The Effect of Exercise on Respiration, Volume 2, p. 812
see The Osmorespiratory Compromise, Volume 2, p. 1389
see Functional Properties of Skeletal Muscle: Work Loops, Volume 1, p. 555

ENERGETIC MODELS

Bioenergetics in Aquaculture Settings *M Jobling* 1664
Bioenergetics in Ecosystems *CP Madenjian* 1675

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

General Principles of Biochemical Adaptations *GN Somero* 1681

TEMPERATURE

Effects of Temperature: An Introduction *PM Schulte* 1688
Measures of Thermal Tolerance *TL Beitinger and WI Lutterschmidt* 1695
Proteins and Temperature *PA Fields* 1703
Mitochondria and Temperature *H Guderley* 1709
Temperature and Excitable Membranes *M Vornanen* 1717
Membranes and Temperature: Homeoviscous Adaptation *CD Moyes and JS Ballantyne* 1725
Heat Shock Proteins and Temperature *S Currie* 1732
Effects of Climate Change *H-O Pörtner and MA Peck* 1738
see Temperature Preference: Behavioral Responses to Temperature in Fishes, Volume 1, p. 758
see Integrated Responses of the Circulatory System to Temperature, Volume 2, p. 1197

HYPOXIA

The Expanding Hypoxic Environment *RJ Díaz and DL Breitburg* 1746
Respiratory Responses to Hypoxia in Fishes *SF Perry* 1751
Anaerobic Metabolism in Fish *Y Wang and JG Richards* 1757
Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia *JG Richards* 1764
see General Principles of Biochemical Adaptations, Volume 3, p. 1681
see Behavioral Responses to Hypoxia, Volume 1, p. 765
see Integrated Responses of the Circulatory System to Hypoxia, Volume 2, p. 1221

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

HAGFISHES AND LAMPREY

Hagfishes *ME Forster* 1771

Lampreys: Energetics and Development *MP Wilkie* 1779

Lampreys: Environmental Physiology *MP Wilkie* 1788

see Hagfish Slime, Volume 1, p. 504

see Functional Morphology of the Brains of Agnathans, Volume 1, p. 16

STURGEON

Physiology of Sturgeon *SI Doroshov and JJ Cech Jr.* 1800

CHONDRICHTHYES

Physiology of Sharks, Skates, and Rays *JS Ballantyne and JW Robinson* 1807

see Functional Morphology of the Brains of Cartilaginous Fishes, Volume 1, p. 26

see The Ear and Hearing in Sharks, Skates, and Rays, Volume 1, p. 262

BONY FISHES

Zebrafish *JG Richards* 1819

Crucian Carp *GE Nilsson* 1824

Life in Hot Water: The Desert Pupfish *SD Hillyard* 1831

Blind Cavefish *Y Yamamoto and WR Jeffery* 1843

see Eel Migrations, Volume 3, p. 1937

AIR-BREATHING FISHES

The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction *JB Graham* 1850

Respiratory Adaptations for Air-Breathing Fishes *JB Graham* 1861

Circulatory Adaptations for Air-Breathing Fishes *JB Graham* 1875

PELAGIC FISHES

An Introduction to the Biology of Pelagic Fishes *D Bernal* 1887

Physiology of Tuna *KA Dickson* 1903

Endothermy in Tunas, Billfishes, and Sharks *BA Block* 1914

see Tracking Oceanic Fish, Volume 3, p. 1928

FISH MIGRATIONS

The Biology of Fish Migration *TR Binder, SJ Cook, and SG Hinch* 1921

Tracking Oceanic Fish *BA Block* 1928

Eel Migrations *D Righton and J Metcalfe* 1937

Pacific Salmon Migration: Completing the Cycle *SJ Cooke, GT Crossin, and SG Hinch* 1945

see Spatial Orientation: How do Fish Find their Way Around?, Volume 1, p. 720

DEEP-SEA FISHES

Deep-Sea Fishes *AP Farrell* 1953

INTERTIDAL FISHES

Intertidal Habitats *PM Schulte* 1959

APPLIED ASPECTS OF FISH PHYSIOLOGY

CELLULAR, MOLECULAR, GENOMICS, AND BIOMEDICAL APPROACHES

Culture of Fish Cell Lines *NC Bols, A Kawano, and LEJ Lee* 1965
Pigment Genes and Cancer Genes *I Braasch and D Liedtke* 1971
Evolution of Fish Genomes *J-N Volff, F Brunet, A Böhne, and D Galiana-Arnoux* 1980
Fish as Model Organisms for Medical Research *JN Cech and LI Zon* 1986
Transgenesis and Chromosome Manipulation in Fish *N Maclean* 1998
Physiology of Triploid Fish *TJ Benfey* 2009
Growth Hormone Overexpression in Transgenic Fish *RH Devlin* 2016
Molecular and Chromosomal Aspects of Sex Determination *KR Siegfried* 2025
Molecular Fish Pathology *CJW Scott, PC Morris, and B Austin* 2032
Germ Cell Migration and Trans Sex *M Kinoshita* 2046
Global Gene Expression Profiling in Fish *AY Gracey* 2055

see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Erythropoiesis in Fishes, Volume 2, p. 992
see Blind Cavefish, Volume 3, p. 1843
see Zebrafish, Volume 3, p. 1819

TOXICOLOGY

The Toxicology of Metals in Fishes *CJ Kennedy* 2061
The Toxicology of Organics in Fishes *CJ Kennedy* 2069
The Effects of Toxicants on Olfaction in Fishes *KB Tierney* 2078

see Anthropogenic Influences on Fish Behavior, Volume 1, p. 783

AQUACULTURE

Physiology of Fish in Culture Environments *CA Myrick* 2084

see Dietary Requirements of Fish Under Culture Conditions, Volume 3, p. 1617
see Bioenergetics in Aquaculture Settings, Volume 3, p. 1664
see Physiology of Triploid Fish, Volume 3, p. 2009
see Manipulation of Fish Phenotype by Parasites, Volume 1, p. 777

INDEX 2091

PREFACE

The *Encyclopedia of Fish Physiology: From Genome to Environment* is the first of its kind. It follows in the strong tradition built by the book series *Fish Physiology* that was pioneered in the late 1960s by Bill Hoar and Dave Randall and is still ongoing; however, the Encyclopedia differs markedly from this book series in three important respects.

Foremost, the Encyclopedia targets a much broader audience, not just the research community. My hope is that the Encyclopedia will serve as a general educational tool for biologists, as well as being of general use as an information resource for those interested in fishes. After all, the Encyclopedia is replete with magnificent examples of how fishes are marvelously adapted to almost every water body on the Earth, no matter how deep, how hot, how cold, how salty, or, in some cases, how dry!

Second, the articles span well beyond just the physiology of fishes. The intent here is to connect physiological functions, environmental factors, behavior, and the genome. This is the case even though the main focus here is on physiology.

Third, and as a result, the breadth of topics covered by the Encyclopedia far exceeds that of any focused volume of the *Fish Physiology* book series. Thus, the Encyclopedia is ambitious in both its content and its target audience.

The Encyclopedia contains a very impressive array of entries, each in the form of a discrete, self-standing article that is typically 3000–4000 words in length, not counting the ancillary material contained in figures, glossary, further reading, etc. In total, there are 252 articles written by over 200 international experts from over 20 countries.

The articles are arranged thematically. The larger thematic sections have an introductory article, written by a section editor, who explains contributions in that theme and places them in a broader and often historical context. Tributes are deservedly given to those who made key scientific advances.

The electronic format of the Encyclopedia was deliberate and a crucial requirement before this project even started. I have to thank Andy Richford for garnering Elsevier's support of this idea, as well as initiating this project when he was an acquisition editor. The electronic format, through 'hot keys', provides an easy and almost instant access to the glossary and related articles. Each article is generously linked to many others in this manner, offering the reader easy digressions or more detailed information and explanations. The 'Further Reading' section associated with each article provides additional resources.

This ambitious opus could not have been possible without the dedication and commitment of a great number of individuals. I had an idea that I have steered to completion, but others did most of the hard work. I am especially indebted to my associate editors, Don Stevens, Joe Cech, and Jeffrey Richards. They embraced my dream early on and stayed with me until completion. Without their wisdom, breadth of knowledge, and hard work, the Encyclopedia could not have come to fruition. I think we worked well together, and, on my part, it was a distinct pleasure to have such enthusiastic support.

I am equally indebted to the section editors: Colin Brauner, Ann Butler, Bruce Carlson, Brandon M Casper, Greg Goss, Jeff Graham, Toshiaki Hara, Sue Holmgren, Norman Maclean, Jay Nelson, Ken Olson, Catharina Olsson, Arthur Popper, Trish Schulte, Bob Shadwick, Kath Sloman, Adam Summers, Matt Vijayan, and Hans-Joachim Wagner. They shaped their section and carried the core of the editorial responsibilities for the contributed articles during the many rounds of review and revisions.

Finally, and importantly, there are over 200 authors and their collaborators, who contributed the articles. My hope is that your rewards go well beyond the recompense, which is a pittance for the time that I know you devoted to generating such marvelous articles. You are thanked for your dedication and trust in the project, and are congratulated for your respective skills and knowledge.

There is another side to the production of this Encyclopedia, and that is the Elsevier team, with whom the editors and authors interacted. This team was headed by Kristi Gomez, the acquisitions editor in San Diego. Milo Perkins, the

development editor in the Oxford office, was our go-to-man after Esmond Collins had set the ball rolling. Milo endured the rough and the smooth, and his rapid responses, as well as his flexible deadlines, were greatly appreciated. Kate Mittell worked quietly and efficiently in the background supporting Milo and was responsible for keeping me on track with the enormous glossary of terms. Nicky Carter, our project manager, superbly rounded out the team. We relied on her for the professional appearance and content of the final copy.

As Editor-in-Chief, I am ultimately responsible for any errors contained herein. If you find any, please let me know so that they can be corrected; likewise, if you feel there are important omissions, please let me know of these. They might be incorporated in future updates.

Bringing this Encyclopedia to its conclusion means one thing for me personally: Miriam, Dustin, Naomi, Nicole and Natalie will all be seeing a lot more of me over the next while! Miriam, your patience throughout was astounding, although it was pushed to the limit at times.

Tony Farrell
Vancouver

GAS EXCHANGE

Respiration: An Introduction

JJ Cech Jr., University of California, Davis, CA, USA

CJ Brauner, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Environment: Water and Air as Respiratory Media
Ventilation and Gas-Exchange Organs
Gas Transport and Exchange
Tissue Respiration
Whole Animal and Techniques in Respiratory Physiology
Further Reading

Glossary

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Carbonic anhydrase A zinc metalloenzyme that reversibly catalyzes the reaction of CO_2 and H_2O to form H^+ and HCO_3^-.

Diffusion Net movement of a solute from an area of higher concentration to an area of lower concentration.

Equilibrium Pertaining to the situation when all forces acting are balanced by others resulting in a stable unchanging system.

Haldane effect Proton binding to hemoglobin (as a function of oxygenation).

Hypoxia Low partial pressures of oxygen in external or internal environments.

Interlamellar cell mass (ILCM) A mass made up of undifferentiated cells and ionocytes, and possibly other cell types, filling up a variable part of the space between the lamellae of fish gills.

Lamellae Also known as secondary lamellae, these are attached in rows to the gill filaments. They are the primary sites for gas exchange in fish gills. Each lamella is made up of two epithelial layers separated by pillar cells. Oxygen is taken up by erythrocytes flowing inside the lamellae from water flowing between the lamellae.

Mitochondria Organelles that produce most of the aerobic energy required by the cell.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Partial pressure The atmospheric pressure exerted by O_2 alone proportional to the total concentration of this gas. It is typically measured in either mmHg (torr) or kPa.

Respiratory cascade A model of gas exchange in which gas is viewed as flowing through a series of resistances from the environment to the tissues or vice versa. The model is based on the analogy of water flowing down a series of cascades with the difference being that gas flow is driven by differences in partial pressure rather than gravity.

Rete Structure consisting of blood vessels arranged to facilitate the exchange of heat or oxygen.

Root effect A property of fish hemoglobin in which protons decrease the maximal oxygen saturation of hemoglobin. For practical purposes, it is defined as a reduction of oxygen saturation at atmospheric oxygen tension.

Ventilation The movement of the respiratory medium (air or water) over the surface of the gas exchanger.

Introduction

Respiration and gas exchange is an essential process to maintain an aerobic existence in all vertebrates, including fishes. The uptake of oxygen (O_2), along with metabolism of organic substrates such as glucose and lipids, is needed to power the biochemical machinery (e.g., in the mitochondria) in cells for body maintenance, as well as for other aerobic functions such as growth, movement, reproduction, and disease resistance (**see also Energetic Models**: Bioenergetics in Aquaculture Settings and Bioenergetics in Ecosystems), all of which are important determinants of fitness.

The complex process of respiration in fish is discussed in detail in this section. It starts with the environment where O_2 and CO_2 move into and out of the animal, respectively, by simple diffusion. Gases diffuse across a gas-exchange organ which represents the interface between the organism and the environment and may consist of skin, gills, and in some cases an air-breathing organ. Ventilation of the respective media (water and in some cases air if an air-breathing organ is present) in conjunction with blood perfusion across the gas-exchange organ, both of which can be altered proportionally depending upon the animal's metabolic state, ensures sufficient gas exchange to meet the demands of the animal.

The circulatory system provides the conduit through which the blood is ultimately delivered to the tissues; hemoglobin (Hb), maintained within the red blood cell, plays a vital role in the transport of both O_2 and CO_2 in all fishes, except icefishes, which represent the only vertebrate that lacks Hb. Hemoglobin is a remarkable molecule and is one of the best-understood proteins in terms of how changes in the environment of the red blood cell alter the tertiary and quaternary structure of Hb to influence the nature in which Hb binds and releases ligands such as O_2, CO_2, and H^+'s in particular. These changes optimize conditions for gas exchange to tissues in general, as well as specifically to the eye and swimbladder allowing for acute vision and buoyancy control respectively in many teleosts.

Finally, blood reaches the tissues where waste products are removed and substrates supplied to cells and mitochondria providing the basics for cellular and mitochondrial respiration, and thus life. The following describes these various steps in respiration in more detail, providing background information and introducing each article that appears within the section.

The Environment: Water and Air as Respiratory Media

Characteristics of the environment can have a profound effect on respiration. Respiratory gas exchange in aquatic environments presents different problems when compared with respiration in air. Water is a dense, viscous medium, which also has a high heat capacity and 20–30-fold lower oxygen concentration (due to low gas solubility) relative to air. Increases in temperature or salinity further decrease oxygen solubility in water, and therefore oxygen content for a given gas pressure as indicated in **Figure 1**.

The P_{O_2} and P_{CO_2} of water can vary dramatically compared to those of atmospheric air. This is because the gases contained in air do not necessarily exchange readily with water. The P_{O_2} of aquatic environments can be zero (anoxia), low (hypoxic), normoxic, or high (hyperoxic), depending on the photosynthetic and respiratory rates of the biotic community and on water circulation characteristics. Some shallow, freshwater habitats may vary between 20 and 40 mmHg P_{O_2} just before dawn to 200–400 mmHg at mid-day. Thus, water can be less saturated and more saturated with oxygen, even on a diel basis. Many fishes, especially those that spend at least part of their lives in shallow habitats, have evolved structural and functional abilities to deal with variable water P_{O_2} values.

Fish in boreal, subpolar, or polar lakes may experience a seasonal challenge of oxygen availability – a

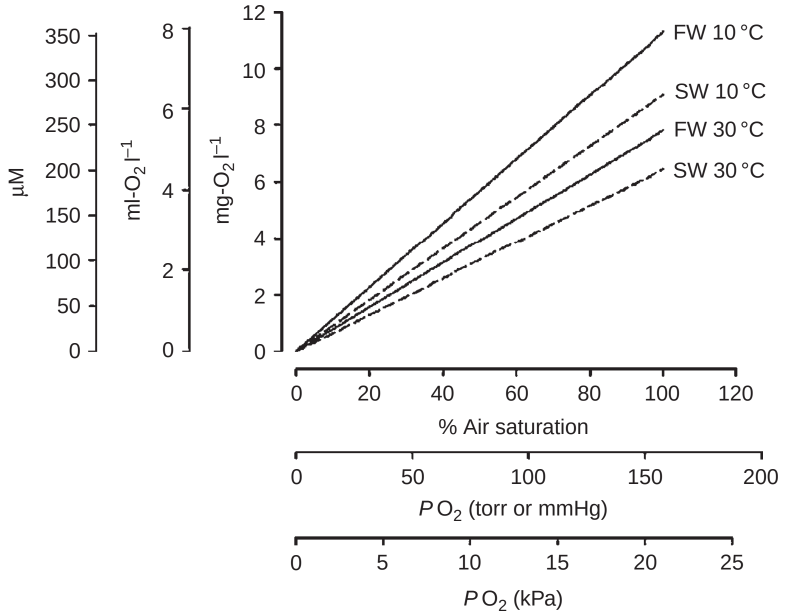


Figure 1 The relationship between partial pressure of oxygen (*x*-axis) and total oxygen content (*y*-axis) at 10 and 30 °C in either freshwater (FW) or seawater (SW). The three legends for each of the axes represent the different units that are used to describe both partial pressure and content in water. Reproduced from Diaz RJ and Breitburg DL (2009) The hypoxic environment. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Volume 27 Hypoxia*, pp. 1–23. Academic Press, with permission from Elsevier.

gradually deepening hypoxia (lowered P_{O_2}, s) during the winter as ice cover seals off gas exchange with the atmosphere and snow cover, along with shortened photoperiods, attenuate incoming light decreasing photosynthetic production of O_2. Extreme winters may prolong these conditions leading to a total consumption of the remaining dissolved O_2 (anoxia) by the lake's biota, leading to a winter fish kill (**see also Hypoxia**: The Expanding Hypoxic Environment).

Fishes living in high-altitude environments must also cope with low-P_{O_2} water. In this case, it is due to the lower total barometric pressure (and the correspondingly lowered partial pressures of the atmospheric gases). Finally, certain types of pollution, including those that introduce excessive nutrients into waterways (eutrophication), typically lead to wider, diel dissolved-O_2 ranges, often including quite hypoxic P_{O_2},s. Thus, fish living and respiring aquatic media are subjected to large and routine changes in O_2 availability relative to air-breathing animals and many are adapted to these potentially extreme conditions.

Ventilation and Gas-Exchange Organs

The physical and chemical characteristics of aquatic environments, specifically low oxygen solubility of water, probably contributed to the evolutionary development of gill structure and function, and to the many mechanisms some fishes use to extract oxygen directly from the air. Article **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs discusses in greater detail the implications of water and air as respiratory media on gas exchange and how various gas-exchange organs differ in terms of efficiency of, and capacity for, gas exchange. The control of gill ventilation is crucial for survival to ensure adequate water flow over the gills. While control of ventilation and ventilatory responses to hypoxia are discussed in other sections, article **Control of Respiration**: The Ventilatory Response to CO_2/H^+ discusses the effect of CO_2 on ventilation and the role of CO_2 in controlling ventilation in fish.

The gill represents the predominant surface for gas exchange, which occurs predominantly across the gill lamellae in adult fish, and a great deal of variability in gill design has evolved among fishes. However, there is also a great deal of plasticity in gill morphology where large changes are observed in some fishes during exposure to hypoxia in particular. In hypoxia, there can be expansion of the total lamellar surface area in some fish species, such as carp, by as much as sevenfold due to the disappearance of an interlamellar cell mass that exists under normoxic conditions. These relatively large and often rapid changes in gill morphology may be more common among fish than previously thought. This exciting, relatively novel finding is discussed in **Ventilation and Animal Respiration**: Plasticity in Gill Morphology.

While the gills are usually the predominant site for gas exchange in adult fish, this is not the case early in development when the total body surface area:volume ratio in larval and juvenile fish is high and gill secondary lamellae in particular have yet to be fully developed. At this point, all gas exchange is across the skin. The fundamental principles associated with gas exchange in aquatic media and their implications for larval fishes are discussed in **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms. This article lays the foundations for a discussion of when the gills become important for gas exchange during development which is discussed in **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions. Interestingly, the gills may take on a more significant role for ionoregulation than for gas exchange early in development, based upon the time that 50% of whole body unidirectional Na^+ uptake and O_2 uptake transitions to the gills. This ontogeny has interesting implications for the evolution of gill function.

Gas Transport and Exchange

Once O_2 has diffused across the gill lamellae, about 98% is reversibly bound to Hb (oxyhemoglobin) contained within the red blood cell. The relatively high affinity of Hb for oxygen helps to maintain the partial pressure gradient for diffusion, maintaining high rates of oxygen uptake at the gills. The importance of Hb-O_2 affinity (usually characterized by the partial pressure at which 50% of the Hb molecules are oxygenated; P_{50}), and the shape of the oxygen equilibrium curve to O_2 uptake and transport are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P50. Once within the red blood cell, O_2 is bound to Hb which consists of two α and two β globin chains. The structure of Hb, models that describe Hb-O_2 binding, and factors that influence O_2 binding in different fish groups are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin.

At the gills, any physically dissolved CO_2 diffuses down its partial pressure gradient to be excreted across the gill lamellae into the water. About 95% of the CO_2 transported in the blood exists as HCO_3^-, mostly in the plasma. The dissolved CO_2, which moves easily across gill epithelia, diffuses across the lamellar epithelium into the aquatic environment, which usually has a high absorbing capacity (i.e., acts as an infinite sink) for CO_2. The corresponding decrease in plasma CO_2 creates conditions for HCO_3^- dehydration ($H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$), which occurs relatively slowly in the plasma but very rapidly in the red blood cell due to high levels of the catalyst carbonic anhydrase. Carbonic anhydrase accelerates the reaction by up to 25 000 times and fish have many different isoforms, which are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange. With continued HCO_3^- dehydration, plasma HCO_3^- is transported into the red blood cell by Cl^-/HCO_3^- exchange and H^+'s are supplied within the red blood cell by Hb, which can act as a buffer, or release H^+'s upon Hb oxygenation known as the Haldane effect. This process continues until the blood leaves the gills which is discussed in greater detail in **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion. The microenvironment of the red blood cell is regulated independently of that in the blood plasma, which is crucial for both O_2 and CO_2 transport. The processes responsible for the regulation of red blood cell pH and volume are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function.

At the tissues, the processes discussed above occur in reverse. CO_2 produced by the tissues diffuses into the blood, where it is hydrated to HCO_3^- and H^+. The H^+'s may be bound by Hb reducing Hb-O_2 affinity by the Bohr effect and reducing Hb-O_2 affinity facilitating O_2 delivery to the tissues. The Bohr effect is thought to have evolved 3 times independently, and in teleosts was accompanied by a reduction in Hb buffer value so that small blood-acid loads exert relatively large effects relative to those in other vertebrates. This is discussed in greater detail in **Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect. The relatively large Bohr effect and low buffer value in teleosts have large implications for the interaction between O_2 and CO_2 exchange, where CO_2 production in the tissues facilitates O_2 delivery via the Bohr effect, and O_2 delivery promotes CO_2 removal via the Haldane effect, the reverse processes occurring at the gills. The implications of this are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange, and taking into account large disequilibrium states that may exist in fish, the benefit to oxygen delivery may be much larger than previously thought.

While the Bohr effect refers to a decrease in Hb-O_2 affinity with a reduction in pH, the Root effect found in the blood of most teleosts refers to a decrease in the O_2-carrying capacity of Hb at low pH so that even at atmospheric O_2 tensions and higher, Hb cannot be fully oxygenated. The Root effect, together with a vascular countercurrent system called a rete and the generation of a localized acidosis, dramatically increases P_{O_2} and facilitates O_2 delivery to the poorly vascularized retinas and to the swimbladder in most teleosts. The molecular basis and evolution of the Root effect are discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems, and the processes through which the Root effect facilitates O_2 delivery to these structures is discussed in **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Tissue Respiration

As blood enters the tissues, O_2 diffuses down its partial pressure gradient into every cell in the body. Adenosine triphosphate (ATP) production by oxidative phosphorylation requires adequate delivery of both oxygen and metabolic fuels to cells and is regulated to meet metabolic demand. The biochemical pathways and fuels used in cellular respiration, along with the influence of the environment and limits to cellular respiration are discussed in **Tissue Respiration**: Cellular Respiration. The ultimate destination for O_2 within the body is the mitochondrion, an organelle found in most cells of all eukaryotic organisms. The mitochondrion is the site of ATP production and is responsible for most of the O_2 consumed by fish. Many of the characteristics of mitochondria are similar among eukaryotes as they arose through endosymbiosis prior to the divergence of plants, fungi, and animals. The basic features of mitochondria are discussed in **Tissue Respiration**: Mitochondrial Respiration, which provides the background for a discussion of some of the unique specializations that are seen in fish which is presented in **Tissue Respiration**: Specializations in Mitochondrial Respiration of Fish.

Whole Animal and Techniques in Respiratory Physiology

The whole is greater than the sum of its parts, and this certainly applies to respiration and the respiratory system in fishes. At each level of biological organization discussed above, respiration in fish is complex. However, adding to this complexity is that all these processes and reactions must be integrated within the whole animal, the level at which natural selection operates. Exercise increases the rate at which all these steps in the respiratory system must operate and is often used as a tool to shed light on which if any steps in the respiratory cascade from environment to tissues may be rate limiting. Changes in respiration during exercise are discussed in **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration and some of the techniques used in the field are described in the section Techniques in Whole Animal Respiratory Physiology.

See also: **Control of Respiration**: The Ventilatory Response to CO_2/H^+. **Energetic Models**: Bioenergetics in Aquaculture Settings; Bioenergetics in Ecosystems. **Hypoxia**: The Expanding Hypoxic Environment. **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration; Specializations in Mitochondrial Respiration of Fish. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Carbonic Anhydrase in Gas Transport and Exchange; Evolution of the Bohr Effect; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; O_2 Uptake and Transport: The Optimal P50; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Plasticity in Gill Morphology; Respiratory Gas Exchange During Development: Models and Mechanisms; Respiratory Gas Exchange During Development: Respiratory Transitions; Techniques in Whole Animal Respiratory Physiology; The Effect of Exercise on Respiration.

Further Reading

Boutilier RG (1990) Vertebrate gas exchange. From environment to cell. In: *Advances in Comparative Environmental Physiology*, vol. 6, 411pp. New York: Springer Verlag.

Dejours P (1988) *Respiration in Water and Air*: Adaptations, Regulation, Evolution, 179pp. Amsterdam: Elsevier.

Diaz RJ and Breitburg DL (2009) The hypoxic environment. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 27 Hypoxia*, pp.1–23. Academic Press.

Perry SF and Tufts BL (eds.) (1998) Fish respiration. In: *Fish Physiology* vol. 17, 356pp. New York: Academic Press.

Randall DJ (1970) Gas exchange in fish. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, Vol. 4*, pp. 253–292. New York: Academic Press.

GAS EXCHANGE

Ventilation and Animal Respiration

Contents

Plasticity in Gill Morphology
Gill Respiratory Morphometrics
The Effect of Exercise on Respiration
Efficiency of Gas Exchange Organs
Respiratory Gas Exchange During Development: Models and Mechanisms
Respiratory Gas Exchange During Development: Respiratory Transitions
Techniques in Whole Animal Respiratory Physiology

Plasticity in Gill Morphology

GE Nilsson, University of Oslo, Oslo, Norway

© 2011 Elsevier Inc. All rights reserved.

Introduction
Mechanisms of Gill Remodeling
Why Remodel the Gills
Gill Remodeling in Other Species
Further Reading

Glossary

Apoptosis The process of programmed cell death.

Edema An accumulation of fluid in the interstitial space. This results when filtration of fluid across the capillary wall exceeds the capacity of lymph drainage.

Hypoxia-inducible factor 1-alpha (HIF-1α) Integrated hypoxia response that activates genes whose protein products either increase O_2 transfer (i.e., erythropoiesis, Hb affinity increases, angiogenesis, etc.) or up regulate metabolic adaptation by regulating anaerobiosis and O_2 consumption rate. HIF-1α induction is an ancient adaptation that first appeared in eukaryotic cells and thus long preceded the origin of metazoans.

Hyperplasia Growth by increase in the number of cells of an organ or tissue.

Ionocytes (mitochondrion-rich cells) A function-specific type of epithelial cell, which expresses ion transporters or enzymes and is responsible for trans-epithelial transport functions of specific ions. In fish gills, ionocytes responsible for ion regulation are named mitochondrion-rich cells based on the characteristic of abundant mitochondria

Lamellae Also known as secondary lamellae, these are attached in rows to the gill filaments. They are the primary sites for gas exchange in fish gills. Each lamella is made up of two epithelial layers separated by pillar cells. Oxygen is taken up by erythrocytes flowing inside the lamellae from water flowing between the lamellae.

Na^+/K^+-ATPase (NKA, the sodium pump) This is a major ATP-consuming ion pump that, directly or indirectly, drives many ion-regulatory processes, including maintaining sodium and ion gradients across cell plasma membranes and being present at high levels in ionocytes.

Introduction

Oxygen uptake in fish gills occurs primarily over the surface of the lamellae (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy and **Gas Exchange**: Respiration: An Introduction), and in some species this area can be altered drastically by plastic morphological changes in the gill structure (gill remodeling). These dramatic changes in gill morphology occur in a rapid (days) and reversible manner. Gill remodeling was first unequivocally demonstrated in the crucian carp (*Carassius carassius*) (**see also Bony Fishes**: Crucian Carp) by J Sollid and co-workers in 2003, and subsequent studies, as well as examination of published data, indicate that this ability could be widespread among fishes.

The initial studies revealed that crucian carp gill filaments have a sausage-like appearance, without protruding lamellae, when the fish are kept in cold (≤20 °C) aerated water. However, when crucian carp are moved to hypoxic water ($[O_2]$ = 6–8% of air saturation) (**Figure 1**(**c**)) or if they are kept at a high temperature (≥25 °C) (**Figure 1**(**d**)), their gills transform into gills with protruding lamellae within a few days. The process is reversible: if the crucian carp are moved back to relatively cold normoxic water for a week, their gill filaments regain the sausage-like morphology. A rise in temperature will have two major consequences for the fish: it will increase the metabolic rate and therefore the need for oxygen, and it will reduce the concentration of oxygen in the water. Like hypoxia, a high temperature will call for an increased capacity to extract oxygen from the water, suggesting that it is the oxygen need, rather than the water oxygen level in itself, that is driving gill remodeling in crucian carp.

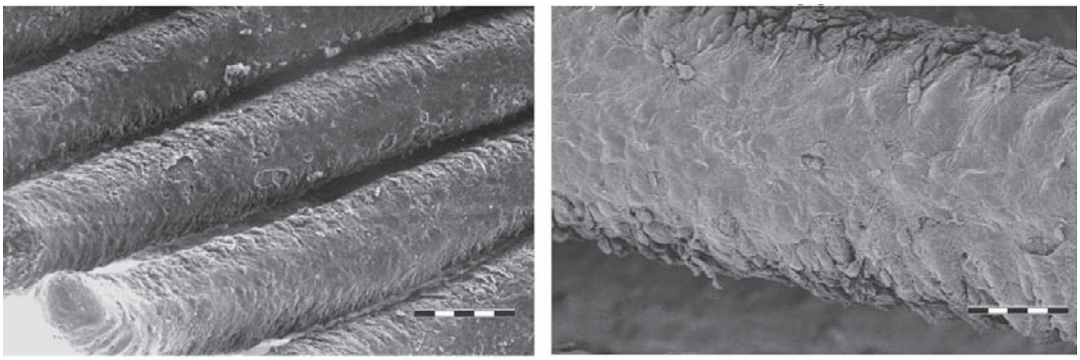
Mechanisms of Gill Remodeling

A series of studies by J Sollid and co-workers showed that the lamellae are always present in crucian carp gills, but during normoxia and low temperature, they are embedded in a cell mass denoted as an interlamellar cell mass (ILCM). In cold normoxic crucian carp, the ILCM completely fills up the space between the lamellae (**Figure 2**(**a**)). It has been shown that an increased rate of apoptosis (programmed cell death or cell suicide) combined with suppressed mitosis is responsible for regression of the ILCM during hypoxia (**Figure 2**(**b**)). The complete transformation of the gill morphology in crucian carp takes between 3 and 7 days at 8 °C.

In crucian carp and the closely related goldfish (*Carassius auratus*), the ILCM appears to be primarily made up of undifferentiated cells, but on top of the ILCM, facing the water, numerous osmoregulatory ionocytes are found. These ionocytes stay on the top of the ILCM, following the ILCM as it retracts in hypoxia (**Figures 3**(**a**) and (**b**)), and the ionocytes do not appear to be affected by the widespread apoptosis. Mitrovic and co-workers recently found that the gill epithelium appears to become less leaky when the

(a) Normoxic crucian carp gill filaments at 8 °C

(b) Normoxic crucian carp gill filament at 8 °C



(c) Hypoxic crucian carp gill filament at 8 °C

(d) Normoxic crucian carp gill filament at 25 °C

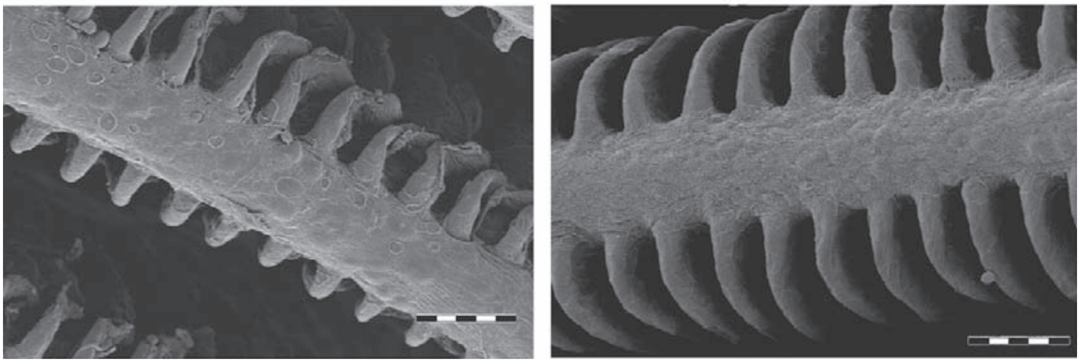


Figure 1 Scanning electron micrographs of gill filaments from crucian carp kept in normoxic water at 8 °C (a)–(b), in hypoxic water at 8 °C (c), or in normoxic water at 25 °C (d). Scale bars are 150 μm in (a) and 50 μm in (b)–(d). From Sollid J, De Angelis P, Gundersen K, and Nilsson GE (2003) Hypoxia induces adaptive and reversible gross-morphological changes in crucian carp gills. *Journal of Experimental Biology* 206: 3667–3673; and Sollid J, Weber RE, and Nilsson GE (2005) Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *Journal of Experimental Biology* 208: 1109–1116.

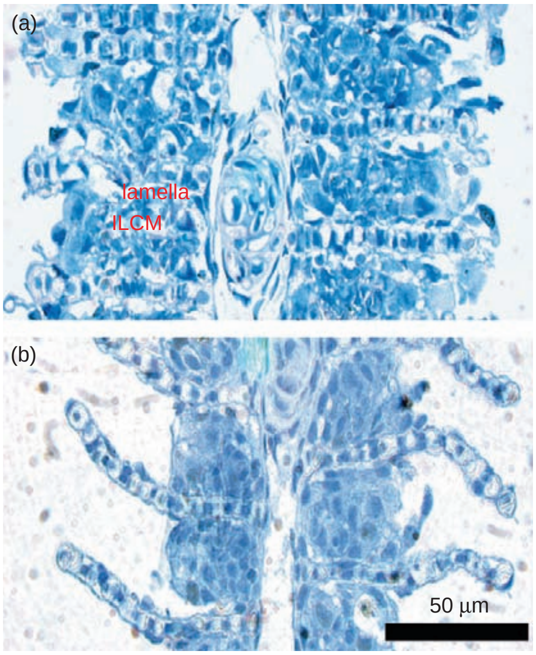


Figure 2 Light micrographs of gills from crucian carp kept in normoxic (a) or hypoxic (b) water at 8 °C. Note that the lamellae are present in both conditions but that a regression of the interlamellar cell mass (ILCM) during hypoxia makes the lamellae protrude, thereby greatly increasing the respiratory surface area. A filament arteriole (with blood clots) is seen running vertically in the center of each micrograph. From Sollid J, De Angelis P, Gundersen K, and Nilsson GE (2003) Hypoxia induces adaptive and reversible gross-morphological changes in crucian carp gills. *Journal of Experimental Biology* 206: 3667–3673.

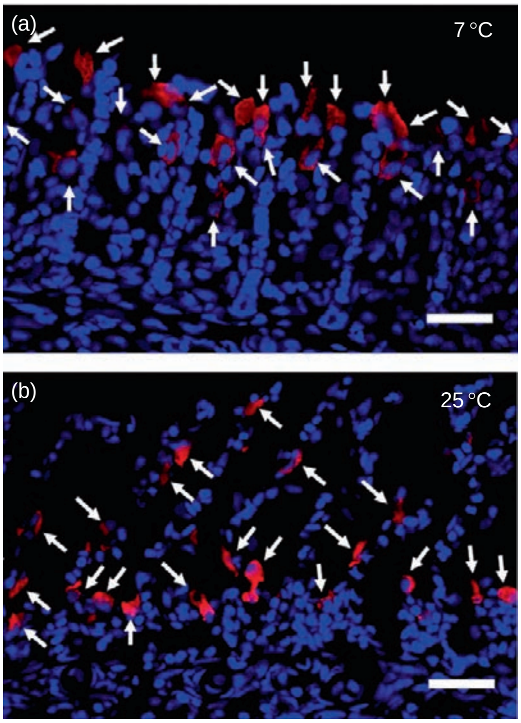


Figure 3 (a, b) Osmoregulatory ionocytes (red staining) stays on top of the ILCM (blue staining) and follows the ILCM as it retracts downward when goldfish are exposed to warm (25 °C) water. Some ionocytes are also attached to the lamellae. Like the ILCM, the lamellae are also stained blue but can be seen as vertical bands of cells attached to the filament at the bottom of the pictures. Ionocytes were stained with antibodies against Na^+/K^+ ATPase. Scale bars are 20 μm. From Mitrovic D and Perry SF (2009) The effects of thermally induced gill remodeling on ionocyte distribution and branchial chloride fluxes in goldfish (*Carassius auratus*). *Journal of Experimental Biology* 212: 843–852.

lamellae are protruding. This decreased permeability may reduce ion fluxes associated with the increased gill surface area.

The molecular signals and mechanisms involved in gill remodeling are not understood. Hypoxia-inducible factor 1-alpha (HIF-1α) is a transcription factor that is generally regarded as a master switch for many hypoxia-induced changes in animals. Increased messenger RNA (mRNA) and protein levels of HIF-1α have been seen in the gills of crucian carp exposed to hypoxia, indicating that HIF-1α could be involved in triggering the regression of the ILCM. However, it has also been found that gill HIF-1α increases with falling temperature during normoxic conditions, when the ILCM is maintained, arguing against a role for this transcription factor in gill remodeling. Moreover, the gill mRNA level of inducible nitric oxide synthase (iNOS), an enzyme induced by HIF-1α and known to trigger apoptosis, appears to be unaffected by hypoxia in crucian carp. Thus, also iNOS is unlikely to be responsible for inducing apoptosis in the ILCM.

Further, it is not clear if the ambient signal (low oxygen or high temperature) for gill remodeling is sensed by the ILCM cells themselves or transmitted to the cells from elsewhere in the body. As the ILCM cells are situated outside the lamellar epithelium, and appear to lack direct contact with nerves or blood vessels, it is unclear how a humoral or neural signal could reach the ILCM cells. Indeed, how these cells receive nutrients for their energy metabolism when they apparently lack a direct blood supply is also not clear.

Why Remodel the Gills

Constitutional species-to-species differences in the surface area of the lamellae have long been known to correlate with the need for oxygen uptake. Thus, the relationship between total lamellar area and body mass differs by more than an order of magnitude in fish species, with highly active fishes having the largest relative lamellar area and sedentary species having the smallest. In addition, the ability to tolerate low oxygen levels (hypoxia tolerance) has been found to correlate with a large lamellar area in fishes, presumably boosting the ability to extract oxygen from the water.

It has also been known for a long time that fishes can regulate their functional respiratory surface area by changing the fraction of the total lamellar area that is perfused with blood (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). This ability allows the fish to cope with rapid fluctuations in oxygen levels and temperature, as well as with matching the momentary needs for oxygen uptake with changes in the activity level. Moreover, ionic or acid–base challenges may cause fishes to adjust the cellular makeup of the gill surface, including the number of ion-pumping mitochondria-rich cells (ionocytes).

The facts that some fish species have a much smaller gill area than others, that fish regulate gill blood-flow patterns, and that some fishes remodel their gills in response to the oxygen demand indicate that there are drawbacks to having a large respiratory surface area (or all fishes would have a well-perfused large gill surface area all the time). Several problems have been suggested to arise from having a large gill surface area. These include:

1. *High costs for ion and acid-base regulation.* These arise from the osmoregulatory compromise, which emphasizes that an increase in the respiratory surface area results in increased water and ion fluxes that has to be counteracted by energetically expensive ion pumping (**see also Role of the Gills**: The Osmorespiratory Compromise).
2. *Increased exposure to toxic substances.* A large lamellar surface area may facilitate the entrance of many naturally occurring toxic substances, such as ammonia, algal toxins, and metal ions. Since the industrial revolution, anthropogenic pollutants can be added to the list of unwanted substances entering fish through the gills.
3. *Increased susceptibility to pathogens.* This also includes microorganisms that enter the fish via the gills and parasites that attach to the gills.
4. *Increased risk of bleeding.* As the whole cardiac output of fish has to pass through the gills, hemorrhage from gill injury could be life threatening, and arguably, large gills are likely to be more vulnerable to injury than small ones.
5. *Reduced capacity for feeding.* Larger gills take up more space in the oral cavity and, therefore, impede feeding.

The life history and physiology of the crucian carp should provide important clues that could help us understand the functions of the extreme gill remodeling shown by this species (**see also Bony Fishes**: Crucian Carp). Compared to most other fishes, the crucian carp is extremely hypoxia tolerant, and even tolerates long periods of anoxia in the winter. In Northern Europe, the crucian carp inhabits small ponds that become hypoxic and finally anoxic for several months every winter. Anoxic crucian carp (and goldfish) turn glycolytically produced lactate into ethanol, which is released to the water over the gills, thereby avoiding lactic acidosis. However, this is an energetically wasteful strategy since a highly energetic hydrocarbon is lost to the water, and it puts the crucian carp in danger of running out of fuel during long anoxic winters, even if it has enormous glycogen stores. It should therefore make the anaerobic period as short as possible, and this could be a major selection pressure for remodeling the gills. In the winter, the anoxic period is preceded by weeks or months of falling oxygen levels, and during this period an increased lamellar surface area allows the crucian carp to maintain aerobic adenosine triphosphate (ATP) production, thereby saving the glycogen stores. Respirometry has shown that crucian carp with protruding lamellae can continue to run aerobic metabolism at lower oxygen levels than crucian carp with the lamellae imbedded in an ILCM.

The extreme hypoxia tolerance of the crucian carp is also reflected in its hemoglobin, which has a record high O_2 affinity. The crucian carp hemoglobin is 50% saturated with O_2 at an oxygen partial pressure (P_{O_2}) of 0.8 mmHg (at 10 °C). The goldfish has almost an equally high blood oxygen affinity. This will not only aid in boosting its oxygen uptake at a very low water P_{O_2}, but also is probably a prerequisite for the extensive gill remodeling displayed by these fishes. Thus, during normoxia, the extremely high hemoglobin oxygen affinity should result in a very large difference in P_{O_2} between water and blood, allowing enough oxygen to diffuse into the blood even when the lamellae are fully covered with a cell mass.

During those seasons of the year when ambient oxygen levels are high (spring, summer, and autumn), covering up the lamellae should allow the crucian carp to reduce its osmoregulatory costs (and possibly its exposure to pathogens and toxins). Hence, more energy can be devoted to growth, reproduction, and rebuilding the glycogen stores.

Gill Remodeling in Other Species

It is now clear that crucian carp and goldfish are not the only ones that can remodel their gills. Among cyprinids, the Qinghai carp (*Gymnocypris przewalskii*) has been found to be able to remodel its gills, reversibly, in a similar fashion as the *Carassius* species, although to a less-extensive extent (**Figure 4**). Moreover, light micrographs published in a study investigating the characteristics of gill Na^+/K^+ ATPase activity in common carp (*Cyprinus carpio*) gills reveal a considerably larger cell mass between the lamellae of carp acclimated to 15 °C compared to carp acclimated to 29 °C (**Figure 5**). This suggests that gill remodeling in response to oxygen and temperature could be widespread among the carp fish family (Cyprinidae),

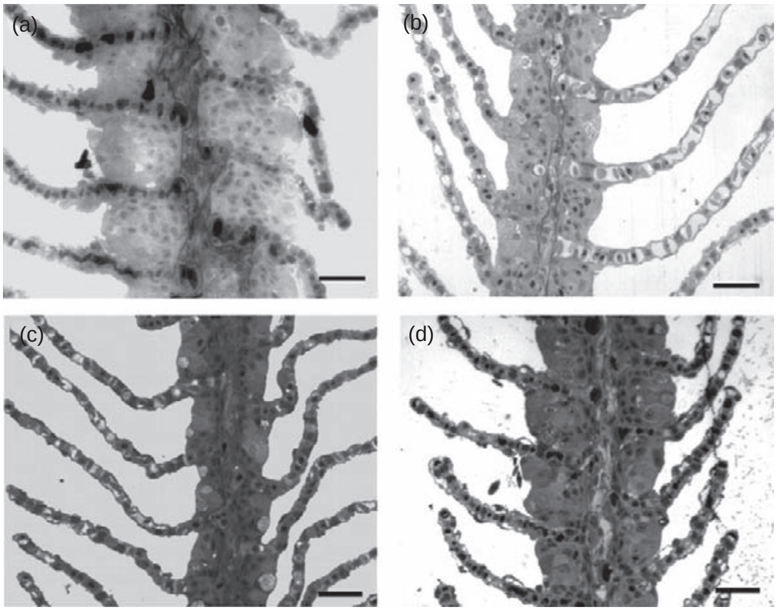


Figure 4 Light micrographs showing gill remodeling in the Qinghai carp (*Gymnocypris przewalskii*). Fish were exposed to normoxia (a), 12 h hypoxia (b), 24 h hypoxia (c), and 12 h normoxic recovery after 24 h hypoxia (d). During hypoxia, the water oxygen level was 0.3 mg l^{-1}. Water temperature was 11–15 °C. Scale bars are 20 μm. From Matey M, Richards JG, Wang Y, *et al.* (2008). The effect of hypoxia on gill morphology and ionoregulatory status in the Lake Qinghai scaleless carp, *Gymnocypris przewalskii*. *Journal of Experimental Biology* 211: 1063–1074.

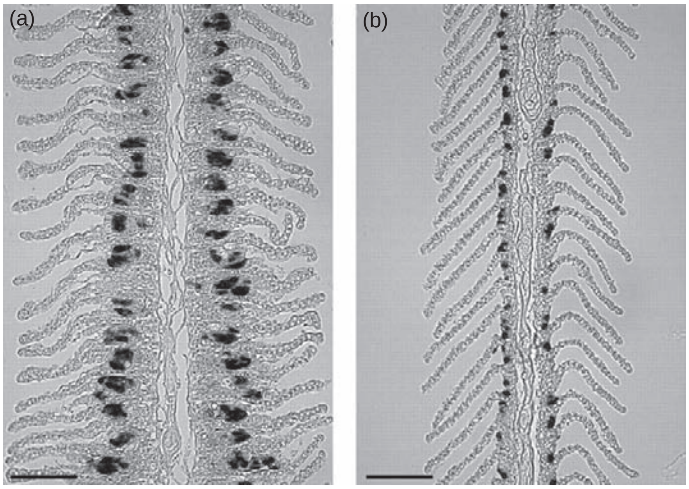


Figure 5 Light micrographs published in a study of Na^+/K^+ ATPase immunoreactivity (dark cells) in common carp (*Cyprinus carpio*) gills, revealing a considerably larger cell mass between the lamellae of carp acclimated to 15 °C (a) compared to carp acclimated to 29 °C (b). Scale bars are 100 μm. From Metz JR, van den Burg EH, Wendelaar Bonga SE, and Flik G (2003) Regulation of branchial Na+/K+-ATPase in common carp *Cyprinus carpio* L. acclimated to different temperatures. *Journal of Experimental Biology* 206: 2273–2280.

although the remodeling rarely may be as extensive as that seen in crucian carp and goldfish.

With regard to other teleost fishes, gill remodeling was recently found in the mangrove killifish (*Kryptolebias marmoratus*) (family Cyprinodontidae) in response to air exposure (**Figure 6**). Its ILCM increases in volume to fill up most of the interlamellar space within a week of air exposure. The process is reversible and may function to increase the ability of the killifish to take up oxygen from air by stabilizing the lamellae. This small fish can venture into the terrestrial environment for several days, taking up its oxygen from the air. Like the cyprinids, the killifish retains the lamellae while it changes the volume of the ILCM (although it is not clear if it is the cell volume or cell number that is changing). Despite their similar Latin family names, the Cyprinodontidae and the Cyprinidae

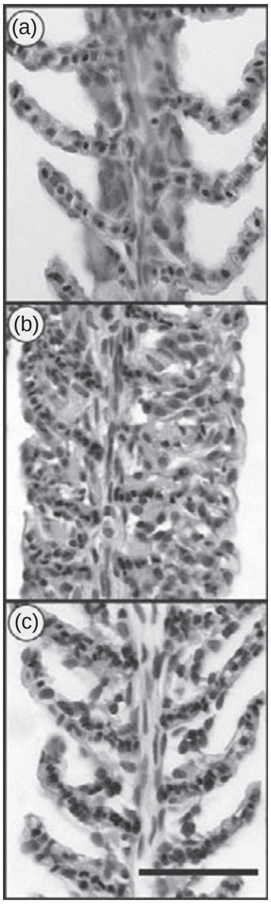


Figure 6 Light micrographs showing gill remodeling in the mangrove killifish (*Kryptolebias marmoratus*) (family Cyprinodontidae) in response to air exposure. The pictures show gills of fish kept in water (a), in air for a week (b), and in water for a week after air exposure (c) (all at 25 °C). Scale bar is 40 μm. From Ong KJ, Stevens ED, and Wright PA (2007) Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. *Journal of Experimental Biology* 210: 1109–1115.

are quite distantly related. They are both within the Teleostei, but cyprinids belong to the superorder Ostariophysi, while the cyprinodontids belong to the superorder Acanthopterygii, with at least 150 million years since they shared a common ancestor.

To some extent, also the European eel (*Anguilla anguilla*) appears to be capable of temperature-induced gill remodeling. It has been noted that the eel have thicker lamellae and filaments when acclimated to 5 °C compared to those acclimated to 25 °C. Micrographs have shown an ILCM that is larger and appears to contain more cells in the cold-acclimated fish (**Figure 7**). The change is relatively modest (the published micrographs suggest an ILCM covering about 20% of the lamellae at 5 °C, compared to 10% at 25 °C). Because eels belong to the teleost subdivision Elopomorpha, it widens further the range of teleosts that are capable of gill remodeling involving changes in the size of an ILCM.

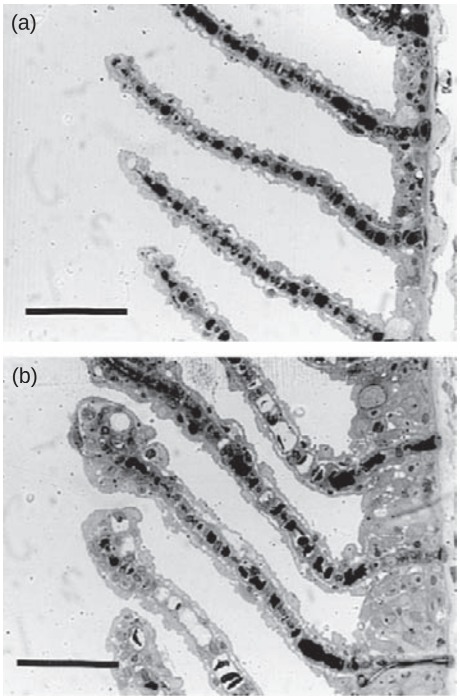


Figure 7 Light micrographs showing a considerably larger cell mass between the lamellae of cold (5 °C) acclimated eel (*Anguilla anguilla*) ((a)) compared to warm (25 °C) acclimated eel ((b)). Also note the striking thickening of the lamellae in cold fish. Scale bars are 5 μm. From Tuurala H, Egginton S, and Soivio A (1998) Cold exposure increases branchial water–blood barrier thickness in the eel. *Journal of Fish Biology* 53: 451–455.

Still, the most striking change in the gill structure of cold-acclimated eel is the considerable thickening of the lamellae (**Figure 7**). Such thickening of the lamellae has also been seen in cold-exposed juvenile largemouth bass (*Micropterus salmoides*) and rainbow trout (*Oncorhynchus mykiss*), species for which dynamic changes in the ILCM so far have not been reported. However, Mueller and co-workers have found that in the brook trout (*Salvelinus fontinalis*), 7–10 days of aluminum exposure at pH 5.2 causes undifferentiated cells and mucus-producing cells to fill up the interlamellar space in a reversible manner. If this is a protective and adaptive response or if a pathological response is not clear, then its histological appearance is strikingly similar to the ILCM of cyprinids. Indeed, there are several other examples of fish that show morphological changes in their gills when exposed to environmental pollutants such as metals and organic chemicals. These changes include hyperplasia (extensive tissue growth), edema (fluid accumulation in the tissue), and fusion of adjacent lamellae. Some of these changes are likely to be protective, functioning to reduce the exposure to chemicals or pathogens, while others may be primarily pathological.

Finally, it could be mentioned that an example of developmental gill remodeling, involving a loss of protruding lamellae, is provided by *Arapaima gigas*, the largest Amazonian fish. From being a water breather, having normal gills with protruding lamellae, early in life, it develops into an obligate air breather, using its swimbladder for oxygen uptake. This coincides with a striking alteration in gill morphology, as its gills no longer have any protruding lamellae. However, this change has never been seen to be reversed, and the lamellae are no longer present under a cell mass as in the reversible remodeling seen in cyprinids, cyprinodonts, and eels.

More examples of reversible gill remodeling among fishes are likely to be described, and it cannot be excluded that this is a widely distributed capacity among fishes in light of the benefits it provides for matching oxygen uptake capacity with oxygen needs in the face of changing environmental conditions. However, we may not find many such extreme examples of gill remodeling as those provided by the crucian carp and goldfish. A complete coverage of the lamellae can probably only occur in fishes possessing extremely high blood oxygen affinities, or in very small fish where the body surface provides a significant route for oxygen uptake. Moreover, the apoptotic and mitotic processes involved probably make gill remodeling best suited for adjusting the respiratory surface area to long-lasting changes in the environment (over weeks or seasons). It is probably more cost effective to adjust gill ventilation and blood flow patterns in response to short-term variations in oxygen needs.

See also: **Bony Fishes**: Crucian Carp. **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Gas Exchange**: Respiration: An Introduction. **Role of the Gills**: The Osmorespiratory Compromise. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs.

Further Reading

Brauner CJ, Matey V, Wilson JM, Bernier NJ, and Val AL (2004) Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *Journal of Experimental Biology* 207: 1433–1438.

Chapman LJ, Galis F, and Shinn J (2000) Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters* 3: 387–393.

Chapman LJ and Hulen KG (2001) Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. *Journal of Zoology* 254: 461–472.

Farrell AP, Kennedy CJ, and Kolok A (2004) Effects of wastewater from an oil–sand-refining operation on survival, hematology, gill histology, and swimming of fathead minnows. *Canadian Journal of Zoology* 82: 1519–1527.

Goss GG, Perry SF, Fryer JN, and Laurent P (1998) Gill morphology and acid–base regulation in freshwater fishes. *Comparative Biochemistry and Physiology – Part A* 119: 107–115.

Matey M, Richards JG, Wang Y, *et al.* (2008) The effect of hypoxia on gill morphology and ionoregulatory status in the Lake Qinghai scaleless carp, *Gymnocypris przewalskii*. *Journal of Experimental Biology* 211: 1063–1074.

Metz JR, van den Burg EH, Wendelaar Bonga SE, and Flik G (2003) Regulation of branchial Na^+/K^+-ATPase in common carp *Cyprinus carpio* L. acclimated to different temperatures. *Journal of Experimental Biology* 206: 2273–2280.

Mitrovic D, Agnieszka D, Nilsson GE, and Perry SF (2009) Physiological consequences of gill remodeling in goldfish (*Carassius auratus*) during exposure to long-term hypoxia. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 297: R224–R234.

Mitrovic D and Perry SF (2009) The effects of thermally induced gill remodeling on ionocyte distribution and branchial chloride fluxes in goldfish (*Carassius auratus*). *Journal of Experimental Biology* 212: 843–852.

Mueller ME, Sanchez DA, Bergman HL, *et al.* (1991) Nature and time course of acclimation to aluminium in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. *Canadian Journal of Fishery and Aquatic Science.* 48: 2016–2027.

Nilsson GE (2007) Gill remodeling in fish – a new fashion or an ancient secret? *Journal of Experimental Biology* 210: 2403–2409.

Ong KJ, Stevens ED, and Wright PA (2007) Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. *Journal of Experimental Biology* 210: 1109–1115.

Schaack S and Chapman LJ (2003) Interdemic variation in the African cyprinid *Barbus neumayeri*: Correlations among hypoxia, morphology, and feeding performance. *Canadian Journal of Zoology* 81: 430–440.

Soivio A and Tuurala H (1981) Structural and circulatory responses to hypoxia in the secondary lamellae of *Salmo gairdneri* gills at two temperatures. *Journal of Comparative Physiology – Part B* 145: 37–43.

Sollid J, De Angelis P, Gundersen K, and Nilsson GE (2003) Hypoxia induces adaptive and reversible gross-morphological changes in crucian carp gills. *Journal of Experimental Biology* 206: 3667–3673.

Sollid J, Kjernsli A, De Angelis PM, Røhr Å.K, and Nilsson GE (2005) Cell proliferation and gill morphology in anoxic crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 289: R1196–R1201.

Sollid J and Nilsson GE (2006) Plasticity of respiratory structures – adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respiratory Physiology and Neurobiology* 154: 241–251.

Sollid J, Rissanen E, Numminen H, *et al.* (2006) HIF-1a and iNOS in crucian carp gills during hypoxia induced transformation. *Journal of Comparative Physiology – Part B* 176: 359–369.

Sollid J, Weber RE, and Nilsson GE (2005) Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *Journal of Experimental Biology* 208: 1109–1116.

Tuurala H, Egginton S, and Soivio A (1998) Cold exposure increases branchial water–blood barrier thickness in the eel. *Journal of Fish Biology* 53: 451–455.

Gill Respiratory Morphometrics

NC Wegner, Scripps Institution of Oceanography, La Jolla, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Gill Morphology
Total Gill Surface Area
Diffusion Distances
Theories on Gill Dimensions
Gill Morphometrics, Fish Habitat, and Metabolic Requirements
Scaling: Fish Gill Morphometrics in Relation to Fish Growth
Further Reading

Glossary

Diffusion capacity Rate of gas transfer across a respiratory surface per unit of gas partial pressure (usually expressed in ml min^{-1} mmHg^{-1}). Dependent on the Krogh coefficient, the respiratory surface area, and the diffusion distance. Also often referred to as the gas transfer factor.

Gill arch Bony or cartilaginous support from which gill filaments extend into the branchial cavities.

Gill filaments (= primary lamellae) Elongated, blade-like structures that extend from the gill arch in the posterior–lateral direction.

Gill lamellae (i.e., secondary lamellae; sing. lamella) Thin folds or plates arising from the gill filaments that contain blood capillaries and serve as the site of gas exchange in the gills of most fishes (singular = lamella).

Gill resistance Resistance to water flow through the gills (= pressure head across the gills divided by the total volumetric flow of the ventilatory stream).

Gill respiratory morphometrics Dimensions of the gills that affect gas transfer between the water and blood.

Krogh coefficient Conductance of a solute (e.g., oxygen) through a particular respiratory medium (e.g., water and blood) or structure (e.g., the water–blood barrier).

Lamellar frequency Number of lamellae per unit length of filament (usually reported per mm). It is also referred to, in some texts, as lamellar density.

Total filament length Sum of the lengths of all filaments in the gills of a fish.

Total gill surface area Area of the gill respiratory epithelium in a fish; Normally reported as the bilateral surface area of all the gill lamellae.

Water–blood barrier thickness Thickness of the tissue barrier through which gases are exchanged between the interlamellar water and blood capillaries. This barrier consists of epithelial cells, a basement membrane, and the flanges of pillar cells. This is also referred to as water–blood barrier distance.

Abbreviations

$\dot{V}_{O_2}$	rate of oxygen uptake (ml O_2 min^{-1})
K	Krogh (= diffusion) coefficient (ml O_2 μm cm^{-2} mmHg^{-1} min^{-1})
A	total gill surface area (cm^2)
ΔP_{O_2}	difference in the partial pressure of oxygen between blood and water at the gills (mmHg)
t	diffusion distance (μm)
D	diffusion capacity (ml O_2 min^{-1} mmHg^{-1})
L_{fil}	total filament length (cm)
n_{lam}	lamellar frequency (number of lamellae per unit length of gill filament) (mm^{-1})
A_{lam}	mean bilateral surface area of an individual lamella (mm^2)
R	gill resistance (Pa s cm^{-3})
μ	dynamic viscosity of water (Pa s)
l	length of the interlamellar channels (= lamellar length) (μm)
d	diameter (= width) of interlamellar channels (μm)
w	width or thickness of a lamella (μm)
h	height of gill lamellae (μm)
b	species-specific scaling exponent, regression coefficient

Introduction

Gills are the primary site of respiratory gas exchange in most fishes. Oxygen acquisition from the environment involves diffusion along a concentration gradient from the ventilatory water stream, across the gill epithelium, and into the blood. The rate of this process is described by the Fick equation:

$$\dot{V}_{O_2} = (K \times A \times \Delta P_{O_2})/t \qquad (1)$$

where $\dot{V}_{O_2}$ is the rate of oxygen uptake, *K* is the diffusion or Krogh coefficient, *A* is the respiratory surface area of the gills, ΔP_{O_2} is the mean difference in the partial pressure of oxygen (=oxygen tension) between the blood and water, and *t* is the diffusion distance (see Abbreviations section for units associated with each term). This equation can be rearranged into the following form:

$$\dot{V}_{O_2}/\Delta P_{O_2} = (K \times A)/t = D \qquad (2)$$

where the ratio of O_2 uptake to the mean difference in ΔP_{O_2} is referred to as the diffusion capacity (*D*). This term is independent of both the metabolic rate of the fish and the oxygen diffusion gradient across the gills and thus provides a useful metric to compare fish species in their ability to acquire environmental O_2 based on gill dimensions, where *D* depends on both the gill area and thickness of the respiratory epithelium (also called the water–blood barrier distance). This article discusses these morphometrics and their constituent parts (i.e., gill area can be subdivided into measures of the filaments and lamellae) in terms of methods for their measurement and theories on optimizing gas exchange, as well as the effects of fish habitat, metabolic demand, and growth on diffusion capacity.

Gill Morphology

Analysis of gill morphometrics with respect to gas exchange requires a basic understanding of fish branchial anatomy (for a comprehensive review of gill structure, the reader is directed to **Design and Physiology of Arteries and Veins**: Branchial Anatomy). Although varying in design and configuration, the gills of all jawed fishes have the same basic structural components: arches, filaments, and lamellae, which are illustrated for a typical teleost fish in **Figure 1**. Most fishes have four gill arches bilaterally positioned on each side of the oral cavity. Each arch supports two rows or sheets of gill filaments, long blade-like structures that extend away from the arch in the posterior-lateral direction (**Figures 1**(**a**) and **1**(**b**)). Together, the two filament sheets constitute a holobranch; each individual row being called a hemibranch (i.e., half a holobranch). In teleosts, the hemibranchs of each arch are largely independent of each other and are connected only near their base by an interbranchial septum (**Figure 1**(**b**)). In elasmobranchs (sharks and rays), this septum extends from the gill arch out to the lateral edge of the fish (where it forms a gill flap), thereby binding the filaments of adjacent hemibranchs along their entire lengths (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy). In both configurations, the filaments of opposing hemibranchs from adjacent arches come together near their tips to form a continuous curtain or sieve through which the entire ventilatory stream passes (**Figure 1**(**b**)).

Each side of the filament blade supports a row of respiratory folds called lamellae (**Figure 1**(**c**)). Their plate-like morphology maximizes surface area while minimizing diffusion distances, thus allowing for effective and rapid gas exchange between blood and water. Blood, which flows in the opposite direction to the ventilatory water stream, is directed through the lamellae by the placement of pillar cells (**Figure 1**(**c**)) that extend across the blood lumen and connect the respiratory epithelium on either side (**Figure 1**(**d**)). The lamellar water–blood barrier is typically composed of two or more layers of epithelial cells, a basement membrane, and the flanges of pillar cells which form the inner lining of the lumen wall (**Figure 1**(**e**)). It may also contain mitochondria-rich cells (involved in osmoregulation and acid–base balance) and goblet cells (associated with mucus production) (**see also Role of the Gills**: Morphology of Branchial Ionocytes); however, in fishes with short diffusion distances, these specialized cells are often confined to the filament epithelium (as shown in **Figure 1**(**e**)).

Total Gill Surface Area

Total gill surface area refers to the area of the respiratory epithelium in a fish, which, in most species, is the bilateral surface of all the lamellae. Gill area (*A*) can thus be estimated using the equation:

$$A = L_{\text{fil}} \times 2n_{\text{lam}} \times A_{\text{lam}} \qquad (3)$$

where L_{fil} is the total length of all the gill filaments, n_{lam} is the lamellar frequency (i.e., the mean number of lamellae per unit length on one side of a filament, which is multiplied by two to account for lamellae on each side), and A_{lam} is the mean bilateral surface area of a lamella. This classical method of estimating gill area takes advantage of the highly organized structure of the gills to methodically sample morphometrics at predetermined locations. With the exception of some flatfishes, the gills on either side of the head are symmetrical; thus, measurements for each dimension are generally only required from one side of the fish.

Total filament length is usually determined by counting all filaments from the gills on one side of the head,

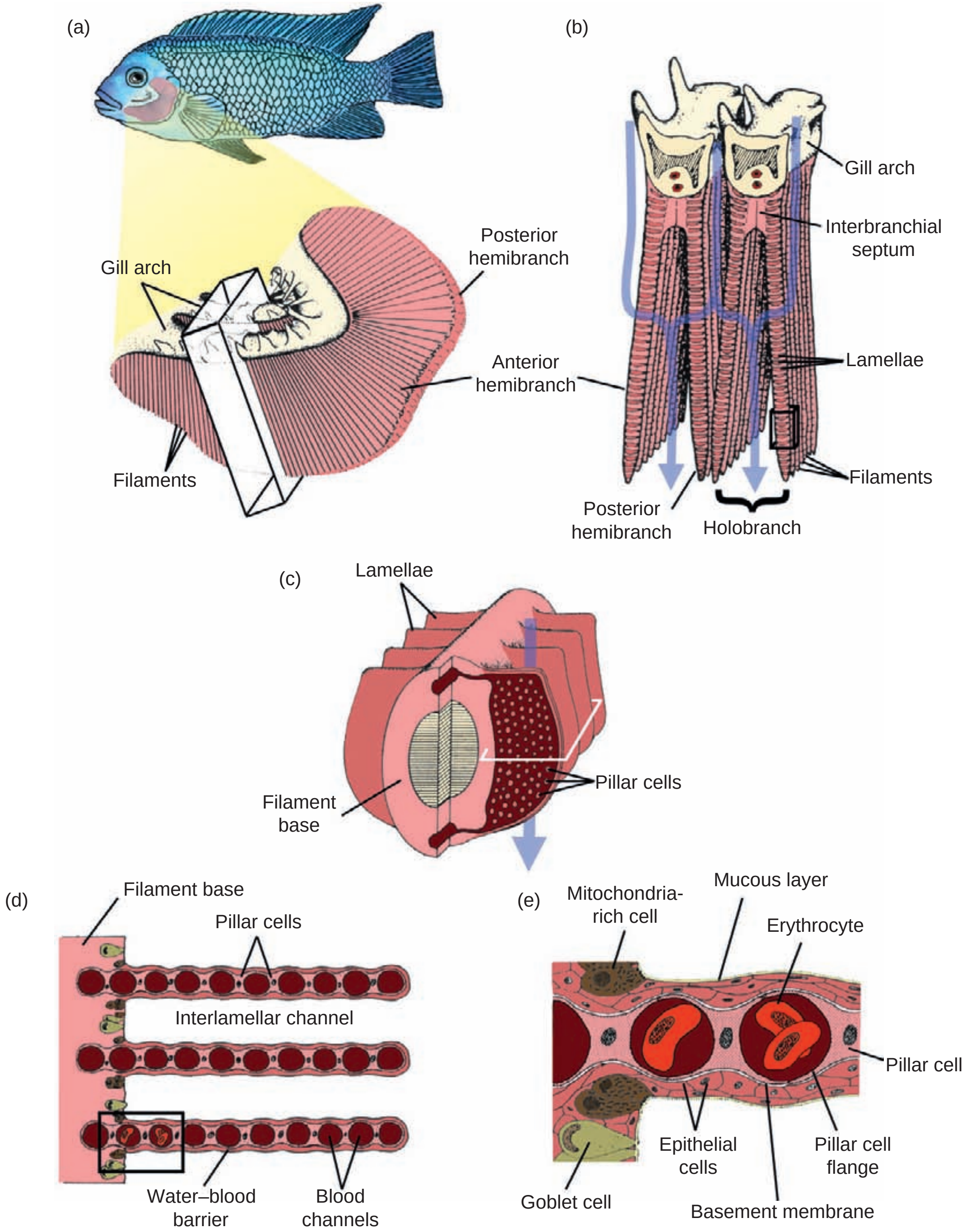


Figure 1 Gill morphology of a typical teleost: (a) anterior two gill arches from the left side of the head; (b) rotated and magnified section from (a) showing the placement and morphology of the two rows of gill filaments extending from each arch; (c) enlarged and rotated section from (b) showing the respiratory lamellae on both sides of a filament; (d) cross section through three adjacent lamellae from (c); (e) enlarged view from (d) showing the detail of the lamellar and filament epithelium. Water flow direction is indicated by blue arrows. Parts of this figure are modified or redrawn from Palzenberger M and Pohla H (1992) Gill surface area of water-breathing freshwater fish. *Reviews of Fish Biology and Fisheries* 2: 187–216.

and, depending on their abundance, dividing them into bins of 10, 20, or more on each arch (**Figure 2**(**a**)). The middle filament from each bin is measured and assumed to represent the mean filament length for that bin. The total length of all filaments in each bin can thus be calculated and these amounts are summed to estimate the total filament length for the gills on one side of the head. This amount is subsequently doubled to determine the total filament length for the entire fish. Lamellar frequency and bilateral surface area measurements are

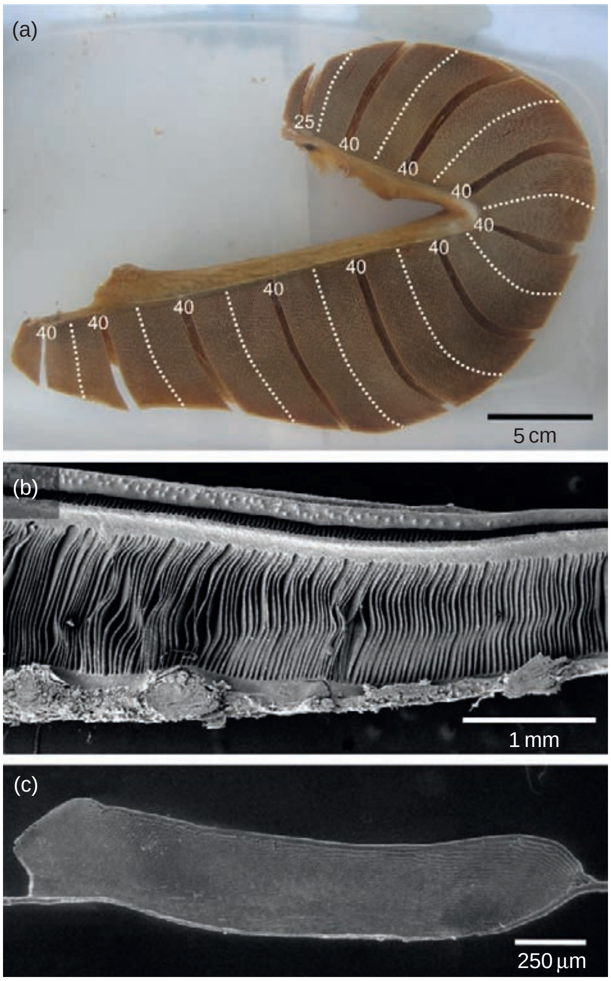


Figure 2 (a) Anterior hemibranch of the third gill arch from a 68-kg striped marlin, *Kajikia audax*, showing the division of 425 filaments into 10 bins of 40 and one bin of 25 (indicated by dotted white lines). The middle filament from each bin has been removed for measurements of lamellar frequency and bilateral surface area. (b) Scanning electron microscope (SEM) image of a selected gill filament used for determination of lamellar frequency. (c) SEM image of a corrosion-cast striped marlin lamella removed for determination of the bilateral surface area.

similarly made on each middle filament and assumed representative of their respective bins. Three measurements of each dimension are typically determined along the length of each filament to account for variation in lamellar shape, size, and spacing. For lamellar frequency, the filament is typically dissected from the arch (**Figure 2**(**a**)) and photographed under magnification near its base, middle, and tip (**Figure 2**(**b**)). Lamellae from these same locations are then dissected from the filament, mounted flat, and photographed to measure lamellar bilateral area (**Figure 2**(**c**)). Lamellar frequency and area measurements from each bin are weighted according to the filament length of that bin before being averaged (thus ensuring lamellar measurements from a bin with a longer filament length will contribute proportionately more toward the mean for the entire gills). Through extensive sampling of lamellar frequency and area on all four gill arches, one arch (usually the second or third) is typically found to be representative of the gills as a whole, and, thus, subsequent n_{lam} and A_{lam} measurements for that species can be made using only that arch.

More recently, gill surface area has also been estimated using stereological techniques. Randomly oriented gill tissue samples are embedded in glycol methacrylate or an alternative, and vertical sections through the tissue are mounted on slides and used to determine total gill volume. Subsequent point counting of filament and lamellar tissue densities using a superimposed grid allows calculation of their relative volumes and surface areas. This method is particularly useful in estimating the gill area of fishes that lack either highly organized and uniform filaments or two-dimensional lamellae. However, there have been few studies comparing the results obtained using stereological techniques with those of the more classic regional sampling method. In addition, stereological methods do not reveal the individual morphometrics contributing to gill area (i.e., L_{fil}, n_{lam}, and A_{lam}).

Diffusion Distances

Oxygen uptake at the gills requires diffusion through the interlamellar water, the water–blood barrier, and the blood plasma before passing through the red blood cell membrane and binding to hemoglobin. Although the widths of the interlamellar channels and lamellar blood vessels thus affect the length of the diffusion pathway, exact measurement of the total diffusion distance is confounded by convective processes of moving media (i.e., the diffusion distance depends on the thickness of boundary layers developing along the lamellar epithelium and other flow-related conditions). Thus, when referring to the diffusion capacity of the gills, emphasis is placed on the thickness of the water–blood barrier.

In most studies, this dimension is determined by viewing lamellar cross sections from multiple gill samples using light, scanning electron, or transmission electron microscopy and calculating the arithmetic mean thickness from measurements immediately overlaying lamellar blood channels (i.e., where the water–blood barrier is generally thin and most O_2 uptake likely occurs). Because diffusion also occurs through longer pathways overlying pillar cells, some studies calculate the harmonic mean thickness, in which averaging the reciprocals of measured lengths includes, but places less emphasis on, longer diffusion distances. In both methods, care must be taken to ensure that thickness measurements are made perpendicular to the plane of the gas-exchange surface, as oblique cross sections result in longer measurements. In order to eliminate the potential for such errors, as well as sampling biases, application of stereological techniques developed for determining the air–blood barrier thickness in mammalian lungs probably provides the most accurate results. This involves vertical sectioning of randomly oriented gill tissue samples and calculates the harmonic mean thickness as $(2/3)l_{\mathrm{h}}$, where l_{h} is the harmonic mean of all length measurements determined along parallel lines of a superimposed grid.

The use of varying techniques to measure barrier thickness can obscure interspecific comparisons (largely because arithmetic means tend to be shorter than harmonic means), and such complications can further be compounded in the calculation of gill diffusion capacity. A frequent error is coupling total gill area with the commonly reported arithmetic mean barrier thickness, which, because it only represents short diffusion distances overlaying blood vessels, causes an overestimation of diffusion capacity. More appropriate is pairing harmonic mean thickness with total gill area and the arithmetic mean with the surface area directly overlying blood channels, which, depending on the size and abundance of the pillar cells, is typically 20–40% lower than the total gill area. Other sources of error in determining gill morphometrics often result from poor tissue sample quality (i.e., gill tissue begins degradation within minutes of air exposure and requires immediate fixation upon collection).

Theories on Gill Dimensions

Despite various methods and difficulties associated with quantifying gill morphology, studies on a wide range of fish species have provided insight into the selective factors sculpting gill morphometrics. Of particular importance is the balance of having a sufficiently large gill area to meet metabolic demands while minimizing branchial resistance and thus the energetic costs of the cyclic buccal–branchial pumping mechanism used by most fishes to ventilate the gills. Because the interlamellar channels are the primary site of both oxygen uptake and gill resistance, this relationship can be examined through the Hagen–Poiseuille equation describing resistance (R) to water flow between parallel plates (i.e., through the lamellae). For a single interlamellar channel

$$R = 12\mu l/d^3 h \tag{4}$$

or for all interlamellar channels in the gills

$$R = 12\mu l(d + w)/d^3 h L_{\mathrm{fil}} \tag{5}$$

where μ is the dynamic viscosity of the water, l is the interlamellar channel length, d is the diameter or width of the channel, w is the width or thickness of a lamella, and h is the lamellar height (dimensions shown in **Figure 3**). In fishes requiring a large gill area, an increase to lamellar length (l) or frequency (thus decreasing d) amplifies gill resistance and, consequently, the energy required to ventilate the gills. In contrast, an increase to lamellar height (h) or to the length of the gill filaments (L_{fil}) decreases gill resistance. Thus, from a purely energetic standpoint, gill area is optimally augmented through large (tall) lamellae and high total filament lengths.

Gill Morphometrics, Fish Habitat, and Metabolic Requirements

With the radiation of fishes into nearly every aquatic and semiaquatic niche, ranging from the open ocean to hypoxic swamps, both gill area and the water–blood barrier thickness can range more than an order of magnitude to meet specific demands associated with a species' physiochemical surroundings and metabolic requirements. As a result, distantly related taxonomic groups show remarkable evolutionary convergence for gill morphology, which has led to the categorization of fishes into different morphological ecotypes. Six major groups are recognized: (1) fast-swimming oceanic species, (2) marine fishes of intermediate activity, (3) sluggish marine species, (4) freshwater fishes,

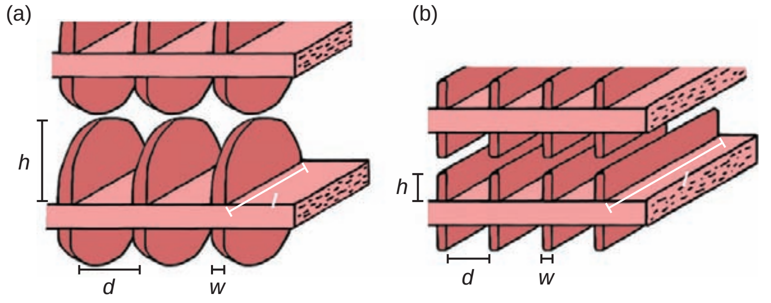


Figure 3 Typical arrangement and shape of the gill lamellae in (a) most teleosts and (b) most fast-swimming oceanic teleosts. *d*, interlamellar channel diameter (= width); *h*, lamellar height; *l*, lamellar length; *w*, lamellar thickness. Modified from Wegner NC, Sepulveda CA, Bull KB, and Graham JB (2010) Gill morphometrics in relation to gas transfer and ram ventilation in high-energy demand teleosts: Scombrids and billfishes. *Journal of Morphology* 271: 36–49.

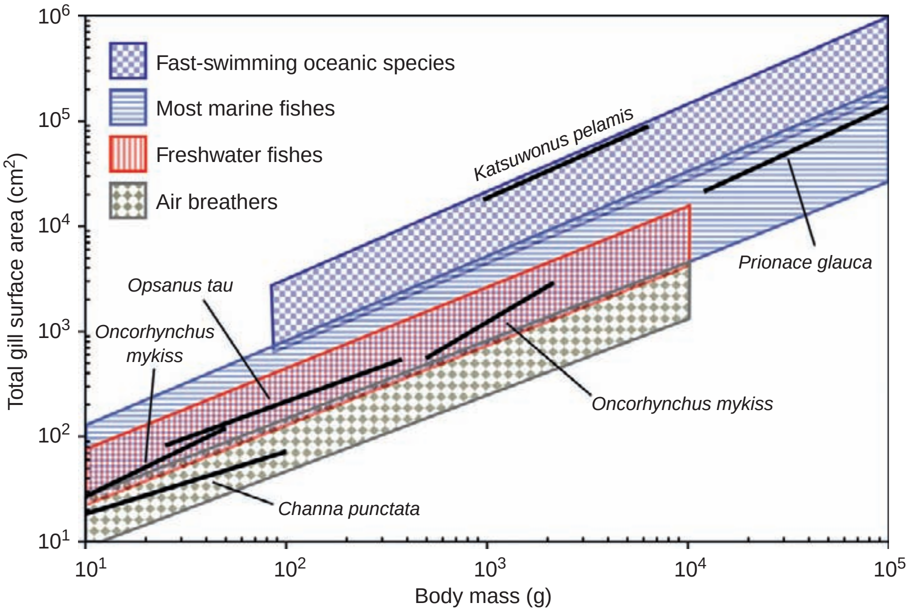


Figure 4 General relationships of gill surface area and body mass for different ecomorphological groups. For visual simplicity, marine fishes of intermediate activity and sluggish marine species are categorized together as most marine fishes. A regression line is shown for a representative of each group: (1) fast-swimming oceanic species, skipjack tuna, *K. pelamis*; (2) marine fish of intermediate activity, blue shark, *P. glauca*; (3) sluggish marine species, oyster toadfish, *O. tau*; (4) freshwater fish, rainbow trout, *O. mykiss*; and (5) air breather, spotted snakehead, *C. punctata*.

(5) air breathers, and (6) hypoxia dwellers. **Figure 4** compares the general range of gill surface area for each ecomorphological group with the exception of hypoxia dwellers, which includes fishes from groups 1–4 showing similar hypoxia-induced changes to gill morphology.

Fast-Swimming Oceanic Species

This group includes tunas and mackerels (family Scombridae), billfishes (Istiophoridae and Xiphiidae), dolphinfishes (Coryphaenidae), some jacks (Carangidae), and sharks of the family Lamnidae (e.g., the shortfin mako, *Isurus oxyrinchus*, and white shark, *Carcharodon carcharias*). These fishes generally have elevated energetic demands associated with fast, continuous swimming and high gill diffusion capacities resulting from large gill areas (which can be an order of magnitude greater than those of other marine fishes) and a thin water–blood barrier (approximately 0.5–1.2 μm). Both large gill areas and short diffusion distances reach their zenith in tunas, which accelerate metabolic processes through the retention of body heat produced by continuous swimming and have the highest O_2 demands of any fish group (**see also Pelagic Fishes**: Physiology of Tuna and Endothermy in Tunas, Billfishes, and Sharks). The largest relative gill area measured to date is that of the skipjack tuna, *Katsuwonus pelamis* (**Figure 4**).

For tunas and other fast-swimming oceanic teleosts, large gill areas generally result from numerous and long gill filaments with high lamellar frequencies (>30 mm^{-1} in some species). The increased number of filaments is achieved through changes to lamellar design, from a semicircular or triangular shape, typical of most fishes (**Figure 3**(**a**)), to a long, rectangular contour with a low profile (height) (**Figures 2**(**c**) and **3**(**b**)), thus allowing filaments to be closely spaced (**Figure 3**(**b**)). This shaping also allows for a marked reduction in the thickness of the water–blood barrier (i.e., low-profile lamellae require less structural support than tall lamellae) contributing to generally thin lamellae (only 5–6 μm in scombrids and billfishes). This reduced lamellar thickness, in conjunction with a narrow spacing between lamellae, increases lamellar frequency, which, in addition to augmenting gill area, decreases diffusion distances and minimizes physiological dead space in the interlamellar channels.

These changes to gill morphology deviate from the theoretical predictions for optimally augmenting gill area; the high frequency and unique shape of the lamellae narrow and lengthen the interlamellar channels (**Figure 3**(**b**)), which, according to Equations (4) and (5), increase gill resistance. Although a high branchial resistance substantially raises the energetic costs of gill ventilation in most fishes, it appears of less concern to fast oceanic species that breathe through ram ventilation, in which branchial flow is driven by fast, forward swimming rather than the pumping of the buccal and branchial cavities. In these fishes, the high branchial resistance associated with this unique morphometric configuration augmenting gill area helps to slow and streamline a fast and turbulent ram-ventilatory stream to create favorable flow conditions for gas exchange in the interlamellar channels.

In contrast, the large gill areas of lamnid sharks are achieved in the predicted manner: long gill filaments with relatively large lamellae. This likely reflects flow constraints imposed by the elasmobranch gill design in which water passing between the gill lamellae must subsequently flow through specialized channels along the interbranchial septum to exit the gill slits (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy). This additional water-flow pathway increases resistance through the elasmobranch gill and appears to preclude the recruitment of a high lamellar frequency to augment gill area (which would further augment branchial resistance). The relatively large lamellae of lamnid sharks appear to require additional structural support through a slightly thicker water–blood barrier ($\sim 1.2\,\mu m$ in the shortfin mako, in comparison to $0.5\,\mu m$ in tunas).

Marine Fishes of Intermediate Activity

This is largely a heterogeneous group of fishes characterized mainly by gill areas that are smaller than those of highly active oceanic species and larger than those of sluggish fishes. Included in this group are most marine fishes studied to date, including many jack species, mullets (Mugilidae), porgies (Sparidae), seabasses (Serranidae), wrasses (Labridae), butterfishes (Stromateidae), croakers (Sciaenidae), puffers (Tetraodontidae), and sharks from the families Sphyrnidae and Carcharhinidae, including oceanic species such as the blue shark, *Prionace glauca* (**Figure 4**). Often referred to as Gray's intermediates (after IE Gray who conducted the first comprehensive comparative study of gill surface areas in the 1950s), the gills of these fishes are largely normal; the gill filaments are of average length and number, and although many of these species are capable of ram ventilation at faster swimming speeds, the lamellae are generally taller and more traditionally shaped than those of the more highly active species. In teleosts, lamellar frequencies typically range from 15 to 25 mm^{-1}, while in elasmobranchs, frequencies of 10–15 mm^{-1} are more common, likely reflecting flow constraints imposed by the interbranchial septum. The thickness of the water–blood barrier usually ranges from 1.5 to $6\,\mu m$.

Sluggish Marine Species

This group includes relatively inactive species usually associated with the benthos or ocean depths that have small gill areas. Fishes from the deep ocean such as the bristlemouths (Gonostomatidae), barbeled dragonfishes (Stomiidae), deepsea tripod fishes (Ipnopidae), and some cusk eels (Ophidiidae) typically have low total filament lengths resulting from generally short and widely spaced filaments. Lamellar frequencies are also reduced, ranging from 7 to 20 mm^{-1}, which minimizes branchial resistance and the energetic requirements of gill ventilation. In some groups, a direct correlation has been found between ocean depth and gill size, in which the smaller gills of deeper-dwelling species likely reflect reduced metabolic demands. In shallower waters, benthic species including flatfishes (Bothidae and Pleuronectidae), some elasmobranchs (Squalidae, Scyliorhinidae, Triakidae, Rajidae, and Torpedinidae), and toadfishes (Batrachoididae) also have reduced gill areas and, in some cases, the water–blood barrier can be quite thick (e.g., the small-spotted catshark, *Scyliorhinus canicula*, $>11\,\mu m$), although this is not necessarily a distinguishing characteristic (e.g., the common sole, *Solea solea*, $<3\,\mu m$). The well-studied oyster toadfish, *Opsanus tau*, has fairly large lamellae, but a small total filament length (resulting largely from only three gill arches on each side of the head) and low lamellar frequency (10–13 mm^{-1}) leading to a small gill area (**Figure 4**), which, when coupled with a relatively thick lamellar water–blood barrier (5–$6\,\mu m$), results in a gill diffusion capacity 100× less than that of a comparably sized tuna.

Freshwater Fishes

The gill surface areas of these fishes are generally smaller than similar species inhabiting the marine environment. For example, the relatively active rainbow trout, *Oncorhynchus mykiss*, has a gill surface area similar to that of *O. tau* (**Figure 4**). This likely reflects several factors, including the reduced osmotic costs associated with life in freshwater and differences in dissolved oxygen levels (i.e., for a given temperature, air-saturated freshwater contains 15–20% more O_2). Despite an increase in O_2 availability, freshwater systems lack high-energy demand fishes comparable to those of the open ocean, which results in a reduced range in gill surface area. The largest gill areas of freshwater fishes thus approach those of marine species of intermediate activity (**Figure 4**) and are often associated with life in hypoxia (see below) or diadromy (the ability to inhabit both marine and freshwater environments). The smaller gill surface areas of freshwater fishes generally result from fairly small lamellae. The water–blood barrier thickness in freshwater fishes typically ranges from 2 to $10\,\mu m$ which is similar to the combined range of intermediate and sluggish marine species.

Air Breathers

In order to exploit hypoxic habitats (**see also Hypoxia**: The Expanding Hypoxic Environment), many fishes have auxiliary air-breathing organs ranging from respiratory epithelia lining the mouth and digestive tract to gas bladders and lungs (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction and Respiratory Adaptations for

Air-Breathing Fishes). Air offers the distinct advantage of containing much more O_2 per unit volume than water and being a less viscous respiratory medium, thus reducing ventilatory costs. The evolution of air breathing is often associated with a reduction in gill area (**Figure 4**), which minimizes the loss of oxygen acquired through an air-breathing organ to hypoxic water (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes). In many species, such as the lungfishes of the genera *Lepidosiren* and *Protopterus*, the gills have become so reduced that drowning occurs if air access is denied. However, most air-breathing fishes are facultative air breathers, meaning the gills are large enough to sustain aerobic metabolism in normoxia and air breathing is used primarily to supplement aquatic respiration under hypoxic conditions or during heightened O_2 demands associated with exercise. The water–blood barrier in air-breathing fishes is often quite thick, especially in amphibious species such as the climbing perch, *Anabus testudineus*, where it can exceed 10 μm and may help to limit gill desiccation and maintain lamellar structural integrity out of water. In nonamphibious air breathers, a thick water–blood barrier may work in conjunction with a small gill area to reduce oxygen loss to the hypoxic environment via the gills. However, many air-breathing fishes maintain relatively thin barriers (i.e, the spotted snakehead, *Channa punctata*, 2.0 μm) indicating the continued importance of the gills in aquatic respiration.

Hypoxia Dwellers

Fishes living in periodic or chronic hypoxia and lacking the ability to breathe air generally show changes to gill morphology that increase diffusion capacity. Specifically, inhabitants of hypoxic swamps, including some African cichlids (Cichlidae), elephantfishes (Mormyridae), cyprinids (Cyprinidae), livebearers (Poeciliidae), characids (Characidae), and trahiras (Erythrinidae), as well as fishes living in the ocean's oxygen minimum zone (OMZ), such as bristlemouths, pearleyes (Scopelarchidae), and bigscale fishes (Melamphaidae) or hypoxic tidepools, including sculpins (Cottidae), usually have larger gill areas than conspecifics or closely related species living in normoxic waters. Unlike high-energy demand teleosts, gill areas in hypoxia dwellers are generally augmented through relatively large lamellae and long gill filaments, thus maintaining low energetic costs for gill ventilation. Few measures of the water–blood barrier thickness exist for such fishes, although it is hypothesized that they should be fairly thin.

Hypoxia-induced changes to gill morphometrics depend on both the severity and periodicity of low O_2 levels. On evolutionary timescales, selective pressures for the exploitation of nutrient-rich waters in the OMZ have resulted in a number of mesopelagic species specialized for life in chronic hypoxia, such as the pearl eye, *Scopelarchoides nicholsi*, which has such long gill filaments they protrude from the opercular openings (**Figure 5**). In environments with prolonged but periodic hypoxia (e.g., many freshwater swamps), phenotypic plasticity in gill morphology allows for adjustment to changing conditions on shorter timescales. For example, African cichlids reared in the laboratory under hypoxic conditions have longer filaments and higher gill surface areas than those raised in normoxia. Such plasticity in gill morphology is epitomized by the crucian carp, *Carassius carassius*, which, when exposed to hypoxia in ice-covered ponds during the winter, can increase gill area by 7.5× in 7–14 days by exposing lamellae normally embedded in an interlamellar cellular mass (**see also Ventilation and Animal Respiration**: Plasticity in Gill Morphology).

Scaling: Fish Gill Morphometrics in Relation to Fish Growth

Gill surface area and its componential dimensions are often reported in relation to fish body mass using log–log plots (**Figure 4**) and the power-law scaling equation:

$$Y = aM^b \tag{6}$$

or log form

$$\log Y = \log a + b \log M \tag{7}$$

where Y is the particular gill morphometric (i.e., A, L_{fil}, n_{lam}, or A_{lam}), a is the intercept value for a 1-g specimen, M is the fish mass, and b is the species-specific slope or scaling exponent (=regression coefficient). As a fish grows, isometric geometry of the gills (i.e., the length/area/volume relationship assuming gill mass increases at the same rate as body mass, $b = 1.0$) predicts that L_{fil} should scale to the one-third (i.e., length/volume, $b = 0.33$), n_{lam} to the negative one-third (length^{-1}/volume, $b = -0.33$), and A_{lam} to the two-thirds (area/volume, $b = 0.67$), which, when added together, sum to the expected scaling exponent for total gill

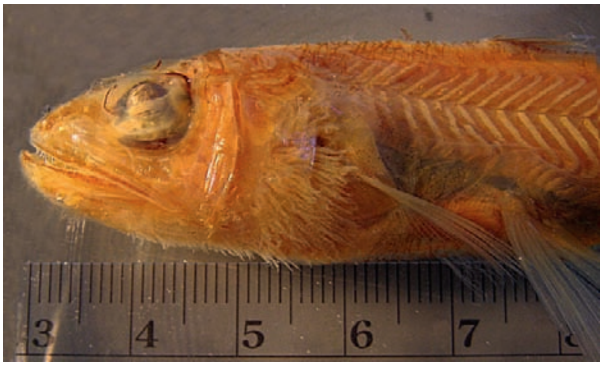


Figure 5 Elongate gill filaments of the pearleye, *Scopelarchoides nicholsi*, extending out the opercular slits. Scale is in cm.

surface area (area/volume, $b = 0.67$). However, in general, measured values do not conform to these geometric predictions, indicating the influence of various physiological processes affecting gill growth. For example, the scaling exponent for gill area ranges from under 0.50 to over 1.00. The mean of this range (0.80) appears to correlate with the mean scaling exponent for fish standard metabolic rate with body mass (0.81), while deviation from the mean may reflect a connection to active metabolic demands (e.g., in continuously swimming fishes which are never at rest) or other variables (**see also Energetics**: Physiological Functions that Scale to Body Mass in Fish). The low scaling exponents of many air-breathing fishes (e.g., *C. punctata*, $b = 0.59$; **Figure 4**) reflect an increased reliance on auxiliary air breathing with growth. At the opposite extreme, the hemoglobin-lacking blackfin icefish, *Chaenocephalus aceratus*, has a scaling exponent of 1.09, which appears to be associated with the increasingly problematic effects of diffusion limitation at larger body sizes.

By incorporating species-specific changes with mass, scaling equations facilitate interspecific comparisons of gill morphometrics and are often extrapolated to compare species of different body size. This has largely replaced methods of early gill studies comparing fishes of different weights through mass-specific gill areas (i.e., gill area/fish mass), which are generally inappropriate because gill area and body mass do not scale isometrically. Although the extrapolation of scaling equations thus provides a more favorable means of comparison, statistically, morphometric relationships can only be compared over a shared (i.e., overlapping) weight range. Errors associated with extrapolating gill area, or other morphometric relationships (especially those established using a limited sample size or weight range), can be seen in **Figure 4** where two independently determined regression lines for *O. mykiss* result in very different scaling equations.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; Respiratory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Energetics**: Physiological Functions that Scale to Body Mass in Fish. **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: The Expanding Hypoxic Environment. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks; Physiology of Tuna. **Role of the Gills**: Morphology of Branchial Ionocytes; The Osmorespiratory Compromise. **Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P50. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Plasticity in Gill Morphology.

Further Reading

Chapman LJ (2007) Morpho-physiological divergence across aquatic oxygen gradients in fishes. In: Fernandes MN, Rantin FT, Glass ML, and Kapoor BG (eds.) *Fish Respiration and Environment*, pp. 13–39. Enfield, NH: Science Publishers.

Da Costa OTF, Predretti ACE, Schmitz A, Perry SF, and Fernandes MN (2007) Stereological estimation of surface area and barrier thickness of fish gills in vertical sections. *Journal of Microscopy* 225: 1–9.

Graham JB (1997) *Air-Breathing Fishes: Evolution, Diversity, and Adaptations*. San Diego, CA: Academic Press.

Graham JB (2006) Aquatic and aerial respiration. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 85–117. Boca Raton, FL: CRC Press.

Gray IE (1954) Comparative study of the gill area of marine fishes. *Biological Bulletin* 107: 219–225.

Hughes GM (1966) The dimensions of fish gills in relation to their function. *Journal of Experimental Biology* 45: 177–195.

Hughes GM (1970) Morphological measurements on the gills of fishes in relation to their respiratory function. *Folia Morphologica* 18: 78–95.

Hughes GM (1972) Morphometrics of fish gills. *Respiration Physiology* 14: 1–25.

Hughes GM (1980) Morphometry of fish gas exchange organs in relation to their respiratory function. In: Ali MA (ed.) *Environmental Physiology of Fishes*, pp. 33–56. New York: Plenum.

Hughes GM (1984) General anatomy of the gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 10A, pp. 1–72. Orlando, FL: Academic Press.

Hughes GM (1984) Measurement of gill area in fishes: Practices and problems. *Journal of the Marine Biological Association of the United Kingdom* 64: 637–655.

Hughes GM and Morgan M (1973) The structure of fish gills in relation to their respiratory function. *Biological Reviews of the Cambridge Philosophical Society* 48: 419–475.

Muir BS and Hughes GM (1969) Gill dimensions for three species of tunny. *Journal of Experimental Biology* 51: 271–285.

Palzenberger M and Pohla H (1992) Gill surface area of water-breathing freshwater fish. *Reviews in Fish Biology and Fisheries* 2: 187–216.

Stevens ED (1992) Oxygen molecules as units to dimension the sieve of fish gills. *Environmental Biology of Fishes* 33: 317–318.

Wegner NC and Graham JB (2010) George Hughes and the history of fish ventilation: From Du Verney to the present. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* 157: 1–6.

Wegner NC, Sepulveda CA, Bull KB, and Graham JB (2010) Gill morphometrics in relation to gas transfer and ram ventilation in high-energy demand teleosts: Scombrids and billfishes. *Journal of Morphology* 271: 36–49.

Weibel ER and Knight BW (1964) A morphometric study on the thickness of the pulmonary air–blood barrier. *Journal of Cell Biology* 21: 367–384.

The Effect of Exercise on Respiration

H Thorarensen, Holar University College, Saudarkrokur, Iceland

© 2011 Elsevier Inc. All rights reserved.

Metabolic Rate and the Cost of Activity
The O_2 Cascade and Transport of Respiratory Gases
Cardiovascular Gas Transport
Cardiac Output
Blood O_2/CO_2 Content
Blood-Flow Distribution
Limitations to Maximum Oxygen Consumption
Further Reading

Glossary

Active metabolic rate (AMR) The maximum O_2 consumption rate measured either in a swim tunnel respirometer or following exhaustive exercise.

Cardiac output (V_b) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Cardiovascular oxygen transport The total amount of oxygen transported through the cardiovascular system ($T_{O_2} = Q \times Ca_{O_2}$).

Critical swimming speed (U_{crit}) The U_{crit} is determined in a swim tunnel where the fish are forced to swim against a current. The current velocity is increased in steps and each step is maintained for a specified time (usually 10 min to 1 h) or until the fish fatigues.

Routine metabolic rate (RMR) The O_2 consumption rate of resting fish is called resting or routine metabolic rate and is usually higher than the SMR.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Metabolic Rate and the Cost of Activity

The locomotion of fish has been broadly classified into three categories depending on the swim intensity. During burst exercise, fish swim at high velocities that can only be maintained for short periods (<20 s) and most of the energy used for the locomotion is generated by anaerobic metabolism. Following these intense bouts of activity, the O_2-consumption rate is temporarily increased, while the fish recover and repay the 'O_2 debt'. A sustained swimming velocity can be maintained for long periods (>200 min) without muscular fatigue and the energy is derived from aerobic metabolism. Between these two extremes is prolonged exercise, which is the swimming velocity that the fish can maintain for 20 s to 200 min. This article reviews cardiac and respiratory function in fish during sustained and prolonged exercise (**Figure 1**).

The energy available for aerobic activity in fish is limited by the scope for activity (SA); the difference between the maximum or active metabolic rate (AMR) and the standard metabolic rate (SMR). Both the SMR and AMR vary with, for example, body mass and ambient temperature, and there are significant differences among species. In general, the SA increases with body mass. The metabolic rate of fish during aerobic activity is usually measured with indirect calorimetry, such as oxygen consumption rate (O_2, **Figure 2**; **see also Ventilation and Animal Respiration**: Techniques in Whole Animal Respiratory Physiology), which can be converted to units of metabolic power output through the oxycalorific coefficient (13.6–14.4 kJ mg^{-1} O_2).

The maximum O_2 consumption rate ($\dot{V}_{O_{2max}}$) is measured while the fish are swimming (**Figure 2**) at or near the critical swimming speed (U_{crit}) or just after they have been chased to exhaustion.

Following feeding, the heat increment (HI; also called the specific dynamic action), which is the metabolic cost of digestion and assimilation, may nearly double the routine metabolic rate (RMR) of fish. The elevation in RMR due to the HI reduces the SA in salmonids, and in less active fish, it may demand most of the metabolic scope. However, in other species, such as sea bass, the $\dot{V}_{O_{2max}}$ is increased postprandially, and the SA is not reduced.

The metabolic cost of swimming increases exponentially as a function of swimming velocity (**Figure 2**). The increase in $\dot{V}_{O_2}$ from rest to maximum varies among fish species, body mass, and ambient temperature. Reported values for the factorial increase in $\dot{V}_{O_2}$ from SMR to $\dot{V}_{O_{2max}}$ range from 5-fold to 20-fold. It is often assumed

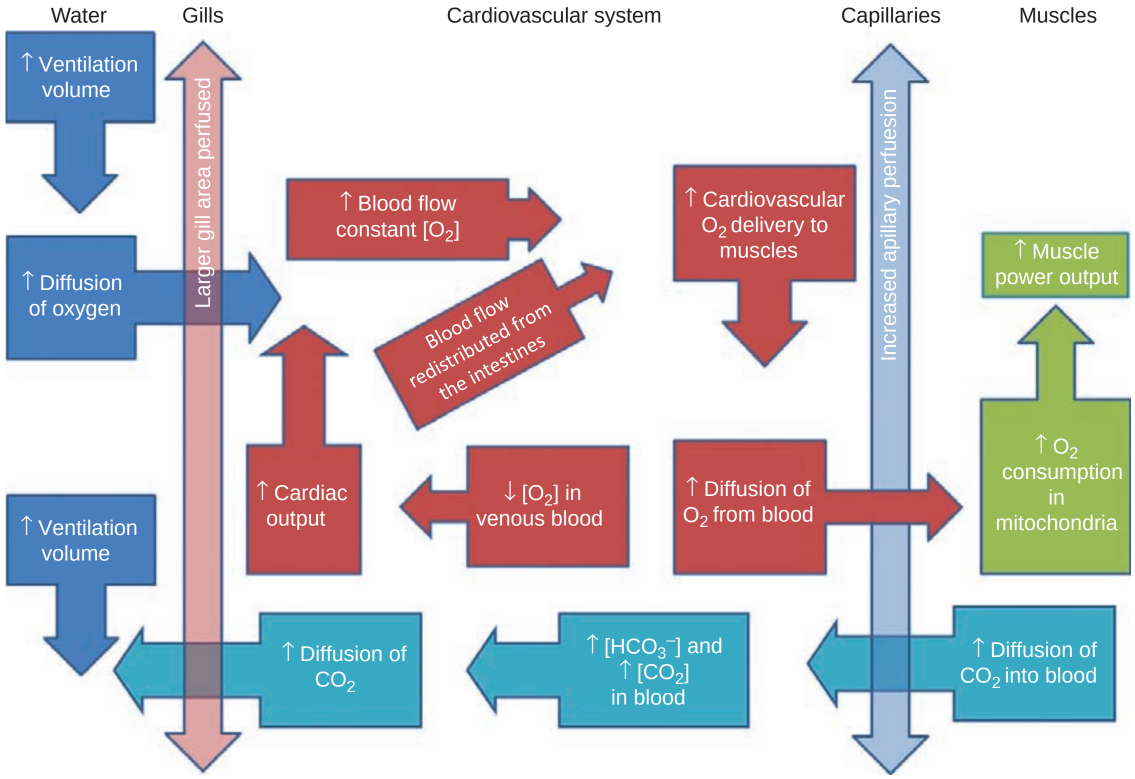

Water
Gills
Cardiovascular system
Capillaries
Muscles
↑ Ventilation volume
Larger gill area perfused
↑ Blood flow constant [O_2]
↑ Cardiovascular O_2 delivery to muscles
Increased apillary perfusion
↑ Diffusion of oxygen
Blood flow redistributed from the intestines
↑ Muscle power output
↑ O_2 consumption in mitochondria
↑ Cardiac output
↓ [O_2] in venous blood
↑ Diffusion of O_2 from blood
↑ Ventilation volume
↑ Diffusion of CO_2
↑ [HCO_3^-] and ↑ [CO_2] in blood
↑ Diffusion of CO_2 into blood

Figure 1 Summary of some of the cardirespiratory changes that support the increased metabolic demands of the locomotory muscles.

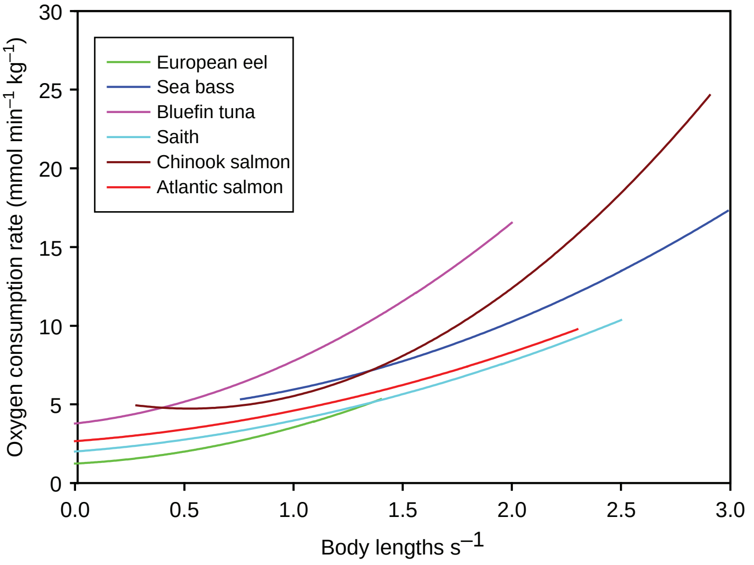


Figure 2 Oxygen consumption rate ($\dot{V}_{O_2}$) of fish swimming at different velocities. The $\dot{V}_{O_2}$ for each species depends on the body mass of the fish and ambient temperature. The $\dot{V}_{O_2}$ (as measured in mmol min^{-1} kg^{-1}) decreases with increased body mass and increases with temperature until the thermal maxima for the species are approached. The body mass of the fish and ambient temperature were as follows: Atlantic salmon (*Salmo salar*), 1 kg 10 °C; European eel (*Anguilla anguilla*), 0.643 kg 18 °C; sea bass (*Dicentrarchus labrax*), 0.325 kg 18 °C; bluefin tuna (*Thunnus thynnus*), 8.3 kg 20 °C; saithe (*Pollachius virens*), 0.485 kg 10 °C; Chinook salmon (*Oncorhynchus tshawytscha*), 0.387 kg 10 °C.

that the increase in $\dot{V}_{O_2}$ above SMR reflects the total energetic cost of activity. However, not all of the increase in $\dot{V}_{O_2}$ with swimming speed reflects the direct cost of locomotion. The energetic cost of the cardiac and the branchial pumps (see below) changes with swimming velocity. Furthermore, other metabolic demands may increase with swimming velocity such as the cost of osmoregulation and protein synthesis. There is also evidence to indicate that SMR is reduced as swimming speed increases due to the redistribution of blood flow. Therefore, the increase in $\dot{V}_{O_2}$ in swimming fish does not necessarily reflect the net cost of locomotion, but instead a combination of factors, some of which will increase the apparent cost of locomotion, such as the increased costs of ventilation and cardiac pumping while others, such as reduced basal metabolic rate, may reduce the apparent cost of locomotion.

Spontaneous routine-swimming activity under natural conditions may demand more energy than suggested by the cost of steady swimming in laboratory swim tunnels. This is mainly because the routine swimming of fish is not steady and rectilinear as in swim tunnels, but interrupted by accelerations and powered turns. The net cost of routine swimming may be three- to fourfold higher than for steady swimming. Therefore, the sustainable swimming speeds estimated in laboratories are likely higher than the routine swimming speed, which can be sustained under natural conditions. The opposite is likely true for burst swimming activity, because swimming tunnels are known to limit the maximum burst swimming capability, and as a result, potentially, the critical swimming speed.

The optimum swimming speed (U_{opt}) is the velocity that maximizes the distance traveled for a unit energy expended. For salmonids, the U_{opt} is close to 1 body length s^{-1}, which appears to coincide with the routine swimming velocity of migrating salmon in the ocean. If foraging success is proportional to the distance covered, there may be substantial gains to be had from swimming at U_{opt} compared with lower velocities. Similarly, during migration, there is a considerable energetic advantage in maintaining U_{opt}.

The O_2 Cascade and Transport of Respiratory Gases

The O_2 consumed by the locomotory muscles is primarily transported to tissues through a sequence of processes that are driven by convection and diffusion (**Figure 1**). The first step is ventilation and then diffusion of O_2 from water across the gill epithelia and into the blood (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). Most of the O_2 transported from the gills to the tissues is bound to the hemoglobin (**Figure 3**; **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). Finally, O_2 diffuses from the blood to the tissues, where it is consumed in the mitochondria (**see also Tissue Respiration**: Mitochondrial Respiration and Cellular Respiration). This transfer of O_2 is appropriately described as the O_2 cascade drawing an analogy with a cascaded waterfall, where O_2 progressively falls down its partial pressure gradient. Some of the O_2 consumed by fish is not carried by the cardiovascular system, but diffuses directly through the skin or is consumed at the gills. Cutaneous O_2 uptake is particularly important during embryonic and larval stages before the gills are fully developed (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions). Adults of water breathing fish may take up 10–20% of the resting O_2 consumption through the skin. However, cutaneous O_2 uptake is not likely to contribute significantly to the increased O_2 demands of exercising muscles.

The respiratory quotient (CO_2 produced/O_2 consumed) is near unity in fish and, therefore, the consumption of O_2 is matched by excretion of CO_2 (**Figure 3**; **see also Tissue Respiration**: Cellular Respiration). The CO_2 diffuses out of muscle cells and into the red blood cells (RBCs), where carbonic anhydrase (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange) converts CO_2 to HCO_3^-. Most of the HCO_3^- then leaves the RBCs through Cl^-/HCO_3^- exchange and is carried in the plasma, while smaller fractions are carried as HCO_3^- in the RBCs or as molecular CO_2 dissolved in plasma. When the blood reaches the gills, the process is reversed and HCO_3^- re-enters the RBCs, is dehydrated by carbonic anhydrase, and diffuses as CO_2 down a partial pressure gradient out of the fish (**See also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion) facilitated by the interaction between O_2 and CO_2 exchange (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

Ventilation and Diffusion across Gills

In resting fish, the buccal and opercular pumps drive water almost continuously across the gills by pressure differences created by alternate changes in the volume of the buccal and opercular cavities. During exercise, the ventilation volume increases in step with or even disproportionately more than $\dot{V}_{O_2}$. Therefore, the cost of ventilation increases with swimming. As swimming speed increases, the mode of ventilation may shift from active to ram ventilation, where most of the power required for ventilation is generated by the locomotory muscles. Some species are obligate ram ventilators and rely entirely on this form of ventilation. In the ram mode of ventilation, the fish adjust ventilation volume by changing the mouth gape. Ram ventilation is more efficient in terms of ventilatory work than the buccal and opercular pumps and reduces the overall cost of ventilation compared with buccal and opercular pumping.

The secondary lamellae are the main gas exchange surfaces of the gills (**see also Design and Physiology of**

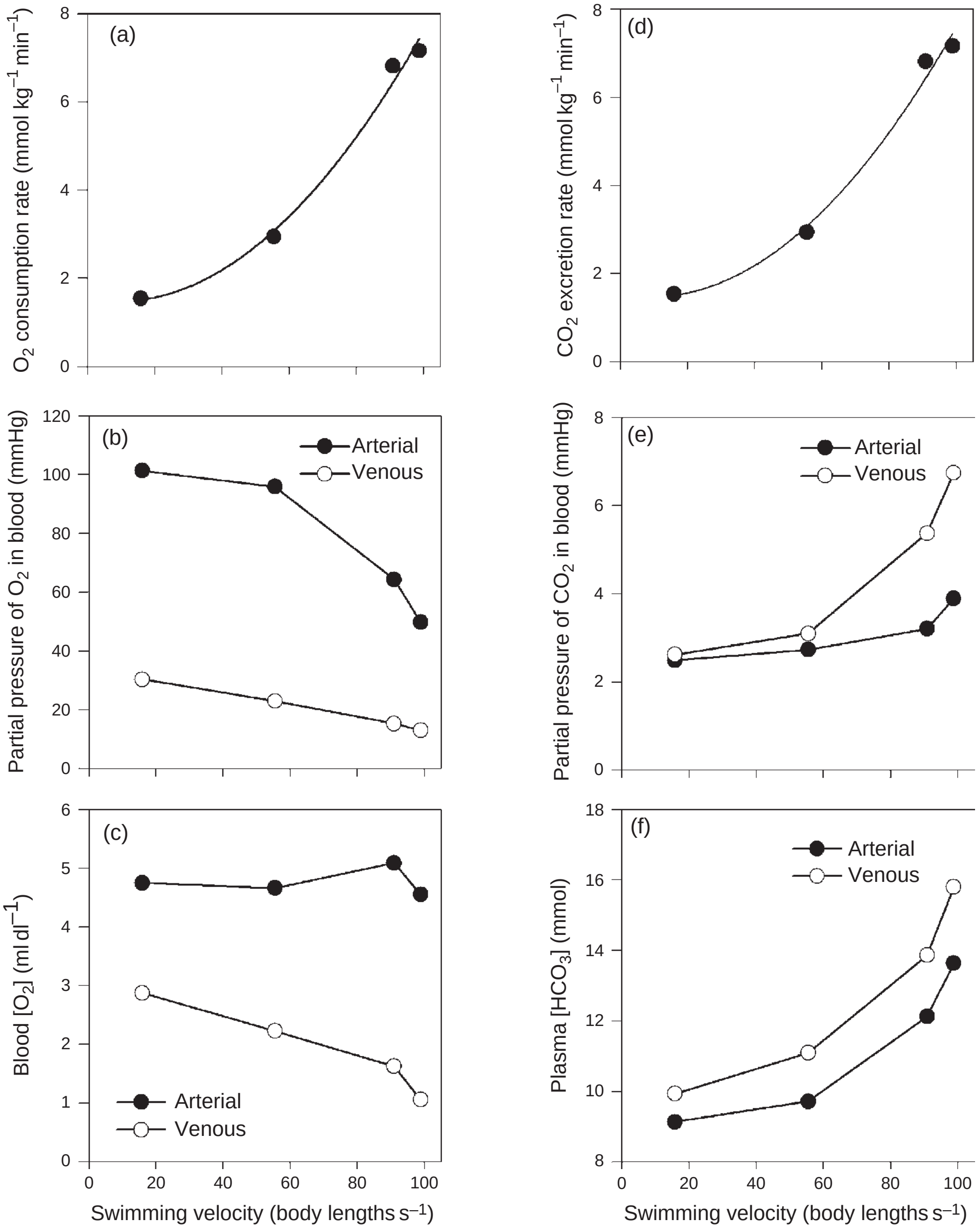


Figure 3 Blood gases in swimming rainbow trout. Oxygen consumption rate (a), partial pressure of O_2 in blood (b), concentration of O_2 in blood (c), CO_2 excretion rate (d), partial pressure of CO_2 in blood (e), and concentration of HCO_3^- in plasma. As swimming velocity increased up to U_{crit}, the cardiac output increased 2.5-fold and the arterial-venous difference in O_2 concentration increased by 1.9-fold. Data replotted from Brauner CJ, Thorarensen H, Gallaugher PE, Farrell AP, and Randall DJ (2000) CO_2 transport and excretion in rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. *Respiration Physiology* 119: 69–82 and Brauner CJ, Thorarensen H, Gallaugher PE, Farrell AP, and Randall DJ (2000). The interaction between O_2 and CO_2 in the blood of rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. *Respiration Physiology* 119: 83–96.

Arteries and Veins: Branchial Anatomy) for uptake of O_2 and excretion of CO_2. Water flows over the lamellae countercurrent to the direction of internal flow of blood. This allows the partial pressure of O_2 in the dorsal aorta (Pa_{O_2}) to be higher than the P_{O_2} of the expired water (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). The rate of diffusion of O_2 between water and blood is proportional to the partial pressure gradients of the gasses at any location across the lamellae. The total conductance for O_2 between water and blood, termed either transfer factor or diffusing capacity, includes the combined conductance of the external water, a boundary layer of water over the gill epithelium, diffusion over the gill epithelium, through plasma, into the RBCs, and the reaction of O_2 with hemoglobin. Furthermore, shunts of water and blood flow from the respiratory surfaces may decrease the apparent conductance as well as regional inhomogenities in perfusion, ventilation, and conductance of the gills. The exact contribution of each factor to the total conductance is not known.

The total conductance for CO_2 between blood and water is higher than that of O_2 and, therefore, a diffusion limitation is less likely for CO_2 than O_2. However, it has been suggested that the rate-limiting step in the excretion of CO_2 is the HCO_3^-/Cl^- exchange at the RBCs (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion).

During exercise, the pattern of blood flow through the gills changes to increase the effective respiratory surface area (**Figure 1**) as well as to reduce the mean diffusion distances between water and blood. At rest, only two-thirds of the lamellae are perfused, mostly in the basal regions of the filaments. The increase in blood pressure in the ventral aorta during exercise recruits the more distal lamellae on each filament, and thus increases the effective respiratory surface area. Furthermore, the higher blood pressure causes proportionately more blood to flow through the central than through the basal region of each lamellae than at rest, which reduces the mean diffusion distance between blood and water. Catecholamines may also change the blood flow pattern of the gills, as well as increase the permeability of the gill epithelium for O_2 during exercise.

There is a good correlation between total gill surface area and $\dot{V}_{O_{2max}}$ of different species of fish (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes). As a result, it has been suggested that total gill surface area is the primary limitation to $\dot{V}_{O_{2max}}$ in fish.

Cardiovascular Gas Transport

The internal O_2 convection is described by the Fick equation:

$$V_{O_2} = Q \times (Ca_{O_2} - Cv_{O_2}) \tag{1}$$

where Q is cardiac output, and Ca_{O_2} and Cv_{O_2} are arterial and venous blood O_2 contents, respectively.

Cardiac Output

The resting Q (Q_{rest}) of fish has been measured in many species of fish and reports range from 6.2 to 132 ml min^{-1} kg^{-1} (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). Most of the variability in Q_{rest} is caused by temperature differences, with higher Q_{rest} occurring at increased temperatures (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature). At temperatures between 8 and 12 °C, the Q_{rest} in teleost fish ranges between 9 and 40 ml min^{-1} kg^{-1}. More active fishes tend to have higher Q_{rest}.

During exercise, Q is increased because of both increased heart rate (HR) and stroke volume (SV) (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming). Many species, for example, salmonids, appear to modulate Q primarily through changes in SV, while changes in HR appear to be less important. However, other species, such as sea bass, modulate Q primarily through changes in HR.

The maximum Q (Q_{max}) of fish during prolonged exercise increases with temperature as does Q_{rest}. Furthermore, active fish also have a higher Q_{max} than sluggish fish. The reported values for the relative increase in Q (Q_{max}/Q_{rest}) during exercise range from no change to about threefold increases. However, there is no clear difference in the relative increase in Q between active fish and sluggish fish during exercise.

The hearts of active fish are larger than the the hearts of less active fish (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and Cardiac Anatomy in Fishes). Moreover, there is considerable plasticity in the heart size of fish and the heart size may increase, for example, in response to exercise training, temperature, and maturation.

Blood O_2/CO_2 Content

The O_2 content of blood depends on hemoglobin concentration, the P_{O_2} of the blood, and the affinity of hemoglobin for O_2. The hemoglobin concentration is generally proportional to hematocrit (Hct, which is the RBC fraction in the blood) and the mean corpuscular hemoglobin concentration. In blood, most of the O_2 is carried bound to hemoglobin and only 4–6% of the total O_2 content is dissolved in the plasma. Because the affinity of hemoglobin for O_2 varies depending on the number of O_2 molecules bound to the hemoglobin, the curve for O_2 saturation of hemoglobin as a function of P_{O_2} is nonlinear (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). The affinity of fish hemoglobin for O_2 is affected by pH, CO_2, nucleotide triphosphate levels, and temperature (**see also Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P50). Reduced pH causes a right shift of the O_2 saturation curve (Bohr shift), and can decrease the O_2 capacity of Hb (Root shift) (**see also** Root **Transport and Exchange of Respiratory Gases in the Blood**: Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). Furthermore, adrenaline can affect intracellular pH and nucleotide triphosphate levels of RBCs and thus modulate the O_2 affinity of hemoglobin (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin).

When fish approach U_{crit}, the Pa_{O_2} may decrease (**Figure 3(b)**), while the Ca_{O_2} remains fairly constant during aerobic exercise (**Figure 3(c)**). By contrast, Cv_{O_2} may decrease by more than 50% (**Figure 3(c)**). The Hct may increase slightly as swimming speed increases both because RBCs are released from the spleen and plasma volume is reduced (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming). However, even if the Hct is increased, the Ca_{O_2} does not change appreciably from resting values.

During aerobic exercise, the delivery of CO_2 from the tissues to the gills increases. The P_{CO_2} and concentrations of CO_2 and HCO_3^- in blood increase, more so in the venous blood (**Figures 3(d)–3(f)**). Although P_{CO_2} increases, blood pH remains unchanged indicating that fish can elevate plasma HCO_3^- either through acid excretion (coupled with HCO_3^- retention) at the gills or kidney, or through direct HCO_3^- uptake across the gills.

Blood-Flow Distribution

As swimming velocity increases, the gas transfer between the gills and the locomotory muscles is elevated both because Q increases and blood flow is redistributed from the intestines and other organs to the muscles (**Figure 1**). The largest shift in blood flow is from the intestines. Little is known about blood-flow redistribution from other tissues such as the skin, but their contribution is likely much less than that from the intestines.

The intestinal blood flow in resting fasted fish is 30–40% of Q_{rest}, although values as low as 10% of Q_{rest} have been reported (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion). As swimming speed increases, gut blood flow is progressively reduced by 30–70%. By contrast, gut blood flow may nearly double in resting fed fish (reported values range from 40% to 155% increase) to support the blood flow and O_2 demands during digestion (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion). The increase in gut-blood flow in fed fish may reduce the blood flow available to exercising muscles, for example, in salmonids, where the cardiovascular system cannot perfuse maximally both muscles and intestines simultaneously. Interestingly, the U_{crit} of some other species, such as sea bass, is not reduced following feeding and their cardiovascular system appears to support simultaneously the O_2 demands of locomotion and the HI.

In addition to providing more O_2 to exercising muscles, the reduced gut-blood flow may also be important for cardiovascular function in swimming fish. There is a significant volume of blood contained in the intestinal veins. The volume of blood in the veins depends on passive distension of the venous walls by blood pressure and/or active changes in venous compliance. Reduced blood pressure or compliance in the intestinal veins could shift blood volume from the gut into the central circulation. This shift in blood volume may be important to increase or maintain central venous pressure in exercising fish and thus the filling pressure of the heart, which, in turn, is important for supporting maximum cardiac output (**see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels).

The systemic blood pressure of fish increases during exercise and this may be important for ensuring maximum perfusion of the gills. The increased resistance in the gut circulation in exercising fish may be important to counteract the reduced resistance to blood flow to the locomotory muscles and thus aid in maintaining arterial blood pressure in swimming fish (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

Limitations to Maximum Oxygen Consumption

The maximum O_2 consumption rate in fish could, in principle, be limited by any step in the O_2 cascade or mitochondrial oxidative phosphorylation pathway (**Figure 1**). Furthermore, substrate availability or transport of ATP to the sites of utilization could also limit $\dot{V}_{O_{2max}}$. Therefore, the limitations of $\dot{V}_{O_{2max}}$ may be a result of the interplay of numerous factors.

There are several lines of evidence that suggest that the primary limitation to $\dot{V}_{O_{2max}}$ in fish resides in the convective transport of O_2 from the gills to the tissues ($T_{O_2} = Q \times Ca_{O_2}$). Both Q_{max} and Ca_{O_2} can directly affect $\dot{V}_{O_{2max}}$. When the Ca_{O_2} in fish is reduced through anemia, reduced hemoglobin O_2 affinity, or exposure to hypoxia, then $\dot{V}_{O_{2max}}$ and swimming performance of fish are decreased. There is a near proportional change in $\dot{V}_{O_{2max}}$ of rainbow trout when the $T_{O_{2max}}$ is varied by experimentally changing the Hct above or below normal values. There is also evidence to indicate that rainbow trout with high Q_{max} have higher $\dot{V}_{O_{2max}}$ and U_{crit} than fish with lower Q_{max}.

When comparing different species of fish, the available data suggest that there is a close correlation between the $T_{O_{2max}}$ and $\dot{V}_{O_{2max}}$. This supports the hypothesis that $\dot{V}_{O_{2max}}$ of fish is primarily limited by $T_{O_{2max}}$. However, the $T_{O_{2max}}$ is clearly not the sole determinant of $\dot{V}_{O_{2max}}$ in fish, and there is ample evidence to suggest that other levels in the O_2 cascade can also affect $\dot{V}_{O_{2max}}$. Some of these are discussed below.

There may be a diffusion limitation in tissues. The Cv_{O_2} is reduced in swimming fish (**Figure 3(c)**), although there is always residual O_2 in venous blood, which suggests that there may be some form of diffusion limitation in tissues. Thus $\dot{V}_{O_{2max}}$ may increase independent of $T_{O_{2max}}$ by increasing the conductance of O_2 from blood to tissues, for example, through increased capillarization.

Another factor that can affect $\dot{V}_{O_{2max}}$ in fish is the size of the gas-exchange area of the gills. Certainly, more-active fish have a larger gill surface area than do less-active fish. Furthermore, experimental reduction in gill surface area will affect gas transfer. At maximum swimming speed, the Pa_{O_2} is reduced (**Figure 3(b)**) and Pa_{CO_2} is increased (**Figure 3(e)**) in some species of fish. These changes in partial pressure can be caused either by insufficient ventilation volume or by diffusion limitations at the gills. Because ventilation volume increases in step with $\dot{V}_{O_2}$ it is unlikely to cause a reduction in Pa_{O_2}. However, the total conductivity for O_2 at the gills may not increase in step with $\dot{V}_{O_2}$, and that would cause a reduction in Pa_{O_2} and an increase in Pa_{CO_2}. This suggests that a diffusion limitation for O_2 and CO_2 may exist across the gills as gas diffusion rates increase with exercise.

It may be useful to draw an analogy between O_2 transfer in fish from water to tissues and an electrical circuit with resistors arranged in series. Each of the factors mentioned above are potential limitations to $\dot{V}_{O_{2max}}$. However, to take the analogy one step further, the relative contribution of each of the 'resistors' to the total resistance to flow of O_2 through the system varies. Therefore, a unit change in any of the larger 'resistors' has a greater effect on O_2 flux than a proportional change in the smaller resistors. Because changes in $T_{O_{2max}}$ of trout can have a substantial effect on $\dot{V}_{O_{2max}}$, T_{O_2} must be one of the larger resistors of the system and possibly the largest, while diffusion limitations for O_2 across the gills and from blood to tissues are significantly smaller. The $\dot{V}_{O_{2max}}$ in mammals also appears to be primarily limited by $T_{O_{2max}}$.

Although the available information points to $T_{O_{2max}}$ as being the primary limitation to $\dot{V}_{O_{2max}}$ in fish, comprehensive studies have only been performed on few species. Considering the great number and diversity of fish species, it is difficult to make generalizations. Furthermore, the concept of symmorphosis states that "functional design (of the respiratory system) is commensurate with functional demand" such that all structures within the respiratory system are approximately matched with the aerobic capacity of an animal. Thus, the gas-exchange area of the gills, heart size, and T_{O_2} are all correlated with $\dot{V}_{O_{2max}}$. Increases in the capacity of a single step in the O_2 cascade can have only limited effects on $\dot{V}_{O_{2max}}$, because other steps in the O_2 cascade will take over as primary limitations for O_2 transport.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy; Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping; Physiology of Capacitance Vessels. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes. **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Digestion; Integrated Responses of the Circulatory System to Temperature. **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Carbonic Anhydrase in Gas Transport and Exchange; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; O_2 Uptake and Transport: The Optimal P50; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Respiratory Gas Exchange During Development: Respiratory Transitions; Techniques in Whole Animal Respiratory Physiology.

Further Reading

Beamish FWH and Trippel EA (1990) Heat increment: A static or dynamic dimension in bioenergetic models? *Transactions of the American Fisheries Society* 119: 649–661.

Brauner CJ and Randall DJ (1998) The linkage between oxygen and carbon dioxide transport. In: Tufts BL and Perry SF (eds.) *Fish Physiology,* vol. 17, pp. 283–319. New York: Academic Press.

Brauner CJ, Thorarensen H, Gallaugher PE, Farrell AP, and Randall DJ (2000) CO_2 transport and excretion in rainbow trout *(Oncorhynchus mykiss)* during graded sustained exercise. *Respiration Physiology* 119: 69–82.

Brauner CJ, Thorarensen H, Gallaugher PE, Farrell AP, and Randall DJ (2000) The interaction between O_2 and CO_2 in the blood of rainbow trout *(Oncorhynchus mykiss)* during graded sustained exercise. *Respiration Physiology* 119: 83–96.

Farrell AP (2007) Cardiorespiratory performance during prolonged swimming tests and potential analytical pitfalls tests with salmonids: A perspective on temperature effects. *Philosophical Transactions of the Royal Society B* 362: 2017–2030.

Farrell AP, Eliason EJ, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835–851.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology,* vol. 12a, pp. 1–88. San Diego, CA: Academic Press.

Gallaugher PE and Farrell AP (1998). Hematocrit and blood oxygen carrying capacity. In: Perry SF and Tufts BL (eds.) *Fish Physiology,* vol. 17, pp. 185–227. New York: Academic Press.

Gamperl AK and Farrell AP (2004) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Kiceniuk JW and Jones DR (1977) The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *Journal of Experimental Biology* 69: 247–260.

Nikinmaa M and Salama A (1998). Oxygen transport in fish. In: Tufts B and Perry SF (eds.) *Fish Physiology,* vol. 17, pp. 141–184. New York: Academic Press.

Randall D and Brauner C (1991) Effects of environmental factors on exercise in fish. *Journal of Experimental Biology* 160: 113–126.

Randall DJ and Daxboeck C (1984) Oxygen and carbon dioxide transfer across fish gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 10A, pp. 263–307. New York: Academic Press.

Sandblom E and Axelsson M (2007) The venous circulation: A piscine perspective. *Comparative Biochemistry and Physiology A* 148: 785–801.

Satchell GH (1992) The venous system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology,* vol. 12a, pp. 141–184. San Diego, CA: Academic Press.

Tufts BL and Perry SF (1998) Carbon dioxide transport and excretion. In: Tufts BL and Perry SF (eds.) *Fish Physiology,* vol. 17, pp. 229–281. New York: Academic Press.

Efficiency of Gas Exchange Organs

H Malte, Aarhus University, Aarhus, Denmark

© 2011 Elsevier Inc. All rights reserved.

Gas Exchangers
Water versus Air as Respiratory Media
The Efficiencies of Gas Exchange
Further Reading

Glossary

Capacitance coefficient Formally defined as $\Delta C/\Delta P$, where C is concentration and P is the partial pressure of a gas. If the gas is present exclusively in physical solution, this is identical to the solubility of the gas.

Convection Bulk movement of a medium, e.g., gas, water, or blood.

Gas partial pressure in a liquid The partial pressure of a gas in blood or water is the partial pressure that a gas phase should have in order to be in equilibrium with that liquid. Thus, it is not a physical partial pressure as defined in a gas mixture.

Parallel inhomogeneity Inhomogeneity in some property, e.g., ventilation or perfusion among the structurally identical units of a gas-exchange organ.

Gas Exchangers

The primary job of the gas-exchange organ is to acquire oxygen from and deliver carbon dioxide to the environment. Therefore, it has a crucial role in the overall transport of gases between the environment and the metabolizing tissues. As outlined in **Figure 1**, this transport has four steps:

1. convective transport of the respiratory medium over the respiratory surface,
2. diffusion across the respiratory surface from medium to blood,
3. convective transport by blood between the respiratory surface and the metabolizing tissues, and
4. diffusion from the capillaries to the mitochondria in the surrounding tissues (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions).

While this overall transport layout is universal among vertebrates, the specific design of the gas-exchange organs is not. Thus, the way blood and media are arranged and flow relative to each other differ considerably among the various groups (**Figure 2**). In terrestrial vertebrates, the lungs are of two main types: the avian, or parabronchial type of lung, and the mammalian lung type. In the former, blood and medium (air) flow in a crosscurrent-like fashion, whereas in the latter, blood perfuses a ventilated pool of air with no specific orientation. Although the latter type differs greatly in sophistication between mammals and reptiles and amphibians, the basic design is the same. This also holds for the air-breathing organs of air-breathing fishes even though these may be of different anatomical origin. Among fishes, a countercurrent arrangement of the blood and water flows is universally found in the gill, although in some air-breathing species this may have been reduced and lost its quantitative importance (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

Water versus Air as Respiratory Media

There are a number of important differences between water and air as respiratory media that have consequences for gas exchange (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions):

1. The density of water is greater than that of air by a factor ~800.
2. The viscosity of water is greater than that of air by a factor ~50.
3. Diffusion coefficients for gases in water are smaller than those in air by a factor ~8000.
4. The capacitance coefficient (solubility) of CO_2 in water is ~30 times greater than that of O_2, whereas in air they are identical.

The density of the medium has importance for the work of breathing in the event that the hydraulic driving pressure (in water-breathing fishes that would be the pressure difference between the buccal and opercular cavities) and the flow are both pulsatile. In this case, the repeated acceleration of the much denser water with each breath

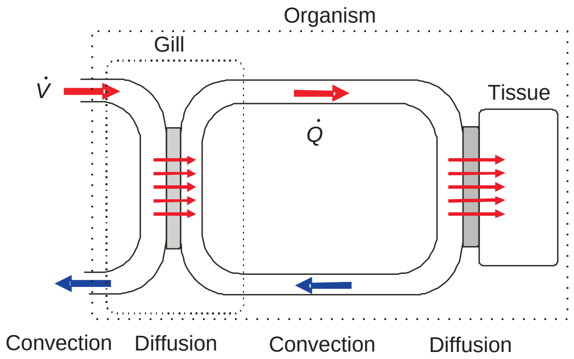


Figure 1 Gas-transport system in a hypothetical organism, illustrating how gas is transported, from the environment to the respiring tissues, by convection and diffusion in an alternating fashion.

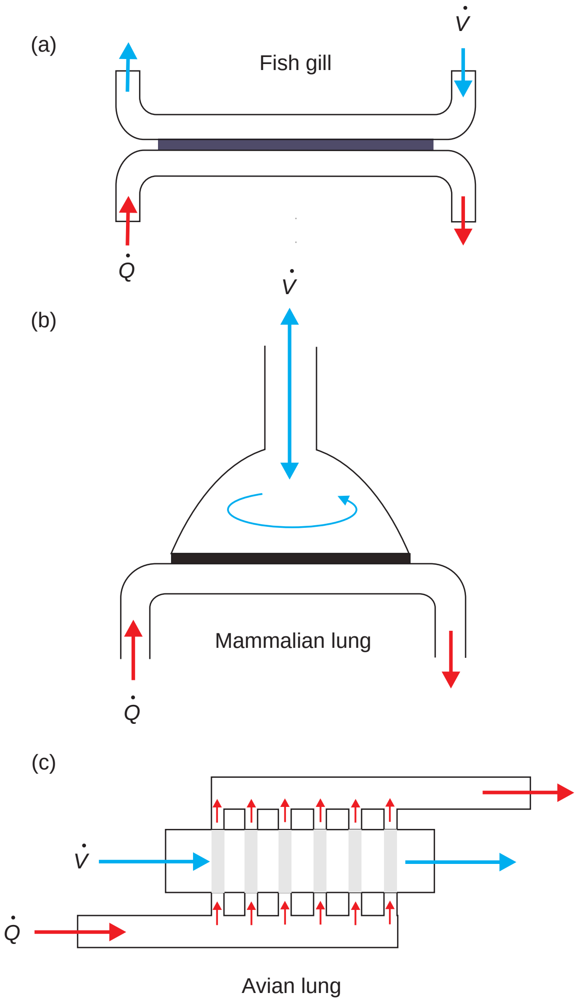


Figure 2 Models of vertebrate gas exchangers. In the fish gill (a), water and blood flow in a countercurrent fashion. In the mammalian lung (b), the blood exchanges gases with a pool of alveolar air that in turn is ventilated with ambient air in a tidal fashion. In the avian lung (c), air flows unidirectionally through the parabronchi and blood flows perpendicular to air flow, termed crosscurrent flow. This system is termed 'ventilated pool' or 'dynamic pool'.

would add to the cost of breathing in fishes. Indeed, the driving pressure is pulsatile in these fishes, and model studies of the pressure/flow relations in the interlamellar space of fish gills indicate that this is the case for flow as well.

The viscosity determines the frictional force that has to be overcome when fluid flows and therefore directly affects the work of breathing. This is independent of the degree of pulsatility of either pressure or flow.

The diffusion coefficients for respiratory gases in the respiratory media might, at first, seem irrelevant since gases are transported from the environment to the respiratory surface by convective transport as outlined in **Figure 1**. However, the risk of buildup of partial-pressure gradients of respiratory gases within the medium close to the respiratory surface is much greater in water than in air. Such gradients will reduce the efficiency of the exchange process. Thus, in fishes, it has been estimated that up to ~25% of the total resistance to diffusion resides in the respiratory water itself.

The capacitance coefficients of O_2, CO_2, and N_2 in water and air are given at 20 °C in **Table 1**. It is apparent that the capacitance coefficient for oxygen is much less in water than in air, which implies that air-saturated water contains correspondingly less oxygen than air contains. Therefore, in order to present the same amount of oxygen at the respiratory surface, much more water has to be moved. To express this quantitatively Fick's principle is applied to the respiratory medium:

$$\dot{M}_{O_2} = \dot{V}\beta_{O_2}(PI_{O_2} - PE_{O_2}) = \dot{V}(CI_{O_2} - CE_{O_2}) \qquad (1)$$

Here $\dot{V}$ is the ventilation rate and β_{O_2} the capacitance coefficient. By introducing the extraction coefficient (the fractional removal of oxygen from the ventilated water, often also referred to as the utilization) $E_{O_2} = (CI_{O_2} - CE_{O_2})/CI_{O_2}$ and rearranging, the following equation is obtained:

$$\frac{\dot{V}}{\dot{M}_{O_2}} = \frac{1}{E_{O_2}\beta_{O_2}PI_{O_2}} = \frac{1}{E_{O_2}CI_{O_2}} \qquad (2)$$

The left-hand side of the equation is termed the convection requirement, which represents the amount of

Table 1 Capacitance coefficients for O_2, CO_2, and N_2 at 20 °C

	O_2	CO_2	N_2
Water	1.82	51.7	0.931
Air	54.7	54.7	54.7

The tabulated values are in μmol·L^{-1}·mmHg^{-1}.
Values for air are calculated as 1/(*RT*), where *T* is the absolute temperature and *R* is the gas constant in L·mmHg·K^{-1}·μmol^{-1} (6.24×10^{-5})
Values for water are taken from *Handbook of Chemistry and Physics*, 73rd edition.

medium required to be moved over the respiratory surface per unit of oxygen taken up. The convection requirement is inversely proportional to the product of the extraction coefficient, the capacitance coefficient, and the inspired oxygen partial pressure. The product of the latter two represents the inspired oxygen concentration. In **Figure 3**, the convection requirement has been plotted as a function of CI_{O_2}, and the approximate value in humans, rainbow trout (*Oncorhynchus mykiss*), and common carp (*Cyprinus carpio*) have been included. An additional value has been included representing rainbow trout and common carp, assuming they have the same low extraction coefficient as humans (and most air breathers). This illustrates the important fact that water breathers tend to have higher extraction coefficients than do air breathers, which to some extent compensates for a high ventilation requirement.

From **Table 1** it is also evident that, while the respiratory gases have identical capacitance coefficients in air, this is not so in water where CO_2 is ~30 times more soluble than O_2. Comparison with the inert gas N_2 shows that this is due to an unusually high capacitance coefficient of CO_2 in water. This fact dictates an important difference between water and air breathers: while the O_2 partial pressures in the blood of air- and water-breathing animals are often similar, water breathers have much lower partial pressures of CO_2 in their blood than air breathers. This can be demonstrated by applying the Fick's principle simultaneously to CO_2 and O_2 transport by the respiratory media:

$$\dot{M}_{O_2} = \dot{V}\beta_{O_2}(PI_{O_2} - PE_{O_2}) \quad (3a)$$

$$\dot{M}_{CO_2} = \dot{V}\beta_{CO_2}(PE_{CO_2} - PI_{CO_2}) \quad (3b)$$

Dividing the CO_2 equation by the O_2 equation, recalling that $\dot{M}_{CO_2}/\dot{M}_{O_2} = RQ$ (respiratory quotient), and rearranging yields

$$PE_{CO_2} - PI_{CO_2} = RQ\frac{\beta_{O_2}}{\beta_{CO_2}}(PI_{O_2} - PE_{O_2}) \quad (4)$$

Thus, the increase in the CO_2 partial pressure of the respiratory medium as it passes over the respiratory surface equals the corresponding fall in O_2 partial pressure times the ratio of the O_2 to CO_2 capacitance coefficient times the *RQ*. The latter is always close to 1.0 (usually between 0.7 and 1.0), so the ratio of capacitance coefficients is crucial. In air, this ratio is 1.0, whereas in water it is ~1/30. This is illustrated in **Figure 4**, which shows the expired CO_2 partial pressure as a function of the expired O_2 partial pressure at an *RQ* of 1.0. It is clear that even if a water breather were able to extract all the oxygen, the expired partial pressure of CO_2 would not exceed 5–6 mmHg. This means that in water breathers the CO_2 partial pressure of blood will be very much lower than in air breathers (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions). **Figure 4** also indicates the approximate ranges of air breathers and water breathers on the lines along with the hypothetical range that would have been observed in air breathers if the oxygen extraction coefficient had been in the same range as that of water breathers.

The differences between air breathers and water breathers, discussed so far, are all due to the different properties of the respiratory media and are completely independent of the nature of the gas-exchange organ.

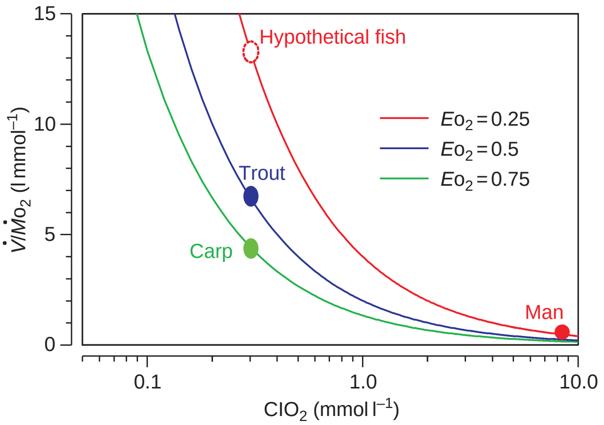


Figure 3 The convection requirement ($\dot{V}/\dot{M}$) as a function of the inspired oxygen concentration. Due to the much lower oxygen-capacitance coefficient of water (here a temperature of 15 °C was assumed) compared to air, the inspired oxygen concentration of air-equilibrated water is much lower than that in air and the convection requirement is correspondingly higher.

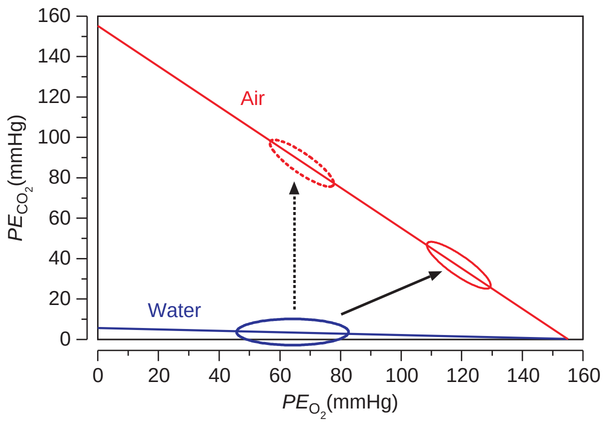


Figure 4 The PE_{CO_2} vs. PE_{O_2} diagram. In air breathers the slope is in the range −0.7 to −1.0 (depending on the respiratory quotient, *RQ*) whereas in water breathers it is only approximately −1/30 due to the very high CO_2 relative to O_2 capacitance coefficient of water. The approximate range of values *in vivo* are indicated by solid circles on the lines for water and air breathers. The dotted circle indicates theoretical values in air breathers with an oxygen-extraction coefficient equal to that of water breathers.

The Efficiencies of Gas Exchange

For the whole organism, the extraction coefficient, as defined earlier, is the appropriate measure of gas-exchange efficiency. This is, however, not a measure of the performance of the gill although it clearly depends on it. There are two measures of the efficiency of the gill as a gas exchanger which can be derived by starting with Fick's principle for water and blood, respectively:

Here, $\dot{V}w$ denotes gill ventilation rate and $\dot{Q}$ cardiac output and βw_{O_2} and βb_{O_2} are the capacitance coefficients of water and blood, respectively. While the relationship

$$\dot{M}_{O_2} = \dot{V}W(CI_{O_2} - CE_{O_2}) = \dot{V}W\beta W_{O_2}(PI_{O_2} - PE_{O_2}) \qquad (5a)$$

$$\dot{M}_{O_2} = \dot{Q}(Ca_{O_2} - C\bar{v}_{O_2}) = \dot{Q}\beta b_{O_2}(Pa_{O_2} - P\bar{v}_{O_2}) \qquad (5b)$$

between concentration and partial pressure in water is linear, in blood it is nonlinear, as described by the oxygen-equilibrium curve. Therefore, βb_{O_2} should be thought of as the slope of the straight line connecting the venous and arterial point on the oxygen-equilibrium curve (**Figure 5**); however, this slope is not constant and depends upon the outcome of the gas-exchange process. Nevertheless, it is a useful way to proceed as seen below. To simplify, conductances for ventilation ($Gv = \dot{V}w\beta_{O_2}$) and perfusion ($Gp = \dot{Q}\beta_{O_2}$) can be included such that the oxygen flux can be described as the product of a conductance and a driving partial-pressure difference:

$$\dot{M}_{O_2} = Gv(PI_{O_2} - PE_{O_2}) \qquad (6a)$$

$$\dot{M}_{O_2} = Gp(Pa_{O_2} - P\bar{v}_{O_2}) \qquad (6b)$$

If we simplify the above equations by multiplying and dividing with the total partial pressure difference available to the gill, we obtain

$$\dot{M} = Gv\frac{(PI - PE)}{(PI - P\bar{v})}(PI - P\bar{v}) = Gv\varepsilon w(PI - P\bar{v}) \qquad (7a)$$

$$\dot{M} = Gp\frac{(Pa - P\bar{v})}{(PI - P\bar{v})}(PI - P\bar{v}) = Gp\varepsilon b(PI - P\bar{v}) \qquad (7b)$$

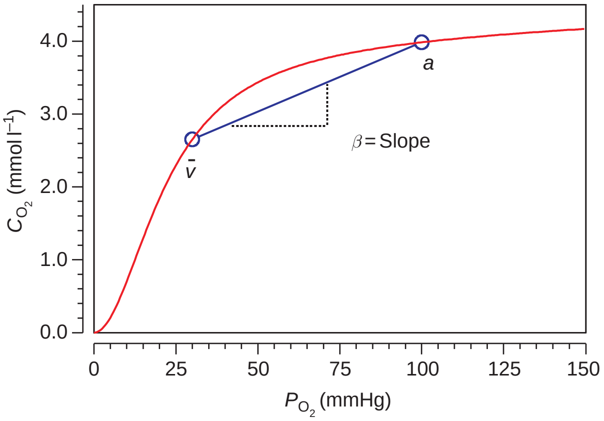


Figure 5 The oxygen-capacitance coefficient of blood βbo_2 can be calculated as the average slope of the oxygen-equilibrium curve between the venous and arterial values, that is, the slope of the secant line connecting them.

Now $\varepsilon w = (PI - PE)/(PI - P\bar{v})$ is the efficiency of the gill seen from the waterside. It expresses how well the ventilated water is deoxygenated by the gill. But, as opposed to the extraction coefficient, it takes into account the fact that PE can never go below $P\bar{v}$. Thus, if PE reaches $P\bar{v}$, the efficiency is 1, and if PE stays at PI, the efficiency is 0. Likewise, $\varepsilon b = (Pa - P\bar{v})/(PI - P\bar{v})$ is the efficiency of the gill seen from the blood side, and it expresses how well the blood is oxygenated by the gill. Obviously, the efficiency, ε_b, attains a value of 1 if $Pa = PI$ and a value of 0 if $Pa = P\bar{v}$. The efficiencies can also, appropriately, be termed the relative partial-pressure differences, because they represent the difference in partial pressure between the input and output on either of the sides viewed relative to the total difference available to the exchanger. From the above equations, it is apparent that the two efficiencies are not independent, that is, $Gv/Gp = \varepsilon b/\varepsilon w$. Thus, if $Gv > Gp$ then $\varepsilon b > \varepsilon w$ and vice versa. In the case where $Gv = Gp$, and therefore $\varepsilon b = \varepsilon w$, the exchanger is said to be matched (discussed in greater detail below).

One can derive an interesting connection between the extraction coefficient and the efficiency of the gill from the waterside:

$$\begin{aligned} E &= \frac{PI - PE}{PI} = \frac{PI - PE}{PI}\frac{PI - P\bar{v}}{PI - P\bar{v}} = \frac{PI - PE}{PI - P\bar{v}}\frac{PI - P\bar{v}}{PI} \\ &= \varepsilon w\frac{PI - P\bar{v}}{PI} = \varepsilon w(1 - P\bar{v}/PI) \end{aligned} \qquad (8)$$

Therefore, the extraction of oxygen from the water certainly depends not only on the performance of the gill as a gas exchanger, but also on the factor (1-Pv/PI), which is independent of the performance of the gill. The mixed-venous oxygen partial pressure, Pv, in the resting condition is species dependent and largely determined by the oxygen affinity of the blood. The higher the oxygen affinity, the lower the $P\bar{v}$ and thus the higher the extraction coefficient of oxygen.

The Efficiency of the Ideal Countercurrent Exchanger

The real fish gill is very complex, and a completely realistic model of gas exchange correspondingly so, with loss of generality as a consequence. A simple model that provides information about the performance, that is, the efficiencies of gas exchange, requires quite a few simplifications:

1. The blood and water flows are perfectly countercurrent and steady (no pulsatility).

2. Blood and water are separated by a uniform diffusion barrier.
3. In the water, as well as in the blood, there are only longitudinal gradients (i.e., no stratification).
4. The blood-gas-equilibrium curves are linear, and there is instantaneous equilibrium between the partial pressures and the concentrations (i.e., no reaction limitation).
5. All respiratory units (secondary lamellae and interlamellar water spaces) are ventilated and perfused at the same rate and have the same diffusion conductance (no $\dot{V}/\dot{Q}$ inequality).

Figure 6 is a graphical representation of such an idealized view of the gill. Performing a mass balance for any respiratory gas on a fine scale, as indicated in the figure, results in two, coupled first-order differential equations:

$$\frac{\mathrm{d}Pw}{\mathrm{d}l} = \frac{Gd}{Gv}(Pw - Pb) \tag{9a}$$

$$\frac{\mathrm{d}Pb}{\mathrm{d}l} = \frac{Gd}{Gp}(Pw - Pb) \tag{9b}$$

Table 2 How to calculate the efficiencies of countercurrent gas exchange

If Gv/Gp ≠ 1

$$\varepsilon b - \frac{Pa - Pv}{PI - P\bar{v}} = \frac{\frac{Gv}{Gp}\left(1 - e^{-\frac{Gd}{Gp}\left(1 - \frac{1}{Gv/Gp}\right)}\right)}{\frac{Gv}{Gp} - e^{\frac{-Gd}{Gp}\left(1 - \frac{1}{Gv/Gp}\right)}}$$

$$\varepsilon w - \frac{PI - PE}{PI - P\bar{v}} = \frac{1 - e^{-\frac{Gd}{Gp}\left(1 - \frac{1}{Gv/Gp}\right)}}{\frac{Gv}{Gp} - e^{\frac{-Gd}{Gp}\left(1 - \frac{1}{Gv/Gp}\right)}}$$

If Gv/Gp = 1

$$\varepsilon b = \varepsilon w = \frac{\frac{Gd}{Gp}}{1 + \frac{Gd}{Gp}}$$

These are obtained by simultaneously solving the two differential equations (9a) and (9b).
Note that at *Gv/Gp* = 1 the general expressions are undefined because both numerator and denominator are then zero.
The solution for the special case *Gv/Gp* = 1 can be obtained from any of the general expressions by applying L'Hospital's rule.

Solving this system of equations yields the partial pressures as a function of length (*l*) along the exchange surface (i.e., *Pw* = *Pw(l)* and *Pb* = *Pb(l)*) and once these (and thereby also the arterial and expired partial pressures, *Pa* and *PE*) are known, the efficiencies, can be calculated. These are listed in **Table 2** Also listed is the solution applicable in the special case of $Gv/Gp = 1$ where the general solutions are undefined. It is noteworthy that although there are three conductances involved (namely the convective, *Gv*, the perfusive, *Gp*, and the diffusive, *Gd*), the efficiencies are completely described by any two ratios of conductances that can be formed from these. As is customary, the solutions given in **Table 2** use the ratios Gd/Gp and Gv/Gp. It is, however, easy to replace one set of ratios with another, since, for example, $Gv/Gp = (Gd/Gp)/(Gd/Gv)$. As evident from **Figures** 7(**a**) and 7(**b**) an increase in the Gd/Gp ratio will increase either efficiency and thus generally improve the conditions for gas exchange. *Gd* for the gill as a whole is given by

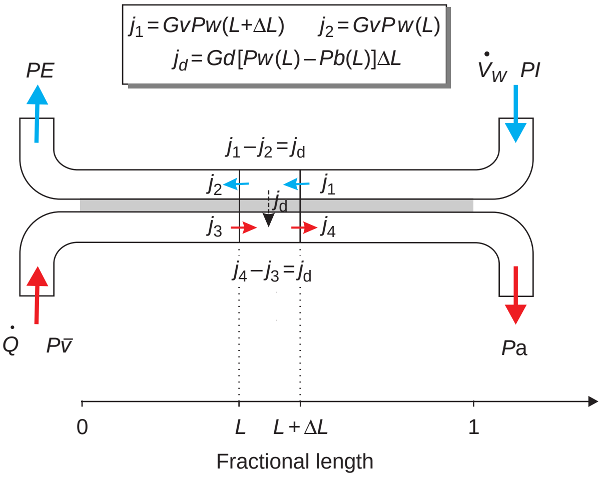


Figure 6 Model view of the gill, illustrating the fundamental mass balance on a small element. On the waterside, the equations of the mass balance have been indicated and letting the length of the element, Δ*L*, tend to zero, will lead to the differential equations (9a) and (9b).

$$Gd = \frac{KA}{t} \tag{10}$$

where *K* is Krogh's diffusion constant, *A* the area of the gill surface, and *t* the thickness of the diffusion barrier. Therefore, it makes perfect sense that an increase in *Gd* (e.g., by increasing area) relative to *Gp* (proportional to blood flow) will increase the performance of the gill as a gas exchanger. Note, however, that a decrease in *Gp* works equally well with respect to increasing efficiencies.

The dilemma of gas exchange in the gill (and any other gas-exchange organ) becomes immediately apparent when comparing how the efficiencies are controlled by the ventilatory to perfusive conductance ratio, Gv/Gp. When this ratio increases, the efficiency on the blood side goes up while that on the water side goes down and vice versa. So, increasing ventilation and thereby *Gv* will

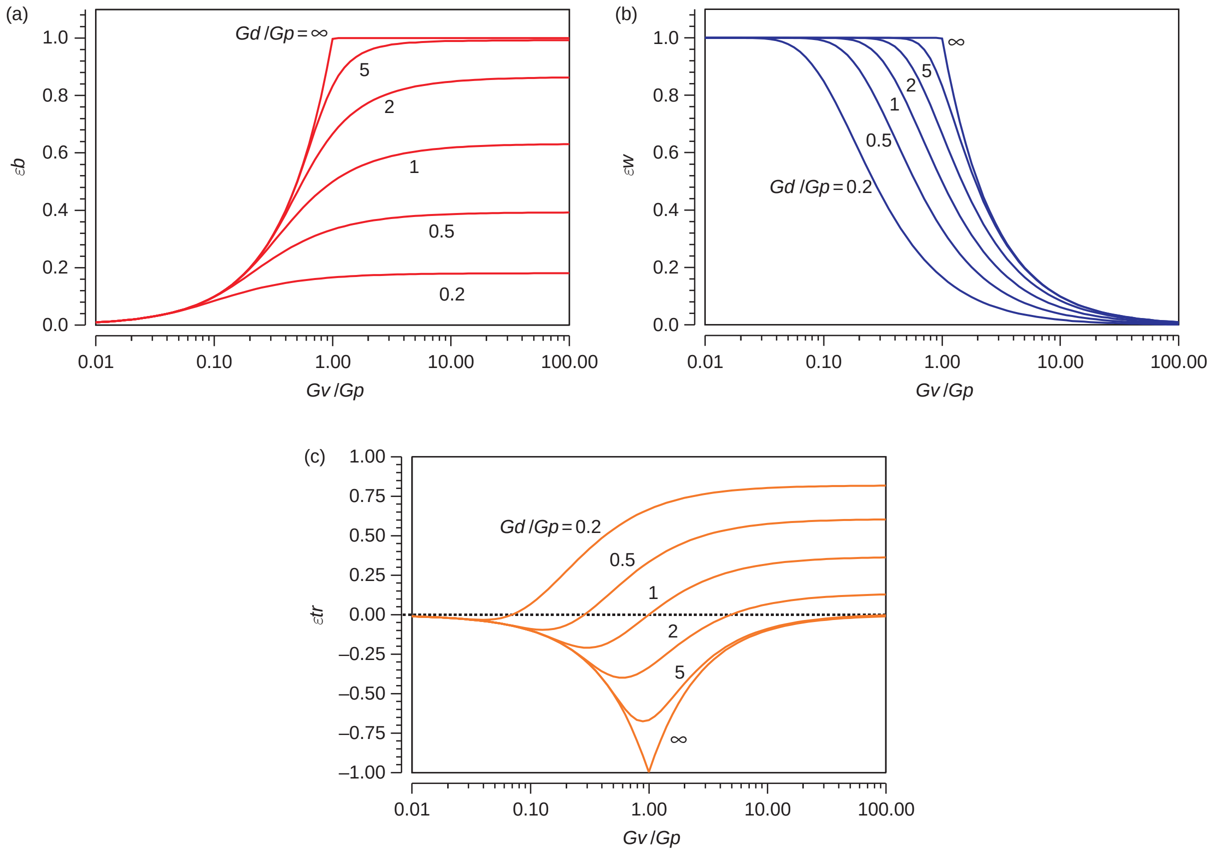


Figure 7 The efficiencies of gill gas exchange as a function of the two dimensionless conductance ratios *Gd/Gp* and *Gv/Gp*. At *Gv/Gp* = 1, the exchanger is matched and an increase in *Gd/Gp* at this point will eventually make the gill a perfect gas exchanger with maximal efficiency on both sides simultaneously. (a) The efficiency or relative partial pressure difference on the blood side, εb. (b) The efficiency or relative partial pressure on the waterside, εw. (c) The total transfer efficiency or partial pressure overlap, εtr. Note that an efficiency of 1 means 100% efficiency.

increase oxygenation of the blood but at the expense of the efficiency of exploitation of the ventilated water.

Inspection of the two figures, however, reveals that there exists one – and only one – perfect compromise where it is possible to have high efficiencies on both sides of the exchanger simultaneously. This compromise is at $Gv/Gp = 1$, and the exchanger is said to be matched at this point. In fact, as Gd/Gp tends toward infinity both efficiencies tend toward 1.0, and the perfect exchange is approached (i.e., the water gives up all its oxygen, and at the same time the blood comes into equilibrium with the inspired water). This situation is better appreciated in **Figure** 7(**c**), which shows $1-\varepsilon w-\varepsilon b$ a function of the conductance ratios. This is termed the total-transfer efficiency. From the definition of the efficiencies, it is given by

$$\varepsilon tr = 1 - \frac{PI - PE}{PI - P\bar{v}} - \frac{Pa - P\bar{v}}{PI - P\bar{v}} = \frac{PE - Pa}{PI - P\bar{v}} \qquad (11)$$

This can take on values from 1 to −1, and when the gill is matched and the diffusion to perfusion conductance ratio tends to infinity, a value of −1 is reached, which is total partial pressure overlap, that is, $Pa = PI$ and $PE = P\bar{v}$. This means that the gill, in theory, is a perfect gas exchanger, provided its ventilation and perfusion are properly matched. This situation comes about entirely due to the countercurrent-exchange principle, which is the only exchange principle that allows for perfect exchange. Thus, even though the bird lung, with its crosscurrent exchange, is very efficient, and may be an important component that allows the bar-headed goose to fly over the Himalayas, it still lags behind the fish gill.

Efficiency versus total exchange

High efficiency of gas exchange is important to minimize the costs of the ventilatory and circulatory pumps. However, there are many situations where total exchange is more important than efficiency, and often the adjustments necessary to increase or to maintain exchange will actually decrease efficiency. The total exchange can be expressed as the product of a total conductance and the total partial pressure difference available to the gill:

$$\dot{M} = G\mathrm{tot}(PI - P\bar{v}) \qquad (12)$$

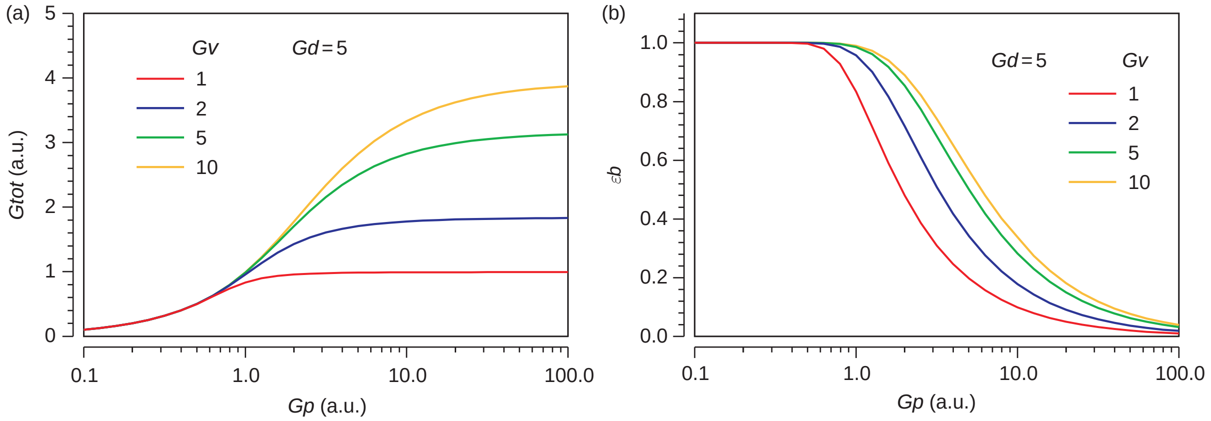


Figure 8 The simultaneous changes in the total conductance (a) and in the efficiency on the blood side (b) resulting from varying the perfusion conductance (G_p) while keeping the diffusion (G_d) and ventilation (G_v) conductances constant.

From eqns (7a) and (7b), it can be seen that the total conductance can be expressed in either of two ways:

$$\mathrm{Gtot} = Gv\varepsilon w = Gp\varepsilon b \qquad (13)$$

As evident, the total conductance depends on the efficiency as well as directly on a conductance in itself. **Figures 8(a)** and **8(b)** illustrate the difference between the total conductance and the efficiency. In **Figure 8(a)**, the total conductance increases as *Gp* increases at four values of *Gv* and one value of *Gd* (all in arbitrary units). At the same time, the efficiency on the blood side of the exchanger actually decreases. Thus, it is possible to increase oxygen uptake over the gill while the exchange efficiency on the blood side decreases. In this particular example, we varied *Gp* which is proportional to cardiac output, but this applies in general and it is important to realize

1. An increase in any of the three conductances alone, or in any combination with any of the other conductances, will always lead to an increase in the total conductance and thereby exchange.
2. This may, at the same time, lead to increases, decreases, or maintenance of the status quo in the efficiencies.

These statements are even more fundamental than it first might seem, because they will apply to any exchange system provided that the blood-gas-equilibrium curves are thermodynamically possible. Thus, in any organism, regardless of the exchange system or oxygen-equilibrium curve and in terms of total exchange between the organism and the surroundings, it never can be advantageous to decrease blood flow, even though this may increase the efficiency.

The effect of the nonlinear nature of the blood-gas-equilibrium curve

The gas-equilibrium curves of blood are nonlinear, and more so for oxygen than for carbon dioxide (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). Thereby *Gp* (for CO_2 as well as for O_2) is no longer a constant, but depends on *Pb*, that is, $Gp(Pb) = \dot{Q}(\partial Cb/\partial Pb)$, where $Cb = Cb(Pb)$ is a function describing the oxygen-equilibrium curve. This generally makes it impossible to solve the differential equations given in eqns 9(a) and 9(b). They may, however, be solved numerically. The nonlinear nature of the oxygen-equilibrium curve has the consequence that, for the same overall (or average) capacitance coefficient, it is more efficient than a linear relationship. This is because the concave shape of the oxygen-equilibrium curve increases the overall partial-pressure difference between water and blood. This is illustrated in **Figure 9(a)**, which shows the calculated oxygen-partial-pressure profiles along the exchange surface in the linear and the nonlinear cases with an identical average capacitance coefficient for oxygen. Along most of the surface, the oxygen partial pressure is kept lower in the nonlinear case, but eventually it exceeds that of the linear case. The greater exchange is, of course, reflected on the waterside of the exchanger where Po_2 falls more steeply and ends at a lower expired Po_2. **Figure 9(b)** shows how this is translated to a Cbo_2 versus Pbo_2 plot. Starting from the same venous point, and with the same average oxygen capacitance coefficient, the arterial oxygen concentration as well as the partial pressure ends at a lower value. The greater the concaveness of the oxygen-equilibrium curve followed during exchange, the greater the benefits. This concaveness, in turn, is determined by the sigmoidicity (the degree of S-shape) of the oxygen-equilibrium curve.

While, in this way, the shape of the oxygen-equilibrium curve affects the efficiency of exchange in the gill, its position, usually defined by the P_{50} (the partial pressure at which the hemoglobin is 50% saturated with O_2), is also very important. The P_{50} affects the overall efficiency of oxygen removal from the water (i.e., the extraction

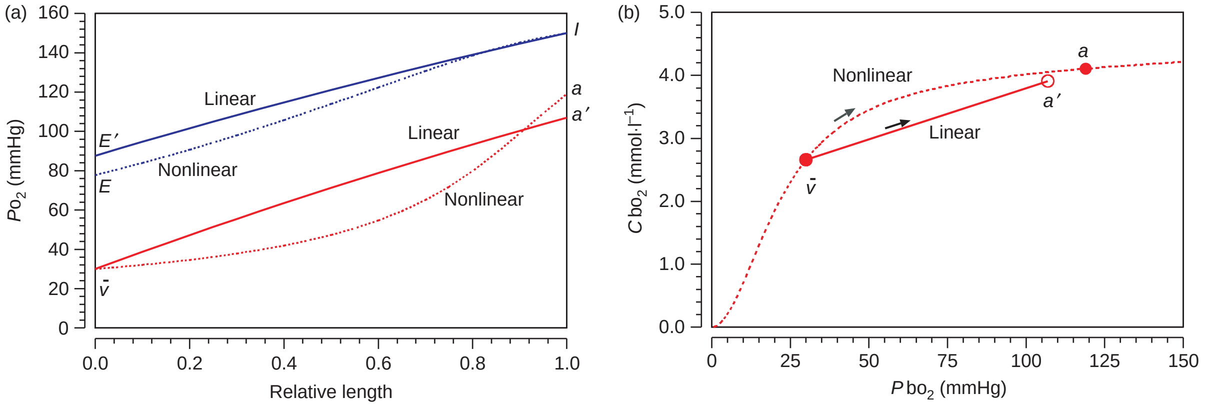


Figure 9 Comparison of oxygen exchange in the gill with linear vs. nonlinear oxygen-equilibrium curves. (a) Oxygen partial pressure profiles in water (blue) and blood (red) along the length of the exchange surface in the event of a linear (fully drawn lines) or nonlinear (broken lines) oxygen-equilibrium curve. The mixed-venous and inspired points are input parameters in the calculation, whereas the arterial and expired points are the results. (b) The nonlinear oxygen-equilibrium curve used in the calculation of the profiles of this example (P_{50} = 22 mmHg, n_H = 2.0) with the mixed-venous and arterial points plotted in. When exchanging oxygen along a linear curve connecting the mixed-venous and arterial points, the resulting arterial point, a′, is at a lower partial pressure and a lower concentration.

coefficient). The higher the hemoglobin-oxygen affinity (the lower the P_{50}), the lower the mixed-venous Po_2 will tend to be, as is evident from eqn (8), and this will tend to increase the extraction coefficient. Thus, common carp, which has a high blood-oxygen affinity relative to that of rainbow trout, has a much higher oxygen-extraction coefficient. This has been shown to be entirely attributed to the differences in the oxygen-equilibrium curves.

For CO_2, the same analysis applies but, because its blood equilibrium curve is much closer to a straight line than that for oxygen, the nonlinearity is of much less significance. In fact, a linear relationship is quite predictive for CO_2.

The effects of pulsatile flow

The gill ventilation by the buccal and opercular pumps as well as cardiac pumping are cyclic processes and the driving pressure differences across the gill for both water and blood are pulsatile as a result. Detailed analyses of the relation between pressure and flow on the waterside as well as on the blood side of the gill indicate that if pressure is highly pulsatile, then so is flow. Everything else being equal, this is expected to decrease the efficiency of gas exchange in the gill, which is also what one finds when modeling this. The degree to which efficiency is reduced is, however, surprisingly small in most fishes including the salmonids and the cyprinids. Only in tunas and mackerels, which have very thin water–blood diffusion barriers and narrow interlamellar water spaces, does this seem to be of significance. In fact, calculations indicate that, for these groups, pulsatility could have a serious impact on the efficiency of gas exchange. Interestingly, these species tend to ram-ventilate (eliminating pulsatility of the water flow but not blood flow), which more or less eliminates the problem.

The effect of parallel imhomogeneities

The gill consists of an immense number of gas-exchange units and, as in mammals, an uneven distribution of the ventilation, perfusion, and diffusion conductances among the units will decrease efficiency compared to the situation with the same overall conductances evenly distributed among the respiratory units. Theoretically, this effect can be serious and more so for oxygen exchange than carbon dioxide, which is due to the greater concaveness of the oxygen-equilibrium curve over the range of exchange. In practice, nothing is known about the degree of inequality in living fishes, but it is probably not as important as in mammals, where gravity effects make it much more difficult to achieve homogeneity.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin. **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions.

Further Reading

Davis JC and Cameron JN (1971) Water flow and gas exchange at the gills of rainbow trout, *Salmo gairdneri. The Journal of Experimental Biology* 54: 1–18.

Dejours P (1981) *Principles of Comparative Respiratory Physiology*. Amsterdam: Elsevier/North-Holland Biomedical Press.

Dejours P (1988) *Respiration in Water and Air. Adaptation–Regulation–Evolution*. Amsterdam: Elsevier.

Hughes GM (1958) The mechanism of gill ventilation in three freshwater teleosts. *Journal of Experimental Biology* 35: 807–823.

Hughes GM (1982) *An Introduction to the Study of Gills.* In: Houlihan DF, Rankin JC, and Shuttleworth TJ (eds.) *Gills,* pp. 1–24. Cambridge: Cambridge University Press.

Hughes GM (1984) General anatomy of gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 10A, pp. 1–72. New York: Academic Press.

Jones DR, Langille BL, Randall DJ, and Shelton G (1974) Blood flow in dorsal and ventral aorta of the cod, *Gadus morhua. American Journal of Physiology* 226: 90–95.

Lomholt JP and Johansen K (1979) Hypoxia acclimation in carp – how it affects O_2 uptake, ventilation and O_2 extraction from water. *Physiological Zoology* 52: 38–49.

Malte H (1989) Pressure/flow relations in the interlamellar space of fish gills: Theory and application in the rainbow trout. *Respiration Physiology* 78(2): 229–241.

Malte H (1992) Effect of pulsatile flow on gas exchange in the fish gill: Theory and experimental data. *Respiration Physiology* 88(1–2): 51–62.

Malte H and Weber RE (1985) A mathematical model for gas exchange in the fish gill based on non-linear blood gas equilibrium curves. *Respiration Physiology* 62(3): 359–374.

Malte H and Weber RE (1987) The effect of shape and position of the oxygen equilibrium curve on extraction and ventilation requirement in fishes. *Respiration Physiology* 70(2): 221–228.

Malte H and Weber RE (1989) Gas exchange in fish gills with parallel inhomogeneities. *Respiration Physiology* 76(1): 129–138.

Piiper J, Dejours P, Haab P, and Rahn H (1971) Concepts and basic quantities in gas exchange physiology. *Respiration Physiology* 13: 292–304.

Piiper J and Scheid P (1975) Gas transport efficacy of gills, lungs and skin: Theory and experimental data. *Respiration Physiology* 23(2): 209–221.

Piiper J and Scheid P (1982) Physical principles of respiratory gas exchange in fish gills. In: Houlihan DF, Rankin JC, and Shuttleworth TJ (eds.) *Gills*, pp. 45–61. Cambridge: Cambridge University Press.

Piiper J and Scheid P (1984) Model analysis of gas transfer in fish gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 10A, pp. 315–359. New York: Academic Press.

Rahn H (1966) Aquatic gas exchange: Theory. *Respiration Physiology* 1: 1–12.

Scheid P and Piiper J (1971) Theoretical analysis of respiratory gas equilibration in water passing through fish gills. *Respiration Physiology* 13(3): 305–318.

West JB (2004) A century of pulmonary gas exchange. *American Journal of Respiratory and Critical Care Medicine* 169: 897–902.

Respiratory Gas Exchange During Development: Models and Mechanisms

PJ Rombough, Brandon University, Brandon, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Respiratory Gases
Properties of Respiratory Gases in Aqueous Solutions
The Respiratory Cascade
Diffusion
Convection
Resistance
Further Reading

Glossary

Aerobic metabolism The generation of metabolic energy where oxygen acts as the final electron acceptor.

Ammonotelic Organisms in which ammonia is the dominant form of nitrogen excretion.

Anaerobic metabolism The generation of metabolic energy by processes that do not require oxygen.

Anoxia The complete absence of dissolved oxygen in water.

Carbonic anhydrase A family of enzymes that reversibly catalyze the conversion of carbon dioxide to carbonic acid.

Conductance The measure of the ease with which a gas moves from one compartment to another. G_x in the gas transfer equation $G_x = M_x/\Delta P_x$, where M_x is the rate of transfer and ΔP_x the partial pressure gradient.

Convection Bulk movement of a medium, e.g., gas, water, or blood.

Diapause A period of quiescence in animals, characterized by cessation of growth and reduction in metabolic activity.

Diffusion Net movement of a solute from an area of higher concentration to an area of lower concentration.

Diffusive boundary layer The layer of semi-stagnant water adjacent to the surface of a gas exchanger in which substances (e.g., dissolved gases) either become depleted or accumulate depending on the direction of net flux.

Egg capsule The nonliving shell encapsulating the eggs of oviparous fish species. It is also called the zona radiata or chorion (although not homologous with the chorion of amniotes).

Forced convection Convection brought about by the action of some external agent (e.g., hydraulic pressure and mechanical energy).

Gas exchanger A structure or region of the body surface heavily involved in and often specialized for respiratory gas exchange.

Metabolic intensity The rate of energy utilization per unit mass (also called mass-specific metabolic rate).

Metabolic rate Energy utilization per unit time.

Natural convection Convection due to density differences in the medium (also called free convection).

Partial pressure The pressure exerted by a particular gas in a mixture of gases.

Perfusion The internal circulation of body fluids.

Perivitelline fluid The fluid in the perivitelline space between the egg capsule and the egg proper (which initially is bounded by the vitelline membrane, hence the name).

Proton trapping The conversion of a lipid-soluble compound (e.g., NH_3) into a water-soluble compound (e.g., NH_4^+) by the addition of a proton. This prevents the water-soluble compound from crossing the plasma membrane by simple diffusion, effectively trapping it on that side of the membrane.

Resistance The sum of the impediments to fluid flow, resistance is increased by vessel length, fluid viscosity, and decreased by increased vessel radius.

Respiratory cascade A model of gas exchange in which gas is viewed as flowing through a series of resistances from the environment to the tissues or vice versa. The model is based on the analogy of water flowing down a series of cascades with the difference being that gas flow is driven by differences in partial pressure rather than gravity.

Rhesus (Rh) glycoproteins Membrane protein that facilitates ammonia transfer.

Temperature–oxygen squeeze The progressive decrease in the effective availability of O_2 experienced by aquatic organisms when faced with rising temperatures. This squeeze occurs because metabolic demand for O_2 increases but O_2 solubility in water decreases as temperature rises.

Ventilation The movement of the respiratory medium (air or water) over the surface of the gas exchanger.

Ventilation–perfusion ratio The flow rate of the respiratory medium through or over the gas exchanger divided by the flow rate of the blood perfusing the gas exchanger.

Respiratory Gases

Developing fish, like adults, depend primarily on aerobic metabolism to support cellular processes. Aerobic metabolism requires a steady supply of oxygen from the environment to the tissues where it serves as the final electron acceptor in the mitochondrial electron-transport chain (**see also Tissue Respiration**: Mitochondrial Respiration). Two gases, carbon dioxide and ammonia, are produced in the tissues as waste products of fuel catabolism. Both gases are toxic and must be removed from the body. In terms of quantity, oxygen exchange is greatest, followed by carbon dioxide and then ammonia. Molar exchange ratios vary depending on species, stage, and diet but for most developing fish they fall in the range of 1.0 for O_2, 0.8–0.9 for CO_2, and 0.05–0.15 for NH_3.

The absolute rate of oxygen consumption typically increases by several orders of magnitude during the course of embryonic and larval development as a direct consequence of the increasing size of the tissue mass (**Figure 1**). Consequently, rates of O_2 consumption are frequently expressed on a mass-specific basis by dividing total O_2 consumption by tissue mass. This measure, termed mass-specific metabolic rate or metabolic intensity, allows the investigator to compare rates of O_2 consumption across different developmental stages. Even so, metabolic intensity typically varies three- to fivefold during the course of embryonic and larval development. This variation appears to be associated with specific developmental events. For example, metabolic intensities are particularly high during epiboly, low just before hatch, and high again toward the end of endogenous feeding. In most species, there does not appear to be any significant trend with respect to age or its covariate, body mass.

Ontogenetic patterns of CO_2 and NH_3 production would be expected to mirror that for O_2 consumption, but with some minor differences reflecting stage-specific differences in energy sources (that would affect molar exchange ratios) and the relative importance of urea versus ammonia as an excretory product for nitrogenous wastes (see below). To date, no one has actually measured CO_2 production throughout the course of development. NH_3 production has been determined in very few cases compared with O_2 consumption, and behaves more or less as expected.

Fish have some capacity for anaerobic metabolism during embryonic and larval development, but the capacity is severely limited by a small carbohydrate reserve (typically <3% of total yolk energy). Carbohydrates are the major fuel during anaerobic metabolism. Some species appear to be able to catabolize amino acids anaerobically, but this ability is probably not widespread. Embryos of some species can tolerate extended periods in complete anoxia, but they do so by entering a hypometabolic state that precludes further development until O_2 is available again. Anoxic tolerance of species that do not normally enter diapause tends to decrease as development proceeds.

Properties of Respiratory Gases in Aqueous Solutions

The concentration of dissolved gas in an aqueous medium is described by Henry's law:

$$C = \alpha P \tag{1}$$

where C is the molar concentration, P the partial pressure, and α the solubility coefficient. Ammonia is the most water soluble of the three major respiratory gases, followed by CO_2 then O_2. Relative solubilities at 20 °C are 22 783 for NH_3, 27.8 for CO_2, and 1.0 for O_2. Gas solubility decreases with increasing temperature, with the effect being somewhat greater for CO_2 than O_2. The magnitude of the temperature effect is relatively large. Water can only hold

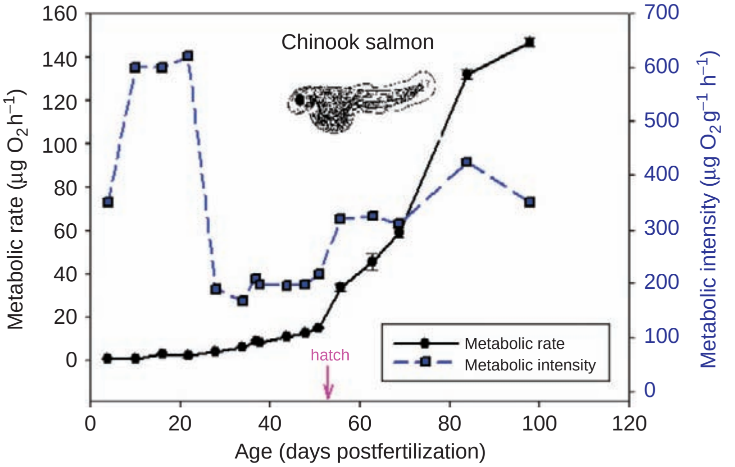


Figure 1 Changes in metabolic rate and metabolic intensity (mass-specific metabolic rate) with age for embryos and endogenous feeding larvae of Chinook salmon at 10 °C. Data from Rombough PJ (1994) Energy partitioning during fish development: additive or compensatory allocation of energy to support growth? *Functional Ecology* 8: 178–186.

~53% as much O_2 and ~38% as much CO_2 at 30 °C as at 0 °C. The lower solubility of O_2 at higher temperatures, in particular, has important physiological consequences. A rise in temperature increases metabolic demand for O_2, but reduces its availability in the environment. It has been proposed that the resulting temperature-oxygen squeeze is what ultimately sets the upper limit of thermal tolerance in fish (**see also Temperature**: Effects of Climate Change and **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature.

The capacity of developing fish to extract O_2 from the environment is generally less than that of adults, which may partially explain their lower thermal tolerance (other factors such as limited capacities for homeoviscous adaptation (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation) and isozyme switching are also important).

Gas solubility normally decreases with increasing salinity. The solubility of O_2 in seawater (35 ppt) is ~81% of that in pure water at 20 °C. This means that marine fish have access to less O_2 than freshwater fish, under air-saturation conditions. It also means that blood plasma (osmolarity ~300–350 mOsm) can hold somewhat less dissolved gas than pure water (~90% for O_2 at 20 °C). Ammonia is somewhat unusual for gases in that its solubility increases slightly with increasing salinity. The solubility of NH_3 in plasma is ~3.4% greater than that in pure water.

The behavior of CO_2 and NH_3 in an aqueous medium is more complex than O_2. In the absence of a specific carrier molecule such as hemoglobin, O_2 is present only in its dissolved form, $O_2(g)$, and is subject to Henry's law. CO_2 and NH_3, in contrast, can combine with water to form ionic compounds that are not subject to Henry's law. When $CO_2(g)$ combines with water, it forms carbonic acid which then dissociates into the bicarbonate ion and a proton: $CO_2(g) + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$. At high pH, the bicarbonate ion can further dissociate into the carbonate ion and another proton: $HCO_3^- \Leftrightarrow CO_3^{2-} + H^+$. The relative concentrations of the various forms depend on the pH of the solution and the presence of various buffers and carrier molecules. At physiologic pH, the dominant form is HCO_3^- (80–90% of total CO_2). Dissolved CO_2 typically represents only a small fraction of the total amount of CO_2 in solution (<5% in plasma) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion). Ammonia can bind with protons in water to form the ammonium ion: $NH_3 + H_3O^+ \Leftrightarrow NH_4^+$. At physiologic pH, the ionized form (NH_4^+) is dominant (>98% total). This is fortuitous because the NH_4^+ is much less toxic than NH_3 (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia and Ureotelism).

The Respiratory Cascade

The underlying principles governing gas exchange during development are the same as those that apply to juveniles and adults (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). The concept of a respiratory cascade is often used to describe the flow of gases between the environment and the tissues (**Figure 2**). This concept breaks overall gas exchange into a series of steps involving diffusive or convective

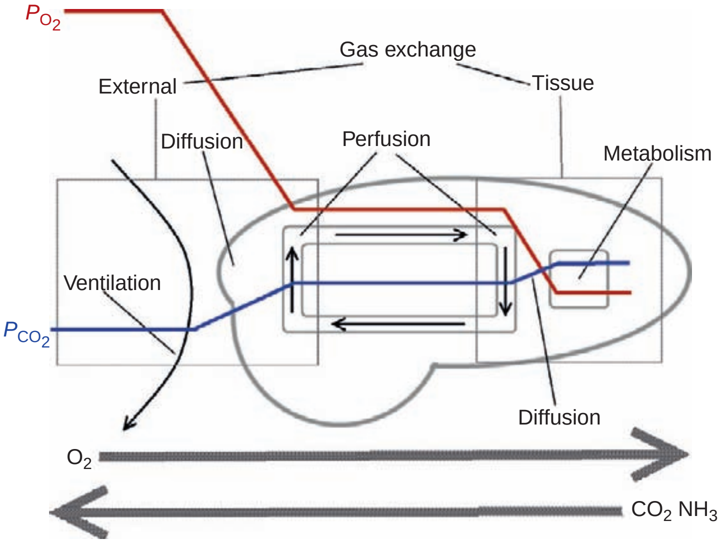


Figure 2 Schematic diagram showing the respiratory cascade for a fish larva. Gas transport is by diffusion and convection. Diffusion takes place externally at the level of the gas exchanger and internally at the tissues. Convection in the external medium is termed ventilation, while movement of the blood is termed perfusion. Diffusion is driven by differences in partial pressure with the direction of the gradient depending on the gas (shown by the large arrows). Representative partial-pressure gradients are shown for O_2 and CO_2. Gradients illustrate general trends and are not to scale (see text for representative ΔP's). Modified from Piiper J (1982) Respiratory gas exchange at lungs, gills and tissues: Mechanisms and adjustments. *Journal of Experimental Biology* 100: 5–22.

transport. In well-developed actively-ventilating larvae, as in adult fish, there are generally considered to be four steps in the respiratory cascade. For O_2 these steps would be: (1) bulk fluid to surface of gas exchanger (skin or gill); (2) surface of gas exchanger to blood in gas exchanger; (3) blood in gas exchanger to blood in tissue; and (4) blood in the tissue to the mitochondrion. Flows of CO_2 and NH_3 would be in the opposite direction. Steps 1 and 3 involve convective transport, while steps 2 and 4 involve diffusive exchange. Gas flux through the system is driven by differences in partial pressure (ΔPx). The total partial pressure difference across the whole system varies depending on the gas, stage of development, and activity (among other things) but generally would be expected to fall in the range of ~100–150 torr (= mmHg) for ΔP_{O2}, ~2 torr for ΔP_{CO2}, and $\sim 20 - 50\,\mu$torr for ΔP_{NH3}.

Dissecting the respiratory cascade is somewhat more complicated for embryos because of the presence of the egg capsule (also called the chorion or zona radiata) and the fact that for about the first one-third of embryonic development there is no blood circulation. The space between the egg capsule and embryo is filled with perivitelline fluid (PVF) that is not stirred generally for the first one-third or so of embryonic development. The extent to which the PVF is stirred during later embryonic stages is variable, depending on factors such as species, temperature, dissolved-O_2 level, and pH. If the PVF is unstirred and there is no blood circulation, the respiratory cascade is essentially reduced to two steps: (1) convective exchange in the bulk fluid and (2) diffusion from the egg capsule through to the mitochondrion. If PVF is stirred and blood is circulated, the respiratory cascade consists of up to six steps: (1) external convection in the bulk medium, (2) diffusion through the egg capsule, (3) convection in the PVF, (4) diffusion through the gas exchanger, (5) internal convection in the blood, and (6) diffusion through the tissues.

Respiratory cascades can be further subdivided if the investigator wishes to look at what is happening in finer detail. One such subdivision that is particularly important during development is the diffusive boundary layer (DBL). The DBL is a layer of semi-stagnant fluid immediately adjacent to a surface such as the skin, gill, or egg capsule. Depending on the direction of flux, gases accumulate or become depleted in this layer. For example, O_2 levels in the DBL adjacent to the skin of a fish larvae would tend to be lower than the free-stream value (**Figure 3**), while CO_2 and NH_3 levels would tend to be

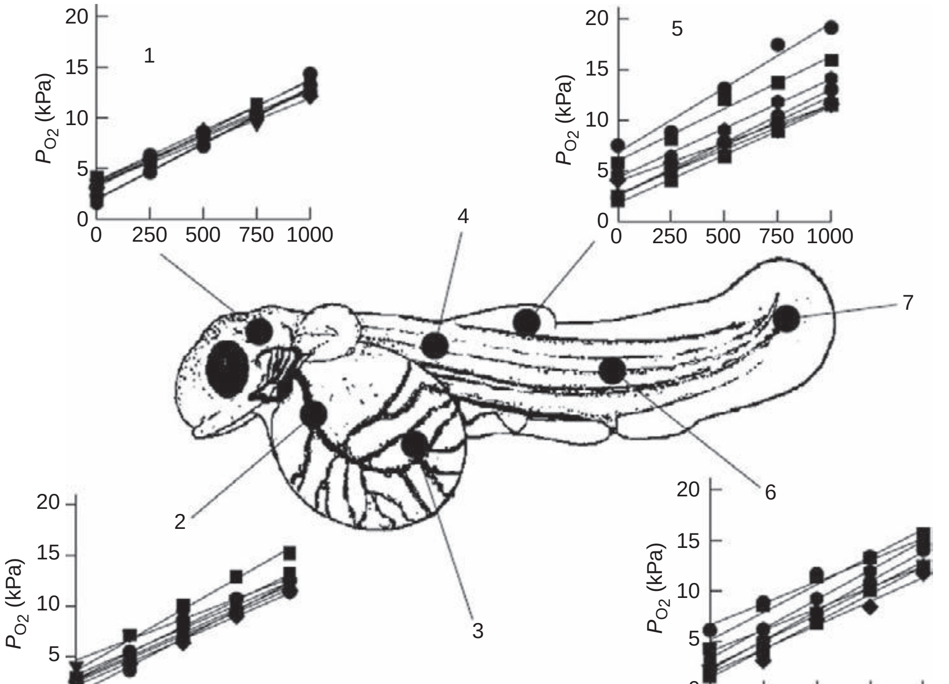


Figure 3 Oxygen partial pressure (P_{O2}) in the DBL surrounding a newly hatched rainbow trout larva in still water. Graphs show values at various distances from the skin surface for seven to eight individuals at locations 1, 2, 5, and 6. Results were similar at locations 3, 4, and 7. The free-stream value >1 cm from the body surface was 20.9 kPa (157 torr). Data from Rombough PJ (1998) Partitioning of oxygen uptake between the gills and skin in fish larvae: A novel method for estimating cutaneous oxygen uptake. *Journal of Experimental Biology* 201: 1763–1769.

higher. Boundary-layer thickness is usually defined as the distance from the boundary surface to where velocity is 99% of the free-stream value. The thickness (*d*) of this layer is proportional to $v^{-1/2}$, where v is the bulk velocity of the fluid flowing past the surface. The exception is where the bulk flow of fluid is constrained by channeling or some similar process, in which case the thickness of the boundary layer cannot exceed half the channel width (e.g., between adjacent gill lamellae). DBLs are present on both sides of a barrier. The thickness of the layer on a given side will depend on the bulk flow rate on that side and the degree of channeling. In the case of most external gas exchange surfaces (e.g., skin or gills), the thickness of the boundary layer at the outer surface is very much greater than at the inner surface. In these cases, the resistance to flux imparted by the inner layer is usually included with the resistance of the gas exchanger. An exception to this would be the egg capsule when both the bulk medium and the PVF are stirred. In this case, there could be a substantial DBL adjacent to the inner as well as outer surface of the capsule. External boundary layers can have a substantial impact on diffusive gas exchange (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs and Respiratory Gas Exchange During Development: Respiratory Transitions).

Diffusion

The planar diffusion of a gas is described by the Fick equation

$$\mathcal{J} = KA(\Delta P/\delta) \qquad (2)$$

where $\mathcal{J}$ is the rate of gas transfer, K the Krogh constant of diffusion, A the area, ΔP the partial pressure gradient, and δ the diffusion distance. Krogh's constant of diffusion is the product of the diffusion coefficient (D) and the Bunsen capacitance coefficient (α).

The diffusion of each gas in a mixture of gases is independent of other gases when it is in the gaseous or dissolved form. However in an aqueous medium, the dominant forms of CO_2 and NH_3 are not as dissolved gas, but in the form of ions (i.e., as HCO_3^- and H^+ or NH_4^+). This makes it possible for the diffusion of these gases to be linked indirectly via chemical reactions between the various ions and/or charge coupling. At this juncture, it is worth pointing out that the movement of ionic compounds such as HCO_3^- and NH_4^+ depends on the electrical as well as the concentration gradient (i.e., the electrochemical gradient). This complication is usually ignored when considering the bulk transfer of respiratory gases but becomes important when considering the implications of gas movement on acid–base balance and ion exchange.

Diffusion in Aqueous Media

Values for D, α, and K for O_2 and CO_2 in pure water at 20 °C are given in **Table 1**. The rate of diffusion in water is inversely proportional to the size of the molecule, which explains the lower diffusion constant for CO_2. The aqueous diffusion coefficient for NH_3 in water is similar to that for CO_2. Values for D of both O_2 and CO_2 increase $\sim 2\%\ °C^{-1}$ due to increased kinetic energy. As mentioned, CO_2 is more soluble in water than O_2 (i.e., $\alpha_{CO2} > \alpha_{O2}$) and displays greater temperature sensitivity. Krogh's constant of diffusion increases with increasing temperature at a rate of $\sim 1\%\ °C^{-1}$ for O_2 but decreases at approximately the same rate for CO_2. K for CO_2 at 0 °C is ~25 times that for O_2; the difference at 30 °C is ~18-fold. The higher K value for CO_2 has important physiological consequences. Given the same partial pressure gradient and resistance, CO_2 will diffuse 18–25 times as fast as O_2. This keeps internal P_{CO2} low (typically 2–5 torr in adult fish; values for developing fish have not been determined) and means that O_2 uptake is far more likely to become limiting than CO_2 excretion (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). The extremely high solubility of NH_3 keeps internal P_{NH3} very low, in the range of 50 μtorr in adult fish (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). P_{NH3} values in the yolk of rainbow trout *Oncorhynchus mykiss* embryos have been reported to be in the range of 10–25 μtorr.

Table 1 Physicochemical parameters for oxygen and carbon dioxide in pure water at 20 °C

Gas	*D (10^{-5} cm^{2}s^{-1})*	*α (nmol cm^{-3} torr^{-1})*	*K (10^{-5} nmol cm^{-1} s^{-1} torr^{-1})*
O_2	2.5	1.82	4.6
CO_2	1.82	51.4	93
CO_2/O_2	0.73	28.2	20.2

D is the diffusion constant, α the Bunsen solubility coefficient, and *K* the Krogh's constant of diffusion.

Diffusion through Membranes

The basic principles governing the diffusion of gases through biological membranes are the same as those for diffusion in an aqueous medium. However, rates of diffusion can be quite different in the two media because of differences in the solubility of gases in the lipid (membrane) and aqueous (water) phases. This is reflected in different values for Krogh's coefficient in the two media. Transport of CO_2 and NH_3 across membranes is potentially problematic because the dominant forms of both gases are ionic: HCO_3^- for CO_2 and NH_4^+ for NH_3. Ions are strongly hydrophilic and, as a result, have low intrinsic membrane permeabilities. Rapid transport of these compounds across membranes requires that they be converted to a more lipid-soluble form or have their movement facilitated by a specific transport carrier.

Oxygen

As far as we know, O_2 only moves across cell membranes by diffusion. Empirically determined values for K_{O2} for tissues of adults of lower vertebrates are typically about one-third the value for K_{O2} in pure water. This value is generally what has been applied to developing fish. However, a recent estimate based on measurements in zebrafish *Danio rerio* embryos suggests that the K_{O2} for embryonic tissue may actually be quite close to the K_{O2} for water. Interestingly, there have been a couple of reports that the K_{O2} for yolk is higher than that for water. The reason for this is unclear. One potential explanation has to do with the high lipid content of yolk. O_2 is considerably more soluble in lipid than in water. A higher K_{O2} for yolk, if confirmed, would mean that the yolk lipids may actually facilitate O_2 transport to embryonic tissues. This could be particularly important prior to the onset of blood circulation.

Carbon Dioxide

Like O_2, CO_2 is highly soluble in lipid and should cross plasma membranes readily. The problem is that most of the CO_2 in internal fluids is in the form of dissociated H_2CO_3. Neither HCO_3^- nor H^+ can cross membranes readily (at least not without the aid of specific transporters). To cross membranes fast enough to keep up with the rate of production, H_2CO_3 must be dehydrated to dissolved CO_2 which can then diffuse across the membrane (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion). This process requires the presence of the enzyme carbonic anhydrase (CA; **see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange), which catalyzes the reaction $CO_2(g) + H_2O \Leftrightarrow H_2CO_3$. Fish express multiple isoforms of CA (at least 16 in zebrafish). The precise roles of the various isoforms are not well understood, but some appear to be involved primarily in CO_2 transport and excretion, some in acid–base balance and ion exchange, and some in other specific processes. The situation is complicated by the fact that CO_2 excretion, acid–base balance, and ion exchange are all intimately interconnected.

In adult fish, CO_2 diffuses out of the tissues into the blood stream where most of it enters red blood cells (RBCs) (**Figure 4**; **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). Inside the RBC, CA catalyzes the hydration of CO_2 to H_2CO_3, which then dissociates into HCO_3^- and H^+. The H^+ ions are buffered by hemoglobin, while the HCO_3^- ions leave the RBC through electroneutral Cl^-/HCO_3^- exchangers. A small amount of CO_2 also appears to be hydrated under the influence of CA in the interstitial fluid. HCO_3^- dissolved in the blood plasma is transported to the gas exchanger, where the process is reversed. HCO_3^- re-enters the RBC and combines with H^+ to form H_2CO_3, which is then dehydrated to CO_2 in the same CA-catalyzed reaction. The CO_2 diffuses down its partial-pressure gradient out of the RBC and across the respiratory epithelium. Some of the CO_2 that enters the external boundary layer is hydrated to HCO_3^- and H^+, where the H^+ may play a role in proton trapping of NH_3. CA activity has been reported in the boundary layer of some species.

It generally has been assumed that the process of CO_2 excretion is similar for developing and adult fish. This is probably true once fish begin to produce hemoglobin. As noted above, hemoglobin inside RBCs plays a central role in adult fish. The problem is that some fish do not start to produce appreciable amounts of hemoglobin until near the end of the larval stage. Even with species such as zebrafish that begin hemoglobin production at a relatively early age (~24 h postfertilization, hpf), there is still a period when neither hemoglobin nor red cells are present. How CO_2 is transported from the tissues to the gas exchanger during this period is unknown. A clue might reside in the apparent high buffering capacity of embryonic tissue, approximately twice that of adult muscle in the case of early zebrafish embryos. Another possibility is that tissue or extracellular CA plays a larger role. Developing fish appear to initiate CA production at an early age. In zebrafish, at least four CA isoforms are expressed by the time the blood begins to circulate. One of these, CAb, which is involved in catalyzing CO_2 hydration/dehydration reactions in RBCs of adult fish, is present as early as 8 hpf (i.e., well before the differentiation of RBCs). Precisely how or even if CAb is involved in CO_2 excretion at such an early age is not known. Based on changes in the respiratory exchange ratio for O_2 and CO_2, CA appears to begin to play an important role in CO_2 excretion in zebrafish between 24 and 48 hpf. Blocking CA activity with various inhibitors at 48 hpf resulted in an approximately 50% reduction in the rate of CO_2 excretion.

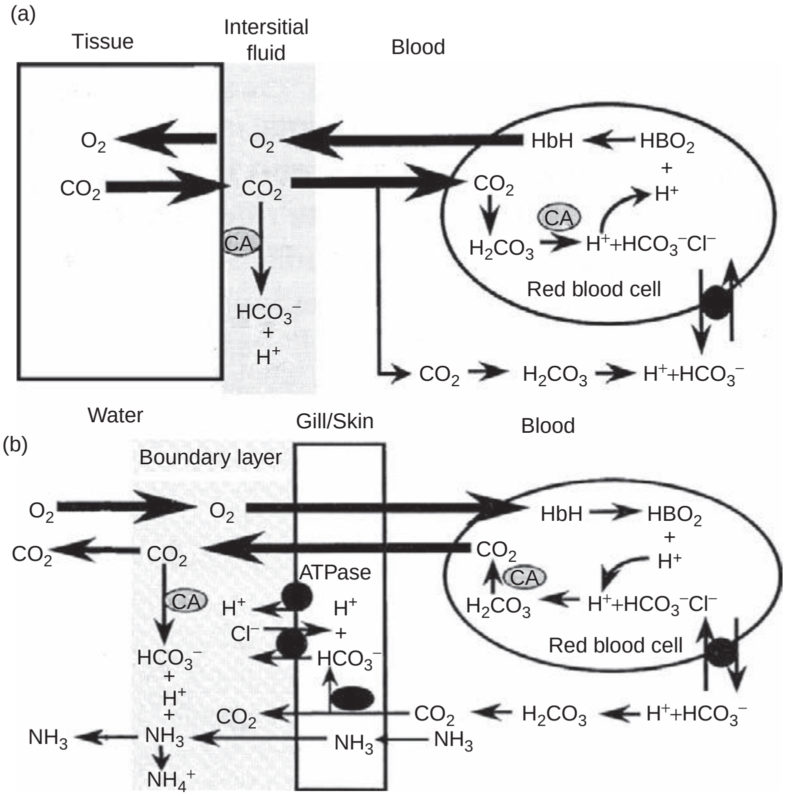


Figure 4 CO_2 excretion in adult fish. (a) Exchange at the tissues. (b) Exchange at the gills or skin. HbH and HBO_2 are deoxygenated and oxygenated hemoglobin, respectively. The enzyme CA catalyzes the hydration/dehydration of CO_2. CO_2 and O_2 transport by Hb are linked by the reciprocal Bohr and Haldane effects. Proton trapping of NH_4^+ in the external boundary layer is a potential linkage between CO_2 and NH_3 transport. Processes are thought to be similar during early life. Modified from Tufts B and Perry SF (1998) Carbon dioxide and acid–base balance. In: Perry SF and Tufts BL (eds.) *Fish Respiration*, pp. 229–281. San Diego, CA: Academic Press.

There has been some speculation in the literature that transport proteins/channels similar to those involved in ammonia transport (see below) may facilitate the transmembrane transport of CO_2. However, recent empirical studies of artificial and natural membranes indicate that simple diffusion through the phospholipid bilayer can fully account for observed CO_2 membrane permeabilities. These studies have shown that the rate-limiting step in transmembrane transport is the near-membrane boundary layer, not the membrane itself.

Ammonia

Dissolved NH_3 can cross membranes by simple diffusion through the lipid bilayer. Ammonia, however, is not particularly soluble in lipid (the oil/water partition coefficient for NH_3 is ~ 1/750 that for CO_2). The impact of this low lipid solubility can be ameliorated to some extent by acid trapping in the external boundary layer, where NH_3 picks up a proton and is converted to NH_4^+. This helps maintain the NH_3 partial pressure gradient across the membrane. Another means of circumventing low lipid solubility is the use of specific membrane transporters (**Figure 5**). This appears to be the major role of the Rhesus (Rh) glycoproteins. Rh proteins are widely distributed in aquatic organisms, including fish, where they appear to facilitate NH_3 transport in a manner similar to the way aquaporins facilitate water transport. Finally, ammonia may be excreted as the ammonium ion either directly as appears to be the case in some marine fishes or coupled to Na^+ uptake, which appears to be more widespread. In general, direct diffusion of NH_3 and Rh-facilitated transport appears to account for the bulk of total ammonia transport in adult fish.

Fish begin to express ammonia transporter (Rh protein) genes relatively early in development. Rh expression has been reported in zebrafish embryos as early as ~1 dpf, the stage blood starts to circulate. Rainbow trout already express relatively high levels of mRNA for several Rh proteins by the eyed stage, nearly half way to hatch. Knock-down of Rh proteins results in about a 50% decrease in the rate of NH_3 excretion in newly hatched zebrafish (3 dpf) demonstrating that even at this early age facilitated NH_3 transport is important. Ammonia excretion across the skin of larval zebrafish appears to be tightly linked to acid secretion illustrating the potential importance of proton trapping of NH_4^+ in maintaining the transepithelial NH_3 gradient.

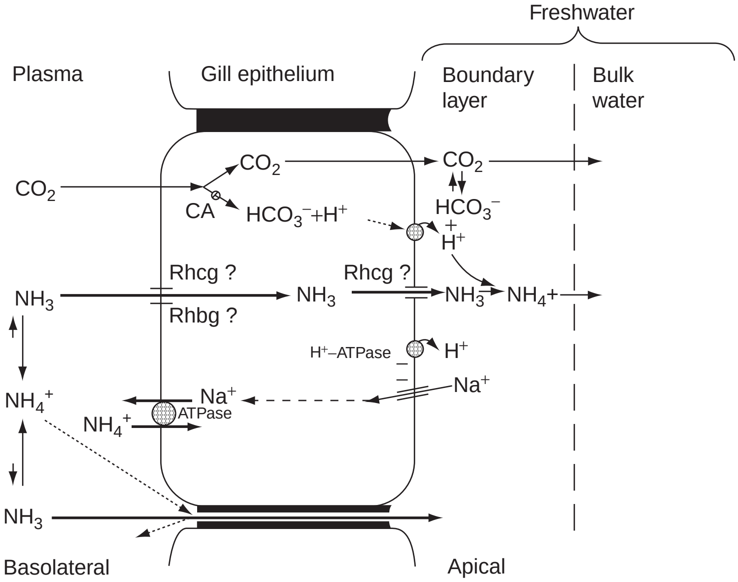


Figure 5 Model of ammonia excretion in freshwater fish. Rhcg and Rhbg are putative Rhesus NH_3 transporters. Note the potential linkage between NH_3 and CO_2 transport via proton trapping of NH_4^+ in the external boundary layer. Modified from Weihrauch D, Wilkie MP, and Walsh PJ (2009) Ammonia and urea transporters in gills of fish and aquatic crustaceans. *Journal of Experimental Biology* 212: 1716–1730.

At this juncture, it is worth pointing out that at least some fish species employ an alternate/complementary strategy to deal with the potential toxicity of NH_3 during development, namely to convert it to urea (**see also Nitrogenous-Waste Balance**: Ureotelism). Urea is less toxic than ammonia, and also is less energetically favorable because its synthesis requires a net input of energy (~4 ATP equivalents/urea). Embryonic urea production occurs even in species that would be considered ammonotelic as adults. In general, urea represents a greater fraction of total N excretion earlier in development. Excretion of a high-energy N-compound during early development suggests that the capacity for direct NH_3 excretion initially may be limited. Two factors, the diffusive resistance imparted by the presence of the egg capsule and a lack of ammonia transporters, have been advanced as reasons for this apparent limitation.

Time

Time is also an important factor when considering diffusion. Diffusion time (t) is $\sim\delta^2$. If the distance is small, diffusion is rapid (e.g., O_2 takes ~0.1 ms to diffuse 1 μm in water), but if the distance is greater than about 1 mm, diffusion is generally too slow to satisfy metabolic demand ($t_{O2} \sim 100$ ms at 1 mm). This means that if gas has to be transported over longer distances, an alternate method must be used.

Convection

Convection is the method used to transport gases over longer distances. Convection can be natural or forced. Natural convection depends on density differences to move the bulk fluid the gas is dissolved in (**Figure 6**). Forced convection requires energy input, usually provided by the organism. Convective transport can be quantified using the equation

$$J = F\beta(\Delta P) \tag{3}$$

where J is the rate of gas transfer, F the flow rate, β the gas capacitance of the medium, and ΔP the difference in partial pressure in the medium entering and leaving the site where gas is exchanged. Flow in

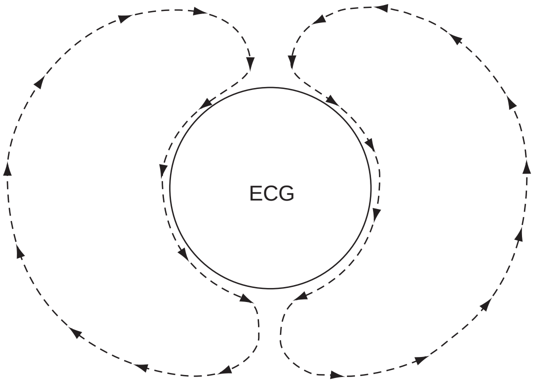


Figure 6 Natural convection of water surrounding a salmon egg. The respiring embryo removes O_2 and adds CO_2 to the DBL. This increases the density of the water in the boundary layer, which falls away from the egg generating toroidal water currents. Modified from O'Brien RN, Visaisouk S, Raine R, and Alderdice DF (1978) Natural convection: A mechanism for transporting oxygen to incubating salmon eggs. *Journal of the Fisheries Research Board of Canada* 35: 1316–1321.

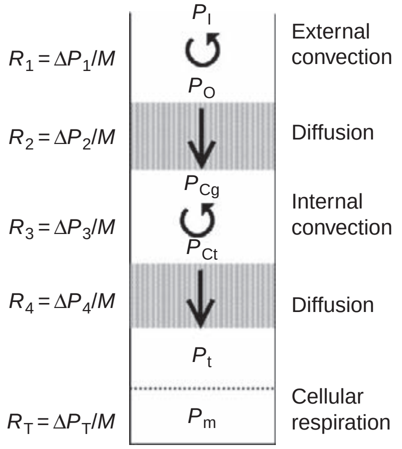


Figure 7 The respiratory-cascade model depicted as a series of convective and diffusive resistances. Partial pressure differences (ΔP) between successive regions are determined by resistance (R) and the rate of production or consumption of the gas (M). Faster stirring of conductive elements reduces ΔP for those elements. Total resistance (R_T) is the sum of all the individual resistances. P_I = free-stream partial pressure, P_O = partial pressure of medium leaving gas exchanger, P_{Cg} = partial pressure in capillary at gas exchanger, P_{Ct} = partial pressure in capillary at tissue, P_t = partial pressure in tissue, and P_m = partial pressure at the level of the mitochondrion. Modified from Otis AB (1987) An overview of gas exchange. In: Farhi LE and Tenney SM (eds.) *Handbook of Physiology: The Respiratory System*, pp. 1–11. Bethesda MD: American Physiological Society.

the external medium (water for most fish) is referred to as ventilation and abbreviated as V. Flow of the internal medium (blood in vertebrates) is referred to as perfusion and abbreviated as Q. For external convection, β is the capacitance of water (βw) which is generally close to the value of the Bunsen coefficient (α). β for internal convection (βb) includes dissolved gas and any gas that is dissociated or chemically bound to carrier molecules in the blood (e.g., hemoglobin). In general, $\beta b > \beta w$, the precise extent depending on the gas, hemoglobin concentration, and buffering capacity of the blood (for details concerning determination of βb, **see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs).

For efficient gas exchange, the ventilation–perfusion ratio ($\dot{V}$: Q) should be balanced so all the gas brought to one side of a gas exchanger can be carried away at the other side. Because β's are different for O_2 and CO_2, $\dot{V}$: Q cannot be optimized for both gases simultaneously. In adult fish, $\dot{V}$: Q is generally close to the optimum for O_2 implying that O_2 transport is most likely to become limiting. There is little information on $\dot{V}$: Q ratios during early life because of the practical difficulty of measuring $\dot{V}$ and Q in very small organisms. Measuring $\dot{V}$ is particularly problematic because there is often not a clear ventilatory flow.

Resistance

The total flux of gas from the medium to the tissues or vice versa (M_x) can be described by the mass transfer equation, usually written as

$$M_x = G_x \Delta P_x \text{ or } M_x = \Delta P_x / R_x \qquad (4)$$

where ΔP_x is the partial pressure gradient, G_x the total conductance, and R_x the total resistance ($R_x = 1/G_x$). Similar equations can be written for each step in the respiratory cascade (**Figure 7**). The overall resistance to gas exchange is the sum of all the individual diffusive or convective resistances in the pathway (i.e., $R_t = R_1 + R_2 + R_3 + \ldots$). The ratio of the resistance of an individual component to total resistance (e.g., R_1/R_t) is a useful indicator of the relative impact of that particular component of the pathway.

See also: **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Tissue Respiration**: Mitochondrial Respiration. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Carbonic Anhydrase in Gas Transport and Exchange; Hemoglobin. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Respiratory Gas Exchange During Development: Respiratory Transitions.

Further Reading

Boutilier RG, Heming TA, and Iwama GK (1984) Appendix: Physicochemical parameters for use in fish respiratory physiology. In: Hoar S and Randall DJ (eds.) *Fish Physiology,* vol. X, pp. 403–430. Orlando, FL: Academic Press.

Braun MH, Steele SL, Ekker M, and Perry SF (2009) Nitrogen excretion in developing zebrafish (*Danio rerio*): A role for Rh proteins and urea transporters. *American Journal of Physiology Renal Physiology* 296: F994–F1005.

Brauner CJ (2008) Acid–base balance. In: Finn RN and Kapoor BG (eds.) *Fish Larval Physiology*, pp. 185–198. Enfield, NH: Science Publishers.

Dejours P (1981) *Principles of Comparative Respiratory Physiology*. Amsterdam: Elsevier.

Gilmour KM, Thomas K, Esbaugh AJ, and Perry SF (2009) Carbonic anhydrase expression and CO_2 excretion during early development in zebrafish *Danio rerio*. *Journal of Experimental Biology* 212: 3837–3845.

Pelster B (2008) Gas exchange. In: Finn RN and Kapoor BG (eds.) *Fish Larval Physiology*, pp. 91–117. Enfield, NH: Science Publishers.

Rombough PJ (1988) Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: Hoar S and Randall DJ (eds.) *Fish Physiology,* vol. XIIA, pp. 59–161. San Diego, CA: Academic Press.

Terjesen B (2008) Nitrogen excretion. In: Finn RN and Kapoor BG (eds.) *Fish Larval Physiology*, pp. 263–302. Enfield, NH: Science Publishers.

Respiratory Gas Exchange During Development: Respiratory Transitions

PJ Rombough, Brandon University, Brandon, MB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Resistance to Respiratory Gas Exchange
Diffusive Boundary Layer
Egg Capsule and PVF
Skin
Gills
Further Reading

Glossary

Aerobic metabolism The generation of metabolic energy where O_2 acts as the final electron acceptor.

Branchial respiration Exchange of respiratory gases across the gill.

Cutaneous respiration Exchange of respiratory gases across the skin.

Diffusive boundary layer The layer of semi-stagnant water adjacent to the surface of a gas exchanger in which substances (e.g., dissolved gases) either become depleted or accumulate depending on the direction of net flux.

Egg capsule The nonliving shell encapsulating the eggs of oviparous fish species, which is also called the zona radiata or chorion (although not homologous with the chorion of amniotes).

Gastrulation Migration and rearrangement of cells after the embryo undergoes cell proliferation to increase cell number (cleavage stage) to create three primary germ layers during early development.

Organogenesis The process whereby the primary germ layers develop into rudimentary organs. Organogenesis continues until the definitive characteristics of the organ are achieved.

Oviparous Laying eggs that develop and hatch externally.

Ovoviviparous Eggs hatch within the mother. Nutrients are from the yolk and the mother provides for gas exchange.

Perivitelline fluid Fluid found in the perivitelline space between the egg membrane or embryonic skin and the egg capsule. Similar, but not identical, to the ambient medium in terms of chemical composition.

Viviparous Giving birth to live young. Mother contributes nutrients as well as providing for gas exchange.

Introduction

Fish, like all animals, begin life as a single cell. This cell ultimately gives rise to all the cells in the body as the developmental program progressively unfolds. Developmental processes post-gastrulation (i.e., for most of development) are powered almost entirely by aerobic metabolism. This means that the embryo needs a continuous supply of O_2. It also has to get rid of the waste products of metabolism, in particular carbon dioxide and ammonia. In adult fish, uptake and removal of these gases is the task of the respiratory system. Developing fish, however, initially do not possess a respiratory system or, indeed, any kind of specialized structure for gas exchange. This article focuses on how fish embryos and larvae obtain O_2 and get rid of CO_2 and NH_3 as well as how the sites and mechanisms by which this is accomplished change as development proceeds.

Fishes comprise the largest group of vertebrates with more than 30 000 extant species in three classes: Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes), and Osteichthyes (bony fishes). As might be expected, they display great diversity in terms of habitat, life history, morphology, and how they handle various physiological problems. This diversity extends to development of the respiratory system. Some of the more unusual respiratory adaptations include the development of temporary external gills by embryos of some cartilaginous (e.g., little skate *Raja erinacea*) and bony fishes (e.g., spined loach *Cobitis taenia*). Viviparous and ovoviviparous fishes develop complex placenta-like structures where gases are exchanged between the embryo and the mother. Air-breathing bony fishes have formed various types of accessory respiratory structures (e.g., labyrinth organs, swimbladders, and lungs) in addition to internal gills (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). For the vast majority of fishes, which are oviparous and water breathing, respiratory development is more straightforward. Early in development, gas exchange takes place solely by diffusion across nonspecialized body surfaces. Later in development, internal gills begin to form and eventually supplant the skin as the major site of gas exchange. The focus of this article is on this transition from cutaneous to predominantly branchial respiration. Emphasis is on O_2 uptake. O_2 is the gas that is most likely to become limiting during development and it is also the gas we know most about. It is assumed that, aside from the direction of flux, exchange patterns for CO_2 and NH_3 are essentially the same as those for O_2 but more research is required to confirm this.

Resistance to Respiratory Gas Exchange

As pointed out in **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs), the flux of gases between an organism and its environment can be viewed as passing through a series of diffusive and convective resistances. For most of embryonic and larval development, diffusive resistances dominate. The diffusive resistance imparted by the boundary layer, the egg capsule, the perivitelline fluid (PVF), body surfaces, notably the skin and gills, and the tissues themselves is particularly important. One of the major trends observed in the ontogeny of the respiratory system is a shift toward greater reliance on convection as a means of transporting gases. This shift is driven by tissue growth, greater diffusion distances, and a relatively reduced surface area for exchange. Tissue growth results in increased metabolic demand for O_2 and concomitant increases in the need to get rid of CO_2 (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion) and NH_3 (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia) as well as increases in the distances over which gases must be transported.

Diffusive flux is described by the Fick equation (see eqn (2) in **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms and Efficiency of Gas Exchange Organs). According to this equation, the major determinants of the quantity of gas exchanged per unit time are Krogh's constant (K), the area of the exchange surface (A), the partial pressure gradient (ΔP), and the diffusion distance (δ). Organisms can do little about K, but they can adjust the value of the other variables to suit their needs. If the intent is to maximize exchange, this can be achieved by having a large surface area, a small diffusive distance, and a large ΔP. It should be pointed out at this juncture that developing fish do not always maximize diffusive flux. Fish not only need to ensure that sufficient O_2 is supplied to the tissues to support aerobic metabolism during embryonic and larval development, but also need to keep tissue P_{O_2} relatively low to minimize the production of oxygen free radicals. Design characteristics of the developing respiratory system that appear to be inefficient in terms of maximizing diffusive fluxes are probably a reflection of this trade-off.

Diffusive Boundary Layer

The diffusive boundary layer (DBL) is the single most important barrier to diffusive gas exchange for much of larval development. The thickness of the boundary layer adjacent to the skin of rainbow trout (*Oncorhynchus mykiss*) in slow-moving water ($\leq 0.01\,\mathrm{cm\ s^{-1}}$) is in the order of $1000\,\mu\mathrm{m}$ during the first half of larval development and represents 60–90% of the total resistance to O_2 flux (**Figure 1**). Increasing water velocity (v) reduces the thickness (d) of the boundary layer ($d \sim v^{-0.5}$), but even at a velocity of $2\,\mathrm{cm\ s^{-1}}$ the boundary layer is still $\approx 300\,\mu\mathrm{m}$ thick and accounts for $\approx$40% of total resistance. A velocity of $2\,\mathrm{cm\ s^{-1}}$ is equivalent to that of water flowing over the gill of a resting adult trout and is much higher than is likely to be encountered by larvae of most species in nature. The thickness of the boundary layer and its impact on O_2 flux in rainbow trout larvae appear to be relatively uniform over the whole surface of the body in slow-moving water ($\leq 0.08\,\mathrm{cm\ s^{-1}}$). This uniformity begins to break down at higher velocities with the boundary layer becoming relatively thinner above more anterior surfaces and relatively thicker in more sheltered regions (this assumes water flow is anterior to posterior, which is normally the case because most larvae are positively rheotactic). Regional differences in the thickness of the boundary layer can also be induced by the movement of the pectoral fins. In 1975, Peterson showed that the rhythmic flutter of the pectoral fins displaced water from behind the opercula of Atlantic salmon (*Salmo salar*). This tended to draw water through the buccal and opercular cavities. Routine swimming reduces the overall thickness of the boundary layer, but probably produces little regional variation because the boundary layer tends to be dragged along with the larvae at the low speeds characteristic of routine swimming. This may not be the case during the startle response (C-starts) when velocities are briefly high enough to move the larvae out of the viscous hydrodynamic regime.

The external boundary layer is also a significant resistance to diffusive gas exchange during embryonic development. Its relative impact, however, does not appear to be as great as for larvae. There are two reasons for this. At the same water velocity, the DBL adjacent to the external surface of the egg capsule tends to be thinner than it is adjacent to the skin of a hatched larva. For example, at a water velocity of $0.01\,\mathrm{cm\ s^{-1}}$, the boundary

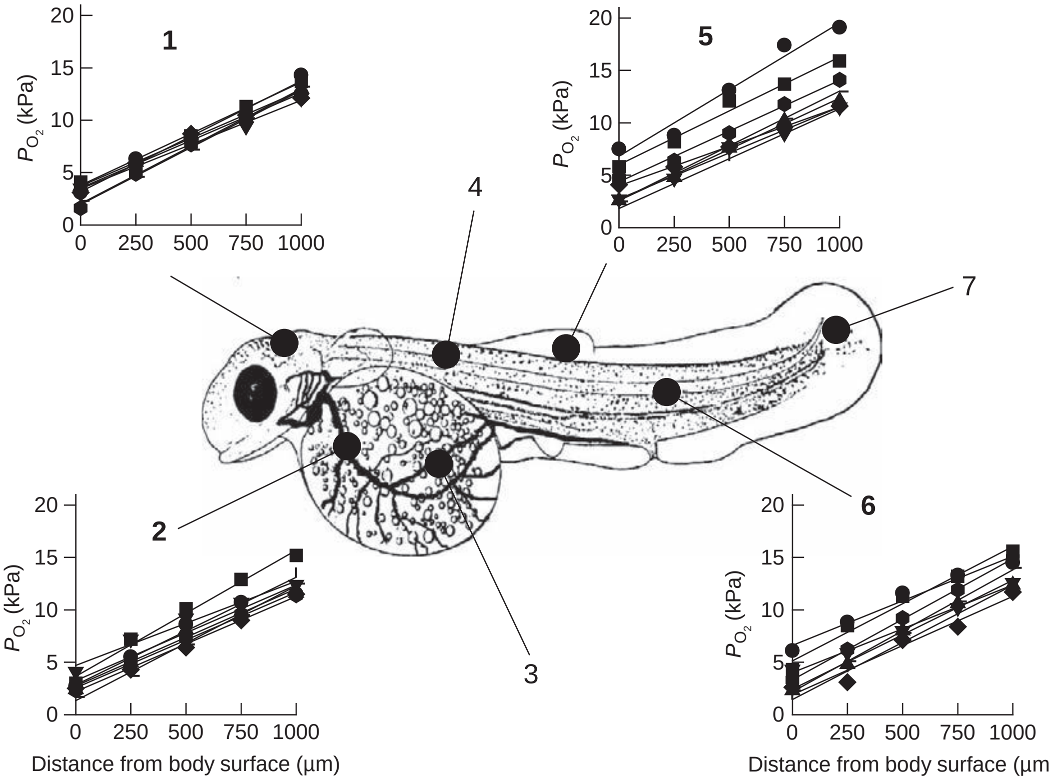


Figure 1 Oxygen profiles at various locations in the diffusive boundary layer surrounding newly hatched rainbow trout larvae in still water. Regression lines represent different individuals. From Rombough PJ (1998) Partitioning of oxygen uptake between the gills and skin in fish larvae: A novel method for estimating cutaneous oxygen uptake. *Journal of Experimental Biology* 201: 1763–1769.

layer external to the egg capsule just prior to hatch is ≈350 µm thick in rainbow trout, while that surrounding the newly hatched larvae only a few days later is >1000 µm thick. This difference probably has something to do with shape because it persists if one compares the thickness of the boundary layer surrounding an artificially decapsulated embryo with that for an intact egg at the same stage of development. The other reason for a lower relative impact prior to hatch has to do with the fact that embryos face an additional resistance to gas exchange not experienced by larvae, namely that imparted by the egg capsule and PVF. Although the absolute resistance of the boundary layer per unit thickness is the same for eggs and larvae, the relative resistance is less in eggs because of these added barriers. That being said, the boundary layer still represents a major fraction of total resistance during the egg stage. In rainbow trout, it accounts for 25–40% of the total resistance of O_2 flux, depending on the stage of development and water velocity. The relative impact of the boundary layer in embryos tends to increase with age but is unaffected by hypoxia. The increase with age is a reflection of increasing metabolic demand for O_2.

The DBL has a significant impact on CO_2 and NH_3 exchange by virtue of the resistance it provides to their excretion. Its negative impact on NH_3 excretion appears to be mitigated to some extent by acting as a site for proton trapping of the NH_4^+ ion (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). The boundary layer surrounding rainbow trout larvae has been shown to be significantly more acidic than the bulk medium as a consequence of the accumulation of CO_2 and its hydration to carbonic acid. This favors the conversion of NH_3 to NH_4^+, which in turn helps maintain the NH_3 partial pressure gradient for diffusive efflux from the body. Dissociation and proton trapping in the boundary layer could potentially impact CO_2 excretion; however, this has not been investigated.

Egg Capsule and PVF

The egg capsule and the PVF together represent a substantial resistance to diffusive gas exchange. Their precise impact is difficult to quantify and would be expected to vary with species and stage of development, but based on the little that is known, their combined resistance would appear to be about the same magnitude as for the external boundary layer (i.e., ≈20–40% of total resistance). Measurements of P_{O_2} gradients in the PVF indicate that the combined resistance of the egg capsule and PVF was equivalent to ≈30–75% of the resistance from the body surface to the mitochondria during cleavage and early gastrulation in loach *Misgurnus fossilis*. In eyed embryos of rainbow trout, the drop in P_{O_2} across the capsule and PVF was about the same as that across the external boundary layer (i.e., ≈40% total resistance). Removing the egg capsule (which also eliminates the PVF) surrounding chinook *Oncorhynchus tshawytscha* embryos shortly before normal hatch decreased total resistance to ≈50%.

The PVF appears to represent more of a barrier to diffusive gas exchange than does the egg capsule. Krogh's constant of diffusion for O_2 (K_{O_2}) is essentially the same for PVF as for water. Estimates for K_{O_2} of egg capsules of different species range from 13% to 87% (mean = 22%) of that for pure water. This means that on a per-unit thickness basis, the capsule has a greater impact than the PVF. The PVF, however, is much thicker than the capsule. Egg capsule thickness varies among species but is generally in the range of 20–60 μm. The thickness of the PVF also varies depending on species but is typically at least several hundred microns thick (e.g., ~200 μm in Atlantic salmon and ~400 μm in loach). Precise estimates of the relative impacts of the two layers are difficult to determine because, unlike the egg capsule, the thickness of the PVF is not uniform around the embryo. Generally, the PVF is thicker adjacent to the ventral surface of the embryo and thinner on the dorsal surface. Ignoring this complication, one would expect the impact of the PVF to be about twice that of the capsule if the PVF is not stirred. This is the situation for about the first one-third of embryonic development. Older embryos stir the PVF. This decreases the overall resistance of the PVF and facilitates gas exchange. Unfortunately, there are no empirical estimates of the magnitude of this effect, and we know too little about the dynamics of PVF flow to model it with any accuracy.

In general, embryos employ two types of movements to stir the PVF: flexing of the trunk musculature and oscillation of the pectoral fins. There is some controversy as to the efficacy of trunk movements, but oscillation of the pectoral fins almost certainly facilitates gas exchange. In Atlantic salmon, fairly high-frequency trunk movements (>100 h^{-1}) begin abruptly during early organogenesis. The frequency of trunk movement declines as development proceeds to reach levels in the range of only 1–2 h^{-1} by late organogenesis. About midway through organogenesis, embryos begin to use their pectoral fins to stir the PVF. The frequency of pectoral fin movements is much greater than trunk movements, in the range of 60–130 min^{-1}. The frequency of pectoral fin movement increases with both increasing temperature and decreasing O_2 level as would be expected if their primary role were to enhance O_2 exchange. Unfortunately, the impact of these ventilatory movements on gas exchange has not been quantified (**Figure 2**).

Skin

In most species, the skin is the only significant site of gas exchange between the organism and its environment during embryonic development. During the earliest stages, gases are exchanged across the vitelline membrane but this is soon replaced by the skin once the primary germ

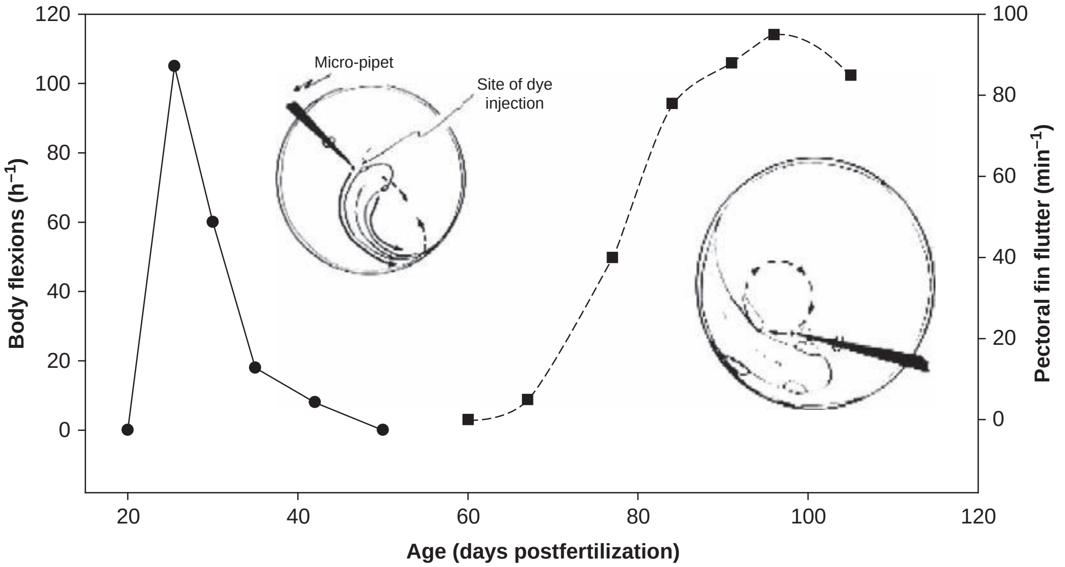


Figure 2 Frequency of body flexions and pectoral fin movements by Atlantic salmon embryos. Body flexions are indicated by the circles and solid line; pectoral fin movements are indicated by the squares and dashed line. Note the difference in frequency timescales (i.e., h^{-1} vs. min^{-1}). The inset drawings indicate the trajectories (indicated by arrows) taken by injected dye in response to body flexion (left) and pectoral fin movements (right). From Peterson RH and Martin–Robichaud DJ (1983) Embryo movements of Atlantic salmon (*Salmo salar*) as influenced by pH, temperature, and state of development. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 777–782.

layers begin to differentiate. As mentioned, embryos of a few species develop temporary external gills while still in the egg capsule, but this is exceptional. The vast majority of species continues to rely on the skin as their sole means of exchanging gases until the internal gills come on line. The stage at which this occurs varies considerably among species. On the one hand, salmon and trout, for example, begin to develop gills before hatch, well before use in gas exchange after hatch. Many marine species, on the other hand, do not initiate gill formation until well into the larval stage, perhaps many weeks after hatch. The transition from cutaneous respiration to primarily branchial (gill) respiration is a gradual process (**Figure 3**). In salmon and trout, for example, it takes several weeks for the transition to occur and even at the end of the transition period, the skin still accounts for a considerable fraction of total O_2 uptake ($\approx$20–30%). In this regard, it is worth noting that the skin in most species remains a significant site of gas exchange (5–30% of total O_2 uptake) even for adults.

The primary reason most developing fish are able to manage without any specialized respiratory structures has to do with their small size. Surface area is a major determinant of exchange capacity of a gas exchanger. The surface area of the skin of an embryo is large in relation to the mass of tissue it has to support. This is because for geometrically similar objects, surface area (A) is proportional to the relevant linear dimension squared, while mass (M) is proportional to the linear dimension cubed. The net result is that the surface-to-mass ratio of an organism tends to decrease with increasing size (i.e., $A \sim M^{2/3}$). In other words, smaller organisms have larger $A{:}M$ ratios than larger organisms. For example, the mass-specific surface area (A/M) of the skin of a newly hatched walleye *Stizostedion vitreum* weighing 2.5 mg is 38-times greater than the mass-specific surface area of the gill of a 1-kg adult. Larvae tend to have higher mass-specific metabolic rates ($\dot{M}_{\mathrm{O}_2}/M$) than adult fish (8.5-times greater in walleye); however, even taking this into account, the effective surface area per unit O_2 uptake of a newly hatched walleye larvae is still 4.5-times larger than for the adult fish.

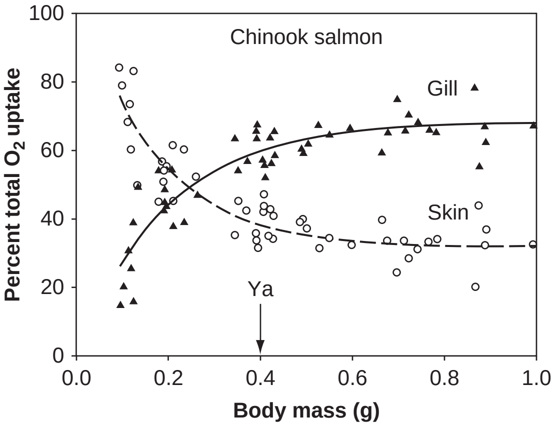


Figure 3 Changes during the course of larval development in the relative amounts of O_2 taken up across the skin and gills of chinook salmon (*Oncorhynchus tshawytscha*). The arrow marked 'Ya' indicates size at yolk absorption. From Rombough PJ and Ure D (1991) Partitioning of oxygen uptake between cutaneous and branchial surfaces in larval and young juvenile chinook salmon *Oncorhynchus tshawytscha*. *Physiological Zoology* 64: 717–727.

Another factor that facilitates cutaneous gas exchange during development is the thinness of the skin. According to the Fick equation, flux is inversely proportional to membrane thickness. The skin of fish embryos and larvae is typically in the order of 10–20 μm thick, although values in the range of 2–3 μm have been reported (e.g., herring (*Clupea harengus*)). These lower values are less than the thickness of the gill blood–water barrier of many adult fish.

The skin is generally well vascularized during late-embryonic and early-larval development. This is particularly true for the yolk-sac epithelium, but this probably has more to do with nutrient mobilization than gas exchange. Regional differences in the degree of vascularization do not appear to translate into differences in O_2 uptake rate. Indeed, the net rate of O_2 uptake across the yolk-sac epithelium, the most densely vascularized region of the skin, was actually slightly lower than the average rate for the rest of the body in Atlantic salmon larvae. In rainbow trout larvae, no significant regional differences in rates of transcutaneous O_2 uptake were recorded in still or slow-moving water. The most likely explanation for the apparent lack of correlation between uptake rates and blood-vessel density is that perfusion and blood gas transport are not limiting in normoxia. This is probably also true for moderate levels of hypoxia. This interpretation is supported by observations that reducing the O_2-carrying capacity of the blood by eliminating hemoglobin has little or no impact on the overall rate of O_2 consumption by zebrafish (*Danio rerio*) larvae under most conditions. It is not until O_2 falls to very low levels that one sees any advantage of having hemoglobin in terms of meeting larval metabolic O_2 demand.

Rates of O_2 uptake across the skin of fish larvae have been determined for only a few species, but they do not appear to be strikingly different from rates of uptake across the skin of adult fish. Area-specific rates of cutaneous O_2 uptake in adult fish are generally in the range of 6–10 μg-O_2 h^{-1} cm^{-2}; reports of transcutaneous uptake rates in salmonid larvae fall in the range of 3–9 μg-O_2 h^{-1} cm^{-2}. This is despite the fact the skin of larvae is considerably thinner (<1/5th) than the skin of adults. The most likely explanation for the relatively low rates of area-specific O_2 uptake that have been reported for larvae is that transcutaneous O_2 uptake is not the limiting step in the O_2 cascade under the conditions in which it has been measured (i.e., in normoxia and relatively early in larval development). Estimates of the effective surface area available per unit O_2 uptake support this contention. A morphometric study of five species indicated that newly hatched larvae had 2.5–6.0 times the cutaneous surface needed to meet their metabolic demand for O_2 in normoxia, assuming that the efficiency of transcutaneous uptake was the same in larvae as it was in adults. This is a conservative estimate because there are reasons to believe that the exchange potential of the skin is actually greater for larvae than for adults (e.g., shorter diffusion distances). One situation in which cutaneous exchange capacity might not be sufficient during early larval development is in profound hypoxia, but the dynamics of this situation have not been investigated. It is possible that in extreme hypoxia some other step in the O_2 cascade (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms) might become limiting before skin surface area.

While cutaneous gas exchange capacity initially may not be limiting, it will eventually become so as the larva continues to grow and mature. There are two major reasons for this: a gradual increase in skin thickness and a gradual decrease in $A:M$ ratios. The larval skin initially is only a few cells thick, but thickens considerably toward the end of the larval period. For example, the skin thickness of Atlantic salmon increases from ≈20 μm at hatch to ≈70 μm by the early fry stage. The skin also tends to mineralize which further decreases its exchange capacity. As discussed previously, $A:M$ ratios tend to decrease with increasing body size. The cutaneous surface area of fish larvae typically expands with increases in body mass at a rate proportional to $\approx M^{0.6}$. The metabolic demand for O_2, in contrast, typically expands at a rate $\approx M^{0.9}$. The net result is a gradual decrease in skin surface area per unit O_2 uptake. At some point, the available surface area on the skin simply becomes too small to keep up with the rate of metabolic expansion. The size at which this occurs varies among species and is also strongly dependent on temperature. Different species have different body shapes and growth trajectories for various parts of the body. The result can be a considerable variation in the species-specific rate of skin expansion. There are also species-specific differences in the rate of metabolic expansion. Temperature has a pronounced impact on larval metabolic rate, both within (mean $Q_{10} \approx 3.0$) and among species (mean $Q_{10} \approx 2.0$), but little or no impact on surface area. As a result, cutaneous respiration tends to become limiting at a smaller size for larvae of warm-water species than for cold-water species. For example, cutaneous O_2 uptake is estimated to become limiting for zebrafish larvae incubated at 28 °C at a body mass of ≈300 μg. Cutaneous uptake in salmonid larvae incubated at 10 °C is not projected to become limiting until they reach a body mass of ≈100 mg. Interestingly, in both groups the size at which cutaneous O_2 uptake is projected to become limiting coincides fairly closely to the size at which gill surface area begins to expand.

Gills

The gills of teleost fishes are anatomically complex structures consisting of, in order of formation, gill arches, gill filaments, and gill lamellae (**see also Ventilation and Animal Respiration:** Gill Respiratory Morphometrics). Gill lamellae are considered the definitive site of gas exchange, but some exchange also occurs across the filaments and arches. The gills are also functionally complex. Functions associated with the adult gill include gas exchange, acid–base balance, iono-regulation, ammonia excretion, hormone production, immune defense, and metabolism of various circulating metabolites. There has been some controversy concerning the order in which the developing gill assumes these various functions. Recent physiological evidence suggests that ion exchange shifts to the gill before gas exchange. This supports the morphological evidence showing that the cells involved in ion exchange (mitochondria-rich cells) appear on the arches and filaments well before lamellae, the definitive site of gas exchange, start to form. Since ion exchange and some components of NH_3 and CO_2 excretion are functionally linked, this raises the possibility that the gill may initially play a more important role in the excretion of these two gases than it does in O_2 uptake.

The stage at which definitive gill development, defined as the appearance of filamental buds, is initiated varies considerably among species (branchial-arch formation is a poor indicator as they are associated with the aortic arches which form early in organogenesis in all species). For example, gill filaments begin to form in rainbow trout several days before hatch. Gill filaments are first observed in zebrafish about 1 day after hatch (**Figure 4**). In Atlantic

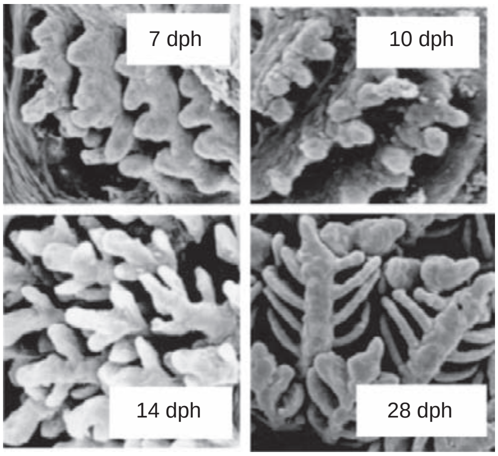


Figure 4 Development of the gills of zebrafish larvae. At 7 days posthatch (dph), filament buds can be seen forming on the gill arches. Lamellar buds first appear at about 10 dph. By 14 dph, 1–3 basal (closest to the gill arch) lamellae have formed. Aside from being smaller, the gill of a 28-dph larva is similar to that seen in juvenile and adult fish.

halibut (*Hippoglossus hippoglossus*), gill filaments do not appear until near metamorphosis, >1 month after hatching. The speed at which gill development proceeds, once initiated, also varies. In some species, the gill assumes its definitive adult appearance within a few weeks, while in other species it may take several months to reach this stage.

The transition from cutaneous to primarily branchial respiration is mainly a result of rapid expansion of the diffusing capacity of the gill rather than a decrease in the diffusing capacity of the skin. Diffusing capacity (*D*) is a measure of gas conductance (the reciprocal of resistance) and can be quantified as K^*A/d, where A is the surface area, d is the diffusion distance, and K is the Krogh's coefficient of diffusion. The absolute diffusing capacity of the skin increases throughout larval development but does so at a slower rate than the rate of metabolic expansion. The net result is that skin surface area per unit O_2 uptake gradually decreases. Gill diffusing capacity, in contrast, increases in both absolute and relative (compared to metabolism) terms. During the initial stages of gill development, there is a very rapid increase in surface area and a smaller, but still significant, decrease in diffusion distance. Allometric mass exponents for gill surface area during the initial period of expansion average about 3.0 across species (i.e., $A \approx M^{3.0}$), but values as high as 7.1 are reported.

Rates of gill expansion gradually diminish as development proceeds and by the end of the larval period they stabilize at levels typical of juvenile and adult fish (i.e., $SA \approx M^{0.8\text{–}0.9}$). Gill surface area per unit body mass is maximal (i.e., higher than at any other life stage, including juvenile and adult) at about this time. The thickness of both gill filaments and lamellae decreases as development proceeds. The magnitudes of these decreases, however, are relatively small. For example, in Atlantic salmon, mean filament diffusion distance decreased from 14 μm at hatch to 11 μm by the early juvenile stage; the corresponding decrease in lamellar diffusion distance was from 3.7 to 2.4 μm.

More important is the shift in the site of branchial gas exchange from the filaments to the lamellae. Whereas filaments accounted for ≈88% of gill surface area in newly hatched Atlantic salmon, they represented <30% of total gill area in young juveniles. The net result was about a 70% decrease in the average thickness of the blood–water barrier, from about 13 to 4.0 μm. Diffusing capacity, which takes into account both *SA* and *d*, has been shown to be a fairly accurate indicator of the fraction of O_2 actually taken up across the gill and skin for most of larval development (**Figure 5**). The exception is very early in larval development where *D* tends to underestimate gill uptake (and overestimate skin uptake). This discrepancy probably reflects differences in how the skin and gills are ventilated.

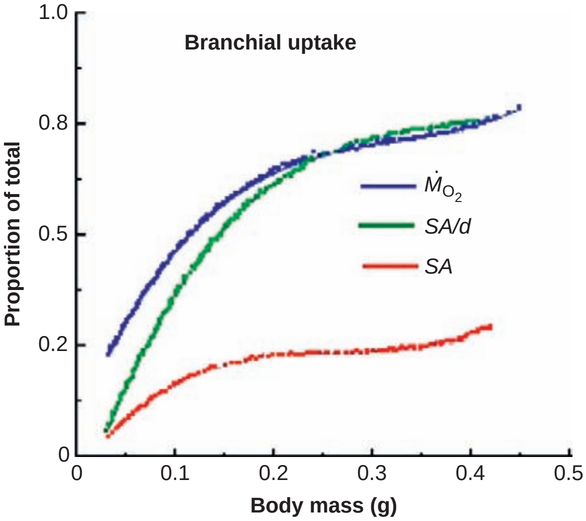


Figure 5 Actual ($\dot{M}_{O_2}$, blue line) vs. estimated fractions of total O_2 taken up across the gill of young Atlantic salmon based solely on relative surface area (*SA*, red) and a combination of surface area and diffusion distance (*SA/d*, green). Surface area alone is a poor predictor of O_2 uptake. The anatomical diffusing factor, *SA/d*, provides a much better estimate, particularly for larger fish. From Wells PR and Pinder AW (1996) The respiratory development of Atlantic salmon. 2. Partitioning of oxygen uptake among gills, yolk sac and body surfaces. *Journal of Experimental Biology* 199: 2737–2744.

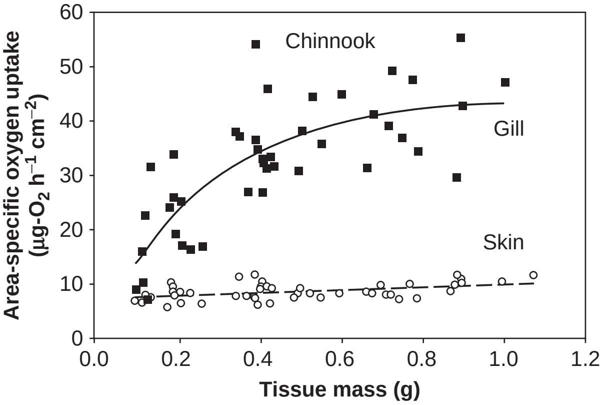


Figure 6 Changes in area-specific O_2 uptake across the skin and gills during the course of larval development in chinook salmon. Area-specific uptakes are initially similar for skin and gills, but by the end of the larval period about 4-times as much O_2 per unit surface area is taken up across the gill as across the skin. From Rombough PJ and Ure D (1991) Partitioning of oxygen uptake between cutaneous and branchial surfaces in larval and young juvenile chinook salmon *Oncorhynchus tshawytscha*. *Physiological Zoology* 64: 717–727.

Branchial gas exchange potentially is more effective than cutaneous gas exchange. In chinook salmon, for example, 4–5 times as much O_2 per unit surface area was taken up across the larval gill once it began to be well ventilated than across the skin (**Figure 6**). Part of this difference is due to shorter blood–water diffusion distances in the gill. More important, however, are differences in how the two surfaces are ventilated. In particular, water in the gill is forced to flow through relatively narrow channels between adjacent filaments or lamellae. This effectively reduces the thickness of the branchial boundary layer to about 30% of channel width. As a result, the effective thickness of the boundary layer adjacent to the gill lamellae of a rainbow trout larvae is only about 1/20th the thickness of the boundary layer adjacent to its skin at the same flow rate (the relative impact on filamental exchange is not as great because of larger interfilamental distances). A thinner boundary layer has the effect of increasing ΔP_{O_2} at the gill surface and, thereby, enhancing flux rates. Channeling has the added advantage of ensuring that water flow in the gill is unidirectional and largely countercurrent to the flow of blood. At low flow rates, countercurrent flow has been shown to result in higher exchange efficiencies than if blood flow and water flow are at some other angle to each other. There is some evidence that fish larvae can direct water flow over portions of their skin surfaces so it is approximately countercurrent to blood flow, but this appears to be limited to relatively small regions of the body and only occurs intermittently.

Gill ventilation initially tends to be intermittent. This does not appear to reflect an inability to detect changes in ambient O_2 levels (the primary driver of gill ventilation in adult fish) or a lack of neuromuscular control. Studies of zebrafish indicate that they possess functional O_2-sensitive neuroepithelial cells on their gills as early as 1-day post-hatch. When newly hatched Arctic char larvae are exposed to hypoxia, they quickly shift from an intermittent to continuous ventilatory pattern. The most likely explanation for early discontinuous breathing is that it is energetically less expensive than regular breathing. The surface area of the skin of newly hatched larvae, which does not need to be ventilated, typically is more than sufficient to meet the metabolic demand in normoxia. Under these circumstances, energy that otherwise would be used to ventilate the gills can be freed up for other purposes. This raises the question of why the gills are ventilated even intermittently in normoxia. A possible explanation has to do with the other functions of the larval gill, in particular its role in ion exchange. Ion exchange is an energetically expensive process supported by aerobic metabolism. For this process to continue, some O_2 must be supplied to the gill. This explanation fits with the observation that although the gill of newly hatched trout larvae is responsible for about 20% of total O_2 uptake, very little, if any, of that O_2 appears to make it into the systemic circulation.

One of the major functional advantages of gill respiration over cutaneous respiration is a better ability to extract O_2 under hypoxic conditions. The initial stages of lamellar expansion, in particular, are associated with decreases in critical O_2 levels (P_c). P_c is the minimum O_2 level at which fish are able to maintain a normal metabolic rate when faced with declining P_{O_2}. The reasons the gill is more effective in hypoxia are twofold. The channeling of water between adjacent filaments and/or lamellae results in a significant reduction in the thickness of the DBL. This effectively increases the P_{O_2} at the exchange surface, which is particularly important at low ambient P_{O_2}. Gill ventilation also provides the fish with more control over how much water can be passed over the exchange surface. Larvae can alter water flow over the skin (e.g., by swimming or pectoral fin movements) but this is relatively ineffective because of the much greater thickness of the DBL and the resulting greater mass of water that needs to be moved. In some (but not all) species, the shift to primarily gill ventilation translates into improved hypoxic tolerance (i.e., survival at low P_{O_2}). The reason not all species respond this way is not that their gills are less efficient. There is a general trend during larval development for tissues to become less tolerant of anoxia and hypoxia. Whereas enhanced O_2 uptake at the gill more than compensates for this increased sensitivity to low P_{O_2} in some species, it does not in other species.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Gill Respiratory Morphometrics; Respiratory Gas Exchange During Development: Models and Mechanisms.

Further Reading

Fu C, Wilson JM, Rombough P, and Brauner CJ (2010) Ions first: Na^+ uptake shifts from the skin to the gills before O_2 uptake in developing rainbow trout, *Oncorhynchus mykiss*. *Proceedings of the Royal Society B* 277: 1553–1560.

Jonz MG and Nurse CA (2006) Ontogenesis of oxygen chemoreception in aquatic vertebrates. *Respiratory Physiology and Neurobiology* 154: 139–152.

Pelster B (2008) Gas exchange. In: Finn RN and Kapoor BG (eds.) *Fish Larval Physiology*, pp. 91–117. Enfield,NH: Science Publishers.

Peterson RH and Martin-Robichaud DJ (1983) Embryo movements of Atlantic salmon (*Salmo salar*) as influenced by pH, temperature, and state of development. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 777–782.

Rombough PJ (1988) Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: Hoar WS and Randall DJ (eds.) *Fish Physiology* vol. XIIA, pp. 59–161. San Diego, CA: Academic Press.

Rombough PJ (1998) Partitioning of oxygen uptake between the gills and skin in fish larvae: A novel method for estimating cutaneous oxygen uptake. *Journal of Experimental Biology* 201: 1763–1769.

Rombough PJ (2004) Gas exchange, ionoregulation and the functional development of the teleost gill. *American Fisheries Society Symposium* 40: 47–83.

Rombough PJ (2007) The functional ontogeny of the teleost gill: Which comes first, gas or ion exchange? *Comparative Biochemistry and Physiology – A Comparative Physiology* 148: 732–742.

Rombough PJ and Ure D (1991) Partitioning of oxygen uptake between cutaneous and branchial surfaces in larval and young juvenile chinook salmon *Oncorhynchus tshawytscha*. *Physiological Zoology* 64: 717–727.

Wells PR and Pinder AW (1996) The respiratory development of Atlantic salmon. 2. Partitioning of oxygen uptake among gills, yolk sac and body surfaces. *Journal of Experimental Biology* 199: 2737–2744.

Techniques in Whole Animal Respiratory Physiology

JJ Cech Jr., University of California, Davis, CA, USA

CJ Brauner, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Apparatus and Techniques
Further Reading

Glossary

Active metabolism The maximum aerobic metabolic rate associated with swimming at the greatest sustainable velocity.

Aerobic scope The capacity of an animal to increase its level of aerobic metabolism beyond that which is required for maintenance alone and thereby support activities such as muscular exercise, growth or reproduction; it sets the capacity for oxygen-consuming physiological processes that an animal may perform at a given time. Aerobic scope is the difference between minimal and maximal oxygen consumption rate.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Respirometer An apparatus used to measure respiratory metabolic rates via indirect calorimetry (e.g., via oxygen consumption or carbon dioxide excretion).

Resting-routine metabolism The metabolic rate for quiescent fish, but not necessarily the lowest rate during the 24-h cycle.

Routine metabolism The metabolic rate including spontaneous movements.

Standard metabolism In ectotherms, this term describes the minimal level of aerobic metabolism (per unit time) required for maintenance functions to sustain life. In this sense, it is analogous to basal metabolism in endotherms, although SMR is strongly influenced by environmental temperature and so it is not always constant within an individual.

Swimming metabolism The metabolic rate measured at some voluntary or forced level of swimming.

Ventilation The movement of the respiratory medium (air or water) over the surface of the gas exchanger.

Ventilation volume The total amount of water pumped over the gills in a period of time (usually 1 min); the product of the rate of breathing multiplied by the volume of water pumped with each breath.

Ventilatory frequency The frequency of buccal or opercular movements.

Ventilatory stroke volume The volume of water pumped per buccal–opercular stroke or movement.

Introduction

Measurements of fish respiratory metabolism and gill ventilation can inform us concerning a fish's physiological state, by providing a quantitative measure of how rapidly energy and oxygen are being converted/used. Data on respiratory metabolic rates are important in the construction of bioenergetic models, and changes in respiratory metabolic and gill ventilatory rhythms can be sensitive indicators of altered environmental conditions or physiological states, and thus can reveal much about a fish's recent and current activity, acclimation, and level of stress. The scope of this article is limited to measurements of respiratory (aerobic) metabolism and gill ventilation.

Respiratory Metabolism Measurement Methods

Metabolic rates can be measured using either direct or indirect calorimetry. Direct calorimetry, the measurement of heat production, is rarely used for fishes because their metabolic and heat production rates are generally low (e.g., compared with those of mammals or birds) and the heat capacity of water is high, which together result in limited measurement sensitivity. Indirect calorimetry, through measurement of oxygen consumption rate ($\dot{M}_{O_2}$), has become the conventional measure for fishes because changes in dissolved oxygen levels over time in water can be determined with relative ease and reliability. The iodometric or Winkler method of measuring O_2 concentrations (mg or mL O_2 L^{-1} H_2O) is highly accurate when fresh reagents are available to fix and titrate water samples and is very useful in the field. Clark-type polarographic electrodes and fiber-optic O_2 sensors (see below) measure O_2 tensions (i.e., partial pressures, P_{O_2}) and are easier and faster to use in the laboratory than the Winkler method. The wide availability and precision of these electrodes have resulted in the rapid expansion of the field of respiration physiology over the past 30 years. Oxygen consumption rate is only an accurate measure of overall metabolism when anaerobic contributions are insignificant. During high-speed sustained and burst swimming or during exposure to hypoxia, metabolic requirements cannot be met aerobically and any deficit is met anaerobically, as indicated by either lactate appearance in the blood or tissues, or an O_2 debt that is repaid during recovery.

In addition to oxygen consumption rate, carbon dioxide (CO_2) production rate can be measured by indirect calorimetry. However, the high solubility of CO_2 in water makes it difficult to measure changes in the partial pressure of CO_2 (P_{CO_2}) accurately with available electrodes, and changes in total CO_2 of the water over time are often difficult to measure against the background CO_2 bound as carbonates and bicarbonates, especially in hard water and saltwater.

Respiratory Metabolism Levels

Aerobic metabolism in fishes can be categorized as standard, resting routine, routine, swimming, and active. Standard metabolism is the minimum metabolic rate for intact fish. Resting-routine metabolism is the rate for quiescent fish, but not necessarily the lowest rate during the 24-h cycle. Routine metabolism is the rate including spontaneous movements. Swimming metabolism rates are measured at some voluntary or forced level of swimming. Active metabolism is the maximum aerobic rate associated with swimming at the greatest sustainable velocity. Aerobic scope is calculated as the difference between active and standard metabolic rates and reflects the aerobic capacity available for everything beyond maintenance, such as growth, reproduction, exercise, and digestion/absorption of food.

The standard (i.e., basal, maintenance, or resting) metabolic rate is the minimum required to sustain life. It should be measured when fish are absolutely quiescent – that is, when they are expending no energy for activity (even random activity), food digestion, reproductive development, growth, or stress responses. Fish should be carefully shielded from changes in water temperature and chemistry and from all disturbances, and they should be given adequate time to habituate to the respirometer. Metabolic rates should be recorded throughout the diel cycle to determine the minimum rate. The innate restlessness of pelagic fishes such as salmon or tuna makes it much more difficult to determine standard metabolism for these species than for demersal or sedentary forms such as flatfishes. Some workers have calculated standard metabolism from data on swimming fish from relationships between swimming velocity and the logarithm of metabolic rate via extrapolation to zero velocity. Besides the statistical problem (i.e., widening confidence limits) associated with extrapolation, the physiology (e.g., via hormone secretions) of active and quiescent fish may be quite different.

The resting-routine metabolic level falls between standard and routine metabolism. Measurements of resting-routine metabolism require a quiescent fish without food in its gut, a respirometer with dimensions that constrain swimming but are not overly confining, and isolation of the fish from laboratory stimuli. However, these measurements do not account for the diel activity cycles that fish normally undergo.

Most metabolic values reported for fishes are routine rates, which represent generally quiescent to moderately active fish. Routine metabolic rates include spontaneous activity, possibly due to daily activity cycles, and they may include swimming at a velocity of up to 1 body length s^{-1}.

Swimming metabolic rates are measured during various voluntary or forced levels of swimming. Active metabolic rates are generally defined as those attained while a fish is swimming at the greatest sustainable velocity for a particular period of time, such as 1 h.

Gill Ventilation

Oxygen uptake across the respiratory surfaces is driven by diffusion. The rate of O_2 diffusion is directly proportional to the partial-pressure gradient (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs and Respiratory Gas Exchange During Development: Models and Mechanisms). Because the movement of ventilation water over the gills replaces the boundary layer of water next to the lamellar epithelium (where O_2 is diffusing inward), thus maintaining the P_{O_2} gradient between water and blood, gill ventilation is an essential respiratory process in most fishes (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). Ventilation volume is the total volume of water forced over the gills. Ventilatory frequency is the frequency of buccal or opercular movements, and ventilatory stroke volume is the volume of water pumped per buccal–opercular stroke or movement. Percentage utilization of oxygen is the percentage of available oxygen removed by respiration from the inspired water.

Apparatus and Techniques

Oxygen Measurements

Oxygen dissolved in water is usually measured electrometrically or titrimetrically (e.g., with Winkler titrations). One common electrometric measurement system uses the Clark-type oxygen electrode. This electrode consists of an anode (e.g., silver–silver chloride) and a cathode (e.g., platinum), which are bathed in an electrolytic solution in the electrode body. An O_2-permeable membrane is stretched across the electrode tip. When a small polarizing voltage is applied across the anode and cathode, O_2 diffusing across the membrane is reduced at the cathode, producing a small electrical current through the electrode. This current is proportional to the P_{O_2} in the water. The current is amplified and displayed on a meter or digital readout; the display equipment varies from battery-powered portable units to bench-top units with computer interfaces. Because of possible drifting, calibration of Clark-type O_2 electrodes should be checked frequently. Some dissolved oxygen meters use fiber-optic oxygen sensors, which may require less-frequent calibration and maintenance and, unlike the Clark-type oxygen electrode, do not consume O_2. Whereas some meter and electrode systems for dissolved oxygen automatically calculate O_2 concentrations (e.g., mg O_2 L^{-1}) from detected O_2 tensions if water temperature and salinity values are known, other units display P_{O_2} (e.g., in millimeters of mercury (=torr) or kilopascals). These can be converted to O_2 concentration (usually mL O_2 L^{-1}) with the aid of a nomogram or oxygen solubility tables and the formula

$$C_{O_2}(\mathrm{ws}) = P_{O_2}(\mathrm{ws})C_{O_2}(\mathrm{AS}) / P_{O_2}(\mathrm{AS}) \tag{1}$$

where $C_{O_2}(\mathrm{ws})$ is the O_2 concentration (mL O_2 L^{-1}) in the water sample, $P_{O_2}(\mathrm{ws})$ the O_2 tension (mmHg) in the water sample, $C_{O_2}(\mathrm{AS})$ the O_2 concentration in water at air saturation (from nomogram or tables), and $P_{O_2}(\mathrm{AS})$ the O_2 tension at air saturation (from eqn (2)). Oxygen concentrations in mL O_2 L^{-1} can be converted to mg O_2 L^{-1}, assuming 1.428 mg O_2 mL^{-1} O_2.

The oxygen tension at air saturation is

$$P_{O_2}(\mathrm{AS}) = 0.2094(P_B - P_{WV}) \tag{2}$$

where 0.2094 is the mole fraction or volumetric fraction of O_2 in the atmosphere, P_B the total barometric pressure (mmHg), and P_{WV} the water vapor pressure at the experimental temperature (mmHg).

Overall, it is imperative that water samples from respirometers be taken with the minimum possible exposure to the atmosphere. Where continuous monitoring of water O_2 tension is desired, fiber-optic oxygen sensors may be placed directly in the respirometer, provided the water volume is adequately mixed. Fiber-optic oxygen sensors or Clark-type electrodes can be housed outside the respirometer in a thermostatically controlled chamber where water is drawn from the respirometer, forced past the electrode, and returned to the respirometer in a closed loop driven by a peristaltic pump.

Body Mass Considerations

Large fish generally consume more O_2 than small fish. However, on a unit-mass basis, small fish consume more O_2 than large fish. This allometric relationship can be described as

$$Y = aX^b \tag{3}$$

or

$$\log_{10} Y = \log_{10} a + b \log_{10} X \tag{4}$$

where Y is the O_2 consumption rate (mg O_2 min^{-1}), a the mass coefficient, X the body mass (g or kg), and b the mass exponent (**see also Energetics**: Physiological Functions that Scale to Body Mass in Fish and **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions).

The log–log plot of X and Y (eqn (4)) is a linear one, but with a slope (b) that usually is less than 1.0. Consequently, to compare metabolic rates of different-sized fish, O_2 consumption rate cannot simply be divided by fish mass (i.e., mg O_2 g^{-1} h^{-1}), and both intra- and interspecific comparisons of respiratory metabolic rates must be conducted carefully. For intraspecific comparisons, it is best if the mean body masses of the two groups are statistically indistinguishable and the ranges around those means are small. If the two groups of fish have significantly different mean masses, one of two other analyses might be appropriate. The first is an analysis of covariance with mass as the covariate, and the other is to determine the actual mass exponent (e.g., $b = 0.81$) for the particular group of fish and divide the raw data by (mass^b). This last technique yields mass-independent metabolic rates that are most correctly analyzed in $\log_{10}$-transformed form. These two analyses are also appropriate when different species of fish are compared, although their regression intercepts (mass coefficients, a; eqns (3) and (4)) may vary (**see also Energetics**: General Energy Metabolism). Finally, mass exponents should be determined over as wide a size range (e.g., one to two orders of magnitude) of homomorphic (i.e., of same body proportions) individuals as possible.

Respiratory Metabolism

A respirometer incorporates a chamber for the fish and a surrounding respiratory medium (usually water, though air or access to air may be appropriate). There are essentially two types of respirometers: closed respirometers, in which the same volume of respiratory medium is continuously used with little replacement, and open respirometers, in which the medium is continuously replaced. Closed respirometers are of two types: static respirometers and swimming respirometers. In static respirometers, the medium is not moved in directed currents, though it may be kept well mixed by gentle stirrers and the fish itself. In swimming respirometers, water flows within the vessel, forcing the fish to swim against a directed current. In contrast, fresh water continually flows into and out of open (or flow-through) respirometers, the object being to maintain water quality. In general, static and open respirometers are used to measure similar aspects of fish respiration, though the measurements are made differently. Swimming respirometers can be quite elaborate and expensive. Whatever system is chosen, the study will progress most efficiently if several replicate setups can be used simultaneously.

Static Respirometers

A static respirometer contains a circulating, fixed volume of water at constant temperature. It may be as simple as a glass jar or flask and a tight-fitting stopper to which water-sampling and water-flushing tubes have been fitted (**Figure 1**), or a probe that has been inserted directly. The jar or other container should be impermeable to gases and at least partially transparent to allow detection of bubbles. Because air contains up to 30 times more O_2 per unit volume than water, bubbles, as potentially a great source of respirometry error, must be removed. A transparent chamber may also help the enclosed fish to visually orient to the environment (e.g., experimental tank or stream bottom) in which the respirometer is placed, and thereby to acclimate to the confines of the respirometer. Some species (e.g., gobies (Gobiidae) and toadfish (Batrachoididae), which have secretive habits), however, acclimate more readily if the vessel is nearly opaque.

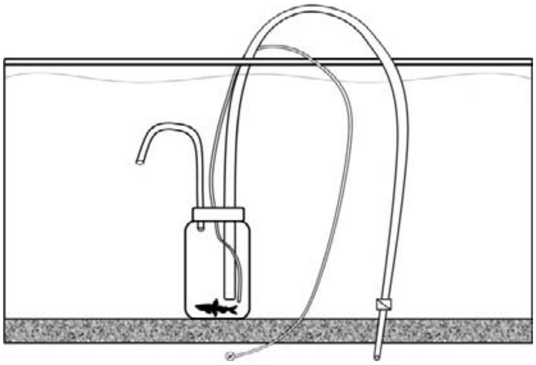


Figure 1 Static, closed respirometer (situated in an aquarium), consisting of a glass jar with rubber stopper through which siphon tubes can flush aquarium water through the respirometer during fish acclimation to the respirometer. The outflow siphon tube is equipped with a tubing clamp (depicted by a rectangle surrounding a diagonal line) to shut off the flushing flow during an experiment. Initial and final water samples are collected in a syringe (not shown) via the small-diameter water-sampling tube and attached stopcock (depicted by a circle surrounding an 'x'). The fish's small movements and gill ventilation circulates the water inside the respirometer. Redrawn by R. Coulter from Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

Measurements of O_2 consumption are calculated from changes in dissolved O_2 concentration over time, taking into account the respirometer volume:

$$\dot{M}_{O_2} = (C_{O_2}(A) - C_{O_2}(B))V/T \qquad (5)$$

where $\dot{M}_{O_2}$ is the O_2 consumption rate (mg O_2 h^{-1}), $C_{O_2}(A)$ the O_2 concentration in water (mg O_2 L^{-1}) at the start of the measurement period, $C_{O_2}(B)$ the O_2 concentration in water (mg O_2 L^{-1}) at the end of the measurement period, V the volume of respirometer (L), and T the time elapsed during measurement period (h).

Corrections for microbial O_2 consumption or production can be made by accounting for the O_2 consumed from a blank (fishless) respirometer with dimensions and tubing lengths similar to those of respirometers containing fish. Some investigators measure microbial O_2 consumption rates in the fish's respirometer after the fish is removed, a correction technique that is especially recommended for extended metabolic studies because microbial dynamics may differ between experimental and blank vessels.

The volume of the respirometer should generally be 30–50 times that of the fish. A respirometer that is smaller than this may stress the test fish and elevate respiratory metabolic rates. In one that is larger, the time required to measure $\dot{M}_{O_2}$ may be prohibitively long and the fish's ventilatory and fin movements will not keep the water mixed well enough to assure the investigator of representative samples in which case additional stirring mechanisms may be required. Submersible pumps add heat energy to the system, which could affect metabolic rates.

Considerable effort should be made to minimize stress. When fish must be moved, they should be transferred quickly from one tank to another using a vessel (e.g., beaker or bucket) that will prevent air exposure. If possible, the water source and quality should be the same in the respirometers' water bath as it is in the fishes' holding tank. The tank containing the respirometers should be at least partially covered to shield test fish from the glare of lights and the movement of people. Fish should be guided carefully into the respirometer opening. The respirometers can be slowly flushed with water from the respirometer tank (e.g., with a siphon, **Figure 1**) while the fish is initially settling down and between experiments. When the measurement period begins, the outflow tubing normally is clamped off and either the decline in O_2 levels within the system is continuously monitored, or initial and final water samples are taken for O_2 analysis. The outflow tube may be used for this purpose, especially if large samples are needed for Winkler titrations, but a small-diameter, heavy-walled sampling tube is recommended if only a few milliliters are needed. The dead-space water filling the sampling tube is easily removed with syringes and a three-way stopcock (**Figure 1**). Small water samples insure that replacement water drawn into the respirometer from the water bath has minimal effect on the final O_2 measurement. Spontaneous activity from fish in routine metabolic states can be quantified using video recordings of fish, especially if the respirometers have grid marks on the walls.

Special static respirometers can be used to isolate the respiratory contributions from different gas-exchange sites. For example, some respirometers may incorporate a rubber sleeve to separate branchial from cutaneous compartments. In each compartment, the water is circulated past an oxygen electrode by a pump, allowing O_2 consumption rates from each gas-exchange site to be measured separately. Special (e.g., L-shaped, with a gas space at the top of its vertical arm) static respirometers that incorporate an air phase can be used to study respiration by fishes with air-breathing organs (e.g., lung and swimbladder). Changes in volume and O_2 content in the gas space allow calculation of instantaneous O_2 consumption rates.

Because fish eventually deplete the O_2 in the enclosed volume of water, the main limitations of the static respirometer concern long-term experiments, especially those in which fish are to be exposed to prolonged stable levels of hypoxia. For metabolic studies under normoxic conditions, measurements must be made before chamber P_{O_2} declines to a critical level. Flushing the respirometer with hypoxic water permits $\dot{M}_{O_2}$ measurements at predetermined hypoxic P_{O_2} ranges.

Dissolved metabolic wastes (CO_2 and ammonia) also increase during static experiments. Automated systems that incorporate intermittent flows have solved some of the problems inherent in long experiments with static respirometers.

Open (Flow-Through) Respirometers

Open respirometers (**Figure 2**) solve many of the problems that arise during long-term studies with static systems. Continuous flows of water over the fish eliminate the need for periodic flushing flows; wastes do not accumulate, and desired characteristics such as oxygen concentration are maintained.

Under stable environmental conditions, oxygen consumption rates are calculated by the equation

$$\dot{M}_{O_2} = (C_{O_2}(I) - C_{O_2}(O))FR \qquad (6)$$

where $\dot{M}_{O_2}$ is the O_2 consumption rate (mg O_2 min^{-1}), $C_{O_2}(I)$ the O_2 concentration in inflowing water (mg O_2 L^{-1}), $C_{O_2}(O)$ the O_2 concentration in outflowing water (mg O_2 L^{-1}), and FR the water flow rate through the respirometer (L min^{-1}).

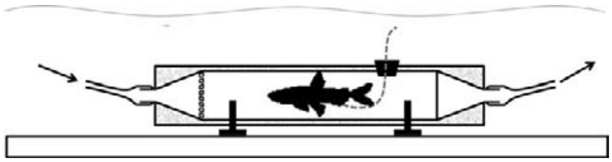


Figure 2 Open (i.e., flow-through) respirometer (situated in a tank of water), consisting of clear acrylic pipe with tapered ends to smooth the slow, continuous flow of water through the respirometer. Note that the inflowing water tube (shown cutoff in this diagram) would be attached (e.g., via a manifold with a port to measure dissolved oxygen) to a constant-head reservoir. The inflowing water would pass through the baffle plate (shown upstream of the fish) in which the perforating holes are drilled at angles to minimize eddy creation in the respirometer. The outflowing water tube (also shown cutoff) would lead to a rack where water could be sampled for dissolved oxygen and captured over a fixed time to measure flow rate. The rubber stopper and dashed line show a possible arrangement for leading an indwelling cannula (e.g., for blood-gas measurements) from the fish out of the respirometer. Not shown is the hatch or removable (rear) end cap through which the fish is inserted and removed from the respirometer. Redrawn by R. Coulter from Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

To avoid using a time-lag correction factor, fish should be quiescent and water conditions should not change quickly in the flow-through respirometer. To check that the desired slug of water moves through the respirometer without mixing or creating major eddies, flow properties can be checked by injecting dye into the inflow water and observing its pattern through the chamber.

Automated designs incorporate computers or timers (e.g., with solenoid valves) to divert water past a calibrated O_2 electrode.

Generally, water flow rates are set so that the difference in oxygen concentration between inflowing and outflowing water is 0.5–1.0 mg L^{-1}. Water flows are typically measured as the weight (1 g = 1 mL) or volume of outflowing water collected per unit time. Water flowmeters may also be used, but they usually are not precise enough for metabolic calculations.

Flow-through respirometers are generally constructed of clear, thick-walled acrylic plastic such as Plexiglas®. Some kind of inert, virtually gas-impermeable tubing (e.g., a heavy-walled transparent vinyl such as Tygon®) directs the inflowing water to this chamber. This tubing should be as short as possible to minimize the buildup of attached organisms, and should be of the same length on multiple respirometers if these are used. It is important to spread the flow from the small-diameter tubing across the larger diameter of the chamber without creating eddies or dead spaces. Smooth-walled cones connecting the two and a baffle plate usually serve this function (**Figure 2**).

Swimming Respirometers

Swimming respirometers usually contain a closed volume of water that is circulated past a fish, inducing the fish to swim. As in static systems, changes in dissolved oxygen concentration through time in the enclosed volume reflect the rate of respiratory metabolism, in this case at a particular swimming velocity. Two basic designs of swimming respirometers are in current use. The most common is the water tunnel or Brett-type respirometer, which consists of a closed loop of pipe containing a centrifugal pump or propeller connected to an electric motor (**Figure 3**). A variable-speed drive on the motor changes the water velocity past the fish. The fish is restricted to its chamber by screens, flow-straightening tubes, or an electrifiable grid at the rear of the chamber. Gradual turns of custom-bent polyvinyl chloride pipe in the tunnel loop and gradual tapers (<25°) in sections connecting the swimming chamber with other pipes minimize turbulence and temperature increases. Alternatively, a closed loop of pipe of constant diameter allows faster water velocities to be achieved with the same-size motor. However, the somewhat larger volume of the constant diameter system will result in longer experiments, due to the slower change in water P_{O_2} because of metabolism.

Water velocity through the swimming chamber is proportional to pump speed, measured in revolutions of the pump shaft per minute. Water velocity should be measured with an electronic flowmeter, and then compared with speed settings on the motor or pump shaft. After this calibration, water velocity can be determined by measuring pump speed with a tachometer. A correction factor for solid blocking effect must be applied if the test fish occupies more than 10% of the cross-sectional area of the swimming chamber. Automated versions of the Brett-type respirometer may incorporate a computer-controlled system that re-oxygenates respirometer water via an artificial lung perfused with O_2 gas between bouts of oxygen-consumption rate measurements.

The second basic swimming respirometer (Blazka) design is based on concentric (coaxial) tubes; the swimming chamber is in the inner tube and water returns between the inner and outer tubes (**Figure 4**). A propeller, driven by a variable-speed electric motor, or a submersible water pump pulls or pushes water past the fish and returns it to the front of the swimming chamber via the outer tube after it is deflected by a domed cap. The spiraling movement imparted to the water by the rotating propeller can be minimized by replacing the conventional propeller with a jet outboard impeller and by incorporating flow-straightening vanes in the outer tube and small-diameter tubes at the ends of the inner tube. The small-diameter tubes, which may vary in diameter from thin-walled 1.25-cm polyvinyl chloride pipe in larger Blazka respirometers to 3-mm plastic soda straws in small ones, also confine the fish to the swimming chamber. The respirometer may also have an electrified wire grid (8–10 V AC) behind the fish to encourage it to swim if required. The relatively small water volume of the Blazka design minimizes the time needed to measure oxygen consumption rates at a particular temperature and swimming velocity.

Metabolic rates of swimming fish have also been estimated from heartbeat, ventilation, and swimming muscle contraction frequencies by telemetry. These telemetric techniques point the way to improved *in situ* metabolic measurements, although activities that require different muscles (i.e., without inserted electrodes) may not yield accurate metabolic estimates.

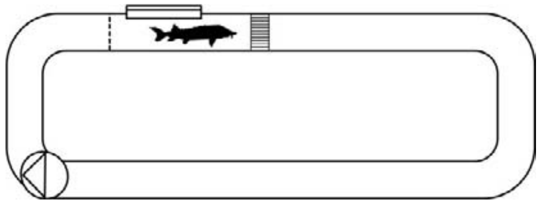


Figure 3 Brett-type, swimming closed respirometer is a loop of pipe (e.g., PVC, with a clear acrylic section for the fish), which incorporates either a propeller or a pump (shown as a circle surrounding a triangle) powered by a variable-speed motor (not shown) to push water past the fish. Flow-straighteners are shown upstream and a screen (dashed line) is shown downstream of the fish. The hatch above the fish, for insertion and removal of the fish, is shown as an acrylic saddle and rubber gasket over the top of the acrylic pipe (not intruding into the smooth, constant diameter of this respirometer). Not shown are sampling tubes for dissolved oxygen measurements. Redrawn by R. Coulter from Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

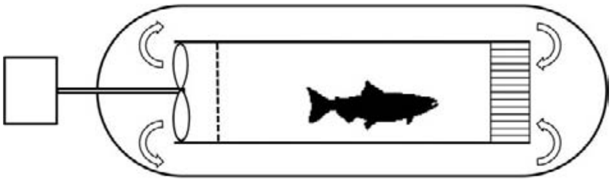


Figure 4 Blazka-type, swimming closed respirometer is an acrylic tube within a larger acrylic tube. A variable-speed motor (rectangle) turns a propeller, drawing water past the fish. The end caps recirculate the water (depicted by the arrows) between the outer and inner tubes, through the flow straighteners (upstream of the fish) and the screen (depicted by the dashed line downstream of the fish). Not shown are the hatches or removable end caps to insert and remove the fish, or the sampling tubes for dissolved-oxygen measurements. Redrawn by R. Coulter from Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

Gill Ventilation

Gill ventilation is accomplished in two ways: water is actively pumped by the buccal–opercular apparatus, or it is passively forced over the gills as fish swim, called ram ventilation. Ram ventilation requires only that a fish maintains a buccal–opercular gape while it swims at or above a threshold velocity, which is thought to conserve energy. Active pumping of gill ventilation water is commonly measured in a respirometer that directs ventilatory water flows into a container or past a flow probe. Respirometers designed for water collection usually incorporate a thin rubber membrane or mask fitted to the fish's head to separate inspired from expired water for measurements of ventilation volume. The fish is situated in a two-chamber (van Dam-type) respirometer with the membrane sealing the barrier between the two chambers except for the fish's head (**Figure 5**). The water levels in both chambers are set to the same height by adjustment of surface drains so that hydrostatic pressure does not aid or inhibit gill ventilation. Water continuously flows into the anterior chamber (any excess flows out through the surface drain) and is moved from the anterior to the posterior chamber only by the fish's active ventilation. As the fish ventilates its gills, water equal to the ventilation volume is displaced from the posterior chamber via the posterior surface drain. This water is collected for a time as a measure of ventilation volume. Ventilatory movements of the buccal or opercular apparatus can be counted visually or detected with immersed electrodes sutured to the external opercula and connected to an amplifier and computer. Measured simultaneously over a specified time period, the frequency of ventilation (F_G, movements min^{-1}) and the volumetric rate of ventilation ($\dot{V}_G$, mL min^{-1}) allow calculation of ventilatory stroke volume (V_S, mL/movement: $V_S = \dot{V}_G/F_G$). Simultaneous measurements of oxygen concentrations in inspired and expired water, sampled near the mouth and gills, permit estimation of the oxygen consumption rate by the Fick equation:

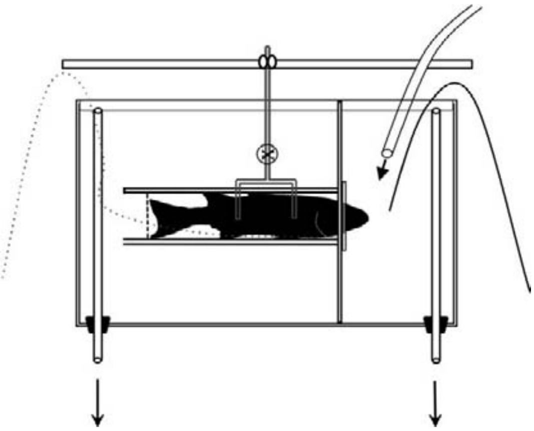


Figure 5 Two-chamber (van Dam-type) respirometer is a clear box (e.g., aquarium), incorporating a clear wall separating the two chambers. The fish is suspended in an acrylic box, so that its head partially protrudes through a hole in a light-gauge latex sheet, which is stretched over a large hole in the separating wall. Thus, the fish acts as a pump to move water from the anterior to the posterior chamber. A constant flow in inflowing water (top, right) enters the anterior chamber, and water not pumped by the fish escapes through the anterior chamber's surface drain (bottom, right). The water displaced by the fish's ventilatory pumping escapes through the posterior chamber's surface drain (bottom left). This flow is collected for a fixed time for the ventilation volume measurement. Note that the two surface drains are adjusted so that the water depths in the anterior and posterior chambers are equal. The solid and dashed lines represent water-sampling tubes for dissolved-oxygen measurements of inspired and expired water, respectively. Redrawn by R. Coulter from Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

$$\dot{M}_{O_2} = \dot{V}_G(C_{O_2}(I) - C_{O_2}(E)) \tag{7}$$

where $\dot{M}O_2$ is the O_2 consumption rate (mg O_2 min^{-1}); $\dot{V}_G$ is the volumetric ventilation rate (mL water min^{-1}); $C_{O_2}(I)$ is the O_2 concentration in inspired water (mg O_2 mL^{-1} water); and $C_{O_2}(E)$ is the O_2 concentration in expired water (mg O_2 mL^{-1} water).

Because these oxygen consumption rates are calculated from oxygen concentrations immediately adjacent to the mouth and gills, they may underestimate total respiration because cutaneous gas exchange can represent an important proportion of total oxygen uptake, even for scaled fishes.

Percentage utilization (%U) of oxygen taken up at the gills can be calculated from the same data. Either oxygen tensions or concentrations can be used in this measure of the gills' oxygen extraction efficiency:

$$\begin{aligned}\%U &= (P_{O_2}(I) - P_{O_2}(E))/P_{O_2}(I) \times 100 \\ &= (C_{O_2}(I) - C_{O_2}(E))/C_{O_2}(I) \times 100\end{aligned} \tag{8}$$

where $P_{O_2}(I)$ is the O_2 tension of the inspired water (mmHg) and $P_{O_2}(E)$ is the O_2 tension of the expired water (mmHg).

The energetic cost of gill ventilation has been determined by comparison of oxygen consumption rates at velocities below and above the transition from active ventilation to passive ram ventilation on sharksuckers, which are similar to remoras, attached to the inside of a tunnel respirometer by their dorsal cephalic suction disks. (*see* **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions).

See also: **Energetics**: General Energy Metabolism; Physiological Functions that Scale to Body Mass in Fish. **Ventilation and Animal Respiration**: Respiratory Gas

Exchange During Development: Models and Mechanisms; Efficiency of Gas Exchange Organs.

Further Reading

Bridges CR and Butler PJ (1989) *Techniques in Comparative Respiratory Physiology: An Experimental Approach*, *Society for Experimental Biology Seminar Series,* p. 351. Cambridge: Cambridge University Press.

Cech JJ (1990) Respirometry. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–363. Bethesda, MD: American Fisheries Society.

Gehrke PC, Fidler LE, MenseDC, and Randall DJ (1990) A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. *Fish Physiology and Biochemistry* 8(1): 61–67.

Steffensen JF (1989) Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Journal of Fish Physiology and Biochemistry* 6: 49–69.

GAS EXCHANGE

Control of Respiration

Contents

Generation of the Respiratory Rhythm in Fish
The Ventilatory Response to CO_2/H^+
Oxygen Sensing in Fish

Generation of the Respiratory Rhythm in Fish

EW Taylor, University of Birmingham, Birmingham, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Central Respiratory Rhythm Generator
Influences from the Midbrain
Mechanoreceptors
Episodic Breathing
Recruitment of Feeding Muscles
Air-Breathing Fishes
Summary
Further Reading

Glossary

Afferent Activity conducted toward the brain, normally sensory. Incoming, e.g., in the case of nucleus A projecting to nucleus B, the neurons in nucleus B would receive an afferent input from nucleus A.

Brainstem A variably defined term that includes the brain regions where most cranial nerves have their origin and/or termination, i.e., the hindbrain and midbrain. Alternatively, the base of the brain extending forward from the spinal cord and including the medulla oblongata and midbrain.

Caudal Toward the tail.

Cell body The major part of a neuron including the nucleus but excluding processes such as axons.

Chemoreceptors Type of cell that is specialized to detect chemical stimuli and convert them into signals within the nervous system.

Cranial nerves Nerves leaving the brain.

Discharge activity Measured electrical activity in nerve fibers.

Efferent Outgoing, e.g., in the case of nucleus A projecting to nucleus B, the axons that arise from neuron cell bodies in nucleus A would be efferent projections of that nucleus. Note that the adjectives efferent and afferent can apply to the same set of axons; the terms are used in reference to the nucleus from which they arise vs. the nucleus to which they project, respectively.

Electromyogram (emg) Measured electrical activity generated during muscle contractions.

Entrain To cause rhythmically active systems to assume an imposed rhythm.

Episodic breathing Bouts of active gill ventilation separated by periods of cessation of ventilation, characteristic of inactive fish in well-oxygenated water.

Mechanoreceptors Sense organs that respond to movement or change in position.

Medulla oblongata Hindmost portion of the brainstem that contains the vagal motor and sensory areas, the respiratory rhythm generator, and cardiovascular control center.

Motor Causing muscles to contract.

Motor nuclei Groups of motor neurons supplying efferent axons to specific motor nerves that innervate particular muscles.

Nucleus of the solitary tract The part of the brain that is a relay center for afferent information providing reflex control of the respiratory and cardiovascular systems.

Phasically active Activity that varies continuously, often in phase with rhythmically active systems such as respiration.

Ram ventilation Ventilation of the gills by swimming forward with the mouth open while suspending activity in the respiratory muscles.

Rostral Toward the head.

Sensory Responding to or relaying information from stimulated sense organs.

Sensory projections Afferent nerves relaying information to specific parts of the brain.

Tonically active Unvaryingly active over long periods.

Transected Cut through or divided, usually referring to surgical intervention.

Introduction

Water contains less oxygen per unit volume than air but is considerably more dense and viscous. Consequently, compared with air breathers, fish have to work harder to move water to obtain the same amount of oxygen and they normally exhibit continuous rhythmical breathing movements. However, the work done to move water is mitigated by using a unidirectional flow to ventilate their gills: in at the mouth and out over the gills (**see also Gas Exchange**: Respiration: An Introduction). This means that the kinetic energy imparted to the water by the respiratory muscles is maintained in one direction, unlike the tidal ventilation of a lung when the direction has to be reversed.

To ensure this rhythmic, unidirectional water flow, gill ventilation requires a complex and coordinated interaction of two muscle pumps: the buccal pump in all fishes and the opercular pump in bony fishes (teleosts) or the analogous septal pump in cartilaginous fishes (elasmobranchs). The synchronized activity of these pumps needs to be controlled and coordinated by the brain. This article is about how the brain generates and coordinates the rhythmic activity of the muscles involved with these pumps.

The muscles used for gill ventilation are inserted around the jaws, the opercular flaps in bony fishes (teleosts) and pharyngeal skeleton supporting the branchial (gill) arches. They are innervated by a dorsal group of cranial nerves exiting from the brainstem. This series of nerves contains sensory fibers and in most cases visceral motor components. The arrangement of these nerves in the dogfish, *Scyliorhinus canicula*, an elasmobranch, is schematized in **Figure 1**.

There are four cranial nerves innervating muscles involved in normal gill ventilation. The Vth cranial nerve, the trigeminal, provides the major innervation to the mouth region of all vertebrates, including the maxillary branch to the upper jaw and mandibular branch to the lower jaw, responsible for motor control of the jaw-closing muscles. The VIIth cranial nerve, the facial, provides the hyomandibular branch to the branchial muscles in the hyoid arch that, in teleosts, includes the opercular muscles; in elasmobranchs, the facial nerve innervates the spiracle, an inhalant opening in front of the gill slits. Together, the IXth cranial nerve, the glossopharyngeal, and the Xth cranial nerve, the vagus, are called the branchial nerves. They innervate respiratory muscles within

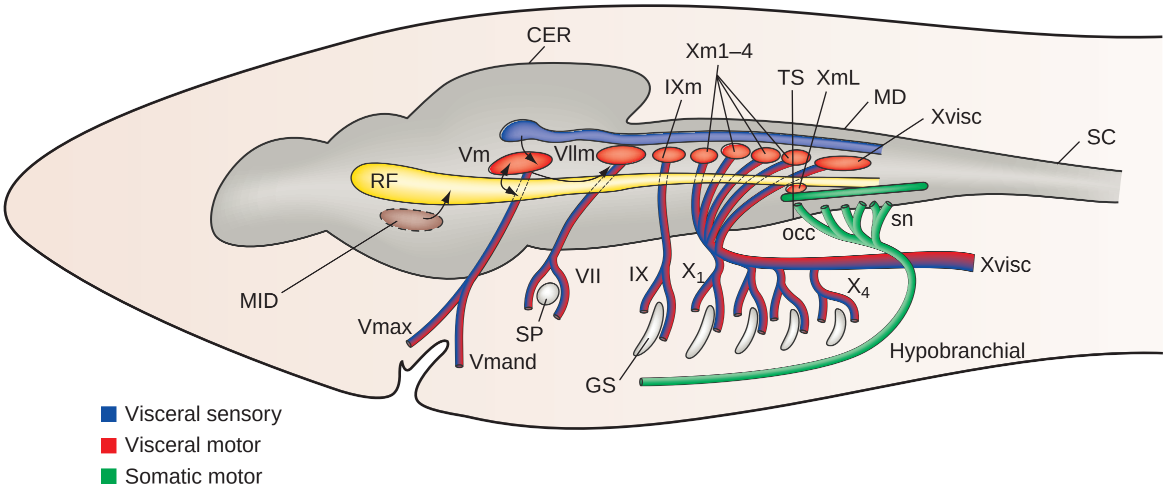


Figure 1 Schematic diagram of the left side of a dogfish to show details of the cranial nerves innervating the respiratory system, together with the location of their motor and some of their sensory nuclei in the brainstem. The nerves and nuclei are color coded and are listed from the most rostral to the more caudal: Red are motor nuclei: Vm, trigeminal nucleus supplying Vmand, mandibular branch of Vth cranial nerve; Vmax, maxillary branch of V; VIIm, facial nucleus supplying VII facial nerve; IXm, glossopharyngeal nucleus supplying IX; Xm1-4, vagal motor nuclei supplying branches of X1-4; Xvisc is the visceral vagus. Blue are sensory nuclei supplying V, VII, XI, and X. Yellow is the reticular formation (RF). Brown represents midbrain nuclei (MID) including the tegmentum. Green is occipital (occ) and anterior spinal (sn) nerves supplying the hypobranchial nerve. TS marks the line indicating the TS shown in **Figure 2**. Arrows indicate possible interactions; details are given in the text. CER, cerebellum; GS, gill slit; MO, medulla oblongata; SC, spinal cord; SP, spiracle.

the gill arches that are important in maintaining the integrity of the gill arches and the curtain of gill lamellae. They also innervate mechanoreceptors and chemoreceptors on the gills that are important in ventilatory control. All of these nerves have their efferent cell bodies and afferent sensory projections located dorso-medially in the brainstem (**Figures 1–3**).

The Central Respiratory Rhythm Generator

The respiratory rhythm driving ventilation is generated in the central nervous system (CNS). It is a feed-forward command signal related to the goal of providing adequate oxygen to fuel the animal's rate of aerobic metabolism (**see also Gas Exchange**: Respiration: An Introduction). Generation of a respiratory rhythm has a lineage as ancient as the evolution of multicellular animals. Evidence suggests that the central nervous mechanisms generating the respiratory rhythm are highly conserved from fish to mammals. Furthermore, the location of the respiratory rhythm generator (RRG) in all vertebrates is concentrated in the brainstem, and specifically the medulla oblongata. If the brainstem is transected to isolate the medulla oblongata, rhythmic ventilatory movements continue in fish and these can resemble the patterns in intact animals, suggesting that the RRG is contained solely in the medulla.

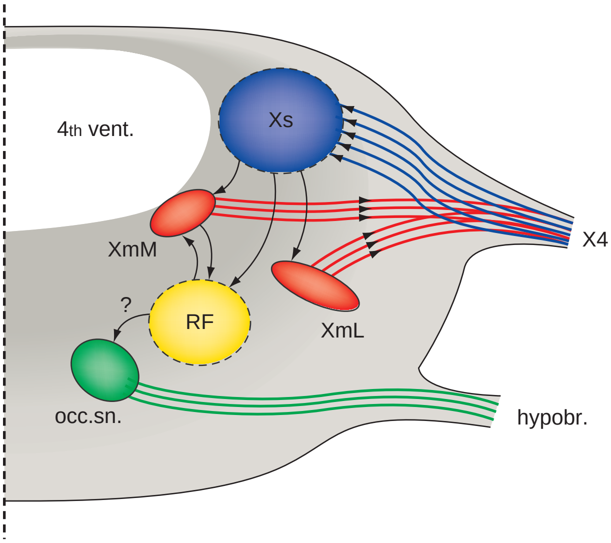


Figure 2 Schematic diagram of TS through brainstem of dogfish at point labeled on **Figure 1** to show spatial and possible functional relationships between nuclei supplying respiratory nerves. Color code and labels as in **Figure 1**. Additional labels: 4th vent. 4th ventricle; XmL, lateral vagal preganglionic neurons; XmM, preganglonic neurons in the dorsal vagal motor nucleus; Xs, dorsal sensory nucleus of vagus; X4, 4th branchial branch of vagus. Arrows indicate possible functional connections; details are given in text.

However, despite years of detailed investigation, the nature of respiratory rhythm generation in all vertebrates, including mammals, remains in dispute regarding the two mechanistic models: pacemaker neurons showing spontaneous rhythmic oscillations or neural networks relying on synaptic interactions. These two mechanisms have been separated functionally by bathing preparations in chloride-free saline. This blocks the synaptic inhibition, intrinsic to neural networks, but is without effect on the spontaneous activity generated by pacemaker neurons.

Various techniques have localized and characterized the RRG in fish. Although each technique has certain limitations, some common features have emerged. Local destruction of neurons in the hindbrain of the tench, *Tinca tinca*, and recordings of respiration-related electrical discharge activity from neural cells in the brainstem of several fishes suggest that an extended, diffusely organized neuronal network within the medulla generates the respiratory rhythm. Recordings of electrical activity in this region and injection of a marking substance to precisely locate where the electrode was recording from, identified a longitudinal strip of neurons with spontaneous, respiration-related bursting activity. These neurons extend dorsolaterally throughout the whole medulla and comprise elements of the Vth, VIIth, IXth, and Xth motor nuclei that drive the respiratory muscles, together with the descending trigeminal nucleus and the diffuse reticular formation (RF). All the motor nuclei are interconnected, and each receives an afferent projection from the descending trigeminal nucleus and has efferent and afferent projections to and from the RF (**Figure 1**). Finally, areas in the midbrain, such as the tegmentum, have efferent and afferent connections with the RF (**Figure 1**). The respiratory rhythm apparently originates in a diffuse respiratory pattern generator in the RF. This remains functional under anesthesia, providing circumstantial evidence that it can work independently of external inputs from the periphery or elsewhere in the CNS, although maintenance of a respiratory rhythm in intact animals generally relies on an element of respiratory drive from peripheral chemoreceptors. There is some evidence that the size of the population of respiratory neurons varies with conditions so that more cells may be active during periods of respiratory distress, such as in hypoxia.

These studies also revealed differences among fishes. Neural tracers injected into branches of the cranial nerves innervating respiratory muscles are transported into the brainstem and accumulate in the cell bodies of neurons providing axons to the injected nerve (**Figures 3(a)–3(c)**). In the brainstem of the dogfish, such studies show that the neurons providing axons to the respiratory branches of each of the cranial nerves have motor nuclei distributed in a sequential series (**Figure 1**). In contrast, in pacu, *Piaractus mesopotamicus*, a teleost, the Vth nerve is located in a separate, more rostral position, while the cell bodies of the cranial nerves V, VII, IX, and X have an

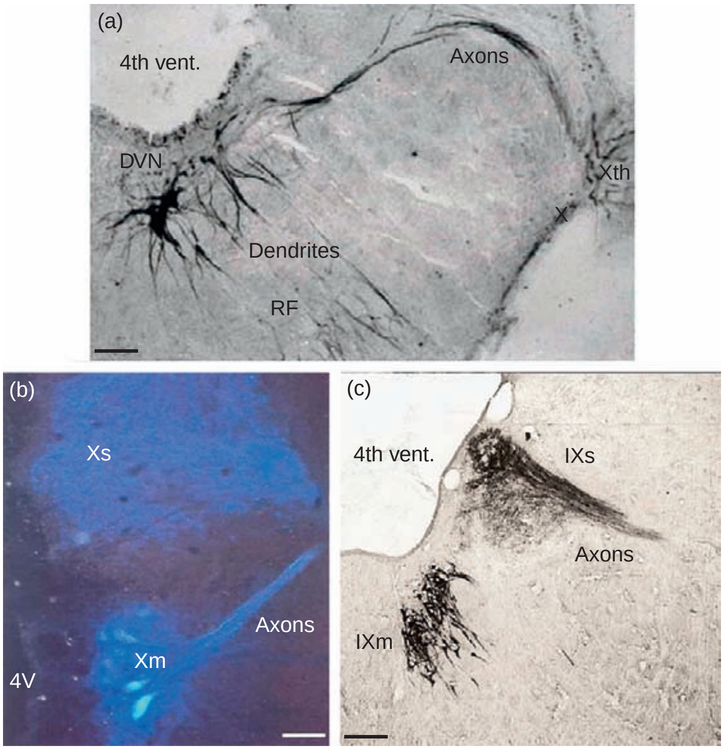


Figure 3 (a) TS of dogfish brainstem 3 mm rostral of obex following injection of cobalt chloride into the right vagus nerve. The darkly stained structures close to the 4th ventricle (4th vent.) are neurons with their cell bodies in the dorsal vagal motor nucleus (DVN), having axons that project out to the X cranial nerve (Xth) and dendrites that project ventrolaterally into an area occupied by the reticular formation (RF). (b) TS of sculpin brainstem to show motoneurons (Xm) in the dorsal vagal motor nucleus close to the 4th ventricle (4V) with axons projecting to the vagus nerve and the projection of the vagus nerve into the diffuse dorsal sensory area (Xs) revealed by injection of the fluorescent neural tracer Fast Blue into a vagal ganglion. (c) TS of dogfish brainstem 4 mm rostral of obex following injection of horse radish peroxidase into the right glossopharyngeal nerve. The darkly stained structures located ventrally, close to the 4th ventricle (4th vent.) are motor neurons (IXm) having dendrites that project ventro-laterally into an area occupied by the reticular formation. The more diffuse area located dorsally is the dorsal sensory area (IXs) with axons projecting to the IXth cranial nerve. Scale bars are 50 μm for (a) and (b) and 25 μm for (c).

overlapping distribution in the caudal brainstem. Simultaneous recordings of neuronal activity from the medulla oblongata and an electromyogram (emg) from the respiratory muscles of carp showed that adjacent neurons have firing patterns related to different muscle sets, suggesting lack of a strict spatial topography. In contrast, recordings of efferent activity from the central cut ends of the nerves innervating the respiratory muscles of the dogfish and the pacu revealed that the Vth cranial nerve fires in advance of the VIIth, IXth, and Xth cranial nerves, with some evidence of sequential firing of this latter series in the dogfish and ray (**Figure 4**). The resultant coordinated contractions of the appropriate respiratory muscles may relate to their original segmental arrangement before the evolution of the brain, head, and jaws with their cranial musculature, each with its separate neural oscillator, an arrangement that is seen during development and apparently retained in the hindbrain of the dogfish in the sequential topographical arrangement of the motor nuclei, including the subdivisions of the vagal motonucleus (**Figure 1**). Based on developmental studies of the role of neural crest cells, a separate origin for the jaws as feeding structures, independent of the visceral arches, has been suggested and this view is supported by a study of marker genes. A possible evolutionary antecedent of the jaws may be the velum of filter-feeding protochordates that provides the major water flow in larval cyclostomes.

Our partial knowledge of the nature and topography of the RRG in fish has been traced back, in evolutionary terms, as far as the jawless cyclostomes. The lamprey, *Petromyzon* sp., has a larval form, the ammocete, which may in its ontogeny recapitulate vertebrate phylogeny because it has a muscular velum that pumps water and uses the pharyngeal clefts to filter-feed. The feeding current flowing unidirectionally through the clefts is generated in part by their ciliated epithelia but there are also muscular pumps associated with the velum and anterior branchial pouches that act against the elastic recoil of the branchial basket. The jawless adult has an oral sucker with which it attaches to fish, such as the salmon. Consequently, its mouth is closed off and it breathes via seven pairs of branchial pouches that are ventilated bidirectionally (i.e., it is a tidal breather). Ventilation is again

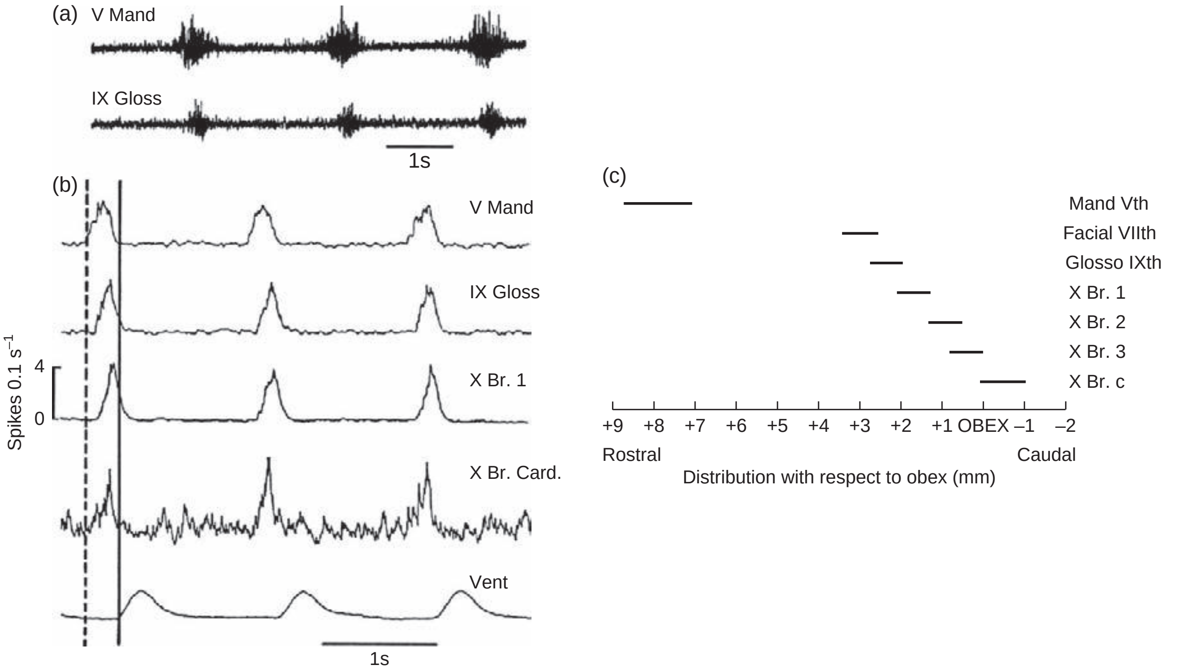


Figure 4 Spontaneous activity in cranial nerves supplying respiratory muscles and the heart in a decerebrate dogfish plus the rostro-caudal distribution of motor nuclei supplying these nerves. (a) Spontaneous bursts of activity recorded simultaneously from the Vth and IXth nerves. (b) Integrated activity recorded from the Vth, IXth, and branches of the Xth supplying a gill slit (X Br 1) and the heart (X Br card), plus movements of a gill septum (Vent). C, rostro-caudal distribution of motor neurons supplying respiratory branches of the Vth, VII, IX, and X plus the cardiac branch of X (XBr.c).

by contraction of the muscles around the branchial basket forcing water out through the pouches, with elastic recoil drawing water back into the pouches. Generation of its respiratory rhythm has provided a model for what may be the ancestral form of the vertebrate RRG.

Spontaneous bursts of respiration-related activity have been recorded from the isolated brainstem of the lamprey. As in teleost and elasmobranch fishes, periodic bursts of activity recorded from motor nuclei supplying the Vth cranial nerve, located in the rostral half of the medulla, precede those recorded from respiratory motor nuclei in the caudal half of the medulla, innervating the VIIth, IXth, and Xth cranial nerves. A brain–gill–velum preparation of the ammocete larva showed that velar pumping was driven by an RRG in the trigeminal region with descending pathways driving the branchial motoneurons; this hierarchy is retained in the adult even though the trigeminal motoneurons are no longer active as the velum ceases to act as a pump. Activity continues in the trigeminal nucleus when it is isolated from the rest of the brain, suggesting that rhythm generation involves neurons located within this region. Recent evidence suggests that rhythm generation in these rostral neurons fits a group pacemaker model as their activity continues in an isolated brainstem, bathed with chloride-free saline. Electrical stimulation of this area excites the other respiratory motoneurons directly and could entrain the respiratory rhythm. These observations suggest that the motor pattern for respiration is at least partly generated and coordinated in the rostral half of the medulla in the lamprey, possibly in the trigeminal nucleus, and is transmitted to respiratory motoneurons through descending pathways. However, the caudal region of the brainstem, including the motor nuclei of VII, IX, and X, is capable of generating rhythmic activity following transection at the level of the Vth nucleus, suggesting a separate rhythm generator possibly responsible for strong contraction of the muscles of the gill pouches or coughs used to clear the branchial basket of obstruction. Midline transaction of the rostral medulla disrupts the normal respiratory rhythm while leaving the coughs unaffected (**Figure 5**). Although the true nature of the RRG remains unresolved, the mechanisms uncovered in the cyclostomes seem to be somewhat similar to those retained in jawed fishes, implying that they utilize a mechanism that may be an ancestral link back to velar ventilation of a feeding apparatus.

Influences from the Midbrain

Although the RRG clearly resides in the medulla oblongata, several investigators have reported modulation of the respiratory rhythm following lesions or stimulation of the midbrain. Therefore, areas outside of the medulla can influence the respiratory rhythm. Two groups of neurons

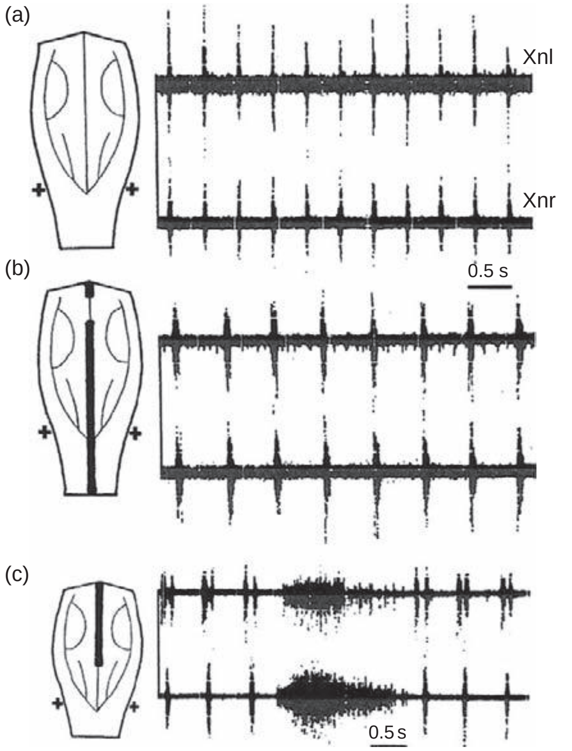


Figure 5 Effects of brain lesions on coordination of respiratory bursts in the isolated brainstem of the lamprey. (a) Synchronized respiratory bursts in left and right vagal roots prior to midline section. (b) Synchronization unaffected by sectioning of all but 0.3 mm of rostral midline. (C) Loss of synchronization after section of the rostral midline with the caudal intact. The cough is still concurrent on both sides. Redrawn from figures 7A, 7B, and 8 in Rovainen CM (1985) Respiratory bursts at the midline of the rostral medulla of the lamprey. *Journal of Comparative Physiology A* 157: 303–309.

that influence respiration have been described in the carp. Neural tracing has revealed type A neurons that have direct connections with the RF. When these neurons are electrically stimulated at frequencies close to the intrinsic respiratory rhythm, the generator is entrained to the imposed rhythm. However, these neurons are not directly involved in sustaining the normal rhythmic ventilation pattern. Activity in these neurons also anticipates the onset of a bout of ventilation during intermittent breathing (see below). A second group, called 'type B neurons', directly influences motor output to the respiratory muscles. Electrical stimulation of these neurons elicits activity in the respiratory muscles and they may act to adjust the balance of activity between the different cranial muscles during gill ventilation. Neural tracing revealed two routes from type B neurons to respiratory motor centers, a direct route and an indirect route via the RF. Electrical stimulation revealed that the either route could induce a rapid twitch response in the respiratory muscles, while the indirect route also has a long latency response that is likely to be integrative. These relationships are summarized in **Figure 6**.

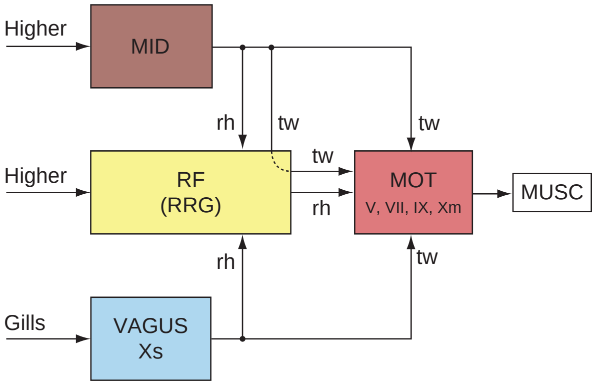


Figure 6 Possible connections in the brainstem controlling respiratory activity in fish. Relationships are described in the text. Color coding and interconnections correspond to **Figures 1** and **2**. MID, midbrain; MOT, peripheral motor control system; MUSC, respiratory muscles; rh, rhythmic influence; RF, reticular formation; RRG, respiratory rhythm generator; tw, twitch response; VAGUS Xs, vagal sensory nucleus receiving afferent input from gill mechanoreceptors. Redrawn from figure 1.5 (1) in Ballantijn CM (1987) Evolution of central nervous control of ventilation in vertebrates. In: Taylor EW (ed.) *Neurobiology of the Cardiorespiratory System*, pp 3–27. Manchester: Manchester University Press.

Mechanoreceptors

The respiratory muscles in fish contain length and tension receptors, in common with other vertebrate muscles, and the gill arches bear a number of mechanoreceptors with various functional characteristics. By recording activity in nerves supplying the gill arches, slow-adapting position receptors on the gill arches and phasic movement receptors on the gill filaments and rakers have been described in the carp. These may function to protect the gill sieve from clogging or damaging material as strong mechanical stimulation of the gill arches is known to elicit forced expulsion of water and suspended material from the mouth in the so-called cough reflex. These mechanoreceptors should reinforce the respiratory rhythm because they will be stimulated by normal ventilatory movements of the gill arches and filaments. Indeed, when gill arches of a lightly anesthetized fish were artificially moved, the respiratory rhythm was regularly reset by the imposed movements in a manner related to the phase of the respiratory cycle at which the movement was imposed, with 1:1 entrainment achieved when the imposed rhythm was close to the natural rhythm. Thus, phasic mechanoreceptor activity likely serves to stabilize the generation of the respiratory rhythm, preventing the central generating circuits from being disrupted by other inputs.

Central stimulation (toward the brain) of nerves innervating respiratory muscles in the carp with short trains of electrical stimuli also entrained the respiratory rhythm to the imposed stimuli (**Figure 7**). Central stimulation of

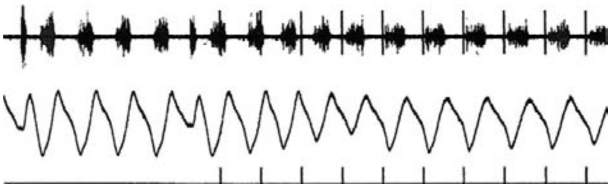


Figure 7 Recruitment of respiratory rhythm by central electrical stimulation of the vagus in the carp. Traces are in descending order: EMG of levator hyomandibulae (identified for the dogfish in **Figure 10**); ventilatory movement of an opercular bone (opening upwards); stimulus marker. Stimuli given at 900 ms led to 1:1 entrainment after the 5th stimulus. Redrawn from figure 7B in De Graaf PJF and Roberts BL (1991) Effects of sensory input on the breathing rhythm of the carp. *Journal of Experimental Biology* 155: 77–91.

branchial branches of the vagus in the dogfish with bursts of electrical pulses entrained the efferent activity in neighboring branchial and cardiac branches. In addition to stimulating ventilatory activity, increased rates of water flow can also inhibit active ventilation. In fast-swimming fishes, such as tuna and some sharks, stimulation of branchial mechanoreceptors by elevated rates of water flow may trigger cessation of active ventilatory movements during ram ventilation, when the mouth is held open to allow a continuous stream of water to ventilate the gills and powered by the locomotory rather than the branchial muscles. This confers the dual advantages of leaving the effort of ventilation to the powerful locomotory muscles and reducing drag.

The branchial branches of the IXth and Xth cranial nerves innervate a range of tonically and phasically active mechanoreceptors as well as chemoreceptors on the gill arches of fish and project directly to a dorsal sensory nucleus lying above the equivalent motor nuclei in the medulla (see **Figures 1**, **2**, and **3**(**b**) and **3**(**c**)). The sensory area, in turn, projects centrally to the respiratory motor nuclei (**Figure 2**). However, sensory fibers from branchial receptors may terminate in different locations within the brainstem and consequently have different affects on integration. Some vagal afferent fibers seem to project to vagal motoneurons innervating branchial muscles by short loops, either directly or via the RF and may be involved in the reflex contraction of adductor muscles on the gill filaments in response to mechanical stimulation of the gill filaments or gill rakers (see **Figure 2**). Stronger stimulation may induce the coughing reflex with simultaneous contraction of respiratory pump muscles that receive inputs from the RF.

Vagal afferent fibers also connect with the trigeminal complex that receives inputs from proprioceptors in the respiratory pump muscles innervated by the trigeminal Vth cranial nerve. As proprioceptive reflexes are involved in entrainment and stabilization of the respiratory rhythm, their inputs must be connected fairly directly with the rhythm-generating neurons. Some recent work indicates that chemoreceptor stimulation, relayed via the vagus, only affects ventilation when the midbrain is intact, implying that the dorsal sensory areas project to the midbrain that then influences the RRG. However, microinjection of glutamic acid into identified areas of the vagal sensory projection in sculpin, identified by injection of a fluorescent tracer (see **Figure 3**(**b**)), elicited specific, highly localized responses, including changes in ventilation frequency and amplitude (**Figure 8**). Glutamate has been identified as the neurotransmitter for afferents into the nucleus tractus solitarius (NTS) in mammals.

Episodic Breathing

Inactive fish often interrupt their normal regular rhythm of gill ventilation in normoxic or hyperoxic waters and

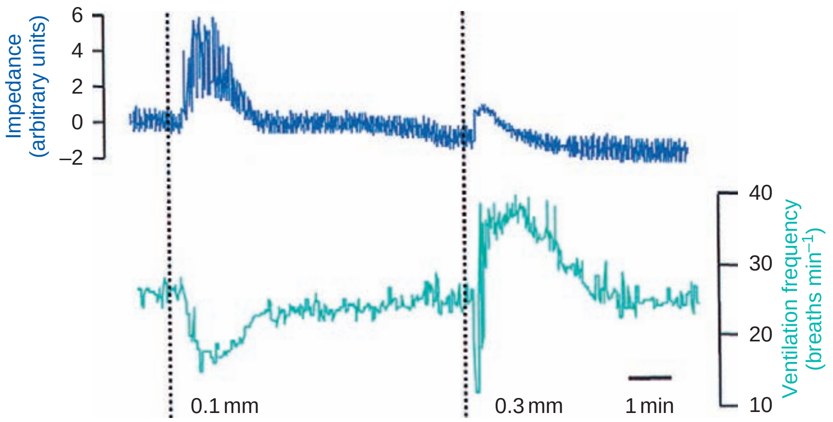


Figure 8 Microinjection of glutamate into the brainstem of the sculpin at two depths 0.2 mm apart, on a single trajectory, elicits very different respiratory responses. Impedence is a measure of ventilatory movements. Redrawn from figure 6 in Sundin L, Turesson J, and Taylor EW (2003) Evidence for glutamatergic mechanisms in the vagal sensory pathway initiating cardiorespiratory reflexes in the shorthorn sculpin *Myoxocephalus scorpius*. *Journal of Experimental Biology* 206: 867–876.

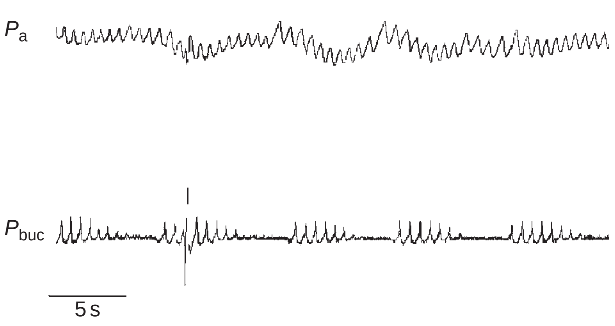


Figure 9 Episodic breathing pattern in undisturbed, normoxic pacu. Heart rate rises during each bout of gill ventilation. Previously unpublished data from C. Leite. P_a, arterial blood pressure; P_{buc}, pressure in the buccal cavity.

exhibit episodic (or intermittent) breathing patterns (see **Figure 9**). As muscles work optimally during bouts of moderate activity, compared to continuous low levels of activity, this may save energy.

Type-A neurons located in the midbrain of carp appear to play a key role in the control of episodic breathing. They typically fire just before the onset of a breathing bout during intermittent ventilation. In addition, during a ventilatory pause, stimulation of this area of the midbrain brings forward the onset of the next breathing bout. However, something other than the absence of activity in these neurons seems to be involved in the cessation of episodes of breathing. This is likely to be the stimulation of an internal receptor by the rise in blood-oxygen level above a threshold for cessation of breathing; however, this remains to be established. Central electrical stimulation of the vagus nerve also initiates ventilation in carp breathing intermittently, which is likely to be a response to stimulation of fibers innervating gill chemoreceptors.

Recruitment of Feeding Muscles

During routine aquatic ventilation in fish, expansion of the oro-branchial cavity relies largely on elastic recoil in the muscles and branchial skeleton (i.e., on the tendency of the branchial skeleton to resume its former shape following its distortion by contraction of the respiratory muscles), particularly in cartilaginous fish such as sharks and in the cyclostomes. However, during the vigorous, forced ventilation following exercise or deep hypoxia, both elasmobranch and teleost fishes recruit additional muscles into the respiratory cycle. These are normally used for active expansion of the oro-branchial cavity to ingest food but can also enhance inspiration of water into the buccal cavity during the mouth-opening phase. These muscles form a complex ventral sheet, inserted between the pectoral girdle, the lower jaw, and the ventral processes of the hyoid and branchial skeleton (**Figure 10**). They are innervated by the hypobranchial nerve, which contains elements of the occipital nerves and the anterior spinal nerves (**Figures 1** and **2**). The hypobranchial nerve in fish is the morphological equivalent of the hypoglossal nerve, which innervates the muscles of the tongue in

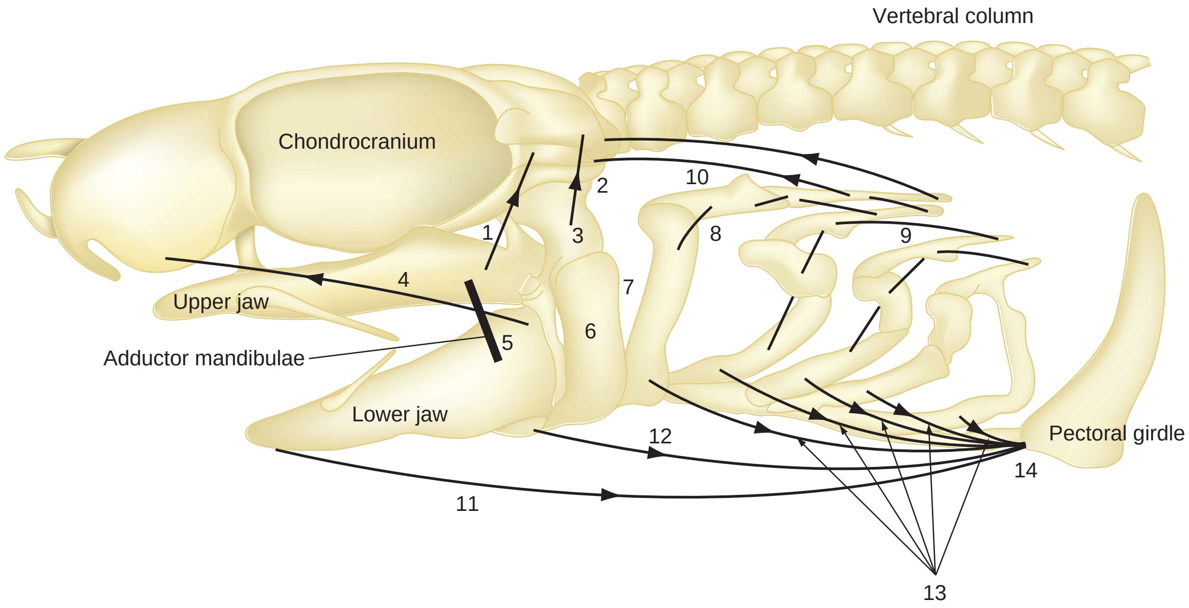


Figure 10 Diagrammatic illustration of the dogfish cartilaginous skull and branchial skeleton to show the insertion of muscles. Respiratory muscles (1–9), are inserted around the jaws and gill arches and are innervated by cranial nerves V, VII, IX, and X. The adductor mandibulae is the main jaw closing muscle. Feeding muscles (11–14) are inserted onto the pectoral girdle and innervated by the hypobranchial nerve complex (occipitals and anterior spinals) They are used to actively expand the branchial basket during forced ventilation.

reptiles, birds, and mammals. These tongue muscles are used for suckling by infant mammals, an activity likely to require its own central oscillator.

Intravenous injection of noradrenaline, which simulates the response of fish to deep hypoxia or intense disturbance, caused a previously silent hypobranchial nerve to fire respiration-related activity. The mechanisms involved in recruitment of hypobranchial motoneurons into the respiratory rhythm have not been studied but the motoneurons supplying the occipital nerves have an overlapping distribution with vagal motoneurons supplying respiratory muscles and with the caudal extension of the RF (see **Figures 1–2**). In the dogfish and ray, rhythmic opening and closing of the mouth occur during ingestion of food, implying the central generation of a feeding rhythm. The respiratory and feeding rhythms in fish are thought to be generated by separate groups of interneurons. It is now well established that in mammals the masticatory and suckling rhythms are generated in the hindbrain, in the RF. The pre-Bötzinger nucleus that forms part of the RF is now considered to be the primary site for respiratory rhythmogenesis in neonatal mammals. As was detailed above, it is the RF that is thought to contain the RRG in fish, indicating the early evolution of a functional role in the generation of rhythmical contractions for this discrete area in the brainstem. Its subsequent retention in the tetrapods was associated with some adaptive changes in its role, possibly associated with the evolution of lung breathing and the need to control contractions of thoracic as well as cranial muscles.

Air-Breathing Fishes

Air-breathing fishes have evolved a variety of air-breathing organs (ABOs) for obtaining oxygen from above the water surface but retain gills, ventilated by cranial muscles, for the uptake of a variable proportion of their oxygen requirements, dependent on species and conditions, and for excretion of most of their carbon dioxide (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). Many facultative air breathers, such as the tarpon (*Megalops cyprinoides*), rely on gill ventilation and restrict blood flow to the ABO in normoxic water but increase rates of air breathing and perfusion of the ABO with hypoxic exposure and particularly during exercise. During these periods, opercular beating becomes imperceptible, indicating cessation of effective gill breathing or a switch to ram ventilation while swimming. Access to air reduces the lactic acid load during burst swimming and prolongs aerobic exercise in tarpon; however, they are able to repay an accumulated oxygen debt during recovery by increased rates of gill ventilation.

In all air-breathing fish, gulping of air at the water surface is achieved through the action of the same muscles as used for feeding or for forced ventilation in water-breathing fishes. These are elements of the jaw musculature, innervated by cranial nerve V, together with the hypobranchial musculature, innervated by occipital and anterior spinal nerves. They function together in a coordinated action either for feeding or for gulping air, actions that are independent of the visceral arches and may derive from their separate evolutionary origins as feeding muscles. In the primitive ray-finned (actinopterygian) fish, the bowfin (*Amia calva*), which utilizes a well-vascularized swimbladder as an ABO, there appear to be two types of air breath, one that involves exhalation followed by inhalation (designated type I air breaths) and one that simply involves inhalation (type II air breaths). Type I breaths are possibly respiratory in nature, whereas type II breaths have a buoyancy-regulating function. Spectral analysis indicates an inherent rhythmicity to type I (i.e., respiratory-related) air breathing, both in normoxia and hypoxia that may be driven by the changes in blood O_2 status during the interbreath interval, rather than by an RRG for air breathing. Control of the switch between ventilation of the gills and the ABO is likely to relate to stimulation of chemoreceptors by reduced oxygen levels at the gills or in the ABO (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). However, the central sites responsible for control of air-breathing reflexes in fish are still unknown.

Reorganization of the CNS associated with the evolution of air breathing has been poorly studied in fish. Actinopterygian air-breathing fishes probably have an RRG for gill ventilation located in the RF of the hindbrain similar to that of water-breathing fish. In the bowfin, catecholamine infusion stimulates gill ventilation, apparently via a central mechanism, but has no effect on air breathing in normoxia or hypoxia, indicating that central sites controlling gill ventilation and air breathing are pharmacologically and possibly spatially different. However, the sequence of events associated with air breathing in the bowfin suggests that the action of air breathing would require little change in the pattern of neural control required for suction feeding and/or coughing, with the exception of control over opening of the glottis at the entrance to the ABO. Neural tracers revealed that the ABO in the bowfin is innervated by vagal motor neurons that may supply the glottis or smooth muscle in the walls of the ABO. An isolated brainstem preparation from the long-nosed gar, *Lepisosteus osseus*, showed two distinct motor patterns in fictive respiratory activity recorded from the root of the Vth cranial nerve, a high-frequency, low-amplitude pattern, associated with gill breathing and a low-frequency, high-amplitude pattern associated with ventilation of the lung during air breathing (**Figure 11**). This latter activity was associated with phasic activation of the Xth cranial nerve. The differing motor patterns and recruitment of vagal motoneurons suggest the presence of two separate oscillators.

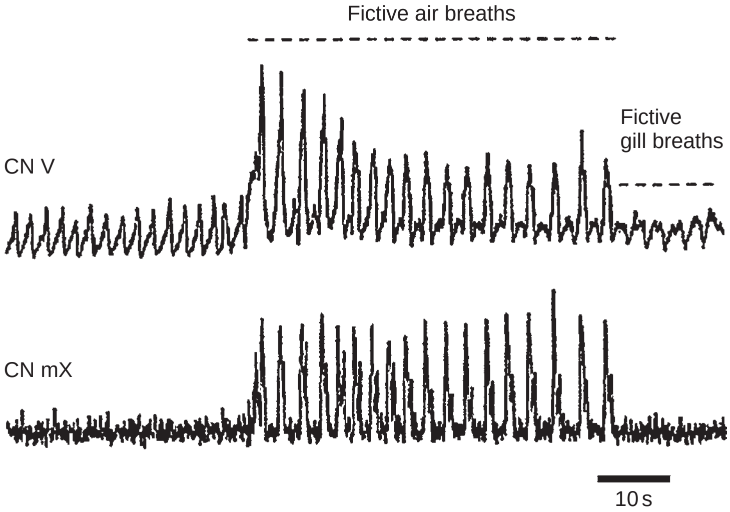


Figure 11 Integrated bursting activity recorded from the roots of the Vth cranial nerve (CNV) and a medial root of X (CNmX) of an isolated brainstem preparation of the gar *Lepisosteus osseus*. Fictive gill breathing is interrupted by a bout of fictive air breathing with stronger bursts in CNV and bursting activity in CNmX. From figure 2B in Kinkead R (2009) Phylogenetic trends in respiratory rhythmogenesis: Insights from ectothermic vertebrates. *Respiration Physiology and Neurobiology* 168: 39–48.

There may be separate origins for air-pumping mechanisms in actinopterygian fishes and the lobe-finned (sarcoptergian) lungfish together with their evolutionary cousins, the amphibians. However, both utilize the same sets of muscles to pump air into the lung, and consequently may possess the same central oscillators. African lungfish (*Protopterus aethiopicus*) may possess two separate central rhythm generators, one for gill ventilation and the other for air breathing. The sequence of airflow in the breathing cycles of lungfish and amphibians, such as bullfrogs, is essentially similar. In amphibians, there is evidence that evolution of air-breathing rhythms may have required a new motor pattern in the CNS rather than one evolved from progressive modification of the branchial rhythm generator. Episodic breathing rhythms were recorded from an isolated brainstem of a bullfrog, implying that they are generated centrally in the absence of patterned inflow from receptors. Microinjection of glutamate into rostral areas of the bullfrog-isolated brainstem in an area of the RF that corresponds to that identified as responsible for respiratory rhythmogenesis in fetal mammals, caused brief breathing episodes. It seems that the neural networks associated with respiratory rhythmogenesis have been well conserved during vertebrate evolution.

Summary

Respiratory rhythmogenesis resides in the brainstem of all vertebrates, including fish, and the isolated medulla oblongata can generate a respiratory rhythm. However, the site of the primary generator is poorly defined and its activity is modulated by centers in the midbrain and stabilized by peripheral inputs from mechanoreceptors on the gills. The respiratory rhythm in fish may become episodic or cease completely during ram ventilation. When oxygen demand is high, feeding muscles can be recruited into the respiratory cycle to provide active inspiration of water during the mouth-opening phase. Air-breathing fish utilize the same muscles to gulp air at the water surface. There is some evidence of separate RRGs for water and air breathing but their properties and locations are unclear

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; Respiratory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes.

Further Reading

Ballantijn CM (1987) Evolution of central nervous control of ventilation in vertebrates. In: Taylor EW (ed.) *Neurobiology of the Cardiorespiratory System*, pp. 3–27. Manchester: Manchester University Press.

De Graaf PJF and Roberts BL (1991) Effects of sensory input on the breathing rhythm of the carp. *Journal of Experimental Biology* 155: 77–91.

De Graaf PJF and Roberts BL (1991) Entrainment of the breathing rhythm of the carp by imposed oscillation of the gill arches. *Journal of Experimental Biology* 155: 93–102.

Hughes GM and Shelton G (1962) Respiratory mechanisms and their nervous control in fish. *Advances in Comparative Physiology and Biochemistry (ed OE Lowenstein)* 1: 275–364.

Juch PJW and Ballintijn CM (1983) Tegmental neurons controlling medullary respiratory neuron activity in the carp. *Respiration Physiology* 51: 95–107.

Kinkead R (2009) Phylogenetic trends in respiratory rhythmogenesis: Insights from ectothermic vertebrates. *Respiration Physiology and Neurobiology* 168: 39–48.

Milsom WK (2008) Evolutionary trends in respiratory mechanisms. In: Poulin MJ and Wilson RJA (eds.) *Integration in Respiratory Control*, pp .293–298. New York: Springer.

Rovainen CM (1985) Respiratory bursts at the midline of the rostral medulla of the lamprey. *Journal of comparative Physiology A* 157: 303–309.

Seymour RS, Farrell AP, Christain K, *et al.* (2007) Continuous measurements of oxygen tensions in the air breathing organ of Pacific tarpon (*Megalops cyprinoides)* in relation to aquatic hypoxia and exercise. *Journal of Comparative Physiology B* 177: 579–587.

Shelton G (1959) The respiratory centre in the tench (*Tinca tinca* L): I. The effects of brain transection on respiration. *Journal of Experimental Biology* 36: 191–202.

Shelton G (1961) The respiratory centre in the tench (*Tinca tinca* L): II. Respiratory neuronal activity in the medulla oblongata. *Journal of Experimental Biology* 38: 79–92.

Shelton G (1970) The regulation of breathing. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. IV, pp. 293–359. New York: Academic Press.

Shelton G, Jones DR, and Milsom WK (1986) Control of breathing in ectothermic vertebrates. In: Fishman AP, Cherniack NS, Widdicombe JG, and Geiger SR (eds.) *Handbook of Physiology*, Section 3, vol. 2, Part 2, pp. 857–909. Bethesda, MD: American Physiological Society.

Sundin L, Turesson J, and Taylor EW (2003) Evidence for glutamatergic mechanisms in the vagal sensory pathway initiating cardiorespiratory reflexes in the shorthorn sculpin *Myoxocephalus scorpius*. *Journal of Experimental Biology* 206: 867–876.

Taylor EW, Campbell HA, Levings JJ, Young MJ, Butler PJ, and Egginton S (2006) Coupling of the respiratory rhythm in fish with activity in hypobranchial nerves and with heart beat. *Physiological and Biochemical Zoology* 79: 1000–1009.

Taylor EW, Jordan D, and Coote JH (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiological Reviews* 79: 855–916.

The Ventilatory Response to CO_2/H^+

WK Milsom, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Reflex Responses
Location of CO_2 Chemoreceptors
Stimulus Specificity
Further Reading

Glossary

Acidosis Decreased pH in water or blood.

Hypercapnia High levels of carbon dioxide in blood.

Hypercarbia High levels of carbon dioxide in water or air.

Hyperoxia High levels of oxygen in water or arterial blood.

Hyperventilation An increase in ventilation.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Normoxia Normal levels of oxygen in water or arterial blood.

Respiratory alkalosis An increase in pH in arterial blood due to excessive ventilation leading to excretion of carbon dioxide.

Ventilation stroke volume The volume of water pumped with each compression of the orobranchial cavity during breathing in fish.

Ventilation volume The total amount of water pumped over the gills in a period of time (usually 1 min); the product of the rate of breathing multiplied by the volume of water pumped with each breath.

Introduction

While there is no doubt that the primary drive to breathe in fish is the need to obtain sufficient oxygen to fuel metabolism, fish also need to excrete the CO_2 that is produced as a by-product of this metabolism in order to regulate pH levels within their tissues around an optimum for enzyme and protein function. These two needs (to obtain oxygen and to excrete CO_2) are tightly linked because CO_2 is produced indirectly in the process of using O_2 to produce adenosine triphosphate (ATP) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

The exchange of these gases takes place primarily at the gills in most fish, as well as across the skin in some species, and through accessory air-breathing organs in others (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). In general, to meet the metabolic demands of the tissues for O_2 uptake and CO_2 excretion, levels of internal (blood) and external (water) O_2 and CO_2 must be sensed so that appropriate ventilatory responses can be generated to match O_2 supply to tissue demand and to match CO_2 excretion to its rate of production.

Adjusting ventilation is one of the most important reflex responses maintaining relatively constant levels of CO_2/H^+ in the tissues. Because of differences in the solubility of oxygen and CO_2 in water (CO_2 is roughly 20 times more soluble), it takes much higher water flow rates over the gills to obtain O_2 from the water than to excrete an equivalent amount of CO_2 into the water. As a result, CO_2 excretion is easily achieved from the ventilation required to obtain oxygen. Regardless, fish respond to increased levels of environmental CO_2 (hypercarbia) and/or decreases in pH (acidosis) by increasing their gill ventilation. Similarly, increases in internal arterial CO_2 (hypercapnia) and decreases in pH associated with increased metabolism also stimulate gill ventilation. Clearly, fish have a ventilatory response to CO_2 and H^+, despite the ease of CO_2 removal across the gills. This response is the topic of this article.

Reflex Responses

Exposure to elevated levels of aquatic CO_2 (hypercarbia) typically elicits an immediate increase in ventilation volume through increases in the volume of water pumped with each breath (stroke volume) and/or breathing frequency. This hypercarbic response is additive with those induced by hypoxia (low oxygen) and hyperoxia (high oxygen). Consequently, exposure to a hypoxic, hypercarbic environment (usually these two occur together in nature) produces a greater response than exposure to either condition alone. This acute, or immediate, response can, in some species, be followed by secondary changes that slowly return ventilation to resting levels despite a sustained hypercarbic exposure.

The increase in the amount of aquatic CO_2 required to elicit a ventilatory response (the threshold level) varies greatly among species. Some species (eel, *Anguilla anguilla*), carp (*Cyprinus carpio*), and tambaqui (*Colossoma macropomum*), and traira (*Hoplias malabricus*)) require fairly high levels of CO_2 (>5% CO_2) before they respond, while others (zebrafish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*)) are very sensitive, responding to very small changes in aquatic CO_2 (<1% CO_2). This finding may reflect species-specific tolerances to CO_2, with ventilatory responses to CO_2 only being elicited at environmentally relevant levels (**Figure 1**).

Similar responses occur in most air-breathing fish. In most species, gill ventilation increases in response to relatively low levels of hypercarbia (<3%) despite relatively stable levels of arterial P_{CO_2}. This is a reasonable response since the majority of CO_2 in air-breathing fishes is excreted via the gills rather than the air-breathing organ (ABO). Even so, in some air-breathing fish, further increases in aquatic CO_2 (>2–10%) inhibit gill breathing and stimulate air breathing, often, also with no accompanying changes in arterial P_{CO_2} (**Figure 2**). Just as in water-breathing fish, it appears that a water-sensing CO_2 receptor stimulates gill ventilation but its input is transformed centrally (in the brain) to excite air breathing and inhibit water breathing with increasing levels of stimulation. Just as with aquatic CO_2, some air-breathing fish show no response to increasing levels of CO_2 in inspired air, while others show an increase in ventilation of the air-breathing organ (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

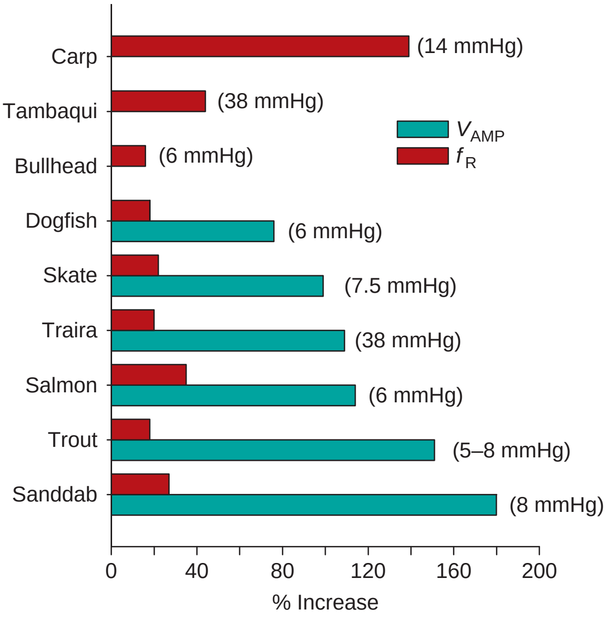


Figure 1 Increase in breathing frequency (f_R) and tidal volume (V_{AMP}) upon exposure to CO_2 (levels shown in brackets for each species). The threshold level of CO_2 required to produce a response, and the magnitude of the response varies between species as a function of factors such as CO_2 tolerance, gill surface area, body weight, activity levels, temperature, and chemosensitivity. The relative contribution of changes in f_R and V_{AMP} to the increase in total ventilation also varies between species. (Adapted from Gilmour KM (2001). The CO_2/pH ventilatory drive in fish. *Comparative Biochemistry and Physiology A*. 130: 219–240.

The hypercapnic ventilatory response in fish appears to be relatively weak when compared to the hypoxic ventilatory response (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes). Thus, hyperoxia reduces ventilation despite the fact that it is accompanied by a rise in arterial CO_2 and a fall in pH. This finding suggests that O_2 delivery in fish is regulated at the expense of CO_2 excretion. This is also true in terrestrial vertebrates, where CO_2 is the primary drive to breathe (e.g., at altitude, mammals hyperventilate despite the production of a significant rise in pH (respiratory alkalosis) due to the heavy breathing). This suggests that while the primary drive to breathe varies between aquatic and terrestrial vertebrates under resting, normoxic conditions, under hypoxic conditions, terrestrial vertebrates behave more like fish because the need to match O_2 supply with O_2 demand predominates.

Location of CO_2 Chemoreceptors

Gill (Branchial) Chemoreceptors

The ventilatory response to CO_2/H^+ in fish arises primarily, if not exclusively, from receptors distributed throughout the gill arches innervated by the IX (glossopharyngeal) and X (vagus) cranial nerves. These chemoreceptors have been identified primarily on the basis of respiratory reflexes to CO_2/H^+ that are eliminated by sectioning the nerves to the gills or removing gills completely (**Figure 3**). However, while it is clear that there are receptors in the gill that monitor the CO_2 in the water, it is not entirely clear whether receptors exist in the gills that can respond specifically to changes in the CO_2 of arterial blood.

Indirect evidence suggests that there are such internal CO_2 receptors. Following exhaustive exercise in normoxic water, ventilation remains elevated although arterial P_{O_2} returns to normal. Arterial P_{CO_2}, however, also remains elevated and pH deceased. Reducing the post-exercise acidosis with carbonic anhydrase injections (an enzyme that enhances CO_2 excretion at the gills (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange)) reduces this hyperventilation. Furthermore, in rainbow trout, intra-arterial injections of CO_2/H^+ stimulate ventilation. These data argue strongly for the presence of

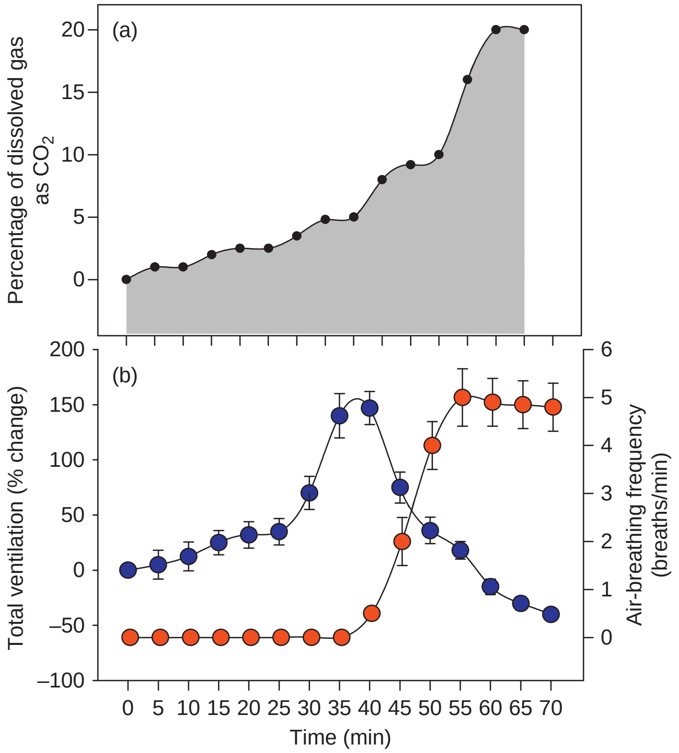


Figure 2 As aquatic levels of CO_2 rise (a), air-breathing fish (Jeju – *Hoplerythrinus unitaeniatus*) switch (b) from gill ventilation (blue symbols) to air breathing (red symbols). Adapted from Boijink C, de L , Florindo L-H, Leite CAC, Kalinin AL, Milsom, WK, and Rantin FT (2010) *Journal of Experimental Biology* 213: 2797–2807.

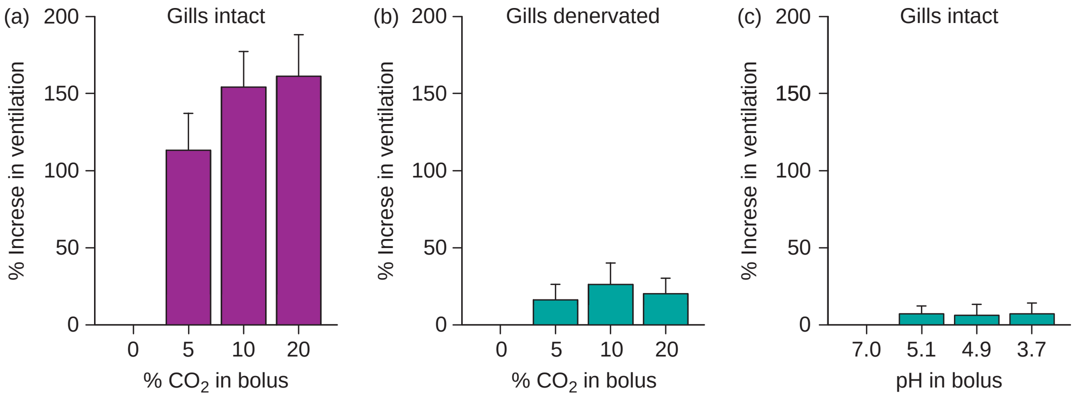


Figure 3 The effects of injecting water over the gills containing different levels of CO_2 (a) before and (b) after denervation of the gills as well as (c) water containing different levels of H^+ (i.e., at different pH) but constant levels of P_{CO_2} in the jeju. Note that the effects of increasing levels of CO_2 are eliminated by denervating the gills and that changing pH has no effect if the P_{CO_2} does not change. (Adapted from de Lima Boijink C, Florindo L-H, Leite CAC (2009) *Journal of Experimental Biology* 213: 2797–2807

internal receptors monitoring changes in the CO_2/H^+ levels in arterial blood. Other lines of evidence, however, suggest that there are no receptors monitoring such changes. In many studies, intra-arterial injections of CO_2/H^+ had no effect on ventilation (in spiny dogfish (*Squalus acanthias*), Atlantic salmon (*Salmo salar*), rainbow trout, tambaqui, and traira). Furthermore, injections of acetazolamide (a drug that blocks carbonic anhydrase) that lead to CO_2 retention and blood acidification also do not alter ventilation (**Figure 4**). This issue remains to be resolved.

Air-breathing fish (chondrosteans, holosteans, and teleosts, as well as the sarcopterygian lungfish) have not been well studied in this respect. Thus, there is no evidence to suggest where the receptors reside that produce responses to CO_2/H^+ in these fish. Based on the location of the receptors responsible for changes in gill ventilation in exclusively water-breathing fish, as well as the receptors sensing aquatic O_2 that stimulate both gill and lung ventilation in the African lungfish, *Protopterus* (peripheral O_2-sensitive chemoreceptors are also CO_2 sensitive in air-breathing vertebrates),

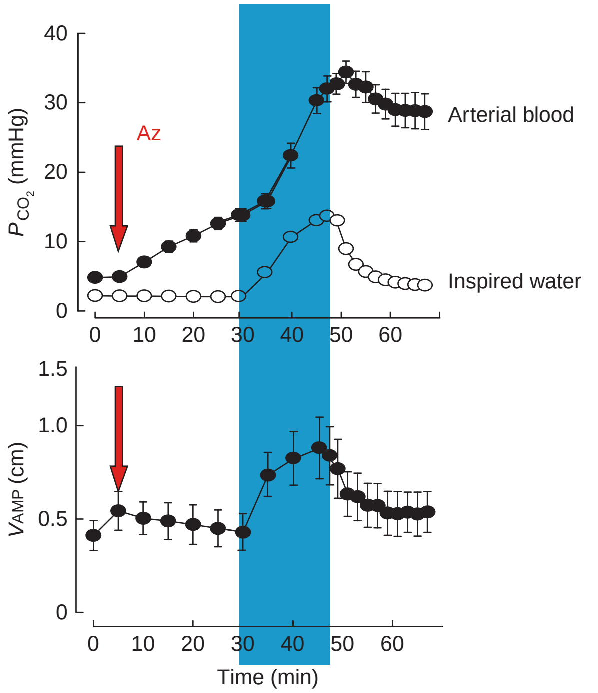


Figure 4 Increasing arterial PCO_2 using the drug azetazolamide (Az; which inhibits CO_2 excretion) has no effect on breathing (ventilatory amplitude, V_{AMP}) but elevating CO_2 tension in the water stimulates breathing. When the CO_2 in the water is returned to normal, breathing slows again despite the fact the arterial CO_2 levels remain elevated. This suggests that all responses are to changes in P_{CO_2} in the external water, not in the blood. (Adapted from Gilmour KM, Milsom WK, Rantin FT, Reid SG, and Perry SF (2005) Cardiorespiratory responses to hypercarbia in tambaqui *(Colossoma macropomum)*: Chemoreceptor orientation and specificity. *Journal of Experimental Biology* 208: 1095–1107.

they will likely be found on all gill arches, but may be more predominant on the first two arches (which have a reduced gas-exchange function in many air-breathing fish).

Extra-Branchial Receptors

There are some indications that extra-branchial CO_2/H^+ chemoreceptors may also exist. There are cases where the increase in ventilation stroke volume elicited by aquatic hypercarbia was not eliminated by branchial denervation and it has been suggested that the sites of the receptors giving rise to the residual response could be located elsewhere in the oro-buccal cavity, which has also been suggested for extra-branchial O_2 chemoreceptors.

Gustatory and Olfactory Receptors

Gustatory chemoreceptors are sensory cells of epithelial origin that are incorporated into taste buds in the oral cavity, palate, gill arches, skin, and barbels (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes). Their afferent sensory information is transmitted to the brain via cranial nerves VII, IX, and X. They respond to both CO_2 and H^+. Stimulation of them leads to a reduction (rather than an elevation) in ventilation amplitude and frequency. It has also been suggested that stimulation of CO_2-sensitive chemoreceptors in the olfactory epithelium inhibits breathing as it does in amphibians, reptiles, and mammals.

The only receptors that have been found to be sensitive to CO_2 in lungs and other air-breathing organs of air-breathing fish are pulmonary stretch receptors. Slowly adapting pulmonary stretch receptors in both the African (*Protopterus*) and South American (*Lepidosiren*) lungfish are inhibited by normal, physiological levels of CO_2, but those in the gar (*Lepisosteus osseus*) and the bowfin (*Amia calva*) are not. There is also evidence that olfactory/gustatory chemoreceptors sensitive to CO_2 in inspired air inhibit breathing.

Central Receptors

Experimental evidence supporting the existence of central (brain) CO_2/H^+ chemoreceptors in water-breathing fish is equivocal. While there is some evidence suggesting that such receptors exist, most studies have failed to find evidence for chemoreceptors that influence breathing within the brain. There is, however, stronger evidence for their existence in air-breathing fish (**Figure 5**). They have been shown, unequivocally, to be present in the Sarcopterygian lungfish, and there is strong evidence for their presence in gar and Siamese fighting fish (*Beta splendens*), but not *Amia* or Alaska blackfish (*Dallia pectoralis*).

To summarize, present data suggest that the ventilatory response to CO_2/H^+ primarily arises from receptors

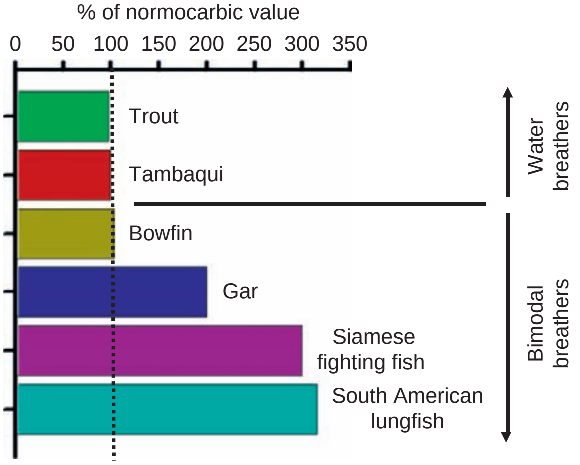


Figure 5 There are no effects of changes in the CO_2/pH of cerebro-spinal fluid on ventilation in water-breathing fish but there are significant increases in bimodal breathing fish (fish that breathe water and air) except for the bowfin (100% is the normal resting level of ventilation).

distributed throughout the gill arches sensitive to aquatic CO_2. Fish gill arches share a common embryological origin with the carotid and aortic arches of tetrapods, and the branchial distribution of water-sensing CO_2 chemoreceptors and water/blood-sensing O_2 chemoreceptors that modulate cardiorespiratory function in fish is phylogenetically consistent with the distribution of peripheral chemoreceptors that monitor only blood CO_2/pH and O_2 in tetrapods.

Stimulus Specificity

Aquatic hypercarbia is accompanied by alteration in water pH, and exposure to this water leads to changes in the P_{CO_2} (hypercapnia) and pH (acidosis) of arterial blood in the fish. Furthermore, these changes in arterial P_{CO_2}/pH induce a Root effect in hemoglobin in many fish, leading to reductions in arterial O_2 content (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). As a result, responses to aquatic CO_2 could be due to changes in any one, or combination of, water CO_2, water pH, arterial CO_2, arterial pH, or arterial O_2 content. It was once thought that a change in arterial O_2 content was the proximate stimulus driving ventilation under hypercarbic conditions, but it is now clear that CO_2/pH directly affects gill ventilation.

Fish respond rapidly when they encounter environmental hypercarbia or injections of CO_2 equilibrated water into the buccal cavity. They respond poorly to injections of water of different pH; environmental acidification has little effect on ventilation in the absence of changes in CO_2. This suggests that the receptors responding to external stimuli respond specifically to changes in CO_2 in the water (**Figure 3**).

To the extent that there is evidence for ventilatory responses arising from receptors responding to internal stimuli (i.e., stimuli in arterial blood), there is a better correlation between the changes in ventilation and changes in arterial pH rather than in arterial P_{CO_2}.

At present, it is thought that the chemosensory cells in the gills are neuroepithelial cells in the branchial epithelium. Certainly, there is strong evidence that neuroepithelial cells in zebrafish sense O_2 by means of a mechanism similar to that in mammalian O_2-chemoreceptors, relying on an O_2-sensitive background K^+ channel (**see also Control of Respiration**: Oxygen Sensing in Fish). The O_2-chemoreceptors of mammals and other tetrapods also exhibit sensitivity to CO_2, raising the possibility that the same situation holds true for fish-gill neuroepithelial cells. In support of this, recent studies have shown that some cultured branchial neuroepithelial cells from zebrafish contain carbonic anhydrase, an enzyme that plays a key role in mammalian CO_2 chemotransduction. Some of these branchial neuroepithelial cells depolarize under hypercapnic conditions. This process also appears to rely on a background K^+ channel, and roughly half of the cells also respond to changes in O_2. Interestingly, this implies that although these receptors may only respond to changes in aquatic CO_2 and not $[H^+]$, at the cellular level, they do this by responding to CO_2-induced changes in intracellular pH.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes. **Control of Respiration**: Oxygen Sensing in Fish. **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes. **Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes. **Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Burleson ML and Smatresk NJ (2000) Branchial chemoreceptors mediate ventilatory responses to hypercapnic acidosis in channel catfish. *Comparative Biochemistry and Physiology A* 125: 403–414.

Coates EL (2001) Olfactory CO_2 chemoreceptors. *Respiration Physiology* 129: 219–230.

Delaney RG and Fishman AP (1977) Analysis of lung ventilation in the aestivating lungfish *Protopterus aethiopicus*. *American Journal of Physiology* 233: R181–R187.

Gilmour KM (2001) The CO_2/pH ventilatory drive in fish. *Comparative Biochemistry and Physiology A* 130: 219–240.

Gilmour KM and Milsom WK (2009) CO_2/H^+ chemoreceptors in the respiratory passages of vertebrates. In: Zaccone G, Cutz E, Adriaensen D, Nurse C, and Mauceri A (eds.) *Structure, Evolution and Function of the Airway Chemoreceptors in the Ertebrates*, vol.436. Enfield, NH: Science Publishers.

Gilmour KM, Milsom WK, Rantin FT, Reid SG, and Perry SF (2005) Cardiorespiratory responses to hypercarbia in tambaqui (*Colossoma macropomum*): Chemoreceptor orientation and specificity. *Journal of Experimental Biology* 208: 1095–1107.

Gilmour KM and Perry SF (2007) Branchial chemoreceptor regulation of cardiorespiratory function. In: Zielinski B and Hara TJ (eds.) *Fish Physiology, Volume 25: Sensory Systems Neurosciencepp*, pp. 97–151. San Diego, CA: Academic Press.

Hughes G and Shelton G (1962) Respiratory mechanisms and their nervous control in fish. *Advances in Comparative Physiology and Biochemistry* 1: 275–364.

Johansen K and Lenfant C (1968) Respiration in the African lungfish, *Protopterus aethiopicus*. II. Control of breathing. *Journal of Experimental Biology* 49: 453–468.

Jonz MG, Fearon IM, and Nurse CA (2004) Neuroepithelial oxygen chemoreceptors of the zebrafish gill. *Journal of Physiology* 560: 737–752.

Lahiri S, Szidon JP, and Fishman AP (1970) Potential respiratory and circulatory adjustments to hypoxia in the African lungfish. *Federation Proceedings* 29: 1141–1148.

McKendry JE, Milsom WK, and Perry SF (2001) Branchial CO_2 receptors and cardio-respiratory adjustments during hypercarbia in Pacific spiny dogfish (*Squalus acanthias*). *Journal of Experimental Biology* 204: 1519–1527.

McKenzie DJ, Burleson ML, and Randall DJ (1991) The effects of branchial denervation and pseudobranch ablation on cardioventilatory control in an air breathing fish. *Journal of Experimental Biology* 161: 347–365.

Milsom WK (1995) Regulation of ventilation in lower vertebrates: Role of CO_2/pH chemoreceptors. In: Heisler N (ed.) *Mechanisms of Systemic Regulation in Lower Vertebrates. Advances in Comparative and Environmental Physiology,* vol. 21, pp. 61–104. Heidelberg: Springer.

Perry SF and Gilmour KM (2002) Sensing and transfer of respiratory gases at the fish gill. *Journal of Experimental Zoology* 293: 249–263.

Perry SF and McKendry JE (2001) The relative roles of external and internal CO_2 versus H^+ in eliciting the cardiorespiratory responses of *Salmo salar* and *Squalus acanthias* to hypercarbia. *Journal of Experimental Biology* 204: 3963–3971.

Perry SF and Reid SG (2002) Cardiorespiratory adjustments during hypercarbia in rainbow trout *Oncorhynchus mykiss* are initiated by external CO_2 receptors on the first gill arch. *Journal of Experimental Biology* 205: 3357–3365.

Sanchez AP, Giusti H, Bassi M, and Glass ML (2005) Acid–base regulation in the South American lungfish *Lepidosiren paradoxa*: Effects of prolonged hypercarbia on blood gases and pulmonary ventilation. *Physiological and Biochemical Zoology* 78: 908–915.

Shelton G, Jones DR, and Milsom WK (1986) Control of breathing in ectothermic vertebrates. In: Cherniack NS and Widdicombe JG (eds.) *Handbook of Physiology, Section 3: The Respiratory System, Vol. II: Control of Breathing, part 2*, pp. 857–909. Bethesda, MD: American Physiological Society.

Thomas S, Fievet B, Barthelemy L, and Peyraud C (1983) Comparison of the effects of exogenous and endogenous hypercapnia on ventilation and oxygen uptake in rainbow trout (*Salmo gairdneri* R). *Journal of Comparative Physiology* 151: 185–190.

Oxygen Sensing in Fish

MG Jonz, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Reflex Responses to Hypoxia
Microscopic Observations of Gill O_2 Chemoreceptors
Chemoreceptor Response to Hypoxia
Conclusions
Further Reading

Glossary

Chemoreceptor A type of cell that is specialized to detect chemical stimuli and convert them into signals within the nervous system.

Depolarization The reduction in electrical potential difference (voltage) measured across a cell membrane. This means the inside of the cell becomes less negative with respect to the outside of the cell. Depolarization occurs during the upsweep of the action potential (opposite of repolarization).

Equilibrium potential The membrane potential (voltage) at which there is no net flow of current for a particular ion. This value can be calculated for any ion using the Nernst equation and is based on the intracellular and extracellular concentrations of that ion.

Ion channel A protein or a group of proteins that form water-filled pores which span the cell membrane. These pores, when open, allow a rapid but selective flow of ions through the cell membrane. Each channel type is specific for one inorganic ion (sodium, calcium, potassium, or chloride).

Neuroepithelial bodies (NEBs) Clusters of neuroepithelial cells that are found in the lung and airway epithelium in neonatal mammals. NEBs are O_2 sensitive.

Neuroepithelial cells (NECs) Within the context of this article, these are cells that exist within the epithelial layer of the gill and are capable of storing and releasing a chemical transmitter from the cytosol. Some authors also refer to these cells as neuroendocrine cells. NECs are the putative oxygen chemoreceptors in aquatic vertebrates.

Oxidative phosphorylation A process in the mitochondrion in which the synthesis of adenosine triphosphate (ATP) is driven by (1) the transfer of electrons along the electron-transfer chain to molecular O_2, and (2) coupling to an electrochemical H^+ gradient.

Partial pressure of O_2 (P_{O_2}) The atmospheric pressure exerted by O_2 alone proportional to the total concentration of this gas. It is typically measured in either mmHg (torr) or kPa.

Postsynaptic Denotes a cell that occurs after the synaptic cleft (an intercellular space) with respect to the flow of information in the nervous system.

Resting membrane potential The measured voltage difference across the plasma membrane for a cell that is not stimulated, but is at rest. The resting potential is based on the concentrations of all charged ions inside and outside of the cell, and also the resting permeability of the membrane.

Introduction

Oxygen is a major element of nearly all important biological macromolecules and played a critical role in the evolution of life on Earth. However, in its diatomic or molecular form, O_2, this gaseous molecule is capable of accepting electrons and participating in oxidation–reduction reactions necessary for the production of metabolic energy within cells. It is this metabolic dependence on O_2 for cellular energy production that defines aerobic organisms and is a characteristic of most eukaryotes.

Another important characteristic of eukaryotes is the presence of numerous mitochondria within the cytosol, for it is within this organelle that O_2 promotes energy production for the entire cell. Oxygen diffuses freely from the cell's cytosol, through both the outer and inner mitochondrial membranes, and finally into the matrix of the mitochondrion, where it acts as the final acceptor of electrons from the electron-transport chain.

The electron-transport chain is a set of proteins that pumps hydrogen ions (H^+) from the mitochondrial matrix to the intermembrane space, where it stores potential energy by establishing an electrochemical gradient of H^+ (i.e., a pH gradient) so that these ions flow passively back to the matrix. It is the energy of H^+ returning to the mitochondrial matrix that is harnessed by another protein complex, an adenosine triphosphate (ATP) synthase, which converts adenosine diphosphate (ADP) to ATP, the currency of cellular energy. This entire process is called oxidative phosphorylation. From the above description, it will be apparent to the reader that O_2 is required for this process. Without it, ATP production in the cell is compromised and this may have dire consequences for the life of the cell (**see also Hypoxia**: Anaerobic Metabolism In Fish and The Expanding Hypoxic Environment). Not surprisingly, the amount of O_2 reaching a cell must be maintained, or even defended, to ensure survival.

If we now advance this discussion to a multicellular organism, such as a vertebrate composed of tissues, organs, and a vascular system, delivery of O_2 to every cell is tightly regulated to avoid ischemic tissue damage and cell death caused by O_2 lack, or hypoxia. But how does a multicellular organism, such as a vertebrate, regulate sufficient uptake of O_2 (e.g., via respiration) to maintain an adequate supply to every cell? More specifically, how is low O_2 in the environment sensed so that this regulatory process can be initiated in the first place? These questions are addressed here.

Most of what is known about the cellular mechanisms of O_2 sensing has come from decades of research on respiratory chemoreceptors of mammals that are sensitive to changes in O_2. Chemoreceptors are cells that are specialized to respond to specific chemicals in their environment. For example, olfactory and lingual epithelia bear chemoreceptors that underlie the sense of smell and taste, respectively. Chemoreceptors are specialized in that they retain a gamut of protein complexes within the plasma membrane, and signaling proteins within the cytosol, that are devoted to detecting specific molecules or even ions. Naturally, chemoreceptors must have access to a sampling environment, and they must also have a means of communicating with other cells so that the chemical message can be relayed to appropriate regions of the central nervous system. Respiratory chemoreceptors that are sensitive to O_2, therefore, are cells that may detect changes in the partial pressure of O_2 (P_{O_2}) in the air, water, or blood, and initiate appropriate physiological changes.

In mammals, there are two important types of respiratory chemoreceptors that regulate changes in ventilation. Type I cells (or glomus cells) are chemoreceptors of the carotid body that sample the chemical content of the blood, such as the partial pressure of O_2 (P_{O_2}). The carotid body is a paired organ found at the bifurcation of the common carotid artery, where it divides into the internal and external carotids. It is the primary O_2-sensing organ in mammals and elicits hyperventilation in response to low arterial P_{O_2}. Neuroepithelial bodies (NEBs) of the airway and lung epithelium are clusters of chemoreceptive cells that also respond to hypoxia, but are believed to play a role in controlling respiration during the neonatal period. As is explained in the following sections, O_2 chemoreceptors of mammals have morphological and physiological properties similar to those of fish. In fact, one of these similar properties is their anatomical location. The site of the mammalian carotid body is derived from the same embryonic structure as the gills, where O_2 chemoreceptors are found in fish. For this reason, we may make some comparisons between O_2 chemoreceptors of mammals and fish to help understand how O_2 sensing might occur in the latter.

Reflex Responses to Hypoxia

In all vertebrates, including fish, a reduction of O_2 in the environment will produce a variety of reflex responses, including hyperventilation, a change in heart rate (in fish, hypoxia slows heart rate; **see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia), and changes in vascular resistance, in order to allow the animal to adapt to changing environmental conditions. These reflex responses, however, are not reviewed here (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes). But it is important to note that studies characterizing the way in which fish respond to hypoxia were instrumental in determining that respiratory chemoreceptors were located within the gill region. However, it was not until extracellular electrical recordings were obtained from the nerves innervating the gills that the presence of gill O_2 chemoreceptors could be verified. When isolated gill arches were subjected to hypoxia, recordings from gill nerves displayed an electrical discharge consistent with hypoxic stimulation of gill chemoreceptors. These same studies also indicated that gill O_2 chemoreceptors may respond to hypoxia detected in the water (externally oriented chemoreceptors) as well as hypoxia detected in the arterial blood supply (internally oriented chemoreceptors).

Microscopic Observations of Gill O_2 Chemoreceptors

Since the early 1980s, studies using both light and electron microscopy have provided morphological evidence implicating a group of cells, called neuroepithelial cells (NECs), as the most suitable candidates for O_2 chemoreceptors in the fish gill. Today, there is physiological evidence to support this view (see following sections). Studies over the last three decades have shown that NECs are found in the gills of nearly every fish species examined, and their morphology is conserved across species.

Neuroepithelial cells of the gills are typically about 10 μm in diameter (depending on species) and are located primarily in the epithelium of the gill filaments. Specifically, NECs reside in a region where they are as close as possible to the constant flow of water over the gills during ventilation – an advantageous position for chemoreceptors to be located (see **Figure 1** for the general orientation of NECs (green) in the gill filaments and **see also Design and Physiology of Arteries and Veins**: Branchial Anatomy). Gill NECs generally do not form clusters but exist as solitary cells, unlike O_2 chemoreceptors of the mammalian carotid body or lung epithelium. **Figure 2** illustrates the general distribution of NECs (green) in the gill filaments of three teleost species.

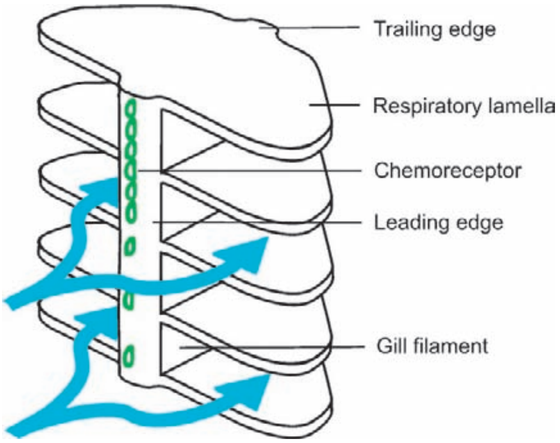


Figure 1 Schematic representation of a teleost gill filament. This illustration shows a region of gill filament that gives rise to several respiratory lamellae, where gas exchange occurs. The leading edge of the filament faces the flow of water (shown by blue arrows) over the gills during ventilation, while the trailing edge represents the filament tissue furthest away. Oxygen-sensitive chemoreceptors (i.e., neuroepithelial cells (NECs), shown in green) reside within the epithelium of the gill filaments, where they may sense changes in oxygen from the water or arterial blood flow.

NECs have been shown, through immunohistochemical experiments, to retain a great number and variety of neuroactive chemicals. These include the neurotransmitter serotonin, enzymes that produce the neurotransmitters nitric oxide and catecholamines, as well as several neuropeptides. In addition, NECs have been shown to retain cytoplasmic synaptic vesicles, which store and release neurotransmitters following an appropriate signal. These morphological characteristics are consistent with the model that NECs are capable of storing and producing neuroactive chemicals within the gill and, potentially, releasing them during a bout of hypoxia. But in order for the release of a neurotransmitter from a chemoreceptor to have a physiological response at the systemic level, for example, hyperventilation or bradycardia, the chemoreceptor must be in a position to communicate with the peripheral nervous system. (**see also Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish).

Evidence from studies using electron and confocal microscopy indicate that gill NECs receive a rich supply of nervous innervation from the glossopharyngeal and vagus cranial nerves. These two nerves are collectively referred to as the gill or branchial nerves. The consensus appears to be that gill NECs receive a mixture of both sensory and motor innervation, the former being most important for transferring a chemical signal from the NECs (i.e., release of a neurotransmitter) to the nervous system. **Figure 3** illustrates the innervation of a gill NEC (green) by a bundle of nerve fibers (red). The bundle of fibers is composed of neuronal axons, the cell bodies of which are likely located within the ganglia of the gill nerves.

The characteristics described above, retention of synaptic vesicles, storage of neurotransmitters, and sensory innervation, provide convincing evidence that gill NECs display all of the morphological requirements expected of O_2 chemoreceptors. Indeed, these characteristics have supported the notion that gill NECs are the phylogenetic precursors of mammalian O_2 chemoreceptors.

Chemoreceptor Response to Hypoxia

Sensing of Hypoxia

Under normal, or normoxic, conditions there is an adequate supply of O_2 to all tissues and cells and respiratory chemoreceptors are relatively inactive. Thus, under these conditions, ventilatory and cardiac rates are at basal

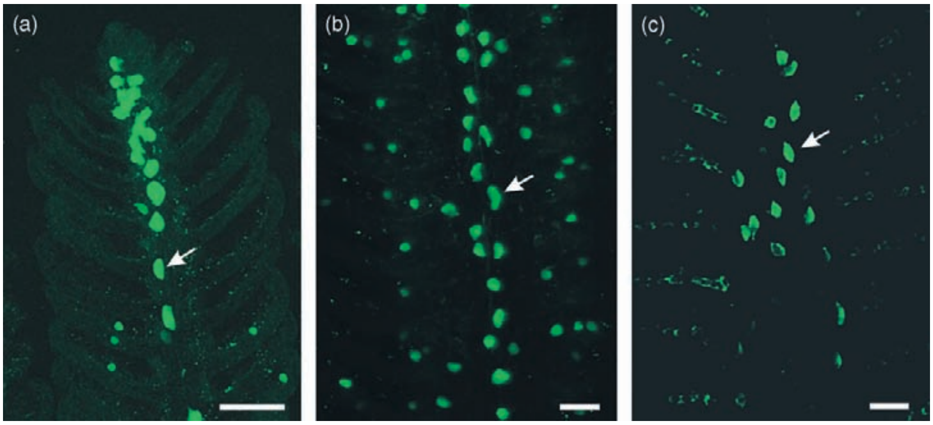


Figure 2 NECs are found in the gill filaments of most teleost fish. NECs (labeled in green and indicated with arrows) are usually solitary and are organized along the longitudinal axis of the filament. NECs are shown in zebrafish, *Danio rerio* (a), goldfish, *Carassius auratus* (b), and rainbow trout, *Oncorhynchus mykiss* (c). The orientation in all three panels corresponds to that of the schematic shown in **Figure 1**. Images are composites of several optical sections taken using a confocal microscope. NECs are colored green because they are labeled with antibodies that identify cells that contain the neurotransmitter, serotonin. Scale = 30 μm.

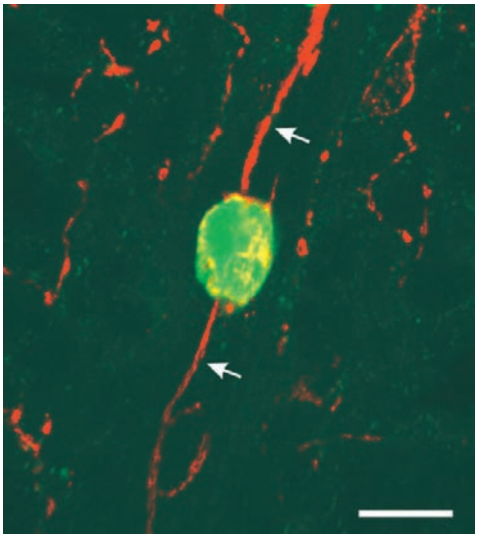


Figure 3 Neuroepithelial cells (NECs) are innervated. A single NEC of the zebrafish gill filament is shown (green) by labeling with serotonin antibodies. This cell receives innervation by nerve fibers (shown in red and indicated with arrows). Scale = 10 μm.

levels. However, once environmental (aquatic, in the case of fish) P_{O_2} begins to decrease, the reflex responses described in the preceding section will be initiated. But how is this drop in environmental P_{O_2} detected or sensed by respiratory chemoreceptors?

Since O_2 is a gas, it will flow down its concentration gradient, crossing epithelial barriers, cell membranes, and into the cytosol (**Figure 4**). For example, if we define water P_{O_2} as 140 mmHg (19 kPa), arterial P_{O_2} as 100 mmHg (13 kPa), tissue P_{O_2} as 60 mmHg (8 kPa), then normal intracellular P_{O_2} of a cell or chemoreceptor would be even less, perhaps 20–30 mmHg (3–4 kPa). It follows, then, that if water P_{O_2} decreases, intracellular P_{O_2} must also decrease. Once the decreased P_{O_2} reaches the cytosol of a chemoreceptor, it may be sensed by a molecule or mechanism that binds or associates with O_2.

In fish, the molecular O_2 sensor that mediates the cellular response to hypoxia is currently unknown. However, there is much evidence from studies on mammalian O_2 chemoreceptors that may provide clues as to the identity of the O_2 sensor in fish. Since it is apparent that respiratory chemoreceptors of mammals and fish appear to be related phylogenetically, we may make a legitimate comparison here. Generally, there are two different hypotheses that have emerged in the recent literature that may explain the location of the O_2 sensors in mammalian chemoreceptors. The sensor may be present in the plasma membrane, or elsewhere in the cytosol, such as the mitochondrion. It is not the author's intent to exhaustively describe here all of the previous or contemporary models for mammalian O_2 sensors, though the curious reader is encouraged to consult the 'Further reading' section at the end of the article for recent reviews. Only a few examples are mentioned here, instead. It should be pointed out here that it is generally accepted in most cases that hypoxia will lead to a decrease in membrane permeability to K^+ ions. However, this matter is discussed in the section entitled 'Transduction of the hypoxic stimulus'.

The membrane hypothesis of O_2 sensing proposes that the molecular O_2 sensor is confined to the plasma

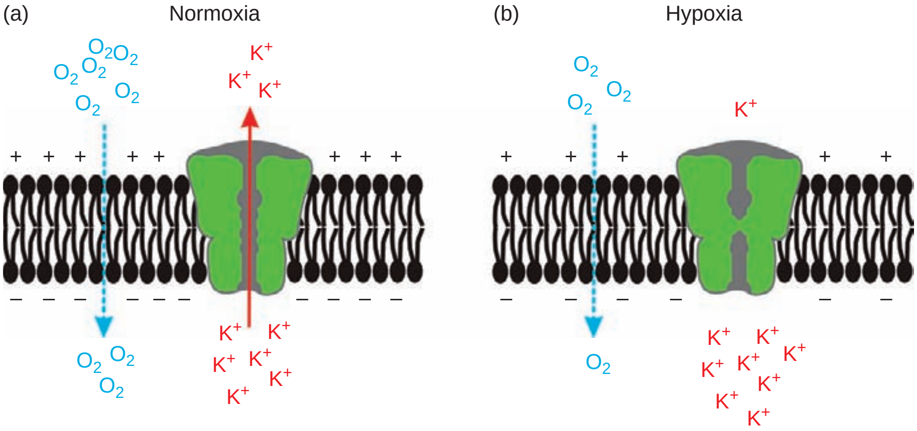


Figure 4 Schematic representation of the current working model of hypoxic chemotransduction in oxygen-sensitive NECs of the fish gill. The normal, or normoxic, condition is shown in (a), while the low oxygen, or hypoxic, condition is shown in (b). Gaseous O_2 diffuses freely across the phospholipid bilayer of the cell membrane (black) down its concentration gradient. Potassium ions (K^+) are transported out of the cell passively through selective ion channels, some of which are O_2 sensitive (green transmembrane structure), down their electrochemical gradient. In (a), O_2-sensitive K^+ ion channels are open, leading to a high membrane permeability of K^+ and a separation of charge across the cell membrane. In this case, the membrane is said to be polarized, such that the inside of the cell is relatively negative compared to the outside of the cell. In (b), a decrease in the partial pressure of O_2 is detected by an unidentified cytosolic O_2 sensor, and a closed conformation of the O_2-sensitive ion channel is favored. This closing of K^+ channels leads to a slight depolarization, as shown by the separation of fewer positive and negative charges across the cell membrane, and represents the conversion of a chemical to an electrical signal.

membrane. Evidence supporting this model was obtained from experiments using carotid body type I cells from rat. An enzyme, called hemoxygenase-2 (HO-2), was identified within the complex of a specific type of membrane-bound K^+ ion channel. In the presence of O_2, HO-2 produces the gas, carbon monoxide (CO), which in turn increases the activity of these ion channels and, consequently, the K^+ ion permeability of the membrane. A decrease in CO production by HO-2 during hypoxia, therefore, will decrease K^+ permeability. Thus, HO-2 is a candidate for an O_2 sensor in the cellular response to hypoxia because, depending on O_2 status, it can affect membrane permeability. The significance of the latter point will become clear as we proceed. This model, however, does not appear to be valid in mouse, suggesting that it may be species specific.

An alternative membrane model suggests that the sensing of hypoxia may occur via a membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and its production of hydrogen peroxide (H_2O_2). In neuroepithelial bodies (NEBs) of the rabbit lung, K^+ permeability is increased by H_2O_2 under conditions of normoxia. But during hypoxia, a decrease in O_2 substrate will lead to decreased K^+ permeability because of decreased H_2O_2 production.

By contrast, there is much evidence to indicate that O_2 sensing is tied to metabolic energy production in the mitochondrion, perhaps at the site of a cytochrome oxidase in the electron-transport chain. There are two models that explain how a decrease in oxidative phosphorylation during hypoxia may be coupled to plasma membrane K^+ permeability in carotid body type I cells. The first proposes that since ATP increases the activity of a specific type of K^+ channel, a decrease in intracellular ATP concentration during hypoxia (because of reduced ATP production by oxidative phosphorylation) would lead to a decrease in membrane K^+ permeability. The second model proposes that a similar drop in ATP production during hypoxia would lead to an increase in the cytosolic AMP/ATP ratio. Increased AMP/ATP would then induce activation of AMP-activated protein kinase and inhibition of O_2-sensitive K^+ channels by phosphorylation. Therefore, these two models predict that the O_2 sensor is associated with energy production in the cell.

As stated already, the literature on cellular mechanisms of O_2 sensing is dominated by experiments on mammalian O_2 chemoreceptors. Recent studies, however, have proposed that hydrogen sulfide (H_2S) may act as a sensor or transducer of intracellular P_{O_2} and mediate responses to hypoxia in vertebrates, including fish. In this model, constitutive levels of intracellular H_2S are balanced by its production and its oxidation by available O_2. The model proposes that during hypoxia, decreased O_2 leads to reduced oxidation of H_2S and its subsequent accumulation in the cell. Although application of exogenous H_2S mimicked hypoxia and led to depolarization in gill NECs, thus indicating a change in membrane permeability, its effects on K^+ channels, or association with a mechanism of transduction, are not yet clear in chemoreceptors.

To summarize, in these models of O_2 sensing in respiratory chemoreceptors, a common theme is that a change in concentration of an environmental chemical (i.e., O_2) is sensed or received by a molecular O_2 sensor that is either confined to the membrane or found within the cytosol. In fact, there is little agreement as to whether a single O_2 sensor is common to respiratory chemoreceptors in all vertebrates, including fish. From the brief description of potential O_2 sensors provided above, the reader may appreciate that this is a highly controversial issue within the field. Indeed, there does not appear to be a universal O_2 sensor – one that is found in respiratory chemoreceptors of all animals. But the very interesting chemosome hypothesis, proposed recently by Prabhakar, states that involvement of not one but multiple O_2 sensors in the cellular response to hypoxia may be advantageous and allow for responses across a broad range of P_{O_2} levels. What happens next, that is, how the low O_2 signal is converted, or transduced, into a form that can be used by the cell, is the subject of the following section.

Transduction of the Hypoxic Stimulus

Having already alluded in the previous section to the fact that the sensing of intracellular hypoxia predominantly results in a decrease in K^+ ion permeability across the cell membrane, we now further discuss this concept with the effects of hypoxia upon membrane-bound ion channels and how these begin a process of chemotransduction that results in a physiological chemoreceptor response.

Excitable cells, such as neurons and chemoreceptors, retain a complement of ion channels within their membrane that each has specific molecular, biophysical, and pharmacological properties. Moreover, each type of membrane ion channel plays a specific physiological role in the cell. For example, the opening of Na^+ channels permits the large influx of positively charged Na^+ ions into the cell and this characterizes an action potential; activation of Ca^{2+} channels allows entry of Ca^{2+} into the cytosol to stimulate neurosecretion; and K^+ channels contribute significantly to setting the resting membrane potential and excitability of the cell, in part, because of the membrane's high permeability to K^+ ions. The latter point is important to the present discussion because it is the membrane's permeability to K^+ ions that is modified during hypoxia.

Very broadly, one can differentiate between K^+ ion channels that are gated (i.e., opened and closed) by changes in voltage across the cell membrane, and those that are not. Voltage-gated K^+ channels serve many important roles in cells, including repolarization following an action potential. But K^+ channels that are not gated by voltage change, the so-called 'leak' or 'background' K^+ channels, are not affected by voltage change, and it is this type of K^+ channel that is particularly important in generation of the resting membrane potential. Importantly, when a cell, such as a chemoreceptor, is at rest, background K^+ channels are open and allow for the continuous efflux (from inside to outside) of K^+ ions (**Figure 4**(**a**)). K^+ ions will tend to move out of the cell under normal physiological conditions because this is the direction of electrochemical flow, as dictated by the distribution of K^+ ions across the cell membrane. This electrochemical directionality is established by the pumping of K^+ ions into the cell via ATP-consuming transporters, which create a gradient. Therefore, because K^+ channels permit an efflux of positively charged K^+ ions, and because of the maintained distribution of K^+ ions across the membrane (i.e., a very negative equilibrium potential for K^+), there will be a separation of electrical charge across the membrane. The end result is that the inside of the cell is more negative relative to the outside of the cell, and the measured difference in voltage across the membrane is called the resting membrane potential. While resting potentials vary considerably from cell to cell, a value of −70 mV (millivolts) is typical. Generation of the resting membrane potential is far more complex than what is described here, and is actually determined by the permeabilities of several additional ions. The reader is, therefore, referred to other sources, such as those cited in the 'Further reading' section.

Given the importance of K^+ channels in influencing membrane potential, it is not surprising that throughout the course of evolution cellular chemoreceptors have acquired mechanisms by which to modify this potential, depending on O_2 status. In the previous section, it was indicated that after hypoxia is sensed by a membrane-bound or cytosolic O_2 sensor, there is a decrease in K^+ permeability of the membrane. Specifically, hypoxia inhibits K^+ channels so that there is a reduced efflux of K^+ ions (**Figure 4**(**b**)). In keeping with the diversity of hypotheses of how O_2 is sensed by the chemoreceptor cell, there are many types of K^+ channels that are targets of modification during hypoxia, such as those that are gated by voltage or even Ca^{2+} ions. There are even channels that are selective for other ions, such as Ca^{2+}, that display O_2 sensitivity. Perhaps the most interesting, however, is that of the background K^+ channel, as discussed earlier, because it is open at resting membrane potentials, rather than like voltage-gated K^+ channels that must first be activated by voltage change before hypoxia may affect its activity. In addition, background K^+ channels that are responsive to hypoxia have been found in type I cells of the mammalian carotid body and NECs of the fish gill, suggesting that this mechanism of chemotransduction may be highly conserved in vertebrates.

Figure 4 illustrates how background K^+ channels from fish or mammalian O_2 chemoreceptors behave during hypoxic stimulation. After a reduction in P_{O_2} is sensed, closure of K^+ channels reduces the membrane permeability to K^+, thereby inducing a relative depolarization of the membrane, or a reduction in the membrane potential. It is this membrane depolarization that characterizes the transduction of the hypoxic stimulus – the conversion of a chemical event to an electrical one. Studies of fish and mammalian chemoreceptors have shown that the electrical response of chemoreceptors exposed to hypoxia is indicative of reduced electrical current carried by K^+ ions through background K^+ channels. **Figure 5** shows an idealized recording using a technique, called whole-cell electrophysiological recording, in which the flow of electrical current that is carried across the cell membrane by ions can be recorded while the experimenter controls the membrane voltage. In this example, a NEC from the fish gill is subjected to a continuous change in membrane voltage from −100 mV to +50 mV (x-axis) while

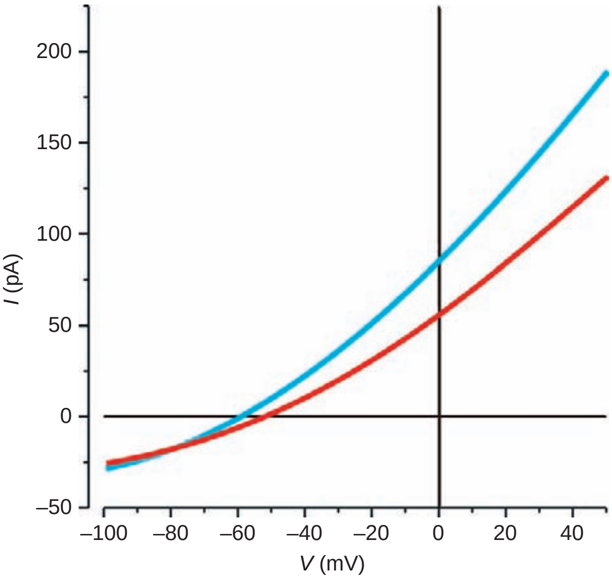


Figure 5 A simplified electrical recording of a neuroepithelial cell (NEC) responding to hypoxia. This illustration is based on actual electrophysiological recordings and demonstrates the current voltage (I–V) relationship observed in these cells. As the experimenter changes the voltage (i.e., membrane potential) across the cell membrane from −100 mV to +50 mV, an increased force pushes more K^+ out of the cell. In a normal, or normoxic, cell this may be recorded as a change in current from about −25 pA to 200 pA (blue line). If the cell is subjected to a lowered partial pressure of O_2, such as in hypoxia, the I–V relationship changes such that less K^+ flows out of the cell because more O_2-sensitive K^+ channels are closed. Compare this with the structural changes illustrated in **Figure 4**.

recording the evoked change in current (*y*-axis). Under control, or normoxic, conditions (blue line) this voltage change elicits a range of current from –25 pA (picoamperes) to about +200 pA. This is known as the current–voltage (*I*–*V*) relationship and reflects the electrical properties of the cell. By convention, in this example, a negative current value indicates an inward flow of current carried predominantly by K^+ ions, while a positive value indicates an outward current. After the cell is exposed to a reduction in P_{O_2} (red line) of the medium surrounding the cell (achieved by displacing O_2 with N_2 gas), there is an obvious decrease in outward current that can be observed at voltages ranging from –40 mV to +50 mV, indicating that the flow of K^+ ions through the membrane was reduced because of inhibition of background K^+ channels.

If we now combine our discussion of O_2 sensors with that of chemotransduction by K^+ channels in the plasma membrane, there is evidence from studies of mammalian type I cells in favor of a link between a metabolic O_2 sensor and inhibition of background K^+ channels during hypoxia. As the activity of background K^+ channels in these cells is regulated by the concentration of ATP and phosphorylation by adenosine monophosphate (AMP)-activated protein kinase, both mitochondrial models of O_2 sensing discussed earlier are plausible. Since the biophysical characteristics of the background K^+ channels found in gill NECs described so far are consistent with those of type I cells, it is reasonable to propose that O_2 sensing and chemotransduction in the fish gill occur in the same way as in the mammalian carotid body.

As a final step in the response of the O_2 chemoreceptor to hypoxia, the cell must now communicate this information to the nervous system to initiate adaptive physiological responses at the systemic level, such as hyperventilation and changes in heart rate that were described in a previous section. For this to occur, the hypoxic signal (which, at this point, exists as an electrical depolarization) must be converted back to a chemical form. In the carotid body, type I cells express plasma membrane Ca^{2+} channels that are voltage sensitive and activated by hypoxia-induced membrane depolarization. Activation of these channels causes a sudden rise in the intracellular concentration of Ca^{2+} ions and this leads to the release of neurotransmitters, stored in cytoplasmic synaptic vesicles, to the extracellular environment (i.e., synaptic cleft) where they can bind to specific receptors on postsynaptic sensory neurons. This is the accepted model for how type I cells detect, transduce, and relay the hypoxic signal to the nervous system. In NECs of the fish gill, Ca^{2+} channels have not yet been studied and so the problem of Ca^{2+}-dependent secretion of neurotransmitter following hypoxic depolarization cannot be addressed for the moment. However, it is plausible that NECs adhere to this conventional model since they do retain the neurotransmitter serotonin and synaptic vesicles (see previous section).

Neurochemistry

Despite the plethora of neurotransmitters and neuropeptides that have been localized to gill NECs across a variety of fish species, there is, unfortunately, very little direct evidence in support of neurochemical transmission between NECs and sensory nerve fibers in the fish gill during hypoxia. It is, therefore, difficult to predict what events occur after NECs are depolarized by hypoxic stimulation and what chemical signals are received by sensory nerves. At the ultrastructural level, hypoxia has been shown to induce degranulation of cytoplasmic vesicles, indicative of neurotransmitter release into the synaptic cleft. Though gill NECs in nearly all fish species are rich in serotonin, the questions of whether serotonin is being released, and if it represents the primary excitatory neurotransmitter that underlies communication from NECs to postsynaptic nerves during hypoxic stimulation, remain unresolved. In addition, no receptors have been identified on postsynaptic nerve terminals that may allow for binding of neurochemicals released from NECs.

If we look again to mammalian mechanisms of O_2 sensing, a variety of neurochemicals play excitatory, inhibitory, and modulatory roles. In the carotid body, acetylcholine, ATP, and dopamine appear to be important neurotransmitters, among others, and their respective receptors have been identified within the carotid body; while in lung NEBs serotonin is more important. In experiments in fish, where isolated perfused gill arch preparations were exposed to exogenous neurotransmitters to stimulate sensory pathways in the gill, acetylcholine had stronger stimulatory effects than serotonin. So, it would seem that a neurochemical explanation of hypoxic signaling in the gill is not straightforward, and may have characteristics similar to both carotid body and lung NEB O_2 sensing. To expand this idea, it may be found that the gill may utilize a variety of excitatory, inhibitory, and modulatory neurochemicals to produce the hypoxic response.

Conclusions

NECs of the gills in fish fulfil all of the requirements to be considered as O_2 chemoreceptors. They respond, physiologically, to hypoxia in the form of K^+ ion channel inhibition, which causes a decrease in permeability to K^+ ions, and membrane depolarization; they retain cytosolic secretory vesicles that store serotonin, among other neurochemicals; and they receive nervous innervation, thus providing a putative sensory pathway for transmission of a signal to the central nervous system. Despite what has been characterized so far, relatively little is known of O_2 sensing by NECs when one considers the wealth of information on mammalian O_2 chemoreceptors. For example, while a transduction mechanism has been identified in the cell membrane in fish, candidates for a molecular O_2 sensor in NECs have not been proposed. Furthermore, the chemical details of how neurotransmission between NECs and sensory nerve cells might occur during hypoxia have not been described. The disparity between the present understanding of O_2 sensing in fish and in mammals has been driven by the significance of the latter in biomedical research. However, as presented in this article, at the cellular level O_2 sensing in fish appears to closely resemble that of mammals, suggesting that the use of fish in this field may be of some benefit to biomedical research. For example, studying the model organism, the zebrafish (*Danio rerio*), may allow researchers access to developmental and genetic tools to uncover the fine details of O_2 sensing at the cellular and molecular level. Fish, like other vertebrates, have acquired elegant strategies for adapting to changing environmental conditions, such as hypoxia, throughout the course of evolution. At the very least, a clear understanding of how O_2 sensing in fish occurs will help explain how all aspects of O_2 sensing may have evolved.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Hypoxia**: Anaerobic Metabolism in Fish; Respiratory Responses to Hypoxia in Fishes; The Expanding Hypoxic Environment. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia.

Further Reading

Bailly YJR (2009) Serotonergic neuroepithelial cells in fish gills: Cytology and innervation. In: Zaccone G, Cutz E, Adriawnsen D, Nurse CA, and Mauceri A (eds.) *Airway Chemoreceptors in the Vertebrates*, pp.62–97. Enfield, NH: Science Publishers.

Buckler KJ (2007) TASK-like potassium channels and oxygen sensing in the carotid body. *Respiratory Physiology and Neurobiology* 157: 55–64.

Burleson ML and Milsom WK (2003) Comparative aspects of O_2 chemoreception: Anatomy, physiology, and environmental adaptations. In: Lahiri S, Semenza GL, and Prabhakar NR (eds.) *Oxygen Sensing: Responses and Adaptation to Hypoxia*, pp. 685–707. New York: Marcel Dekker.

Cutz E, Fu WX, Yeger H, Pan J, and Nurse CA (2009) Oxygen sensing in mammalian pulmonary neuroepithelial bodies. In: Zaccone G, Cutz E, Adriawnsen D, Nurse CA, and Mauceri A (eds.) *Airway Chemoreceptors in the Vertebrates*, pp. 269–290. Enfield, NH: Science Publishers.

Falkowski PG and Godfrey LV (2008) Electrons, life and the evolution of earth's oxygen cycle. *Philosophical Transactions of the Royal Society B* 363: 2705–2716.

Fu XW, Wang D, Nurse CA, Dinauer MC, and Cutz E (2000) NADPH oxidase is an O_2 sensor in airway chemoreceptors: Evidence from K^+ current modulation in wild-type and oxidase-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America* 97: 4374–4379.

Goldstein SAN, Backenhauer D, O'Kelly I, and Zilberberg N (2001) Potassium leak channels and the KCNK family of two-P-domain subunits. *Nature Reviews Neuroscience* 2: 1–11.

Hille B (2001) *Ionic Channels of Excitable Membranes*, 3rd edn. Sunderland: Sinauer Associates.

Jonz MG, Fearon IM, and Nurse CA (2004) Neuroepithelial oxygen chemoreceptors of the zebrafish gill. *Journal of Physiology* 560: 737–752.

Jonz MG and Nurse CA (2009) Oxygen-sensitive neuroepithelial cells in the gills of aquatic vertebrates. In: Zaccone G, Cutz E, Adriawnsen D, Nurse CA, and Mauceri A (eds.) *Airway Chemoreceptors in the Vertebrates*, pp. 1–30. Enfield, NH: Science Publishers.

López-Barneo J, Ortega-Sáenz P, Pardal R, *et al.* (2009) Oxygen sensing in the carotid body. *Annals of the New York Academy of Sciences* 1177: 119–131.

Nurse CA (2005) Neurotransmission and neuromodulation in the chemosensory carotid body. *Autonomic Neuroscience: Basic and Clinical* 120: 1–9.

Olson KR (2008) Hydrogen sulfide and oxygen sensing: Implications in cardiorespiratory control. *Journal of Experimental Biology* 211: 2727–2734.

Perry SF, Jonz MG, and Gilmour KM (2009) Oxygen sensing and the hypoxic ventilatory response. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology*, vol. 27, pp. 193–253. Burlington: Academic Press.

Prabhakar NR (2006) O_2 sensing at the mammalian carotid body: Why multiple O_2 sensors and multiple transmitters? *Experimental Physiology* 91: 17–23.

Varas R, Wyatt CN, and Buckler KJ (2007) Modulation of TASK-like background potassium channels in rat arterial chemoreceptor cells by intracellular ATP and other nucleotides. *Journal of Physiology* 583: 521–536.

Williams BA and Buckler KJ (2004) Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type I cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 286: L221–L230.

Williams SE, Wootton P, Mason HS, *et al.* (2004) Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. *Science* 306: 2093–2097.

Wright SH (2004) Generation of resting membrane potential. *Advances in Physiology Education* 28: 139–142.

Wyatt CN and Evans AM (2007) AMP-activated protein kinase and chemotransduction in the carotid body. *Respiratory Physiology and Neurobiology* 157: 22–29.

GAS EXCHANGE

Transport and Exchange of Respiratory Gases in the Blood

Contents
Red Blood Cell Function
Hemoglobin
O_2 Uptake and Transport: The Optimal P_{50}
Carbonic Anhydrase in Gas Transport and Exchange
Carbon Dioxide Transport and Excretion
Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange
Evolution of the Bohr Effect
Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder
Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems
Hemoglobin Differentiation in Fishes

Red Blood Cell Function

M Nikinmaa, University of Turku, Turku, Finland

© 2011 Elsevier Inc. All rights reserved.

Introduction
Membrane Transport, Erythrocyte pH, and Volume
Control of Erythrocytic NTP Concentration
Role of Erythrocytes in Regulating Capillary Function
Further Reading

Glossary

Anion exchanger The major membrane protein of most erythrocytes. With its help, the half-time of chloride and bicarbonate equilibration between erythrocytes and plasma is decreased from a couple of hours to seconds in fish; the protein's transport function enables rapid buffering of acid loads by hemoglobin.

Beta-adrenergically stimulated sodium/proton exchange This ion-transport pathway is unique for teleost erythrocytes and plays a role in controlling red cell pH.

Heme oxygenase An enzyme with high erythrocytic activity, which plays a role in hemoglobin breakdown with carbon monoxide as one end product.

Organic phosphate metabolism Fish erythrocytes produce energy, mainly aerobically.

Oxygen-dependent ion transport Many ion transporters of the erythrocytic membrane respond to changes in ambient oxygen level. Often, it appears that it is not molecular oxygen as such, but reactive oxygen species which cause the apparent oxygen sensitivity.

Volume regulation After osmotic swelling, erythrocytes lose water mainly via osmotic coupling to net potassium, chloride, and taurine efflux, and after osmotic shrinking gain water via sodium and chloride influx.

Introduction

The major function of erythrocytes is to transport oxygen from the respiratory epithelium to the sites of consumption (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Most of the oxygen transported is carried bound to hemoglobin, the major erythrocytic protein at 10–20 mM concentration (concentration given as monomeric globin; 2.5–5 mM tetramers). A hemoglobin concentration of 20 mM is close to the solubility limit of the protein. This hemoglobin level increases the maximal amount of oxygen carried in blood close to 30-fold as compared to a situation in which oxygen would only be carried dissolved.

Aspects of the red cell function that affect hemoglobin function (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin) are particularly important from the respiratory point of view. The major physiological regulators of hemoglobin-oxygen affinity are pH, erythrocyte volume, and organic phosphate concentration. The first two are influenced by ion transport, and the last by energy metabolism.

Hemoglobin also acts as a major buffer in the body. The buffering requires that extracellular acids have access to hemoglobin. This becomes possible when bicarbonate movements are facilitated by the function of the anion exchanger.

In addition to hemoglobin function, erythrocytes play a role in the regulation of capillary function, also important for oxygen transport and thereby the respiratory function of erythrocytes.

The signaling molecules involved both in the regulation of membrane transport and in the regulation of capillary function in fish are poorly known. Gaseous signaling molecules that are known include molecular oxygen, reactive oxygen species (ROS), nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). The potential for erythrocytes to be involved in gaseous signaling is apparent, because their iron stores, which can participate in the free-radical metabolism (Fenton reactions), are very high and because hemoglobin breakdown has heme oxidase enzyme, with CO as one end product, as one component. The transport of nitrite and nitrate into the erythrocytes, and reactions of these nitrogenous compounds (interconversion, and conversion to NO), including the formation of nitrosohemoglobin, are involved in the regulation of capillary diameter and thereby tissue oxygenation. Notably, the amount of oxygen available to erythrocyte constituents is high. Even when all the other cells of the body are suffering from the lack of oxygen, there is enough oxygen in the erythrocytes (bound to hemoglobin) for their metabolism.

This article reviews respiratory aspects of erythrocyte function focusing on: (1) membrane transport and its regulation, because red cell pH and volume are major effectors of hemoglobin function; (2) metabolic pathways, which are involved in the control of erythrocyte organic phosphate level; and (3) the possible role of erythrocytes in the regulation of vascular tone.

Membrane Transport, Erythrocyte pH, and Volume

The scheme of the control of intracellular pH of fish erythrocytes is shown in **Figure 1**, and that of red cell volume regulation in **Figure 2**. Both are mainly influenced by ion transport across the erythrocyte membrane. Also, the ability of erythrocytes to buffer extracellular acid loads depends on rapid bicarbonate transport across the membrane facilitated by anion exchanger. Cyclostomes, that is, hagfish and lampreys, are devoid of rapid anion transport across the erythrocytic membrane. The anion exchange via the Band 3 protein of elasmobranch and teleost erythrocytes shortens the half-time for bicarbonate equilibration across the cell membrane from 1–2 h to seconds (the exact time depends on temperature). The anion exchange plays an important role in carbon dioxide transport (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; **Figure 3**) and affects chloride and bicarbonate distribution between blood plasma and the erythrocyte. Both of these functions are important in controlling erythrocytic pH. In addition, the anion exchange is the major pathway for membrane transport of sulfate and phosphate.

The sodium/proton exchanger extrudes protons from the red blood cell and can increase intracellular pH. It is present in all fish erythrocytes except those of hagfish. Depending on the species, it can be activated by extracellular acidification, osmotic disturbances, and adrenergic stimulation, and its activity is increased by a decrease in oxygen tension. The major signal for activation in lampreys appears to be acidification, as for most sodium/proton exchangers of other cell types. The presence of adrenergically stimulated sodium/proton exchanger is unique for teleost erythrocytes, in some of which adrenergic stimulation is the major activatory signal for sodium/proton exchange, although in some teleost species the exchanger appears to be mainly involved in volume regulation after osmotic disturbances. Elasmobranch erythrocytes, the hemoglobins of which have much higher buffering capacity at the physiological pH range than teleost hemoglobins, do not have adrenerically stimulated sodium/proton exchange.

Teleost fish erythrocytes possess beta(3)-type adrenergic receptors, which appear to be coupled to sodium/proton exchange. The sodium/proton exhanger of teleost erythrocytes harbors a cAMP-responsive cytoplasmic site. The adrenergic sodium/proton exchange is able to displace protons from electrochemical equilibrium even in the presence of robust anion exchange, because the exchanger will continue to carry out a net extrusion of protons as long as the activity of the exchanger remains higher than the rate of uncatalyzed extracellular dehydration of carbonic acid to carbon dioxide and water. The changes in extracellular pH are observed both *in vitro* and *in vivo* if catecholamine levels are elevated. As an example, immediately after a fish is rendered hypoxic, its plasma pH increases as a result of hyperventilation. Thereafter, a marked extracellular acidification ensues (within tens of seconds), which reflects the stimulation of the sodium/

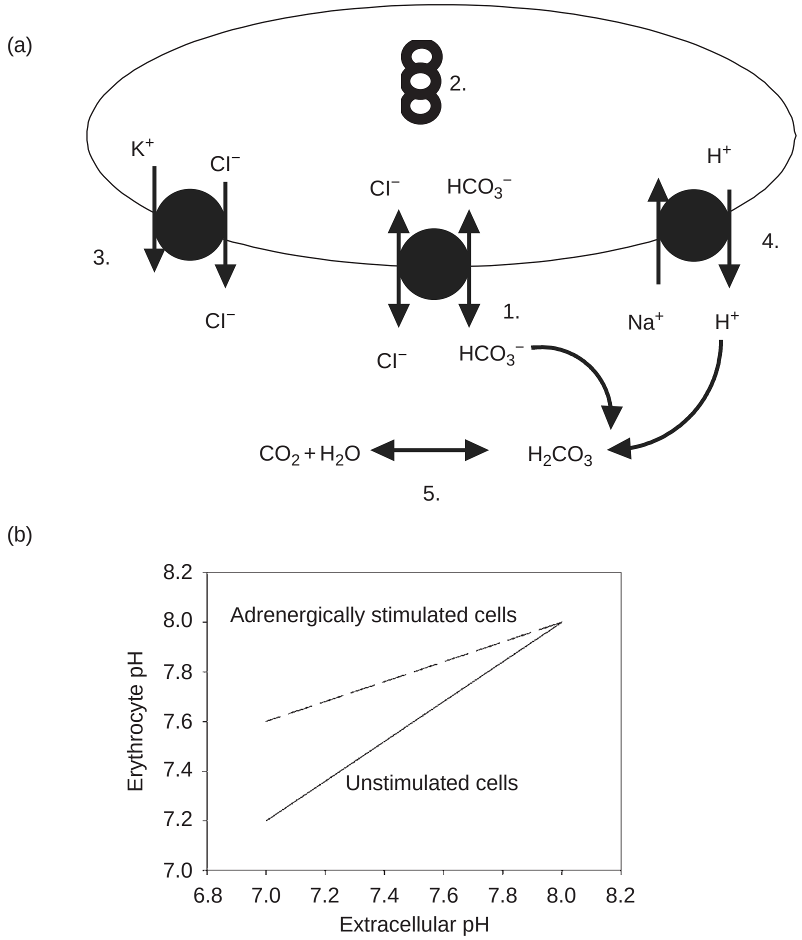


Figure 1 Control of erythrocytic pH in fish. (a) At rest, protons are passively distributed across the erythrocyte membrane as a result of rapid bicarbonate transport across the membrane (1). The intracellular compartment is more acid than the extracellular one. The intracellular pH in teleost erythrocytes is typically 7.3–7.4 at a resting extracellular pH of 7.8. When the extracellular compartment is acidified, the pH of the intracellular compartment decreases less than that of the extracellular one, because the negative charge of intracellular impermeable molecules decreases with decreasing pH. Thus, the bicarbonate ratio (inside/outside) increases, and the difference between extracellular and intracellular pH decreases. The decrease of the difference in pH depends on the properties of intracellular impermeable molecules (2). Typical values for the slope of pH (intracellular) vs. pH (extracellular) are 0.7–0.9. In the intracellular compartment, the hydration reactions of carbon dioxide and the dehydration reactions of carbonic acid (bicarbonate and protons) are catalyzed by carbonic anhydrase, increasing the reaction rates 50 000–100 000 times, compared with the extracellular compartment where the hydration/dehydration reactions proceed at the uncatalyzed rate (with a halftime up to several minutes at physiological conditions for fish). At high oxygen tensions the potassium chloride co-transport (3) is activated, and both potassium and chloride ions leave the cell because of the prevailing potassium gradient (*c*. 100 mM in the erythrocytes and 2–3 mM in blood plasma). The excreted chloride ions re-enter the cell via the anion exchanger in exchange for bicarbonate. Because bicarbonate (=base equivalent) leaves the cell, it is acidified. When teleost erythrocytes are adrenergically stimulated (see **Figure 4**), or respond to an osmotic disturbance, the sodium/proton exchange (4) is activated. Because of the prevailing sodium gradient (*c*. 140 mM in the extracellular compartment, and 30-40 mM within the erythrocytes), the transporter will carry out a net proton extrusion. The net proton extrusion occurs as long as the rate of proton extrusion is greater than the rate of extracellular dehydration of carbonic acid (5). Thereafter, proton extrusion proceeds at the same rate as bicarbonate influx, and no further pH changes occur. (b) The relationship between erythrocyte and plasma pH in unstimulated (solid line) and adrenergically stimulated (dashed line) erythrocytes.

proton exchange by catecholamines and the release of H^+ from the red blood cells. Afterwards the extracellular pH tends to increase. Since the rate of extracellular uncatalyzed dehydration of carbonic acid is important in affecting adrenergic pH changes, an addition of extracellular carbonic anhydrase will make the observed changes in the pH gradient across the erythrocytic membrane smaller or abolish them altogether without reducing the effects of adrenergic stimulation on cell volume.

The sequence of events occurring in adrenergically stimulated teleost erythrocytes is shown in **Figure 4**. Two properties of the adrenergically stimulated sodium/proton exchange make proton extrusion and increased intraerythrocytic pH particularly suited for

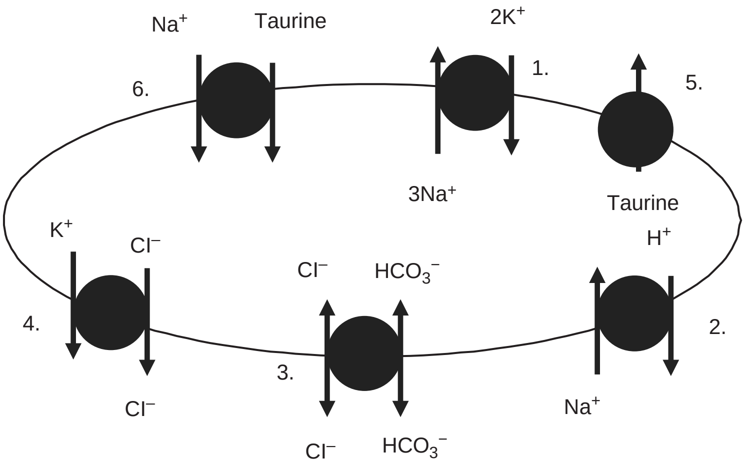


Figure 2 The mechanisms of volume regulation in erythrocytes. At rest, the red cell volume is maintained constant. Usually, the proportion of water is between 65% and 70%. The steady-state volume is maintained by the following mechanism: intracellular, impermeable (negative) polyions, mainly organic phosphates and hemoglobin, and their counterions generate an osmotic pressure difference across the membrane. Unopposed, this would lead to continuous inward water flux, until the cell would burst. However, the cell membrane is made functionally impermeable to one of the positive ions, sodium, as a result of the sodium pump (1) function. The pump extrudes the ions at the same rate as they enter the cells (1). When the environment becomes hyperosmotic and the erythrocyte shrinks, the sodium/proton exchange is activated (2), sodium enters the cell, and osmotically obliged water follows. The sodium/proton exchange is coupled to the chloride/bicarbonate exchange (3). Bicarbonate leaves the cell and chloride enters, drawing osmotically obliged water. The sodium and chloride, and consecutive water, movements into the cell restore the cell volume. If the environment becomes hypertonic, and the erythrocyte swells, the original volume is restored by the activation of potassium chloride co-transport (4) or taurine efflux (5), followed by osmotically obliged water. Very high (several tens of mM) taurine levels within the erythrocytes are reached, as the sodium-dependent taurine transporter (6) is active in the cells.

the regulation of hemoglobin-oxygen affinity during acute stress. First, the activity of the exchanger increases with decreasing oxygen tension. Second, the activity of the exchanger increases with decreasing pH. The adrenergically activated sodium/proton exchange is usually associated with very small buffering capacity of hemoglobin. Consequently, for a given proton extrusion activity, the changes in erythrocytic pH are much smaller in elasmobranch, which lack this mechanism and have large buffering capacity of hemoglobin, than in teleost fish.

Another much-studied transport pathway of teleost erythrocytes is potassium chloride co-transporter. The transport pathway has been studied mainly because of its putative role in cellular volume regulation, although its activity will also influence erythrocyte pH. The activity of the exchanger increases with increasing oxygen tension. Also, the sodium potassium chloride co-transport pathway is apparently oxygen sensitive. Transport via this pathway decreases with increasing oxygen tension. From the very fragmentary data available, it appears that the pathway is not present in all teleosts.

While the original suggestion about the oxygen sensitivity of ion transport in erythrocytes, that is, that binding of oxygen to hemoglobin influences ion transport either directly or indirectly, may hold for mammalian erythrocytes, it appears that the apparent oxygen sensitivity of ion transport in fish erythrocytes is caused by ROS, especially hydroxyl radicals. Erythrocytes are suitable for the regulation of ion transporter activity by hydroxyl radicals, because they contain large iron stores, and ions of iron are involved in Fenton reaction generating hydroxyl radicals. Generally, the hydroxyl radical level increases with increasing oxygen tension. Hydroxyl radicals inhibit the adrenergic sodium/proton exchanger and activate the potassium/chloride transporter.

Of the three cation transport pathways potentially affecting red cell pH and volume, and thereby the oxygen affinity of erythrocytic hemoglobin, only the sodium/proton exchange activity has unequivocally been shown to be important in environmental regulation of blood oxygen transport. The increased hemoglobin-oxygen affinity, largely as a result of adrenergically increased intraerythrocytic pH (increased erythrocyte volume may play a smaller role), is important in exercise, hypoxia, and other forms of stress where catecholamine levels are elevated. Depending on the species, the observed *in vivo* effects may be caused either by noradrenaline or by adrenaline. Because the effects are rapid and dependent on both oxygen tension and erythrocytic pH, which can vary in different parts of circulation, a possibility remains that the response is of different magnitude in different parts of circulation during circulatory timescale. However, the possibility of rapid pH changes, occurring in erythrocytes after adrenergic stimulation and interacting with different oxygen tensions, has been little studied because of technical difficulties.

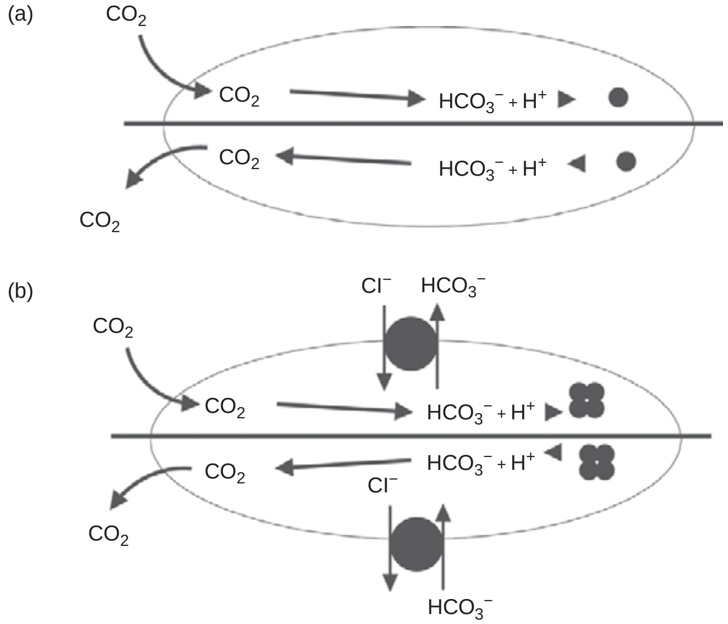


Figure 3 Schematic representations of carbon dioxide transport and excretion in fish. (a) Cyclostomes. In tissues carbon dioxide is formed, and diffuses down its concentration gradient to erythrocytes, where it is hydrated in carbonic anhydrase-catalyzed reaction to bicarbonate ions and protons. The protons are taken up by hemoglobin upon deoxygenation. The effectiveness of proton uptake is increased by increasing Haldane effect, and the amount of bicarbonate ions in solution increases by increasing in pH. In gills the sequence of events is reversed. Protons are given up from hemoglobin upon oxygenation; bicarbonate and protons are dehydrated to form carbon dioxide in a reaction catalyzed by carbonic anhydrase. The carbon dioxide formed diffuses down its concentration gradient out of erythrocytes and the animal. (b) Elasmobranch and teleost fish. The carbon dioxide formed in tissues diffuses to erythrocytes, where it is hydrated in carbonic anhydrase-catalyzed reaction to bicarbonate and protons. Bicarbonate ions leave the cell via the anion exchanger, and protons are taken up by hemoglobin upon deoxygenation. In gills, the sequence of events is reversed.

An increase in potassium chloride co-transport will decrease erythrocytic pH and erythrocytic volume. It has been suggested that such a decrease would facilitate oxygen release in hyperoxic conditions by reducing hemoglobin-oxygen affinity.

Control of Erythrocytic NTP Concentration

Organic phosphates affect hemoglobin-oxygen affinity by direct binding of the phosphate to hemoglobin, and indirectly by affecting intraerythrocytic pH (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin and Evolution of the Bohr Effect). An increase in erythrocytic organic phosphate concentration decreases and a decrease increases intracellular pH because organic phosphates are impermeable anions, which decrease bicarbonate (the major intracellular buffer) level at equilibrium. In fish, the major organic phosphates are the nucleoside triphosphates (NTPs): adenosine triphosphate (ATP) and guanosine triphosphate (GTP). ATP is the major end product of energy production. The potential pathways of the formation and breakdown of ATP and GTP are shown in **Figure 5**.

Because fish erythrocytes are nucleated and contain mitochondria, their energy metabolism is mainly aerobic respiration. The oxygen consumption of rainbow trout erythrocytes is approximately 150 μmol L^{-1} cells per hour. The oxygen consumption of white blood cells is about 20 times higher than that of erythrocytes whereby they make up approximately half of whole blood oxygen consumption.

The energy metabolism of fish erythrocytes can be supported by glucose, lactate/pyruvate, and amino acids, notably glutamate. It appears that at rest glucose may be the major metabolic substrate with the contribution of other substrates increasing with physical exercise. Virtually, all teleost erythrocytic membranes lack a glucose transporter, but monocarboxylate transporters (transporting, e.g., lactate and pyruvate) and various amino acid transporters are present.

Since the net negative charge of the impermeable breakdown products of NTPs is similar to that of the parent compounds, organic phosphates only increase intracellular pH if their total concentration in erythrocytes decreases as a result of the environmental challenge. Whereas the ATP level of erythrocytes is relatively stable and generally high (several millimolar), the GTP concentration varies from nearly undetectable to values markedly exceeding ATP concentration. However, although the regulation of hemoglobin-oxygen affinity by ATP (NTPs), and the

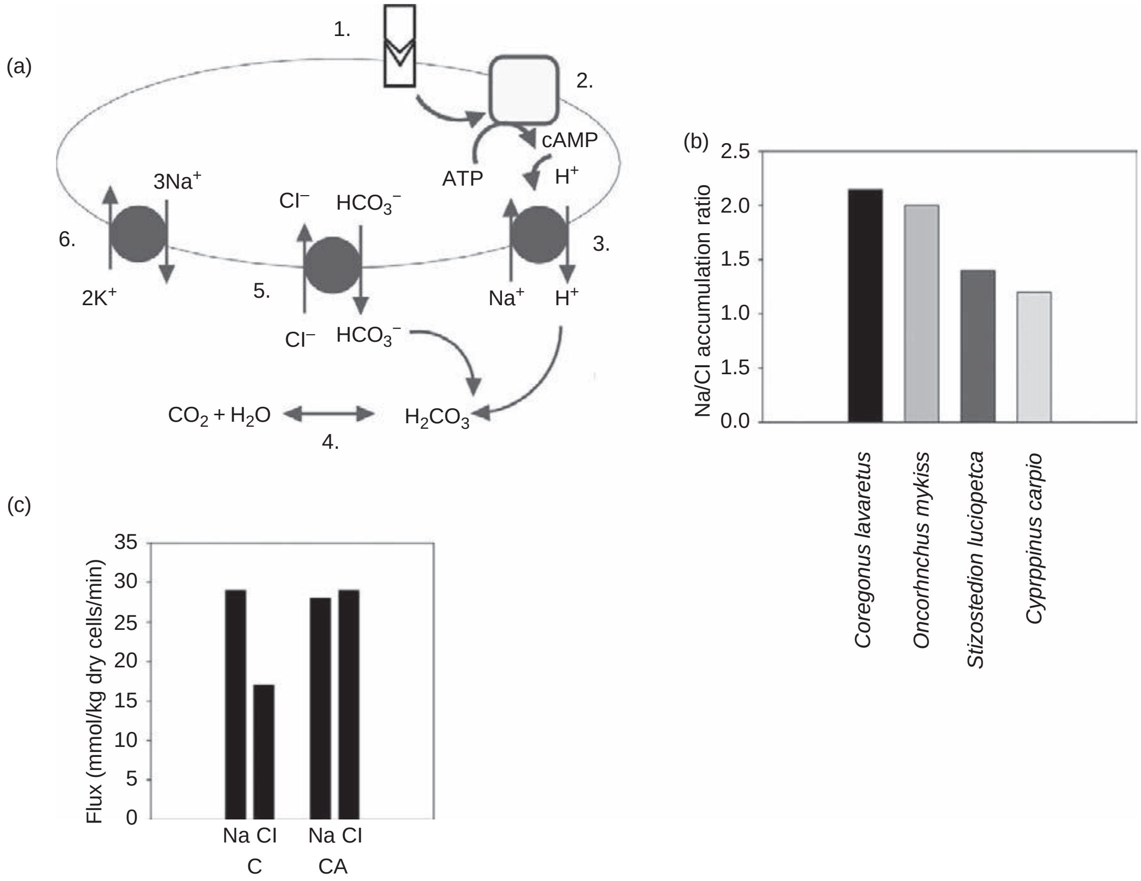


Figure 4 The mechanism of erythrocytic adrenergic response. (a) Beta-adrenergic compounds bind to the beta(3)-type receptors (1). This activates adenylate cyclase and cAMP is formed (2) activating the sodium/proton exchange (3). The increase of intraerythrocytic pH depends on the activity of the sodium/proton exchanger and on the buffering capacity of hemoglobin. The larger the exchanger activity and the smaller the buffering capacity, the larger the pH change. The intracellular pH changes occur as long as the rate of proton extrusion is greater than the rate of uncatalyzed dehydration of carbonic acid to carbon dioxide (4). The cell volume continues to increase with the coupling of proton and bicarbonate extrusion (5). The increased sodium influx causes increased sodium pump activity (6), and the ATP level tends to decrease. (b) The sodium/chloride accumulation ratio in some fish species after maximal adrenergic stimulation (time 30 min, 10 μM adrenaline, pH 7.1). The greater the ratio, the greater will be the adrenergic activity of the sodium/proton exchanger and the increase in erythrocyte pH. (c) The adrenergic net proton extrusion of rainbow trout erythrocytes (as given by the difference in sodium and chloride fluxes in the first 2 min of stimulation with 10 μM isoproterenol; (c) the net proton extrusion is inhibited by adding extracellular carbonic anhydrase (3 g l^{-1}; specific activity of the enzyme 2500 Wilbur-Anderson units/mg; CA), as a result of increased bicarbonate efflux (indicated here as increased chloride influx).

acclimation of oxygen transport in hypoxia were described in early 1970s for fish, the mechanisms by which ATP and GTP concentrations are regulated remain unclear. The suggestion that the ATP level would be adjusted as a result of direct oxygen-dependent changes in oxidative phosphorylation does not fit the profiles of oxygen dependence of ATP levels and oxidative phosphorylation. The activities of the enzymes in the catabolic or anabolic pathways of guanosine phosphates, possibly controlling GTP levels, are generally not known for fish. The rate-limiting step of GTP production may be at the level of guanosine monophosphate and its conversion to diphosphate by guanosine monophosphate kinase. The fragmentary information available suggests that the activity ratio of guanosine monophosphate kinase/adenosine monophosphate kinase is much higher in species (e.g., eel) with high GTP concentration than in those (e.g., hagfish, dogfish) with low GTP level. However, even if this is the case, the regulation of the GTP production/breakdown is unknown. What are the factors affecting anabolic/catabolic enzyme activities? Is transcriptional regulation of enzyme production involved?

Role of Erythrocytes in Regulating Capillary Function

Red cell function may also influence oxygen transport to tissues by affecting blood flow through capillaries (**see also Design and Physiology of Arteries and**

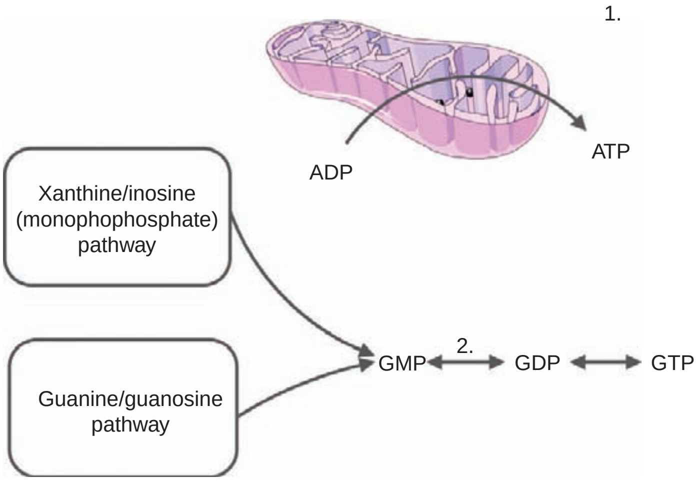

1.
ADP
ATP
Xanthine/inosine (monophophosphate) pathway
Guanine/guanosine pathway
GMP
2.
GDP
GTP

Figure 5 Schematic representation of the possible pathways of GTP and ATP metabolism. The ATP produced is mainly produced as an end product of aerobic metabolism from ADP in mitochondria (1). The GTP level may be regulated by guanosine monophosphate kinase activity (2).

Veins: Physiology of Resistance Vessels). Erythrocytes may, in this case, act as oxygen sensors. The regulation of capillary diameter may involve ATP release from the erythrocytes, which is oxygen dependent at least in mammals, nitric oxide signaling, and/or their interactions. ATP release from fish erythrocytes has been observed, but hitherto the effect of oxygen on the release has not been demonstrated.

It is generally accepted that NO plays a primary role in the regulation of capillary function. The NO-dependent regulation of capillary function may involve hemoglobin. Hemoglobin reacts with NO to form nitrosohemoglobin, which can be converted back to nitric oxide and hemoglobin, especially in hypoxic conditions, and the liberated NO can affect capillary endothelium. While most of the data available concern human erythrocytes, NO metabolism in fish erythrocytes has also been investigated. Notably, the cysteine residue cys93 of the beta-chain of hemoglobin is the site where primary nitrosohemoglobin formation occurs. Thus, fish hemoglobins with serine substituted for cysteine will not have this primary place for NO regulation.

That oxygen-dependent and NO-dependent phenomena interact has been shown in several cell types, including mammalian erythrocytes. However, the possible interactions between NO and oxygen have not been explored in detail in fish. For example, oxygen-dependent ion transport has usually been studied in conditions that virtually preclude NO production. Thus, the possibility remains that the apparently oxygen-sensitive ion transport of fish erythrocytes plays a role in the regulation of capillary function. Recently, it has also become apparent that H_2S is an important regulator of blood vessel function. At present, the importance of erythrocytes in affecting capillary H_2S concentration is not known, although it is clear that hemoglobin reacts with sulfide and that species differ markedly, both in the conversion of sulfide to the end products and in sulfide tolerance.

See also: **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Evolution of the Bohr Effect; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin.

Further Reading

Bogdanova A, Berenbrink M, and Nikinmaa M (2009) Oxygen-dependent ion transport in erythrocytes. *Acta Physiologica* 195: 305–319.

Cossins AR and Gibson JS (1997) Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells. *Journal of Experimental Biology* 200: 343–352.

Hamasaki N and Okubo K (1996) Band 3 protein: Physiology, function and structure. *Cellular and Molecular Biology* 42: 1025–1039.

Hladky SB and Rink TJ (1977) pH equilibrium across the red cell membrane. In: Ellory JC and Lew VL (eds.) *Membrane Transport in Red Cells*,pp.115–135. London: Academic Press.

Jensen FB (1989) Hydrogen ion equilibria in fish haemoglobins. *Journal of Experimental Biology* 143: 225–234.

Jensen FB (2009) The dual roles of red blood cells in tissue oxygen delivery: Oxygen carriers and regulators of local blood flow. *Journal of Experimental Biology* 212: 3387–3393.

Nickerson JG, Dugan SG, Drouin G, Perry SF, and Moon TW (2003) Activity of the unique beta-adrenergic Na^+/H^+ exchanger in trout erythrocytes is controlled by a novel beta(3)-AR subtype. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 285: R526–R535.

Nikinmaa M (1990) *Vertebrate Red Blood Cells*. Berlin: Springer.

Nikinmaa M (1992) Membrane transport and the control of haemoglobin-oxygen affinity in nucleated erythrocytes. *Physiological Reviews* 72: 301–321.

Nikinmaa M and Salama A (1998) Oxygen transport in fish. In: Perry SF and Tufts BL (eds.) *Fish Respiration*,pp.141–184. New York: Academic Press.

Nikinmaa M and Tiihonen K (1994) Substrate transport and utilization in fish erythrocytes. *Acta Physiologica Scandinavica* 152: 183–189.

Olson KR (2005) Vascular actions of hydrogen sulfide in nonmammalian vertebrates. *Antioxidants and Redox Signalling* 7: 804–812.

Ryter SW, Otterbein LE, Morse D, and Choi AMK (2002) Heme oxygenase/carbon monoxide signaling pathways: Regulation and functional significance. *Molecular and Cellular Biochemistry* 234: 249–263.

Val AL (2000) Organic phosphates in the red blood cells of fish. *Comparative Biochemistry and Physiology A – Molecular and Integrative Physiology* 125: 417–435.

Walsh PJ, Wood CM, and Moon TW (1998) Red blood cell metabolism. In: Perry SF and Tufts BL (eds.) *Fish Respiration*,pp.41–73. San Diego, CA: Academic Press.

Hemoglobin

M Nikinmaa, University of Turku, Turku, Finland

© 2011 Elsevier Inc. All rights reserved.

Hemoglobin Structure
Binding of Oxygen by Hemoglobin
Factors Affecting the Oxygen-Binding Properties of Hemoglobin
Temperature Effects
Hemoglobin Oxidation
Further Reading

Glossary

Adenosine triphosphate (ATP) An almost universal carrier of chemical bond potential energy; fish use ATP made from catabolism of foodstuff or body reserve molecules to fuel energy-dependent processes.

Allosteric regulation A regulatory molecule affects the binding of a primary molecule via binding to a different place in the protein from that of the binding site of the primary molecule.

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Guanosine triphosphate (GTP) The major erythrocytic organic phosphate of some fish. Usually its influence on hemoglobin-oxygen affinity is greater than that of ATP.

Haldane effect Proton binding to hemoglobin (as a function of oxygenation).

Heterotropic interaction Binding of a molecule affects the binding of molecules of different type.

Hill plot A logarithmic transformation of the oxygen equilibrium curve. From Hill plot chain-to-chain interaction, the oxygen affinity of the T-state and the oxygen affinity of the R-state can be seen.

Homotropic interaction Binding of a molecule affects the binding of consecutive molecules of the same kind.

Methemoglobin Oxidized hemoglobin which is unable to bind oxygen and in which heme iron is in ferric state.

Nucleoside triphosphates (NTPs) Many measurements of erythrocyte organic phosphates, originally thought to measure ATP, actually measure all NTPs.

Oxygen equilibrium curve It gives hemoglobin oxygen saturation as a function of oxygen tension.

Root effect A property of fish hemoglobin in which protons decrease the maximal oxygen saturation of hemoglobin. For practical purposes it is defined as a reduction of oxygen saturation at atmospheric oxygen tension.

R-state hemoglobin Relaxed (oxy) hemoglobin, at high oxygen tensions hemoglobin assumes relaxed state which has higher oxygen affinity than T-state hemoglobin.

T-state hemoglobin Tense (deoxy) hemoglobin, the form is the more stable hemoglobin form in the absence of oxygen (e.g., organic phosphates bind preferentially to T-state hemoglobin).

Hemoglobin Structure

The hemoglobin of most fishes is a tetrameric molecule with a molecular weight of 60 000–70 000 Da. It consists of four globin chains, two α- and β-chains. The globin chains have 140–160 amino acids and their molecular weights are between 15 000 and 17 000 Da. Every globin chain can bind one oxygen molecule. Thus, altogether four oxygen molecules can be bound, meaning that blood with fully oxygen-saturated hemoglobin contains 10–20 mM oxygen molecules, depending on the precise hemoglobin concentration of blood (**see also Blood**: Cellular Composition of the Blood).

Every globin chain contains eight helical portions (denoted A–H), which are folded on each other to form a globular tertiary structure. The folds may be either abrupt or more gradual. The α-like chain appears to be the more ancestral globin chain type. The ancestral vertebrates, hagfishes and lampreys, still have only one type of hemoglobin chain (although several different structures may be present). In these cyclostomes, hemoglobin can be either monomeric or aggregate to di- or tetramers. Notably, the single hemoglobin chain of cyclostomes is markedly different from both α- and β-chains of other vertebrates. In addition to α- and β-chains of adult hemoglobins, all vertebrates appear to have embryonic/larval (and mammals also fetal) globin chains. In teleost fish, the embryonic/larval ξ-globin chains seem to be produced in primitive erythrocytes and the adult-type globin chains in definitive erythrocytes. Tetrameric hemoglobins can be considered to be formed of two $\alpha\beta$ dimers (designated as $\alpha_1\beta_1$ and $\alpha_2\beta_2$) with rigid contacts between the chains, whereas the other existing contacts between chains ($\alpha_1\beta_2$ and $\alpha_2\beta_1$) are more flexible.

The globin chain with its 140–160 amino acids serves to make the binding of oxygen to heme iron reversible. Heme is situated in a hydrophobic pocket which is formed in the crevice lined by E, F, and G helical portions of the molecule. In all globins studied, the oxygen-binding heme group is covalently bound to a histidine residue in the F-helix (F8). Also, a phenylalanine at the interface of C and D helices (Phe CD1) is present in all studied globins. The phenylalanine is situated at the entrance of heme pocket, and makes an important contribution to its hydrophobic nature. In almost every vertebrate hemoglobin, the interaction of oxygen and heme iron is regulated also by a histidine residue in the E-helix (E7). A notable exception is hagfish, which has glutamine in this position. A change of only one or a few amino acids in the globin chains is required to change the three-dimensional structure of the hydrophobic heme pocket so that the access of oxygen to heme iron is affected, whereby the oxygen-binding properties of hemoglobin will be changed.

A major feature of teleost fish hemoglobins is their multiplicity. In some species, more than 10 hemoglobin components can be separated electrophoretically. The electrophoretically different hemoglobins may have different functional properties, as exemplified by the eel and rainbow trout. The cathodically migrating hemoglobins are characterized by a reverse Bohr effect (at pH range 7–8 in the absence of organic phosphate binding), whereas the anodic hemoglobins have a marked Bohr effect.

Another important feature of teleost fish hemoglobins is that they have very few titratable histidines in the globin chains (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). Whereas teleost fish have four to five histidines per globin chain, the other vertebrates including elasmobranch fish typically have double this amount (e.g., the dogfish *Squalus* has nine in the α-chain and 10 in the β-chain). Thus, whereas most tetrameric hemoglobins have more than 35 histidine residues, teleost hemoglobins usually have less than 25. In addition to teleost fish, lampreys similarly have very few histidine residues in the single globin chain (e.g., the major hemoglobin component of *Petromyzon marinus* has only two, those in the heme pocket). The small number of titratable histidines is associated with a large dependence of intraerythrocytic pH on oxygen tension (large Haldane effect). With a decrease of oxygen tension from atmospheric (i.e., 150–160 torr) to zero pressure, the intraerythrocytic pH may increase by more than 0.3 units.

Fish hemoglobins characteristically have serine substituted for cysteine in the position 93 (F9) of the β-globin chain. This substitution was originally thought to be an important reason for the Root effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect), but now the cysteine residue is considered to be the primary site of nitrosohemoglobin formation. Fish hemoglobins also have the end-terminal amino acid of the α-chain acetylated. Because this prevents direct carbon dioxide binding to the residue, carbamino formation in fish hemoglobins is markedly reduced (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion).

Binding of Oxygen by Hemoglobin

The oxygen binding by hemoglobin is normally described using the oxygen equilibrium curve (OEC, or oxygen dissociation curve; **Figure 1**). In the curve, the relationship is shown between the percentage (or fractional) saturation of hemoglobin with oxygen (*y*-axis) and the (blood) oxygen tension (*x*-axis). Often, the Hill plot (**Figure 2**) is used to describe the oxygen binding by hemoglobin. The Hill plot is a logarithmic transformation of the oxygen equilibrium curve. The oxygen equilibrium of hemoglobin can be described using the empirical equation:

$$S/(1-S) = K_A P^n_{O2}$$

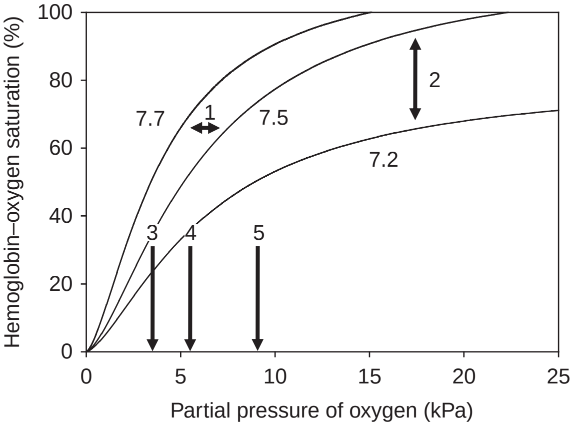


Figure 1 Oxygen equilibrium curve (OEC, or oxygen dissociation curve, ODC). The *y*-axis gives the oxygen saturation of hemoglobin (either in % or as fractional saturation) and *x*-axis the partial pressure of oxygen. Three hypothetical oxygen equilibrium curves (at pH 7.7, 7.5, and 7.2) are drawn. Arrow 1 indicates Bohr effect, i.e., a pH-induced decrease of hemoglobin–oxygen affinity without a decrease in maximal oxygen saturation. Arrow 2 indicates Root effect, i.e., a pH-induced decrease in the apparent maximal oxygen saturation of hemoglobin. Arrows 3, 4, and 5 indicate P_{50} values for the three oxygen equilibrium curves. P_{50} value is the partial pressure of oxygen at which hemoglobin is 50% saturated, and is commonly used to describe the oxygen affinity of hemoglobin.

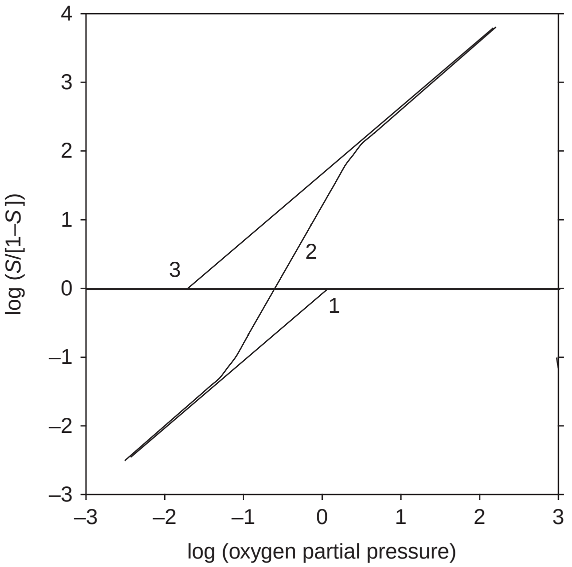


Figure 2 Hill plot. A logarithmic transformation of the oxygen equilibrium curve. The lower portion of the plot, which has a slope of 1, shows oxygen binding to the T-state hemoglobin. The numerical value of this, P_{50} value for T-state hemoglobin, is obtained by extending the plot to 50% oxygen saturation of hemoglobin (log(*S*/[1 − *S*]) = 0, where *S* is fractional oxygen saturation (1). The slope of the middle part of the plot gives the *n* (=Hill constant) (2), which indicates the cooperativity of oxygen binding (i.e., interaction between globin chains). The upper part of the plot has a slope of one, and shows the oxygen binding to the R-state hemoglobin, the numerical value of which can be obtained by extending the plot to 50 % oxygen saturation (3).

where S is the fractional oxygen saturation of hemoglobin, K_A the association constant for the overall hemoglobin-oxygen reaction, P_{O_2} the blood (solution) oxygen tension, and n the Hill coefficient. The Hill coefficient numerically represents the extent of the sigmoidal nature of the oxygen equilibrium curve, with higher n values reflecting more sigmoid oxygen equilibrium curves. The sigmoidicity of OEC indicates interaction between oxygen binding sites, for example, binding of oxygen to one site increases the likelihood of oxygen binding to the following site(s). The Hill coefficient gives an indication of the number of interacting oxygen binding sites (individual hemoglobin chains). The oxygen affinity of hemoglobin is often described using the P_{50} value instead of the whole OEC (P_{50} refers to oxygen partial pressure at which hemoglobin is 50% oxygen saturated). This value gives the oxygen tension at which hemoglobin is 50% saturated with oxygen. The two values, P_{50} and n, give a reasonable description of the oxygen-binding behavior of hemoglobin.

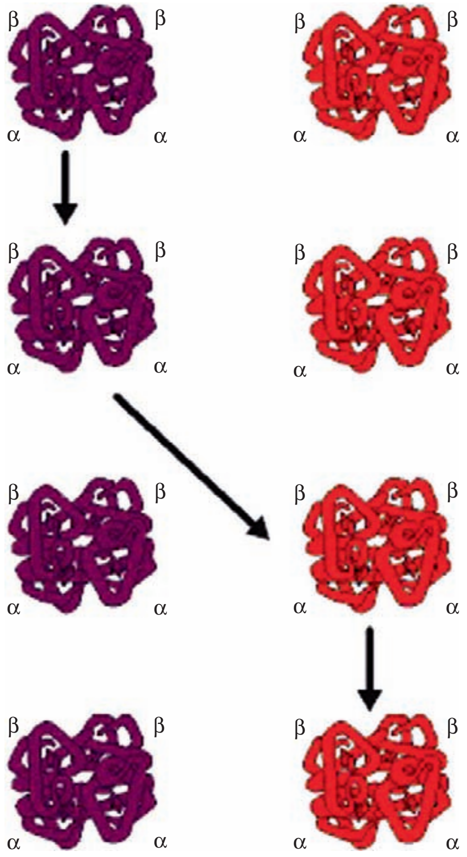


Figure 3 A schematic representation of T- and R-state hemoglobins. In the absence of oxygen, the probability of hemoglobin being in T-state (molecules on the left) is at least 10^6 times greater than that of the R-state (molecules on the right). Binding of organic phosphates (ATP and GTP; binding to a crevice between β-chains of T-state) and protons stabilizes the T-state as the bonds keeping the molecule in T-state are strengthened. Binding of oxygen first to hemes of α-globin chains increases the probability of occurrence of R-state hemoglobin. When an adequate amount of oxygen has bound (often after binding of two molecules of oxygen), the probability of the occurrence of the R-state becomes greater than that of the T-state. Arrows indicate the probable conformational changes of hemoglobin molecule upon oxygenation.

As a simplification, hemoglobin can exist in two conformations: tense (T-) or deoxy state and relaxed (R-) or oxy state (**Figure 3**). In actual fact, many intermediate conformations occur, that is, the T-form and R-form assume different conformations depending on the amount of bound oxygen. In simple terms, binding of oxygen by hemoglobin can be described in the following manner. When no oxygen is bound, virtually all hemoglobin molecules are in T-state, which has low oxygen affinity. In the Hill plot, this is seen as the lower asymptote of the plot. The oxygen affinity (P_{50} value) of T-state hemoglobin is obtained by extending the asymptote to the 0-value of $\log[S/(1-S)]$. Initially, oxygen seems to bind solely to α-chains, which have significant oxygen affinity also in the T-state hemoglobin. Binding of oxygen (to T-state) hemoglobin causes strain on the molecule, which is relieved when the molecule is converted from T-state to R-state. In most cases, this occurs when two molecules of oxygen have been bound to hemoglobin (i.e., α-chains have bound oxygen). In the Hill plot this is seen as a marked increase in the slope of the line describing the oxygen binding. At this time (10–90% oxygen saturation), the slope of Hill plot gives the n value, which indicates the number of interacting chains. Most often, n_{50} (i.e., Hill coefficient at 50% oxygen saturation of hemoglobin) is given as a measure of chain-to-chain interactions. At high oxygen saturations, any further oxygen-binding takes place to the R-state molecule. As a result, there is no interaction between globin chains (i.e., binding of oxygen to one site does not affect the structure of any other chains so that binding of oxygen to them would be affected), whereby the slope of Hill plot is one. The oxygen affinity (P_{50} value) of R-state hemoglobin is again obtained by extending the asymptote to the 0-value of $\log[S/(1-S)]$.

Factors Affecting the Oxygen-Binding Properties of Hemoglobin

The effect of oxygen on the binding of consecutive oxygen molecules is an example of homotropic interactions, where a molecule affects the binding of consecutive molecules of the same type. Although homotropic interactions are important in influencing the oxygen-binding properties of hemoglobin, physiological regulation of hemoglobin function relies mainly on heterotropic interactions, where different molecules affect the oxygen binding behavior. A prime example of heterotropic interactions is the binding of organic phosphates to a site which is different from the oxygen-binding site. The binding of organic phosphates to their binding site tends to affect the structure of the oxygen-binding site so that the accessibility of oxygen to and from the site (and thereby oxygen-binding properties) is affected. This constitutes an example of allosteric effect. Because hemoglobin assumes two major conformations which have different oxygen affinities, the effect of different factors on oxygen binding behavior can be reduced to the factors affecting the probability of the occurrence of the different conformations.

Proton Effects

The three-dimensional structure of hemoglobin is generated and maintained mainly by salt bridges, hydrogen bonds, and van der Vaals bonds. Protons affect all of these bonds, thus being major effectors of the three-dimensional structure of hemoglobin molecule. The structural effects of protons stabilize the T- (deoxy) state of hemoglobin. Thus, at physiological pH values a decrease in pH shifts the OEC to the right, that is, decreases hemoglobin-oxygen affinity (increases P_{50} value; **Figure 1**). This is called the (alkaline) Bohr effect. The effect was originally observed more than 100 years ago as the effect of carbon dioxide on hemoglobin-oxygen affinity and thus actually predates the formulation of pH concept. The Bohr effect is described in detail elsewhere (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect), so only the basic principles are given here. The magnitude of the Bohr effect is usually given by the Bohr constant ($\text{BC} = \Delta\log P_{50}/\Delta\text{pH}$), which varies greatly from positive values in the absence of organic phosphates in the cathodically migrating eel and trout hemoglobins to negative values exceeding −1, using P_{50} values in torr (=mmHg). In general, the Bohr effects of teleost fish and lamprey hemoglobins are large and those of elasmobranchs and hagfish small. Whereas in tetrameric teleost fish hemoglobins the Bohr effect must be due to proton binding to specific residues, and consecutive effects on oxygenation; in lampreys, it is due to oxygenation-linked aggregation of hemoglobin chains. There appears to be a negative correlation between the number of histidine residues and Bohr effect: the Bohr effect is large if the number of histidine residues per globin chain is small. The strength of the Bohr effect can also be given as the number of protons taken up upon deoxygenation. This is usually called the Haldane effect, and is an example of linked functions (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Whereas human and many mammalian hemoglobins take up maximally two protons per molecule, teleost fish hemoglobins maximally take up four. While most of the residues involved in generating the Bohr effect in fish still remain unknown, the role played by the C-terminal histidine residue of the β-chain is clear. In the cathodic hemoglobins of eel and rainbow trout, which are essentially devoid of Bohr effect, this residue is replaced by phenylalanine. In Bohr-effect hemoglobins, the hemoglobin can be fully saturated with oxygen at atmospheric oxygen tension. The Hill coefficient is, furthermore, pH insensitive.

In addition to the Bohr effect, a pronounced effect of protons in fish hemoglobins is the Root effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems and Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). In Root effect hemoglobins (**Figure 1**), full oxygen saturation is not reached, and the Hill-coefficient value decreases with decreasing pH, ultimately to values below 1. The simplest explanation for this is that binding of oxygen to the (α-chains of) T-state hemoglobin causes conformational changes to the molecule that prevent oxygen binding to the remaining β-chains. So far, the amino acid residues involved in the extreme stabilization of T-state in the Root effect hemoglobins have not been clarified, but the available information suggests that amino acids in the $\alpha_1\beta_2$-contact region may play a role.

Anion, Particularly Organic Phosphate Effects

Anions bind to the cavity between the β-chains of the T-state in tetrameric hemoglobins. Hagfish and lampreys are devoid of anion effects, because their hemoglobins do not form stable tetramers. The effectiveness of binding increases with the number of (hydrogen) bonds that the compound can form with the hemoglobin. The most important anions affecting hemoglobin function in fishes are the organic phosphates adenosine triphosphate (ATP) and guanosine triphosphate (GTP). In the hemoglobin of rainbow trout, the amino acid residues involved in organic phosphate binding have been clarified and involve the amino terminal glutamic acid, a lysine in the EF transition (EF6) and arginine in the H-helix (H21). Since GTP can form one hydrogen bond more than ATP, its influence on hemoglobin function is greater than that of ATP. In some fish hemoglobins, however, the effect of ATP is as great as that of GTP. While the structural basis of this is unknown, it is possible that the structure of the cavity between β-chains is affected.

The binding of organic phosphates stabilizes the T-state of hemoglobin. Thus, an increase in nucleoside triphosphate (NTP) concentration decreases the hemoglobin-oxygen affinity (**Figure 4**). In addition to affecting the hemoglobin-oxygen affinity, the binding of organic phosphates to hemoglobin increases *n* value.

Temperature Effects

Binding of oxygen to hemoglobin is exothermic. The apparent heat of oxygenation (ΔH_{app}) is given by the equation: $\Delta H_{\text{app}}=2.303R(\Delta \log P_{50}/\Delta[1/T])$. An increase in temperature will decrease hemoglobin-oxygen affinity of most hemoglobins. The apparent heat of oxygenation includes the actual heat of heme oxygenation and also the heat of solution of oxygen in water, the heats of conformational changes, and heats of ionization (proton and anion binding and release, including organic phosphate binding and release). This being the case, the apparent heats of oxygenation of pure hemoglobin solutions and hemoglobin in blood can be markedly different. In the latter, the large endothermic release of Bohr protons and of organic phosphates when oxygen is bound decreases the overall apparent heat of oxygenation. This indicates that there is a need to study both isolated hemoglobins and native red blood cells to gain a full understanding of how and why temperature affects the oxygen equilibria of hemoglobin.

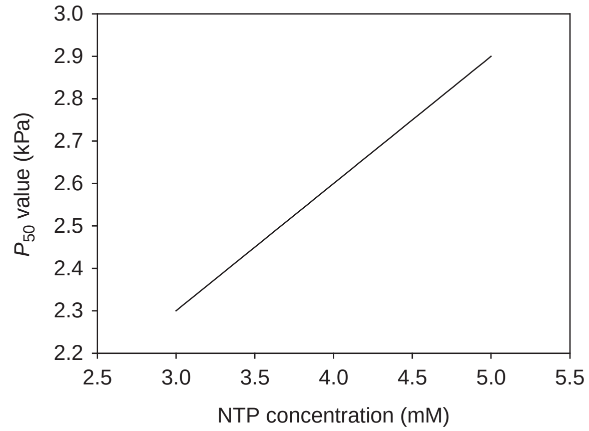


Figure 4 The relationship between hemoglobin-oxygen affinity (as given by the P_{50} value) and organic phosphate concentration in rainbow trout. Figure based on data from Soivio A, Nikinmaa M, and Westman K (1980). The blood oxygen binding properties of hypoxic *Salmo gairdneri*. *Journal of Comparative Physiology B* 136: 83–87.

The hemoglobin–oxygen affinity of some tunas and some sharks is either temperature insensitive, or their hemoglobin–oxygen affinity may even increase with increasing temperature. The fish with the overall lack of heat of oxygenation of hemoglobin or reversed temperature dependency of oxygenation are warm-bodied fishes. As a result of the nonexistent or even reversed temperature dependence of hemoglobin–oxygen affinity, these hemoglobins will not lose oxygen despite the marked temperature gradient (up to 20 °C from the peripheral tissues to the core). The hemoglobins of some tunas, the best-known warm-bodied fishes, have exothermic apparent heat of oxygenation when the first oxygen molecules are bound to hemoglobin, virtual lack of temperature dependence of oxygen binding at near 5% hemoglobin-oxygen saturation, and endothermic reaction when the last oxygen molecules bind. The endothermic reaction may be the result of pronounced endothermic proton release when the last oxygen molecules are bound to hemoglobin. In some species, marked reduction of temperature dependency of oxygen binding is achieved via organic phosphates: in the presence of ATP the oxygen affinity of hemoglobin is temperature insensitive or may even increase with increasing temperature. A physiological role of temperature-independent oxygen binding (or its reversed temperature dependency) for warm-bodied (heterothermic) fish is to minimize the loss of oxygen (from hemoglobin) during temperature increase (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks).

Hemoglobin Oxidation

The reversible binding of oxygen by hemoglobin requires that the heme iron is unstable in the ferric state. In free heme groups, iron is readily oxidized to ferric iron. In simplified terms, one can state that the major reason for embedding the heme in the protein moiety is to prevent irreversible oxidation. The less hydrophobic the heme environment is (i.e., the higher its oxygen affinity), the more likely the oxidation to ferric iron is. Of the specific amino acid residues the highly conserved histidine in the position E7 plays a major role in protecting the heme iron from oxidation.

The oxidation of heme iron results from an attack of nucleophiles. In undisturbed conditions the biologically most important nucleophiles are water and hydroxyl ions. Autoxidation occurs preferentially, if hemoglobin is in the deoxy-(T-)state. Because His(E7) both inhibits the access of water in the heme pocket and stabilizes heme–oxygen interaction after it has occurred, the major causes of hemoglobin oxidation are counteracted. The rate of hemoglobin autoxidation increases with increasing temperature, and probably with decreasing pH. Also, various chemicals such as nitrite cause hemoglobin oxidation. Notably, in contrast to autoxidation, nitrite-induced oxidation occurs more readily in oxy- than in deoxyhemoglobin.

The oxidized hemoglobin (called methemoglobin) has a three-dimensional structure that resembles the R-state more closely than the T-state. Similarly, partial oxidation shifts the equilibrium between T- and R-state of hemoglobin toward the R-state, whereby the oxygen affinity of the nonoxidized hemoglobin chains increases.

See also: **Blood**: Cellular Composition of the Blood. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Evolution of the Bohr Effect; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M (2006) Evolution of vertebrate haemoglobins: Histidine side chains, specific buffer value and Bohr effect. *Respiratory Physiology and Neurobiology* 154: 165–184.

Berenbrink M, Koldkjær P, Kepp O, and Cossins AR (2005) Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Brauner CJ and Berenbrink M (2007) Gas transport and exchange. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 26, Primitive Fishes*, pp. 213–282. San Diego, CA: Academic Press.

Brittain T (2005) Root effect hemoglobins. *Journal of Inorganic Biochemistry* 99: 120–129.

Dickerson RE and Geis I (1983) *Hemoglobin: Structure, Function, Evolution and Pathology,* 176pp. Menlo Park, CA: Benjamin Cummings.

Jensen FB (2004) Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. *Acta Physiologica Scandinavica* 182: 215–227.

Jensen FB, Fago A, and Weber RE (1998) Hemoglobin structure and function. In: Perry SF and Tufts BL (eds.) *Fish Physiology, Vol. 17: Fish Respiration*, pp. 1–40. San Diego, CA: Academic Press.

Nikinmaa M (1990) *Vertebrate Red Blood Cells,* 262pp. Berlin: Springer.

Nikinmaa M (1997) Oxygen and carbon dioxide transport in vertebrate erythrocytes. An evolutionary change in the role of membrane transport. *Journal of Experimental Biology* 200: 369–380.

Nikinmaa M (2001) Haemoglobin function in vertebrates: Evolutionary changes in regulation in hypoxia. *Respiratory Physiology* 128: 317–329.

Nikinmaa M (2006) Gas transport. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 153–174. Boca Raton, FL: CRC Press, and Taylor and Francis.

Perutz M (1990) *Mechanisms of Cooperativity and Allosteric Regulation in Proteins,* 101pp. Cambridge: Cambridge University Press.

Perutz MF (1990) Mechanisms regulating the reactions of human haemoglobin with oxygen and carbon monoxide. *Annual Review of Physiology* 52: 1–25.

Riggs AF (1988) The Bohr effect. *Annual Review of Physiology* 50: 181–204.

Val AL (2000) Organic phosphates in the red blood cells of fish. *Comparative Biochemistry and Physiology Part A* 125: 417–435.

Weber RE (2000) Adaptation for oxygen transport: Lessons from fish. In: di Prisco G, Giardina B, and Weber RE (eds.) *Hemoglobin Function in Vertebrates. Molecular Adaptation in Extreme and Temperate Environments*, pp. 24–37. Rome: Springer.

Weber RE and Fago A (2004) Functional adaptation and its molecular basis in vertebrate hemoglobins, neuroglobins and cytoglobins. *Respiratory Physiology and Neurobiology* 144: 141–159.

Weber RE and Jensen FB (1988) Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Review of Physiology* 50: 161–179.

Wells RMG (2009) Blood–gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. In: Richards JF, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 27, Hypoxia, Richards*, pp. 255–299. San Diego, CA: Academic Press.

Wyman J, Jr. (1964) Linked functions and reciprocal effects in hemoglobin: A second look. *Advances in Protein Chemistry* 19: 223–286.

O_2 Uptake and Transport: The Optimal P_{50}

T Wang and H Malte, Aarhus University, Aarhus, Denmark

© 2011 Elsevier Inc. All rights reserved.

Introduction
Conclusions
Appendix
Further Reading

Glossary

Capacitance coefficient Formally defined as $\Delta C/\Delta P$, where C is concentration and P is the partial pressure of a gas. If the gas is present exclusively in physical solution, this is identical to the solubility of the gas.

Convection Bulk movement of a medium, e.g., gas, water, or blood.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Introduction

A primary function of the blood in fish is to transport oxygen (O_2) from the gills to the metabolizing tissue where respiration takes place (**see also Tissue Respiration**: Cellular Respiration and Mitochondrial Respiration). The convective transport of O_2 by the blood is performed by the cardiovascular system, and the vast majority of O_2 is transported by hemoglobin through reversible binding to the iron moiety (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). The uptake of O_2 from the water across the gills and the delivery of O_2 from the systemic capillary to the mitochondria are driven by diffusion alone, and diffusion is solely governed by differences in the O_2 partial pressure (P_{O_2}) (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). Thus, at the gills, a high blood-O_2 affinity would maintain a high P_{O_2} gradient across the branchial epithelium and thereby facilitate loading. Conversely, a low blood-O_2 affinity would serve to elevate the P_{O_2} in the capillary and thereby increase O_2 delivery to the tissue (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Thus, the optimal blood-O_2 affinity reflects a compromise that simultaneously must meet the demands of O_2 loading at the gills and unloading at the tissues. A theoretical optimal blood-O_2 affinity is calculated by mathematical modeling of the gas-exchange system as described below. However, a serious caveat of this approach is the lack of complete data sets, and some of the input parameters for the models accordingly must be estimated based upon a number of assumptions.

The Blood-Oxygen Equilibrium Curve

Blood O_2 binding is usually described by the relationships between the blood P_{O_2} and O_2 concentration or hemoglobin-O_2 saturation (HbO_2 saturation), where the latter is expressed as percentage of the O_2-carrying capacity of the hemoglobin (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). A typical blood-O_2 equilibrium curve (OEC) is shown in **Figure 1**. As seen from this figure, there is a sigmoidal relationship between O_2 concentration and P_{O_2}, due to cooperativity in O_2 binding, and it is also apparent that the maximal change in O_2 concentration occurs slightly below the P_{O_2} that results in 50% saturation of the hemoglobin (P_{50}). The P_{50} of blood is often used to quantify blood-O_2 affinity. This P_{50} value is physiologically significant because a great deal of O_2 can be unloaded at the tissues with only a small change in P_{O_2} (steep part of the OEC). Therefore, fairly constant conditions for O_2 unloading are maintained. The sigmoidal shape of the OEC helps to increase the P_{O_2} gradient for both O_2 loading at the gills and O_2 unloading within the capillaries and represents a functionally important property of blood-O_2 binding.

It is well established that the O_2 affinity of a given species is determined by the intrinsic functional properties of the hemoglobin molecule as well as by the intra-erythrocytic concentrations of numerous effectors

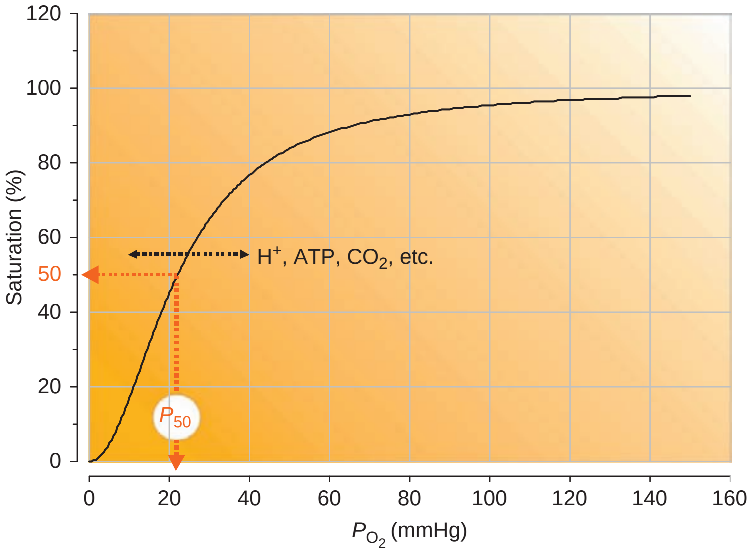


Figure 1 A typical sigmoidal O_2-equilibrium curve (OEC). The P_{50} is the partial pressure of O_2 at which hemoglobin is 50% saturated with O_2. The position (and thus P_{50}) of the O_2-equilibrium curve can be modified by variation in the intraerythrocytic concentration of certain effectors, such as H^+, CO_2, or ATP (or GTP), that bind to hemoglobin.

that modify the intrinsic properties by binding to hemoglobin. The most notable of these effectors are protons (H^+), CO_2 and, not the least, organics phosphates, which, in fish, are primarily adenosine triphosphate (ATP) and guanosine triphosphate (GTP). (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). Both ATP and GTP reduce blood-O_2 affinity and provide a means for the fish to alter the blood-O_2 binding characteristics without having to express other hemoglobin molecules with different intrinsic binding properties. Many studies on a variety of fish species show that blood-O_2 affinity increases within hours after exposure to hypoxic conditions due to a reduction in red-blood-cell ATP and/or GTP levels. These reductions in organic phosphates facilitate O_2 uptake at the gills in response to a reduction in environmental O_2 levels.

The blood-O_2 affinity varies considerably among different fish species. As a general trend, fish inhabiting waters that are either chronically hypoxic, or where hypoxia occurs on a regular basis, tend to have higher blood-O_2 affinities than fishes inhabiting well-oxygenated environments. An increased blood-O_2 affinity in a hypoxic environment ensures high arterial-O_2 saturation and hence is expected to enhance systemic O_2 delivery.

Many studies have described the blood-O_2 affinity in fish, and there are numerous interpretations regarding how the specific O_2 affinity may represent an adaptation to various environments or behaviors. While most of these interpretations are intuitively appealing, it must be emphasized that they remain largely untested and hence not verified. As evident from the mathematical model presented below, the functional correlates of blood-O_2 affinity in a given species can only be evaluated if other physiological parameters, such as the diffusive capacities of the gills and the systemic circulation as well as cardiac output are included in the analysis. As a caveat, it should also be remembered that the blood-O_2 affinity may be correlated with physiological functions other than O_2 delivery *per se*. For example, in some endothermic fish, such as some sharks and tunas, temperature effects may interact to determine functional characteristics of blood-O_2 binding.

The Optimal Blood-Oxygen Binding Affinity

Because O_2 consumption of resting animals is likely to be independent of O_2 delivery, and hence P_{O_2} of the capillary blood, it is reasonable to argue that the exact position of the OEC, within a reasonable range of blood-O_2 affinities, has no influence on resting metabolism. However, when metabolism and the cellular need for O_2 increase, O_2 delivery becomes a limiting factor. Thus, it is not unreasonable to assume natural selection selected for an O_2 affinity that maximizes O_2 delivery during elevated metabolic rate and hence enables the maximal rate of O_2 consumption ($\dot{V}_{O_2max}$) possible. As outlined below, it is relatively straightforward to model $\dot{V}_{O_2max}$.

The O_2-transport cascade of fish can be described as a schematic of four steps, as illustrated in **Figure 2** (**see also Ventilation and Animal Respiration**: The Effect of Exercise on Respiration). As the first step,

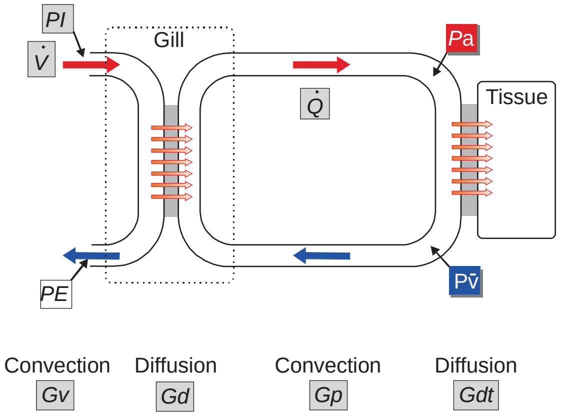


Figure 2 A schematic representation of the O_2-transport cascade from the surrounding water to the mitochondria where respiration takes place. Oxygen is transported in four steps, alternately by convection and diffusion, as indicated. Water is transported to the gill with a flow ($\dot{V}$) and a conductance, (*Gv*). Diffusion from water to blood is characterized by a diffusion conductance (*Gd*). The blood O_2 is transported with a blood flow ($\dot{Q}$) and a conductance (*Gp*), and finally diffusion from the blood to the tissue is characterized by a conductance (*Gdt*). Water enters the gills with an inspired partial pressure (*P*I) and leaves with an expired partial pressure (*P*E). In the model the arterial and mixed-venous partial pressures (*P*a and Pv, respectively) as well as PE are all dependent variables.

water is transported by convection across the gills (gill ventilation (Gv)), followed by diffusion across the gills to the blood (Gd) (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). Then, blood is convectively transported by the cardiovascular system to the peripheral circulation in the tissue (Gp), where the final diffusive step takes place as the O_2 reaches the mitochondria of the respiring cells (**see also Tissue Respiration**: Cellular Respiration and Mitochondrial Respiration).

A Quantitative Model of the Oxygen-Transport Cascade to Predict the Optimal Blood-Oxygen Affinity

To specifically evaluate the optimal blood-O_2 affinity (optimal P_{50}) to maximize $V_{O_2\text{max}}$ in fish, we constructed a simple mathematical model. This model incorporates a counter-current exchanger in the gills (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs), convective O_2 transport within the blood (**see also Ventilation and Animal Respiration**: The Effect of Exercise on Respiration) and diffusion from blood to tissue. We modeled how $V_{O_2\text{max}}$ is influenced by P_{50} and sigmoidicity of the OEC at several levels of inspired P_{O_2}. The equations used for the model are explained in the Appendix section, which also includes an overall description of the iterative procedure used to solve the various equations simultaneously. Input parameters consisted of data from rainbow trout (*Oncorhynchus mykiss*) – the only species of teleost studied in sufficient detail to provide the necessary physiological parameters. The specific parameters used in the model are listed in **Table 1**.

The results of the model are shown in **Figure 3**, where $V_{O_2\text{max}}$ is depicted as a function of P_{50} for various inspired P_{O_2} values in the two upper panels. The predictions during severe hypoxia (P_{O_2} of 10 and 20 mmHg) are expanded in the upper right-hand panel. The analysis clearly shows that $V_{O_2\text{max}}$ decreases with a reduction in inspired P_{O_2} as the level of hypoxia becomes more severe, which is consistent with *in vivo* observations. At all levels of inspired O_2, very high O_2 affinity (as indicated by a low P_{50}) causes $V_{O_2\text{max}}$ to decrease, while $V_{O_2\text{max}}$ only decreases a little as P_{50} increases above physiologically relevant values. As inspired O_2 levels are decreased, the maximal $V_{O_2\text{max}}$ occurs at lower P_{50} values showing that increased O_2 affinity does safeguard O_2 delivery, but there is still a wide plateau, where P_{50}

Table 1 Constants used in the calculations of the effect of P_{50} (P_{O_2} at which hemoglobin is 50% saturated) on maximal oxygen uptake in rainbow trout

Name	*Symbol*	*Unit*	*Value*
Hemoglobin concentration	C_{Hb}	mmol L^{-1}	1.0
Capacitance coefficient, water	βw_{O_2}	µmol L^{-1} $mmHg^{-1}$	2.24
Capacitance coefficient, blood	βb_{O_2}	µmol L^{-1} $mmHg^{-1}$	1.99
Diffusion conductance, gill	*Gd*	µmol min^{-1} kg^{-1} $mmHg^{-1}$	1.60
Diffusion conductance, tissue	*Gdt*	µmol min^{-1} kg^{-1} $mmHg^{-1}$	4.64
Cardiac output	$\dot{Q}$	mL min^{-1} kg^{-1}	53
Ventilation	$\dot{V}w$	mL min^{-1} kg^{-1}	1730

Cardiac output, ventilation, and hemoglobin concentration are from measurements on rainbow trout performing maximal exercise at 10 °C and the capacitance coefficients (solubilities), are literature values reported for 10 °C. The diffusion conductances, *Gd* and *Gdt,* given in the table are result of a preliminary test in which their values were adjusted so that the arterial and venous P_{O_2} values, coming out of the model using a P_{50} of 22 mmHg, corresponded roughly to the values measured on rainbow trout performing maximal exercise at 10 °C.

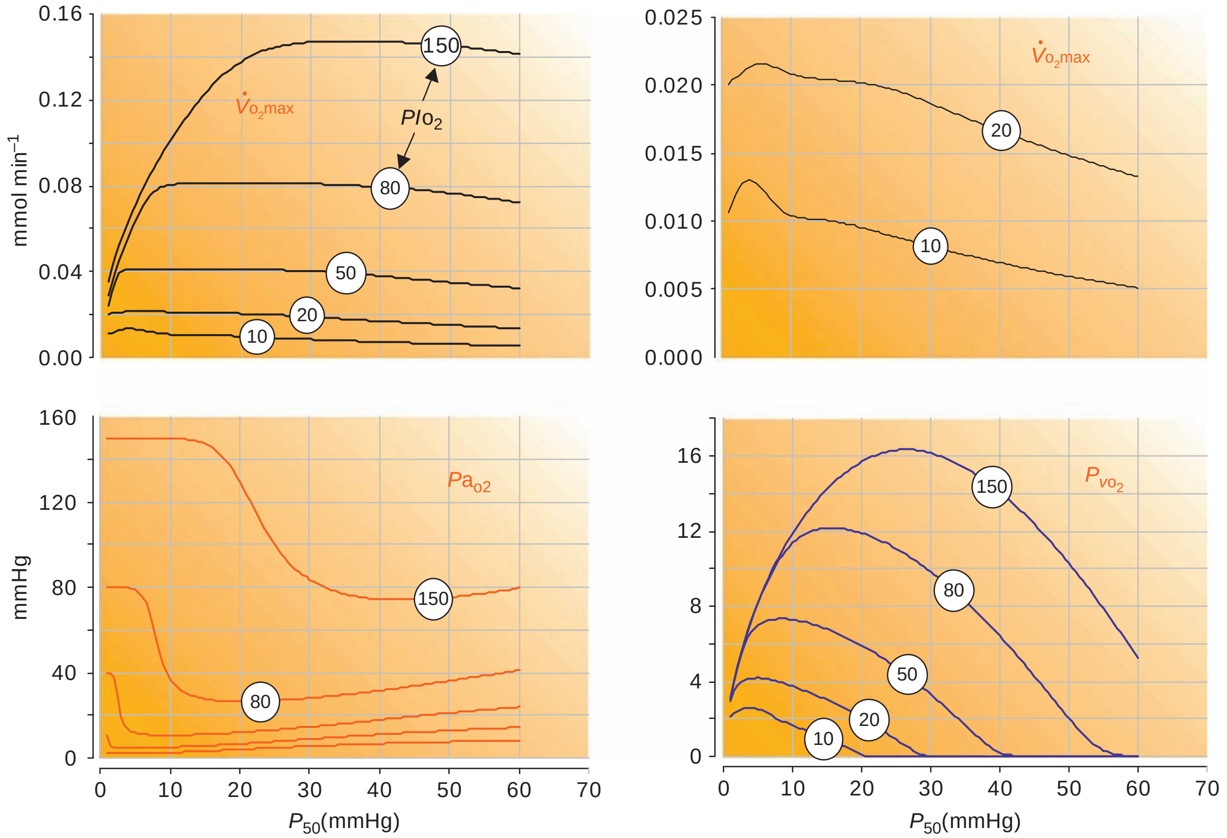


Figure 3 The effect of blood P_{50} (mmHg) on maximal O_2 consumption rate (V_{O_2max}), and resulting arterial and mixed-venous O_2 partial pressures (Pa_{O_2} and Pv_{O_2}) calculated using the model in the appendix. V_{O_2max}, Pa_{O_2} and Pv_{O_2} were calculated at a number of inspired O_2 partial pressures (PI_{O_2}), where the PI_{O_2} is indicated by numbers contained within small circles that refer to each entire respective curve.

has little effect on the predicted V_{O_2max}. The underlying mechanisms for the effects of P_{50} on V_{O_2max} can be understood, at least in part, from the calculated changes in arterial and venous P_{O_2} values shown in the two lower panels. As P_{50} increases, the P_{O_2} gradient over the gills is reduced and the blood does not reach full equilibrium with the water, which is evident from the fall in arterial P_{O_2} as P_{50} increases. This relationship reflects that low blood-O_2 affinity (high P_{50}) impairs O_2 loading at the gills, while the simultaneous reduction in venous P_{O_2} shows how unloading in the tissues benefits from the reduced blood-O_2 affinity. The low venous P_{O_2} values at the very high affinities (low P_{50}) are a consequence of the high affinity, so that venous O_2 concentration actually remains high (not shown in the figure).

It is important to realize that the arterial and venous O_2 levels (i.e., saturation, P_{O_2}, and concentration) are dependent variables of all the input parameters. Thus, the model ignores the normal physiological regulation of arterial blood gases, which, at first, may be regarded as a limitation of the model. However, we model only maximal O_2 uptake, and therefore there is no room for regulation of arterial blood gases – everything is at its maximum. Despite the great variability in the partial pressures of the arterial gases as P_{50} changes, there is remarkably little change in maximal O_2 uptake.

Conclusions

The overall conclusion of the model is that the optimal P_{50} decreases with inspired P_{O_2}. However, the optimum is very broad, and P_{50} only modestly influences the predicted V_{O_2max}. The model also shows that the relationship between P_{50} and V_{O_2max} is asymmetrical, such that V_{O_2max}, at most levels of inspired P_{O_2}, declines more at low, relative to high P_{50} values. Only in severe hypoxia ($P_{O_2} < 20$ mmHg; approximately the P_{50} of the blood used in these calculations) is there a pronounced effect on V_{O_2max} of increasing blood-Oxygen affinity (decreasing P_{50}).

Our analysis shows that a P_{50} below 25 mmHg reduces V_{O_2max} in normoxic water, but that an increased affinity does confer advantages in severe hypoxia. This pattern is consistent with the general finding that active fish with high $V_{O_2\mathrm{max}}$ tend to have lower blood-O_2 affinities than hypoxia-tolerant fish.

Appendix

The Equations Used to Predict the Optimal Blood-Oxygen Affinity

At the respiratory surface of the gill, the exchange of O_2 is described by two differential equations – one for water and one for blood:

$$\frac{\mathrm{d}Pw}{\mathrm{d}l} = \frac{Gd}{Gv}(Pw - Pb) \qquad (1a)$$

$$\frac{\mathrm{d}Pb}{\mathrm{d}l} = \frac{Gd}{Gp}(Pw - Pb) \qquad (1b)$$

Here, Pw and Pb are the P_{O_2} values in water and blood, respectively. l refers to the length coordinate along the exchange surface and Gd, Gp, and Gv are the diffusion, perfusion, and ventilation conductances, respectively. These equations are derived from mass balance and simply express that, at any point and on either side of the exchanger, the change in P_{O_2} along an infinitesimal length of surface is proportional to the amount of O_2 diffusing across the barrier (for a more detailed account of this and of the meaning of the different terms **see Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs)

The diffusion conductance is given by

$$Gd = \frac{KA}{t} \qquad (2)$$

Here K is Krogh's diffusion constant, A the area and t the thickness. In this model, we fix Gd at a constant and maximal value. The ventilation conductance is given by

$$Gv = \dot{V}w\beta w \qquad (3)$$

where $\dot{V}w$ is the ventilation volume and βw_{O_2} is the capacitance coefficient of O_2 in water (also called solubility). In the following calculations, we fix this at a constant and maximal value. The perfusion conductance is defined in a similar manner

$$Gp = \dot{Q}\beta b \qquad (4)$$

where $\dot{Q}$ is cardiac output and βb the O_2 capacitance coefficient of blood. Due to the inherently nonlinear nature of the O_2-equilibrium curve the latter is, however, not a constant but depends on the blood's P_{O_2}, Pb. Formally it is defined as the slope of the relation between concentration and partial pressure, that is,

$$\beta b = \frac{\mathrm{d}C_b}{\mathrm{d}P_b} \qquad (5)$$

The relation between hemoglobin-O_2 saturation and P_{O_2} is most conveniently described by the Hill equation, which gives a satisfactory description of the relationship with only two constants:

$$Sb = \frac{Pb^n}{P_{50}^n + Pb^n} \qquad (6)$$

Here, P_{50} is the blood P_{O_2} that results in 50% saturation of the hemoglobin and n (the so-called Hill coefficient) is a parameter describing the sigmoidicity of the curve. Taking the physically dissolved O_2 into account, the relation between concentration and partial pressure becomes

$$Cb = \beta b\, Pb + 4C_{Hb}\frac{Pb^n}{P_{50}^n + Pb^n} \qquad (7)$$

where C_{Hb} is hemoglobin concentration.

Gas exchange in the tissue is described by

$$\frac{\mathrm{d}P_b}{\mathrm{d}l} = \frac{Gdt}{Gp}(P_b - P_t) \qquad (8)$$

Here, Gdt is the blood to tissue diffusion conductance, which is determined mainly by the capillarity and Pt is tissue P_{O_2}. As we are only concerned about the maximal O_2 uptake we set Pt at 0.

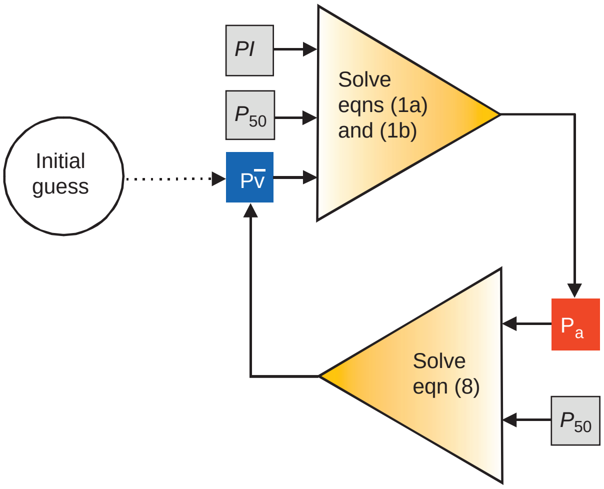


Figure 4 Diagrammatic illustration of the way the equations describing O_2 transport in the gill and in the tissue were simultaneously solved. For any given combination of the O_2 affinity (P_{O_2} that results in 50% saturation of the hemoglobin, P_{50}) and the inspired O_2 partial pressure (PI), an initial guess of the mixed-venous P_{O_2} was entered into the model and was used in the solution of the equations for gas exchange in the gill (eqns (Ia) and (Ib)). This resulted in an arterial O_2 partial pressure, Pa, (and an expired, PE, as well), which then was used in the solution of the equation for gas exchange in the tissue (eqn(8)). This resulted in a new mixed-venous P_{O_2}, which then could be used in the initiation of a new cycle, and so forth. This was repeated until Pa and Pv no longer changed.

How We Solved the Equations

Because we investigate how the O_2 affinity of the blood affects the maximal O_2 uptake, the input to the model is the P_{50} and the output is the O_2 uptake. In this situation, the arterial and venous P_{O_2} values as well as concentrations are all dependent variables that will automatically adjust to values dictated by the constraint that O_2 uptake over the gills must be matched exactly by the consumption in the tissues. In order to solve eqns (1a), 1(b), and (8) simultaneously, we adopted the iterative procedure outlined in **Figure 4** and applied the constants of **Table 1**.

See also: **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration. **Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; Red Blood Cell Function. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; The Effect of Exercise on Respiration.

Further Reading

Brauner CJ and Wang T (1997) The optimal oxygen dissociation curve: A comparison of hypoxic hypoxia and anemia. *American Zoologist* 37(1): 101–108.

Davis JC and Cameron JN (1971) Water flow and gas exchange at the gills of rainbow trout, *Salmo gairdneri. Journal of Experimental Biology* 54: 1–18.

Dejours P (1981) *Principles of Comparative Respiratory Physiology*. Amsterdam: Elsevier/North-Holland Biomedical Press.

Jones DR, Langille BL, Randall DJ, and Shelton G (1974) Blood flow in dorsal and ventral aorta of the cod, *Gadus morhua. American Journal of Physiology* 226: 90–95.

Kiceniuk JW and Jones DR (1977) The oxygen transport system in trout (*Salmo gairdner*) during sustained exercise. *Journal of Experimental Biology* 69: 247–260.

Malte H and Weber RE (1985) A mathematical model for gas exchange in the fish gill based on nonlinear blood gas equilibrium curves. *Respiration Physiology* 62(3): 359–374.

Malte H and Weber RE (1987) The effect of shape and position of the oxygen equilibrium curve on extraction and ventilation requirement in fishes. *Respiration Physiology* 70(2): 221–228.

Piiper J, Dejours P, Haab P, and Rahn H (1971) Concepts basic quantities in gas exchange physiology. *Respiration Physiology* 13: 292–304.

Piiper J and Scheid P (1984) Model analysis of gas transfer in fish gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 10(A), pp. 315–359. New York: Academic Press.

Wang T and Hicks JW (2002) An integrative model to predict maximum O_2 uptake in animals with central vascular shunts. *Zoology* 105: 42–53.

Weber RE and Jensen FB (1988) Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Review Physiology* 50: 161–179.

Wells RMG (1990) Hemoglobin physiology in vertebrate animals: A cautionary approach to adaptationist thinking. In: Boutilier RG (ed.) *Advances in Comparative and Environmental Physiology: Vertebrate Gas Exchange from Environment to Cell,* vol. 6, pp. 143–161. Berlin: Springer.

Wells RMG (2009) Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology*, vol. 27, pp. 255–299. New York: Academic Press.

Carbonic Anhydrase in Gas Transport and Exchange

KM Gilmour, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Carbonic Anhydrase – The Enzyme
CA Isoforms
Methodological Approaches for Discerning CA Function
Physiological Functions of CA in Gas Transport and Exchange
Summary and Conclusions
Further Reading

Glossary

Acetazolamide A sulfonamide (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) that readily crosses cell membranes and inhibits carbonic anhydrase by binding to the zinc ion in the active site of the enzyme.

Acid–base balance The regulation of constant pH in the body fluid compartments by means of a suite of physiological responses for the excretion and/or accumulation of acid–base equivalents, and usually by adjusting P_{CO_2} and/or HCO_3^- levels.

Acid–base equivalents H^+ and HCO_3^- or substances that release or bind H^+ when dissolved in water.

Active site The portion of an enzyme that binds the substrate and catalyzes its reaction.

Benzolamide A sulfonamide (5-benzenesulfonamido-1,3,4-thiadiazole-2-sulfonamide) that crosses cell membranes relatively slowly. It inhibits carbonic anhydrase by binding to the zinc ion in the active site of the enzyme.

Carbonic anhydrase (CA) A zinc metalloenzyme that reversibly catalyzes the reaction of CO_2 and H_2O to form H^+ and HCO_3^-.

Dehydration reaction The reaction of HCO_3^- and H^+ to form CO_2 and H_2O.

Electrometric ΔpH assay A technique used to assess CA activity in which changes in pH are measured following the addition of CO_2 to a buffer containing CA. The rate of change of pH in the presence of the CA represents the rate of the catalyzed reaction since one proton is produced for each molecule of CO_2 that is hydrated.

Hydration reaction The reaction of CO_2 and H_2O to form carbonic acid (H_2CO_3), which rapidly dissociates to H^+ and HCO_3^-.

Isoform Different forms of the same protein produced from related genes or arising from the same gene by alternative splicing.

Orthologs The homologous genes (i.e., sharing a common evolutionary ancestor) that are found in different species.

Pillar cell A spool-shaped cell found in the fish gill lamellae. It has a trunk-like body that separates the epithelial layers of the lamella and cytoplasmic flanges that spread out and attach to neighboring flanges so as to delimit the vascular space.

Transmembrane proteins Proteins that span the plasma membrane.

Introduction

Carbonic anhydrase (CA) is an enzyme that catalyzes the reversible reactions of carbon dioxide and water: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$. Because the dissociation of carbonic acid (H_2CO_3) to protons (H^+) and bicarbonate ions (HCO_3^-) is very rapid, the reactions are typically summarized as: $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$. The hydration ($CO_2 + H_2O \rightarrow H^+ + HCO_3^-$) and dehydration ($H^+ + HCO_3^- \rightarrow CO_2 + H_2O$) reactions proceed at a moderate pace even in the absence of a catalyst, but can become limiting to rapid physiological processes. For example, as blood passes through the gills of fish, HCO_3^- ions are dehydrated to molecules of CO_2 that then diffuse across the gills into the ventilated water (**Figure 1**). The uncatalyzed conversion of HCO_3^- to CO_2 may require more than 1 min to approach completion, yet the blood remains in the gills for only 1–3 s. It is red blood cell (RBC) CA that enables effective CO_2 excretion, increasing the rate of CO_2 hydration/dehydration reactions up to 25 000-fold so as to allow conversions between HCO_3^- and CO_2 to reach near completion in milliseconds. The importance of RBC CA to CO_2 excretion is clearly illustrated by the decrease in CO_2 excretion and elevation of blood CO_2 tension that occurs when RBC CA is inhibited (**Figure 2**).

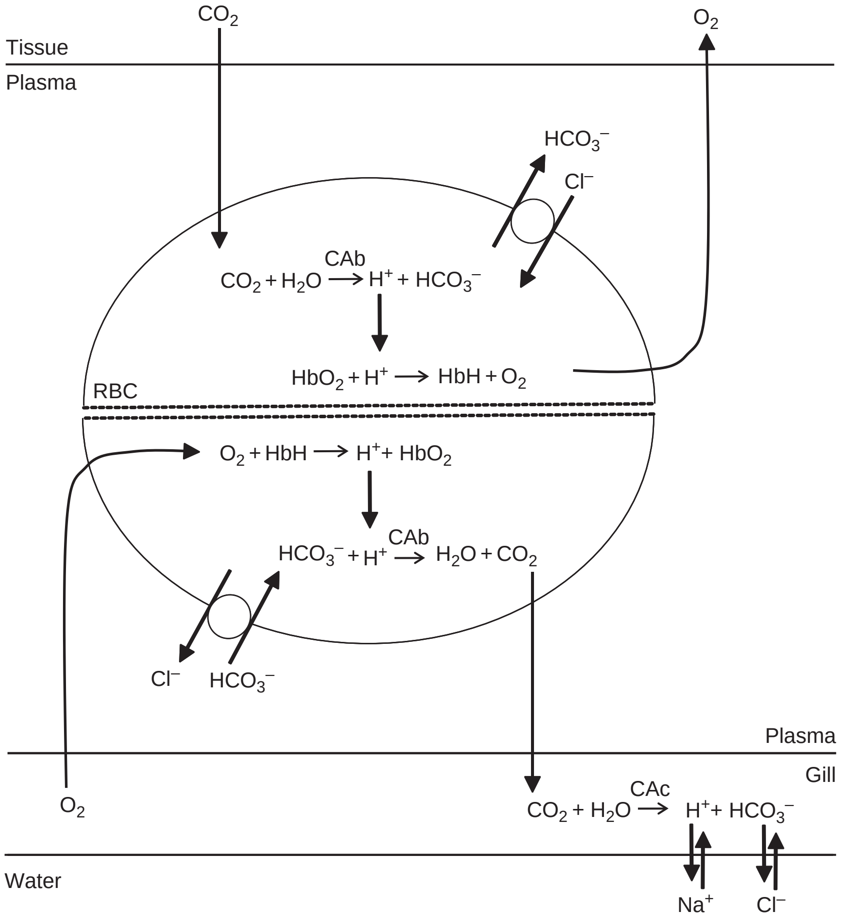


Figure 1 A schematic depicting the role of red blood cell (RBC) carbonic anhydrase (CAb) and gill epithelium cytosolic CA (CAc) in CO_2 excretion and acid–base balance, respectively, in a teleost fish. CO_2 diffuses from the tissues into the RBC where it is hydrated in the presence of CAb. Protons are buffered by hemoglobin (Hb) while HCO_3^- ions are removed to the cytoplasm by the anion exchanger band 3. These reactions are reversed at the gill. CO_2 that diffuses into the gill epithelium may be hydrated in the presence of CAc to provide H^+ and HCO_3^- used as counterions in, respectively, Na^+ and Cl^- uptake.

CA was, in fact, discovered (in 1932) in RBCs as a result of the realization that the uncatalyzed rate of HCO_3^- dehydration was too low to support CO_2 excretion during the time blood spent at the gas-exchange surface. Perhaps because it was first discovered in RBCs, or because RBCs provide a readily accessible and rich source of the enzyme, CA is often considered to be primarily involved in gas transfer, specifically CO_2 excretion (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion). The reality, however, is that CA plays a key role in a multitude of physiological processes ranging from calcification through metabolism and gas transfer to ionic regulation and acid–base balance. These varied roles reflect both the direct and indirect consequences of catalyzed CO_2 reactions: for example, enhanced rates of HCO_3^- supply for metabolism or acid–base equivalents for acid–base balance, as well as faster movement of CO_2 through fluid compartments and across membranes. The differing demands of the many roles played by CA have resulted in the existence of multiple CA isoforms that vary in molecular sequence, kinetic properties, tissue distribution, and subcellular localization. With few exceptions, these isoforms share in common a catalytic mechanism reflecting specific molecular structures.

Carbonic Anhydrase – The Enzyme

Three genetically unrelated families (α, β, and γ) of CA isoforms exist, but only the α-genes appear to be present in vertebrates. The α-CAs are monomeric metalloenzymes that employ a two-step, ping-pong catalytic mechanism centered around a zinc ion at the active site. Hydration is initiated when a zinc-bound hydroxide ion attacks the carbon atom of a CO_2 molecule held in a hydrophobic pocket adjacent to the active site. The product of this nucleophilic attack is a zinc-bound bicarbonate ion, which is then displaced by a water molecule:

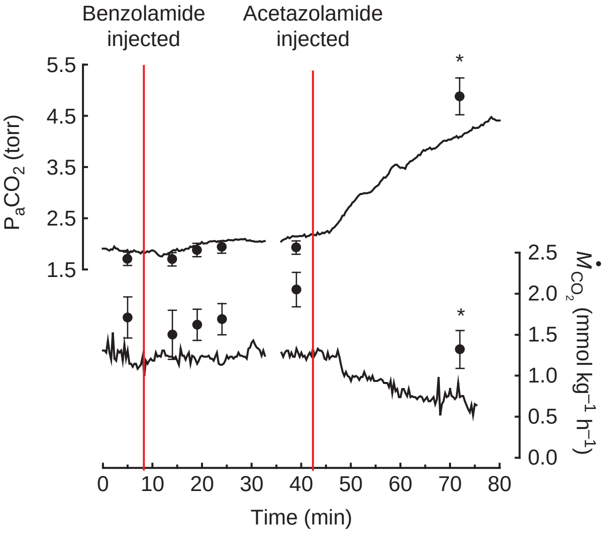


Figure 2 Injection of benzolamide into rainbow trout (*Oncorhynchus mykiss*) is without effect on CO_2 excretion ($\dot{M}$ CO_2) or the arterial partial pressure of CO_2 (P_aCO_2). Acetazolamide treatment, on the other hand, significantly reduces $\dot{M}$ CO_2 resulting in a substantial increase in P_aCO_2. The symbols represent mean data for five to six fish while the lines are representative recordings from a single fish. An asterisk indicates a significant difference from the value prior to CA inhibitor injection. Data are redrawn from Gilmour KM, Perry SF, Bernier NJ, Henry RP, and Wood CM (2001) Extracellular carbonic anhydrase in dogfish, *Squalus acanthias*: A role in CO_2 excretion. *Physiological and Biochemical Zoology* 74: 477–492

$$\mathrm{EZn\text{-}OH^- + CO_2 \leftrightarrow EZn\text{-}HCO_3^- + H_2O \leftrightarrow EZn\text{-}H_2O + HCO_3^-} \qquad (1)$$

The advantage of this pathway is its speed; the direct formation of HCO_3^- via this route is about five orders of magnitude faster than the uncatalyzed reaction involving the formation and subsequent dissociation of carbonic acid.

With water coordinated to the zinc ion, the enzyme is inactive. To regenerate the zinc-bound hydroxide ion for the next round of catalysis, a proton must be transferred from the zinc-bound water to the solution buffer (B):

$$\mathrm{EZn\text{-}H_2O \leftrightarrow EZn\text{-}OH^- + BH^+} \qquad (2)$$

This proton transfer appears to be the rate-limiting step in the catalytic mechanism; in some of the catalytically very active isoforms (e.g., mammalian CA II and CA IV; see below), the process is assisted by a proton shuttle, a histidine residue located at the entrance of the active site (His 64).

The molecular structures that support this catalytic activity are in general conserved across both isoforms and species (**Table 1**), suggesting that the catalytic mechanism described above, which was deduced largely from the study of a limited number of mammalian isoforms, applies broadly to all α-CAs. Where differences in these molecular structures do occur, they have in some cases been found to account for differences in catalytic activity among isoforms. The zinc ion is coordinated in the active site by three histidine residues (His 94, His 96, and His 119), each of which has an associated ligand (Gln 92, Asn 244, and Glu 117) that provides a stabilizing hydrogen bond. This motif is called the zinc-binding site and is conserved across the α-CA gene family except in the CA-related proteins (CA-RPs; see below), which lack catalytic activity. Adjacent to the zinc-binding site is a series of six nonpolar amino acids, the substrate-association pocket. The residues of this motif form a hydrophobic pocket responsible for isolating and guiding the substrate, CO_2, toward the zinc-bound hydroxide ion. Orientation of the zinc-bound hydroxide ion is supported by a third motif, the threonine-199 loop. Finally, the proton-shuttle residue (position 64) facilitates regeneration of the active form of the enzyme by accepting a proton from the zinc-bound water molecule and donating it to solution buffers. High-activity CA isoforms typically exhibit a histidine residue in this location substitution of which is associated with decreased catalytic activity.

CA Isoforms

Our knowledge of the α-CA gene family is most detailed for mammals, where 16 isoforms have been identified. For most of these isoforms, at least partial information on their kinetic properties, susceptibility to inhibitors, tissue distribution, and physiological function is available. Among the mammalian CA isoforms, three groups may be distinguished. The intracellular CAs include five cytosolic isoforms (I, II, III, VII, and XIII) as well as the two CA V homologs found in mitochondria. The extracellular CAs include five membrane-associated isoforms (IV, IX, XII, XIV, and XV) as well as the only isoform that is secreted, CA VI. The membrane-associated isoforms may be further subdivided into those anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage (IV and XV), and those expressed as single-pass transmembrane proteins (IX, XII, and XIV). The final group includes three isoforms (VIII, X, and XI) that lack catalytic activity and are termed CA-RPs.

The mammalian CA complement provides a framework within which fish CA isoforms may be considered (**Figure 3**). Although fish orthologs of many of the mammalian CA isoforms have been discovered through homology-based cloning approaches and/or mining of genomic databases, our knowledge of many of these isoforms is limited to their molecular sequence. Much work remains to characterize the kinetic properties, inhibitor sensitivity, and tissue distribution of these isoforms, as well as to determine their physiological function(s). Moreover, research effort to date has focused on a handful of fish species yet fish as a group show tremendous diversity. Describing and understanding patterns of

Table 1 A comparison across carbonic anhydrase (CA) isoforms of the amino acid residues that make up the active site pocket of the enzyme

	Residue number																																			
	Zinc-binding site						*Substrate association pocket*						*Threonine-199 loop*		*H^+ shuttel*	*Remainder of active site*																				
	92	**94**	**96**	**117**	**119**	**224**	**121**	**141**	**143**	**198**	**207**	**209**	**106**	**199**	**64**	**7**	**29**	**61**	**62**	**65**	**66**	**67**	**69**	**91**	**107**	**131**	**145**	**192**	**194**	**200**	**201**	**202**	**204**	**206**	**211**	**246**
		Z	Z		Z																															
CA I	Q	H	H	E	H	N	V	L	I	L	V	W	E	T	H	Y	S	N	V	S	F	H	N	F	H	L	G	W	Y	H	P	P	H	S	I	R
CA II	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	N	E	I	H	F	G	W	Y	T	P	P	L	C	V	R
CA III	Q	H	H	E	H	N	V	I	V	F	I	W	E	T	K	Y	S	N	N	T	C	R	V	R	H	F	G	W	Y	T	P	P	E	C	L	R
CA XIII	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	S	S	F	N	D	R	H	F	G	W	Y	V	P	P	L	S	V	R
CA VII	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	V	Q	D	K	H	F	G	W	Y	T	P	P	S	S	V	R
Lamprey	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	S	E	K	H	F	G	W	Y	T	P	P	F	S	V	R
Dogfish	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	S	Y	S	N	T	S	V	Q	D	R	H	F	G	Y	Y	T	P	P	F	S	V	R
Gar	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	Q	D	R	H	F	G	W	Y	T	P	P	L	S	V	R
Trout CAb	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	Q	G	K	H	F	G	W	Y	T	P	P	L	S	V	R
Trout CAc	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	Q	T	K	H	F	G	W	Y	T	P	P	L	S	V	R
Zfish CAb	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	Q	D	R	H	F	G	W	Y	T	P	P	L	S	V	R
Zfish CAc	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	F	Q	S	K	H	F	G	W	Y	T	P	P	L	S	V	R
CA IV	Q	H	H	E	H	N	V	I	V	L	V	W	E	T	H	Y	S	N	N	S	V	M	L	K	H	V	A	F	Y	T	P	T	D	K	V	R
CA IX	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	H	S	N	N	S	V	Q	T	L	H	V	A	F	Y	T	P	P	A	G	V	R
CA XII	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	S	V	K	N	T	H	A	A	Y	Y	T	P	P	N	T	V	R
CA XIV	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	N	T	V	Q	S	A	H	L	G	F	Y	T	P	T	Y	Q	V	R
CA XV	Q	H	H	E	H	N	V	F	I	L	V	W	E	T	H	Y	S	N	N	T	V	L	R	L	H	M	A	Y	Y	T	P	G	E	A	V	R
Df IV	Q	H	H	E	H	N	V	L	V	L	V	W	E	T	H	Y	S	N	D	S	V	Q	T	V	H	I	G	Y	Y	T	P	G	F	A	V	R
Trout IV	Q	H	H	E	H	T	V	V	V	L	V	W	E	T	R	Y	S	N	N	T	V	Q	D	I	H	L	G	Y	Y	T	P	P	E	A	V	R
Zfish IV	Q	H	H	E	H	T	V	V	V	L	V	W	E	T	H	Y	S	N	N	T	V	Q	N	Q	H	L	G	F	Y	T	P	G	A	A	I	R

CA sequences were individually aligned against human CA I (GenBank accession AAH27890) to identify the residues of the active site pocket; amino acid residues have been grouped according to molecular motif (see text). Within the zinc-binding site, residues marked Z are those that coordinate the zinc ion. Cytosolic CA isoforms are presented in the top group, with membrane-bound CA isoforms below. Among the cytosolic isoforms: CA II, CA III, and CA XIII represent consensus sequences among human, mouse, and rat sequences for these isoforms; human CA VII (AAL78167) was used; lamprey (*Petromyzon marinus*), dogfish (*Squalus acanthias*), and gar (*Lepisosteus osseus*) represent CA sequences cloned from lamprey (AAZ83742), dogfish or gar (AAM94169) blood; and two cytosolic isoforms, blood specific and general cytosolic, are presented for each of the teleost species, rainbow trout (*Oncorhynchus mykiss*, respectively AAP73748 and AAR99329) and zebrafish (*Danio rerio*, respectively NP_571185 and NP_954685). Among the membrane-bound isoforms: human CA IV (NM_000717), CA IX (NM_001216), CA XII (BC001012), and CA XIV (012113) and mouse CA VX (AF231113) are included as mammalian representatives; CA IV isoforms from dogfish (Df; AAZ03744), rainbow trout (AAR99330), and zebrafish (zfish; AAH78387) are included as fish representatives. The numbers present in the column head are residue numbers.

inter-specific diversity in CA isoforms as well as the properties and physiological functions of these isoforms represent an additional challenge.

As in mammals, fish CA isoforms belong to the α-CA gene family and fall into three distinct groups. CA-RPs have been identified that correspond to mammalian CA VIII and X (two forms) but not XI. Within the extracellular isoform grouping, fish orthologs corresponding to the secreted form, CA VI, and the transmembrane CAs IX, XII, and XIV have been identified. Among the GPI-linked membrane-associated isoforms, however, the picture that is emerging for fish is more complex than that

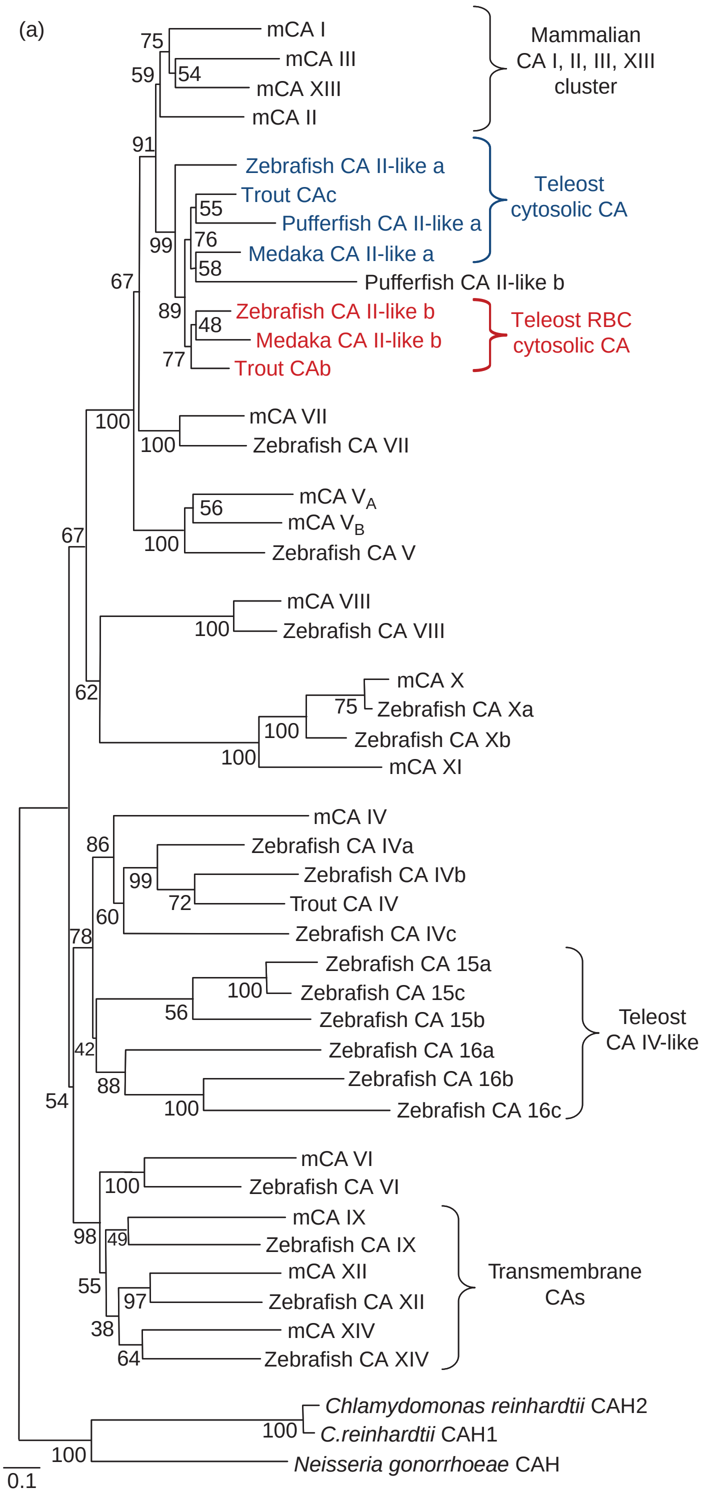


Figure 3 (*Continued*)

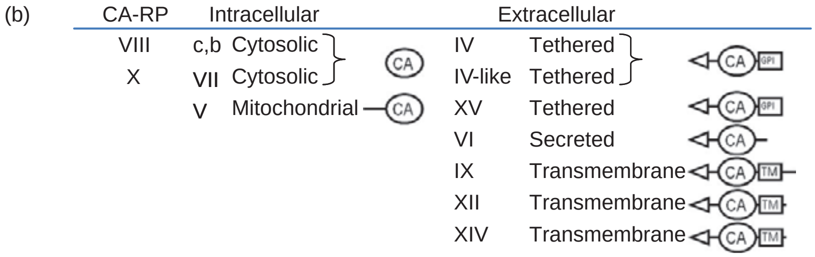


Figure 3 (a) A summary of the phylogenetic relationships among mammalian (mouse, m) and fish (medaka *Oryzia latipes*, pufferfish *Tetraodon nigroviridis*, rainbow trout *Oncorhynchus mykiss*, and zebrafish *Danio rerio*) CA isoforms. (b) A summary of the CA isoforms found in fish illustrating in addition the presumed domain structure of each isoform, where TM indicates transmembrane domain and GPI indicates a glycosylphosphatidylinostiol anchor. (a) Modified from consensus tree created by Lin T-Y, Liao B-K, Horng J-L, *et al*. (2008) Carbonic anhydrase 2-like a and 15a are involved in acid–base regulation and Na^+ uptake in zebrafish H^+-ATPase-rich cells. *American Journal of Physiology* 294: C1250–C1260.

in mammals. In addition to CA IV, at least some fish species possess two CA IV-like isoforms, with all of these isoforms showing three variants, for a total of nine CA IV-like isoforms. A CA XV-like isoform has been identified in another fish species. More information is needed to develop an understanding of the physiological significance of this CA IV diversity in fish.

The intracellular isoform grouping is also a point of significant divergence between fish and mammals. Like mammals, fish possess both the mitochondrial CA V (although possibly only one rather than the two forms found in mammals) and CA VII, the cytosolic CA that is thought to be basal to the remaining cytosolic isoforms. Whereas the CA I, II, III, and XIII gene cluster then arose in mammals, with CA II as the workhorse in both CO_2 excretion and acid–base regulation, fish appear to possess either one (e.g., lamprey, *Petromyzon marinus*) or two (e.g., teleost fish) cytosolic CAs. The emergence of two cytosolic CAs in teleost fish reflects a whole genome duplication of the teleost common ancestor that subsequently gave rise to two closely related cytosolic CA isoforms that appear, based on the limited data available, to differ in tissue distribution, kinetic properties, and physiological function. One isoform is expressed predominantly in RBCs and exhibits a higher catalytic efficiency than the second form, which is more widely distributed but shows little RBC expression. These differences suggest that the higher-activity RBC isoform has been tailored to favor CO_2 excretion while the slower isoform, which shows moderate to high expression in kidney and gill, respectively, contributes to acid–base and ionic regulation.

Methodological Approaches for Discerning CA Function

The existence of numerous CA isoforms and the (presumed) differences among these isoforms in kinetic properties, subcellular localization, and tissue distribution reflect the varied physiological functions of CA. Within fish, however, our knowledge of the distribution, kinetic properties, and physiological functions of specific CA isoforms is limited. With the availability of sequence information, it is possible to examine the tissue distribution of CA messenger ribonucleic acid (mRNA) expression, but the localization of CA protein is made challenging by the scarcity of fish CA isoform-specific antibodies. The kinetic properties of CA isoforms can be determined by assessing the dependence of CA activity on substrate concentration; a similar approach can be adopted to evaluate sensitivity to different CA inhibitors.

The most widely used technique for assaying CA activity is the electrometric ΔpH approach, which involves measuring changes in pH in response to the addition of CO_2 to a buffer containing the CA source of interest. The rate of change of pH in the presence of the CA source represents the rate of the catalyzed reaction because one proton is produced for each molecule of CO_2 that is hydrated. Isolation of subcellular fractions by differential centrifugation followed by assays of CA activity can yield information on what CA isoform(s) is present. For example, CA activity that persists when membrane fractions are isolated and washed (to remove any cytosolic CA that may have loosely associated with membranes during isolation) is likely due to a membrane-linked CA isoform. If the CA activity is resistant to denaturation by the detergent sodium dodecylsulfate (SDS), then this deduction is strengthened because membrane-associated CA isoforms typically are more stabilized by intramolecular disulfide linkages than are cytosolic CAs. Finally, phosphatidylinositol-specific phospholipase C (PI-PLC) can be used to cleave a GPI linkage, releasing GPI-linked CA from its membrane association and allowing GPI-linked membrane-associated CA to be distinguished from transmembrane isoforms.

These types of approaches have been used in combination with *in vivo* work or tissue preparations to elucidate the function of CA in different physiological systems.

A standard approach is to assess physiological function before and after selective inhibition of the CA isoform thought to be involved. The two main classes of CA inhibitors are metal-complexing anions and sulfonamides, both of which exert their effects by binding to the zinc ion of the enzyme, preventing it from playing its essential role in the catalytic mechanism. Consequently, developing CA inhibitors that are selective for a particular isoform has proved to be challenging. One successful approach has involved manipulating the size or charge of the inhibitor to limit its ability to cross plasma membranes, thereby conferring the ability to distinguish between intra- and extracellular isoforms. For example, whereas the sulfonamide inhibitor aminobenzolamide rapidly crosses membranes and therefore inhibits both intra- and extracellular CA isoforms, a polymerized form, polyoxyethylene-aminobenzolamide (F3500), is restricted to the extracellular environment by virtue of its large size (molecular weight ~3500 Da).

Physiological Functions of CA in Gas Transport and Exchange

Creative use of the tools described above – sequence information, localization of gene expression, protein or activity, assessment of kinetic properties and/or inhibitor sensitivities, and application of inhibitors *in vivo* or in reduced preparations – has enabled the role of CA isoforms to be identified for a variety of physiological processes. Particular effort has been focused on addressing the roles of CA isoforms in gas exchange, acid–base balance, and ion transport – physiological processes that are inextricably linked in fish.

CA plays an essential role in CO_2 excretion (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion and Red Blood Cell Function). In the RBC, cytosolic CA (the blood-specific isoform in teleost fish) catalyzes the hydration reaction to facilitate loading of CO_2 into the blood at the tissues. Effective removal of CO_2 from the blood during its brief residence in the gills is made possible by CA-catalyzed HCO_3^- dehydration to molecular CO_2 (**Figure 1**). Inhibition of RBC CA by acetazolamide impairs CO_2 excretion, raising blood CO_2 tension and causing a respiratory acidosis (**Figure 2**). In elasmobranch fish, a second CA isoform also contributes to CO_2 excretion. CA IV is anchored to the plasma membrane of gill pillar cells (**Figure 4**; **see also Design and Physiology of Arteries and Veins**: Branchial Anatomy) where it catalyzes the dehydration of HCO_3^- to molecular CO_2 within the plasma, thereby providing an alternative pathway to the RBC for HCO_3^- dehydration (**Figure 4**). By providing protons for plasma HCO_3^- dehydration, the relatively high plasma-buffering capacity of elasmobranch fish is important in allowing this pathway to contribute to CO_2 excretion. Evidence supporting this dual pathway for CO_2 excretion in elasmobranch fish comes from the selective inhibition of extracellular CA using F3500 or low concentrations of the slowly permeating sulfonamide benzolamide, which raises blood CO_2 tension and lowers pH in elasmobranch but not teleost fish (**Figures 2** and **4**); teleost fish lack branchial CA IV.

CA also contributes to acid–base balance and ion transport in fish. Active salt uptake at the gill in freshwater fish is necessary to offset salt loss by diffusion to the dilute external environment. Branchial cytosolic CA (the general cytosolic isoform in teleost fish) catalyzes the intracellular hydration of CO_2 to provide the H^+ and HCO_3^- used as counterions for the active uptake of, respectively, Na^+ and Cl^- ions (**Figures 1** and **4**; **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes); Na^+ and Cl^- uptake are reduced when branchial cytosolic CA is inhibited using permeant sulfonamides such as acetazolamide. Compensation of acid–base disturbances is achieved through manipulation of these same exchanges. That is, H^+ and HCO_3^- effluxes (which are of course linked to Na^+ and Cl^- influxes, respectively) are differentially modified so as to adjust plasma HCO_3^- levels, thereby correcting pH. The role of CA in such acid–base compensation has been confirmed by demonstrations of reduced acid–base equivalent excretion following CA inhibition.

In freshwater fish the kidney contributes to both ionic and acid–base regulation by reabsorption of salt and HCO_3^-, respectively, from the filtrate (**see also Role of the Kidneys**: The Kidney). Again, CA is involved (**Figure 5**). Reabsorption of HCO_3^- from the filtrate relies on both membrane-associated and cytosolic CA isoforms. Filtered HCO_3^- ions are dehydrated to CO_2 in the presence of luminal membrane-associated CA; CA IV is present but other membrane-linked CA isoforms may also be involved. Molecular CO_2 diffuses into the cell where it is hydrated, a reaction catalyzed by cytosolic CA (the general cytosolic isoform in teleost fish), resulting in HCO_3^- that is exported across the basolateral membrane to the blood, and H^+ that is recycled across the apical membrane into the lumen to participate in HCO_3^- dehydration. This process results in the net translocation of HCO_3^- from lumen to blood, and is adjusted according to the requirements of the fish for acid–base regulation. Inhibition of either cytosolic CA (using acetazolamide) or luminal extracellular CA (using F3500) impairs HCO_3^- reabsorption.

Marine teleost fish actively excrete salt across the gill while drinking seawater to offset diffusional salt gain and osmotic water loss driven by their hyperosmotic environment (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

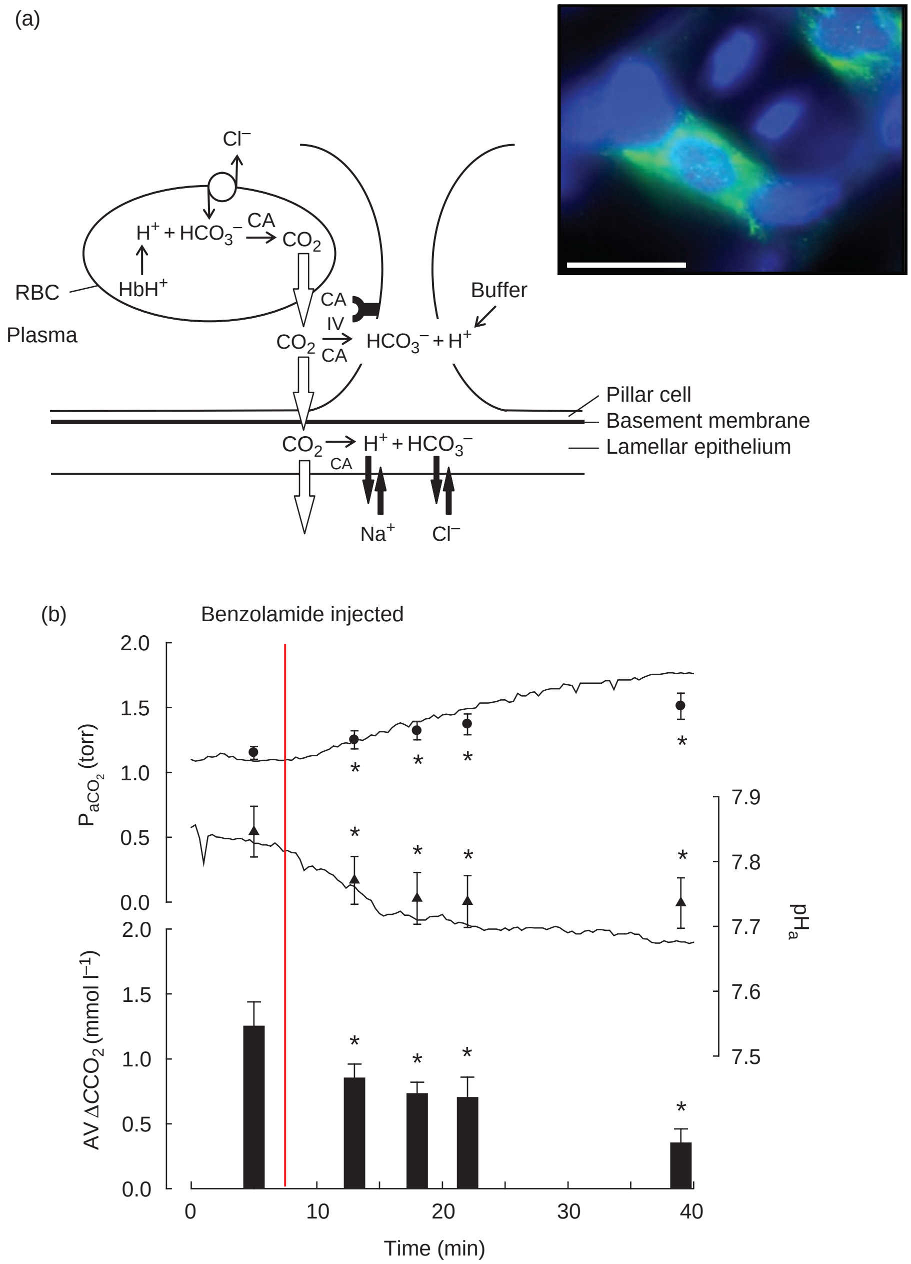


Figure 4 (a) A schematic depicting the roles of red blood cell (RBC) carbonic anhydrase (CA) and pillar cell CA IV in CO_2 excretion in an elasmobranch fish. The possible contribution of gill epithelium CA to acid–base balance is also illustrated. This model of CO_2 excretion in an elasmobranch fish differs from the teleost model in allowing for substantial HCO_3^- dehydration in the plasma catalyzed by pillar cell CA IV. The inset micrograph presents a representative light micrograph in which immunohistochemistry has been used to localize dogfish CA IV (green immunofluorescence); nuclei were visualized using 4′,6′-diamidino-2-phenylindole. Scale = 5 μm. (b) The contribution of extracellular CA to CO_2 excretion in dogfish (*Squalus acanthias*) is demonstrated by injecting benzolamide to selectively inhibit extracellular CA, which results in a significant impairment of HCO_3^- removal from the blood at the gills (ΔC_{CO_2}; calculated as arterial–venous differences in blood HCO_3^-) leading to a significant rise in the arterial partial pressure of CO_2 (P_{aCO_2}) and a concomitant fall in arterial pH (pH_a), unlike the situation for a teleost fish as depicted in **Figure 2**. The symbols represent mean data for five to seven fish while the lines are representative recordings from a single fish. An asterisk indicates a significant difference from the value prior to benzolamide injection. (a) Modified from Gilmour KM and Perry SF (2010) Gas transfer in dogfish: A unique model of CO_2 excretion. *Comparative Biochemistry and Physiology A* 155: 476–485. (b) Modified from Gilmour KM, Perry SF, Bernier NJ, Henry RP, and Wood CM (2001) Extracellular carbonic anhydrase in dogfish, *Squalus acanthias*: A role in CO_2 excretion. *Physiological and Biochemical Zoology* 74: 477–492.

Water absorption across the intestinal epithelium relies in part on base excretion into the intestinal lumen, a process that involves at least two CA isoforms (**Figure 6**). Cytosolic CA (the general cytosolic isoform) catalyzes the hydration of CO_2 to provide HCO_3^- ions that are exported across the apical membrane in exchange for Cl^-.

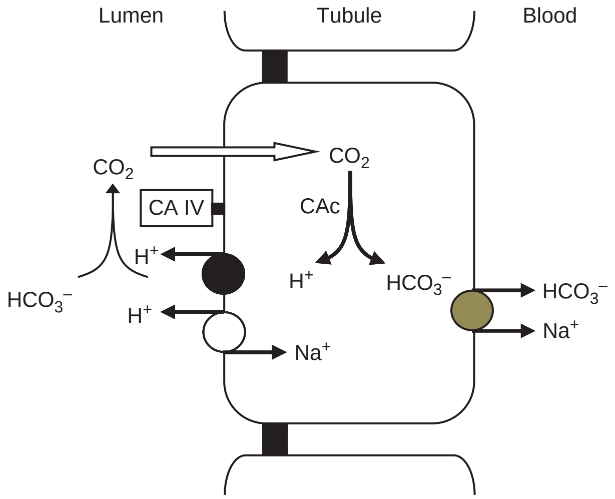


Figure 5 A schematic representation of the contribution of CA to HCO_3^- reabsorption by the teleost kidney proximal tubule. Luminal CA IV (and/or other membrane-bound isoforms) catalyzes the dehydration of filtered HCO_3^- by H^+ provided by Na^+/H^+ exchange and/or H^+-ATPase. The CO_2 so formed diffuses into the cytosol and is hydrated in the presence of cytoplasmic CAc, with HCO_3^- exiting basolaterally via a Na^+/HCO_3^- co-transporter and H^+ being recycled across the apical membrane. Modified from Gilmour KM and Perry SF (2009) Carbonic anhydrase and acid–base regulation in fish. *Journal of Experimental Biology* 212: 1647–1661.

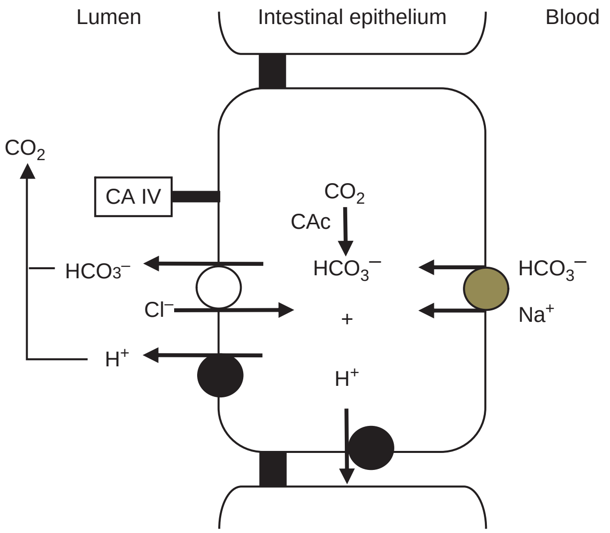


Figure 6 A schematic representation of the contribution of CA to HCO_3^- secretion by the marine teleost anterior intestine. Cytosolic CA (CAc) catalyzes the hydration of CO_2 to HCO_3^-, which fuels the apical anion exchanger driving HCO_3^- secretion into the intestinal lumen. Protons may exit the cell either apically or basolaterally via H^+-ATPase. In the lumen, CA IV (and/or other membrane-bound isoforms) catalyzes the dehydration of secreted HCO_3^-, a process that helps to maintain the gradient for HCO_3^- secretion into the lumen. Modified from Grosell M, Genz J, Taylor JR, Perry SF, and Gilmour KM (2009) The involvement of H^+-ATPase and carbonic anhydrase in intestinal HCO_3^- secretion in seawater-acclimated rainbow trout. *Journal of Experimental Biology* 212: 1940–1948.

In the lumen of the intestine, at least some HCO_3^- ions are dehydrated to CO_2 in a reaction catalyzed by CA IV and possibly other membrane-associated CA isoforms. The inhibition of either extracellular CA (using F3500) or both isoforms (using permeant sulfonamide inhibitors) reduces intestinal base excretion, attesting to the importance of CA in this process (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation).

Summary and Conclusions

Fish express a diversity of CA isoforms that contribute to gas exchange, acid–base balance, and ion transport, as well as many other physiological processes. Our knowledge of the diversity, distribution, function, and regulation of these CA isoforms is increasing and will continue to improve as better tools, including selective CA inhibitors and fish species- and isoform-specific CA antibodies, become available.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **Role of the Kidneys**: The Kidney. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Red Blood Cell Function.

Further Reading

Chegwidden WR and Carter ND (2000) Introduction to the carbonic anhydrases. *EXS* 90: 13–28.

Esbaugh A, Perry SF, Bayaa M, *et al.* (2005) Cytoplasmic carbonic anhydrase isozymes in rainbow trout *Oncorhynchus mykiss*: Comparative physiology and molecular evolution. *Journal of Experimental Biology* 208: 1951–1961.

Esbaugh A and Tufts BL (2006) The structure and function of carbonic anhydrase isozymes in the respiratory system of vertebrates. *Respiratory Physiology and Neurobiology* 154: 185–198.

Georgalis T, Gilmour KM, Yorston J, and Perry SF (2006) The roles of cytosolic and membrane bound carbonic anhydrase in the renal control of acid–base balance in rainbow trout *Oncorhynchus mykiss*. *American Journal of Physiology* 291: F407–F421.

Gilmour KM, Bayaa M, Kenney L, McNeill B, and Perry SF (2007) Type IV carbonic anhydrase is present in the gills of spiny dogfish (*Squalus acanthias*). *American Journal of Physiology* 292: R556–R567.

Gilmour KM and Perry SF (2009) Carbonic anhydrase and acid–base regulation in fish. *Journal of Experimental Biology* 212: 1647–1661.

Gilmour KM, Perry SF, Bernier NJ, Henry RP, and Wood CM (2001) Extracellular carbonic anhydrase in dogfish, *Squalus acanthias*: A role in CO_2 excretion. *Physiological and Biochemical Zoology* 74: 477–492.

Grosell M, Genz J, Taylor JR, Perry SF, and Gilmour KM (2009) The involvement of H^+-ATPase and carbonic anhydrase in intestinal HCO_3^- secretion in seawater-acclimated rainbow trout. *Journal of Experimental Biology* 212: 1940–1948.

Henry RP (1996) Multiple roles of carbonic anhydrase in cellular transport and metabolism. *Annual Review of Physiology* 58: 523–538.

Henry RP and Heming TA (1998) Carbonic anhydrase and respiratory gas exchange. In: Perry SF and Tufts BL (eds.) *Fish Respiration*, pp 75–111. San Diego, CA: Academic Press.

Henry RP and Swenson ER (2000) The distribution and physiological significance of carbonic anhydrase in vertebrate gas exchange organs. *Respiratory Physiology* 121: 1–12.

Lin T-Y, Liao B-K, Horng J-L, *et al.* (2008) Carbonic anhydrase 2-like a and 15a are involved in acid–base regulation and Na^+ uptake in zebrafish H^+-ATPase-rich cells. *American Journal of Physiology* 294: C1250–C1260.

Lindskog S and Silverman DN (2000) The catalytic mechanism of mammalian carbonic anhydrases. In: Chegwidden WR and Carter ND eEdwards YH (eds.) *The Carbonic Anhydrases: New Horizons*, pp. 175–195. Basel, Switzerland: Birkhäuser Verlag.

Maren TH (1967) Carbonic anhydrase: Chemistry, physiology, and inhibition. *Physiological Reviews* 47: 595–781.

Rahim SM, Delaunoy J-P, and Laurent P (1988) Identification and immunocytochemical localization of two different carbonic anhydrase isoenzymes in teleostean fish erythrocytes and gill epithelia. *Histochemistry and Cell Biology* 89: 451–459.

Supuran CT (2008) Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nature Reviews Drug Discovery* 7: 181.

Swenson ER (2000) Respiratory and renal roles of carbonic anhydrase in gas exchange and acid–base regulation. *EXS* 90: 281–341.

Carbon Dioxide Transport and Excretion

DJ Randall, City University of Hong Kong, Hong Kong, China

© 2011 Elsevier Inc. All rights reserved.

Introduction
Transfer of Gases to and from the Blood
Regulation of RBC pH
Patterns of Carbon Dioxide Excretion
The Effect of Temperature and Variations in Environmental CO_2 Levels
Further Reading

Glossary

Arterial Pertaining to blood that has been oxygenated.

Band-III protein anion exchanger The major membrane protein of most erythrocytes. With its help, the half-time of chloride equilibration between erythrocytes and plasma is decreased from a couple of hours to seconds in fish.

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Carbamino compounds These are formed when carbon dioxide reacts with $-NH_2$ groups on proteins – particularly hemoglobin – to form carbamino compounds ($-NHCOO^-$).

Carbonic anhydrase A zinc metalloenzyme that reversibly catalyzes the reaction between CO_2 and H_2O to form H^+ and HCO_3^-.

Chloride shift The exchange of HCO_3^- and Cl^- ions across the red-blood-cell (RBC) membrane to maintain charge balance within the cell.

Equilibrium Pertaining to the situation when all forces acting are balanced by others, resulting in a stable unchanging system.

4-Acetamido-4′-isothiocyano-2,2′-disulfonic stilbene (SITS) A disulfonic stilbene derivative that inhibits most RBC band III proteins.

Haldane effect Proton binding to hemoglobin (as a function of oxygenation).

Jacob–Stewart cycle The cycling of CO_2 and bicarbonate across the RBC system, resulting in the transfer of acid between the plasma and the cell interior.

Na^+/H^+exchanger The protein involved in the transfer of sodium in exchange for a proton across a cell membrane.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

RBC sodium/proton exchanger An ion-transport pathway unique for teleost erythrocytes that plays a role in the control of red cell pH.

Rete mirabile (or simply *rete*, plural *retia mirabilia*) – Structure consisting of blood vessels arranged to facilitate the exchange of heat or oxygen.

Root effect Property of hemoglobins in some fishes such that, in the presence of acid, it is impossible for hemoglobin molecules to be completely saturated with oxygen, even at extremely high oxygen partial pressures.

Swimbladder A gas-filled bladder found in most but not all teleost fish.

Venous Pertaining to blood that has passed through tissues having had some oxygen removed for metabolism in fish.

Introduction

Fish take up oxygen and metabolize substrates, producing carbon dioxide, ammonia, and water. Carbon dioxide and especially ammonia are toxic and cannot be allowed to accumulate in the body, and any excess must be excreted. An increase in CO_2 acidifies body fluids. Elevated CO_2 levels inhibit growth and high levels cause narcosis, probably because of disturbances to the acid–base balance. Carbon dioxide reacts with water to form carbonic acid (H_2CO_3), a weak acid that dissociates into bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}). Carbon dioxide also reacts directly with hydroxyl ions (OH^-) to form bicarbonate. The proportion of CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-} in solution depends on the pH, temperature, and the ionic strength of the solution. In fish blood at normal pH the ratio of CO_2 to carbonic acid is approximately 1000:1, and the ratio of CO_2 to bicarbonate ions is about 1:20. Bicarbonate is, therefore, the predominant form of CO_2 in fish blood. The carbonate content may approach 5% of the total CO_2 content of the blood, especially at low temperatures. As partial pressure of carbon dioxide (PCO_2) or pH increases, the major change is an increase in the bicarbonate content of the blood. The pH of red blood cells (RBCs) is less than that of plasma, but because PCO_2 is in equilibrium across the cell membrane, bicarbonate levels are lower in erythrocytes than in plasma. In addition, plasma volume is usually greater than erythrocyte volume; thus, it follows that most of the bicarbonate in the blood is in plasma.

In addition to forming bicarbonate ions, carbon dioxide also reacts with $-NH_2$ groups on proteins – particularly hemoglobin – to form carbamino compounds ($-NHCOO^-$). Carbamino groups are more likely to form when hemoglobin is oxygenated. The extent of carbamino formation increases with the concentration of CO_2 and the number of terminal $-NH_2$ groups available. The terminal $-NH_2$ group of the α-chain of teleost fish hemoglobins, however, is acetylated, blocking the formation of carbamino groups. In addition, organophosphates bind at the same terminal $-NH_2$ group of the β-chain that forms carbamino groups, so organophosphate binding reduces carbamino formation. High pH reduces organophosphate binding and so augments carbamino formation by making more $-NH_2$ groups available. Because fish erythrocytes often have high organophosphate levels as well as acetylated α-chains, teleost fish rely less on carbamino formation for CO_2 transport than mammals. The hemoglobin α-chains of elasmobranchs are not acetylated, and therefore they have more carbamino CO_2 compared with teleosts.

The sum of all forms of CO_2 in the blood – that is, molecular CO_2, H_2CO_3, HCO_3^-, CO_3^{2-} and carbamino compounds – is referred to as the total CO_2 content of the blood. The total CO_2 content varies with PCO_2 and the relationship can be described graphically in the form of a CO_2 dissociation curve.

Most cell membranes are permeable to CO_2 and NH_3 but relatively impermeable to HCO_3^- and NH_4^+ ions. Blood pH in fish and other vertebrates is midway between the negative logarithm of the dissociation constants (pK's) of the carbon dioxide–bicarbonate and ammonia–ammonium reactions. A body pH that is midway between these pK's ensures adequate rates of excretion by diffusion of both CO_2 and NH_3. Because these pK's vary with temperature, so does the normal pH of blood. Plasma pH falls from about 7.9 to 7.6 as temperature increases from 5 to 30 °C, ensuring adequate rates of excretion of both CO_2 and NH_3 over a range of temperatures.

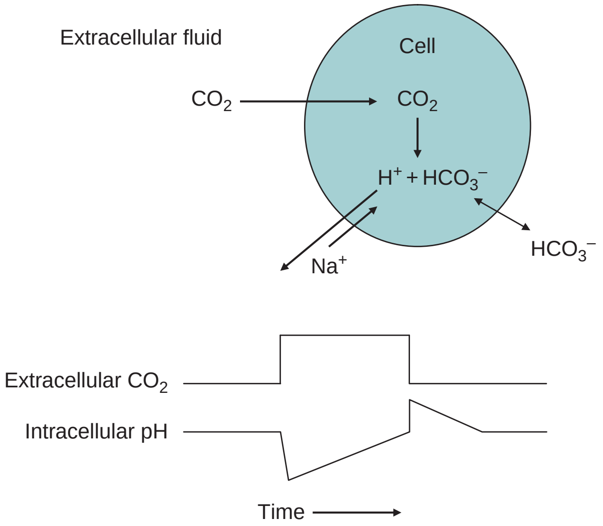


Figure 1 Following an increase, extracellular CO_2 will diffuse into and rapidly acidify cells. A reduction in extracellular CO_2 will have the opposite effect. Proton and bicarbonate excretion will gradually return the cellular pH to normal.

Transfer of Gases to and from the Blood

Carbon dioxide diffuses from the tissues into the blood, is transported in the blood, and diffuses across the gill surface into the environment. In most cases, membranes are much more permeable to CO_2 than to HCO_3^- and to CO_3^{2-}; thus, most of the transfer is as dissolved, gaseous CO_2. The movement of CO_2 will acidify the compartment into which it moves and alkalinize the compartment it leaves (**Figure 1**). In the tissues, CO_2 enters the blood and either is hydrated to form HCO_3^- or reacts with $-NH_2$ groups of hemoglobin and other proteins to form carbamino compounds. The reverse process occurs when CO_2 is unloaded from the blood at the respiratory surface. The greatest change is in the HCO_3^- concentration; changes in the levels of CO_2 and carbamino compounds usually represent less than 20% of total carbon dioxide excretion.

The uncatalyzed CO_2/HCO_3^- hydration/dehydration reactions are slow, requiring several seconds or even minutes at low temperatures. Such reaction rates are relatively slow compared with the time that blood within capillaries spends in contact with the tissue. However, in the presence of the enzyme carbonic anhydrase (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange), this reaction approaches equilibrium in much less than a second. Carbonic anhydrase also facilitates carbon dioxide transfer, referred to as facilitated diffusion of CO_2 which results from the simultaneous diffusion through the epithelium of bicarbonate and protons, the latter also augmented by release from buffers. Because carbonic anhydrase is present in RBCs of teleost fish but not in the plasma, most CO_2 hydration/dehydration occurs in the erythrocytes. In addition, the oxygenation of hemoglobin releases H^+'s, further increasing CO_2 production from bicarbonate in the RBC; conversely, deoxygenation results in the binding of H^+'s to hemoglobin, increasing bicarbonate levels, reducing the change in PCO_2 and pH as CO_2 enters the blood; this phenomenon is termed the Haldane effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect and Red Blood Cell

Function). Many fish hemoglobins show a large Haldane effect.

On entering the blood from the tissues, CO_2 diffuses into RBCs (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function), where HCO_3^- is formed rapidly in the presence of carbonic anhydrase (**Figure 2**). As the HCO_3^- levels within erythrocytes rise, HCO_3^- ions move into the plasma across the RBC membrane. Charge balance within the cell is maintained by anion exchange; as HCO_3^- ions leave the RBCs, there is a net influx of Cl^- ions from the plasma into the cells, a process called the 'chloride shift'. Teleost and elasmobranch RBCs, unlike many other cells, are very permeable to both Cl^- and HCO_3^- because their membranes have a high concentration of an anion carrier, band-III protein, which binds Cl^- and HCO_3^- and transfers them in opposite directions across the membrane. This anion exchange is passive and depends on concentration gradients to drive the process, which can occur in either direction, so bicarbonate can flow out of the erythrocyte in the tissues and into the erythrocyte at the respiratory surface. The fish RBC anion exchangers have a similar molecular size and drug sensitivity similar to that described for mammals; however, they operate at higher rates and are less temperature sensitive than human band III, as would be expected for fish living in changing temperatures where operating temperatures are usually much colder than that of mammalian RBCs. Band-III anion exchanger in carp (*Cyprinus carpio*) RBCs, however, appears to be insensitive to disulfonic stilbene derivatives such as 4-acetamido-4′-isothiocyano-2,2-disulfonic stilbene (SITS), whereas all other teleost band-III proteins investigated are inhibited by SITS. The band-III proteins also vary between fish showing differences in rates of anion exchange. Operation of the anion exchange may be pH and hemoglobin-oxygenation sensitive, but this is not certain.

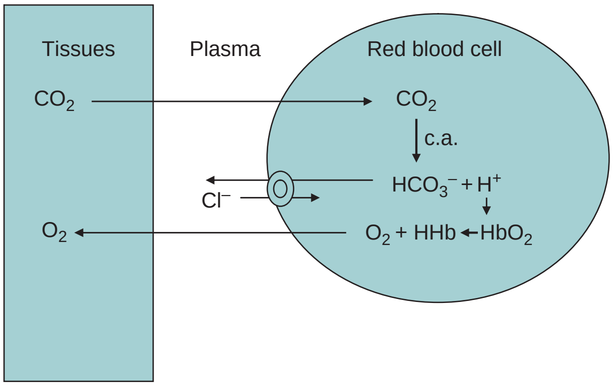


Figure 2 CO_2 diffuses into the red blood cell (RBC) and, in the presence of carbonic anhydrase (c.a.), rapidly acidifies the RBC. Bicarbonate is exchanged for chloride via band-III protein in the RBC membrane. Protons bind to hemoglobin and enhance the release of oxygen.

Excretion of CO_2 in teleost fish is limited by the rate of bicarbonate–chloride exchange across the erythrocyte membrane. The surface-to-volume ratio of erythrocytes, as well as their capacity for bicarbonate–chloride exchange mediated by band-III protein, will be important in determining the rates of carbon dioxide excretion. RBCs from rainbow trout (*Oncorhynchus mykiss*) are larger than RBCs from humans and have a much higher concentration of band-III protein in their membranes, which presumably compensates for the increased cellular volume and offsets, at least to some degree, the effects of a lower body temperature on anion-exchange rates. Even so, anion exchange is slower across trout RBCs at 15 °C than across human RBCs at 38 °C. However, transit times for erythrocytes through the gills are longer than those in the lungs, allowing more time for anion exchange across the RBC. Anion exchange rates are faster in turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morhua*) than in rainbow trout and in European eels (*Anguilla anguilla*), which have similar, slower rates of exchange. However, hematocrit and blood-transit time through the gills also vary. Because all these fish are extant, one can presume that these features are to some extent correlated; it is probable that the rapid rates of anion exchange across RBC membranes are coupled to fast gill-blood-flow transit times. Despite these considerations, it is still not clear why different species have evolved RBCs of such different sizes. Those animals with large RBCs also have large cells generally. Thus, cell size may have been selected for reasons other than for efficient gas transfer and may be unrelated to gas-transfer rates. For instance, triploid salmon (*Oncorhynchus tshawytscha*) have RBCs 1.5 times the size of those of their diploid cousins (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Physiology of Triploid Fish) but have the same hemoglobin concentration and swim just as fast, indicating that their efficiency of gas transfer is comparable even though RBC size is different. Large blood cells may not mean longer diffusion distances because the cells are flat and may present their flat surface to the gill endothelium.

Cyclostome erythrocytes lack band-III protein. In these animals, bicarbonate stays within the RBCs, and there is no anion transfer between the erythrocyte and the plasma with changes in $[CO_2]$. In lampreys, like teleosts, there is no carbonic anhydrase activity in the plasma or on the gill endothelium. Carbonic anhydrase is in the RBC, and bicarbonate dehydration in the RBC accounts for about 80% of CO_2 excretion. Lamprey blood has a large Haldane effect and the protons produced by hemoglobin oxygenation promote bicarbonate deydration. The RBCs have a Na^+/H^+ exchanger and proton excretion from the RBC may enhance bicarbonate formation in the tissues. The lamprey Na^+/H^+

exchanger, however, is catecholamine insensitive. Hagfishes also appear to lack physiological levels of band-III protein, but they have a smaller Haldane effect and no RBC Na^+/H^+ exchange. Thus, there is a smaller bicarbonate change in each hagfish RBC flowing through the gills compared with that of lampreys. Some hagfish species (e.g., *Eptatretus*) have a larger Haldane effect and so may have increased bicarbonate excretion from each RBC. It appears that the pattern of CO_2 excretion is similar in lampreys and hagfish, but much less is known about the situation in hagfish. For example, it is not known if there is carbonic anhydrase activity available to plasma on the gill endothelium.

In teleost fishes, such as the trout, the plasma perfusing the gills is not exposed to carbonic anhydrase. In these animals, almost all excretion of CO_2 occurs through the RBCs and is tightly coupled to O_2 uptake through proton production by oxygenation of hemoglobin (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Interestingly, many teleost hemoglobins have a relatively low buffering capacity because of a reduced number of the histidine side chains that normally are involved in buffering (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). This means that protons released during oxygenation are available for bicarbonate dehydration, facilitating the tight coupling of oxygen and carbon dioxide transfer within the RBCs. This tight coupling also ameliorates the oscillations in blood pH during CO_2 transfer. The proton pump in the apical membrane of the gills of freshwater fishes excretes acid and develops an electrochemical gradient that brings sodium into the gill epithelium. The activity of the proton pump is pH dependent, and oscillations in blood pH could have a marked effect on the pump if they were rapidly transferred into the gill epithelium. Thus, the structure of the hemoglobin molecule is finely tuned not only to the animal's requirements for oxygen and carbon dioxide transfer, but also for ion transfer across the respiratory epithelium and pH regulation. On the other hand, elasmobranchs, such as dogfish (*Squalus acanthias*), have lower levels of erythrocytic carbonic anhydrase than do trout and have only a loose coupling between oxygen and carbon dioxide flux in the RBCs. These fishes have significant levels of carbonic-anhydrase activity in the blood plasma as well as a broad distribution in the gill epithelium, facilitating carbon dioxide excretion from plasma directly across the gill epithelium.

Regulation of RBC pH

In most vertebrate RBCs, unlike most other cells, hydrogen ions are passively distributed across the membrane, and the membrane potential maintains a lower pH inside the RBC than in the blood plasma (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). A sudden addition of acid to the plasma (e.g., following anaerobic production of H^+'s) results in a fall in erythrocyte pH. The acid is transferred from the plasma to the interior of the erythrocyte not by diffusion of H^+, but by bicarbonate–chloride exchange. The addition of H^+'s to the plasma causes the PCO_2 to increase as HCO_3^- is converted to CO_2, which then diffuses into the RBC and is converted to HCO_3^-, thereby reducing intracellular pH. Bicarbonate then diffuses out of the cell via the chloride–bicarbonate exchange mechanism. Thus, in erythrocytes, the CO_2/bicarbonate system operates to transfer acid from the plasma to the cell interior, sometimes referred to as the Jacob–Stewart cycle.

Cells become acidotic during hypoxia and following burst-swimming activity. This proton production causes a drop in plasma pH, which can be very large in fish because nearly two-thirds of their bodies are made up of white muscle fibers all of which can be activated during burst swimming. If this pH drop was transferred to the RBC, oxygen and CO_2 transfer would be seriously impaired in teleost fish, especially those with a large Root-shift hemoglobin (where a reduction in blood pH reduces blood-oxygen-carrying capacity; **see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems). Many teleosts, however, have a Na^+/H^+ exchange mechanism in the RBC membrane to regulate RBC pH, preventing a Root-off shift and maintaining hemoglobin oxygenation in the face of a plasma acidosis (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). Proton excretion is coupled to sodium uptake and is stimulated in oxygenated blood and by increased circulating catecholamine levels. Thus, teleost RBC Na^+/H^+ exchange is augmented during hypoxia, violent activity, and stress by an increase in blood catecholamine levels. The subsequent increase in RBC pH results in an increase in RBC bicarbonate, which moves out into the plasma in exchange for chloride. Thus, the net effect of Na^+/H^+ activation is an increase in RBC pH and NaCl content, the latter causing water to enter and the cell to swell (**Figure 3**).

Bicarbonate levels are high in plasma leaving the gills because RBC-anion exchange is the rate-limiting process in CO_2 excretion. Plasma-bicarbonate dehydration in the gills and in arteries is uncatalyzed in teleost fish. Slow uncatalyzed plasma-bicarbonate dehydration continues to form CO_2 as the blood flows away from the gills, resulting in a gradual rise in plasma pH. The formation of CO_2 in plasma and its subsequent entry into the RBC cause a fall in red cell pH. This in turn causes a rise in partial pressure of oxygen (PO_2) via the Root-off shift as blood flows from

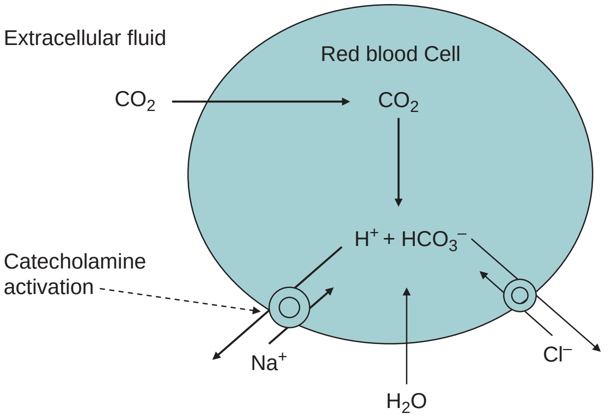


Figure 3 Catecholamines activate proton excretion coupled to sodium uptake in many teleost red blood cells. The increase in pH causes an increase in bicarbonate and the stimulation of chloride–bicarbonate exchange. The result is an uptake of NaCl and water, causing the RBC to swell.

the gills to the tissues. The transit time for blood flow to the tissues must be of the order of a few seconds, sufficient for the CO_2/bicarbonate system to approach equilibrium. Such an increase in PO_2 would augment oxygen delivery to the tissues. Activation of RBC Na^+/H^+ exchange reduces plasma pH which further enhances the magnitude of the disequilibrium in the bicarbonate dehydration reaction in plasma flowing away from the gills (**Figure 4**). Arterial blood flowing to a region where there is carbonic anhydrase imbedded in the capillary endothelium would immediately catalyze plasma bicarbonate dehydration and cause a rapid rise in CO_2. CO_2 will diffuse into the RBC and lower pH, generating a Root-off shift which will further enhance tissue oxygenation (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

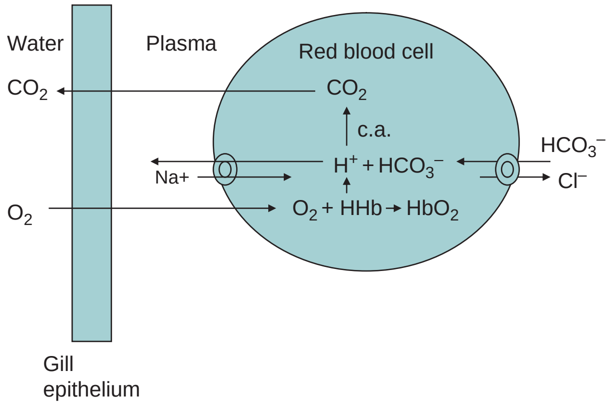


Figure 4 CO_2 diffuses into the water and bicarbonate is removed from the RBC more rapidly than it can enter. Na^+/H^+ exchange removes protons produced by hemoglobin oxygenation, creating a large plasma bicarbonate dehydration disequilibrium in the absence of carbonic anhydrase (c.a.) in the plasma. As blood flows away from the gills, plasma CO_2 increases raising RBC pH and blood PO_2 via the Root-off shift.

Patterns of Carbon Dioxide Excretion

The pattern of CO_2 excretion is different between elasmobranchs and teleosts. Movements of oxygen and carbon dioxide are closely linked in teleost fish, the coupling occurring in the RBC due to the presence of carbonic anhydrase in the erythrocyte and not in the plasma. Teleosts' hemoglobin has reduced buffering but large Bohr and Root shifts to further enhance this coupling. Elasmobranchs have carbonic anhydrase in both plasma and RBC and, consequently, the coupling of CO_2 and oxygen is much less pronounced, the Root shift is absent, and there is no Na^+/H^+ exchange across the RBC membrane. Hemoglobin buffering is much larger in elasmobranch hemoglobin than that in teleosts. As discussed earlier, lamprey have Na^+/H^+ but no anion exchange in the RBC membrane; hagfish lack both Na^+/H^+ and anion exchange in the RBC membrane. Clearly, the pattern of CO_2 excretion in teleosts varies from that observed in terrestrial vertebrates with the pattern in elasmobranchs being most similar to that of terrestrial vertebrates. The teleost pattern is one in which CO_2 and O_2 transfer are highly coupled and may result in augmented transfer of oxygen into the tissues. This has been clearly shown in the swimbladder and eye of teleosts and may be the case in many other tissues (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

Carbon dioxide is involved in gas secretion into the swimbladder (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). The swimbladder is filled with gas in most fish and has several functions including buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). If a fish swims deeper, the water pressure increases and the bladder is compressed. The fish must add gas at pressure to maintain buoyancy. Some teleost fish have a bladder without a duct to the gut connecting it to the environment. The only way gas can get into this bladder in these fish is from the blood. The bladder is filled with oxygen at pressures that increase by 1 atm with every 10-m increase in depth; that is, the bladder can be filled with oxygen at many atmospheres pressure, and the oxygen has diffused across the gills into the blood at no more than 0.2 atm and from the blood into the bladder. There are gas glands situated in the bladder wall with a very extensive blood supply from which oxygen can diffuse into the bladder. The arterial blood supply to the gas gland forms a countercurrent exchange with venous blood leaving the gas gland, the arterial and venous vessels consisting of a large number of capillaries forming a so-called rete mirabile (marvelous network). A gas gland produces acid which drives oxygen from the hemoglobin raising PO_2 levels in the gas gland and oxygen diffuses into the bladder. Oxygen can also diffuse from venous blood leaving the gas gland across the rete mirabile into the arterial capillaries. This serves to retain oxygen in the bladder and allows the buildup of very high oxygen levels. Acid produced in the gas gland is transferred into the RBC by CO_2 which is also recycled through the rete. A similar mechanism occurs in the teleost eye, which also has very high oxygen levels in the ocular fluid. It is clear that CO_2 is very much involved in the transfer of oxygen at high pressure into the bladder and the eye of teleost fish (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). It is possible that CO_2 plays a similar role in the delivery of oxygen to many other tissues in teleost fish, for example, muscle, but there is little evidence for or against this mechanism (see above).

There is some bicarbonate excretion across the apical membranes of the gill but this constitutes only a small portion of CO_2 excretion. In addition, not all CO_2 is excreted across the gills. Marine teleosts are more dilute than the surrounding seawater and continually lose water across their body surfaces. They drink seawater to compensate for the water loss but must excrete the ionic load contained in the seawater. Marine fish excrete calcium and magnesium as calcite (calcium carbonate), producing chalk-like white feces. Thus, a component of CO_2 excretion occurs as carbonate across the gut of marine teleosts. The proportion excreted via the gut is probably around 1–5% of total CO_2 excretion by the fish. The associated protons are presumably excreted across the gills. The white fecal pellets sink to depths of the ocean and eventually dissolve at high pressure. Thus, fish acidify the surface layers of the ocean and contribute to carbonates taken to depth. Fish may contribute somewhere between 3% and 15% of marine carbonate production.

The Effect of Temperature and Variations in Environmental CO_2 Levels

In an aquatic fish, body PCO_2 levels are only a few torr (i.e., mmHg) above ambient. This is because of the countercurrent nature of the flows of blood and water at the gills and the absence of any large diffusion distances. Water flow to supply the oxygen requirements of the fish is more than sufficient to keep CO_2 levels in the blood around 2 or 3 torr and excretion is limited by the rate of Cl^-/HCO_3^- exchange across the red-cell membrane. Although increases in CO_2 cause increases in gill ventilation, fish cannot regulate blood CO_2 levels, and therefore blood pH, via changes in ventilation in a manner similar to that in terrestrial vertebrates. Oscillations in water CO_2 are transferred into the fish and pH compensation occurs via changes in bicarbonate, resulting from changes in proton and/or bicarbonate excretion across the gills and/or the kidney; that is, pH regulation in fish is not mediated by changing CO_2 levels via ventilation (**see also Control of Respiration**: The Ventilatory Response to CO_2/H^+) but rather by the excretion of acid or base into the environment.

The aquatic environment is often hypoxic and many fish supplement their oxygen supply by breathing air. Air breathing in fish involves an air-breathing organ (ABO) that is not usually the gills (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). Gills collapse when air exposed and become nonfunctional, except in a few fish such as *Amia* and mudskippers with more rigid gills. The ABO can be a modified buccal cavity, swimbladder, gut, or skin. Fish predation occurs when they rise to the surface to breathe air, especially by birds. Thus, fish tend to take large infrequent air breaths resulting in large oscillations in blood-oxygen levels. The circulation of the ABO is in series with the gills such that venous blood is pumped first through the gills and then to the ABO. Much of the CO_2 is removed from the blood during passage through the gills, and then oxygen is added from air in the ABO; that is, many air-breathing fish excrete CO_2 into the water via the gills and take up oxygen from the air via an ABO. Many of these fish live in hypoxic waters, and so there are selective pressures to reduce the size of the gills to prevent oxygen loss from the blood as it passes through the gills. A consequence of smaller gills is greater CO_2 retention by the fish, but nevertheless the larger CO_2 gradient across the gills maintains an adequate CO_2 excretion rate. Consequently, air-breathing fish often have higher blood CO_2 levels than completely aquatic fish. Although CO_2 levels can oscillate within a fish and vary among fish, pH is adjusted to a similar value in all fish by adjustment of bicarbonate.

Temperature can have a marked effect on body pH despite blood pH regulation at a given temperature. Fish increase plasma pH with a decrease in temperature (e.g., at 5 °C they have a plasma pH of 7.9–8.0). Adequate excretion rates of both CO_2 and NH_3 can be maintained when blood pH is regulated. Tissues are generally less alkaline than plasma. For example, the intracellular pH of erythrocytes is about 0.5 pH units less than the pH of plasma, and the intracellular pH of muscle cells is about 7.0, but all tissues increase pH with decreasing temperature. Temperature has a marked effect on the p*K* values of plasma proteins and the CO_2/HCO_3^- system, with the p*K* values increasing as temperature decreases. The temperature-induced changes in plasma pH offset the temperature-dependent changes in the p*K* of plasma proteins so that the extent to which the plasma proteins dissociate remains unchanged. The important point is that if body pH changes with temperature in the same way as the p*K* of proteins, then the charge on proteins should remain unchanged. If there is little or no change in the net charge on proteins, their function will be retained over a wide range of temperatures. The apparent p*K* (pK_{app}) of the CO_2 hydration–dehydration reaction changes less with temperature than does blood pH; the ratio of CO_2 to HCO_3^- in the blood must be changed to regulate pH if the charge on proteins is to remain constant. Fish are unable to modulate CO_2 levels, but bicarbonate levels increase as temperature decreases to regulate pH.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Physiology of Triploid Fish. **Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange; Evolution of the Bohr Effect; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M, Koldkjaer P, Kepp O, and Cossins AR (2005) Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Bernier NJ, Brauner CJ, Heath JW, and Randall DJ (2004) Oxygen and carbon dioxide transport during sustained exercise in diploid and triploid chinook salmon *(Oncorhynchus tshawytscha). Canadian Journal of Fisheries and Aquatic Sciences* 61(9): 1797–1805.

Brauner CJ and Randall DJ (1998) The linkage between oxygen and carbon dioxide transport. In: Perry SF, Tufts B, Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology,* vol. 17, pp. 283–319. New York: Academic Press.

Brauner CJ, Thorarensen H, Gallaugher P, Farrell AP, and Randall DJ (2000) CO_2 transport and excretion in rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. *Respiratory Physiology* 119(1): 69–82.

Brauner CJ, Thorarensen H, Gallaugher P, Farrell AP, and Randall DJ (2000) The interaction between O_2 and CO_2 in the blood of rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. *Respiratory Physiology* 119(1): 83–96.

Gilmour KM (1998) Causes and consequences of acid–base disequilibria. In: Perry SF and Tufts BL (eds.) *Fish Physiology, Vol. 17: Fish Respiration*, pp. 321–348. San Diego, CA: Academic Press.

McKenzie DJ, Wong S, Randall DJ, Egginton S, Taylor EW, and Farrell AP (2004) The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *Journal of Experimental Biology* 207: 3629–3637.

Pelster B and Randall DJ (1998) The physiology of the Root effect. In: Perry SF and Tufts BL (eds.) *Fish Physiology, Vol. 17: Fish Respiration*, pp. 321–348. San Diego, CA: Academic Press.

Perry SF and Gilmour KM (1996) Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch. Squalus acanthias and a teleost. *Oncorhynchus mykiss*. *Journal of Experimental Biology* 199: 2105–2118.

Randall DJ (1998) Factors influencing the optimization of hemoglobin oxygen transport in fish. In: Weibel ER, Taylor CR, and Bolis L (eds.) *Principles of Animal Design: The Optimization and Symmorphosis Debate*, pp. 195–201. Cambridge: Cambridge University Press.

Randall DJ, Holeton GF, and Don Stevens E (1967) The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *Journal of Experimental Biology* 46: 339–348.

Tufts BL and Perry SF (1998) Carbon dioxide transport and excretion. In: Perry SF and Tufts BL (eds.) *Fish Physiology, Vol. 17: Fish Respiration*, pp. 229–281. San Diego, CA: Academic Press.

Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange

CJ Brauner and JL Rummer, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Basis for the Interaction between O_2 and CO_2: Bohr–Haldane Effect
Theoretical Bohr–Haldane Coefficient Optimal for Oxygen Delivery
Nonlinear Bohr–Haldane Effect within the OEC
Implications of Non-Steady-State Conditions for the Interaction between O_2 and CO_2 Exchange
Concluding Remarks
Further Reading

Glossary

Arterial Adjective pertaining to blood that has been oxygenated.

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Carbonic anhydrase A zinc metalloenzyme that reversibly catalyzes the reaction between CO_2 and H_2O to form H^+ and HCO_3^-.

Equilibrium Pertaining to the situation when all forces acting are balanced by others resulting in a stable unchanging system.

Haldane effect Proton binding to hemoglobin (as a function of oxygenation).

Hypercarbia High levels of carbon dioxide in water or air.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Partial pressure The pressure that one gas would have if it alone occupied the same volume at the same temperature as the mixture.

Root effect Property of hemoglobins in some fishes such that, in the presence of acid, it is impossible for hemoglobin molecules to be completely saturated with oxygen, even at extremely high oxygen partial pressures.

Venous Adjective pertaining to blood that has passed through tissues having had some oxygen removed for metabolism.

Introduction

All animals produce about the same amount of CO_2 as O_2 consumed through the process of metabolism. O_2 is taken up from the environment and delivered to the tissues by the blood, and CO_2 is released by the tissues and transported by the blood for release to the environment. Hemoglobin (Hb), encapsulated within the red blood cell (RBC), plays a vital role in both O_2 and CO_2 transport in the blood of all vertebrates (with the exception of icefish, the only vertebrate lacking Hb). This article focuses on the nature of the interaction between O_2 and CO_2 at the level of Hb, a topic that has been well studied since the dual role of Hb was discovered in the early 1900s.

Oxygen uptake at the gas-exchange organ (referred to as the gills from this point forward, although the skin and various air-breathing organs can contribute to gas exchange in some fishes) facilitates CO_2 removal through the Haldane effect, where Hb-oxygenation releases H^+'s that combine with HCO_3^- to form CO_2, which diffuses into the environment (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion, Carbonic Anhydrase in Gas Transport and Exchange, and Red Blood Cell Function). CO_2 removal at the gills and the associated increase in blood pH results in an increase in Hb-O_2 affinity, which increases the driving force for O_2 diffusion across the gills, ultimately facilitating O_2 uptake via the Bohr effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). At the tissues, the reverse occurs. CO_2 diffusion into the blood creates an acidosis that facilitates O_2 delivery to the actively metabolizing tissues via the Bohr effect. Oxygen delivery facilitates CO_2 uptake by Hb, and thus CO_2 removal from the tissues via the Haldane effect. Thus, there is an intimate interaction between O_2 and CO_2 transport at both the gills and the tissues at the level of Hb within the RBC, which is determined in part by the magnitude of the Bohr and Haldane effects.

Tremendous diversity in Hb characteristics exists within the fishes, making this group of animals particularly interesting for investigating the interaction between O_2 and CO_2 exchange. Some fish possess very small, even nonexistent Bohr and Haldane effects with relatively high Hb-buffer values, substantially limiting the interaction between O_2 and CO_2 exchange. However, the majority of fishes (teleosts) possess large Bohr and Haldane effects (as well as a Root effect, where oxygen-carrying capacity of the blood is reduced at low pH) and low Hb-buffer values, collectively resulting in tightly coupled O_2 and CO_2 exchange. Furthermore, many teleost fishes exhibit a nonlinear Bohr and Haldane effect over the region of the oxygen-equilibrium curve (OEC), which has further implications for the nature of the interaction between O_2 and CO_2 exchange.

Basis for the Interaction between O_2 and CO_2: Bohr–Haldane Effect

The interaction between O_2 and CO_2 exchange is largely determined by the Bohr and Haldane effects as discussed above; however, their respective magnitudes are important in determining the nature of the interaction. The Bohr effect describes how the affinity of Hb for O_2 is affected for a given change in the H^+ concentration (pH) of the blood. It is calculated as follows:

$$\text{Bohr coefficient } = -\Delta \log P_{50}/\Delta p\text{H} \qquad [1]$$

where P_{50} refers to the partial pressure of O_2 (P_{O_2}) at which 50% of the Hb molecules are oxygenated.

The Haldane effect describes how the affinity of Hb for H^+'s and CO_2 is affected by changes in Hb-O_2 saturation. It is calculated as follows:

$$\text{Haldane coefficient} = \Delta\text{H}^+ \qquad [2]$$

where ΔH^+ refers to the moles of H^+'s released per mole of O_2 bound to Hb.

Although the Bohr and Haldane effects are often discussed in terms of their respective roles relative to O_2 and CO_2 dynamics at the level of the Hb, they are actually mirror images of the same phenomenon. While the Bohr effect describes changes in Hb-O_2 affinity that arise from a change in H^+ concentration, the Haldane effect describes the changes in Hb–H^+ affinity that arise from a change in P_{O_2}, and therefore Hb-O_2 saturation. Thus, the Bohr and Haldane effects are linked functions, as has been recognized by the classic Wyman linkage equation:

$$(\log P_{O_2}/\text{pH})\,Y = (\text{H}^+/Y)\text{pH} \qquad [3]$$

where Y refers to Hb-O_2 saturation and H^+ refers to the number of protons bound per heme molecule. Assuming that the shape of the OEC is symmetrical and H^+ release is linear with Hb-binding O_2, which is often the case in vertebrates (but not always, as described below in section Nonlinear Bohr–Haldane Effect), the linkage equation is often reduced to the following:

$$-\Delta\log P_{50}/\Delta\text{pH} = \Delta\text{H}^+ \qquad [4]$$

The point is that the Bohr and Haldane coefficients are numerically equivalent, and will be referred to as the Bohr–Haldane coefficient, and reported as a positive value from this point forward. Further, this relationship has been experimentally validated. Air-breathing animals typically have moderate Bohr–Haldane coefficients (i.e., 0.35), while most teleosts have relatively large Bohr–Haldane coefficients (0.5 to >1.0). The numeric value has large implications for the nature of the interaction between O_2 and CO_2 exchange *in vivo*, as described in the following section.

Theoretical Bohr–Haldane Coefficient Optimal for Oxygen Delivery

The potential benefit to tissue-O_2 delivery associated with the Bohr effect is quantified as the product of the pH change associated with metabolic CO_2 production during blood transit through a tissue (arterial–venous pH change (pH_{a-v})) and the magnitude of the Bohr–Haldane coefficient. A large Bohr effect is often assumed to convey a greater benefit to tissue O_2 delivery, but this will only be true if the pH change described above is sufficient. Associated with a large Bohr effect is a large Haldane effect; therefore, upon deoxygenation, Hb will bind H^+'s, thereby reducing the magnitude of the pH_{a-v} and consequently the expression of the Bohr effect at the tissues. In 1983, Lapennas conducted an analysis to determine the optimal Bohr coefficient for O_2 delivery under steady-state conditions. Assuming that the pH_{a-v} arises from tissue CO_2 production (and associated conversion to HCO_3^- and H^+) and that most animals have a tissue respiratory quotient (RQ) between 0.7 and 1.0 (moles of CO_2 produced per mole of O_2 consumed; **see also Tissue Respiration**: Cellular Respiration), a Bohr–Haldane coefficient of 0 will result in the largest pH change within the tissues. This would be due to the absent Haldane effect and associated H^+ binding upon deoxygenation, but would have no effect on O_2 delivery due to the lack of pH sensitivity of the Hb (i.e., no Bohr effect). Conversely, a Bohr–Haldane coefficient equivalent to the RQ (e.g., 1.0) will result in no pH change due to the increased H^+ binding associated with the large Haldane effect. That is, all protons produced by CO_2 production at the tissues will be bound by Hb as O_2 is released to the tissues, and, despite the presence of a pH-sensitive Hb with a large Bohr effect, there may be no benefit to O_2 delivery due to the lack of pH_{a-v}. Furthermore, a Bohr–Haldane coefficient greater than RQ may result in a reverse pH_{a-v}, during blood transit, which actually could impair tissue-O_2 delivery.

Lapennas determined that the optimal Bohr coefficient for O_2 delivery under steady-state conditions (and with many assumptions) is 0.5 × RQ. This represents a compromise between pH sensitivity of the Hb and the resulting pH change that occurs during capillary blood transit. Because many air-breathing vertebrates have Bohr–Haldane coefficients of 0.35 (which is very close to optimal if RQ is assumed to be 0.7), he concluded that their Hbs have been optimized for O_2 delivery (**Figure 1**). Given that most teleost fish possess Bohr–Haldane coefficients much greater than 0.5 × RQ (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect), it has been assumed that, under steady-state conditions in most tissues, fish Hbs may be optimized for CO_2 transport and acid–base homeostasis rather than tissue-O_2 delivery. This clearly does not apply to the unique structures within the swimbladder and eye, where there exists a tremendous potential for generating and localizing an acidosis, which in conjunction with the Root effect and associated large Bohr–Haldane coefficients generates incredibly high O_2 tensions (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems and Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). However, in other tissues, given Lapennas' assumptions, a very large Bohr–Haldane coefficient would not benefit tissue-oxygen delivery. Despite Lapennas' many assumptions, his analyses serve as an interesting framework for hypothesizing how different Bohr–Haldane coefficients within and between species may influence the interaction between O_2 and CO_2 exchange *in vivo*.

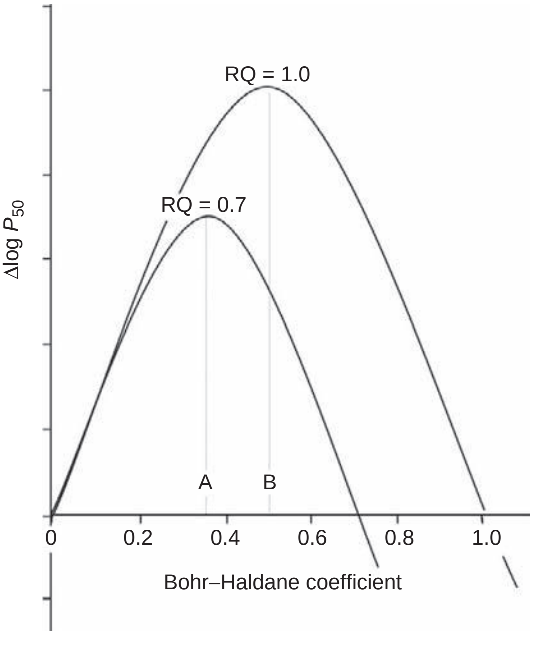


Figure 1 The optimal Bohr–Haldane coefficient: theoretical Bohr shifts, as described by a change in P_{50} (Δlog P_{50}) during blood capillary transit using two respiratory quotients (RQs). Units have been omitted intentionally from the *y*-axis, because the magnitude of this response will vary by species, depending on Hb buffer values. A and B indicate Bohr–Haldane coefficients optimal for O_2 delivery for RQ values of 0.7 and 1.0, respectively. Each curve crosses the *x*-axis at both zero and the RQ, two points at which Lapennas suggests there will be no benefit to O_2 delivery associated with the Bohr–Haldane effect. Modified from Lapennas GN (1983) The magnitude of the Bohr coefficient: Optimal for oxygen delivery. *Respiration Physiology* 54(2): 161–172.

Nonlinear Bohr–Haldane Effect within the OEC

The assumption for many models depicting vertebrate Hb function is that the magnitude of the Bohr–Haldane coefficient is relatively constant across the entire OEC. Although this may be true for most air-breathing vertebrates, it is not the case in several fish species, where most of the Bohr–Haldane effect occurs in the upper reaches of the OEC (between 50% and 100% Hb-O_2 saturation) with very little expression below 50% Hb-O_2 saturation. The nonlinear Bohr–Haldane effect is typically associated with species that possess Root-effect Hbs, perhaps suggesting that nonlinearity is common among teleosts. When fish use different regions of the OEC for gas exchange *in vivo*, implications for a nonlinear Bohr–Haldane effect influencing the interaction between O_2 and CO_2 exchange become interesting.

The entire Bohr–Haldane effect may be exploited in resting fish, where venous Hb–O_2 saturation levels rarely fall below 50%. In resting rainbow trout *Oncorhynchus mykiss*, the Bohr–Haldane coefficient calculated over the region of the OEC used *in vivo* is approximately 1.0, a value close to RQ. Therefore, CO_2 excretion at the gills and CO_2 and acid–base transport at the tissues will be maintained because Hb will bind all H^+'s released from the tissues during O_2 delivery. However, when fish are forced to swim, the arterial–venous O_2 difference increases, requiring a greater region of the OEC to be used for gas exchange. The magnitude of the Bohr–Haldane coefficient calculated over the region of the OEC used for gas exchange during exercise is reduced to a value of 0.4–0.5, remarkably close to the value deemed optimal for O_2 delivery by Lapennas. Accordingly, during periods of increased activity and therefore muscle-O_2 demand, the nonlinear Bohr–Haldane effect may be important for optimizing O_2 delivery. Thus, the nature of the interaction between O_2 and CO_2 exchange, if a nonlinear Bohr–Haldane effect is present, changes with exercise intensity and the region of the OEC exploited for gas exchange.

Implications of Non-Steady-State Conditions for the Interaction between O_2 and CO_2 Exchange

Most of the discussion to this point has assumed steady-state, equilibrium conditions; however, it is unlikely that such conditions exist *in vivo,* because gas exchange consists of a complex combination of blood flow, boundary layers, chemical reactions, and diffusion. For example, if CO_2 from the tissues diffuses into the blood faster than O_2 diffuses to the tissues, a large Bohr–Haldane coefficient could facilitate O_2 delivery during blood capillary transit. The only way to determine whether this occurs *in vivo* is by direct measurement, which is very difficult. In the only published study to date where this has been conducted in fish, P_{O_2} was measured in real time via an optode implanted in the red muscle of resting rainbow trout. Results indicate a much higher tissue P_{O_2} than in mammals. Despite an *in vivo* Bohr–Haldane coefficient of 1.0 under resting conditions (far greater than the optimal value determined as described above), it could be that general O_2 delivery is enhanced in rainbow trout, and perhaps other fish species. Clearly, additional studies of this nature are required to investigate this further.

In fish, the greatest disequilibrium state at the level of the RBC is likely associated with catecholamine-stimulated Na^+/H^+ exchange. During stress, or when Hb-O_2 saturation falls below 50%, metabolic CO_2 and H^+ production may be elevated. Catecholamines such as adrenaline and noradrenaline are released into the circulation and bind to β-adrenergic receptors on the RBC membrane. Through adenylate cyclase, which activates 3′,5′-cyclic monophosphate (cAMP), the β-adrenergic Na^+/H^+ exchange (βNHE) on the RBC membrane is activated. The carbonic anhydrase (CA)-catalyzed hydration of CO_2 inside the RBC produces H^+ and HCO_3^-; the former are removed in exchange for Na^+ via the βNHE to restore pH_i and therefore Hb-O_2 affinity, but at the cost of decreasing plasma pH. The HCO_3^- is removed via anion exchange for Cl^-. The resulting osmotic gradient activates the Na^+, K^+ pump, and osmotically obliged water enters the RBC, causing the cell to swell (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion and Red Blood Cell Function). This process is thought to have evolved to protect O_2 uptake at the respiratory surface during a general acidosis in species possessing Root-effect Hbs, where an acidosis drastically decreases not only Hb's affinity but also carrying capacity for O_2. However, this is also an example of a disequlibrium state where O_2 and CO_2 transport dynamics do not follow steady-state models.

The adrenergically activated βNHE elevates pH_i but only in the absence of plasma-accessible CA. If CA were accessible to the plasma, this would short-circuit the RBC βNHE, because H^+'s would combine with HCO_3^- to form CO_2 and back-diffuse into the RBC, decreasing pH_i (**Figure 2**). Fish lack plasma-accessible CA at the gills and thus adrenergically activated βNHE protects O_2 uptake at the respiratory surface; however, CA may be plasma accessible in some tissues, where the CA IV isoform (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange) is bound to endothelial cells and plasma oriented. When the βNHE is activated during stress, and blood passes through capillaries possessing CA, the RBC βNHE is short-circuited, resulting in a much larger pH_{a-v} than which would otherwise occur, greatly facilitating O_2 delivery to the tissues.

Short-circuiting of RBC βNHE has been validated *in vitro.* When CA is added to adrenergically stimulated, mildly acidified RBCs in a closed system, it results in a rapid acidification of RBC pH_i and a dramatic increase in P_{O_2}. Accordingly, short-circuiting of RBC βNHE *in vivo* could potentially have large effects. In rainbow trout implanted with an O_2 optode in red muscle, which allows real-time tissue P_{O_2} monitoring, inducing a mild acid–base disturbance by exposing fish to elevated environmental CO_2 levels (hypercarbia) results in a significant increase in tissue P_{O_2}. However, when plasma-accessible CA is subsequently inhibited following the addition of a non-membrane-permeable CA inhibitor to the blood, the hypercarbia-induced increase in tissue P_{O_2} is abolished, indicating that short-circuiting of RBC βNHE may be operational *in vivo* and may greatly facilitate O_2 delivery. Therefore, although rainbow trout have a large Bohr–Haldane effect, a large pH_{a-v} in the RBC may occur via exploiting disequilibrium states *in vivo.* Furthermore, this system would operate with every pass through the tissues, effectively harnessing the general acidosis to create a RBC pH_{a-v}, which will be localized to tissues that possess plasma-accessible CA.

Concluding Remarks

The intricate relationship between O_2 and CO_2 exchange has been described in past models that assume equilibrium conditions, which link the Bohr–Haldane relationship to the RQ in order to make predictions as to optimal O_2 delivery or CO_2 transport and acid–base homeostasis. However, equilibrium conditions probably never occur *in vivo.* In addition, teleosts possess a large Bohr–Haldane coefficient, which according to Lapennas' theory may be detrimental to tissue O_2 delivery. Yet, real-time measurements confirm elevated muscle P_{O_2} in rainbow trout, suggesting that a mechanism is in place to enhance O_2 delivery in fish. Teleosts' unique Root-effect Hbs convey a great potential for O_2 delivery, and possessing a nonlinear Bohr–Haldane effect allows different parts of the OEC to

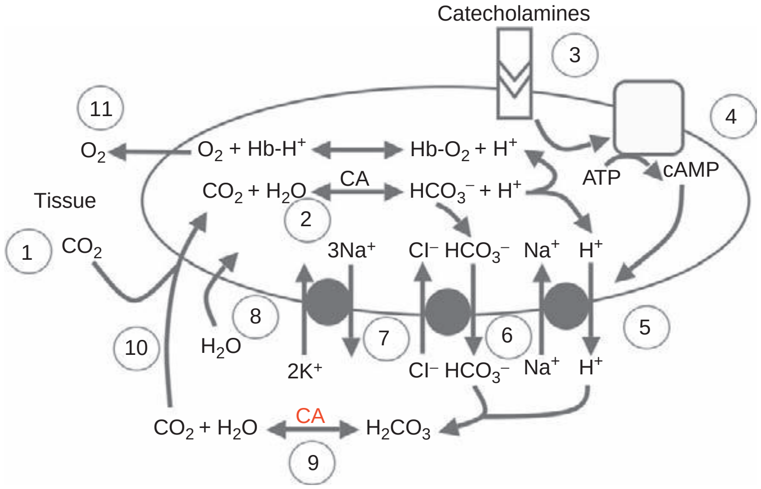


Figure 2 Short-circuiting the β-adrenergic response at the red blood cell: With the advent of an acidosis or when metabolic CO_2 production is high, the red blood cell (RBC) intracellular pH (pH_i) can decrease substantially. Metabolic CO_2 enters the RBC (1) and is catalyzed by carbonic anhydrase (CA) to form HCO_3^- and H^+ (2), the latter of which will bind hemoglobin (Hb), thus releasing O_2. Catecholamines such as adrenaline and noradrenaline are released into the circulation to bind to receptors on the RBC membrane (3), activating adenlylate cyclase and 3′,5′-cyclic monophosphate (cAMP) (4), which activates the Na^+, H^+ exchanger (βNHE) (5). As protons (H^+) are removed from the RBC in exchange for Na^+, pH_i increases and Hb–O_2 affinity is restored. Bicarbonate (HCO_3^-) is removed from the RBC in exchange for Cl^- via the anion exchanger (6), resulting in an osmotic gradient that activates the Na^+, K^+ pump (7). Osmotically obliged water enters the cell (8) resulting in RBC swelling. As long as βNHE rates are high and CA is not accessible to the plasma, this results in an increase in RBC pHi. However if CA is plasma accessible (9), which may occur in the tissues, H^+ and HCO_3^- removed from the RBC would be catalyzed to form CO_2 that would back-diffuse into the RBC (10). This would short-circuit the original protective function of the βNHE mechanism and favor O_2 unloading from the Hb (11), which in the case of Root-effect Hbs would be substantial, creating enhanced O_2 delivery localized to tissues possessing plasma-accessible CA.

be utilized under different conditions. Indeed, the potential exists, and during non-steady-state conditions such as intense exercise or hypoxia exposure, adrenergic stimulation of the RBC βNHE creates the acid–base disequilibrium needed to maximize this potential. Plasma-accessible CA in select locations within metabolizing tissue may be localizing the acidosis by short-circuiting the βNHE, therefore facilitating O_2 delivery to tissues to a much greater degree than that in air-breathing vertebrates. Root-effect Hbs evolved long before the eye and swimbladder retia, structures typically associated with the unique pH-sensitive Hb, suggesting a general use for such great O_2 delivery potential (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems). Along with the increase in and nonlinearity of the Bohr–Haldane effect, teleosts appear to greatly facilitate O_2 transport with only moderate whole-blood pH changes.

See also: **Tissue Respiration**: Cellular Respiration. **Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange; Carbon Dioxide Transport and Excretion; Evolution of the Bohr Effect; Hemoglobin; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M, Koldkjaer P, Kepp O, and Cossins AR (2005) Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307(5716): 1752–1757.

Brauner CJ and Randall DJ (1998) The linkage between oxygen and carbon dioxide transport. In: Perry SF and Tufts B (eds.) *Fish Physiology, Volume 17: Fish Respiration*, pp. 283–319. New York: Academic Press.

Brauner CJ, Thorarensen H, Gallagher P, Farrell AP, and Randall DJ (2000) The interaction between O_2 and CO_2 exchange in rainbow trout during graded sustained exercise. *Respiration Physiology* 119: 83–96.

Brauner CJ, Wang T, Val AL, and Jensen FB (2001) Non-linear release of Bohr protons with haemoglobin–oxygenation in the blood of two teleost fishes: Carp (*Cyprinus carpio*) and tambaqui (*Colossoma macropomum*). *Fish Physiology and Biochemistry* 24(2): 97–104.

Jensen FB (1989) Hydrogen ion equilibria in fish haemoglobins. *Journal of Experimental Biology* 143: 225–234.

Lapennas GN (1983) The magnitude of the Bohr coefficient: Optimal for oxygen delivery. *Respiration Physiology* 54(2): 161–172.

McKenzie DJ, Wong S, Randall DJ, *et al.* (2004) The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *Journal of Experimental Biology* 207(21): 3629–3637.

Nikinmaa M (1990) *Vertebrate Red Cells: Adaptations of Function to Respiratory Requirements*. Berlin: Springer.

Tufts B and Perry SF (1998) Carbon dioxide transport and excretion. In: Perry SF and Tufts B (eds.) *Fish Physiology, Volume 17: Fish Respiration*, pp. 229–281. New York: Academic Press.

Wyman J (1973) Linked functions and reciprocal effects in haemoglobin: A second look. *Advanced Protein Chemistry* 19: 223–286.

Evolution of the Bohr Effect

M Berenbrink, University of Liverpool, Liverpool, UK

© 2011 Elsevier Inc. All rights reserved.

Discovery and Definition of the Bohr Effect
Distinction between a Bohr Effect and a Root Effect
Factors Modulating the Magnitude of the Bohr Effect in Vertebrates
Occurrence of the Bohr Effect in Animals
Molecular Mechanism(s) of the Bohr Effect in Vertebrate Hbs
Benefits and Evolution of the Bohr Effect
Further Reading

Glossary

Allosteric binding Binding of an effector molecule to a protein site that is different from the active site.

Bohr effect The property of vertebrate hemoglobins whereby changes in pH affect the hemoglobin-oxygen affinity, supporting loading (in the lungs/gills) and unloading (in the tissues) of oxygen from hemoglobin.

Equilibrium Pertaining to the situation when all forces acting are balanced by others, resulting in a stable unchanging system.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Root effect The property of hemoglobins of some fishes such that in the presence of acid, it is impossible for the hemoglobin molecule to be completely saturated with oxygen, even at extremely high oxygen partial pressures.

Discovery and Definition of the Bohr Effect

The Bohr effect generally describes the effect of pH on the blood-O_2-binding affinity. It is named after the eminent Danish respiratory physiologist Christian Bohr, who in 1904 together with his pupils, Karl Hasselbalch and the later Nobel prize winner August Krogh, published their observations on the effect of the CO_2 tension (i.e., partial pressure) on the binding of O_2 by dog blood. They showed that an increase in carbon dioxide partial pressure (P_{CO_2}) decreased the affinity of the blood for O_2 and realized immediately the physiological importance of this effect. They predicted that what became to be known as the Bohr effect would enhance O_2 delivery to the tissues, where CO_2 was released into the blood, and facilitate O_2 uptake in the lungs, where CO_2 was discharged from the blood. They also observed that the effect of CO_2 on blood-O_2 binding decreased at oxygen partial pressure (P_{O_2}) values approaching those of air, where the blood approached complete O_2 saturation and the effect of P_{CO_2} became negligible. The Bohr effect occurs in the blood of many vertebrates and, in **Figure 1**, the original data of Bohr and colleagues are replotted in the form of a series of blood-O_2 binding curves with increasing P_{CO_2} and compared with data on carp (*Cyprinus carpio*) blood.

The effect of CO_2 on blood-O_2 binding can be mimicked by lactic acid and it is now known that the Bohr effect is mainly due to the hydrogen ions that are generated in the hydration of CO_2 and subsequent dissociation into bicarbonate and protons:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \qquad (1)$$

Although the concept of pH was only introduced after its original discovery, the Bohr effect is now generally assessed by determining the pH-dependence of the blood-O_2 affinity. When pH is decreased within the physiological pH range, blood exhibiting a Bohr effect requires a higher P_{O_2} for half-maximal O_2 saturation (the P_{50}).

Distinction between a Bohr Effect and a Root Effect

In many fishes, CO_2 or low pH exert an additional effect on blood-O_2 binding. In 1931, Root discovered that the effect of CO_2 on blood-O_2 binding persists in some marine fishes at high P_{O_2} values, such that blood cannot be fully saturated with O_2 even at atmospheric P_{O_2} values. This latter feature is widespread among ray-finned fishes, including teleosts, and is known as the Root effect. The

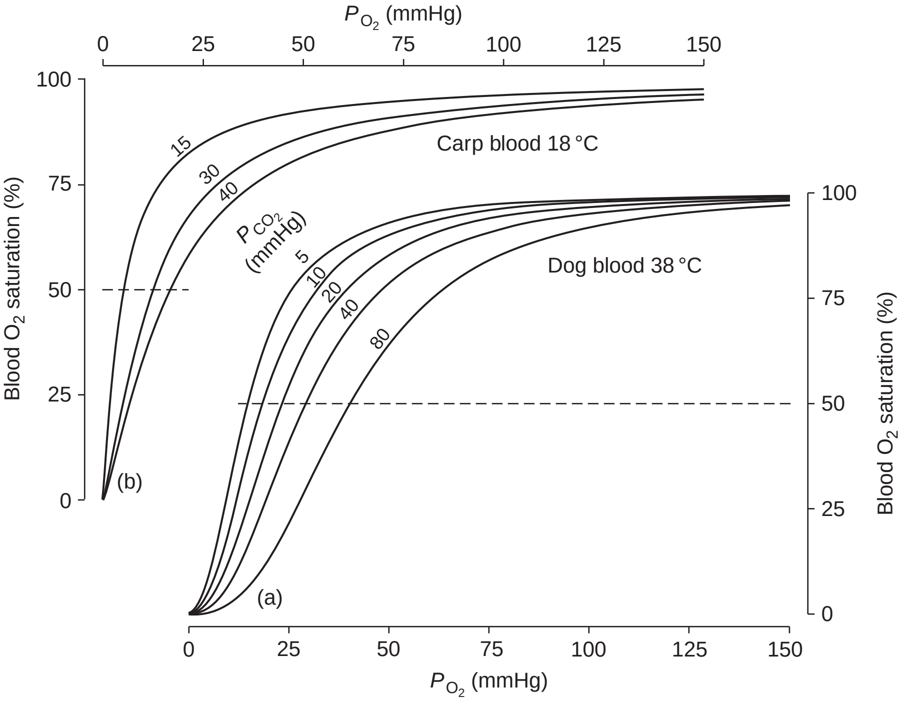


Figure 1 Effect of carbon dioxide partial pressure (P_{CO_2}) on blood-oxygen binding in dog (a) and carp (b) blood. In both cases, increasing P_{CO_2} increases the oxygen partial pressure (P_{O_2}) at half-maximal saturation (dashed lines). Curves are fits to the original data reported (a) by Bohr C, Hasselbalch K, and Krogh A (1904) *Skandinavisches Archiv für Physiologie* 16: 402–412 and (b) by Wastl H (1928) *Biochemische Zeitschrift* 197, 363–380.

Root effect is an important component of the mechanism for O_2 secretion in the swimbladder and eye of many fishes (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder).

Figure 2 shows a series of blood-O_2 binding curves of the marine goosefish *Lophius americanus* at different pH values to illustrate the difference between the Bohr effect and the Root effect. Between pH 8 and 7, a decrease in blood pH shifts the O_2-binding curve toward the right so that higher P_{O_2} values are needed for half-maximal O_2 saturation. Extension of the measurement range for P_{O_2} up to 750 mmHg (close to 1 atmosphere of O_2) shows that pH in this range does not affect the maximal O_2-binding capacity, because the blood can be still 100% saturated at high P_{O_2}. This indicates a Bohr effect. After further acidification below pH 7, high P_{O_2} values fail to fully saturate the blood as indicated by the asymptotic maximum of the curves falling below 100%. This is typical of the Root effect. A more detailed analysis of the curves shows that below pH 7 the curves are no longer S-shaped and approach the shape of a rectangular hyperbola. This loss of cooperative O_2 binding is not seen in Bohr-effect hemoglobins (Hbs) and is another distinguishing characteristic of the Root effect.

Factors Modulating the Magnitude of the Bohr Effect in Vertebrates

The magnitude of the Bohr effect can vary within a species, depending on the type of Hbs and their microenvironment inside the red blood cell. It is most often quantified by the change in the logarithm of P_{50} per change in pH ($\Delta\log P_{50}/\Delta$pH). Usually the Bohr effect shows a maximum within the physiological pH range inside red blood cells and declines at higher and lower pH values. At very low pH values, the Bohr effect can be reversed, meaning that a decrease in pH actually increases Hb-O_2 affinity. This is called a negative or acid Bohr effect as compared to the normal or alkaline Bohr effect.

The Bohr effect is generally reduced by low organic-phosphate concentrations and in many species also by low chloride concentrations and/or elevated P_{CO_2}. The blood of many fish species contains more than one Hb type and these can differ in their functional properties. Thus, the blood of salmonids, such as rainbow trout *Oncorhynchus mykiss*, and of eels, such as the European eel *Anguilla anguilla*, contains Hb components that can constitute about 20–40% of the total Hb and that lack, or even show a reverse Bohr effect, thereby reducing the Bohr effect of the composite Hb system.

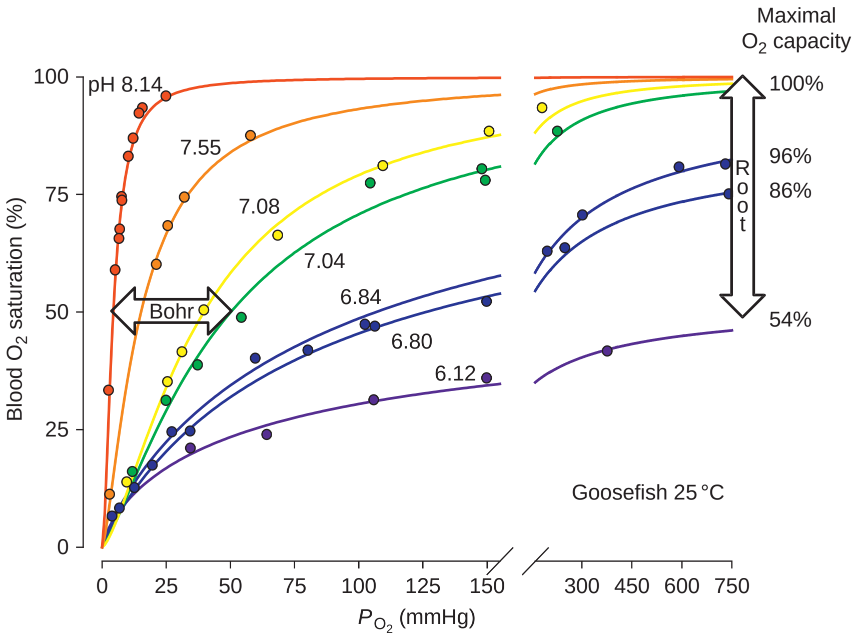


Figure 2 Effect of pH on whole-blood-oxygen binding in a teleost, the goose fish. Above pH 7, the blood displays a classical Bohr effect, namely a decrease in pH increases the oxygen partial pressure (P_{O_2}) required for half-maximal oxygen (O_2) saturation but 100% blood O_2 saturation is still achieved at high P_{O_2}. Below pH 7, a Root effect is displayed: a decrease in pH increases the P_{O_2} at half-maximal saturation and decreases the apparent maximal O_2 capacity. Modified from Green AA and Root RW (1933) *Biological Bulletin* 64: 383–403.

Another source for (apparent) variability lies in the reference pH that is used for Bohr-effect assessments. Blood pH measurements essentially assess the pH of the plasma. The intracellular pH of red blood cells in the vicinity of Hb is usually lower than that of the plasma and any change in plasma pH is not completely transferred to intracellular pH, particularly when red cell pH is regulated by the activation of ion regulatory pathways (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). Therefore, even when other factors, such as P_{CO_2} and the concentrations of chloride, Hb, and organic phosphate are kept the same, the whole-blood Bohr effect underestimates the true effect of pH on Hb-O_2 affinity, which is obtained by relating it to intracellular pH or the buffer pH of Hb solutions.

Occurrence of the Bohr Effect in Animals

A Bohr effect of similar magnitude to that originally found in dog blood by Bohr and colleagues is also found in blood of humans, other mammals, and most tetrapods (exceptions include tadpoles and a caecilian). The effect is of approximately similar size in lungfishes, e.g., *Lepidosiren paradoxa*, the coelacanth *Latimeria chalumnae*, and basal members of the ray-finned fishes such as bichir, sturgeon, and gar (*Polypterus senegalus, Acipenser ruthenus*, and *Lepisosteus platyrhincus*, respectively).

Most teleost fishes have a particularly strong Bohr effect. However, in elasmobranchs and hagfishes, elevated P_{CO_2} or a pH decrease has only a small effect on blood-O_2 affinity. In lampreys, the Bohr effect is as pronounced as in teleosts. The Bohr effect is not only found in bloods containing intracellular Hb but also found in the giant extracellular Hbs of annelid worms, hemocyanins of cephalopods and crustaceans, and in the hemerythrins of brachiopods. The latter two groups of respiratory pigments have quite different protein structures compared to globins and the O_2-binding sites consist of Cu atoms and non-heme-bound Fe, respectively. The fundamental differences in the nature of the O_2-binding site and/or in the protein structure of the diverse invertebrate respiratory pigments and vertebrate Hbs already indicate that the Bohr effect in animal bloods evolved several times and by quite different molecular mechanisms.

Molecular Mechanism(s) of the Bohr Effect in Vertebrate Hbs

The pH-induced change in O_2 affinity involves allosteric binding of protons to Hb at specific sites, which then decrease the O_2 affinity of the O_2-binding sites. The molecular mechanism of the Bohr effect has been most extensively studied on human Hb. According to the two-state allosteric model, tetrameric Hb of jawed vertebrates exists in equilibrium between two different

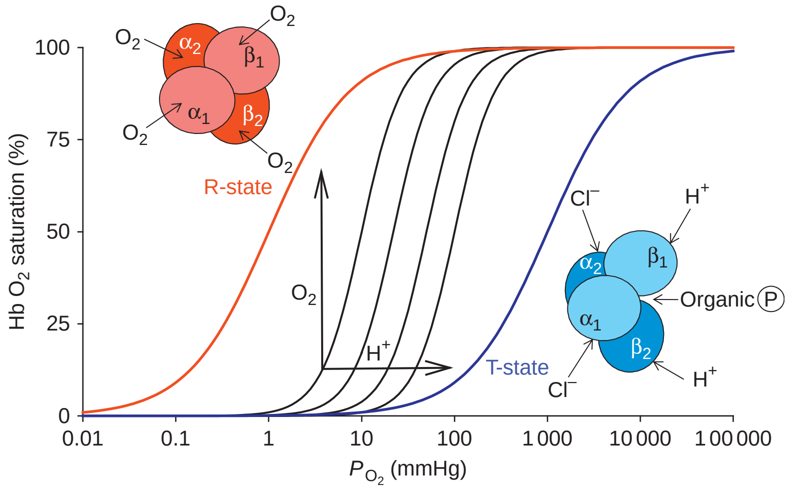


Figure 3 Schematic explanation of the Bohr effect according to the two-state allosteric model. Hb tetramers exist in equilibrium between the R-state and T-state conformations, which have inverse affinities for O_2 and H^+ (indicated by arrows). High P_{O_2} promotes the transition (black lines) from the low-O_2-affinity T-state to the high-O_2-affinity R-state (red line), which is the basis for cooperative O_2 binding (increased steepness of black curves at intermediate O_2 saturations). Protons stabilize the low-O_2-affinity T-state (blue line). This increases P_{50} by delaying the T- to R-state transition. Chloride and organic phosphates enhance the effect of protons by additional stabilization of the T-state. Note that the R-state and T-state O_2-binding curves are really rectangular hyperbolas when depicted on a nonlogarithmic *x*-axis scale.

conformations that differ in their intrinsic O_2 affinity: a tense (T-state) conformation with low O_2 affinity and a relaxed (R-state) conformation with a high O_2 affinity (**Figure 3**). The T-state prevails at low P_{O_2} values and has a relative high affinity for protons, organic phosphates, and chloride, while the R-state is favored at high P_{O_2} and has a lower affinity for protons, organic phosphates, and chloride.

The two-state allosteric model is an oversimplification, because there is increasing evidence that the O_2 affinity in the R-state and T-state itself can also vary with pH. However, the model is useful in explaining the cooperative nature of Hb-O_2 binding by the shift from a low-O_2-affinity T-state at low P_{O_2} to a high-affinity R-state at higher P_{O_2} values. It accounts for the Bohr effect by simply assuming that an increase in the proton concentration shifts the equilibrium between the two Hb conformations toward the low-O_2-affinity T-state, thereby lowering the overall O_2 affinity of the system. Using the two-state allosteric model, it is easy to see how elevated chloride or organic phosphate concentrations could enhance the Bohr effect by additionally stabilizing the T-state conformation.

The magnitude of the Bohr effect can also be expressed in terms of the number of protons that can be additionally bound by, and thereby stabilize, the low-O_2-affinity T-state conformation compared to the high-O_2-affinity R-state conformation. Under standardized conditions of temperature, pH, chloride, CO_2, and organic phosphate concentrations, the number of these so-called Bohr protons can be used to compare the magnitude of the Bohr effect between different species. The molecular sites on Hb where these Bohr protons bind, and thereby the molecular mechanism of the Bohr effect, have been intensively studied. Recently, it has become clear that even within vertebrates no single mechanism can account for the Bohr effect observed in mammals, teleost fishes, and lampreys.

Mammals

In mammals, in general, about two Bohr protons per Hb tetramer are additionally bound by the low-O_2-affinity T-state, compared to the R-state Hb. In humans, the terminal histidine amino acid residue in position 146 of the beta chain of the Hb tetramer is the major site for the binding of Bohr protons, accounting for 63% of the Bohr effect under standard conditions (**Figure 4(a)**). Another 22% of the Bohr effect is contributed by proton binding to other histidine residues at different positions, with three histidine residues in the alpha chain and two in the beta chains having a moderate positive effect and two other beta-chain histidine residues having a moderate negative contribution to the Bohr effect. The remaining 14% of the total Bohr effect can be explained by non-histidine Bohr-proton-binding sites. Thus, in the T-state, chloride binding to the amino group of the N-terminal amino acid in the alpha chains allows additional binding of Bohr protons.

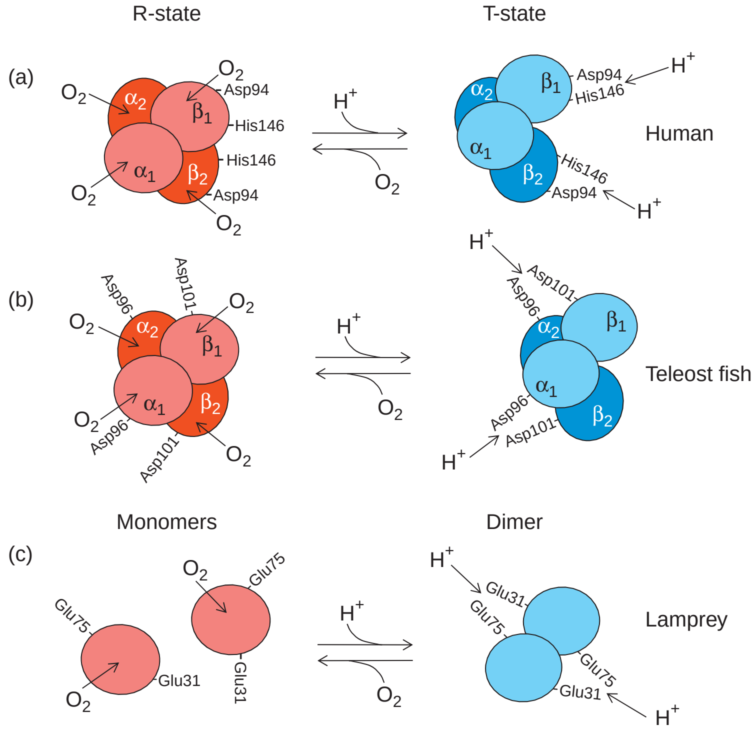


Figure 4 Molecular mechanisms of the Bohr effect in vertebrate Hbs, schematically indicating major proton-binding sites that stabilize low-O_2-affinity conformations over high-O_2-affinity conformations. (a) In human Hb, the Bohr effect is largely caused by proton binding to the imidazole side chain of the terminal histidines (His) in the two beta chains. This allows formation of a salt bridge with an aspartic-acid (Asp) residue in the same chain, which stabilizes the low-O_2-affinity T-state (blue). This interaction is not possible in the high-O_2-affinity R-state (red). (b) In teleost fishes, the pH sensitivity of Hb-O_2 binding is largely caused by stabilization of the T-state through proton binding at a different site. Two Asp residues at the alpha–beta interface between the two dimers (shaded light and dark) that make up the Hb tetramer form a negative cluster in the T-state conformation that allows proton binding. (c) In lamprey Hb, the low-O_2-affinity dimer is stabilized over the high-O_2-affinity monomers by proton binding at two clusters of glutamic acid (Glu) residues at the dimer interface. Amino acids are numbered according to their position in the 2 alpha- and 2 beta-globin chains (α_1, α_2 and β_1, β_2).

Teleost Fishes

In most teleost fish Hbs, the Bohr effect is twice as strong as in mammals because up to four additional protons are bound, thereby stabilizing the low-O_2-affinity T-state. However, the overall Bohr effect in the blood of some teleost species is not higher than in mammals due to the presence of additional Hb components with no, or even a reverse, Bohr effect, as in eels and trout (see above). Like in mammals, and indeed tetrapods, fish Hbs with a Bohr effect generally possess a histidine at position 146 in their beta chains. Studies on carp Hb, in which this histidine was chemically removed, suggest that this treatment may reduce the Bohr effect by 50%. However, structural studies on several other fish Hbs suggest that histidine beta 146 is not involved in the Bohr effect in these species. Teleost fishes further have a much-reduced histidine content in their alpha- and beta-globin chains, so that most of the other histidines implicated in the Bohr effect in human Hb are missing. In addition, the terminal amino group of the alpha chain is chemically blocked, so that the chloride-dependent binding of Bohr protons at this site that occurs in human Hb is also impaired. Thus, the majority of the Bohr protons in teleosts must bind at other sites than in human Hb.

Indeed, in Hb of tuna (*Thunnus thynnus*), carp, Antarctic emerald rockcod (*Trematomus bernacchii*), and in Hb IV of trout, about half of the Bohr protons may bind to two clusters of aspartic-acid residues that are formed in the T-state conformation at the interface between the two alpha–beta dimers in the tetramer (**Figure 4**(**b**)). The other half of the Bohr-proton-binding sites are still debated and may vary among the Hbs of different teleost species. In addition, the presence of the two aspartic-acid residues at the dimer interfaces appears a necessary, but not sufficient, condition for contributing to a Bohr effect because these residues are also present in Hbs that show no Bohr effect as well as in human Hb, where their local protein environment prevents the formation of a cluster and thus proton binding at this site. It is also unclear which of the different Bohr-proton-binding sites discussed above are merely contributing to the pH-induced shift in Hb-O_2 affinity and which are also involved in the reduction of the maximal O_2-binding capacity and the loss of cooperativity that characterize the Root effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems). Thus, the molecular mechanism of the large Bohr effect in teleosts remains incompletely explained, although it is clearly different from the mechanism in both humans and lampreys, as discussed subsequently.

Lampreys

Lamprey Hbs have a large Bohr effect that approaches the size of the effect in some teleosts. Yet, their Hbs have none of the amino acid residues that are involved in the Bohr effect in human Hb. Lamprey Hbs further differ from all other higher vertebrates by comprising a type of globin chain that exists in a monomer–dimer equilibrium (**Figure 4(c)**). The monomer has a higher O_2 affinity than the dimer. Similar to the two conformational states of tetrameric Hb discussed above, proton binding to the dimer stabilizes the low-O_2-affinity form. Structural and mutational studies have shown that about 50% of the large Bohr effect in lamprey Hb can be accounted for by proton binding to two clusters of glutamic-acid residues that are formed during dimer formation at the interface between the two monomers.

These case studies show that the Bohr effect in mammals, teleosts, and lampreys is achieved by three entirely different mechanisms and, therefore, the Bohr effect likely evolved independently at least 3 times. Specific differences in the influence of chloride, organic phosphates, and CO_2 on the Bohr effect in bird Hbs relative to mammalian Hbs may suggest even a fourth case of independent Bohr-effect evolution in vertebrates.

Benefits and Evolution of the Bohr Effect

The presence of a Bohr effect allows more-efficient blood-O_2 transport because under the acidic conditions in the tissue capillaries, where CO_2 or lactic acid are released into the blood, the Hb-O_2 affinity is decreased. The right-shift of the O_2-binding curves allows increased offloading of O_2 without compromising venous P_{O_2}. Thereby the O_2-diffusion gradient from the blood to the tissues can be maintained. In the absence of a Bohr effect,

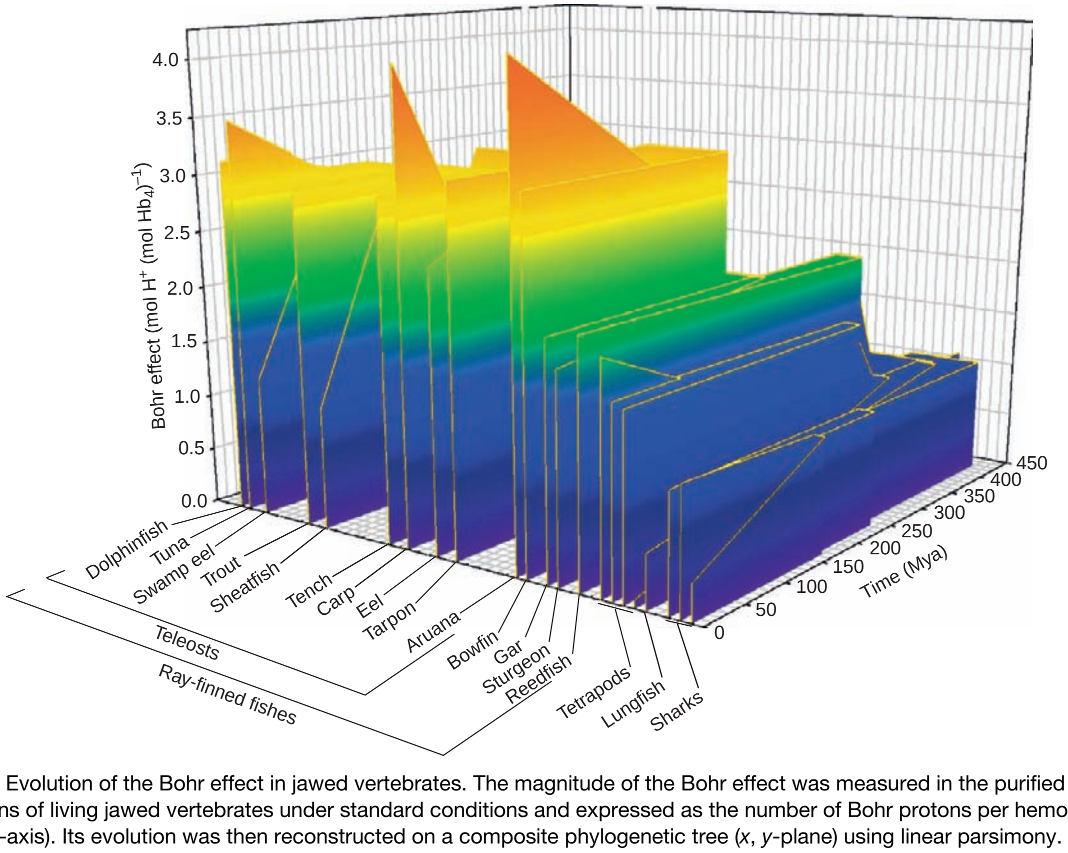


Figure 5 Evolution of the Bohr effect in jawed vertebrates. The magnitude of the Bohr effect was measured in the purified hemoglobins of living jawed vertebrates under standard conditions and expressed as the number of Bohr protons per hemoglobin tetramer (*z*-axis). Its evolution was then reconstructed on a composite phylogenetic tree (*x*, *y*-plane) using linear parsimony. The reconstruction shows an increase in the Bohr effect in tetrapods and early ray-finned fishes about 350 million years ago (Mya). A second increase occurred 250 Mya in the ancestor of bowfin and teleosts. Dolphinfish *Coryphaena hippurus*, swamp *eel Monopterus albus*, sheatfish *Silurus glanis*, tench *Tinca tinca*, tarpon *Megalops cyprinoides*, aruana *Scleropages jardinii*, bowfin *Amia calva,* reedfish *Erpetoichthys calabaricus*. Modified from Berenbrink M, Koldkjær P, Kepp O, and Cossins A (2005) Evolution of O_2 secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

increased O_2 offloading in the tissue capillaries requires a decrease in venous P_{O_2}, which decreases the driving force for O_2 diffusion into the tissues. As noted by Bohr and colleagues in 1904, the effects of CO_2 on O_2 binding at the elevated P_{O_2} values in lung blood are minimal, ensuring that O_2 loading is not impaired by, for example, elevated environmental CO_2 levels.

The Bohr effect also aids in blood-CO_2 transport, as the uptake of Bohr protons by Hb in tissue capillaries shifts the equilibrium between CO_2 and bicarbonate (see eqn (1)) in such a way that deoxygenated blood carries more bicarbonate than oxygenated blood at constant P_{CO_2} (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion and Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

These two well-known benefits for blood O_2 and CO_2 transport may have selected for the multiple evolution of a Bohr effect in the respiratory pigments of the otherwise so-very-different cephalopods, crustaceans, annelid worms, brachiopods, and vertebrates. However, the Bohr effect evolved more than once even in vertebrates. Increasing evidence suggests that the Hb monomers of lampreys do not resemble the ancestral globin of higher vertebrates before the latter became duplicated and evolved into alpha and beta globins. Thus, the very different Bohr-effect mechanism in lampreys and the tetrameric Hb of higher vertebrates may not be unexpected.

Surprisingly, as detailed above, the molecular mechanism of the Bohr effect in mammals and teleost fishes now suggests that the Bohr effect evolved independently in these two groups as well. This is independently supported by a reconstruction of the evolution of the Bohr-effect magnitude on a phylogenetic tree of jawed vertebrates. As explained above, the magnitude of the Bohr effect is quite variable even within a species. Therefore, such comparisons need to be made under carefully standardized conditions, to exclude as much as possible nonheritable variation in the data. When this is done, it is seen that the magnitude of the Bohr effect has dynamically changed during the 450-million-year history of jawed vertebrates (**Figure 5**).

The analysis suggests that the last common ancestor of jawed vertebrates, some 450 Mya, had a low Bohr effect similar to the values still seen today in living sharks and lungfish. Around 300 Mya, a Bohr effect with a magnitude of around two Bohr protons per tetramer had evolved in tetrapods and in the last common ancestor of ray-finned fishes. This can still be seen in today's mammals and in the

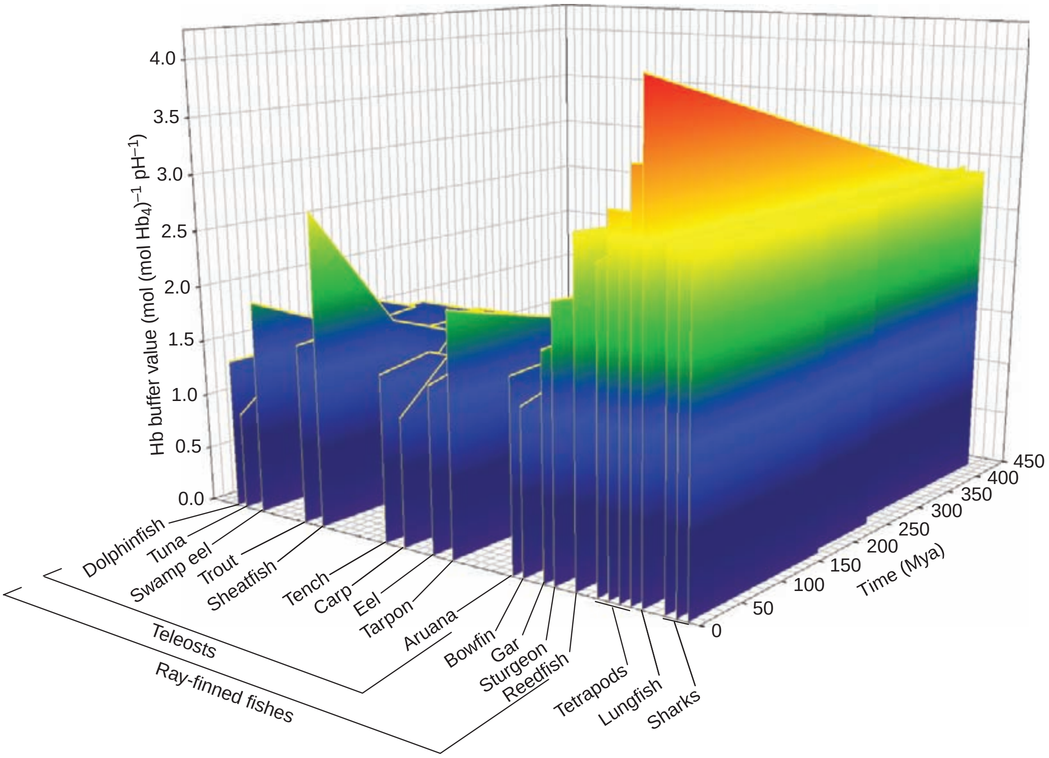


Figure 6 Evolution of hemoglobin (Hb) buffer properties in jawed vertebrates. The Hb buffer value (moles of protons needed to decrease the pH of a 1 molar solution of tetrameric Hb by 1 pH unit, *z*-axis) was measured in living species and its evolution reconstructed on a phylogenetic tree (*x*, *y*-plane) by linear parsimony. Hb buffer value was high in the last common ancestor of jawed vertebrates 450 million years ago (Mya) and substantially decreased during the evolution of modern ray-finned fishes. Reconstructed using data from Berenbrink M, Koldkjær P, Kepp O, and Cossins A (2005) Evolution of O_2 secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

surviving members of basal ray-finned fish lineages, such as reedfish (*Erpetoichthys calabaricus*), sturgeon, and gar. At 250 Mya, a second increase of the Bohr effect occurred in ray-finned fishes, namely in the last common ancestor of the bowfin (*Amia calva*) and teleost fishes. Most teleost fishes have retained this large Bohr effect of around four Bohr protons per Hb tetramer until today.

It has been mentioned earlier that teleost Hbs contain fewer histidine amino acids in their alpha and beta globin chains relative to other jawed vertebrates. The imidazole side group of histidine is the most important proton buffer group in proteins. Interestingly, the evolutionary increase of the Bohr effect in teleosts is closely paralleled by a decrease in the proton buffer value of their Hbs (**Figure 6**). This means that during the evolution of ray-finned fishes, blood pH may have become more prone to change for a given acid-loading event at the same time as the pH sensitivity of Hb-O_2 affinity increased via the Bohr effect. Thus, a given lactic-acid load or increase in P_{CO_2} in blood passing tissue capillaries has a much greater effect on Hb-O_2 affinity in teleost fishes compared to, for example, mammals for two reasons. First, because the Bohr effect is about twice as high in teleosts, and, second, because blood pH is much more weakly buffered in teleost fishes compared to mammals.

Water-breathing fishes have much lower blood P_{CO_2} levels than air breathers (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). Based on the heightened CO_2 sensitivity of teleost fish blood, it was once suggested that the early vertebrates emerging onto dry land in the Devonian first had to evolve stronger blood acid–base buffering and Hb with a reduced CO_2 sensitivity. However, the evolutionary reconstruction of the Bohr effect and Hb-buffer values now suggest that the fish ancestors of the first tetrapods emerging onto land never had low Hb-buffer values nor a Bohr effect as large as today's teleost fishes.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes. **Gas Exchange**: Respiration: An Introduction. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; O_2 Uptake and Transport: The Optimal P_{50}; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M (2006) Evolution of vertebrate haemoglobins: Histidine side chains, specific buffer value and Bohr effect. *Respiratory Physiology and Neurobiology* 154: 165–184.

Berenbrink M, Koldkjær P, Kepp O, and Cossins A (2005) Evolution of O_2 secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Brauner CJ and Berenbrink M (2007) Gas transport and exchange. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Fish Physiology*, vol. 26, pp. 214–270. San Diego, CA: Academic Press.

Jensen FB, Fago A, and Weber RE (1998) Hemoglobin structure and function. In: Perry SF and Tufts BL (eds.) *Fish Physiology*, vol. 17, pp. 1–40. San Diego, CA: Academic Press.

Lukin JA and Ho C (2004) The structure–function relationship of haemoglobin in solution at atomic resolution. *Chemical Reviews* 104: 1219–1230.

Nikinmaa M (1990) Vertebrate red blood cells. In: Bradshaw SD, Burggren W, Heller HC, *et al.* (eds.) *Zoophysiology*, vol. 28, 262 pp. Berlin: Springer.

Qiu Y, Maillett DH, Knapp J, Olson JS, and Riggs AF (2000) Lamprey hemoglobin: Structural basis of the Bohr effect. *Journal of Biological Chemistry* 275(18): 13517–13528.

Yokoyama T, Chong KT, Miyazaki G, *et al.* (2004) Novel mechanism of pH sensitivity in tuna haemoglobin: A structural explanation of the Root effect. *Journal of Biological Chemistry* 279(27): 28632–28640.

Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder

W Waser, University of Turku, Turku, Finland

© 2011 Elsevier Inc. All rights reserved.

Definition of the Root Effect
Functional Role of the Root Effect
Physiological Significance – Eye and Swimbladder
Ocular Blood Supply and Pseudobranch
Further Reading

Glossary

Arterial Pertaining to blood that has been oxygenated.

Avascular Pertaining to the lack of blood vessels or capillaries.

Bohr effect The property of vertebrate hemoglobins, whereas changes in pH affect the hemoglobin-oxygen affinity, supporting loading (in the lungs/gills) and unloading (in the tissues) of oxygen from hemoglobin.

Choroid Pertaining to a vascular structure behind the retina; so-called because it resembles the chorion, the membranous structure that surrounds an embryonic mammal and contributes to the development of the placenta.

Hemibranch A half branch (gill arch) with only one row of filaments.

Operculum/opercular The bony gill cover of teleost fish/pertaining to gill cover.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Pseudobranch A reduced mandibular gill arch, in elasmobranchs the spiracular gill, in teleosts located on the inside of the operculum. In teleosts with ocular oxygen secretion, blood is supplied to the eye only via the pseudobranch.

Rete A Structure consisting of densely packed blood vessels, mostly with a countercurrent blood flow scheme, arranged to facilitate the exchange of, for example, heat or oxygen.

Root effect The property of hemoglobins of some fishes such that in presence of acid it is impossible for the hemoglobin molecule to be completely saturated with oxygen, even at extremely high oxygen partial pressures.

Venous Pertaining to blood that has passed through tissues and has had some of its oxygen removed for metabolism.

Definition of the Root Effect

The Root effect is predominantly defined as the reduced oxygen (O_2) carrying capacity of hemoglobin at low pH values, even at atmospheric O_2 partial pressures (P_{O_2}). This is in contrast to the Bohr effect, which is defined as the reduction in hemoglobin-O_2 affinity caused by a reduced pH (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). In general, vertebrate hemoglobin is fully saturated with O_2 at atmospheric P_{O_2}. Due to cooperativity between its four subunits, tetrameric hemoglobin (Hb_4) exhibits a sigmoidal O_2-binding curve (saturation plotted against P_{O_2}, **Figure 1**), with the P_{50} depicting the P_{O_2} of half saturation. The pH effect, termed Bohr effect, affects O_2 affinity, shifting the O_2-binding curve, that is, the P_{50}, horizontally to the left (increased pH), or to the right (reduced pH). Since the horizontal shift of the O_2-binding curve occurs around the P_{50} and therefore only at a fraction of the atmospheric P_{O_2}, the saturation levels at this high P_{O_2} are not affected. The Root effect, however, describes the pH-dependent vertical shift of the O_2-binding curve (**Figure 1**). It not only decreases saturation up to atmospheric O_2 levels, but also continues to be effective even at extremely high P_{O_2} of several hundred atmospheres, severely limiting the O_2-carrying capacity of the blood.

The Root effect is almost exclusively found in teleost fish. Different from humans, who have only one type of hemoglobin, rainbow trout (*Oncorhynchus mykiss*), for example, has four types of hemoglobin, which are readily distinguishable by gel electrophoresis. Only hemoglobin type IV, the electrophoretically anodic type, shows the distinct reduction in O_2-carrying capacity at low pH, while the more cathodic hemoglobins, types I–III, do not exhibit this effect. Depending on the relative content

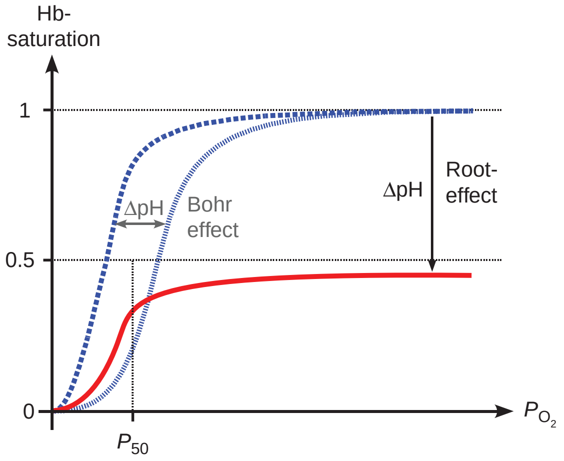


Figure 1 O_2-binding curves showing the Bohr effect and Root effect. The Bohr effect describes pH-mediated changes in the O_2 affinity of hemoglobin, delineated by a horizontal shift of the binding curve around the P_{50} (blue lines). The Root effect describes a reduction in O_2-carrying capacity of the hemoglobin at low pH (red line), prohibiting complete saturation of hemoglobin with O_2 even at high P_{O_2}.

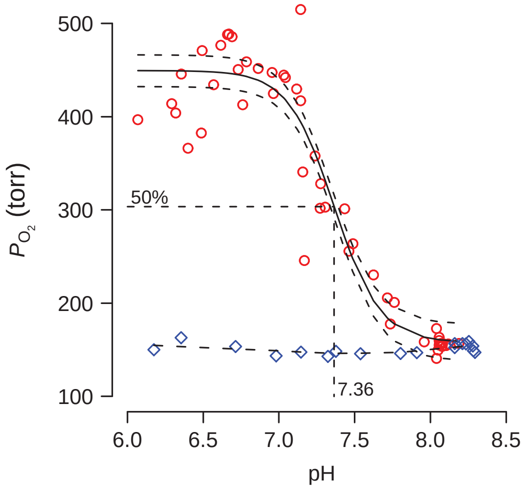


Figure 2 Root effect expressed as an increase in P_{O_2} following acidification of sealed blood samples. Red symbols: trout blood, blue symbols: human blood. Adapted from Waser W and Heisler N (2005) Oxygen delivery to the fish eye: Root effect as a crucial factor for elevated retinal P_{O_2}. *Journal of Experimental Biology* 208:4035–4047.

of the Root-effect-sensitive hemoglobin, at a low pH, a significant, and in some species the larger, proportion of the hemoglobin therefore is unavailable for O_2 binding and hence O_2 transport. The actual magnitude of the Root effect is species specific and can produce up to an 80% reduction of hemoglobin saturation. Apart from teleosts, the Root effect has also been demonstrated in several nonteleost species, some sharks, *Xenopus laevis* (African clawed frog) and some cephalopod hemocyanins. In teleosts, the Root effect has a clear physiological (see next section) and evolutionary explanation (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems), but in other animals its function is unclear.

Functional Role of the Root Effect

Despite its universal definition in textbooks and review articles as the reduced O_2-carrying capacity of hemoglobin, this is a confusing term for the actual functional role of the Root effect. Teleosts do not control the total amount of O_2 transported in the blood via the adjustment of pH, as might be surmised from its definition. Arterial blood is instead typically fully saturated with O_2 and the crucial aspect of the Root effect concerns the fate of O_2 when this fully saturated blood is acidified within a closed system, that is, it is the effect of unloading hemoglobin-bound O_2 on arterial P_{O_2} (**Figure 2**). The concomitant reduction of hemoglobin-saturation levels via acidification is only secondary.

Gases in Solution

According to Henry's gas law

$$C_{O_2} = \alpha_{O_2} P_{O_2} \tag{1}$$

the concentration of physically dissolved O_2 depends on the solubility coefficient α and the P_{O_2}. O_2 that is chemically bound to hemoglobin does not affect this equation. Therefore, at an atmospheric P_{O_2} of 20.9 kPa (156.6 mmHg) and with a solubility coefficient α of about 13 $\mu mol\, l^{-1}\, kPa^{-1}$ (1.7441 $\mu mol\, l^{-1}\, mmHg^{-1}$ for blood plasma at 15 °C), a total of 272 $\mu mol\, l^{-1}$ of O_2 will be physically dissolved in blood plasma. However, blood can hold >20 times more O_2 per unit volume because it contains hemoglobin. O_2 concentration of fully saturated blood would be 6.272 $mmol\, l^{-1}$ in a rainbow trout with an Hb_4 concentration of about 1.5 $mmol\, l^{-1}$.

The activation of the Root effect via acidification causes the release of some of the O_2 chemically bound to hemoglobin, which in a closed system increases the amount of physically dissolved O_2 in the plasma. This additional physically dissolved O_2 now significantly affects Henry's equilibrium

$$P_{O_2} = \frac{C^{Pl}_{O_2} + C^{Hb}_{O_2}}{\alpha_{O_2}} \tag{2}$$

between concentration, solubility, and P_{O_2}. Accordingly (eqn (2)), it causes a rise in P_{O_2} (**Figure 2**).

The Root effect is therefore more correctly described as the increase in P_{O_2} following acid-mediated desaturation of oxy-hemoglobin.

Single-Pass Root Effect

The amount of O_2 released following the activation of the Root effect depends on the proportion of Root-effect-sensitive hemoglobin, which is species specific. In rainbow trout, for example, Hb_4 IV, the Root-effect-sensitive hemoglobin comprises about 60% of the total hemoglobin concentration of 1.5 mmol l^{-1}. With the Root effect fully activated, 3.6 mmol l^{-1} of the 6 mmol l^{-1} of the O_2 bound to hemoglobin will be released. This then raises the concentration of physically dissolved O_2 over 10-fold from 272 μmol l^{-1} to 3.872 mmol l^{-1}. This, in turn, increases P_{O_2} from the atmospheric value of 20.9 to 298 kPa, or 2.94 atm.

Of course, the circulation is not a closed system and O_2 is free to diffusion down its partial pressure gradient. As a result of the Root effect, there is a 10-fold increase in this gradient in the above example, which allows more O_2 to diffuse than would otherwise be possible. In addition, the tissues are also exposed to higher P_{O_2} values.

The final delivery of O_2 to cells and mitochondria is realized via diffusion according to Fick's law:

$$\dot{M}_{O_2} = K_{O_2}\,\Delta P_{O_2}\,\frac{A}{L} \qquad (3)$$

The amount of O_2 delivered per unit time depends primarily on the P_{O_2} gradient ΔP_{O_2} and the diffusion distance L, because the diffusion coefficient K and exchange area A are fixed by tissue composition and geometry restraints. Therefore, increasing P_{O_2} via the Root effect allows for O_2 delivery over far greater distances than would be possible with normal P_{O_2}, allowing an increase in size. In addition, capillary density can be reduced, with the blood vessels taking up less space in a tissue. Conversely, a high P_{O_2} is detrimental due to O_2 toxicity. In humans, O_2 becomes toxic at partial pressures above 1.8 atm, and teleosts have been shown to die of O_2 toxicity at about 2 atm of O_2. Curiously, this occurs at less than the maximal P_{O_2} attainable by full Root-effect activation in rainbow trout.

Physiological Significance – Eye and Swimbladder

Providing a mechanism to increase blood and hence tissue P_{O_2} far above atmospheric values via acidification of O_2-saturated blood, the Root effect has great functional and physiological significance for two organs in teleosts, the retina in the eye and the swimbladder.

Retina

As a neuronal tissue, the retina has one of the highest metabolic rates of all tissues and depends on a high and constant supply of O_2. It is rather surprising, therefore, that the ancestral mode of nourishing the retina in vertebrates, as seen in fish, does not include an intraretinal blood supply, that is, vascularization of the retinal layers. This avascular state of the retina exists in many teleosts, with the blood supply being limited to the choriocapillaries outside of the pigment epithelium. Furthermore, an extra challenge concerning O_2 supply exists in many teleosts because their retina is significantly thicker than those of other vertebrates. Diffusion distances can be up to 6 times larger than the ~140 μm observed in the vascularized retina of mammals. Hence, to maintain O_2 supply over this greater diffusion distance, the P_{O_2} gradient needs to be increased.

Modeling of O_2 diffusion into actively O_2-consuming tissues shows that the required ΔP_{O_2} is proportional to the square of the diffusion distance. Doubling diffusion distance requires a fourfold increase in P_{O_2}. Therefore, a sixfold increase in diffusion distance, as observed in some teleosts' retinas, requires a 36-fold increase in P_{O_2}. Starting from arterial P_{O_2} of about 13.3 kPa (100 mmHg), an elevated partial pressure of 480 kPa (3600 mmHg, 4.73 atm) would be needed to maintain O_2 delivery. This huge gradient is almost achieved by the Root effect, but is still 60% higher than the maximally attainable P_{O_2} in rainbow trout blood by complete activation of the Root effect, indicating that other processes are necessary to further increase the blood P_{O_2} to properly supply the eye.

Countercurrent Multiplication

The process and structure boosting the Root effect to achieve P_{O_2} levels sufficiently high for retinal oxygenation and swimbladder filling is a countercurrent multiplication in special vascular structures, the choroid rete mirabile (for the eye) and the (swimbladder) rete mirabile. **Design and Physiology of Arteries and Veins**: The Retia. Briefly, the blood vessels supplying the O_2-secreting tissues are arranged in a closed-loop, countercurrent vascular arrangement, with the inflowing and outflowing blood coming into very close, large-surface, contact within the rete mirabile (**Figure 3**, crm), an arrangement of a huge number of densely packed arterial (inflow) and venous (outflow) capillaries. In the eye, this choroid rete mirabile lies within the eyecup between retina and sclera, encircling the optic nerve. After passage through the arterial capillaries of the choroid rete mirabile, the blood flows through the choriocapillaries (**Figure 3**, chc) underlying the retina. Since most of the blood from the

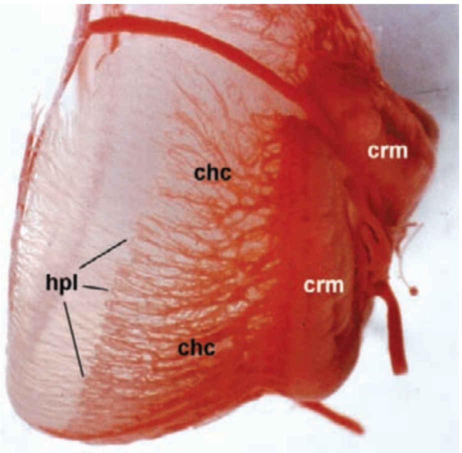


Figure 3 Lateral view of a corrosion cast of the ocular vascular system of an Atlantic cod eye. crm, choroid rete mirabile; chc, choriocapillaries; hpl, hairpin loops of choriocapillaries.

choriocapillaries returns via the venous capillaries of the choroid rete mirabile, the choriocapillaries constitute the apex of the vascular closed-loop system (**Figure** 3, hpl), where the Root effect is activated by acidification of the blood causing a rise in P_{O_2} directly adjacent to the retina. Elevated P_{O_2} in the venous blood returning through the choroid rete mirabile is then transferred to the inflowing arterial blood, preloading the blood with physically dissolved O_2. Within the choriocapillaries, the Root effect is then again activated, now in the preloaded blood, elevating P_{O_2} in excess of the already-raised P_{O_2}. The combination of the closed-loop countercurrent exchange system with an active concentration step at the loop's apex effectively multiplies the single-pass Root effect and generates very high P_{O_2} **see Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes for further details. This multiplication effect easily provides the 60% increase in P_{O_2} needed beyond that of the Root effect alone.

Swimbladder

The second organ in teleost fish where an elevated P_{O_2} is needed is the swimbladder. For a detailed treatment of the topic, **see Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. In contrast to the eye, where O_2 remains physically dissolved, rendering the gas-secretion process independent of hydrostatic pressure, the swimbladder is filled with gas, mainly O_2. P_{O_2}, therefore, equals the surrounding hydrostatic pressure, which changes by about 1 atm per 10-m depth change and reaches several hundreds of atmospheres at several kilometers of depth. Gas pressure in the swimbladder is therefore much higher and much more variable than in the eye, posing far more demanding requirements on the gas-secretion processes.

Ocular Blood Supply and Pseudobranch

Vascular Supply to the Eye

The ocular rete mirabile is supplied with arterial blood only indirectly, via a special organ, the pseudobranch (see below). In rainbow trout (and Atlantic cod, *Gadus morhua*, **Figure 4**), the ventral efferent artery of the first gill arch (**Figure 4**, b), carrying freshly oxygenated blood, constitutes the afferent pseudobranch artery, providing this organ with O_2-saturated blood. After passage through the pseudobranch's vascular structures, the efferent pseudobranch artery (**Figure 4**, e), connected to its contralateral counterpart by a small commissura (**Figure 4**, f), becomes the ophthalmic artery (**Figure 4**, g), supplying the ocular rete mirabile with blood. In other species, the arterial blood supply of the pseudobranch can also, or alternatively, originate at the cephalic circle. In contrast, the swimbladder receives arterial blood via the dorsal aorta and celiac artery.

The Pseudobranch

The teleost pseudobranch is a reduced mandibular gill arch, located on the inside of the operculum (**Figure 5(a)**), and homologous to the spiracular hemibranch of elasmobranchs. Among teleosts, pseudobranch structure ranges from free, gill-like structures fully exposed to the water (e.g., flounder, *Platichthys flesus*,

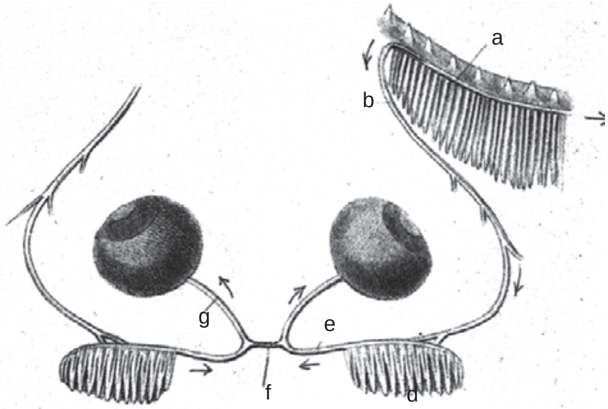


Figure 4 Drawing of Müller,1839, of the vascular connections from the gill, via the pseudobranch, to the eye of Atlantic cod. (a) first gill arch, (b) efferent ventral gill artery carrying oxygenated blood, (d) pseudobranch, (e) efferent pseudobranch artery, (f) commissura between left and right efferent pseudobranch artery, and (g) ophthalmic artery.

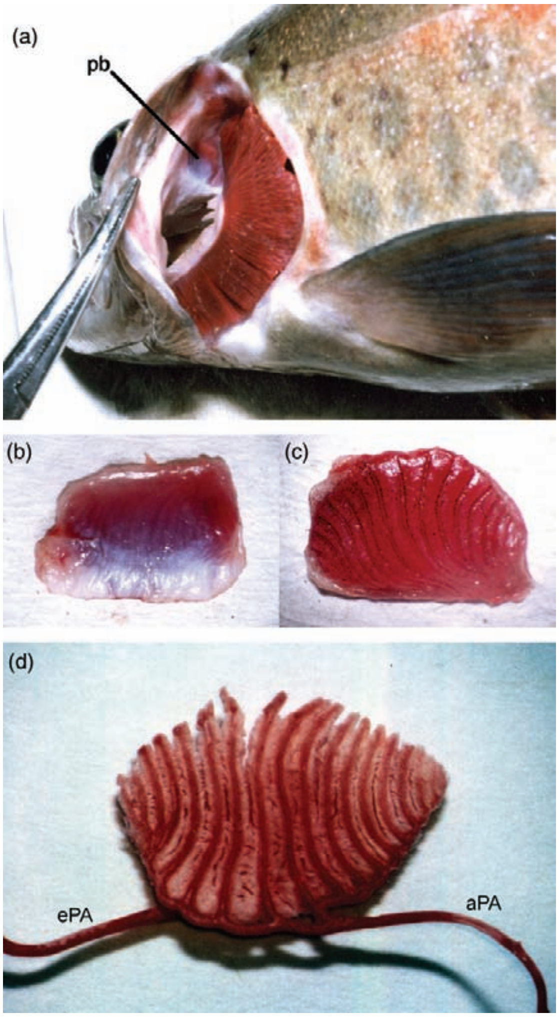


Figure 5 Rainbow trout pseudobranch. (a) Location at the dorsal articulation of the operculum. pb, pseudobranch. (b) Outer and (c) inner surface of freshly excised pseudobranch. In Rainbow trout, the pseudobranch is covered by epithelium that leaves only the very tips of the filaments exposed. The inner surface is only loosely attached to the underlying muscle and provides a better view of the filaments. (d) Corrosion casts of pseudobranch vasculature (left pseudobranch, inner surface). aPA, afferent pseudobranch artery (= efferent ventral gill artery); ePA, efferent pseudobranch artery (= ophthalmic artery). Filamental arteries (dark red) and lamellae (light red, in between filamental arteries) are clearly visible.

black goby, *Gobius niger*), to glandular pseudobranchs, with fused lamellae, deeply buried in the opercular tissue, and without any contact to the external medium (e.g., Cyprinidae, Atlantic cod, and killifish, including *Fundulus*).

Being derived from a former gill arch, the internal morphology of the pseudobranch still closely resembles that of a hemibranch (**Figures 5(b)**–**5(d)**), even in the more glandular types. The pseudobranch exhibits one row of filaments, each bearing numerous secondary lamellae (**Figure 5(d)**). Filaments contain the filamental cartilage, filamental artery and vein, and a venous sinus.

Pseudobranch lamellae also resemble gill lamellae, with the internal blood space held open by pillar cells. Lamellae in free pseudobranchs are spaced so that water flow between the lamellae is possible. In the more glandular-type pseudobranchs, which are mostly completely covered by opercular epithelium, the lamellae are fused, obliterating the interlamellar space and rendering diffusional exchange between water and blood all but impossible. In contrast to gills, pseudobranchs contain a unique cell type, termed 'pseudobranchial cells'. These cells are characterized by many tubular invaginations of their basal membranes associated with numerous mitochondria, pointing to a large capacity for active transmembrane transport processes.

Pseudobranch Function

Based on neurophysiological experiments, a number of different sensory functions (e.g., baro-, osmo-, iono-, and O_2-receptor) have been proposed for the pseudobranch. Denervation or ablation of the pseudobranch, however, did not impair circulatory, respiratory, or osmoregulatory parameters. Endocrine and glandular functions have also been attributed to the pseudobranch, but could not be corroborated in investigations. The most notable features of the pseudobranch are its gill-like lamellae and its peculiar position within the blood supply pathway to the choroid rete mirabile. These observations not only have led early (**Figure 4**) to suggestions that the pseudobranch plays a role for vision but also have stimulated recent research into the possible role of the pseudobranch for ocular O_2 secretion.

The conserved, gill-like anatomical layout of the pseudobranch provides a large-surface-contact area between blood and tissue. In combination with the special mitochondria-rich pseudobranchial cells, this would suggest a high capacity for active membrane transport processes. These transport processes could potentially regulate some aspects of blood chemistry en route to the retina. Since the neural tissue of the retina could be damaged by locally low pH values that are necessary for the Root effect, a hypothesis that has been advanced is that the pseudobranch might preadjust blood pH in the blood flowing to the eye. This would lower blood pH toward a Root-effect threshold value and would enable O_2 secretion in the choriocapillaries with only a minimum local acidification, thereby avoiding damage of the retina due to high rates of acid production.

Key enzyme activity patterns, lactate production rates, and respiratory quotient values support this hypothesis, as do high concentrations of vacuolar H^+-ATPase in pseudobranchial cells. In fact, acid secretion by isolated pseudobranchial cells *in vitro* has been demonstrated. However, experiments undertaken to directly measure an acidification effect of the pseudobranch on the passing blood provided ambiguous results. When the pseudobranch was perfused *in situ*, with pH values of the perfusate varying over a large range, above and below the threshold value for the Root effect, no adjustment of perfusate pH by the pseudobranch could be demonstrated. The physiological function of the pseudobranch, having been studied only in a few teleost species, therefore remains an unsolved mystery.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Swimbladder Function and Buoyancy Control in Fishes. **Design and Physiology of Arteries and Veins**: The Retia. **Gas Exchange**: Respiration: An Introduction. **Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems.

Further Reading

Bridges CR, Berenbrink M, Müller R, and Waser W (1998) Physiology and biochemistry of the pseudobranch: An unanswered question? *Comparative Biochemistry and Physiology A* 119(1): 67–77.

Brittain T (1987) The Root effect. *Comparative Biochemistry and Physiology B* 86: 473–481.

Chase J (1982) The evolution of retinal vascularization in mammals. A comparison of vascular and avascular retinae. *Ophthalmology* 89: 1518–1525.

Copeland DE (1980) Functional vascularization of the teleost eye. *Current Topics in Eye Research* 3: 218–280.

Kern G, Bösch ST, Unterhuber E, and Pelster B (2002) Mechanism of acid secretion in pseudobranch cells of rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 205: 2943–2954.

Laurent P and Dunel-Erb S (1984) The pseudobranch: Morphology and function. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. XB, pp. 285–323. New York: Academic Press.

Maetz J (1956) Le rôle biologique de l'anhydrase carbonique chez quelques Téléostéens. *Bulletin Biologique France, Belgique (Paris)* 40: 1–129.

Mölich A, Waser W, and Heisler N (2009) The teleost pseudobranch: A role for preconditioning of ocular blood supply? *Fish Physiology and Biochemistry* 35: 273–286.

Müller J (1839) Vergleichende Anatomie der Myxinoiden. III. Über das Gefäβsystem. *Abhandlungen der Deutschen Akademie der Wissenschaften Berlin* 175–303.

Pelster B and Randall DJ (1998) The physiology of the Root effect. In: Perry SF and Tufts B (eds.) *Fish Respiration,* vol. 17, pp. 113–139. New York: Academic Press.

Pelster B and Weber RE (1991) The physiology of the Root effect. *Advances in Comparative and Environmental Physiology* 8(2): 51–77.

Root RW (1931) The respiratory function of the blood of marine fishes. *Biological Bulletin* 61: 427–456.

Waser W and Heisler N (2005) Oxygen delivery to the fish eye: Root effect as a crucial factor for elevated retinal PO_2. *Journal of Experimental Biology* 208: 4035–4047.

Waser W, Schmitz A, Perry SF, and Wobschall A (2005) Stereological analysis of blood space and tissue types in the pseudobranch of the rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry* 31: 73–82.

Wittenberg JB and Haedrich RL (1974) The choroid rete mirabile of the fish eye. II. Distribution and relation to the pseudobranch and to the swimbladder rete mirabile. *Biological Bulletin* 146: 137–156.

Wittenberg JB and Wittenberg BA (1962) Active secretion of oxygen into the eye of fish. *Nature* 194: 106–107.

Wittenberg JB and Wittenberg BA (1974) The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: Comparison with the swimbladder rete mirabile. *Biological Bulletin* 146: 116–136.

Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems

M Berenbrink, University of Liverpool, Liverpool, UK

© 2011 Elsevier Inc. All rights reserved.

Discovery of the Root Effect
The Root Effect is Only Loosely Defined
Variations in the Magnitude of the Root Effect
Mechanism of the Root Effect
Physiological Roles of the Root Effect
Evolution of the Root Effect and Rete Systems
Further Reading

Glossary

Allosteric binding Binding of an effector molecule to a protein site that is different from the active site.

Bohr effect The property of vertebrate hemoglobins whereby changes in pH affect the hemoglobin-oxygen affinity, supporting loading (in the lungs/gills) and unloading (in the tissues) of oxygen from hemoglobin.

Choroid Pertaining to a vascular structure behind the retina, so-called because it resembles the chorion, the membranous structure that surrounds an embryonic mammal and contributes to the development of the placenta.

Countercurrent Flowing in opposite directions.

Equilibrium Pertaining to the situation when all forces acting are balanced by others resulting in a stable unchanging system.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Rete mirabile (or simply rete, plural retia mirabilia) Structure consisting of blood vessels arranged to facilitate the exchange of heat or oxygen.

Root effect The property of hemoglobins of some fishes such that in the presence of acid, it is impossible for the hemoglobin molecule to be completely saturated with oxygen, even at extremely high oxygen partial pressures.

Vascular Pertaining to blood vessels.

Vascularized Supplied by blood vessels.

Discovery of the Root Effect

The Root effect is named after Raymond W. Root, who in 1931 described a peculiar characteristic of the blood-oxygen (O_2)-binding curve in marine fishes. He observed that elevated carbon dioxide (CO_2) partial pressures (Pco_2's) prevented the blood of the sea robin *Prionotus carolinus*, toadfish *Opsanus tau*, and mackerel *Scomber scombrus* from becoming fully saturated with O_2 at the O_2 partial pressure (P_{O_2}) of air. Previous work by Christian Bohr and colleagues on mammalian blood had shown that upon elevating CO_2, increasingly higher P_{O_2} values were required for 50% O_2 saturation of the blood (P_{50} values). In contrast to marine fishes, however, close to 100% blood O_2 saturation could still be obtained in mammals when the P_{O_2} was elevated to the level of air (150–160 mmHg) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect. Root observed that in his fishes the O_2-binding curve at high P_{CO_2} appeared to approach an upper asymptotic maximum that was distinctly lower than 100%. This was later confirmed in experiments where the P_{O_2} was elevated to as high as 140 atm of pure O_2 and still 100% oxygen saturation could not be reached in some (but not all) of the investigated fishes. Root also demonstrated that the effect of P_{CO_2} could be reproduced by addition of lactic acid and that low pH was the crucial parameter that produced the apparent loss of the maximal O_2 carrying capacity of fish blood. **Figure 1** illustrates the difference between the so-called Bohr and Root effect using the original data on goosefish *Lophius americanus* blood.

Careful analysis of the O_2-binding curves of fish blood at different pH reveals that not only the asymptotic maximum, but also the general shape of the curves changes with pH. Thus, in the example of the goosefish in **Figure 1**, two families of curves can be distinguished. Between pH 8 and 7 the curves are slightly S-shaped or sigmoid; below pH 7, they take the shape of a rectangular hyperbola. The S-shape is due to cooperative O_2 binding, meaning that binding of O_2 to the first of the four

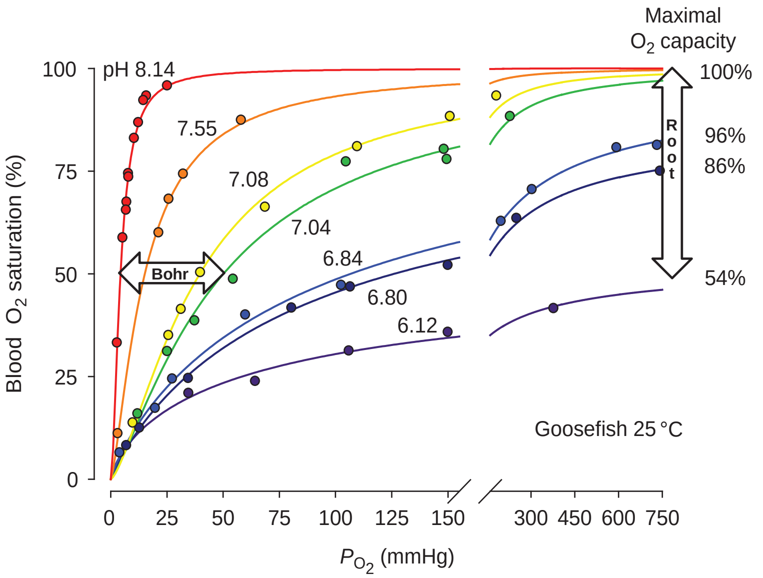


Figure 1 Effect of pH on blood O_2 binding in a marine teleost, the goosefish. Above pH 7, the blood displays a classical Bohr effect, namely a decrease in pH increases the P_{O_2} required for half maximal O_2 saturation (P_{50}) but close to 100% saturation is still achieved at high P_{O_2} (note different scale after axis break). Below pH 7, a Root effect is displayed: a decrease in pH increases the P_{50} and decreases the indicated apparent maximal O_2 capacity as well. Similarly colored lines and symbols refer to the same pH value, as indicated. Modified from Green AA and Root RW (1933) The equilibrium between hemoglobin and oxygen in the blood of certain fishes. *Biological Bulletin* 64: 383–403.

O_2-binding heme groups in the hemoglobin (Hb) tetramer induces structural changes in the globin subunits that increase the O_2 affinity of the other heme-binding sites. The loss of cooperativity of O_2 binding at low pH in the blood of many fishes is another characteristic of the Root effect that distinguishes it from the Bohr effect.

The Root Effect is Only Loosely Defined

There are no strictly defined and commonly accepted criteria for distinguishing whether blood of a given species has a large Bohr effect or rather a small Root effect. Theoretically, all blood or Hb O_2-binding curves only asymptotically approach, but never reach, 100% saturation, even in the case of a Bohr effect. Only few Hbs have been tested for pH-sensitive O_2 binding at P_{O_2} values higher than 1 atm of pure O_2, and it is difficult to predict whether close to 100% O_2 saturation will, or will not be reached at high-enough P_{O_2} values based on extrapolation of data obtained at low P_{O_2}.

The pH-induced decrease in the cooperativity of O_2 binding is of great general interest for understanding protein function, but this criterion for the Root effect is rarely used by whole organism biologists. For practical reasons, the presence of a Root effect is commonly accepted by respiratory physiologists and biochemists alike, when blood or Hb O_2 saturation under air-equilibration ($P_{O_2} \sim 150$ mmHg) and at high pH, which is often taken as the maximal O_2-binding capacity, is considerably reduced by low pH. 'Considerably' is again not defined, but in this context could mean more than in humans where low pH under these conditions causes a decrease in O_2 saturation to approximately 95%.

Variations in the Magnitude of the Root Effect

The extent by which acidification decreases the apparent maximal O_2-binding capacity varies with pH. In **Figure 2**, the O_2 saturation of goosefish blood at various P_{O_2} values from **Figure 1** is plotted as a function of pH. At the P_{O_2} of air (150 mmHg) and above pH 7.5, a decrease in pH has hardly any effect on maximal blood O_2 saturation. Between pH 7.5 and 6.5, blood O_2 saturation rapidly falls with acidification, and below pH 6.5 a lower plateau of blood O_2 saturation is reached. The shape and lower plateau of the curve relating pH and O_2 saturation clearly depend on the P_{O_2} used for comparison. At typical arterial P_{O_2} for many fishes of 80 mmHg, the level of the lower plateau is decreased and O_2 saturation begins to drop already at higher pH values. Conversely, under pure O_2 ($P_{O_2} \sim 750$ mmHg) the lower plateau of O_2 saturation is increased and lower pH values are needed to reach it.

The pH dependence of the Root effect also differs between species, even when compared at the same P_{O_2}. Thus, at atmospheric P_{O_2}, (1) the pH at which blood O_2 saturation falls most precipitously (midpoint pH), (2) the steepness of the curve at midpoint pH, and (3) the lower plateau of O_2 saturation, all differ between the bloods of

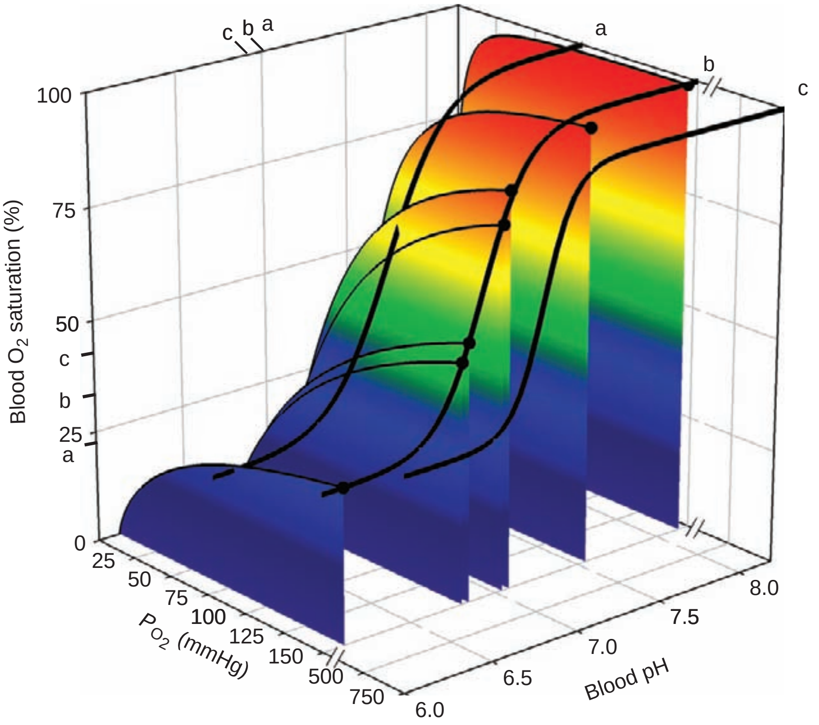


Figure 2 Low pH-induced decrease of Hb O_2 saturation as a function of P_{O_2}. In going from P_{O_2} 80 to 150 and 750 mmHg (black curves a–c), the lower plateau of blood O_2 saturation progressively increases and the pH required for half maximal effect more and more decreases, as indicated by the respective letters on the axes. O_2-binding curves are from Figure 1. Black curves are sigmoid fits of O_2 saturation as a function of pH at constant P_{O_2}. Note axis break and different scale after P_{O_2} 150 mmHg.

different species, such that no two species show the same curve. For example, at P_{O_2} 150 mmHg, blood O_2 saturation in the sea robin is half maximally reduced at pH 7.5, whereas goosefish blood needs to be acidified to pH 7.0 to show the same level of pH-induced deoxygenation (**Figure 3**). Similarly, the pH dependence of the Root effect

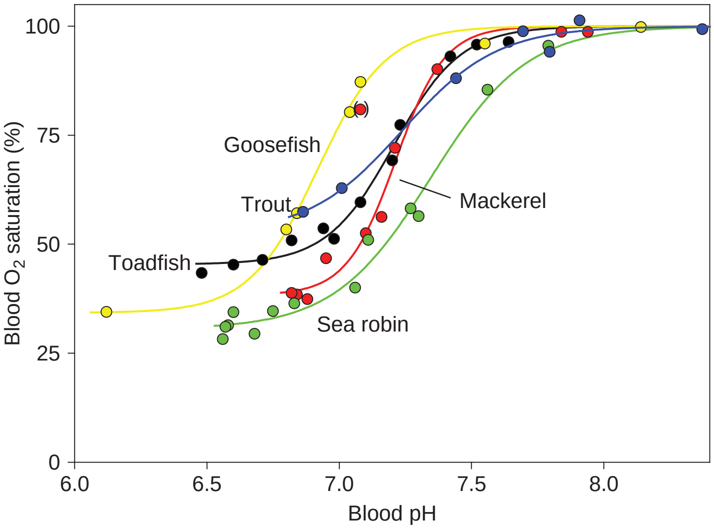


Figure 3 Species differences in the pH dependence of the Root effect, which is here taken as the acid-induced decrease in O_2 saturation of air-equilibrated blood or red blood cell suspensions. Species differ in the saturation plateau that is reached at low pH, the midpoint pH, and the maximal steepness of the curve relating O_2 saturation to pH. Similarly colored lines and symbols refer to the same species, as indicated. Goosefish data re-plotted from Figure 1, trout *Oncorhynchus mykiss* data from Berenbrink M, Völkel S, Heisler N, and Nikinmaa M (2000) O_2-dependent K^+ fluxes in trout red blood cells: The nature of O_2 sensing revealed by the O_2 affinity, cooperativity and pH dependence of transport. *Journal of Physiology* 526: 69–80. All other data from Root RW (1931) The respiratory function of the blood of marine fishes. *Biological Bulletin* 61: 427–455.

in mackerel blood is much steeper than in sea robin, although the two species show about the same magnitude of the Root effect, that is, acidification leads to the same lower plateau of blood O_2 saturation. Finally, the magnitude of the Root effect is distinctly lower in the toadfish than in the sea robin (*c.* 55% vs. 70% maximal acid-induced deoxygenation, respectively) What is the basis for these differences?

The Root effect is a property of Hb inside the red blood cells (RBCs) and depends on the intracellular pH of these cells. Therefore, pH regulatory mechanisms that prevent a plasma acidosis to be transferred to the intracellular pH of RBCs may weaken or even mask the presence of a Root-effect Hb in some fishes. Thus, the β-adrenergically activated sodium, hydrogen exchanger in the RBC membrane of many teleost fish species maintains intracellular pH under exercise-induced acidosis (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function and Hemoglobin).

Many fish species express functionally different Hb isoforms in their RBCs, some of which show the Root effect, whereas others are non-Root-effect Hbs. Thus, the anodic (at high pH negatively charged) Hbs of salmonid fishes and eels, which make up about 60% of the total Hb, show a Root effect, while the cathodic (positively charged) Hbs do not. Differences in the relative proportion of these Hbs between species will change the maximal extent of the overall Root effect in these bloods. The pH at which the lower O_2 saturation plateau is reached is increased by elevated concentrations of small intracellular effector molecules like chloride and the organic phosphates adenosine triphosphate and guanosine triphosphate. The extent to which protons can decrease Hb O_2 saturation finally depends on the general O_2 affinity of the O_2-binding sites of Hb, the H^+ affinity of the H^+-binding sites, and the molecular mechanism of Hb that links the two together and causes H^+ binding to release O_2. This is encoded in the molecular structure of the protein and thereby an inherited property that depends on the genetic makeup of each species.

Mechanism of the Root Effect

Hb is arguably one of the most-studied proteins and elucidating how its structural features on the molecular level allow its functional diversity across vertebrates is of great value in understanding protein function in general. However, the molecular mechanism by which the affinity and cooperativity of O_2 binding of many fish Hbs are so drastically reduced at low pH has remained an enigma, despite more than two decades of research. Only recently significant progress in elucidating the molecular mechanism of the Root effect has been obtained.

The Two-State Allosteric Model of Hb and the Root Effect

Initially, the Root effect was interpreted in the framework of the two-state allosteric model of Hb. This was based on the structural observations of Nobel laureate Max Perutz and his colleagues, who found that the structure of oxy Hb and deoxy Hb differed in important aspects. Hbs of jawed vertebrates generally exist as tetramers. These are formed by two dimers which are each formed by the aggregation of one α and one β globin chain. Contact sites within these dimers are denoted $\alpha1$–$\beta1$ (or $\alpha2$–$\beta2$) contacts. Contact sites between the two dimers in the Hb tetramer are denoted $\alpha1$–$\beta2$, $\alpha1$–$\alpha2$, or $\beta1$–$\beta2$ contacts (**Figure 4(a)** and **4(b)**). Each globin subunit carries a heme group with a Fe^{2+} center that is the binding site for O_2. Structural changes in the globin chains can alter the heme environment and thereby the affinity of the O_2-binding sites. Perutz and colleagues observed that the conformation of the Hb tetramer changes upon releasing O_2. The two α–β dimers rotate relative to each other and move slightly away from each other, opening a central cavity between the $\beta1$ and $\beta2$ subunits. This deoxy conformation is assumed to have a fixed, low O_2 affinity, is stabilized by internal salt bridges, and is called the tense, T-state conformation. Conversely, the oxy conformation is assumed to have a fixed high O_2 affinity, has fewer internal salt bridges, and is called the relaxed, R-state conformation. In the two-state allosteric model, the binding of the first O_2 molecules to the low O_2-affinity T-state conformation is assumed to promote the allosteric shift toward the high O_2-affinity R-state conformation. This T-state to R -state transition gives rise to cooperative O_2 binding. Several other allosteric effector molecules, such as chloride, protons, and organic phosphates, have a higher affinity to the T-state conformation, and binding of these effectors to the T-state impedes the transition from the T-state to the R-state conformation. This results in higher P_{50} values and shifts the overall O_2-binding curve toward the right. According to the two-state allosteric model, the Root effect results from an over-stabilization of the T-state conformation by proton binding at low pH. Thereby the transition to the R-state is blocked and Hb becomes fixed in the low O_2-affinity T-state. This would explain incomplete Hb O_2 saturation under air or even pure O_2 and also account for the loss of cooperative O_2 binding at low pH, which are both observed in Root-effect Hbs. The model also explains the observation that in the presence of chloride and organic phosphates, protons exert a stronger effect on Hb O_2 saturation, as all these allosteric effectors work together in stabilizing the T-state and impeding the transition to the R-state.

On the molecular level, the principal proton-binding site that stabilizes the T-state was thought to be the same

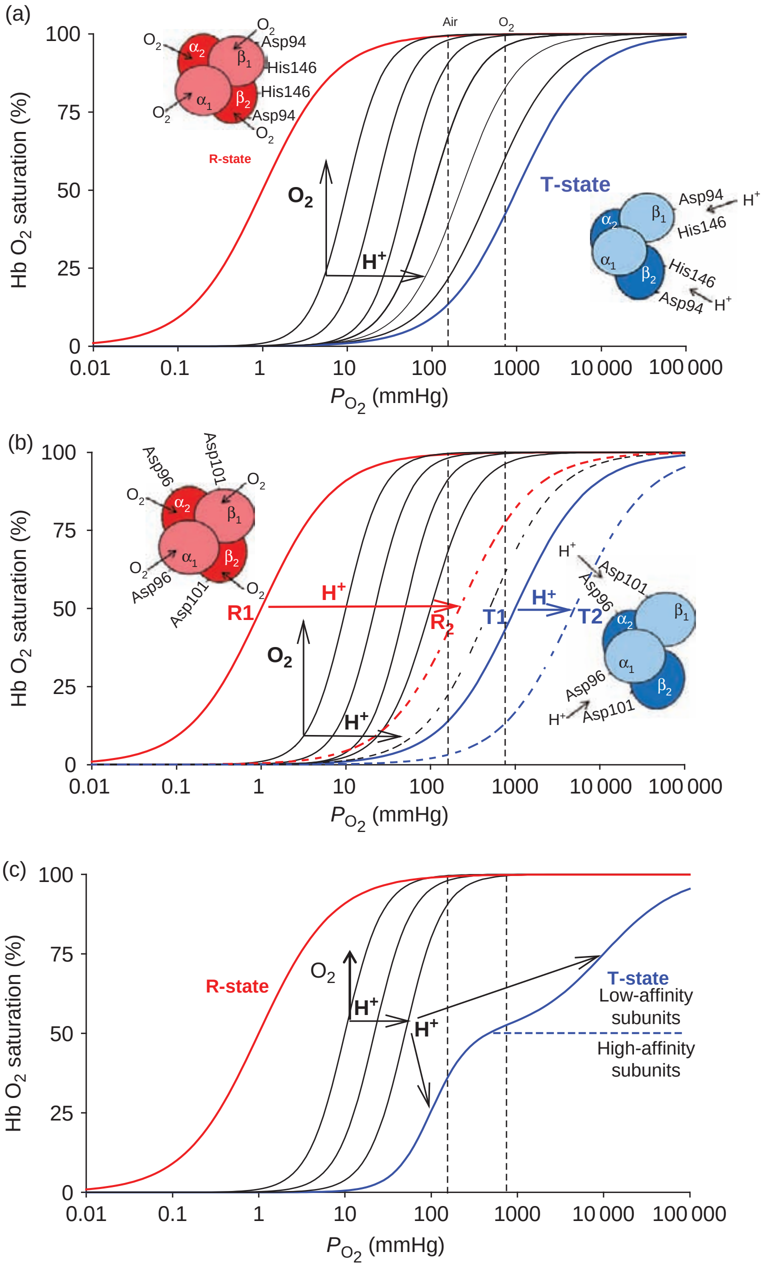


Figure 4 Different perspectives on the molecular mechanism of the Root effect, explaining the loss of cooperative O_2 binding and incomplete Hb O_2 saturation at low pH, even under air or pure oxygen (dotted vertical lines). (a) According to the two-state allosteric model, Hb tetramers exist in equilibrium between the R-state and T-state conformations, which have inverse affinities for O_2 and H^+ (indicated by arrows). High P_{O_2} promotes the transition (black lines) to the high O_2-affinity R-state (red line) and causes cooperative O_2 binding (increased steepness of curves at intermediate O_2 saturations). Protons stabilize the low O_2-affinity T-state (blue line) by binding to residues in the β-chains similar to those principally responsible for the Bohr effect in human Hb. This increases P_{50} by delaying the T–R transition. Strong stabilization of the T-state at very low pH abolishes cooperative O_2 binding. (b) The multistate model expands the two-state allosteric model by allowing pH-induced changes in O_2 affinity within the R- and T-states (substates R1, R2 and T1, T2). Low pH stabilizes the low O_2-affinity R and T substates, decreasing P_{50} and cooperativity. T-states are stabilized by proton binding to sites at the $\alpha1$–$\beta2$ interface, which differ from the sites causing the Bohr effect in human Hb. (c) In some species at low pH, α- and β-subunits in the T-state have widely different O_2 affinities, causing apparent anti-cooperative O_2 binding (flattening of the curve at intermediate O_2 saturations). Note logarithmic x-axis scales which cause sigmoid appearance also of the rectangular hyperbolas characterizing the R- and T-state O_2-binding curves.

as the site responsible for the major part of the Bohr effect in human Hb, namely the terminal histidine residue of the β chain (**Figure 4**(a); **see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). This histidine side chain, when protonated, forms a strong salt bridge with an aspartate residue of the same chain in the T-state, but not R-state, which influences the heme environment and causes a reduced O_2 affinity. Certain amino acid substitutions in Root effect Hbs that were found in the vicinity of the β terminal histidines were thought to cause much stronger proton binding and salt-bridge formation in the T-state of Root-effect Hbs than in human Hb and to thereby cause the Root effect. Thus, the Root effect was merely considered an enhanced or exaggerated Bohr effect of human Hb.

This elegantly simple explanation of the Root effect is, however, challenged by several sets of observations. First, analysis of precise O_2-binding curves at different pH and very high and very low O_2 saturation, which allows determination of the O_2-binding affinity of the first and the fourth O_2 and thereby of the T- and R-state, revealed different O_2-binding affinities within the T-state conformations, that is, the O_2-affinity of the T-state is not fixed and is affected by proton binding within this conformational state. Second, structural analyses revealed that the salt bridge of the β-terminal histidine, which is crucial for the Bohr effect in human Hb, is not formed in several fish Hbs that nevertheless display a strong Root effect. Third, the Root effect has been observed in Hb with arginine as the β-terminal amino acid residue, although the side chain of arginine is unable to bind protons at low pH. Finally, mutations in human Hb, which were designed to produce the Root effect according to the above mechanism, failed to do so and in fact increased Hb O_2 affinity, rather than reducing it. Thus, the two-state allosteric model of the Root effect needed to be expanded and the search for the structural explanation of the Root effect continued.

Multistate Model of the Root Effect

Later structural studies on Root-effect Hbs in the R-state (oxy) conformation and the T-state (deoxy) conformation have revealed a new proton-binding site that is only formed in the T-state conformation between two aspartate amino acid residues at the $\alpha1$–$\beta2$ interface **Figure 4**(**b**). Binding of a shared proton at this site is assumed to stabilize the T-state, impede the shift to the R-state, and to be responsible for 50% of the Root-effect magnitude. Further structural studies on Northern bluefin tuna *Thunnus thynnus* Hb at low and high pH indicate additional modulation of O_2 affinity by proton binding within the T-state. Thus, a specific histidine residue of the β-chain picks up a proton at low pH and forms a salt bridge with a neighboring aspartate of the same chain. This causes structural changes in the vicinity of the heme group of that β-subunit that are predicted to change its O_2 affinity without altering the overall conformational state of the tetramer.

Earlier structural studies on Hb of the teleost spot (*Leiostomus xanthurus*) have been taken to suggest proton binding at low pH also to the R-state conformation that may cause its destabilization and transition to the low O_2-affinity T-state. (**Figure 4**(**b**)) However, it is not clear whether proton binding in this case is also associated with O_2 affinity changes within the R-state. This is certainly a possibility based on recent experiments on human Hb.

Subunit Differences in Oxygen Binding at Low pH

The reduction of cooperative (sigmoidal) O_2 binding that is seen in Root-effect Hbs at low pH has been ascribed to Hb being locked under this condition in a T-state conformation, in which the O_2 affinity is assumed to be fixed and similar in all four binding sites. In some Root-effect Hbs, binding of O_2 to the first half of heme groups seems to impede further O_2 binding to the other half at low pH, indicating apparently anti-cooperative O_2 binding. This may, however, be due to distinctly different O_2-binding affinities between the α- and β-subunits of the tetramer at low pH (**Figure 4**(**c**)). This is supported by structural data on T-state Hbs at high and low pH, which indicate two different proton-binding sites in the α- and β-chains, that alter the heme environment, and presumably O_2 affinity, differently in the T-state α- and β-chains.

Taken together, all these structural and functional studies suggest that the strong effect of protons on the Hb O_2 affinity in Root-effect Hbs is due to a complex mechanism involving pH-induced changes within and between conformational stages that are related to proton-binding sites that are quite different from those involved in the Bohr effect of human Hb.

Physiological Roles of the Root Effect

The Root effect allows an acid-triggered O_2 release from the Hb inside red blood cells even at super-atmospheric P_{O_2} values, where Hb of other vertebrates remains fully O_2 saturated. This property forms the basis for O_2 secretion into the fish swimbladder for buoyancy regulation and for generating elevated P_{O_2} values for enhanced O_2 diffusion from the capillary system underlying the retina into the poorly vascularized fish retina itself (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes). However, some teleost fishes, such as herring *Clupea harengus* and trout *Oncorhynchus mykiss*, are unable to secrete O_2 into their swimbladders, while others, such as the eel *Anguilla rostrata*, are unable to generate high P_{O_2} values at their retinas. Clear evidence for such ability is the presence in the swimbladder or at the retina of P_{O_2} values above those of air. Thus, determining whether or not acidification of air-equilibrated blood or Hb of a given fish species decreases O_2 saturation is a good indicator of whether a species may be able to secrete O_2, regardless of whether this is by a strong Bohr effect or a Root effect.

Furthermore, greatly elevated blood P_{O_2} values are generally associated with a vascular counter-current exchange system known as the rete mirabile (Latin: wonderful network, plural: retia mirabilia; (**see also Design and Physiology of Arteries and Veins**: The Retia). Upon local blood acidification in the choroid capillaries behind the retina or in the swimbladder capillaries, the Root effect allows release of Hb-bound O_2 even at already elevated P_{O_2} (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). The rete mirabile increases the efficiency of this process in two ways. First, it prevents washout of the released O_2 by allowing back-diffusion of O_2 from the venous capillaries into the arterial capillaries of the rete mirabile. This serves to augment the initial P_{O_2} increase elicited by acidification and the Root effect. Second, the rete mirabile keeps the blood acidification localized by allowing CO_2 back-diffusion from the venous site to the arterial site of the rete mirabile. This is important because in the case of a general blood acidosis, blood O_2 loading in the fish gills may be impaired in the presence of a Root effect. However, a rete mirabile in the absence of a Root effect may be disadvantageous, because venous blood with low P_{O_2} leaving a tissue may cause a diffusive shunt of O_2 from arterial to venous capillaries in the rete mirabile, thereby compromising tissue O_2 supply. Perhaps for this reason heat-exchanging retia mirabilia are usually comprised of large-diameter capillaries, which allow sufficient heat transfer but are less efficient in gas exchange compared to retia mirabilia comprised of low capillary diameter.

Evolution of the Root Effect and Rete Systems

Evidently, efficient O_2 secretion requires a Root effect and a rete mirabile. However, the two components appear disadvantageous on their own. As their simultaneous evolution seems unlikely, the question arises, which of the two components came first and how its negative aspects were avoided during the evolution of O_2 secretion? Once O_2 secretion had evolved in one organ, it may have facilitated its evolution also at the other organ. Thus, finding out whether O_2 secretion first evolved in the eye or in the swimbladder may allow identifying the initial selection pressures that led to its existence.

Reconstructions of the evolution of the ocular (choroid) rete mirabile and the swimbladder rete mirabile suggest that the choroid rete evolved first and just once about 250 million years ago in the ray-finned fishes, more precisely in the last common ancestor of the bowfin *Amia calva* and teleosts (**Figure 5**). Most living teleosts thus possess a choroid rete mirabile, although it has been secondarily lost in members of several different groups of fishes, such as in the elephantfishes *Campylomormyrus numenis* and *Gnathonemus petersii*, the European eel *Anguilla anguilla*, some loaches and catfishes, and the swamp eel *Monopterus albus*. In addition to these species, the choroid rete mirabile has also been lost in four lineages of Antarctic fishes of the suborder Notothenioidei. **Figure 5** also indicates the magnitude of the Root effect in each species by giving the maximum acid-induced decrease in the percentage O_2 saturation of air-equilibrated hemolysates at physiological temperature. It is seen that most living teleosts have Root-effect magnitudes of 40% desaturation or more.

The Root effect is only ever reduced below that level in species that have lost the choroid rete mirabile and thereby also lost the ability for elevating retinal P_{O_2} above air. In some species without a choroid rete, such as elephantfishes, the European eel, and some loaches, the Root effect is only slightly reduced. Separate evolutionary reconstructions have revealed that these groups have all independently evolved a swimbladder rete mirabile. The persistence of a reduced Root effect in these three separate groups strongly suggests that the Root effect is used to secrete O_2 into their swimbladders. The almost total loss of the Root effect in catfishes and the swamp eel, which do not possess choroid or swimbladder retia mirabilia, supports this conclusion (**Figure 5**).

Some basal ray-finned fish lineages that never evolved a choroid or swimbladder rete mirabile nevertheless have Root effects as high as 25–40% (sturgeon *Acipenser baeri* and gar *Lepisosteus platyrhincus*, respectively).

Indeed, the evolutionary reconstruction of Root-effect magnitude suggests that the Root effect of early ray-finned fishes gradually increased in the lineage leading to teleosts, attaining values of up to 40% already before the first occurrence of a choroid rete mirabile.

What may then have been the driving force behind the evolution of the Root effect? It very likely was not the generation of super-atmospheric P_{O_2} values, as these usually require that the washout of O_2 released from Hb is minimized by back-diffusion from venous to arterial capillaries in a rete mirabile. In the absence of a rete mirabile, acid-induced O_2 secretion via the Root effect is also difficult because CO_2 generated during blood acidification cannot be recycled via the rete mirabile. This would then entail large amounts of acid entering the general circulation, potentially impeding sufficient O_2 loading in the gills because the Root effect could prevent full Hb saturation even in fully air-equilibrated water.

However, studies on the Hb buffer values of teleosts and basal ray-finned fishes have shown that species such as reedfish *Erpetoichthys calabaricus*, sturgeon, and gar have

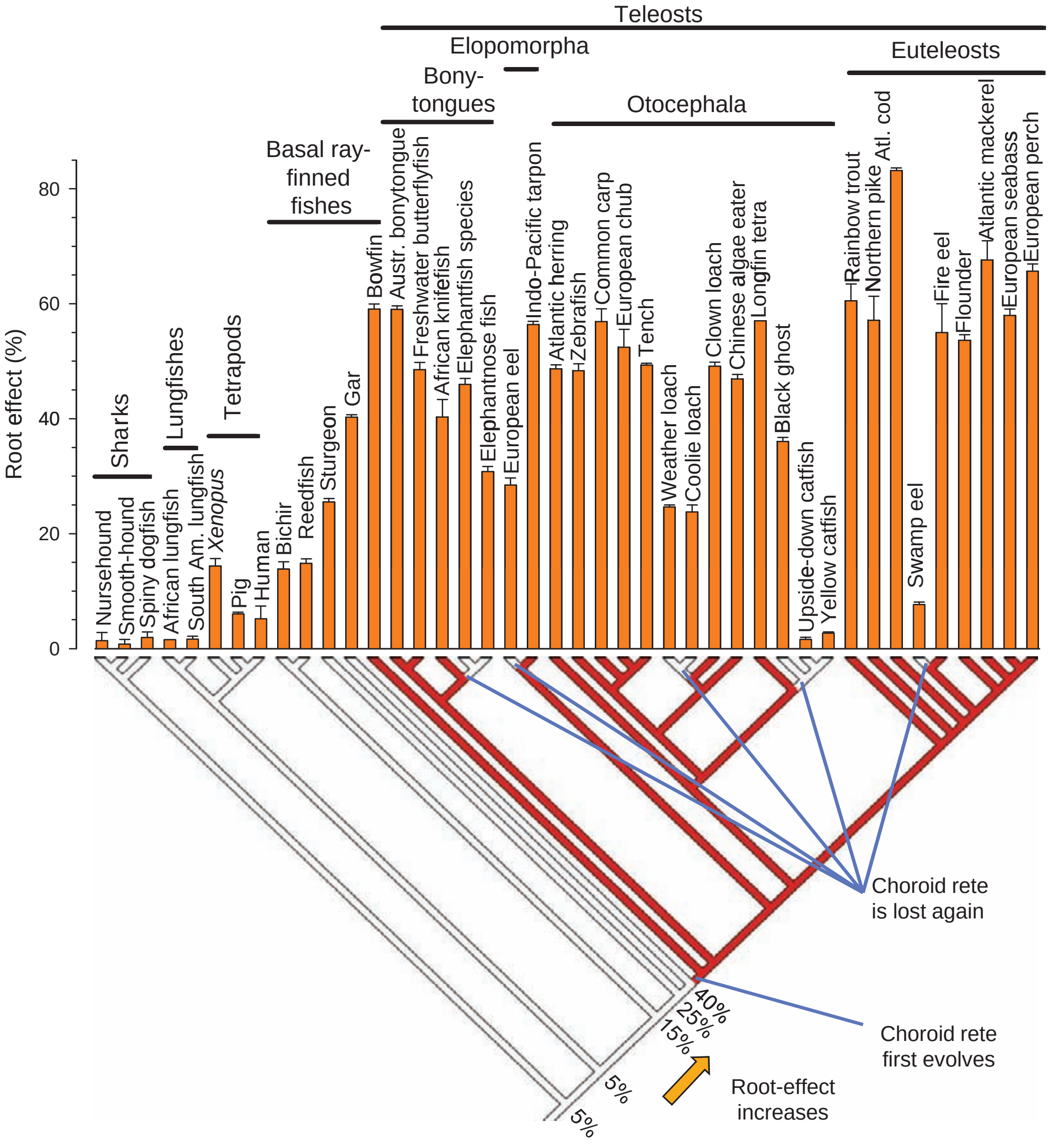


Figure 5 Evolution of the choroid rete mirabile and the magnitude of the Root effect. The presence (red branches) and absence (white branches) of the choroid rete mirabile have been mapped onto a phylogenetic tree of selected jawed vertebrates. The magnitude of the Root effect (mean values and SEM) has been estimated as maximum acid-induced deoxygenation of air-equilibrated hemolysates at physiological body temperatures. Evolutionary reconstruction of these data indicates that the Root effect increased before the evolution of a choroid (or swimbladder) rete mirabile (arrow). However, in living teleosts, the Root effect only ever decreases below 40% in species that have lost the choroid rete mirabile, indicating a functional link. Major taxonomic groups are labeled with bars. Scientific species names from left to right are: Sharks-*Scyliorhinus stellaris, Mustelus asterias, Squalus acanthias*; lungfishes -*Protopterus* sp., *Lepidosiren paradoxa*; tetrapods - *Xenopus laevis, Sus scrofa, Homo sapiens*; basal ray-finned fishes - *Polyterus ornatipinnis, Erpetoichthys calabaricus, Acipenser baeri, Lepisosteus platyrhincus, Amia calva*; bony tongues - *Scleropages jardinii, Pantodon buchholzi, Xenomystus nigri, Campylomormyrus numenius, Gnathonemus petersil*; Elopomorpha - *Anguilla anguilla, Megalops cyprinoides*; Otocephala - *Clupea harengus, Danio rerio, Cyprinus carpio, Leuciscus cephalus, Tinca tinca, Misgurnus anguillicaudatus, Pangio kuhlii, Botia macracanthus. Gyrinocheilus aymonieri, Brycinus longipinnis, Apteronotus albifrons, Synodontis greshoffi, Pelteobagrus fulvidraco*; euteleosts - *Oncorhynchus mykiss, Esox lucius, Gadus morhua, Monopterus albus, Mastacembelus erythrotaenia, Platichthys flesus, Scomber scombrus, Dicentrarchus labrax, Perca fluviatilis*. Modified from Berenbrink M, Koldkjær P, Kepp O, and Cossins A (2005) Evolution of O_2 secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

about twice as high Hb buffer values than typical teleosts. Similarly high Hb buffer values in the South American lungfish *Lepidosiren paradoxa*, and sharks indicate that this may have been the ancestral state of ray-finned fishes and jawed vertebrates in general (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). This means that blood pH was comparatively well buffered when the Root effect first evolved and that a general acidosis was less likely to impair full Hb oxygenation in the gills.

Why did the Root effect evolve then? To answer the question, it is important to remember that the lack of achieving full O_2 saturation in Root-effect Hbs even at the P_{O_2} of air or higher is only seen at low pH values and that these may be rarely achieved in well-buffered blood and without an acid-conserving rete mirabile. At the pH values normally occurring in the general circulation of fishes, Root-effect Hbs behave similar to the Bohr-effect Hbs of mammals (**Figure 1**), even if the Hb-O_2-binding affinity usually shifts twice as much upon a given change in pH in Root-effect Hbs. Thus, although the molecular mechanism for the pH-induced shift in Hb O_2 affinity is quite different in Bohr- and Root-effect Hbs, they may have evolved in the mammal and ray-finned fish lineages for the same purpose, namely improved efficiency of general gas transport by increased O_2 unloading in metabolic active, acid-producing tissues and increased O_2 loading in relatively CO_2 poor gill blood. This idea is consistent with the multiple evolution of a Bohr effect in other animal groups with entirely different respiratory pigments, such as in lampreys, cephalopods, and crustaceans (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect).

The later evolution of retia mirabilia, first behind the retina and later several times independently at the swimbladder, then allowed evolution of O_2 secretion, by enabling the localized generation of low blood pH, and thereby the exploitation of the special characteristics of Root-effect Hbs.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. **Design and Physiology of Arteries and Veins**: The Retia. **Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect; Hemoglobin; Red Blood Cell Function; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M (2007) Historical reconstructions of evolving physiological complexity: O_2 secretion in the eye and swimbladder of fishes. *Journal of Experimental Biology* 209: 1641–1652.

Berenbrink M, Koldkjær P, Kepp O, and Cossins A (2005) Evolution of O_2 secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Berenbrink M, Völkel S, Heisler N, and Nikinmaa M (2000) O_2-dependent K^+ fluxes in trout red blood cells: The nature of O_2 sensing revealed by the O_2 affinity, cooperativity and pH dependence of transport. *Journal of Physiology* 526: 69–80.

Brauner CJ and Berenbrink M (2007) Gas transport and exchange. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Fish Physiology,* vol. 26, pp. 214–270. Amsterdam: Academic Press.

Brittain T (2005) Root effect hemoglobins. *Journal of Inorganic Chemistry* 99: 120–129.

Farmer M, Fyhn HJ, Fyhn UEH, and Noble RW (1979) Occurrence of Root effect hemoglobins in Amazonian fishes. *Comparative Biochemistry and Physiology B* 62: 115–124.

Green AA and Root RW (1933) The equilibrium between hemoglobin and oxygen in the blood of certain fishes. *Biological Bulletin* 64: 383–403.

Jensen FB, Fago A, and Weber RE (1998) Hemoglobin structure and function. In: Perry SF and Tufts BL (eds.) *Fish Physiology,* vol. 17, pp. 1–40. San Diego, CA: Academic Press.

Root RW (1931) The respiratory function of the blood of marine fishes. *Biological Bulletin* 61: 427–456.

Verde C, Berenbrink M, and di Prisco G (2008) Evolutionary physiology of oxygen secretion in the eye of fishes of the suborder Notothenioidei. In: Bolognesi M, di Prisco G, and Verde C (eds.) *Dioxygen Binding and Sensing Proteins: A Tribute to Beatrice and Jonathan Wittenberg*, pp. 49–66. Milan: Springer.

Hemoglobin Differentiation in Fishes

C Verde, D Giordano, R Russo, and G di Prisco, National Research Council (CNR), Naples, Italy

© 2011 Elsevier Inc. All rights reserved.

Introduction
Fish Hemoglobin Functional Differentiation
Further Reading

Glossary

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Orthologous genes Genes in different species that are similar to each other because they originated from a common ancestor.

Root effect The complete loss of cooperativity (indicated by a Hill coefficient equal to 1), and thereby the inability to saturate the ligand sites at low pH even at high O_2 pressure in the hemoglobin molecule, is a distinctive property with respect to the Bohr effect, and is referred to as the Root effect.

Introduction

Few proteins have been studied as widely as hemoglobin, and recent discoveries on its structure–function relationship keep stimulating interest. Hemoglobins are found in bacteria, protists, fungi, plants, and animals. The different and widespread hemoglobins appear to be encoded by orthologous genes, thereby implying that they are descended from an ancient, common ancestral gene.

Genome sequencing suggests that the evolutionary tree of the family of globin proteins extends back 1800 million of years, a time when the O_2 began to accumulate in the atmosphere. The atmospheric O_2 content reached its present levels only about 540 million years ago. At that time, a hemoglobin-like ancestor was likely adapted for scavenging excessive O_2 concentration and/or in detoxification of O_2.

Hemoglobins are also ancient proteins that probably evolved from enzymes used to protect tissues against toxic O_2 levels. Fish hemoglobin, which is a tetramer of two identical α and β globin chains, is a relatively recent adaptation of the hemoglobin molecule to widely different environmental conditions. The evolution of a simple O_2-binding globin protein into multi-unit hemoglobin protein and then its combination with the development of the circulatory system greatly enhanced the ability of blood to transport O_2 to metabolizing cells.

The expression of α and β chains and their post-translational assembly into $\alpha_2\beta_2$ tetramers is fundamental to the function of most vertebrate hemoglobins. In all vertebrates, there is a strong evolutionary pressure favoring the expression of equal amounts of the two types of chains, because all the functional properties displayed by hemoglobin, for example, cooperativity, pH sensitivity, and anionic regulation, occur only in the $\alpha_2\beta_2$ tetramers. In fish, the expression of multiple α and β chains is the basis for functional adaptations because the single chains by themselves do not show the sophisticated allosteric properties of the assembled tetramer.

Fish hemoglobins have, for many years, attracted the attention of biochemists and physiologists for the huge spectrum of functional properties they display. Adaptive hemoglobin structural and functional variations are necessary because fish are influenced to a great extent by their environments. Fish hemoglobins function at the interface between organism and environment; consequently, this molecule can identify key links between molecular and ecophysiological adaptations. Fish experience temporal and spatial variations in O_2 availability, salinity, ionic composition, pH, and temperature. Fish hemoglobins are especially interesting because the gills are in contact with a medium endowed with higher O_2 and lower carbon dioxide tensions when compared to the situation in the alveoli of mammalian lungs. In fish, as well as in most vertebrates, cellular processes rely on rapid responses to external stimuli. The basic molecular events associated with these processes are the protein structural conformational transitions involved. Therefore, fish hemoglobins deserve special attention to elucidate these processes at the atomic and molecular level.

The Basic Structure of Fish Hemoglobins

While fish hemoglobins have evolved structural and functional diversity to adapt to very diverse environments, a large number of amino-acid residues are well conserved in the predominantly helical structure. Since all animal hemoglobins share the same heme group differences in their properties, including O_2 affinity, electrophoretic mobility, and pH sensitivity, must ultimately reside in the differences among a few specific amino-acid residues of the primary structure.

The three-dimensional structure of vertebrate hemoglobin was determined half a century ago. The resolved structure revealed basic principles of protein architecture, with the ≈150-residue globin chain wrapped around the single heme to form the hydrophobic pocket site. In a typical fish hemoglobin, as well as in most vertebrates, iron performs its function in the ferrous state. It is well known that modern vertebrate globins have evolved to control multiple properties simultaneously, including affinity for the heme group, iron-redox potential, dependence of O_2 affinity on pH, temperature, and effector concentration. Heme is chemically highly active and has ancient roots. To remain soluble, heme needs to be surrounded by a hydrophobic environment that is possible with a few structural protein arrangements, the most common being the globin fold. Vertebrate evolution has conserved distinctive globin folds (the so-called 3-on-3 α-helical sandwich) characteristic of myoglobin, despite a rather large sequence divergence and particularly if one considers the other two vertebrate lineages comprising neuroglobins and cytoglobins, recently discovered, which occur widely in vertebrates including fishes (**Figure 1**).

Fish hemoglobin is a hetero-tetramer (**Figure 2**). In all vertebrates, the primary structures of α and β globins are approximately 50% identical, suggesting a common precursor in ancestral vertebrates. Globins are paired in two dimers, $\alpha_1\beta_1$ and $\alpha_2\beta_2$. In fish hemoglobins, the α and β chains have 142 and 147 residues. Humans have one residue less in each of the chains, 141 and 146, respectively. The basic unit is the globin polypeptide chain, associated with a planar complex of ferrous iron and protoporphyrin IX (heme). The globin chain contains seven or eight α-helical segments, named from A to H starting from the N terminus. The nonhelical segments between helices are named AB, BC, CD, etc. The only residues common to all globins are proximal histidyl F8 and phenylalanyl CD1. On the distal ligand site of the heme, distal His E7 and Val E11 are normally present.

Affinity and Cooperativity in Fish Hemoglobins

Hemoglobins are endowed with specific ligand-binding affinity and generally exhibit ligand-binding cooperativity. The reversible reaction of O_2 binding to the four subunits of hemoglobin is nonlinear and does not correspond to a simple equilibrium. The curve

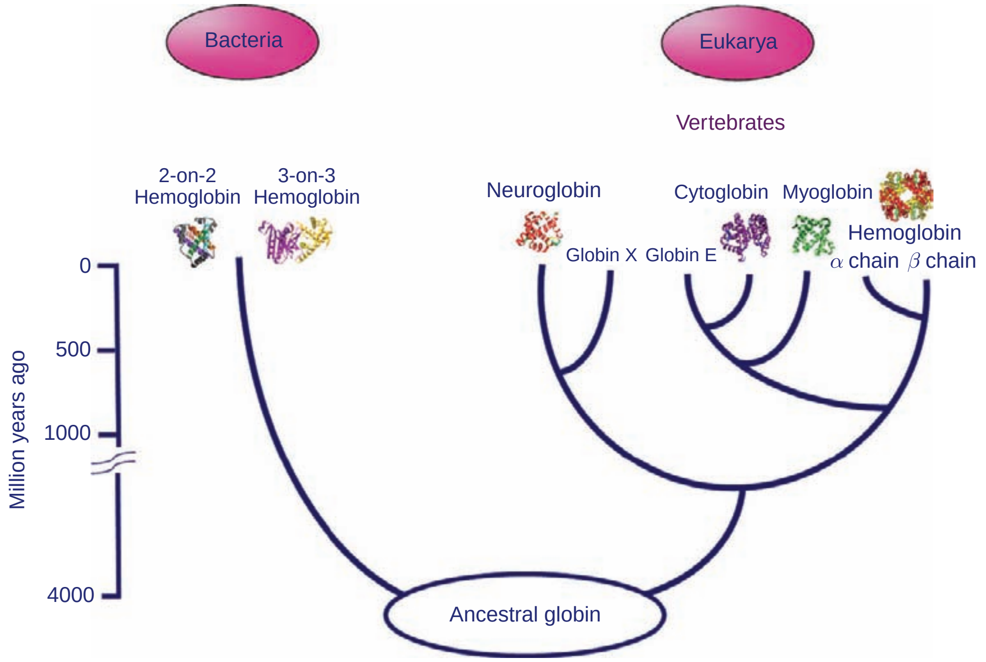


Figure 1 A simplified phylogenetic tree of vertebrate globins. From Vinogradov S, Hoogewijs D, Bailly X, *et al*. (2005) Three globin lineages belonging to two structural classes in genomes from the three kingdoms of life. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11385–11389.

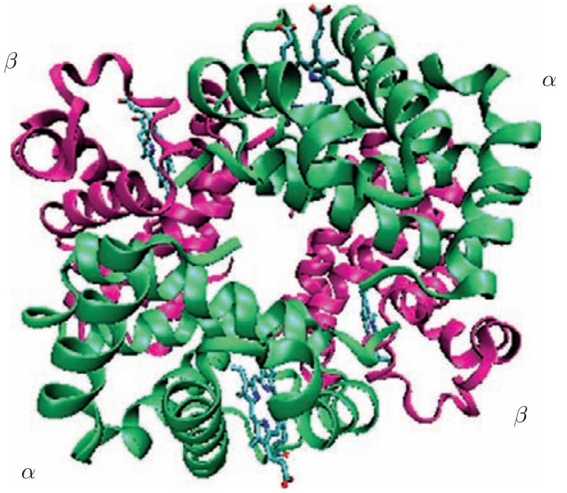


Figure 2 Hemoglobin of *Trematomus bernacchii* (pdb code 2h8D).

describing O_2 binding in blood as a function of its partial pressure is sigmoidal (**Figure 3**), indicating a cooperativity between the subunits (homotropic interactions, see below). The binding of the first O_2 molecule increases the affinity for the second molecule, and so forth. The specific mechanisms whereby inter-subunit interactions lead to a sigmoidal O_2-binding curve have long been the subject of scientific debate. All models presented to date are based on the interplay of tertiary structural changes induced by O_2 binding within a subunit. The overall affinity of hemoglobin for O_2 is expressed as the gas partial pressure required to achieve half-saturation (P_{50}). In a noncooperative system (e.g., myoglobin), where the O_2-binding curve as a function of the gas partial pressure is hyperbolic, this value corresponds to the equilibrium constant for the dissociation reaction.

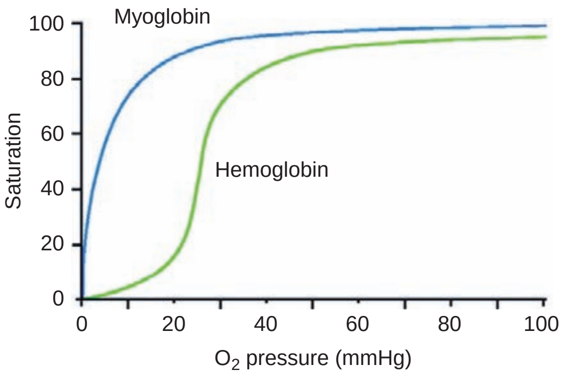


Figure 3 Fractional saturation of myoglobin and hemoglobin. The hyperbolic curve of myoglobin binding is typical for a noncooperative process; the sigmoidal curve of hemoglobin binding is typical for cooperativity.

Affinity and cooperativity may be adaptively modified in different hemoglobins to provide adequate O_2 supply under a wide variety of environmental and physiological conditions. Many of the functional differences observed in fish hemoglobins may be interpreted in terms of substitutions of amino-acid residues, although others are due to changes in the composition of the medium in which the protein works. Changes in levels of physiological effectors also affect binding properties and modify the affinity. Fishes display great ability to control the modulator concentrations.

Changes in the concentration of hemoglobin within a red blood cell (RBC) is another strategy for environment adaptation. Thus, fish may vary the synthesis of hemoglobins and, selectively, the expression of some but not all hemoglobin genes; for further discussion, the reader is referred to the section entitled ‘Fish hemoglobin functional differentiation’.

Allostery and the Monod, Wyman, and Changeux Model

After more than a century of experimental, theoretical, and computational studies, there is no general agreement on the mechanisms underlying the fine regulation of hemoglobin in fish. The crystal structures of myoglobin and hemoglobin provided the first basis to understand the relationship between protein structure and function. The four-subunit quaternary structure also prompted the classic theories and structural studies of allosteric conformational change. The main concept of the two-state allosteric model of Monod, Wyman, and Changeux (MWC) is that the molecule can only exist in two quaternary states, corresponding to a low-affinity structure (T, tense) and a high-affinity structure (R, relaxed) (**Figure 4**). According to the MWC model, cooperative O_2 binding arises due to a shift from the T to the R structure as O_2 binding increases. The pioneering achievements of Perutz identified that distinct structural T and R states are associated with the deoxygenated and the oxygenated form of the protein, respectively. On this basis, a stereochemical model for the allosteric transition was proposed. Although Perutz’s overall view of allosteric transition is still valid, more recent crystallographic analyses have clearly shown that tetrameric hemoglobins possess a larger repertoire of structural states. However, despite the large amount of data accumulated on the endpoints of the transition, structural information on the intermediate forms of the process is still rather limited. Some interesting features of these states have recently been unveiled by a variety of techniques, but the three-dimensional models are unavailable as yet.

Complex and sophisticated molecular mechanisms have evolved to regulate fish O_2 transport in response to pH, carbon dioxide, organic phosphates, and temperature

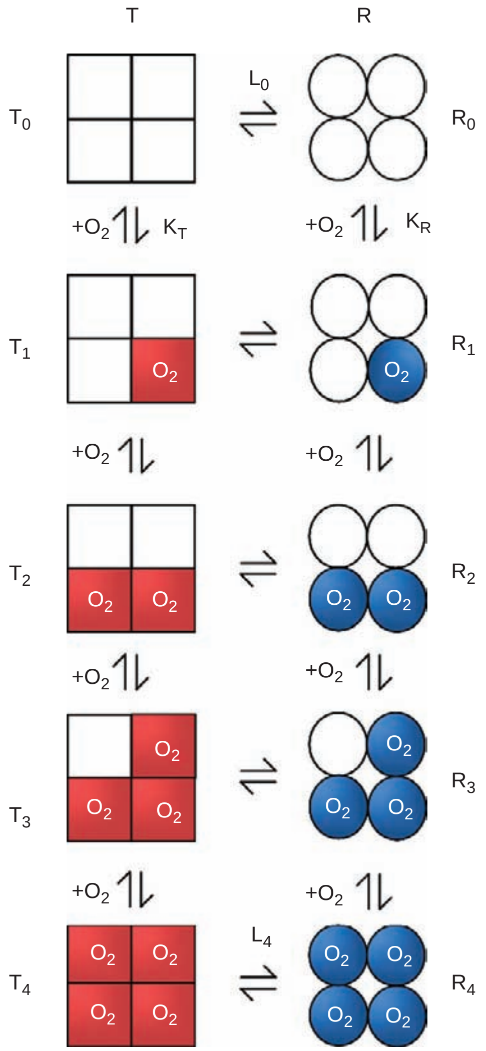


Figure 4 The hemoglobin tetramer is assumed to be in one of the two symmetric conformations: T state with low affinity (K_T) and R state with high affinity (K_R). In each conformation, there are five states subscripted by the number of O_2 bound. The equilibrium constant between the fully deoxygenated states is L_0 and between the fully oxygenated states is L_4.

effect. In allosteric molecules, the reactivity of the active site is modulated, through conformational equilibria, by binding of ligands to other sites of the same molecule. In vertebrates such as fish, the allosteric interactions may be either homotropic, if occurring among sites which bind the same ligand (i.e., O_2), or heterotropic, if the sites bind different ligands which affect O_2 affinity. The heterotropic effectors (phosphates and protons) regulate hemoglobin by modulating O_2 affinity, thus strengthening or weakening the T or R states.

The major effectors in fish are adenosine triphosphate (ATP) and protons. Organic phosphates preferentially bind to the T structure in the central cavity between the two β chains and decrease O_2 affinity.

The Bohr and the Root Effects

A decrease in O_2 affinity at lower physiological pH values is known as the alkaline Bohr effect. Decreasing the pH of blood therefore releases O_2 from hemoglobin. The acid Bohr effect, that is, an increase of O_2 affinity upon further pH decrease, may occur at pH lower than 6.0.

The physiological relevance of the Bohr effect is clear when one considers that highly active tissues release acidic metabolites (carbon dioxide and H^+). This release enhances O_2 unloading at tissues. Physiological concentrations of organic phosphates in RBCs increase the Bohr effect. The magnitude of the Bohr effect may vary among vertebrate classes. In fish, it is generally low in elasmobranchs and high in teleosts.

The Root effect corresponds to extreme pH sensitivity. In Root-effect hemoglobins, when the pH is lowered, the O_2 affinity decreases to such an extent that hemoglobins cannot be fully saturated even at very high O_2 pressure and the cooperativity is totally lost (**Figure 5**). The Root effect is almost exclusively present in teleosts.

The Root effect dictates to what extent the O_2 tension can be raised in an acid-producing tissue. Thus, its physiological role is to secrete O_2 against high gas pressures into the swimbladder, the poorly vascularized retina and possibly skeletal muscle as a result of local blood acidification. A countercurrent capillary system can amplify this effect in the swimbladder and eye. The swimbladder and the retina possess a specialized acid-producing tissue associated with the countercurrent capillary system, the *rete mirabile* (known as choroid *rete* in the eye). Among nonteleosts, the choroid *rete* is present only in *Amia* and evolved just once in fishes, in the clade comprising Amia and Teleostei. The choroid *rete* is well developed in the basal families of the dominant Antarctic perciform suborder Notothenioidei. In Antarctic fish families, many species have lost the *rete*, and the discovery of reduced and microscopic *retia* suggests that this loss may occur gradually. However, in notothenioids, the few species possessing hemoglobins without Root effect, as well as hemoglobin-less Channichthyidae, also lack the choroid *rete*.

The Structural Basis of the Root Effect

The ongoing debate on the structural interpretation of the Root effect envisages the possibility that in fish hemoglobins the classical MWC model is an oversimplified explanation of the pH modulation. C-terminal His in 146β appears to be involved in the Root effect in some hemoglobins but not in all. Moreover, a fundamental difference between Root-effect hemoglobins and hemoglobins with normal Bohr effect (and no Root effect) is that in the former hemoglobins, the $\alpha_1\beta_2$ interface remains stable in the T state upon oxygenation, whereas

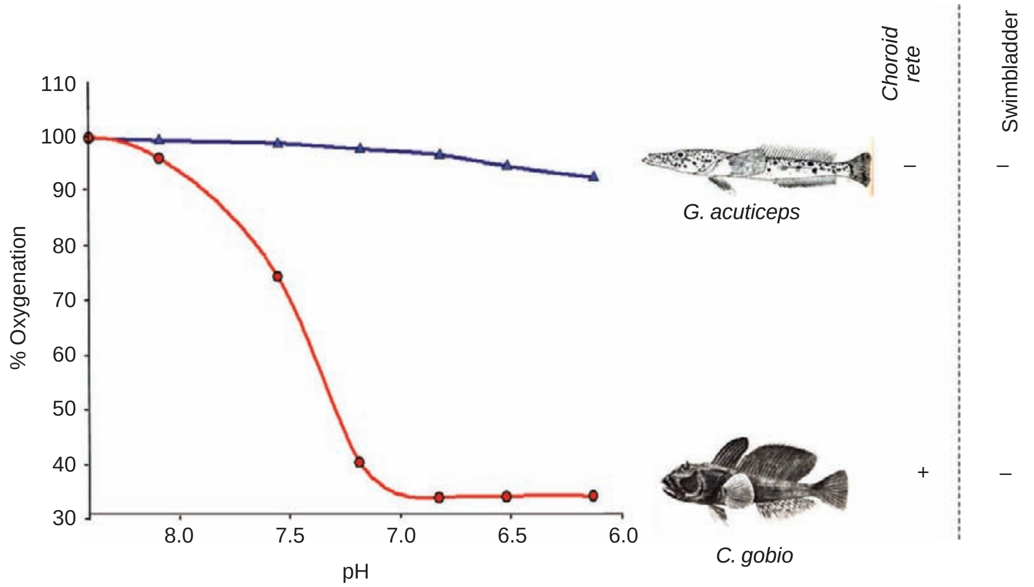


Figure 5 Presence and absence of the Root effect in hemoglobins of the Antarctic fish *Cottoperca gobio* and *Gymnodraco acuticeps*. Presence and absence of swimbladder and choroid *rete* are indicated. The O_2 saturation (expressed as percentage of oxygenation) is reported as a function of pH.

in the latter the switch to the R state occurs. Studies on the Root-effect hemoglobin of the spot, *Leiostomus xanthurus,* have suggested that destabilization of the R state (rather than stabilization of the T state) at low pH, inducing an R/T transition, may provide a molecular basis for the Root effect. Recently, O_2-binding experiments under a wide range of medium conditions have brought about a new model of allosteric regulation of hemoglobin, the global allostery model. This model highlights the role of heterotropic effectors in altering the tertiary structures of both T and R states and consequently in modulating the O_2 affinity and the Bohr/Root effect. Thus, it is feasible that the Root effect not only depends on the primary structure, but also on how and to what extent hemoglobin binds effectors in the T and R states. The pH modulation of Antarctic fish hemoglobins was attributed to changes in the tertiary structure within the T state, supporting the global allostery model. The crystal structure of the deoxygenated form of the cathodal hemoglobin from *Trematomus newnesi* (endowed with the Root effect) has been particularly illuminating to explain the proton heterotropic effect. The amino-acid sequence is characterized by extremely low histidyl content. The deoxygenated structure exhibits a hydrogen bond between Asp 95α and Asp 101β, stabilized by Asp 99β. Therefore, this structural motif *per se* is sufficient to generate the Root effect, and can be considered as the minimal structural requirement needed for designing Root-effect hemoglobins. The role played by the histidyl residues in modulating the strength of the Root effect has been highlighted by the crystal structures of tuna *(Thunnus thynnus)* and *Trematomus bernacchii* hemoglobins. Other authors, using a different approach and perspective, also highlight the role of histidyl residues in the molecular mechanisms of the Bohr and Root effects.

After more than three decades, it is virtually impossible as yet to ascribe the presence or absence of the Root effect to a single structural explanation. Indeed, the situation appears highly complex, and is probably linked to the combination and interplay of a number of factors in the architecture of the tetramer in a given species.

Fish Hemoglobin Functional Differentiation

Hypoxia

Fish live in environments that often show large variations in O_2 levels (the Southern Ocean is an exception). Hypoxia is common in aquatic habitats because O_2 dissolved in water (which can be in small amounts) is rapidly used up by aquatic organisms, and replacement requires either exchange at the interface with the atmosphere or photosynthesis.

Hypoxia tolerance is widely spread in the animal kingdom and probably arose independently at multiple times during evolution. Among the water-breathing teleosts, Cyprinidae are particularly hypoxia tolerant. These fish have evolved suitable adaptations in response to hypoxia, including hemoglobins with exceptionally high O_2 affinities, in contrast to those endowed with low affinity frequently observed in active fish living in well-aerated water. These examples highlight how the intrinsic properties of hemoglobins allow environmental and metabolic adaptations.

The first line of response to hypoxia is physiological, aimed at improving O_2 delivery through adjustments in capillarity, blood volume, or RBC mass. Complementary to the physiological adjustments are the biochemical adaptations in the molecule. Hemoglobin adaptations to hypoxia may include (1) increase in blood content, (2) changes in O_2 affinity, and (3) changes in regulation.

How and where hypoxia is sensed to allow response still remains a puzzle, since the response to environmental hypoxia is not the same as that to O_2 limitations due to swimming. For more than half a century, various, often conflicting hypotheses have considered the role of body size in hypoxia tolerance in fish. Recent evidence suggests that body size *per se* probably has little or no impact on O_2-uptake capacity during hypoxia; however, larger individuals have clear advantages over smaller ones, because the latter will much faster run out of glycogen or reach lethal levels of anaerobic end products. However, larger fish have a greater absolute O_2 demand.

Hemoglobin Multiplicity

Many fish express multiple hemoglobins with different O_2-binding properties, in order to meet O_2 demand under changing environmental conditions or metabolic challenges, rather than relying on a single respiratory protein. Multiplicity is also common in elasmobranchs. As suggested by some authors, multiple hemoglobins may protect against deleterious mutational changes in the globin genes and provide higher total hemoglobin concentration in the RBC (according to the phase rule, in a saturated solution, multiple proteins afford higher total concentration than a single protein).

Multiple hemoglobins may occur at different developmental stages; in genetically different strains of the same species, polymorphism of allo-hemoglobins also occurs, increasing the number of components. Most of these, however, cannot be properly characterized, due to either low levels of expression, or inadequacy of available purification techniques in the frequent cases of microheterogeneity.

Multiplicity is frequently found in fish that cope with highly variable conditions, whereas the presence of a single dominant hemoglobin is usually associated with stable conditions. For example, most Antarctic notothenioids are sluggish and have a single major hemoglobin, whereas temperate fish generally show high multiplicity. Multiple hemoglobins can finely tune O_2 delivery in response to environmental variability and/or variations in metabolic demands. The O_2-rich habitat of the Antarctic sea, in contrast, may have been permissive following the deletion of the globin genes in some Antarctic species such as icefish.

Unlike Antarctic notothenioids, many Arctic fish display multiplicity, usually interpreted as a sign of phylogenetic and molecular adaptation. For example, the spotted wolffish, *Anarhichas minor* (suborder Zoarcoidei), contains three functionally distinct major hemoglobins. High hemoglobin multiplicity, again with three major hemoglobins, is broadly found in the family Gadidae, in *Arctogadus glacialis* (Arctic cod), *Boreogadus saida* (polar cod), and *Gadus morhua* (Atlantic cod). For the Arctic ichthyofauna, it may be advantageous to maintain multiplicity, considering the need to adapt to a much more variable environment than the Antarctic one, where the Antarctic Polar Front maintains permanently stable conditions, including lower temperatures.

The well-documented adaptations in blood O_2-binding properties in response to environmental conditions comprise intraspecific adaptations (e.g., changes in organic phosphate concentrations in the RBC occurring in individuals) as well as interspecific adaptations (differences in hemoglobin structure and heterogeneity between species).

The functional differences in hemoglobins, due to gene duplications common in fish, are often minor and sometimes have not been shown to have adaptive physiological relevance. While cyprinids and cichlids (class I fish) possess only the electrophoretically anodal hemoglobin with relatively low O_2 affinity and marked Bohr and Root effects, the class II fish (comprising eels, salmonids, and some catfishes) additionally possess cathodal hemoglobins with high isoelectric points ($pI \geq 8.0$), high O_2 affinity, and low pH sensitivity (small Bohr effect).

The pH-insensitive hemoglobins generally carry a net charge different from that of Root-effect hemoglobins and migrate toward the cathode in electrophoresis experiments. They generally lack the histidyl residue at the *C* terminus of the β chain. Cathodal hemoglobins may function as O_2 carriers when the function of anodal components is impaired by acidification and hypoxia. During respiratory or metabolic acidosis, O_2 binding to anodal hemoglobins may be hampered by their strong Bohr and Root effect; however, O_2 transport may rely on pH-independent cathodal components often found in fishes with active lifestyle. The bluefish (*Pomatus saltatrix*), a highly active, globally distributed predator, contains highly pH-sensitive hemoglobins (Root-effect hemoglobins), while other hemoglobins show little or no pH dependence. The Root-effect hemoglobins are used for proton-driven O_2 unloading into the swimbladder to produce neutral buoyancy. However, the advantage in possessing pH-sensitive and -insensitive hemoglobins may vary in different fish.

The readers are cautioned that describing anodal hemoglobins as having marked Bohr and Root effect, and cathodal hemoglobins as having low pH sensitivity, is an oversimplification. In notothenioids, the situation is exactly reversed (**see** section 'The Structural Basis of the Root Effect').

Changes in globin gene expression during fish ontogeny have been described in many teleosts. In the rainbow trout, the embryonic and adult hemoglobins display different functional properties, with higher O_2 affinity and reduced Bohr effect in the former. Non-Bohr-effect hemoglobins may provide the embryo with enhanced O_2-carrying capacity over a wide pH range. In the rainbow trout, the change in hemoglobin composition was correlated with an apparent change in erythroid cell population. In general, the abundance of highly pH-sensitive anodal hemoglobins relative to pH-insensitive cathodal hemoglobins may vary in some fish with age and stress. The changes in hemoglobin composition may be very complex, as described in the Arctic charr, *Salvelinus alpinus*, with different numbers of components in embryos and in later stages. The exact functional meaning of these shifts, as well as the advantages in possessing a large number of globin genes in the early stages of development, remain poorly understood. Unfortunately, the biochemical characterization of embryonic fish hemoglobins is hampered by the small amount of protein. Hemoglobin switching was demonstrated for the globins of lampreys, suggesting that the switch mechanism was already present in the ancestral globin gene locus before the event of duplication generating the α- and β-globin loci.

See also: **Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Red Blood Cell Function; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Berenbrink M, Koldkjaer P, Kepp O, and Cossins AR (2005) Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* 307: 1752–1757.

Brittain T (2005) Root effect hemoglobins. *Journal of Inorganic Biochemistry* 99: 120–129.

Cheng C-HC and Detrich III, HW (2007) Molecular ecophysiology of Antarctic notothenioid fishes. *Philosophical Transaction of the Royal Society London B Biological Sciences* 362: 2215–2232.

Dettaï A, di Prisco G, Lecointre G, Parisi E, and Verde C (2008) Inferring evolution of fish proteins: The globin case study. *Methods in Enzymology* 436: 535–566.

di Prisco G, Eastman JT, Giordano D, Parisi E, and Verde C (2007) Biogeography and adaptation of Notothenioid fish: Hemoglobin function and globin-gene evolution. *Gene* 398: 143–155.

Mazzarella L, Bonomi G, Lubrano MC, *et al.* (2006) Minimal structural requirement of Root effect: Crystal structure of the cathodic hemoglobin isolated from *Trematomus newnesi. Proteins* 62: 316–321.

Mazzarella L, Vergara A, Vitagliano L, *et al.* (2006) High resolution crystal structure of deoxy hemoglobin from *Trematomus bernacchii* at different pH values: The role of histidine residues in modulating the strength of the Root effect. *Proteins* 65: 490–498.

Monod J, Wyman J, and Changeux JP (1965) On the nature of allosteric transitions: A plausible model. *Journal of Molecular Biology* 12: 88–118.

Perutz MF (1970) Stereochemistry of cooperative effects in hemoglobin. *Nature* 228: 726–739.

Perutz MF (1983) Species adaptation in a protein molecule. *Molecular Biology and Evolution* 1: 1–28.

Riggs A (1988) The Bohr effect. *Annual Review of Physiology* 50: 181–204.

Verde C, Balestrieri M, de Pascale D, *et al.* (2006) The oxygen transport system in three species of the boreal fish family Gadidae. *Journal of Biological Chemistry* 283: 22073–22084.

Verde C, Vergara A, Giordano G, Mazzarella L, and di Prisco G (2008) Hemoglobins of fishes living at polar latitudes: Current knowledge on evolutionary and structural adaptation in a changing environment. *Current Protein and Peptide Science* 9: 578–590.

Vinogradov S, Hoogewijs D, Bailly X, *et al.* (2005) Three globin lineages belonging to two structural classes in genomes from the three kingdoms of life. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11385–11389.

Weber RE, Fago A, Val AL, *et al.* (2000) Isohemoglobin differentiation in the bimodal-breathing Amazon catfish *Hoplosternum littorale*. *Journal of Biological Chemistry* 275: 17297–17305.

Weber RE and Jensen FB (1988) Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Review of Physiology* 50: 161–179.

Yokoyama T, Chong KT, Miyazaki G, *et al.* (2004) Novel mechanisms of pH sensitivity in tuna hemoglobin: A structural explanation of the Root effect. *Journal of Biological Chemistry* 279: 28632–28640.

GAS EXCHANGE

Tissue Respiration

Contents

Cellular Respiration
Mitochondrial Respiration
Specializations in Mitochondrial Respiration of Fish

Cellular Respiration

GB McClelland, McMaster University, Hamilton, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Cellular Metabolism
Fuel and O_2 Delivery to Tissues
Cell Membrane Transport
Cytosolic Transport
Mitochondrial Transport
Cellular Respiration in Fish
Environmental and Energetic Stress
Limits to Cellular Respiration
Novel Species
Conclusions
Further Reading

Glossary

Basal metabolic rate Oxygen consumption in a nonexercising, nondigesting, awake animal. Standard metabolic rate is used for fish because they are rarely motionless.

Lipids Group of naturally occurring hydrophobic compounds including fats, oils, waxes, phospholipids, and others.

Nonesterified fatty acids (NEFAs) Free fatty acids, not in the form of glycerol esters.

Oxidative phosphorylation (OXPHOS) Aerobic ATP production using the energy in a proton electrochemical gradient to phosphorylate ADP via the F_1F_o ATPase.

Q_{10} or temperature coefficient Factorial change in the rate of a biochemical reaction or process with a 10 °C increase in temperature.

Triacylglycerol (TAG) Storage lipid consisting of three fatty acids linked to a glycerol backbone.

Cellular Metabolism

The main goal of cellular respiration is to produce energy in the form of adenosine triphosphate (ATP) at the same rate at which ATP is consumed by cellular processes (**Figure 1**), that is, balancing ATP supply and demand. Cellular respiration, through oxidative phosphorylation (OXPHOS), constitutes the main oxygen-consuming and ATP-producing processes, and whole-animal metabolic rate is the sum of respiration from all tissues. ATP production by OXPHOS requires an adequate delivery of both oxygen and metabolic fuels to cells. While the delivery of oxygen depends on the environmental levels of oxygen (e.g., atmospheric or dissolved oxygen) and the pathway from gas transfer organs to tissues, fuels are typically drawn from the stores already within the body. Although many cells still rely on the delivery of exogenous fuels (lipids, carbohydrates, and proteins) from other tissues that store large amounts of these substrates (e.g., lipids are stored in fat cells or adipocytes), most cells have their own stores to draw upon.

The various cell types that make up the different tissues in vertebrates show considerable variation in their energetic demands and their aerobic scope, where

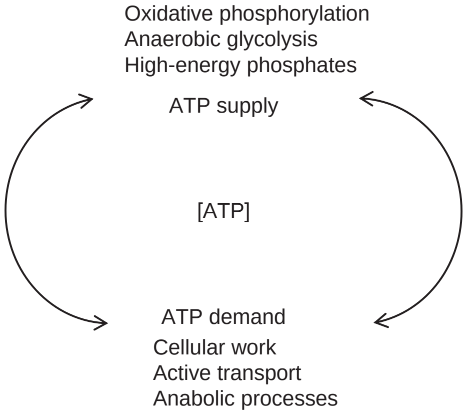


Figure 1 ATP homeostasis is maintained through the balance between ATP-supply pathways and ATP-demand pathways.

aerobic scope is defined as the difference between basal and maximal rates of aerobic ATP production. For example, in most animals, skeletal muscle has the largest aerobic scope (~100-fold). Basal metabolism also differs between tissues affecting their relative contribution to whole body basal metabolic rates (BMRs). Mammalian and fish muscles, despite having a rather low resting metabolic rate, contribute about 20–30% to BMR because they constitute the largest proportion of body mass (approximately 40–60%). For this reason, we use muscle cells (myocytes) as a primary model to discuss cellular metabolism and respiration (**Table 1**).

Cellular metabolism ultimately aims at maintaining homeostatic ATP concentrations despite dynamic fluctuations in ATP demand. Cellular processes that consume ATP include all active ion transport processes, whether it is to maintain membrane potentials (e.g., Na^+/K^+-ATPase), to transport calcium for actin–myosin cross-bridge cycling (e.g., Ca^{2+}ATPase) or for neurotransmitter release, to name a few. Protein turnover, the balance between protein synthesis and degradation, can also be a major ATP-consuming process, especially during the periods of growth. Other energy-consuming processes in vertebrates include urea synthesis and gluconeogenesis. Therefore, to balance all these ATP-consuming reactions there must be a corresponding equivalent rate of ATP synthesis. As discussed subsequently, this involves the coordinated delivery of oxygen and metabolic fuels to cells.

Table 1 The relative contribution of various tissues to basal or routine metabolic rate in a typical mammal and fish

	Relative metabolism (% total metabolism)	
Tissue	*Fish*	*Mammal*
Gill/ lung	19	<16[a]
Liver	13	21
Brain	1	20
Heart	4	9
Kidneys	5	8
Muscle	30	22

[a] Includes bones, skin, intestines, and lungs.

Cellular processes in most vertebrates are supplied energy by aerobic ATP production via OXPHOS (**Figure 2**). OXPHOS uses oxygen as a terminal acceptor for electrons that travel down the electron transport chain (ETC). The electrons are donated to the ETC by molecules termed 'reducing equivalents', namely nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), and it is the breakdown of the food consumed and fuels stored onboard that are the ultimate source of these electrons.

In vertebrates, the major fuels for cell metabolism are lipids, carbohydrates, and proteins. Major sources of lipid for ATP synthesis are the fatty acids in plasma known as

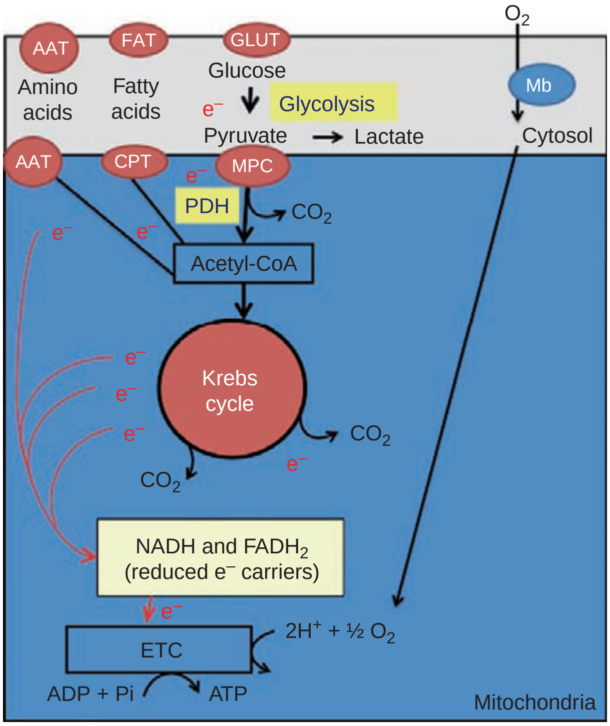


Figure 2 The pathway for oxygen from capillary to mitochondria and the major ATP-supply pathways from amino acids, glucose, and fatty acids. The three major fuels enter cells through specific transporters and enter the cytosol. Entry into the mitochondria for complete oxidation also involves specific transporters. All three fuels enter the Krebs cycle as acetyl-CoA. Electrons are transferred to reducing equivalents $FADH_2$ and NADH at various steps in catabolism. The reducing equivalents donate the electrons to the ETC and ultimately to oxygen as the terminal electron acceptor. AAT, amino acid transporter; FAT, fatty acid translocase; GLUT, glucose transporter; CPT, carnitine palmitoyltransferase; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; and ETC, electron transport chain.

nonesterified fatty acids (NEFAs). Further, lipids can be stored in large amounts in adipose tissue or liver as triacylglycerol (TAG); these reserves can be mobilized by the process of lipolysis and then released into the circulation as NEFA. Most cells have their own TAG reserves but at much lower concentrations than the specialized storage organs. However, these intracellular stores represent a readily available source of fuel for ATP production.

Carbohydrates are stored mainly in liver as glycogen, consisting of monomers of glycosyl units. Once broken down by the glycolytic pathway, glucose is released into the circulation for oxidation by other tissues. As is the case for lipids, most cells also have their own store of glycogen that is readily available for use but exists in much smaller concentrations in extraheptatic tissues. Glucose can also be produced in some tissues from specific precursors (e.g., alanine, glycerol, and lactate) via the process of gluconeogenesis. In most vertebrates, the liver and, to a lesser extent, the kidneys are the major gluconeogenic tissues.

Protein turnover rates are higher in fish than mammals but vary depending on many factors, including growth or feeding status. Since we focus here on fuel catabolism for ATP production, we have to consider the potential of amino acids to provide energy for cellular metabolism. When proteins are broken down by the ubiquitin–proteasome pathway in mammals and by lysosomes in fish, amino acids are liberated and may serve as a fuel source. In mammals, amino acids are not a major source of fuel for metabolism; they only contribute to a small percentage of total ATP production at rest and even less so during exercise. In contrast, protein metabolism may play a more important role in fish, as discussed later.

Fuel oxidation can be determined by measuring the rates of cellular $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to determine respiratory quotients (RQs) for tissues or respiratory exchange ratio (RER) for whole-animal respiration. Defined as the ratio of $\dot{V}_{CO_2}/\dot{V}_{O_2}$, these ratios are unique to the three major metabolic fuels due to their stoichiometries of oxidation, at 1.0 for carbohydrates, 0.71 for lipids, and 0.81 for proteins (**Table 2**).

Fuel and O_2 Delivery to Tissues

Fuel and oxygen delivery share a convective transport step in the circulation, and the rate of delivery of both depends on cardiac output and their respective concentrations in the blood and/or plasma (eqns (1) and (2)):

$$T_{O_2} = \dot{Q} \times C_{aO_2} \qquad (1)$$

$$\text{Fuel delivery} = \dot{Q} \times (1 - H_{ct}) \times [\text{Fuel}] \qquad (2)$$

where T_{O_2} is the oxygen transport rate, C_{aO_2} is the oxygen concentration of arterial blood, $\dot{Q}$ is the cardiac output, H_{ct} is hematocrit fuels refers to carbohydrates, lipids, and amino acids.

They differ in a number of ways:

1. Oxygen is mainly carried bound to hemoglobin and fuels are principally circulating dissolved in the plasma, although some, notably hydrophobic NEFAs, must be bound to a carrier protein. Indeed, NEFA circulatory transport rates depend on the plasma concentration of the carrier protein albumin, the number of binding sites available for NEFA, and the plasma flow rate ($\dot{Q}_{\text{plasma}}$).
2. Unlike metabolic fuels, oxygen cannot be stored to any great extent in the body. For this last reason it has been speculated that the circulatory system has evolved to favor oxygen over fuel delivery.
3. Probably more importantly is that oxygen enters the cell by simple diffusion and the net flux across cell membranes depends on surface area for transport, permeability coefficient of oxygen in the membrane, and is inversely related to the thickness of the diffusion barrier. Membrane transport of fuels, on the other hand, depends on transmembrane proteins (transporters). The surface area for transport thus depends on the density of each fuel-specific transporter.

The rate of tissue oxygen consumption ($\dot{V}_{O_2}$) can be determined empirically by using the Fick equation (eqn (3); **see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs):

$$\dot{V}_{O_2} = \dot{Q} \times (C_{aO_2} - C_{VO_2}) \qquad (3)$$

Table 2 Stoichiometries of oxygen and carbon dioxide used to infer fuel use via gas-exchange measurements in air or water

Fuel	*CO_2 (mol)*	*O_2 (mol)*	*Respiratory exchange ratio (RER)*
Glucose ($C_6H_{12}O_6$)	6	6	1
Palmitoyl-stearoyl-oleaoyl-glycerol ($C_{55}H_{104}O_6$)[a]	55	78	0.71
Protein[b]	4.1	5.1	0.81

[a] Representative triacylglycerol.

[b] Average amino acid.

RER = $\dot{V}_{CO_2}/\dot{V}_{O_2}$.

where $\dot{V}_{O_2}$ is the rate of oxygen consumption, $\dot{Q}$ is the cardiac output, and $C_{aO_2} - C_{VO_2}$ is the arterial-venous difference in oxygen concentration.

This equation describes how tissue $\dot{V}_{O_2}$ depends directly on cardiac output and the arterial to venous difference in oxygen content. It is assumed that all of the oxygen that has entered the cells is consumed in OXPHOS or other minor oxygen-consuming reactions (e.g., P450 enzymes). In contrast, although a modified version of the Fick equation (eqn (4)) can be used to determine cellular uptake of fuels, generally not all of the fuels taken up by a tissue become directly oxidized:

$$\text{Fuel uptake} = \dot{Q}_{\text{plasma}} \times (a - v)\text{fuel} \qquad (4)$$

where fuels entering cells is the product of plasma flow ($\dot{Q}_{\text{plasma}}$) and the arterial-venous difference for carbohydrates, lipids, or amino acids.

For instance, in mammals, only approximately 50% of the NEFA taken up by muscle will eventually be oxidized in that tissue. This highlights the many different fates for a fuel once it enters a cell. In addition to being broken down and transferring electrons to reducing equivalents for electron donation to the ETC, NEFA can be re-esterified and stored as TAG, incorporated into membranes, and can act as signaling molecules or as ligands for nuclear receptors (e.g., the peroxisomal proliferator-activated receptors, PPARs). In contrast, most of the glucose that enters muscle cells during exercise ultimately is oxidized. At rest or in other cell types, glucose entering a cell can undergo gluconeogenesis and be incorporated into glycogen for storage. The fate of fuels in a cell often depends on the ability of cells to either oxidize the fuel or incorporate it into cellular stores, which depends on whether that cell has: (1) a high density of mitochondria, (2) enzymes in specific metabolic pathways, and (3) high membrane-transporter density for metabolites and other metabolic intermediates. A good example is the slow-oxidative skeletal muscles in both mammals and fish, which have a low capacity for gluconeogenesis but a high capacity for glucose and NEFA oxidation.

Cell Membrane Transport

Oxygen uptake into cells occurs by simple diffusion across a diffusion barrier consisting of the capillary endothelium and the target cell membrane. The rate of net flux is directly related to the total surface area for transport, the partial pressure gradient for oxygen to enter the cell, and is inversely proportional to the thickness of the diffusion barrier. Fuels rely on facilitated diffusion for uptake involving fuel-specific transporter systems, both at the cellular and at the mitochondrial membranes (**Figure 2**). However, for both oxygen and fuels the surface area for transport can vary, in part, due to the amount of capillarization a tissue receives (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis).

Many fuel transport systems have been identified in both mammals and fish. NEFA transport at the cell membrane occurs via three possible transporters, fatty acid translocase (CD36/FAT), fatty-acid-binding protein plasma membrane (FABPpm), and fatty acid transport protein (FATP). Current evidence in mammals supports CD36/FAT as the major transport pathway for NEFA. Diffusion gradients for NEFA to enter the cell are maintained by the conversion of NEFA to fatty acyl-coenzyme A (CoA) by acyl-CoA synthase (ACS) or, possibly, by the membrane-bound FATP.

Glucose enters cells through a family of transporters called 'glucose transporters' (GLUTs), of which there are several members in mammals and many homologs in fish. In mammals, the GLUT transporters have tissue-specific distributions, for example, with GLUT 2 occurring in liver, GLUTs 1 and 4 in muscle, and GLUT 1 in red blood cells. Plasma glucose levels are under tight hormonal control in mammals, with release and uptake being continuously adjusted. Fish, on the other hand, are considered to be glucose intolerant because they have high circulating glucose even when insulin levels (the major glucose homeostasis hormone in mammals) are high. Glucose uptake into cells depends on the maintenance of an inward diffusion gradient. This is controlled by the enzyme hexokinase, which phosphorylates glucose as it enters the cell and keeps intracellular glucose concentrations low.

A host of other membrane transporters exist to facilitate the transport of other metabolites. The metabolic intermediates (lactate, pyruvate, and ketone bodies) are transported by a family of membrane transporters called the 'monocarboxylate transporters' (MCTs), of which there are now 12 members known in mammals. In fish, at least nine MCTs have been identified and found to facilitate transport of both lactate and pyruvate, although it has been speculated that there may be functional divergence between some fish and mammal MCTs. To vary membrane transport capacity, rapid recruitment of transport proteins from the intracellular stores to the plasma membrane can adjust transporter densities. This has been demonstrated in mammals for both CD36/FAT and GLUT 4. In addition, gene expression can be induced for many of these transporters in response to chronic energetic or environmental stress.

Cytosolic Transport

Since diffusion over long distances is often too slow to support biological processes or because some molecules are poorly soluble in aqueous solutions, carrier proteins exist in the intracellular fluid. Oxygen is poorly soluble in water and is typically associated with various storage/carrier proteins. Myoglobin (Mb) is a monomeric oxygen-binding protein that occurs in heart and muscle cells. Each Mb binds a single oxygen molecule and the amount of Mb correlates with the oxidative potential of the tissue. Mb does not demonstrate cooperative binding like hemoglobin (Hb) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin) but its affinity for O_2 is much higher ($P_{50} \sim 3$ torr), which facilitates offloading of O_2 from the blood to the cells. Mb can also increase the rate of diffusion in the cytosol by several-fold. In muscle, Mb is never 100% deoxygenated even with maximal oxygen consumption. For this reason, Mb is considered an O_2 buffer for intracellular $[O_2]$, which remains homeostatic. Mb binds oxygen in the areas of high P_{O_2} and releases it in the areas of low P_{O_2}, thus aiding diffusion, in addition to the diffusion gradient (although thought to be shallow) from the cell membrane to cytochrome oxidase that aids intracellular O_2 transport. Moreover, it has been hypothesized that intracellular lipids increase O_2 diffusion rate because O_2 has a greater solubility in fat. Interestingly, an additional hypothesis proposes that an intracellular perfusion system is responsible for cytosolic transport for both oxygen and fuels.

Hydrophobic NEFAs are converted to acyl-CoAs in the cytosol and rely on cytosolic fatty acid binding proteins (FABPs) for transport to the mitochondria for breakdown by β-oxidation. Homologous FABPs have been identified in mammals, birds, and fishes. FABP increases NEFA solubility in the cytosol by ~700-fold and FABP is abundant in highly aerobic tissues. Interestingly, the whole NEFA–FABP complex moves within the cell rather than the NEFA moving from one FABP molecule to another, and this association is thought to increase the rate of diffusion through the cytosol by ~17-fold. FABPs also tend to aggregate close to mitochondria, with the majority bound to the cytosolic side of the outer mitochondrial membrane. Similarly, newly formed FA-CoAs are bound to an acyl-CoA-binding protein (ACBP) for transport to the mitochondrial membrane. When glucose crosses the cell membrane, it is rapidly converted to glucose-6-phosphate by hexokinase and enters glycolysis. The rates of glucose diffusion in the cytoplasm are adequate to support glucose oxidation, and the enzymes of glycolysis are often strategically located so that the pyruvate generated can quickly enter the mitochondria. As the oxidation of lipids, carbohydrates, and amino acids occurs in the mitochondrial matrix, these fuels must be transported across the mitochondrial membranes.

Mitochondrial Transport

Oxygen enters the mitochondria by simple diffusion and relies on the partial pressure gradient between the cytosol and cytochrome oxidase. Matching fuel transport into mitochondria relies on specific mitochondrial membrane transporters (**Figure 2**). Pyruvate generated by glycolysis in the cytosol crosses the mitochondria membrane via a mitochondrial pyruvate carrier (MPC) and is further converted to acetyl-CoA by pyruvate dehydrogenase complex (PDH). Entry of lipids into the mitochondria begins with the conversion of FA-CoA into a fatty-acylcarnitine by carnitine palmitoyltransferase (CPT)I on the inner side of the outer mitochondrial membrane. This is generally considered a major controlling step in mitochondrial fatty acid oxidation. Fatty-acylcarnitine is transferred into the mitochondrial matrix by carnitine acyltransferase (CAT) and converted back to FA-CoA by CPTII on the inner surface of the inner mitochondrial membrane. FA-CoA enters β-oxidation generating acetyl-CoA. Acetyl-CoA produced from glucose, NEFA, and amino acid catabolism enters the Krebs cycle.

Along with oxygen and fuels, the reducing equivalents NADH and $FADH_2$ generated in the cytosol need to be transferred into the mitochondria. Because the cell membranes are impermeable to these molecules, a number of redox shuttles exist for their membrane transfer. These include the malate–aspartate shuttle and the glycerol-3 phosphate shuttle. Recent evidence suggests that a lactate–pyruvate shuttle may exist in peroxisomes, but its role in mitochondrial redox shuttling has not been firmly established.

Cellular Respiration in Fish

Most, but not all, of the aspects of cellular respiration outlined above have been examined in fish. Probably the biggest difference between mammals and fish are the fuels used to power muscle during exercise and their plasticity in response to environmental and energetic stress.

Fuel usage during exercise is fairly well described in mammals. Low-intensity aerobic exercise is primarily fueled by lipids, and as intensity increases carbohydrates become a more important component of total metabolism. Some evidence points toward a roughly similar pattern occurring in fish, but with a higher contribution of protein metabolism to power swimming. In fact, measurements of NEFA turnover and glucose uptake suggest that these fuels do not meet all of the muscle energy requirements.

Protein (amino acids) and lipoproteins are two fuel sources that appear to play a more important role for energy production in fish than in mammals. Lipoproteins represent a very high proportion of energy in circulating lipids, and migrating salmon show a dramatic decrease (~27-fold) in circulation of lipoproteins over the course of their annual migration. Furthermore, in fish, a large part of resting metabolism is covered by protein catabolism (10–20%). The importance of proteins as a metabolic substrate also markedly increases in fish during extended periods of starvation or during migration. Migrating salmon lose about half of their white muscle mass over the course of a 1000+ km migration (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). This represents a release of approximately 1200 g of protein that are broken down to amino acids, principally by lysosomes. These amino acids play several roles for the migrating salmon: they provide intermediates for the Krebs cycle to ensure continued OXPHOS, glucogeogenic precursors (alanine) to form glucose used during spawning, and finally amino acids that can be used as oxidative fuels. Like mammals, fish red muscle expresses the enzyme branched-chain oxoacid dehydrogenase for the oxidation of branched-chain amino acids (leucine, isoleucine, and valine). In fact, levels of this enzyme are higher in fish red muscle than in mammalian liver, suggesting the increased importance of amino acids to muscle metabolism in fish. In addition, excretion of nitrogenous wastes from protein breakdown is less energetically expensive in fish than in mammals (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia).

Unlike mammals, most fish do not have a great ability to oxidize ketone bodies. In this respect, one notable exceptional group is the elasmobranchs (sharks and rays), as they rely on ketone bodies to power muscle contraction. This is probably a compensatory adaptation of this group for the inability to transport NEFA in the circulation and to oxidize NEFA to any great extent in muscle. As a result, a high level of lipid oxidation in the livers of elasmobranchs ensures an adequate supply of ketones for extrahepatic tissues.

Environmental and Energetic Stress

Acute changes in oxygen and fuel delivery occur in response to changes in environmental temperature and oxygen and also with changes in energetic demand with the transition from rest to exercise. Since both oxygen and fuels share a convective transport step, increased cardiac output increases oxygen and fuel delivery to tissues to support an increase in metabolic rate during exercise or to enhance oxygen delivery during hypoxia. In fish, metabolic rate will increase by a factor of 2 or 3 with a 10 °C increase in temperature ($\dot{Q}$ of 2–3; **see also Temperature**: Effects of Temperature: An Introduction). Acutely, this is met with corresponding changes in oxygen and fuel delivery as kinetic processes respond directly to temperature. For example, an acute drop in temperature reduces resting heart rate and cardiac output in fish and consequently decreases fuel delivery to working tissues. Cold also increases the viscosity of intracellular fluids and decreases the diffusion rate of metabolites from the cell membrane to mitochondria. In addition, lower temperature can increase Mb affinity for O_2 and slow down dissociation and rate of diffusion. Interestingly, fish have Mb that rapidly binds and releases O_2 at greater rates than mammals and this aids in cold adaptation. Acute hypoxia and low environmental temperature cause distinct changes in plasma concentrations and turnover of NEFA and glucose in rainbow trout (*Oncorhynchus mykiss*). NEFA turnover declines with both hypoxia and temperature, while glucose appearance increases only in hypoxia. More importantly, in trout, turnover and plasma concentration are not correlated, which highlights the pitfalls of using concentration measurements to predict fuel kinetics (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation).

Chronic exposure to low temperature in fish and other ectothermic animals demonstrates distinct compensatory responses (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature). This is especially dramatic in eurythermal fish that undergo seasonal changes in temperature. These fish will increase the aerobic capacity of cells by increasing mitochondrial density. This compensation for the decelerating effect of cold temperature is thought to aid in oxygen delivery by reducing the diffusion distance from capillary to mitochondria. Chronic cold-temperature exposure also causes an increase in tissue capillarity (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis) and cardiac hypertrophy, both of which aid in enhancing oxygen delivery. Fish muscles also respond to energetic stress brought about by repeated contractions (i.e., swim training). This also increases the aerobic capacity of the muscle to deal with higher energetic demands. Interestingly, distinct molecular mechanisms lead to cold- and contraction-induced remodeling in fish, and these regulatory pathways are divergent from the typical mammalian response to exercise training. There is a possibility that these differences in the regulation of fuel use in fish are due to their evolutionary history. As a group, fish experienced several large-scale genome duplication events that resulted in the presence of multiple forms of important regulatory proteins for which mammals only possess a single version (**see also Tissue Respiration**: Mitochondrial Respiration).

Limits to Cellular Respiration

The upper limit to cellular respiration is set by the upper limits to oxygen and fuel delivery, and the oxidative capacity of the cell. The upper limit to oxygen delivery is set by the oxygen transport system, namely the upper limit to gill diffusion capacity, blood oxygen-carrying capacity, and cardiac output. The upper limit to fuel delivery is a bit more complex since fuels are stored intracellularly, essentially right next to the mitochondria. Therefore, it is the rate of reducing equivalent production and capacity of the ETC for electron flux that limit the fuel side of the equation. Interestingly, some animals seem to have reached an upper limit to density of ETC components. For example, hummingbirds and bees have extremely high densities of inner mitochondria membrane that are packed with ETC proteins. However, because of their relatively low metabolic rate compared to an endotherm of the same size, fish do not ever reach this level of cristae density. Looking across body size in fish or at so-called high-performance fish such as tuna, gives us an idea of how increased energy demand is met by energy supply pathways. Tuna, billfish, and other predatory fish have very high metabolic rates that can approach those of similar-sized endotherms. These fish have oxygen and fuel supply systems that are upregulated to support these high energetic demands. For example, tuna have glucose turnover rates that are similar to those of similar-sized mammals. High turnover rates are partially the result of mammalian-like cardiac outputs (see **Design and Physiology of the Heart**: Physiology of Cardiac Pumping) in these high-performance fish species (**see also Pelagic Fishes**: Physiology of Tuna).

Novel Species

There are two very interesting groups of fish that can be thought of as models to investigate alternative oxygen or fuel delivery systems. The first has already been discussed and is the altered lipid metabolism of elasmobranchs. The second is the altered oxygen delivery system in the channichthyid icefish. Both of these examples can be thought of as natural knockout models with elasmobranchs lacking a key β-oxidation enzyme and icefish lacking oxygen transport proteins. The elasmobranchs circumvent their inability to oxidize NEFA in muscle by upregulating ketone body metabolism. The species of icefish lacking Hb and/or Mb compensate by decreasing diffusion distances through increased tissue vascularization, mitochondria densities, and lipid content. Moreover, icefish must maintain large blood volumes and heart masses to achieve much higher cardiac outputs than other similar-sized fish (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). Many, but not all, of these adaptations are thought to be the result of high nitric oxide (NO) levels. High levels result from the lack of Hb and Mb, proteins that would normally break down NO – a molecule involved in angiogenesis and mitochondrial biogenesis (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature).

Conclusions

The adequate transport of oxygen and fuels to working cells is essential for the homeostatic regulation of intracellular ATP concentrations. Both the oxygen- and fuel-delivery pathways share a convective transport step in the circulation. Thus, cardiac output and tissue capillarization play important roles in their supply to tissues. While the pathway for oxygen is roughly linear from environment to mitochondria, the pathway for fuels can be considered branched. The increased complexity of fuel-delivery systems stems from their multiple storage sites both intra- and extracellular. Besides, fuels must rely on specialized transport proteins to cross membranes and then specific metabolic pathways for their catabolism. Special adaptations may exist in fish to maintain adequate oxygen- and fuel-delivery rates. This can take the form of unique physiologies in species that show distinct rearrangements of specific fuel pathways or in the oxygen cascade.

See also: **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle. **Gas Exchange**: Respiration: An Introduction. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature. **Membranes and Temperature**: Homeoviscous Adaptation. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Pelagic Fishes**: Physiology of Tuna. **Temperature**: Effects of Temperature: An Introduction. **Tissue Respiration**: Mitochondrial Respiration; Specializations in Mitochondrial Respiration of Fish. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs.

Further Reading

Hochachka PW (1999) The metabolic implications of intracellular circulation. *Proceedings of the National Academy of Sciences of the United States of America* 96: 12233–12239.

McClelland GB (2004) Fat to the fire: The regulation of lipid oxidation with exercise and environmental stress. *Comparative Biochemistry and Physiology – Part B* 139: 443–460.

McClelland GB, Craig PM, Dhekney K, and Dipardo S (2006) Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *Journal of Physiology, London* 577: 739–751.
Mommsen TP (2004) Salmon spawning migration and muscle protein metabolism: The August Krogh principle at work. *Comparative Biochemistry and Physiology – Part B* 139: 383–400.
Moon TW (2001) Glucose intolerance in teleost fish: Fact or fiction? *Comparative Biochemistry and Physiology – Part B* 129: 243–249.
Moyes CD and West TG (1995) Exercise metabolism of fish. In: Hochachka PW and Mommsen T (eds.) *Biochemistry and Molecular Biology of Fishes,* vol. 4, pp. 367–392. Amsterdam: Elsevier.
Oikawa S and Itazawa Y (2003) Relationship between summated tissue respiration and body size in a marine teleost, the porgy *Pagrus major*. *Fisheries Science* 69: 687–694.
Sidell BD and Hazel JR (1987) Temperature affects the diffusion of small molecules through cytosol of fish muscle. *Journal of Experimental Biology* 129: 191–203.
Sidell BD and O'Brian KM (2006) When bad things happen to good fish: The loss of hemoglobin and myoglobin expression in Antarctic icefishes. *Journal of Experimental Biology* 209: 1791–1802.

Relevant Websites

http://marinelife.about.com – About.com: Marine Life; Elasmobranch.
http://www.earthlife.net – Earthlife Web; Fish.
http://www.fbs.leeds.ac.uk – University of Leeds; School of Biochemistry and Molecular Biology: Oxidative Phosphorylation Home Page.

Mitochondrial Respiration

CD Moyes, Queen's University, Kingston, ON, Canada

CMR Le Moine, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Importance of the Endosymbiotic Origin of Mitochondria
Mitochondrial Structure
Basic Features of OXPHOS
Conclusions
Further Reading

Glossary

Complex I The first enzyme of the electron transport system (ETS). It oxidizes reduced nicotinamide adenine dinucleotide (NADH) and transfers electrons to ubiquinone. It is also known as NADH dehydrogenase.

Complex II The first enzyme of the ETS. It oxidizes reduced flavin adeninine dinucleotide ($FADH_2$) and transfers electrons to ubiquinone. It does not pump protons. It is also part of the tricarboxylic acid cycle and, when considered in this role, is called succinate dehydrogenase.

Complex III A proton-pumping complex that accepts electrons from ubiquinol (reduced ubiquinone) and transfers them to cytochrome *c*.

Complex IV The final enzyme of the ETS. It accepts electrons from cytochrome *c* and transfers them to oxygen, forming water. Like complexes I and III, this is a proton pumping complex. It is also known as cytochrome *c* oxidase, or COX.

Complex V The multiprotein mechanoenzyme that uses the energy of the proton motive force to generate ATP within the mitochondria. It is also known as the F_1F_o ATPase.

Creatine phosphate An energy store alternative to adenosine triphosphate (ATP). It is also known as phosphocreatine.

Electron transport system (ETS) A series of four multiprotein complexes (I, II, III, IV) and two mobile electron carriers (ubiquinone, cytochrome *c*) that transfer electrons from reducing equivalents to molecular oxygen to form water. It was previously called the electron transport chain, or ETC.

Mitochondria Cellular organelles that act as the powerhouse of the cell and produce the chemical energy source adenosine triphosphate, using oxygen as the final electron acceptor.

mtDNA The mitochondrial genome composed of circular DNA encoding critical subunits of OXPHOS and the tRNA and rRNA required for translation within the organelle.

Oxidative phosphorylation (OXPHOS) The coupling of oxidation in the ETS and phosphorylation by the F_1F_o ATPase.

Proton motive force (PMF) The electrochemical gradient across the inner mitochondrial membrane, produced by the ETS and used by the F_1F_o ATPase.

Reducing equivalents Molecules derived from vitamins that transfer electrons in redox reactions, mainly NADH, $FADH_2$ and reduced flavin mononucleotide ($FMNH_2$).

Introduction

Mitochondria are the main consumers of oxygen within the body and thus mitochondrial energy metabolism is the primary reason why fish breathe. Most of the impressive respiratory and circulatory adaptations described elsewhere in this encyclopedia are in place primarily to deliver oxygen to mitochondria. Consequently, understanding how mitochondria respire is at the core of most aspects of fish physiology.

Mitochondria are membranous organelles found within most eukaryotic cells. They have important roles in biosynthesis, but we focus here on their role in oxygen-dependent energy production. Animals consume complex food and break it down to macromolecules (carbohydrates, lipids, proteins, and nucleic acids). Multicellular organisms, including fish, further metabolize some of these dietary products into forms that can be imported by cells for energy production. Carbohydrates are converted to monosaccharides

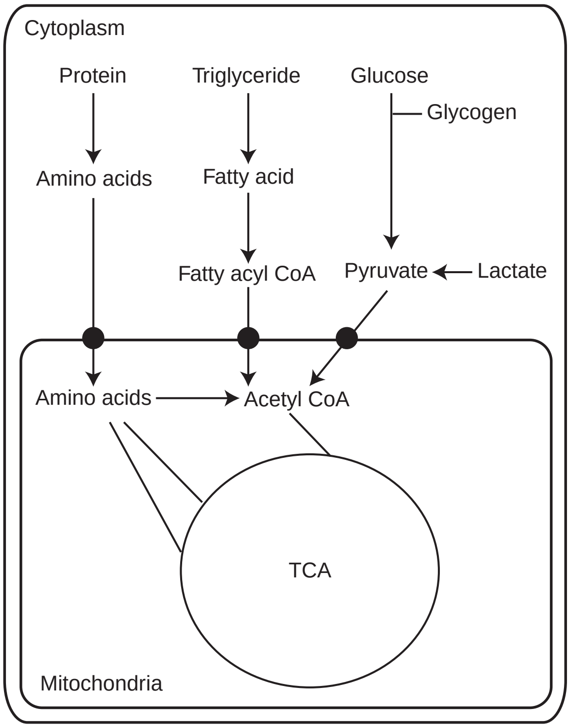


Figure 1 Production of mitochondrial substrates by cells.

(glucose), lipids are converted into fatty acids, and proteins are broken down to amino acids; nucleic acids have a minor role in energy production. These molecules – glucose, fatty acids, and amino acids – can then be metabolized by the cell to produce the substrates for mitochondria (**Figure 1**). Mitochondria metabolize their substrates using the enzymes of the tricarboxylic acid (TCA) cycle, producing reducing equivalents (nicotinamide adenine dinucleotide (NADH), flavin adeninine dinucleotide ($FADH_2$)). These in turn are oxidized by the electron transport system (ETS), which consumes oxygen while producing a proton gradient across the mitochondrial membrane. This electrochemical gradient can be utilized by the F_1F_o ATPase to produce adenosine triphosphate (ATP). The combination of electron transfer reactions (oxidation) and ATP synthesis (phosphorylation) is oxidative phosphorylation, or OXPHOS. This pathway is completely dependent on oxygen. Thus, the main reason why animals breathe is to get oxygen to mitochondria for efficient ATP synthesis. Without oxygen, cells must rely on preformed energy stores (ATP, creatine phosphate) and the fast, but inefficient, glycolytic pathway.

Many general aspects of mitochondrial structure and function are shared among all eukaryotic organisms. The basic structure of mitochondria, the nature of OXPHOS, the complement of enzymes, and the genetics, organization, and regulation are all fairly conserved among animals and thus much of what follows in this article can apply equally well to any species. However, evolution has led to surprising diversity in the specific features of mitochondria and these have been particularly well studied in fish in relation to evolution and development. The specializations of fish are discussed in **Tissue Respiration**: Specializations in Mitochondrial Respiration of Fish.

Importance of the Endosymbiotic Origin of Mitochondria

More than 1 billion years ago, life on the Earth was limited to prokaryotes, probably similar to the eubacteria and archaebacteria alive today. These living prokaryotes possess a remarkable array of metabolic strategies that allow them to survive even in the harshest environments on the planet. One of the earliest events in the evolution of more complex life was the invention of a membrane surrounding and protecting the genetic material (DNA), giving rise to the first eukaryote. This organism was probably unable to use oxygen, but entered into a functional relationship with another ancient organism, something resembling a modern alpha-proteobacterium. According to the endosymbiosis theory, the host cell engulfed the bacterium, and instead of digesting it as food, it negotiated a functional relationship that enabled the host cell/endosymbiont to utilize oxygen in energy metabolism. Over generations, the relationship changed with the endosymbiont/mitochondrion surrendering control over its own regulation to the host, even losing most of its genetic machinery to the host nucleus.

This ancient origin of mitochondria explains the conservation of many mitochondrial properties among diverse taxa. The basic features of mitochondria are similar among animals, plants, and fungi because mitochondria appeared in an ancestor shared more than a billion years ago. Indeed, many characteristics of mitochondria, such as genetic regulation and protein structure, remain similar to those of the modern purple bacteria. When talking about mitochondria function in an encyclopedia about fish, it is helpful to understand that many properties are highly conserved among all vertebrates, all animals, and indeed, all eukaryotes. Thus, the description of mitochondria that follows could apply equally to any animal because of the way mitochondria are built. Despite this general similarity, there are unique features of mitochondrial structure and function that have evolved, accounting for important differences between species and tissues.

Mitochondrial Structure

Most traditional depictions of mitochondria show the organelle to exist as ovals, about the size of bacteria, suggesting that mitochondria exist within a cell as multiple discrete entities. However, as revealed by three-dimensional reconstructions and whole cell imaging, it is now clear that mitochondria in many tissues exist as a connected membranous network, or reticulum. It is thought that a reticulum allows mitochondria to act like a giant electrical cable, allow a membrane potential to be transmitted throughout the entire network.

If one were to look inside a living cell, one would see the mitochondria moving throughout the cell. They are carried along cytoskeletal tracks (actin and microtubules) by motor proteins (myosin, kinesin, and dynein). In neurons, mitochondria travel along the axon, moving between the cell body and the axon terminus. In other cells, the mitochondria are taken to semi-permanent locations and mounted on the cytoskeletal framework. In muscle, separate populations of mitochondria exist beneath the cell membrane (subsarcolemmal) and others woven between bundles of myofibrils (interfibrillar).

Whether mitochondria exist as discrete organelles or extensive networks is determined by suites of GTPases and other proteins. Dynamin-like proteins pinch off pieces of the network, whereas mitofusins extend the network by recruiting individual mitochondria into the reticulum. This remodeling process is generally thought to provide the cell with a capacity to identify defective mitochondrial regions and send them to autophagosomes for degradation. It may also be important when tissues need to reduce mitochondrial content as a means of regulating bioenergetics.

The main structural features of mitochondria are seen in cross section. Surrounding the mitochondria is the outer mitochondrial membrane. Inside this membrane is the tightly folded inner mitochondrial membrane. Where the inner membrane is arranged into overlapping leafs, the layers are called cristae. The intermembrane space is between the inner and outer membranes, and the matrix is the space inside the inner mitochondrial membrane. Each of these four compartments has a characteristic complement of proteins, including transporters and enzymes (**Figure 2**).

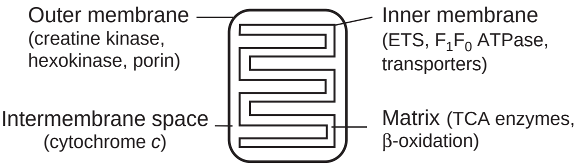


Figure 2 Mitochondrial compartments.

The outer mitochondrial membrane delimits the mitochondria. It is similar in composition to the cell's internal membrane system (cell membrane/endoplasmic reticulum/Golgi apparatus). As with most membranes, it has a relatively low permeability to small molecules. However, inserted into the outer membrane are large channels called porins. These channels permit the free movement of molecules smaller than about 10 000 Da into and out of the intermembrane space according to their chemical gradients. This means that metabolites, such as ATP, pyruvate and fatty acids, as well as ions, can cross the outer membrane but large proteins, such as cytoplasmic enzymes and cytochrome *c*, cannot. Due to this permeability, the intermembrane space is similar in solute composition to cytoplasm but distinct in terms of protein profile. The outer membrane also possesses some intrinsic proteins embedded within the lipid bilayer and peripheral proteins reversibly associated with the membrane.

The inner mitochondrial membrane is unusual in many respects. The membrane is unusually rich in proteins; as much as 80% of the membrane mass is protein, with the remainder lipid. In addition, the profile of phospholipids that make up its bilayer is very distinct from the outer membrane. Some classes of phospholids are unusually abundant, but there is also a phospholipid (cardiolipin) that occurs in this inner membrane and nowhere else in the cell. Cardiolipin has an important role in regulating many of the membrane-bound proteins of the inner membrane. Furthermore, the folded arrangement of the inner membrane into many layers (cristae) greatly increases the surface area available to insert complexes of OXPHOS. In most tissues, 1 cm^3 of mitochondria contains about 20–40 m^2 of cristae surface area.

The matrix, the innermost compartment of mitochondria, is a fluid in which the various metabolites associated with the TCA and OXPHOS are dissolved. The composition of mitochondrial matrix is very different from that of the cytoplasm and intermembrane space. It is very rich in proteins, including the enzymes of the TCA cycle. In fact, proteins are so abundant that most of the water within the matrix is bound to the proteins to form their hydration shells. Without much free water within the matrix, it has been suggested that many of the reactions within the TCA cycle involve substrate channeling from one enzyme to the next. The matrix also contains the numerous circles of mitochondrial DNA (mtDNA). They are collected together in bunches that are associated with specific integral membrane proteins of the inner membrane.

Mitochondrial Biogenesis

Over the course of eukaryote evolution, there was a transfer of power between the organelle and the host cell. In animals, most of the proteins needed by

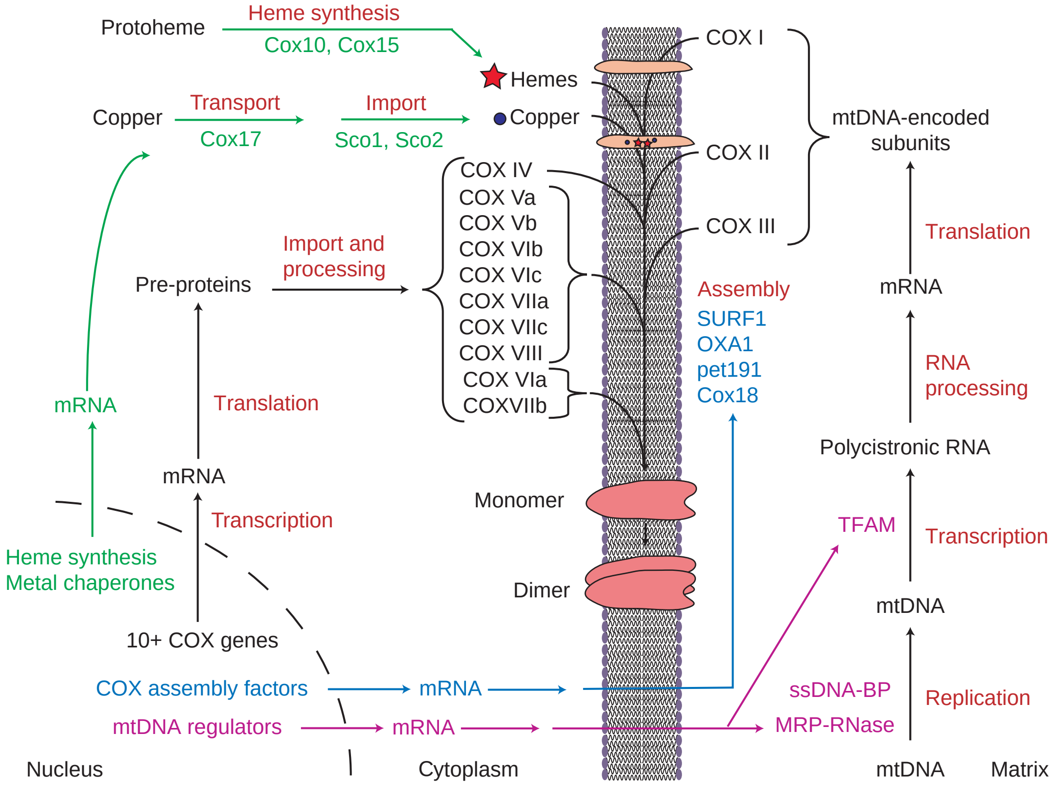


Figure 3 COX synthesis. Diagram includes COX subunit genes (black) encoded by nucleus (IV–VIII) and mtDNA (I, II, III), as well as proteins involved in metal and heme transport (green), transcriptional regulators (pink), and assembly factors (blue). Modified from Moyes CD and Hood DL (2003) Origins and consequences of mitochondrial variation. *Annual Reviews of Physiology* 65: 177–201.

mitochondria are encoded by nuclear genes. However, there is a core group of 13 proteins that are encoded by mtDNA. In addition to the protein-coding genes, mtDNA encodes the ribosomal and transfer RNA necessary for translation within the organelle. The organization of the mitochondrial genome is more similar to that of bacteria than a eukaryote nucleus. This bigenomic location presents a formidable problem for cells that want to alter their mitochondrial quantitative or qualitative properties. For example, consider the complexity of producing a single enzyme of the ETS (**Figure 3**). Cytochrome *c* oxidase (COX) is a 13-subunit metalloprotein that is produced from three mtDNA-encoded genes and 10 nuclear-encoded genes. When the cell needs to change the content of COX, it must produce the individual subunits in the correct proportions. For many of the nuclear-encoded subunits, there are multiple isoforms; so, in reality, the nucleus must control the expression of almost 20 distinct genes. Once the proteins are made in the cytoplasm, they need to be imported into the mitochondria, along with the metals and heme needed to produce the metalloprotein. While these events are happening outside the mitochondria, inside the mitochondria mtDNA-encoded genes are being regulated to produce the right amount of the three remaining subunits. Once all of the precursors are in place, the enzyme is assembled in a stepwise manner, a complex process mediated by suites of assembly proteins.

Coordination of the nucleo-cytoplasmic and mitochondrial processes of mitochondrial biogenesis is mediated in large part at the level of transcription. The hundreds of nuclear genes encoding mitochondrial proteins are regulated by a small suite of transcriptional regulators. These proteins bind onto promoters of individual genes and control expression as networks of genes. The most prominent of these transcriptional regulators are nuclear respiratory factor-1 (NRF-1), NRF-2, and their common regulator peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1). The role of these regulatory factors is best studied in mammals, where increases in the levels of one or more of these proteins parallel increases in mitochondrial enzyme content.

Basic Features of OXPHOS

The process by which mitochondria use oxygen and make ATP is a combination of two independent pathways. These pathways, oxidation and phosphorylation, are functionally coupled through shared sensitivity to the electrochemical gradient across the mitochondrial inner membrane.

Oxidation by the ETS

The ETS accepts electrons from reducing equivalents, passes them down a chain, ultimately to oxygen to form water (**Figure 4**). The first step in this process is the oxidation of a reducing equivalent, NADH or $FADH_2$. NADH is produced in a number of mitochondrial reactions, but the most important sources are the pyruvate dehydrogenase complex (PDC) and the three TCA cycle dehydrogenases: isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase, and malate dehydrogenase. The NADH from these reactions is oxidized by complex I, a large complex composed of more than 40 subunits encoded by both the nucleus and mtDNA. Electrons are transferred within the complex and donated to ubiquinone, the first mobile electron carrier of the ETS. Ubiquinone is also an electron acceptor for other dehydrogenases that use flavin nucleotides as internal electron acceptors. Complex II, or succinate dehydrogenase, is a flavin adeninine dinucleotide (FAD)-linked enzyme that donates electrons arising from succinate oxidation. The fatty acid oxidation enzyme fatty acyl CoA dehydrogenase is a flavin mononucleotide (FMN)-linked enzyme that is another source of electrons for ubiquinone. Regardless of the source of electrons for ubiquinone, the reduced ubiquinone (ubiquinol) is oxidized by complex III, which transfers electrons to the second mobile electron carrier, the heme protein cytochrome *c*. Reduced cytochrome *c* transfers its electrons to complex IV (cytochrome *c* oxidase, or COX). Finally, complex IV transfers electrons to oxygen to form water.

This basic pattern of electron transport is similar among all animals and its purpose is to provide energy to pump protons into the intermembrane space from the matrix.

Proton Motive Force

During electron transport through the chain of complexes, some of the energy that is released from oxidation of reducing equivalents is used to pump protons out of the mitochondrial matrix. Complexes I, III, and IV each pump protons; complex II and the other flavin-linked dehydrogenases do not. The resulting proton gradient is a source of energy that can be used for various purposes. There is chemical energy associated with the pH gradient (delta pH) as the proton concentration is higher outside the mitochondria. There is also an electrical energy associated with the membrane potential (delta psi) with the inside of the mitochondria being more electronegative. This electrochemical gradient, known as the proton motive force (PMF), drives many transport processes but perhaps the most important is ATP synthesis.

Phosphorylation

The PMF is the driving force for ATP synthesis by the mechanoenzyme complex V, or F_1F_o ATPase. When protons flow through a channel in the enzyme, the movement spins the protein, much like wind drives a turbine. The mechanical movement of this rotor provides the energy to add an inorganic phosphate group to adenosine diphosphate (ADP) to form ATP. Like most enzymes, but unlike most ATPases, this enzyme can run backward within the cell; ATP hydrolysis can

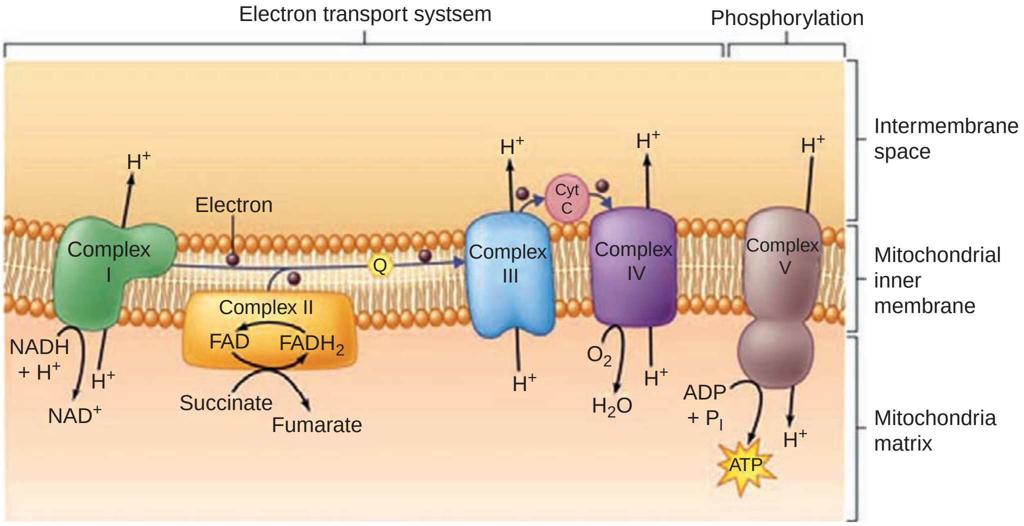


Figure 4 Electron transport and OXPHOS. Reproduced from Moyes CD and Schulte PM (2008) *Principles of Animal Physiology*. San Francisco, CA: Pearson/Benjamin Cummings.

be the driving force to cause the enzyme to eject protons out of the mitochondria. This capacity enables depolarized mitochondria to regenerate their PMF. ATP synthesis by this enzyme is possible only when both substrates (phosphate and ADP) are available and the mitochondria possess sufficient PMF to drive the reaction.

Coupling and Uncoupling

The PMF is produced by the oxidation–reduction cycles of the ETS and it is utilized in the phosphorylation of ADP by the F_1F_o ATPase. These two processes – oxidation and phosphorylation – may involve separate proteins but they share sensitivity to the PMF. One can think of the PMF as a product of the ETS, and when this product is high, it inhibits the ETS, leading to a low rate of oxygen consumption regardless of the availability of the reducing equivalent substrates. When mitochondria synthesize ATP, the PMF is diminished, allowing greater rates of electron transport and oxygen consumption. Thus, oxidation by the ETS is coupled to phosphorylation. This relationship is the main reason why animals consume less oxygen at rest and more oxygen during activity.

In mitochondria that are well coupled, oxygen consumption reflects the rate of ATP synthesis. However, there are instances where respiration occurs independently of ATP synthesis, that is, uncoupled respiration. Uncoupled mitochondria consume oxygen but produce less ATP. If, for example, mitochondria sustain damage such that there is a loss in the integrity of the inner membrane, protons flow freely down their concentration gradient. In such circumstances, a lower PMF permits ETS activity but is insufficient to drive ATP synthesis.

On the surface, uncoupling respiration would seem to have no upside. It is a waste of chemical energy because fuels are consumed but less ATP is produced. However, in some circumstances this can be beneficial because the chemical energy that would have been transferred from reducing equivalents to ATP is instead lost as thermal energy. In other words, uncoupled mitochondria generate heat. In endotherms, such as mammals and birds, the inner mitochondrial membrane is inherently leaky, and their mitochondria are less coupled. For a given amount of ETS activity (oxygen consumption) less ATP is produced than would be in mitochondria from ectotherms, like fish. This membrane leakiness is an important source of heat for endothermy. Mammals are also capable of inducing uncoupling through synthesis and activation of a specific uncoupling protein (UCP) known as thermogenin. This protein would appear to be a mammalian invention, and though all eukaryotes possess their own UCPs, nonmammalian UCP homologs do not generate significant heat. Though several species of fish are able to generate and retain metabolic heat, none of the pathways appear to involve uncoupling of mitochondria. For example, tuna and lamnid sharks generate heat through muscle activity, and billfish heater organs produce heat through futile cycling of Ca^{2+} (**see also Pelagic Fishes**: Physiology of Tuna and Endothermy in Tunas, Billfishes, and Sharks).

Regulation of Mitochondrial Respiration Rate

The regulation of mitochondrial ATP production is complex because so many different substrates and products can influence OXPHOS. In muscle, most of the control of respiration probably resides in the substrates and products of the ATPase. In resting trout white muscle, mitochondria respire at very low rates due to a high ratio of ATP/ADP. Though there is a need for some mitochondrial ATP to support diverse cellular activities, the low respiration rate under these resting conditions results from both basal ATP demands and the effects of mitochondrial proton leak. Both processes dissipate the PMF and permit ETS activity, although only the former results in ATP synthesis. With high-intensity exercise, ATP must be hydrolyzed by the actinomyosin ATPase, increasing both ADP and phosphate levels in muscle. The increases in these substrates stimulate ATP synthesis, which dissipates the PMF and permits the consumption of oxygen by the ETS. In this way, muscle cells can regulate the rates of ATP production in response to ATP demand.

Although regulation of oxygen consumption by the substrates and products of the ATPase is the most common scenario, there are situations where OXPHOS is regulated by other substrates, such as NADH and $FADH_2$. The importance of redox control has been best studied in mammals but it is likely that redox regulation of OXPHOS can play a role in respiratory control of some fish tissues. Under some circumstances, the desired rate of respiration can be suppressed by oxygen limitations within the cell. This has two main effects on mitochondria. First, with a deficiency in this important substrate, ETS activity is reduced and the PMF suffers, reducing the rate of ATP synthesis. Second, the reduction in electron flow through the ETS accelerates the spontaneous production of reactive oxygen species (ROS). In impairing ETS activity, complexes spend more time in the reduced state, and under these conditions, molecular oxygen can steal electrons to form superoxide. This ROS initiates a cascade of free radical production that damages DNA, lipids, and proteins.

Fuel Selection

At the level of the whole animal, the choice of which fuels to burn for energy is controlled at multiple levels and involves hormones, the gastrointestinal tract, energy stores, and enzyme regulation at the storage and target tissues (**see also Tissue Respiration**: Cellular Respiration). Thus, much of the regulation of fuel selection occurs prior to mitochondrial exposure to its carbon substrates. At the level of mitochondria, the main factors that affect fuel selection are fuel availability and regulation of one main enzyme that affects the choice between fatty acids and pyruvate, PDC. Low energy levels are reflected in reductions in NADH/NAD, acetyl CoA, and ATP/ADP. These metabolites and metabolite ratios regulate PDC activity both allosterically and covalently, through effects on PDC kinases (inhibition) and PDC phosphatases (activation).

Conclusions

Many aspects of mitochondrial structure and function are broadly similar among diverse species because of the origins of mitochondria in a common ancestor shared by eukaryotes more than a billion years ago. Mitochondria, in muscle, exist as a dynamic organelle network, with regional specializations that distinguish mitochondria in subsarcolemmal and interfibrillar regions of the myofiber. Regulation of mitochondrial rate in response to physiological energy demands occurs largely through substrates and products of the OXPHOS. Probably, the most important regulatory step in regulation of mitochondrial rate under changing environmental and physiological conditions is the F_1F_o ATPase, particularly through effects of the concentration of ADP. Under some conditions, oxygen limitations impair mitochondrial respiration, compromising ATP synthesis and exacerbating ROS production. When oxygen levels are insufficient to support metabolism, the membrane potential is lost. Thus, animals and tissues use complex pathways (**see also Hypoxia**: Anaerobic Metabolism in Fish, Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia, and The Expanding Hypoxic Environment) to ensure that oxygen uptake at the respiratory epithelium and oxygen delivery by the cardiovascular system are sufficient to permit tissues to use OXPHOS to generate the ATP required for cell survival.

See also: **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: Anaerobic Metabolism in Fish; Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia; The Expanding Hypoxic Environment. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks; Physiology of Tuna. **Tissue Respiration**: Cellular Respiration; Specializations in Mitochondrial Respiration of Fish.

Further Reading

Arthur PG, Keen JE, Hochachka PW, and Farrell AP (1992) Metabolic state of the *in situ* perfused trout heart during severe hypoxia. *American Journal of Physiology* 263: R798–R804.

Chen H and Chan DC (2009) Mitochondrial dynamics – fusion, fission, movement, and mitophagy – in neurodegenerative diseases. *Human Molecular Genetics* 18: R169–R176.

Moyes CD (2003) Controlling muscle mitochondrial content. *Journal of Experimental Biology* 206: 4385–4391.

Moyes CD and Hood DL (2003) Origins and consequences of mitochondrial variation. *Annual Reviews of Physiology* 65: 177–201.

Moyes CD and Schulte PM (2008) *Principles of Animal Physiology*. San Francisco, CA: Pearson/Benjamin Cummings.

Ohno S (1999) Gene duplication and the uniqueness of vertebrate genomes *circa* 1970–1999. *Seminars in Cell and Developmental Biology* 10: 517–522.

Scarpulla RC (2008) Nuclear control of respiratory chain expression by nuclear respiratory factors and PGC-1-related coactivator. *Annals of the New York Academy of Sciences* 1147: 321–334.

Steinke D, Hoegg S, Brinkmann H, and Meyer A (2006) Three rounds (1R/2R/3R) of genome duplications and the evolution of the glycolytic pathway in vertebrates. *BMC Biology* 4: 16.

Suarez RK (1998) Oxygen and the upper limits to animal design and performance. *Journal of Experimental Biology* 201: 1065–1072.

Specializations in Mitochondrial Respiration of Fish

CD Moyes, Queen's University, Kingston, ON, Canada

CMR Le Moine, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Genetic and Genomic Variation
Mitochondrial Structure and Function
Conclusions
Further Reading

Glossary

Coevolution The parallel evolutionary change of structurally or functionally related proteins.

Complex I The first enzyme of the electron transport system (ETS) when oxidizing reduced nicotinamide adenine dinucleotide (NADH). Electrons taken from NADH are transferred to ubiquinone. It is also known as NADH dehydrogenase.

Complex II The first enzyme of the ETS when oxidizing succinate. Electrons taken from succinate are transferred to FAD within the enzyme. The resulting reduced flavin adeninine dinucleotide ($FADH_2$) is oxidized, with the electrons transferred to ubiquinone. It does not pump protons. It is also part of the tricarboxylic acid cycle and, when considered in this role, is called succinate dehydrogenase.

Complex III A proton-pumping complex that accepts electrons from ubiquinol (reduced ubiquinone) and transfers them to cytochrome *c*.

Complex IV The final enzyme of the electron transport system. It accepts electrons from cytochrome *c* and transfers them to oxygen, forming water. Similar to complexes I and III, this is a proton-pumping complex. Also known as cytochrome *c* oxidase, or COX.

Complex V The multiprotein mechanoenzyme that uses the energy of the proton motive force to generate ATP within the mitochondria. It is also known as the F_1F_O ATPase.

Creatine phosphate An energy store alternative to ATP. Also known as phosphocreatine.

Electron transport system A series of four multiprotein complexes (I, II, III, IV) and two mobile electron carriers (ubiquinone and cytochrome *c*) embedded in or associated with the inner mitochondrial membrane that transfer electrons from reducing equivalents to molecular oxygen to form water, and in so doing forms an electrochemical gradient. Previously called the electron transport chain, or ETC.

Mitochondrion A cellular organelle that acts as the 'powerhouse' of the cell and produces the chemical energy source adenosine triphosphate, using oxygen as the final electron acceptor.

mtDNA The mitochondrial genome composed of circular DNA-encoding critical subunits of oxidative phosphorylation and the tRNA and rRNA required for translation within the organelle.

Oxidative phosphorylation (OXPHOS) The coupling of oxidation in the ETS and phosphorylation by the F_1F_O ATPase.

Positive selection The rapid accumulation of mutations in the sequence of a gene that can translate into changes of the structure and function of the resulting protein.

Proton motive force (PMF) The electrochemical gradient across the inner mitochondrial membrane, produced by the ETS and used by the F_1F_O ATPase.

Reducing equivalents Molecules derived from vitamins that transfer electrons in redox reactions, mainly NADH and $FADH_2$.

Introduction

The basic features of mitochondrial metabolism are shared among eukaryotes are discussed in **Tissue Respiration**: Cellular Respiration. However, all species have some specializations that modify the basic unit of a mitochondrion to build an organelle that is better suited to its role in those species. Thus, in fish there are many examples of peculiarities in mitochondrial structure and function that fine-tune mitochondrial properties in ways that are better suited to their lifestyle. In some cases, the differences are quantitative. For example, a fish might possess more of an otherwise-unremarkable enzyme, conferring greater enzymatic capacity. In other cases, the differences are qualitative, where a fish might have a distinctly different approach to mitochondrial structure or function. In this article, we discuss the many ways that mitochondria from a fish differ from the basic plan shared by eukaryotes. In some cases, these are traits of individual taxa of fish, and can be attributed to the adaptive radiation among fishes. In other cases, fish as a group demonstrate differences from other vertebrates that arose as a result of the evolutionary history of vertebrates. Although mitochondria are essential to diverse cellular systems and organismal functions, most research in this area has been conducted on muscle tissue because of the critical role of mitochondria in supporting animal locomotion.

Genetic and Genomic Variation

Diversification of fish over evolutionary time has led to variation in the primary sequence of genes encoding mitochondrial enzymes and regulators of genes encoding mitochondrial products. Such variation can be used to infer evolutionary relationships, and test specific hypotheses in biogeographics and population genetic, making the important assumption that most substitutions are selectively neutral or nearly so and do not have significant consequence for mitochondrial function. In an increasing number of cases, however, genetic differences among lineages have important consequences for mitochondrial function.

Bigenomic Evolution

Mitochondrial DNA (mtDNA) sequence data have played a central role in animal molecular phylogenetics. Such data have proved particularly useful in inferring relationships among relatively closely related groups because mtDNA evolves, on average, faster than comparable nuclear genes. All of the oxidative phosphorylation (OXPHOS) complexes (except complex II) are composed of subunits derived from the nucleus and mtDNA as described in **Tissue Respiration**: Cellular Respiration. If nuclear and mtDNA-encoded subunits for a given complex evolve at different rates, there is the greater potential for mtDNA mutations to disrupt the enzyme assembly and function. Defects in complex structure and function will incur a heavy selective penalty, and thus divergence in DNA sequence of these genes is predicted to be modest and comprised of synonymous substitutions (i.e., those not resulting in a change in the amino acid residue encoded by a codon). This is called purifying selection. However, there are numerous recent examples of groups of taxa where mtDNA sequences have diverged much more than expected (accelerated evolution) or have a preponderance of mutations that are nonsynonymous, resulting in amino acid substitutions.

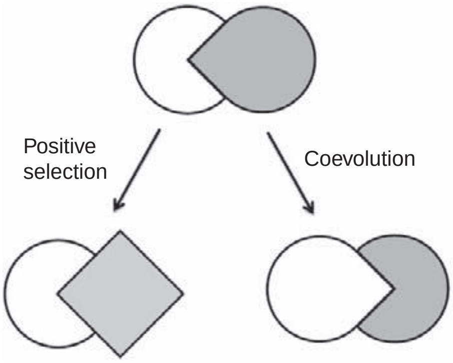


Figure 1 Positive selection and coevolution of multimeric proteins. When separate genes encode subunits of a multimeric protein, there is the potential for different types of structural evolution. In coevolution, both interacting subunits experience changes in structure, but the effects counteract and there is no net change in structure. In positive selection, one subunit experiences a significant change in structure, altering the enzyme global structure and function.

Accelerated mitochondrial evolution and such nonsynonymous mutations have been described for a number of taxonomic groups that have evolved unusual patterns of energy metabolism: snakes, primates, and scombrid fish (tunas and their relatives). Some mutations might change protein sequence, but do so in a way that is functionally neutral. However, amino acid substitutions typically result in one of two situations (**Figure 1**). Positive selection might change the structure in a way that alters assembly, stability, or kinetics of the protein. In many cases, a mutation occurs in one part of a protein (that in isolation would have important structural consequences for the complex), but a second gene also experiences a significant mutation that negates the impact of the first mutation. The net effect of these two compensatory mutations is maintenance of the structure and function of the whole complex. This pattern of coevolution is seen in many of the genes of cytochrome *c* oxidase (COX) in a variety of animals.

Though there is abundant sequence information of mitochondrial genes, an experimental challenge has been to assess the functional consequences of the genetic variation. The impact of mtDNA variation has been difficult to test directly because it is not currently technically feasible to perform site-directed mutagenesis studies on mitochondrial proteins; however, the availability of crystal structures of mitochondrial proteins and related analytical work can give insight on the impact of genetic changes.

Gene and Genome Duplication

Many aspects of metabolism are fine-tuned through the use of isoforms of enzymes (isozymes). For example, tissues can express different lactate dehydrogenase isozymes; one form is well suited to oxidize lactate while the other isoform is better suited to produce lactate. Though different versions of enzymes can arise through alternative splicing of the mRNA from a single gene, it is more common for isozymes to arise from different genes within the same genome (paralogs). The repertoire of paralogs in a given animal arises from ancient duplications of individual genes or entire genomes. Thus, many of the differences between fish and other vertebrates are a reflection of a combination of gene and genome duplications, shortly followed by a division of tasks of the duplicated isoforms (subfunctionalization), diversification of duplicates where one acquires new functions (neofunctionalization), or the loss of a duplicated gene (pseudogenization).

Early in vertebrate evolution, two genome duplication events are thought to have occurred (**Figure 2**). The first duplication (1R) probably occurred in an early agnathan ancestor around the origin of lamprey and hagfish. The second genome duplication (2R) occurred prior to the divergence of chondrichthians. After these duplications, the common ancestor to sharks, fish, and tetrapods shed some duplicate genes, as did the ancestors to individual lineages. A third large-scale duplication event occurred early in teleost evolution (3R), such that most teleosts possess more copies of select genes than do ancient bony fish, cartilaginous fish, and tetrapods. Finally, select lineages of teleosts (e.g., salmonids) have experienced additional genome duplications (4R) resulting in various degrees of polyploidy.

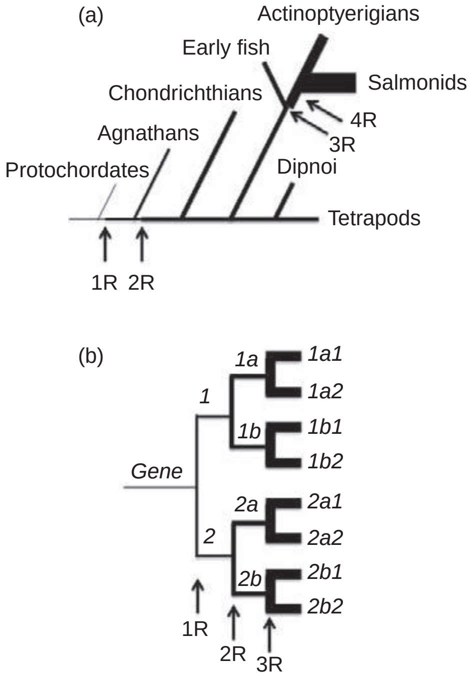


Figure 2 Gene and genome duplications in fish. (a) It is thought that whole-genome duplications (R) have occurred repeatedly over vertebrate history, beginning just prior to the origins of agnathans. (b) These genome duplications increase the number of paralogs of enzymes, giving rise to genes that are usually expressed in tissue-specific patterns.

As a consequence of these events,

1. lamprey and hagfish generally have more copies of genes that are single genes in invertebrates;
2. cartilaginous fish, bony fish, and tetrapods have more gene copies than extant agnathans;
3. derived teleosts have more gene copies than basal teleosts; and
4. select teleosts have more genes than their sister taxa.

Though many studies have explored the impact of these gene duplications on processes such as embryonic development and muscle formation, the significance of genome duplications on metabolism is largely unexplored. For example, most of the genes encoding enzymes of the glycolytic pathway show a pattern of diversity that is consistent with the 1R/2R/3R/4R series of gene duplications. In many cases, the extra copies have evolved to be tissue-specific isoforms. The importance of gene and genome duplications on mitochondrial function has only recently been studied, and like the glycolytic pathway there is an underlying influence of the 1R/2R/3R events on gene families for mitochondrial proteins.

Most of the subunits of tricarboxylic acid cycle enzymes are encoded by single genes, which clearly contrasts with most glycolytic enzymes/enzyme subunits that have multiple isoforms. For whatever reasons, duplicated genes for most mitochondrial proteins have been lost after genomic duplication events. In contrast, there are many examples of isoforms of subunits of OXPHOS complex genes. Many of the duplicates have been retained, with divergence, from the 1R and 2R genomic events. In addition, fish have retained many of the gene duplicates arising from the 3R genome duplication and subsequent lineage-specific genomic events. The functional consequences of the fish OXPHOS subunit variants have yet to be established.

Mitochondrial Structure and Function

In any particular fish, almost every cell possesses a nearly identical genome. Nonetheless, each tissue builds its mitochondria with tissue-specific properties. This developmental plasticity is an important component of mitochondrial function. Thus, it is not surprising that when comparing different species, structural and functional differences accrue. Many important differences in mitochondrial structure and function are obvious when comparing different groups of fish or comparing fish with other vertebrates.

Fuel Selection

One unusual aspect of mitochondrial function in fish, relative to other vertebrates, is fuel selection in muscles. Tetrapod muscles oxidize a range of substrates but, in most circumstances, they rely on some combination of fatty acids (from triglyceride) and pyruvate (from carbohydrates). Under starvation conditions, glucose levels decline and the liver converts some fatty acid to ketone bodies (acetoacetate, beta-hydroxybutyrate) to enable the vast lipid stores to be utilized by tissues such as brain. Under these circumstances, peripheral tissues such as muscle also rely on ketone bodies. In bony fish, however, muscles have little capacity to use ketone bodies as oxidative fuel. The circulating levels of ketones in the blood change little even during prolonged starvation, and mitochondria from fish muscle do not seem to use ketones to any significant extent (**see also Tissue Respiration**: Cellular Respiration).

Like bony fish, chondrichthians (e.g., sharks, rays, and skates) also have an unusual muscle fuel preference. Though muscles of bony fish rely on fatty acids for energy production, shark muscles cannot oxidize fatty acids. Instead, mitochondria from chondrichthian muscle rely on ketone bodies as their lipid source. Ketone bodies are higher in shark blood and change little with starvation. Furthermore, little fatty acid is carried in shark blood and their heart quickly succumbs in experiments where isolated hearts are given fatty acids.

Inner Membrane Surface Area

The inner mitochondrial membrane is the home for the proteins of OXPHOS, and within the membrane the proteins are very closely packed. Proteins constitute almost 80% of the mass of the inner mitochondrial membrane, which has important implications for mechanisms by which evolutionary and developmental mitochondrial specializations arise. For example, if a fish could benefit from an increase in maximal mass-specific mitochondrial OXPHOS capacity (adenosine triphosphate (ATP) produced per minute per gram tissue), then it would need to increase the OXPHOS specific activity within the tissue. Because the inner mitochondrial membrane is densely packed with OXPHOS complexes, there is little potential to increase OXPHOS capacity by increasing the amount of OXPHOS protein in a given amount of inner membrane. Thus, an increase in OXPHOS specific activity can arise through (1) an increase in the amount of inner mitochondrial membrane contained within a volume of mitochondria or (2) an increase in the volume of mitochondria within a volume of tissue (**Figure 3**). The two strategies can be distinguished through mitochondrial morphometric analysis. Mitochondrial images are taken using transmission electron microscopy and ultrastructural features measured directly on the images. The results of the morphometric analyses lead to measures of inner mitochondrial membrane surface area per mitochondrial volume (Sv(im,mt)) and mitochondrial volume per volume of muscle fiber (Vv(mt,mf)).

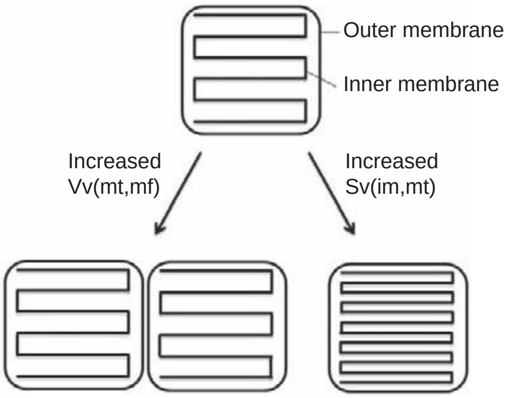


Figure 3 Strategies for changing mitochondrial capacity. Mitochondrial cristae content (surface area) is tightly packed within a volume of mitochondria. Some strategies for increasing mitochondrial inner membrane in a volume of tissue rely upon increasing mitochondrial content (Vv(mt,mf)). Other strategies increase the surface area of mitochondrial inner membrane contained within a given volume of mitochondria (increased Sv(im,mt)).

Most vertebrate muscles have Sv(im,mt) values between 20 and 40 m^2 of inner mitochondrial membrane per cubic centimeter of mitochondria. The reason for this is probably related to constraints on intramitochondrial dimensions and diffusion distances. When inner membrane is packed this tightly, there is barely enough room for a couple of enzyme molecules between opposing layers of inner membrane. The fact that this basic design feature of muscle mitochondria is similar across broad taxa infers that the main way animals alter OXPHOS capacity is by changing the volume of mitochondria within the fiber. However, there are a few high-performance species of vertebrates – tuna, hummingbird, and pronghorn antelope – that have been shown to possess mitochondria with surface area to volume ratios that are twice as high, in the 60–70 $m^2\ cm^{-3}$ range. It is thought that this elevated surface area is an important mechanism by which these species increase OXPHOS capacity within the muscle. Conversely, some polar fish have very low inner-membrane surface area ($<20\ m^2\ cm^{-3}$), though mitochondrial content is relatively normal. Recall that mitochondria in muscle occur as a reticulum and it is thought that this arrangement of mitochondria – abundant mitochondria with relatively few cristae – facilitates diffusion of oxygen into the depths of the muscle.

Mitochondrial Content of Muscle

Even in those species where mitochondrial surface area is within the typical range, differences in OXPHOS capacity can arise through changes in mitochondrial content, typically assessed as Vv(mt,mf). Differences are seen in development, where muscle fibers achieve a mitochondrial content that is appropriate for their fiber type. Thus, slow-twitch (i.e., red) muscle has a higher mitochondrial content than fast-twitch (i.e., white) muscle. This difference in mitochondrial content between fiber types of an animal is seen in all vertebrates. However, while the mitochondrial enzyme activities of tetrapod muscle fibers differ only about two- to threefold between locomotor muscle fiber types, fish red and white muscle mitochondrial enzyme activities differ by much more, 5- to 10-fold.

The main reason for the disparity in mitochondrial enzyme capacities between red and white fibers is a difference in mitochondrial volume per fiber. In most fish species, about 25–30% of the volume of red muscle is occupied by mitochondria, while in the same species white muscle mitochondria typically occupy less than 5% of myofiber (**see also Design and Physiology of the Heart**: Cellular Ultrastructure of Cardiac Cells in Fishes).

Interestingly, there is little difference in the mitochondrial content of red muscle among fish species. The mitochondrial volume in red muscle of the fastest and slowest species is consistently between 25% and 30% of the myofiber volume. The main difference in oxidative muscles between species appears to be the relative proportion of the red muscle itself. In active fish species, red muscle constitutes a much greater proportion of total muscle mass than in inactive species. Fish red muscles generally have higher mitochondrial volumes than do tetrapod locomotor muscles, which have Vv(mt,mf) values that are typically less than 5%.

A paradigm where differences in mitochondrial content are seen in diverse animals is the effect of body mass. Allometric scaling of metabolism, first identified more than 100 years ago by Rubner, reflects the effects of body size on aerobic respiration. Larger animals have greater rates of oxygen consumption (milliliter of O_2 per minute per animal), but less than would be expected if the parameters increased in direct proportion. Thus, larger animals have lower mass-specific metabolic rates (milliliter of O_2 per minute per kilogram). It has also been shown that the mitochondrial enzyme activities also show scaling, though to a lesser extent than metabolic rate. In the case of fish, the data are more complex. Meta-analysis of scaling of respiration suggests no single pattern of the effects of body size on metabolic rate. Likewise, there are complex patterns of size-dependent muscle enzyme activity. In general, however, many fish show evidence of scaling of respiration and muscle mitochondrial enzymes (**see also Energetics**: Physiological Functions that Scale to Body Mass in Fish).

The underlying mechanisms that cause muscles of different-sized fish to produce their appropriate metabolic phenotype remain uncertain. There are questions about how a muscle cell regulates its genes in a coordinated manner to ensure that all of the required gene products are at the required levels. The size-dependent patterns seen within a species as it grows (ontogenetic scaling) and between species differing in mass (phylogenetic scaling) may arise through different mechanisms. However, it is likely that the factors that determine the expression of mitochondrial genes in an individual also play a role in manifestation of the differences seen between species.

Control of Mitochondrial Biogenesis

Like other metabolic pathways, mitochondria respond to environmental and physiological challenges to maintain energy homeostasis. In many cases, fish experience the same type of remodeling responses seen in mammals. For example, changes in muscle-mitochondrial content and fuel selection are seen in response to exercise training and diet. Perhaps the best-studied response is the muscle remodeling that occurs in response to temperature. This process is reviewed in more detail in **Temperature**: Mitochondria and Temperature but, in brief, acclimation to cold temperature and acclimatization to winter conditions both induce mitochondrial proliferation in the locomotor muscles of many fish species. Cold acclimation in striped bass (*Morone saxatilis*) and goldfish (*Carassius auratus*) induces a near doubling of mitochondrial content in white muscle, and a lesser increase in red muscle. While salmonids show a similar but attenuated pattern, centrarchids (bass and sunfish) do not appear to exhibit the typical cold-induced mitochondrial proliferation seen in other species. The reasons for the species-specific responses are not yet known.

Changes in mitochondrial content in response to environmental and physiological challenges appear to be driven by the same family of transcriptional regulators that have been implicated in mammals: nuclear respiratory factor 1 (NRF1) and NRF2. A role for peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC1α), a master regulator of mitochondrial content in mammals, is less clear in fish. In goldfish, NRF1 mRNA levels are a good predictor of mitochondrial gene expression and enzyme levels in different tissues and in response to changes in diet and temperature. Such a finding would be predicted from studies on mammals, but the same studies would also predict that PGC1α mRNA would change in parallel. In contrast, in the goldfish study, PGC1α did not change in parallel to NRF1 (**Figure 4**), suggesting that it is playing a different role in fish than mammals. The reason for this difference may be related to the structure of PGC1α. The region of the protein that interacts with NRF1 in mammals appears to have a series of insertional mutations in fish that may disrupt the interaction. In contrast to PGC1α, a different member of the gene family, PGC1β, may play the role of master controller in fish. The levels of PGC1β mRNA are much better predictors of mitochondrial gene expression in goldfish tissues.

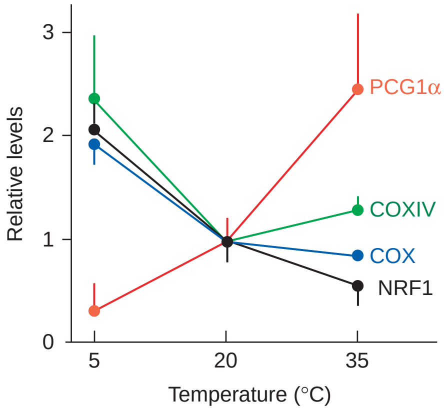


Figure 4 Patterns seen in expression of mitochondrial genes. Cold acclimation of goldfish leads to mitochondrial proliferation in white muscle, as indicated by elevations in cytochrome *c* oxidase (COX) specific activity. This change is paralleled by changes in mRNA for nuclear-encoded subunit COXIV, and by mRNA for nuclear respiratory factor 1 (NRF1), a putative regulator of COX gene expression. The expression of PGC1α, another putative regulator of mitochondrial gene expression in mammals, decreased in the cold when other elements of mitochondrial biogenesis increased. Data from LeMoine CM, Genge CE, and Moyes CD (2008) Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature. *Journal of Experimental Biology* 211: 1448–1455.

Membrane Properties

Though most studies of mitochondrial function focus on enzyme levels, many aspects are influenced by the membrane properties. Integral membrane proteins often have structural relationships with membrane lipids, and changes in the lipids can influence protein function. For example, the electron transport system enzyme COX has cardiolipin molecules that are fully integrated into its structure. Removal of the lipid moiety dramatically affects COX enzymatic properties and it has been presumed that changes in cardiolipin structure would more subtly affect its kinetics. Cold acclimation and winter acclimatization also affect the profile of phospholipids in the membrane. Such changes influence membrane fluidity much like the effects seen in the plasma membrane. Unlike the plasma membrane, the inner mitochondrial membrane is devoid of cholesterol, which has important influences on membrane fluidity and permeability in response to temperature.

Conclusions

Many aspects of mitochondrial structure and function are broadly similar among diverse species. Whether comparing mitochondria between fish and other animals, between fish species, or in a single species in response to environmental or physiological challenges, the main differences are usually qualitative; some species or tissues have more of otherwise similar mitochondria. However, important qualitative differences do exist. For example, bony fish and cartilaginous fish differ from each other and from tetrapods in fuel selection. Most teleosts also possess the remnants of repeated duplication events, leading to the presence of many gene duplicates with still undiscovered consequences to mitochondrial metabolism. Regulation of mitochondrial rate in response to physiological energy demands occurs largely through substrates and products of the F_1F_O ATPase, particularly the concentration of adenosine diphosphate. Under some conditions, oxygen limitations impair mitochondrial respiration, compromising ATP synthesis and exacerbating reactive oxygen species production.

See also: **Energetics**: Physiological Functions that Scale to Body Mass in Fish. **Gas Exchange**: Respiration: An Introduction. **Temperature**: Mitochondria and Temperature. **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration.

Further Reading

Arthur PG, Keen JE, Hochachka PW, and Farrell AP (1992) Metabolic state of the *in situ* perfused trout heart during severe hypoxia. *American Journal of Physiology* 263: R798–R804.

Davies R and Moyes CD (2007) Allometric scaling in centrarchid fish: Origins of intra- and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. *Journal of Experimental Biology* 210: 3798–3804.

Egginton S and Sidell BD (1989) Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *American Journal of Physiology* 256: R1–R9.

Guderley H (2004) Metabolic responses to low temperature in fish muscle. *Biological Reviews* 79: 409–427.

LeMoine CM, Genge CE, and Moyes CD (2008) Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature. *Journal of Experimental Biology* 211: 1448–1455.

Moyes CD (2003) Controlling muscle mitochondrial content. *Journal of Experimental Biology* 206: 4385–4391.

Moyes CD, Buck LT, and Hochachka PW (1990) Mitochondrial and peroxisomal beta-oxidation in elasmobranchs. *American Journal of Physiology* 258: R756–R762.

Moyes CD and Hood DL (2003) Origins and consequences of mitochondrial variation. *Annual Reviews of Physiology* 65: 177–201.

Moyes CD, Mathieu-Costello OA, Brill RW, and Hochachka PW (1992) Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus pelamis*) and a slow (*Cyprinus carpio*) fish. *Canadian Journal of Zoology* 70: 1246–1256.

Moyes CD, Schulte PM, and Hochachka PW (1992) Recovery metabolism in fish white muscle: The role of the mitochondria. *American Journal of Physiology* 262: R295–R304.

Ohno S (1999) Gene duplication and the uniqueness of vertebrate genomes *circa* 1970–1999. *Seminars in Cell and Developmental Biology* 10: 517–522.

Richards JG, Sardella BA, and Schulte PM (2008) Regulation of pyruvate dehydrogenase in the common killifish, *Fundulus heteroclitus*, during hypoxia exposure. *American Journal of Physiology – Regulatory, Integrative, and Comparative Physiology* 295: R979–R990.

Sidell BD (2000) Life at body temperatures below 0 degrees C: The physiology and biochemistry of Antarctic fishes. *Gravity and Space Biological Bulletin* 13: 25–34.

Steinke D, Hoegg S, Brinkmann H, and Meyer A (2006) Three rounds (1R/2R/3R) of genome duplications and the evolution of the glycolytic pathway in vertebrates. *BMC Biology* 4: 16.

Suarez RK (1998) Oxygen and the upper limits to animal design and performance. *Journal of Experimental Biology* 201: 1065–1072.

Treberg JR, Crockett EL, and Driedzic WR (2006) Activation of liver carnitine palmitoyltransferase-1 and mitochondrial acetoacetyl-CoA thiolase is associated with elevated ketone body levels in the elasmobranch *Squalus acanthias*. *Physiological and Biochemical Zoology* 79: 899–908.

Tschantz DR, Crockett EL, Niewiarowski PH, and Londraville RL (2002) Cold acclimation strategy is highly variable among the sunfishes (Centrarchidae). *Physiological and Biochemical Zoology* 75: 544–556.

CIRCULATION

Contents

The Circulatory System: An Introduction
Circulatory System Design: Roles and Principles

The Circulatory System: An Introduction

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

A Historical Perspective on Circulatory Systems
Circulatory Systems in Fishes Today
Further Reading

Glossary

Action potential A small and transient change of voltage across the cell membrane when the negative resting potential momentarily becomes nearly zero volts and quickly returns to the original level in a few milliseconds. The action potential is possible because of the activity of ion channels, embedded in the cell membrane, selectively allowing certain inorganic ions to pass through their pore.
Artery A blood vessel leading away from the heart.
Capillary A vessel that connects the arterial and venous systems, whose wall is composed of endothelial cells and connective tissue.
Cardiac output Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.
Diastole A period of the cardiac cycle when cardiac muscle is relaxed or relaxing. It is associated with filling the heart with blood.
Excitation–contraction coupling The term used to describe the progression from membrane excitation with an action potential to the rise of the calcium transient to cell contraction.
Heart A valved, muscular pump that propels fluid, usually blood or lymph, around the circulatory system.
Medulla oblongata Hindmost portion of the brainstem that contains the vagal motor and sensory areas, the respiratory rhythm generator, and cardiovascular control center.
Resistance The sum of the impediments to fluid flow, resistance is increased by vessel length and fluid viscosity, and decreased by increased vessel radius.
Rete Structure consisting of densely packed blood vessels, mostly with a countercurrent blood flow scheme, arranged to facilitate the exchange of, for example, heat or oxygen.
Secondary circulation A system of blood vessels in fishes that originate as arterio-arterial connections and which normally supplies tissues such as the skin, scales, and gills with blood containing very few red blood cells.
Stroke volume The volume of blood ejected into the ventral aorta by a single contraction of the ventricle(s).
Systole A period of the cardiac cycle when cardiac muscle is contracted or contracting. It is associated with the ejection of blood from the heart.
Vein A blood vessel leading toward the heart.

A Historical Perspective on Circulatory Systems

Anyone who attempts to write on the circulatory system, especially that of fishes where the diversity extends to over 25 000 species of fishes, should do so with some trepidation. In addition to this enormous diversity, intellectual giants have been writing on the circulatory system for over two millennia, dating back to the sixth century BC. These writers reveal the progression of scientific discovery, which was supported by careful, often meticulous observation and experimentation. As new discoveries emerged, ideas necessarily changed, but sometimes reluctantly.

The written records on the circulation date back to Greek philosophers concerned with the four elements (air, fire, water, and earth) and the 'natural, vital, and animal spirits'. At that time, the idea of two types of blood vessels (phlebos: arteries and veins) first emerged, as did the Hippocratic document entitled *On the Heart*. However, blood was viewed as moving back and forth in blood vessels by tides and pulsations until Galen, a second-century physician and philosopher, correctly identified the importance of one-way cardiac and vascular valves, which directed the flow of blood from arteries to veins. Even so, how blood moved between the arterial and venous blood vessels remained a mystery for over a millennium. Furthermore, Galen's idea of the heart as the center of our being and the source of 'vital spirit' withstood scientific and theological challenges for 14 centuries. In fact, the unorthodox view of the heart's meaning and function contributed to Michael Servetus, a sixteenth-century theologian, being burned at the stake! Today, nearly two millennia later, Galen's central tenet for the heart still retains some metaphorical currency given that only love can break your heart!

During the centuries following Galen, the anatomy of the heart and blood vessels was detailed using the naked eye, as exquisitely illustrated by Leonardo da Vinci. The seventeenth century, however, saw the emergence of a new age where new technologies propelled scientific discovery. For example, Malpighi's lenses, followed by Leeuwenhoek's more powerful ones, allowed capillaries to be finally visualized. These observations solidified the idea that circulatory system comprised a closed system of blood vessels. The physical connections between arteries and veins had been proposed nearly two millennia earlier by Erasistratos, but the technology to see these connections did not exist. Harvey, often referred to as the founding father of the modern-day experimental circulatory physiology and who had performed experiments to show that blood did indeed flow from arteries to veins, dispelled the Galenian idea of a vital spirit. Harvey's work was remarkable and extensive. He performed experiments on over 80 species, measuring blood pressure and the output of blood from the heart, as well as aspects of its control. Hales' treatise, entitled *Haemastaticks,* followed in the eighteenth century. It contains details of blood pressure, cardiac output, and blood viscosity measurements. Poiseuille then provided a mathematical equation to predict the flow of viscous fluids in narrow tubes. Ludwig's kymograph appeared in the nineteenth century, paving the way to continuously record mechanical events such as contractions of the heart and blood vessels. The working life of kymographs as a teaching and research tool extended to the undergraduate days of the author in the 1970s!.

The turn of the twentieth century saw Frank's and Starling's discovery of the remarkable intrinsic mechanical property of the heart: the heart can empty with sufficient blood pressure to propel blood around the circulatory system by greatly amplifying the stretch of its walls caused by the small amount of energy in the venous circulation. However, this intrinsic property remained without a mechanistic explanation for several decades. At the same time, Krogh and his wife were breaking new ground in their examination of the relationship between capillaries and tissues. They clearly showed that oxygen moved into and out of capillaries by diffusion and, in doing so, rejected Bohr's theory of oxygen secretion. Around the same time, the first physiological experiments were being made on blood pressure in fishes, building on an extremely rich history of anatomical studies of their hearts and blood vessels, which had amassed in the German, French, and English literature. In 1904, Greene reported the first measurements of arterial blood pressure in a fish, showing that it was about half of that in humans. The first measurements of cardiac output in fishes had to await the inventiveness of Hart, who tied two sutures around the heart, pulling them tight after systole or diastole to determine cardiac stroke volume as the difference of the two volumes.

Circulatory Systems in Fishes Today

The series of articles in this section provides a synthesis of current knowledge concerning circulatory design and function of fishes. It begins with an introductory article (**see also Circulation**: Circulatory System Design: Roles and Principles) that describes the roles of a circulatory system. Principles of hydrodynamics and circulatory design are explained alongside the equations that predict the movement of blood through blood vessels.

The powerhouse of the circulatory system is the heart, which serves as a muscular organ that rhythmically contracts with each heartbeat. The gross anatomy (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes) and the variations in form of fish hearts are explained, as is with the cellular structure of cardiac muscle (**see also Design and Physiology of the Heart**: Cellular Ultrastructure of Cardiac Cells in Fishes). Fish hearts have their own neurons and neurotransmitters (**see also Design and Physiology of the Heart**: Intracardiac Neurons and Neurotransmitters in Fish), and some have a coronary circulation (**see also Design and Physiology of the Heart**: The Coronary Circulation). The heart works as a pump, generating blood pressure and hence blood flow. The basic physiology of cardiac function (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping) is accompanied by details on how the cardiac action potential is generated (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart). The action potential an electrical event generated by an orchestrated, highly controlled series of ion movements across cardiac cell membranes that ultimately produce a contraction of all cardiac muscle cells – termed excitation–contraction coupling. Central to excitation–contraction coupling is the movement of calcium into the cytoplasm (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux) and then the binding of calcium to troponin C to initiate contraction of the contractile proteins (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element), namely actin, myosin. As it turns out, the fish heart is exquisitely sensitive to stretch when its chambers are filled (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping) and cellular mechanism for this is explained (**see also Design and Physiology of the Heart**: Cardiac Cellular Length-Tension Relationship).

The heart pumps blood around a closed system of blood vessels. The varied patterns and forms of the circulatory system in fishes are described (**see also Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns), while noting the important phylogenetic differences that exist for the major groupings of fishes, namely the cyclostomes, the primitive fishes, the elasmobranchs, and the teleosts. Special consideration is given to the major types of blood vessels, the outflow vessels from the heart (**see also Design and Physiology of the Heart**: The Outflow Tract from the Heart), the resistance vessels (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels), the capillaries (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis), and the veins (**see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels), because their form is closely allied to their function. While the circulatory system of fishes is termed closed, describing the fact that capillaries connect the arterial and venous system, the capillaries are designed to facilitate fluid and gas exchange with tissues (**see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels). This fluid exchange must be carefully controlled and balanced (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange).

The gastrointestinal circulation of fishes receives at least a quarter of cardiac output even in nondigesting fish and therefore is given separate attention (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation). Special consideration is also given to the anatomy of the branchial circulation (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy) and gill morphometrics (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics), which are responsible for gas and ion exchange with water. Air-breathing fishes have specialized circulatory adaptations to permit dual breathing of air and water (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes).

The cellular composition of blood is described (**see also Blood**: Cellular Composition of the Blood), along with information on how the primary cell type in blood, the red blood cell, is produced (**see also Blood**: Erythropoiesis in Fishes). More detail on gas transport in the circulatory system, which is the primary role of the red blood cell, is found in **Gas Exchange**: Respiration: An Introduction. Beyond gases (oxygen, carbon dioxide, and ammonia), the circulatory system plays critical roles in distributing hormones and then removing them from the circulation (**see also Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation).

A circulatory adaptation apparently unique to fishes is the secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy), which supplies certain tissues with blood largely devoid of its cellular components (**see also Blood**: Cellular Composition of the Blood). Associated with the secondary circulation and the lymphatics (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy) are additional pumps. These so-called accessory hearts (**see also Design and Physiology of the Heart**: Accessory Hearts in Fishes) impart additional potential energy to blood to help return it back to the central heart. A special arrangement of arteries and veins, called rete, promotes highly efficient gas and heat exchange between the arterial and venous vessels (**see also Design and Physiology of Arteries and Veins**: The Retia).

Obviously, fine control of the circulatory system is an essential requirement for a life support system. This control occurs through autonomic neural control of the heart (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes) and resistance vessels (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). The autonomic cardiovascular control centers are located in the medulla oblongata of the brain (**see also Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish). These centers are ultimately responsible for integrating circulatory control (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System), which then allows fish to appropriately respond to the circulatory demands of digestion (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion), exercise (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming), hypoxic environments (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia), and changes in temperature (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature). As might be expected, there is intimate interaction between the control of respiration and circulation with the brain (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish), especially during hypoxia (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes).

We have a solid foundation of knowledge on the function and anatomy of the circulatory system of fishes. However, much remains to be discovered beyond the few dozen species studied so far in any detail. Indeed, remarkable circulatory adaptations are still likely to emerge as more research is performed. Beyond this attraction, discoveries for fish may find applications in medicine.

When trying to understand circulatory form and function, there may be wisdom in stepping back from the complexities of a Ferrari sports car (humans) and temporarily considering a model T Ford (fishes). For example, a comparative approach to the debate surrounding "the mixing of pneuma and blood in the left ventricle to create the vital spirit", which lasted nearly 1000 years, might have advanced much faster than it did by considering the simpler, single circulatory design of fishes rather than the deluxe, double circulation of mammals.

See also: **Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes. **Blood**: Cellular Composition of the Blood; Erythropoiesis in Fishes. **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; Physiology of Capacitance Vessels; Physiology of Resistance Vessels; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis; Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of the Heart**: Accessory Hearts in Fishes; Action Potential of the Fish Heart; Cardiac Anatomy in Fishes; Cardiac Cellular Length-Tension Relationship; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Cellular Ultrastructure of Cardiac Cells in Fishes; Intracardiac Neurons and Neurotransmitters in Fish; Physiology of Cardiac Pumping; The Coronary Circulation; The Outflow Tract from the Heart. **Gas Exchange**: Respiration: An Introduction. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish; Integrated Control of the Circulatory System. **Integrated Response of the Circulatory System**: Hormone Metabolism in the Circulation; Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Digestion; Integrated Responses of the Circulatory System to Hypoxia; Integrated Responses of the Circulatory System to Temperature. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: Cardiovascular System*, pp. 89–139. San Diego, ca: Academic Press.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 1–88. San Diego, CA: Academic Press.

Fishman AP and Richards DW (1982) *Circulation of the Blood: Men and Ideas,* 859 pp. Bethesda, MD: American Physiological Society.

Greene CW (1904) Physiological studies of the Chinook salmon. *Bulletin of the United States Fish Commission* 24: 431–456.

Hart JS (1943) The cardiac output of four fresh-water fish. *Canadian Journal of Research* 21: 77–84.

Hoar WS, Randall DJ, and Farrell AP (1969–2010). *Fish Physiology,* vols. 1–30. San Diego, CA: Academic Press.

Krogh A (1959) *The Anatomy and Physiology of Capillaries*, 2nd edn., 422 pp. New York, NY: Hafner Publishing.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology*, 3rd edn., pp.119–152. Boca Raton, FL: CRC Press.

Satchell GH (1991) *Physiology and Form of Fish Circulation,* pp. 1–235. New York, NY: Cambridge University Press.

Stevens ED and Randall DJ (1967) Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *Journal of Experimental Biology* 46: 307–315.

Circulatory System Design: Roles and Principles

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Function of the Cardiovascular System
Operating Principles of the Cardiovascular System
Hemostatics and Hemodynamics
Building a Cardiovascular System
Functional Definitions of Blood Vessels
Further Reading

Glossary

Baroreceptors Receptors in the walls of blood vessels that monitor blood pressure.

Compliance The ability of a blood vessel to stretch. Compliance is defined mathematically as the slope of the change in volume of the vessel divided by the change in pressure, i.e., $\Delta V/\Delta P$.

Convection Bulk movement of a medium, e.g., gas, water, or blood.

Diastole The condition of the heart at rest; diastolic pressure is the lowest blood pressure.

Fahraeus effect The observation that hematocrit in small vessels is significantly lower than that in large vessels.

Fahraeus–Lindqvist effect The condition in small blood vessels where viscosity becomes less dependent on hematocrit.

Hematocrit The percentage of packed red blood cells contained in a volume of blood after centrifugation.

Hydraulic pressure Pressure imparted on a solution by a piston or pump. Blood pressure is a form of hydraulic pressure.

Hydrostatic pressure Pressure due to the height of a column of fluid directly above the reference point. This pressure exists in both static and flowing fluids and can be either added to, or subtracted from, the hydraulic pressure, where the reference point is the heart.

Newtonian fluid A fluid such as water whose viscosity remains constant at all flow rates.

Non-Newtonian fluid A fluid such as blood whose viscosity changes with flow rate.

Oncotic pressure Unique form of osmotic pressure created by unequal protein concentrations across a membrane or capillary, also called colloid osmotic pressure.

Osmotic pressure The hydrostatic pressure produced by the different concentrations of two solutions on opposite sides of a semipermeable membrane.

Portal circulation An anatomical condition where there are two resistance networks in parallel.

Resistance The sum of the impediments to fluid flow; resistance is increased by increased vessel length and fluid viscosity, and decreased by increased vessel radius.

Suction pump A pump that creates negative pressure and pulls fluid into it.

Sump pump A pump that fills passively and only pumps when fluid flows into it.

Systole The condition of the heart during contraction; systolic pressure is the maximum arterial blood pressure.

Transmural pressure The pressure difference between the inside and the outside of a vessel.

Viscosity The ease with which a liquid will flow under an applied pressure; molasses is more viscous than water.

Function of the Cardiovascular System

Molecules move from one location to another three ways – diffusion, carrier-mediated transport, and convection. Diffusion is a random process capable of moving many molecules but it is only efficient over short distances, typically no further than several cells. Furthermore, the net movement of molecules can only occur down a concentration gradient. Carrier-mediated transport is similar to diffusion in that it is for only short distances, typically across cell membranes or epithelia, and serves to move molecules across otherwise impermeable barriers or allow them to be concentrated against an electrochemical gradient. Convection is the movement of molecules in a current and is the only efficient way to move molecules over considerable distances.

The purpose of the cardiovascular system is the convective movement of molecules over the considerable distances that separate the different cells of the body. The best-known activity of the cardiovascular system is the movement of nutrients from sites of uptake such as the respiratory organs (gills) or gastrointestinal (G.I.) tract to the cells, or removal of metabolic wastes and delivery of these excretory products to the gills, kidney and liver. However the cardiovascular system is also used for other functions. These include the body's signaling system through transport of hormones, the body's defense mechanism through transport of immune response elements, and the dissipation, or retention, of heat from the animal's core to the environment. Because blood is a liquid, the body must also have mechanisms to prevent it from leaking out when a vessel is cut (clotting mechanisms) and counter-mechanisms to prevent excessive or spontaneous clotting.

Operating Principles of the Cardiovascular System

In very general terms the operation of the cardiovascular system is quite analogous to the water supply of many cities. A pump (or heart) pumps water (or blood) from an underground reservoir, usually an aquifer, to an elevated reservoir such as a water tower that is higher than the houses. In this process the mechanical energy of the pump is converted into potential energy in the water tower. The pressure that is generated by the difference in height between the reservoir and the buildings (a hydrostatic pressure) ensures that under normal circumstances the water pressure supplied to all houses is the same. Thus, each house has the opportunity to use water depending upon its need, that is, drinking, washing, watering the lawn, etc. As long as there is adequate water in the reservoir, the pressure will remain constant and intermittent water usage by one house will not affect water supply or delivery pressure to the other houses. However, in times of crisis such as drought or excess demand, there also has to be a higher authority empowered to prioritize water use and reduce water use by less critical applications such as washing the car or watering the lawn. This ensures adequate water supply for critical organizations such as the fire department, hospitals, and for drinking water.

The cardiovascular system operates by analogous principles. The heart pumps blood into the aorta and major arteries and this arterial pressure is the potential energy supplied to all organs (houses). Each organ has the opportunity to regulate its own blood flow based on its immediate need, usually metabolic demand, and can do so as long as the arterial pressure remains adequate. This is termed local regulation. A crisis can occur if the pump (heart) becomes weak (after a heart attack) or if there is not enough blood coming to the heart to pump (venous return: e.g., excessive loss of blood and high gravitational forces), or if the demand by one of the tissues becomes excessive. In any one of these situations the higher authority (central nervous system) has the power to restrict blood flow to less critical organs such as the gastrointestinal tract, skin, or skeletal muscle in order to ensure that blood flow to the organs most vital to immediate survival, the heart and brain, and sometimes exercising skeletal muscle, is not compromised. This is termed remote regulation and the central nervous system makes these decisions based largely on information it receives from pressure receptors (baroreceptors) located in the walls of the arteries, veins and heart. However, unlike the water pump in a city, the fish heart is not a very good suction pump and must therefore depend on blood pressure in the veins to ensure adequate filling of the heart (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). This entails additional control mechanisms that regulate blood volume and venous pressure.

Hemostatics and Hemodynamics

In order to understand the principles of the cardiovascular system, it is important to first understand some basic properties of fluids at rest (hemostatics) and in motion (hemodynamics).

Fluids at Rest: Hemostatics

Unlike many molecules that have specialized proteins to help them move across membranes or epithelia, there are no known carriers for water and the only way water moves from one location to another is down a pressure gradient. There are three forms of pressure – hydrostatic, hydraulic, and osmotic.

Hydrostatic pressure

Hydrostatic pressure is determined by the weight of fluid directly above a point of reference; the latter depends on the distance between the top of the water and the reference point and the density of the fluid (**Figure 1**(**a**)). The pressure at the reference point (P) is then $P = \rho g h$, where ρ is the density of the fluid (in g L^{-1}), g is the gravitational acceleration constant (9.8 m s^{-2}), and h is the height (in m) from the reference point to the top of the water. There is an interesting dichotomy here, however, because as fish descend deeper into water, the hydrostatic pressure on the outside of the fish is offset by the hydrostatic pressure in the fish's cardiovascular system (**see also Design and Physiology of Arteries and Veins:** Physiology of Capacitance Vessels). Thus, actual blood pressure in a fish (and in physiological experiments) is always measured relative to the surface of the water. Furthermore, the only time hydrostatic pressure is important in fish is

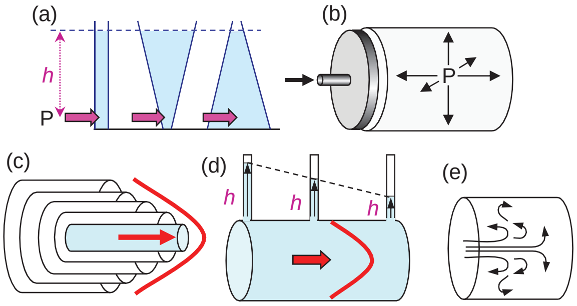


Figure 1 Pressure and flow in the cardiovascular system. (a) Hydrostatic pressure is determined by the height (*h*) of a column of water above a reference point (arrow) irrespective of the container shape. The hydrostatic pressure is the same at all arrows. (b) Hydraulic pressure (*P*) is exerted by a piston or pump and at any point in a fluid is the same in all directions. (c) Newtonian fluids flow through a tube in concentric cylinders with the flow through the outer cylinder the slowest due to frictional drag on the walls of the tube. (d) Hydraulic pressure in fluid flowing down a tube decreases due to the frictional loss of energy. At any point along the tube, the hydraulic pressure is equivalent to the hydrostatic pressure (*h*) which would force fluid up a vertical standpipe. (e) Turbulent flow results when lamellar flow is disrupted such as branch points in vessels and around the heart valves; this may increase viscosity but is not normally a feature of the peripheral circulation.

when they are out of water (except at depths of greater than 1000 m; (**see also Deep-Sea Fishes**).

Hydraulic pressure

Hydraulic pressure is pressure supplied by a pump, in this case the heart. Hydraulic pressure is exerted in all directions in a blood vessel (**Figure 1**(**b**)). Hydraulic pressure will be examined in greater detail in subsequent sections.

Osmotic and oncotic pressure

Osmotic pressure is the pressure caused by water at different concentrations due to the dilution of water by dissolved molecules (solute), notably salts and nutrients. Osmotic pressure is very important in bony fish where the difference in osmotic pressure between the body fluids (typically 300 milliosmoles per liter; mosm L^{-1}) and the environment (<1 mosm L^{-1} in freshwater and 1000 mosm L^{-1} in saltwater) can create substantial forces that, if otherwise uncontrolled, can cause volume overload in the former and dehydration in the latter. For instance, a difference of 10 mosm across the membrane can create a pressure difference of 190 mmHg, which is approximately 6–8 times the arterial blood pressure of a fish. A unique form of osmotic pressure called oncotic or colloid osmotic pressure is presumed to exist across the capillaries. This pressure is due solely to the difference in protein concentration between plasma and interstitial fluid because proteins are presumed to be the only solute molecules that do not freely cross capillaries. While oncotic pressure is important in maintaining fluid balance across mammalian capillaries, its importance in transcapillary fluid balance in fish is controversial.

Blood pressure definitions

Blood pressure can be measured anywhere in the circulation and usually the location is specified, that is, arterial pressure, venous pressure, etc. Arterial pressure is not constant but varies with the cardiac cycle (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). Systolic pressure is the maximum pressure and occurs with ejection of blood from the heart (systole). Diastolic pressure is the minimum pressure and occurs when the heart is at rest (diastole). Pulse pressure is the difference between systolic and diastolic pressure, and mean blood pressure is the average pressure. In many fish the oscillations in arterial pressure take on a sawtooth pattern and the mean pressure is the arithmetic average of systolic and diastolic pressure (i.e., mean pressure = (systolic + diastolic) 2^{-1}). In other fish the diastolic period is longer than systolic and mean pressure is the time-integrated average pressure weighted toward the diastolic. The formula derived for mammals, mean = (systolic + 2 diastolic) 3^{-1}, is often used in these instances.

Fluids in Motion: Hemodynamics

Perfect Newtonian fluids

The movement of fluid down a tube (flow) is directly related to the pressure gradient (ΔP) along the tube and inversely related to the resistance (R) to flow (i.e., $F = \Delta P R^{-1}$). This is Darcy's law, which is the hemodynamic equivalent of Ohm's law for electricity. The ΔP is the difference between the input and output pressures and is an absolute requirement for fluid to flow down a tube or blood vessel. Newtonian fluids flow down a tube as concentric cylinders, the outermost cylinder moves at the slowest rate due to friction against the wall, and the innermost cylinders move progressively faster as they slide along their neighbors (**Figure 1**(**c**)). This is called laminar flow and it has a parabolic profile (red lines in **Figures 1**(**c**) and **1**(**d**)). Resistance is due to the fractional energy loss as fluid interacts with the wall of the blood vessel which in turn results in a progressive decrease in pressure (**Figure 1**(**e**)). Resistance encompasses all of the factors that impede flow and, for a Newtonian (i.e., 'pure' fluid such as water), can be described by the relationship

$$R = \frac{\eta l}{r^4}\frac{8}{\pi}$$

where η is the viscosity of the fluid, l is the length of the tube, r is the radius of the tube, and $8\ \pi^{-1}$ is a geometric constant. Substituting these values for resistance back into Darcy's law produces the Hagen–Poiseuille equation

$$F = \frac{\Delta P r^4}{\eta l}\frac{\pi}{8}$$

Because length and viscosity do not normally vary substantially, it is clear from this equation that the major determinants of flow through a tissue are the vessel radius and pressure gradient. The considerable effect that a change in vessel radius has on flow is evident from a simple calculation: a twofold increase in vessel radius will cause a 16-fold decrease in resistance, and therefore increase flow 16 times. Turbulent flow results when lamellar flow is disrupted (**Figure 1**(**e**)) and it will increase the apparent viscosity. Turbulence may be found around vascular branch points or the heart, but is not a common feature of the peripheral circulation.

Imperfect non-Newtonian fluids

Blood is considered a non-Newtonian fluid primarily because the presence of red blood cells changes the viscous properties of blood. Hematocrit is the percent volume of blood occupied by the red blood cells, that is, if 100 ml of whole blood contained 35 ml of packed red blood cells, the hematocrit would be 35 (**Figure 2**(**a**); **see also Blood:** Cellular Composition of the Blood). In larger vessels such as arteries and veins, the viscosity of blood increases as hematocrit increases and at each hematocrit blood viscosity increases even more at very low flow (**Figure 2**(**b**)). **Figure 2**(**c**) shows the distribution of red cells in the blood vessel at different flow rates. At low flow (small arrow) the red cells mingle and bump into the walls of the vessel and each other, thereby increasing viscosity. At higher flow rates red blood cells become concentrated in the center of the vessel, a phenomenon known as axial streaming, and viscosity is reduced as blood flow becomes more laminar. In small blood vessels the red blood cells may have to deform to enter the vessel (**Figure 2**(**d**)) and any increase in red blood cell size or decrease in flexibility of the red blood cell membrane will increase the effective viscosity. Once inside small vessels, however, blood viscosity is far less dependent on hematocrit than it was in large vessels, a phenomenon known as the Fahraeus–Lindqvist effect. It is also interesting to note that the hematocrit in vessels ≤300 μm diameter is less than that found in large arteries and veins, often as low as 10. This phenomenon is known as the Fahareus effect.

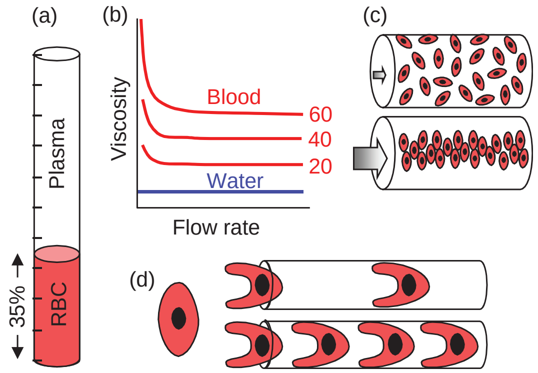


Figure 2 Non-Newtonian properties of blood. (a) The hematocrit is the percent volume of blood occupied by red blood cells (RBCs), in most fish around 35%. (b) The viscosity of blood increases as hematocrit increases and at each hematocrit viscosity increases at very low flow rates. The viscosity of water, a Newtonian fluid, is lower than that of blood and independent of flow rate. (c) At low flow rates red blood cells bump into the walls of the vessel disrupting laminar flow and increasing hematocrit. At higher flow rates, the red cells become concentrated in the center of the vessel (axial streaming) and viscosity is reduced. (d) Red blood cells must typically deform to enter smaller capillaries. An increase in the size of the red blood cell or decrease in flexibility of the red blood cell membrane will increase blood viscosity. Once inside small vessels, blood viscosity becomes considerably less dependent on hematocrit (the Fahraeus–Lindqvist effect).

Vascular Circuits: In-Series and In-Parallel Resistance

Vascular circuits such as electrical circuits can be found in series and in parallel (**Figure 3**). With in-series circuits, the total resistance of the system is equal to the sum of the individual resistances: $R_{\mathrm{T}} = R_1 + R_2 + R_3$. It is evident that as additional circuits are added, total resistance increases arithmetically. With in-parallel systems total resistance is calculated from: $1R_{\mathrm{T}}^{-1} = 1R_1^{-1} + 1R_2^{-1} + 1R_3^{-1}$. As additional circuits are added in parallel, total resistance decreases. In-series circuits are not as common in cardiovascular systems because of the increased pressure that must be generated to perfuse vascular beds in tandem. The most notable in-series circuit in the fish cardiovascular system is the gills and systemic circuit, although there

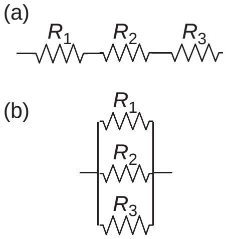


Figure 3 Resistance patterns in blood vessels. (a) Resistances in series. The total resistance (R_{T}) for vessels in-series is the sum of their individual resistances: $R_{\mathrm{T}} = R_1 + R_2 + R_3$. (b) Resistances in parallel. The total resistance for vessels in-parallel is $1R_{\mathrm{T}}^{-1} = 1R_1^{-1} + 1R_2^{-1} + 1R_3^{-1}$. Increasing the number of resistors in-series increases the total resistance, whereas increasing the number of resistors in-parallel decreases total resistance.

are other systems such as the hepatic portal, real portal, and pituitary.

Building a Cardiovascular System

A basic cardiovascular system can be constructed using the analogy of a simple bicycle tire as shown in **Figure 4**. For the moment the contribution of the respiratory circulation will be ignored and the arterial and venous components will be assigned to the left and the right side of the tire. When the tire is open (vented) to the environment (panel A), arterial pressure (P_a) equals venous pressure (P_v) and both pressures are equal to atmospheric pressure (P_{ATM}). Because arteries do not collapse, and veins are not completely collapsed, there will always be some blood remaining in the circulation. This volume of blood is called the unstressed volume (USV). Because the USV does not exert any pressure on the system, it is essentially hemodynamically inert. Remarkably, the USV in both fish and mammals is around 60% of the total blood volume.

In panel B, an air pump inflates the tire and now P_a and P_v are greater than P_{ATM} but still equal to each other because there is no flow. P_a and P_v are now equal to the mean circulatory filling pressure (P_{MCFP}), which by definition is the pressure that exists throughout the cardiovascular system when there is no flow and pressure has equilibrated throughout. The volume of air (or blood) that has been added to the system is called the stressed volume (SV). Compliance (C) is an index of how readily the walls of the tube can distend to contain more blood. Compliance is calculated from the ratio of the change in volume to a given change in pressure (i.e., $C = \Delta V \Delta P^{-1}$). In panel B, the increase in volume is the same as the stressed volume, and the increase in pressure is the same as the mean circulatory filling pressure, so compliance is equal to the ratio of the SV to the mean circulatory filling pressure (i.e., $C = \mathrm{SV}\ P_{MCFP}^{\ -1}$). Rearranging this equation it can be seen that the mean circulatory filling pressure is the ratio of SV to compliance, $P_{MCFP} = SVC^{-1}$.

In panel C, the bicycle tire becomes more like a cardiovascular system by modification of arterial and venous compliance and addition of a heart. In both fish and mammals, venous compliance (C_v) is approximately 20 times greater than arterial compliance (C_a). This allows more blood to be stored in the veins than in the arteries; in mammals approximately 80% of systemic blood is in the veins, 15% in arteries, and 5% in capillaries; however, these proportions are not known for fish. This also means that P_{MCFP} is far more dependent on C_v, and the equation can now be rewritten to reflect this: $P_{MCFP} = V_s\ C_v^{\ -1}$.

The vertebrate heart is more like a sump pump than a suction pump. That is, it is relatively inefficient in sucking blood into it and therefore cardiac filling and cardiac output are more dependent upon extracardiac factors that deliver blood to it, namely P_v, although the rigid pericardium in some fish assists in developing moderate cardiac suction. Because P_v is very close to P_{MCFP}, the most important determinant of cardiac filling and cardiac output is $V_s\ C_v^{\ -1}$. Any increase in this ratio (an increase in V_s or a decrease in C_v) will favor an in increase in cardiac output and vice versa. V_s is subjected to a variety of factors that can affect blood volume or flow into the venous

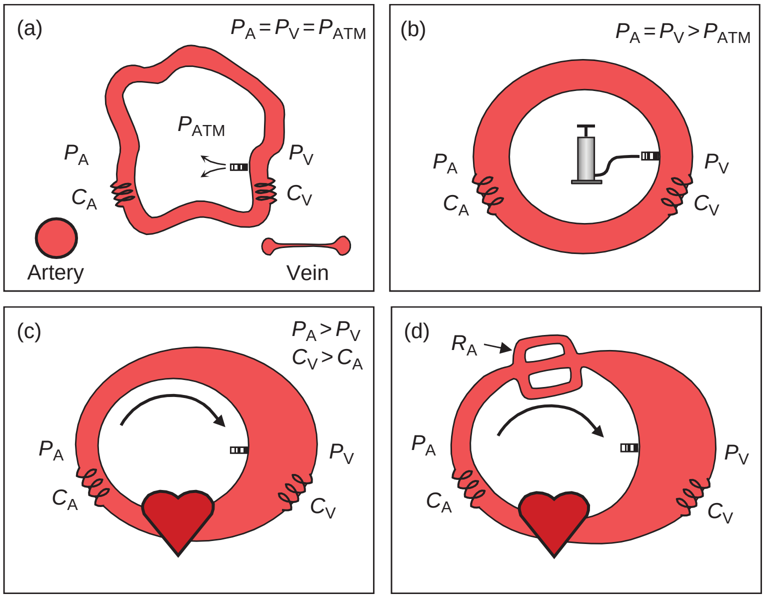


Figure 4 Development of a simple cardiovascular system using a bicycle tire. Abbreviations: C_A, C_V, arterial and venous compliance, respectively; P_A, P_V, P_{ATM}, arterial, venous, and atmospheric pressure, respectively; R_A, arterial vascular resistance. See text for details.

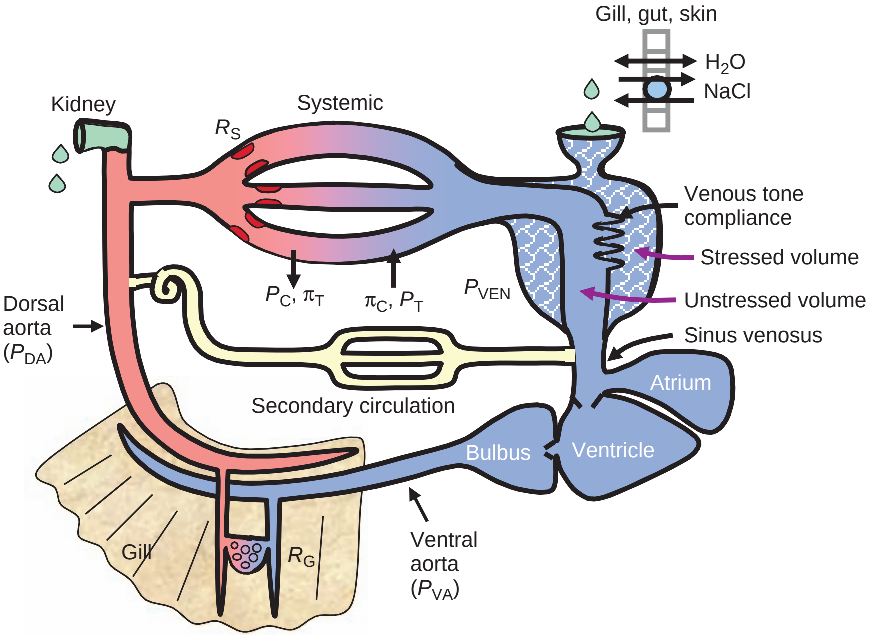


Figure 5 General features of the fish cardiovascular system. Abbreviations: P_{VA}, P_{DA}, P_{VEN}, hydraulic pressure in ventral aorta, dorsal aorta, and central vein, respectively; P_C, P_T, hydraulic pressure in capillary and tissue interstitial fluid, respectively; R_G, R_S, gill and systemic vascular resistance, respectively; π_c, π_T, oncotic pressure in capillary and tissue interstitial fluid, respectively. See text for details. Reproduced from Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, pp. 119–152. Boca Raton, FL: CRC Press.

circuit (see **Figure 5**) and C_v is dependent upon the elastic properties of the veins and can be further modified, increased, or decreased, by active dilation or constriction of these vessels. There is a simple axiom that the mammalian heart cannot be the direct cause of an increase in cardiac output, but when it fails it can be the direct cause of a decrease in cardiac output. This holds quite well for fish as well.

This simple cardiovascular system is completed by the addition of organs and their capillary networks (panel D). The major resistance to flow in the cardiovascular system resides in the small arteries and arterioles within the tissues (R_a) and these are all in-parallel circuits. Adding more tissues will decrease total resistance, not increase it. R_a can be controlled locally by the specific needs of the tissues or remotely by nerves and hormones in response to changes in blood pressure, analogous to the city water system (see above). This system also has some degree of intrinsic responsiveness in that a local decrease in R_a in response to a tissue's need to increase blood flow will send more blood into the venous circulation which in turn increases P_v, cardiac filling, and ultimately cardiac output.

Figure 5 shows the integrated fish cardiovascular system. Blood is pumped from a single ventricle into the ventral aorta and from there passes through the gill circulation before continuing on to the dorsal aorta and then to the systemic circulation. Because gill resistance (R_g) and systemic resistance (R_s) are in series, pressure in the ventral aorta (P_{VA}) and gill capillaries exceeds pressure in the dorsal aorta (P_{DA}) and systemic capillaries. Total blood volume (both stressed volume and USV) is determined by factors that affect the net exchange of water across the gill, gut, and skin, and by urinary output by the kidney, and by transcapillary fluid movement. In mammals, the factors that affect transcapillary movement of fluid include capillary and tissue hydraulic pressure (P_C and P_T, respectively) and capillary and tissue oncotic pressure (π_C and π_T, respectively). As described above, the importance of these factors in transcapillary movement in fish has not been established (**see also Design and Physiology of Capillaries and Secondary Circulation:** Circulatory Fluid Balance and Transcapillary Exchange). The secondary circulation is an alternative pathway, perhaps the progenitor of the lymphatics (**see also Design and Physiology of Capillaries and Secondary Circulation:** Secondary Circulation and Lymphatic Anatomy). Venous pressure and therefore filling of the heart are determined by the stressed blood volume, venous compliance, and, to a lesser extent, by fluid entering from the arterial side (inversely related to R_S) and ventricular suction.

Functional Definitions of Blood Vessels

Blood vessels are most commonly categorized according to their structure and location (i.e., arteries, arterioles, capillaries, venules, and veins), but they can also be categorized by their primary function. Compliance (aka elastance or windkessel) vessels dampen the arterial pulse. The intermittent ejection of blood from the heart stretches the elastic tissue in these vessels converting some of the potential energy of hydraulic pressure generated during systole into mechanical stretch. During diastole, when there is no blood flowing out of the heart the elastic recoil of these compliance of vessels transfer energy back into hydraulic pressure. This helps maintain a positive arterial pressure and blood flow down these vessels even when the heart is at rest. The bulbus (or conus in some fish) arteriosus is specifically designed for this function although the ventral aorta, dorsal aorta, and other vessels between the heart and systemic tissues also contribute (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart). Interestingly, this arterial pulse is not dampened out by the gills and is transmitted all the way through the dorsal aorta and into the systemic tissues. Conductance vessels, as their name implies, are conduits for blood transfer. Many of these serve dual roles as compliance vessels. Resistance vessels are the small arteries and arterioles that constitute the greatest impediment to flow. Active regulation of their diameter, by both local and remote mechanisms, is the primary means of regulating both tissue perfusion and arterial blood pressure. Exchange vessels are typically capillaries and small venules, although gas exchange may occur across small arterioles as well. The rest of the cardiovascular system exists for the sole purpose to deliver blood to these vessels. Capacitance vessels are the venules and veins and they conduct blood back to the heart, and through active contraction or relaxation, they are also the major determinants of the mean circulatory filling pressure, and ultimately cardiac filling.

See also: **Design and Physiology of Arteries and Veins:** Anatomical Pathways and Patterns; Physiology of Capacitance Vessels. **Design and Physiology of Capillaries and Secondary Circulation:** Circulatory Fluid Balance and Transcapillary Exchange; Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of the Heart:** Physiology of Cardiac Pumping. **Hormonal Control of Metabolism and Ionic Regulation:** The Hormonal Control of Osmoregulation in Teleost Fish.

Further Reading

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 89–120. San Diego, CA: Academic Press.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac function in fish. *Physiological Zoology* 64: 1137–1164.

Forster ME, Lomholt JP, Franko-Dossar F, Welsch U, and Potter IC (1998) Part seven. The cardiovascular system. In: Jørgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 235–273. London: Chapman and Hall.

Guyton AC, Coleman TG, Cowley AW , Jr., *et al.* (1972) Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *American Journal of Medicine* 52: 584–594.

Guyton AC, Jones CE, and Coleman TG (1973) *Circulatory Physiology: Cardiac Output and Its Regulation*. Philadelphia, PA: W.B. Saunders.

Lai NC, Graham JB, Dalton N, Shabetai R, and Bhargava V (1998) Echocardiograph and hemodynamic determinations of the ventricular filling pattern in some teleost fishes. *Physiological Zoology* 71: 157–167.

Marshall WS and Grosell M (2006) Ion transport, osmoregulation, and acid–base balance. In: Evans DE and Claiborne JB (eds.) *The Physiology of Fishes*, pp. 177–230. Boca Raton: FL: CRC Press.

Olson KR (1992) Blood and extracellular fluid volume regulation: Role of the renin–angiotensin system, kallikrein–kinin system, and atrial natriuretic peptides. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part B: The Cardiovascular System*, pp. 136–232. San Diego, CA: Academic Press.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, pp. 119–152. Boca Raton, FL: CRC Press.

Priede IG (1976) Functional morphology of the bulbus arteriosus of rainbow trout (*Salmo gairdneri* Richardson). *Journal of Fish Biology* 9: 209–216.

Rothe CF (1991) Vascular capacitance. In: Dulbecco R (ed.) *Encyclopedia of Human Biology*, 2nd edn., vol. 8, pp. 757–765. San Diego, CA: Academic Press.

Rothe CF (1993) Mean circulatory filling pressure: Its meaning and measurement. *Journal of Applied Physiology* 74: 499–509.

Rowell LB (1993) *Human Cardiovascular Control*. New York: Oxford University Press.

Sandblom E and Axelsson M (2007) The venous circulation: A piscine perspective. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* 148: 785–801.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

Satchell GH (1992) The venous system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 141–179. San Diego, CA: Academic Press.

Satchell GH (1999) Distinctive attributes of the circulation of elasmobranch fishes. In: Hamlett WC (ed.) *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes*, pp. 218–237. Baltimore, MD: The Johns Hopkins University Press.

Taylor EW, Jordan D, and Coote JH (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiological Reviews* 79: 855–916.

CIRCULATION

Blood

Contents
Cellular Composition of the Blood
Erythropoiesis in Fishes

Cellular Composition of the Blood

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Separating Blood Cells
Red Blood Cells
White Blood Cells
Further Reading

Glossary

Anemia A state of reduced numbers of red blood cells, usually associated with bleeding.

Blood A tissue that moves around the body within blood vessels, serving many transport functions.

Erythrocyte The most abundant cell found in the blood of nearly all fish. It contains the respiratory pigment hemoglobin and is commonly called a red blood cell because of its color.

Granulocyte A medium-sized white blood cell with variously shaped nuclei and a cytoplasm-containing granules that can perform phagocytosis.

Hematocrit The percentage of packed red blood cells contained in a volume of blood after centrifugation.

Leucocrit The percentage of packed white blood cells contained in a volume of blood after centrifugation.

Leukocyte The name given to a blood cell that does not contain the respiratory pigment hemoglobin. It is commonly called a white blood cell because of its lack of color.

Lymphocyte A type of white blood cell involved in cellular defense and immunity. Granular lymphocytes are called natural killer cells and small lymphocytes produce immunoglobulins (B cells) or toxic granules to pathogens (T cells).

Mean cell hemoglobin concentration The average concentration of hemoglobin in red blood cells. It is usually estimated from the whole blood hemoglobin concentration divided by the proportion of red blood cells in the blood.

Monocyte The largest white blood cell, produced in the head kidney that will be mobilized as a response to inflammatory signals and mature to macrophages.

Plasma The extracellular fluid that often comprises the majority of blood. Its composition varies enormously among fishes.

Red blood cell The most abundant cell found in the blood of nearly all fish. It contains the respiratory pigment hemoglobin.

Thrombocyte The smallest white blood cell, often called a platelet, with an important role in blood clotting.

White blood cell The name given to a blood cell that does not contain the respiratory pigment hemoglobin.

Introduction

Blood is a tissue contained within the circulatory system that serves many transport functions. It has three major components: red blood cells (RBCs, erythrocytes), white blood cells (WBCs, leukocytes), and the extracellular space around these cells, the plasma (**Figure 1**). Blood differs from most of the other body tissues in that its extracellular volume (that occupied by the plasma) is typically twice as large the intracellular volume (that

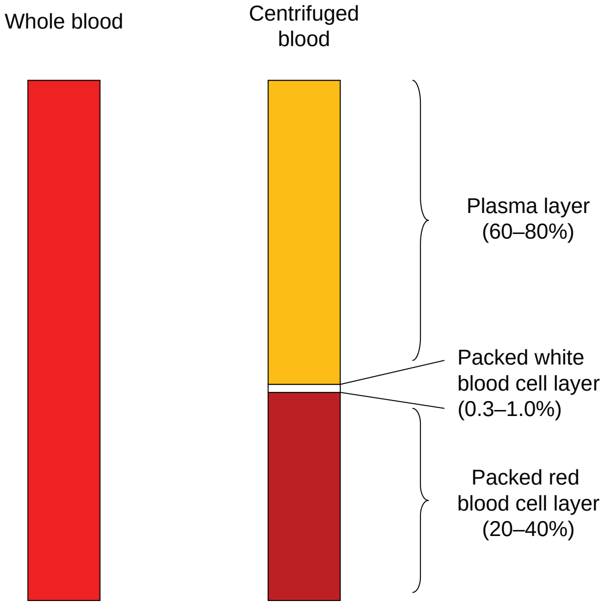

Whole blood
Centrifuged blood
Plasma layer (60–80%)
Packed white blood cell layer (0.3–1.0%)
Packed red blood cell layer (20–40%)

Figure 1 Schematic representation of the cellular composition of fish blood.

occupied by the cells). While RBCs normally remain within the circulatory system, WBCs can routinely move from within the blood, across the endothelium, and into the interstitial space between cells to perform defense functions.

Blood is primarily a transport medium for gases and solutes that include oxygen, carbon dioxide, ammonia, nutrients, and hormones. Simply put, the concentrations of the various components of blood are regulated. Some that are tightly regulated (homeostatic roles) include electrolyte (ion) concentrations, pH, arterial oxygen and carbon dioxide tensions, and osmolarity. This tight regulation stems from the fact that blood composition influences most aspects of a fish's physiology. Consequently, plasma analysis is a routine diagnostic tool used to monitor a fish's physiological status in much the same way as physicians monitor the plasma of human patients. Changes in plasma composition can indicate a disturbed physiology (e.g., stress) or an excessive environmental challenge. Similarly, because RBC and WBC concentrations are also well regulated, changes to WBCs can indicate a pathological situation and anemic fish are not expected to perform well.

Blood composition, however, can vary within normal physiological limits. For example, nutrients (amino acids, fatty acids and lipids, and glucose) and wastes (ammonia) obviously become elevated during feeding. Similarly, certain hormones and yolk nutrients increase during reproductive development. Venous blood and arterial blood also differ considerably in certain respects, especially during exercise, when blood gases, pH, nutrients, and RBCs may change considerably. Such dynamic changes in blood composition are normally resolved within minutes, hours, or days. This article focuses only on the characteristics and variability of RBCs and WBCs.

Separating Blood Cells

The two primary blood cell types can be separated based on differences in cellular density. When a blood sample is centrifuged in a tube, the more dense RBCs (because of a higher iron content; **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin) pack to the bottom of the tube as a red-colored layer (**Figure 1**). On top sits a thin, white, or buff layer of WBCs. WBCs are distinguished from RBCs by their lack of hemoglobin and lower cellular density. Above the cells lies the plasma, which may have on its surface droplets of fat that are less dense than water. The relative composition of blood is represented by the relative heights of the packed layers of RBCs, WBCs, and plasma. Hematocrit (Hct) is the term used for the percentage of the column of blood occupied by RBCs. Leucocrit (Lct) is the term used for the percentage of the column of blood occupied by WBCs. Typically, fish blood comprises 60–80% plasma, 20–40% RBCs, and 0.5–2.0% WBCs (**Figure 1**). However, in special cases, Hct can exceed 50% or RBCs can be completely absent (Hct = 0%).

Red Blood Cells

Fish RBCs are elliptical or ovoid. Like those of amphibians and reptiles, they have a nucleus, which not only contrasts with circulating mammalian RBCs but also results in a larger cell dimension. RBC dimensions vary among fish species, typically within a range of 10–20 μm for length and 6–10 μm for width. Fish lineages that have undergone gene duplication, and fish made triploid (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Evolution of Fish Genomes, Physiology of Triploid Fish, and Transgenesis and Chromosome Manipulation in Fish), have proportionately larger nuclear and RBC sizes.

The most important role of RBCs is the transport of oxygen by the high concentrations of the respiratory pigment hemoglobin found in the cytoplasm. Hemoglobin (**see also Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P50) greatly increases the blood's capacity to transport oxygen, which is poorly soluble in the plasma. RBCs also play an important role in pH regulation (acid–base balance – **see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion, Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange, and Red Blood Cell Function) because hemoglobin binds hydrogen ions and carbon dioxide. The presence of the enzyme carbonic anhydrase (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange) in the cytoplasm is critical for effective carbon dioxide transport (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Clearly, the concentration (and type, **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin) of hemoglobin in blood has an enormous impact on the blood's capability to transport gases and buffer blood pH.

The blood hemoglobin concentration is under the control of the hormone erthythropoietin, which regulates the regular replacement of old or damaged cells with the new ones through the process of erythropoiesis (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin Differentiation in Fishes and **Blood**: Erythropoiesis in Fishes). The main hematopoietic tissues in fish are the thymus, spleen and kidney, and erythropoiesis.

Across fish species, the routine concentration of both the RBCs contained in blood (as reflected by Hct), and the hemoglobin within a single RBC can vary substantially (**Figure 2**). Both phylogeny and other considerations contribute to this variability. Furthermore, blood hemoglobin concentration undergoes temporary and dynamic changes according to the fish's physiological state, largely through changes in Hct.

Hematocrit

Blood hemoglobin concentration is typically linearly related to Hct (**Figure 2(a)**). Hct varies enormously across fish species. Most fish have an Hct between 10% and 40%. The Hct of elasmobranch species tends to be at the lower end of this range (between 10% and 20%). Teleosts generally have a higher Hct (between 25% and 40%), and a value <20% would be considered anemic for say rainbow trout. With normal Hct varying from 0% to >50% among fishes, it is hardly surprising that factors other than phylogeny influence Hct. Athleticism and blood viscosity are very important in this regard. Temporary changes in Hct primarily involve RBC release from a storage site (the spleen) and secondarily involve fluid movements (**Figure 3**).

Athleticism

Athletic fishes are characterized by a circulatory system with a high Hct and cardiac output (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming). The high Hct of athletic fishes has likely been selected based on the high oxygen need for an active lifestyle since a higher Hct increases arterial oxygen concentration. In support of this idea, the Hct of benthic flatfishes can be half than found in athletic fishes. Also, experimentally induced anemia typically reduces swimming performance and maximum oxygen uptake (**Figure 4**). However, the fact that a higher-

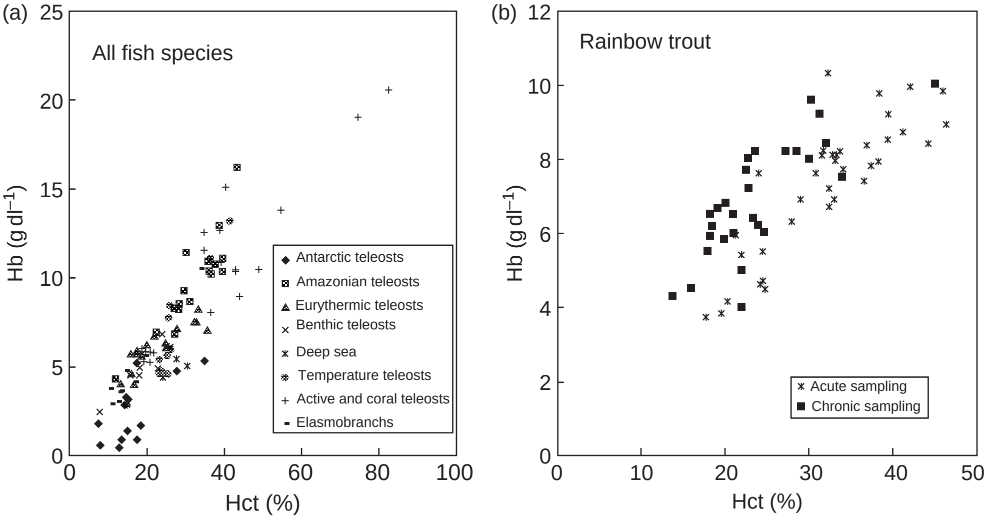


Figure 2 A meta-analysis of Hct values reported for (a) various fish species and (b) rainbow trout (*Oncorhynchus mykiss*). These relationships show the variability in Hct, the expected linear relationships between Hct and blood hemoglobin concentration, and systematic difference for Hct between two sampling methods; hemoglobin concentration at a given Hct in rainbow trout was typically higher for blood samples taken via venopuncture than via a cannula. Reproduced from Gallaugher P and Farrell AP (1998) Hematocrit and blood oxygen carrying capacity. In: Perry SF and Tufts B (eds.) *Fish Respiration, Fish Physiology*, vol. 17, pp. 185–227. San Diego, CA: Academic Press.

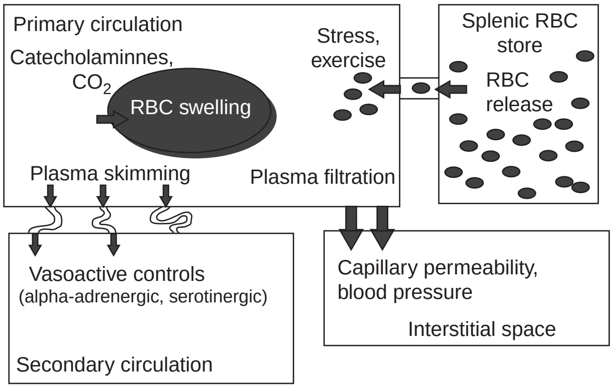


Figure 3 A schematic representation of some of the physiological factors that can influence hematocrit in fish blood. Reproduced from Gallaugher P and Farrell AP (1998) Hematocrit and blood oxygen carrying capacity. In: Perry SF and Tufts B (eds.) *Fish Respiration,* Fish Physiology, vol. 17, pp. 185–227. San Diego, CA: Academic Press.

than-normal Hct (35–45%) in rainbow trout does not improve swimming (**Figure 4**) suggests that functions in addition to oxygen transport during exercise play a role in setting Hct.

Blood viscosity

The heart generates blood pressure to overcome vascular resistance (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping) and blood viscosity directly affects vascular resistance (Poiseuille's law; **see also Circulation**: Circulatory System Design: Roles and Principles and **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). Thus, the heart does more work with each and every heartbeat whenever blood viscosity is elevated. Since blood viscosity increases with Hct and decreases with temperature, it makes energetic sense to keep Hct to the minimum required for oxygen transport during locomotion, especially under cold conditions.

An extreme example of how the combined effects of Hct and low temperature on blood viscosity have resulted in wonderful adaptations involves Antarctic fishes. These extreme adaptations, which include a complete absence of RBCs as well as large, temporary increases in Hct, were likely possible only in the stable, cold (<1°C), and oxygen-rich water of Antarctica and with a lifestyle that was not highly active. Some Antarctic Nototheniids have survived the deletion of the gene for hemoglobin, which means their blood lacks RBCs and hemoglobin. These fishes pump extraordinarily high volumes of plasma around their circulatory system at low pressure using a very large ventricle adapted to accommodate an extraordinarily large cardiac stroke volume. Consequently, Hct and temperature are intimately linked to ventricular design through the effects of blood viscosity on cardiac work. (Interestingly, experimentally induced anemia and cold can trigger rapid ventricular growth in rainbow trout). Other Antarctic fishes, such as *Pagothenia borchgrevinki* and *Notothenia rossi*, have adapted to a very low routine Hct (14% and 6%, respectively) compared with other teleost species. These fishes, however, can double Hct, but only when it is absolutely necessary to temporarily boost oxygen transport (e.g., hypoxia, stress, and exhaustive exercise), because they routinely store an unusually large number of RBCs in their spleen.

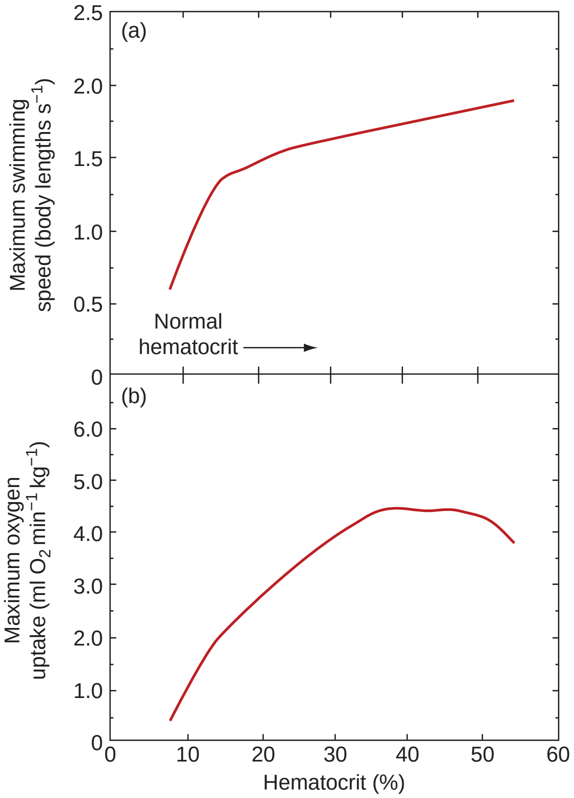


Figure 4 A. is supposed to be shaped a bit like a hockey stick B. is supposed to be shaped like part of a bell-shaped curve with a flattened top. The results of experimentally manipulating hematocrit in rainbow trout on maximum prolonged swimming performance and maximum oxygen uptake. Adapted from Gallaugher P, Thorarensen H, and Farrell AP (1995) Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respiration Physiology* 102: 279–292.

Temporarily changing Hct

Fish use rapid increases in Hct to temporarily increase oxygen transport during hypoxia, stress, and exercise. In these situations, RBCs stored in the spleen are released into the circulation under sympathetic nervous control (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes; **Figure 3**). While a few Antarctic fishes double Hct in this manner, most fish change Hct by just a few percent. RBCs are also released during anesthesia. Thus, when the spleen in anesthetized rainbow trout was surgically tied off, RBCs became trapped in the circulation, raising routine Hct by 8%. During exercise, the difference in Hct between these surgically treated fish and control fish disappeared because the exercise stimulated a similar 8% increase in Hct in control fish. Bleeding and blood sampling likely deplete the splenic store of RBCs before Hct decreases.

Minor changes in Hct occur when plasma, or some of its fluid, moves out of the primary circulation without any accompanying RBCs (**Figure 3**). Transcapillary fluid exchange, which is primarily influenced by capillary permeability and capillary blood pressure, is common to all vertebrates (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange, **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels, and Secondary Circulation and Lymphatic Anatomy). However, a fluid movement unique to fishes is that through the convoluted arterial vessels that connect the primary and secondary circulations (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy). Normally, these narrow vessels preferentially allow plasma but not RBCs into the secondary circulation (termed plasma skimming). Consequently, the secondary circulation has a very low Hct (<<10%). Increased arterial blood pressure enhances plasma skimming, while vasodilation of the connecting vessels admits RBCs. RBCs, entry into the secondary circulation was first visualized in the gills of the European eel (*Anguilla anguilla*) and more recently in the skin of the zebrafish (*Danio rerio*) and the glasscatfish (*Kryptopterus bicirrhis*), two small fish species with relatively transparent bodies. Hypoxia stimulates RBC movement into the secondary circulation of the skin and under the control of nitric oxide release (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). The red flush of skin and scales of anesthetized and heat-challenged fish could also reflect RBCs accumulating in the skin. Whether such RBC movements just deplete splenic RBC stores or decrease Hct in the primary circulation as well is unknown.

These physiological adjustments to Hct can have implications for accurately measuring routine Hct in fish, even as simple as the Hct measurement technique is. For example, stressing rainbow trout while sampling blood (e.g., venopuncture without anesthesia) will overestimate normal Hct due to RBC swelling and/or splenic RBC release. Conversely while blood vessel cannulation techniques can avert stress, blood loss during or after the surgery becomes a possibility. As a result, Hct in rainbow trout is known to be systematically different using these two sampling methods, with Hct being typically higher for blood samples taken via venopuncture than via a cannula (**Figure 2(b)**).

RBC Hemoglobin Concentration

The concentration of hemoglobin within an RBC is usually expressed as mean cell hemoglobin concentration (MCHC). MCHC varies about twofold, between 170 and 340 g L^{-1}, over a wide range of fish species and conditions (physiological and environmental). MCHC in fish is lower than that in mammals, in part because the nucleus of a fish RBC takes up a considerable portion of the cell volume. MCHC estimated from the whole blood hemoglobin concentration divided by the proportion of RBCs in blood (i.e., Hct/100).

MCHC decreases temporarily for two primary reasons. One is that fish RBCs are prone to swelling. For example, RBCs can take on water when blood catecholamine concentrations rise during stress (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). Swelling can change Hct by ~5%, which means that in such situations an Hct measurement alone would overestimate MCHC to the same degree. RBC swelling is typically recognized when Hct increases but blood hemoglobin concentration does not.

The second reason for a decrease in MCHC is that fish release immature RBCs into the circulatory system during erythropoiesis (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin Differentiation in Fishes and **Blood**: Erythropoiesis in Fishes). Thus, a fish recovering from anemia will have a disproportionate number of younger RBCs and a lower MCHC. Young RBCs continue to manufacture hemoglobin while circulating, increasing MCHC over time. There are simple methods to isolate RBCs of different ages. Centrifugation packs older RBCs lower in the RBC layer than younger RBCs because of a higher hemoglobin concentration and density.

White Blood Cells

As in mammals, fish WBCs have primary roles in cellular defense and immunity. These roles result in WBCs being found in tissues outside of the blood, as well as their three primary production sites (thymus, spleen, and kidney). Here, the focus is on the WBCs routinely found in fish blood.

Fish regulate the number of circulating WBCs, despite their miniscule representation in fish blood (Lct = 0.3–1.0%). As a result, Lct is a relative coarse measure of any change in WBC abundance. Even so, an elevated Lct is often used as an indicator of pathology because it can easily double. Toxicants and stress can also depress Lct.

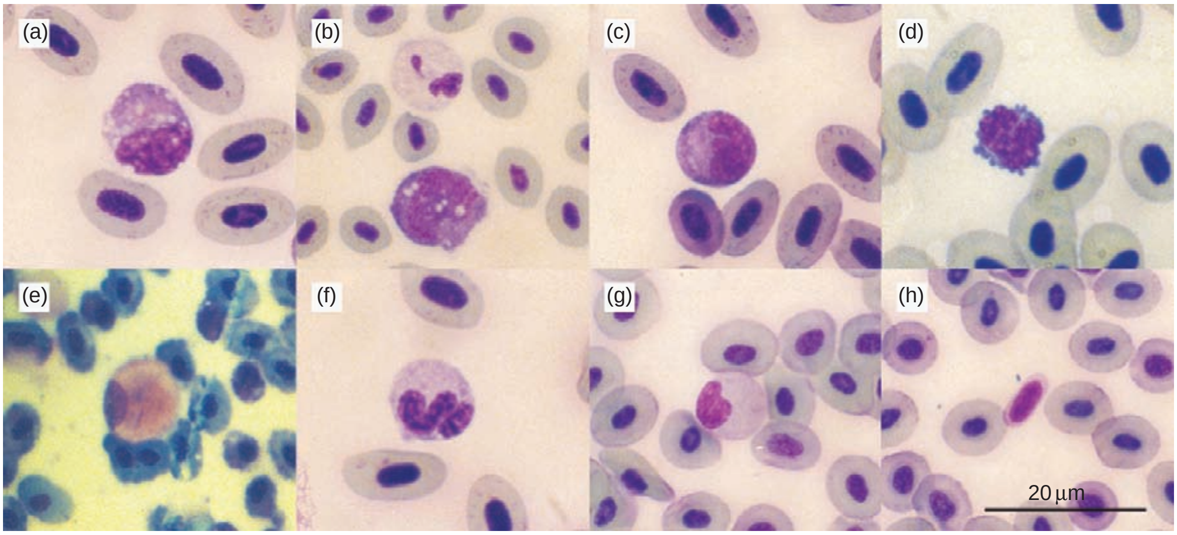


Figure 5 A selection of the types of leukocytes found in four different teleost fish (fathead minnow – *Pimephales promelas*, Pacific herring – *Clupea pallasi*, brook stickleback – *Culea inconstans*, and juvenile coho salmon – *Oncorhynchus kisutch*): (a) monocyte from coho salmon; (b) neutrophil (top) and monocyte (bottom) from Pacific herring; (c) basophilic staining cell from coho salmon; (d) lymphocyte from coho salmon; (e) eosinophilic staining cell from Pacific herring; (f) neutrophil from coho salmon; (g) neutrophil from fathead minnow; and (h) thrombocyte from fathead minnow. The more numerous elliptical cells are nucleated red blood cells. All cells stained with modified Wright–Giemsa and photographed at 1000. Reproduced from Tierney KB, Farrell AP, and Kennedy CJ (2004) The differential leucocyte landscape of four teleosts: Juvenile *Oncorhynchus kisutch*, *Clupea pallasi*, *Culea inconstans*, and *Pimephales promelas*. *Journal of Fish Biology* 65: 906–919.

Microscope counting chambers afford a more accurate measure of WBCs (the number per mm^3 of blood). Typically, there are thousands of WBCs per mm^3 compared with millions of RBCs per mm^3 of blood. Despite their low abundance, WBCs are about 20 times more metabolically active than RBCs (RBCs consume about $150\,\mu mol\,h^{-1}\,L^{-1}$ cells). Consequently, WBC metabolism will account for about 25% of the oxygen consumed by blood if Lct is 1% and Hct is 30%.

The four major WBC types in fish blood (**Figure 5**) are:

1. lymphocytes that play a role in immuno-competence;
2. thrombocytes that are involved in blood clotting;
3. granulocytes that have unknown functions; and
4. monocytes that are phagocytic.

Typically, lymphocytes and thrombocytes dominate over granulocytes, while monocytes are rare. The roles of the different WBC types, as well as their characteristics and proportions, are less well understood for fishes than mammals. Even so, WBCs can be more abundant in fish blood than in human blood.

The WBC types are distinguished in counting chambers and in dried blood smears after staining cells with dyes. Distinguishing features include overall cell size and the size and shape of the nucleus (**Figure 5**). A differential blood count assigns percentages to the proportions of WBC types in a blood sample. Expensive and automated electronic cell counting devices are also used. Criteria used to identify fish WBCs are based on those established for mammals. Even so, inconsistencies exist in the fish literature, partially because WBC appearance is confusing (e.g., small lymphocytes and thrombocytes have a nearly identical appearance), and partially because there are differences with mammalian WBCs.

Lymphocytes

Lymphocytes are small (5–8 μm) or large (12 μm). Regardless of size, about two-thirds of the cell is taken up by a purple-stained nucleus. They often have a serrated appearance due to folds in the membrane called pseudopodia. Lymphocytes are produced primarily in the thymus, and secondarily in spleen and kidney. As in mammals, fish have T-type and B-type lymphocytes. B-lymphocytes contain the antigen immunoglobulin (Ig) on their cell membrane, which is consistent with roles in antibody production and immuno-competence. Cortisol, which is released during stress, can depress lymphocyte numbers.

Thrombocytes

Thrombocytes, the smallest (5–8 μm) WBCs, take on different appearances (a spiked, spindle-cell, ovoid, and lone nucleus). Their cytoplasm stains lightly, whereas the nucleus stains dark purple and occupies the majority of the cell. Thrombocytes have a variable abundance (**Figure 6**). Great care is needed to distinguish

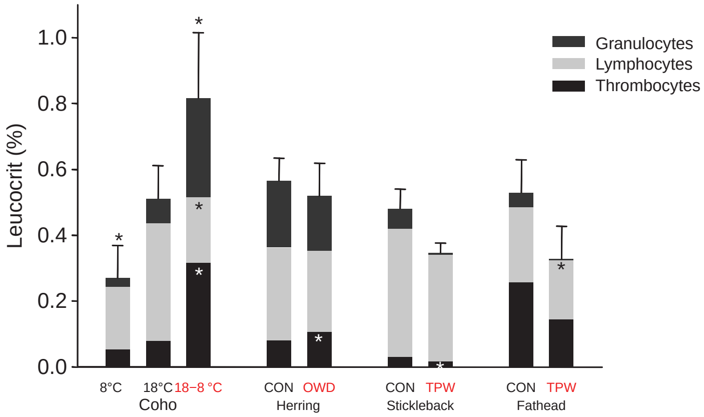


Figure 6 The white blood cell composition of fish expressed as a fraction of the total packed white cell volume (leucocrit). Control values for coho salmon acclimated to both 8 °C and 18 °C are compared with 18 °C-acclimated coho salmon exposed to an acute 96 h decrease in temperature (18–8 °C). Control Pacific herring held in seawater are compared with Pacific herring exposed to oil–water dispersion (OWD). Control stickleback and fathead minnows were sampled following wild capture and are compared with fish held for 48 h in the same tailings pond water (TPW). Lct values with asterisk are significantly different (t-test, á = 0.05). Reproduced from Tierney KB, Farrell AP, and Kennedy CJ (2004) The differential leucocyte landscape of four teleosts: Juvenile *Oncorhynchus kisutch*, *Clupea pallasi*, *Culea inconstans*, and *Pimephales promelas*. *Journal of Fish Biology* 65: 906–919.

thrombocytes with a lone or an ovoid nucleus from small lymphocytes, a mistake that could greatly elevate thrombocyte numbers. The lack of Ig on thrombocytes is perhaps their best distinguishing factor. Thrombocytes are involved in blood clotting (**Figure 7**).

Granulocytes

Granulocytes are 12–15 μm in diameter and can be confused with large lymphocytes. However, the nucleus occupies no more than about a third of the cell and can be mono-, bi-, or multi-lobed. The cells have circular or elliptical shapes and the stained cytoplasm takes on a variety of hues, textures, and shapes when stained, being sometimes referred to as heterocytes or neutrophils. The cytoplasm contains many vacuoles and stains from blue (basophilic) through transparent (neutrophilic) to red (eosinophilic); neutrophils are typically dominant. Depending on the species, eosinophils and basophils can be rare, or both subtypes can approach 10% of the WBC count. The roles of granulocytes are unclear, but reportedly they are phagocytic and respond to inflammation. An increased percentage of granulocytes in stressful situations results in part because lymphocyte numbers decrease. Granulocytes appear to be produced in the kidney and spleen.

Monocytes

Monocytes are infrequent (<1% of WBCs). If they account for 0.1%, of WBCs, on average, at least 1000 WBCs must be viewed just to observe just one monocyte. However, their large size (20 μm diameter) makes them easy to identify. The cytoplasm stains blue and contains vacuoles. The nucleus is typically kidney shaped and occupies more than half of the cell. Monocytes are phagocytic and chemotactic. They possibly mature to become the phagocytic tissue macrophages as part of a nonspecific defense. The respiratory burst activity

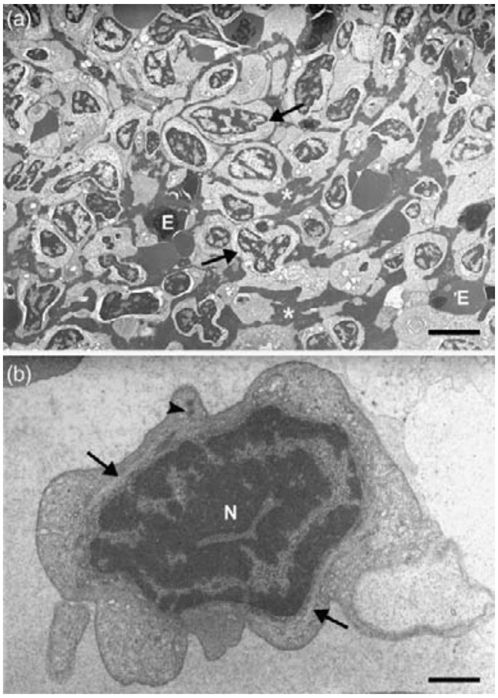


Figure 7 Thrombocytes in the gills of Chinook salmon infected with the microsporidian *Loma salmonae*: (a) a gill arteriole fully obstructed with thrombocytes (arrows), erythrocytes (E), and fibrin (*) (bar, 6 μm); (b) ultra-structural features of a salmonid thrombocyte including an extensive microtubule network (arrows) and small membrane bound granules (arrowhead), (N, nucleus) (bar, 1 μm). Photo courtesy of Jan Lovy, Atlantic Veterinary College, University of Prince Edward Island, Canada.

measured in kidney tissue macrophages is often used to diagnose a sick fish.

Each WBC type is present in variable amounts depending on the species, and according to a fish's physiological or pathological state, and even the season of the year. The variability of granulocytes and thrombocytes among species is particularly evident in **Figure 6**. These data show that Lct doubled by increasing lymphocyte numbers with warm acclimation of coho salmon (*Oncorhynchus kisutch*). In contrast, an acute cooling stress (18–8 °C) increased Lct even further, but this was because granulocyte and thrombocyte numbers increased whereas lymphocyte numbers decreased. High levels of the adrenocorticotrophic hormone and cortisol, typically found in blood during stress (**see also Hormonal Responses to Stress**: Hormone Response to Stress), are known to reduce lymphocyte numbers, leading to a low WBC count (leukopenia). **Figure 6** also illustrates that exposure to tailing water decreased Lct in both brook stickleback (*Culaea inconstans*) and fathead minnow (*Pimephales promelas*), but exposure to water-soluble oil fractions had no effect on either Lct or WBC composition of Pacific herring (*Clupea pallasi*). Furthermore, granulocytes, thrombocytes, and lymphocytes all decreased in brook stickleback, but only granulocytes and thrombocytes decreased in fathead minnow. Thus, we appear some distance from being able to characterize WBC responses across fish species.

In summary, the cellular composition of blood is regulated, dynamic, and multifunctional.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Blood**: Erythropoiesis in Fishes. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Evolution of Fish Genomes; Transgenesis and Chromosome Manipulation in Fish; Physiology of Triploid Fish. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange; Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels; Physiology of Resistance Vessels. **Hormonal Responses to Stress**: Hormone Response to Stress. **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Carbonic Anhydrase in Gas Transport and Exchange; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; Hemoglobin Differentiation in Fishes; O_2 Uptake and Transport: The Optimal P50; Red Blood Cell Function.

Further Reading

Blaxhall PC and Daisley KW (1973) Routine haematological methods for use with fish blood. *Journal of Fish Biology* 5: 771–781.

Ellis AE (1976) Leucoytes of fish: A review. *Journal of Fish Biology* 11: 453–491.

Franklin CE, Davison W, and McKenzie JC (1993) The role of the spleen during exercise in the Antarctic teleost *Pagothenia borchgrevinki*. *Journal of Experimental Biology* 174: 381–386.

Gallaugher P, Axelsson M, and Farrell AP (1992) Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. *Journal of Experimental Biology* 171: 301–314.

Gallaugher P and Farrell AP (1998) Hematocrit and blood oxygen carrying capacity. In: Perry SF and Tufts B (eds.) *Fish Respiration,* Fish Physiology, Vol 17, pp 185–227 San Diego, CA: Academic Press.

Gallaugher P, Thorarensen H, and Farrell AP (1995) Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respiration Physiology* 102: 279–292.

Graham MS and Fletcher GL (1983) Blood plasma viscosity of winter flounder: Influence of temperature, red cell concentration, and shear rate. *Canadian Journal of Zoology* 61: 851–858.

Houston AH and Murad A (1995) Erythrodynamics in fish: Recovery of the goldfish *Carassius auratus* from acute anemia. *Canadian Journal of Zoology* 73: 411–418.

Jensen LDE, Cao R, Hedlund E-M, *et al.* (2010) Nitric oxide permits hypoxia-induced lymphatic perfusion by controlling arterial–lymphatic conduits in zebrafish and glass catfish. *Proceedings of the National Academy of Sciences of the United States of America* 106: 18408–18413.

Olson KR (1984) Distribution of flow and plasma skimming in isolated perfused gills of three teleosts. *Journal of Experimental Biology* 109: 97–108.

Tierney KB, Farrell AP, and Kennedy CJ (2004) The differential leucocyte landscape of four teleosts: Juvenile *Oncorhynchus kisutch*, *Clupea pallasi*, *Culea inconstans*, and *Pimephales promelas*. *Journal of Fish Biology* 65: 906–919.

Wells RMG and Forster ME (1989) Dependence of blood viscosity on haematocrit and shear rate in a primitive vertebrate. *Journal of Experimental Biology* 145: 483–487.

Wells RMG, Macdonald JA, and diPrisco G (1990) Red-blooded Antarctic fishes: A rheological comparison of the haemoglobin-free icefishes, *Chionodraco kathleenae* and *Cryodraco antarcticus*, with a red-blooded nototheniid, *Pagnothenia bernacchii*. *Journal of Fish Biology* 36: 595–609.

Yamamoto KI (1991) Increased arterial O_2 content in exercised yellowtail (*Seriola quinqueradiata*). *Comparative Biochemistry and Physiology – Part A* 98: 43–46.

Erythropoiesis in Fishes

C Verde, D Giordano, R Russo, and G di Prisco, National Research Council (CNR), Naples, Italy

© 2011 Elsevier Inc. All rights reserved.

Introduction
Further Reading

Glossary

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Erythropoiesis Formation of red blood cells in the developing vertebrate embryo.

Evolutionary mutant model An organism naturally expressing a phenotype that mimics the clinical features of human diseases.

Hematopoiesis The process by which the cellular elements of the blood are formed.

Hematopoietic stem cells Responsible for production of all mature blood cells and including red cells, myeloid cells (neutrophils and macrophages), mast cells, megakaryocytes, and B- and T-lymphocytes.

Introduction

Here, we review erythropoiesis in fish and identify important questions yet to be addressed. Fishes account for approximately 50% of all vertebrate species and are suitable organisms for analyzing erythropoiesis. Complex genetic manipulations in zebrafish, an excellent model for studying development, have been important in the elucidation of erythropoiesis in fish. The similarities between erythropoiesis in fish and mammals are not limited to only conservation of genes. All major blood-cell types and the molecular signaling pathways are highly conserved between fish and mammals. Furthermore, analysis of evolutionary mutant models, such as the Antarctic icefish (devoid of hemoglobin and red blood cells (RBCs)), offers additional insights to tackle human disease and to identify novel genetic factors involved in erythropoiesis.

Hematopoiesis in vertebrates (including fish) occurs in two successive waves, that is, primitive (embryonic) and definitive (adult or even larval). Hematopoiesis invokes a hierarchy in which hematopoietic stem cells (HSCs) give rise to multipotential progenitors, which, in turn, generate precursors committed to differentiation along selected lineages.

The formation of RBCs in the developing vertebrate embryo becomes of vital importance as circulation is established and simple diffusion across the body can no longer satisfy increasing O_2 demand. Since RBCs are the first blood cells to appear during embryogenesis, the study of erythropoiesis is important for studying hematopoiesis. Moreover, analysis of the mechanisms by which proteins, such as globins, are highly and specifically expressed in developing RBCs has served as paradigm for transcriptional regulation in higher eukaryotes and has also shed light on the basis of inherited human disorders of hemoglobin production.

The process of hematopoiesis is generally conserved throughout vertebrate evolution, whereas, remarkably, the site of hematopoiesis is not conserved. For instance, the site of adult hematopoiesis in fish is the kidney and the thymus. The frog forms adult blood in the liver, and birds and mammals form blood in the bone marrow. In the frog, *Rana temporaria*, the site of hematopoiesis switches between liver and bone marrow, depending on the season.

Erythropoiesis

Erythropoiesis is the process whereby a fraction of primitive multipotent HSCs becomes committed to the red-cell lineage. Erythropoiesis involves highly specialized functional differentiation and gene expression. The main role of RBCs is to carry O_2 in the blood by the hemoglobin molecule. Therefore, erythropoiesis needs to be tightly regulated to maintain homeostasis and to meet changes in O_2 supply and demand.

The principal factor in the regulation of erythropoiesis is the hormone erythropoietin (Epo), essential for definitive erythropoiesis in all vertebrates studied to date. Epo binds the erythropoietin receptor (EpoR) on erythroid progenitors, stimulating a conserved intracellular signaling program that regulates vertebrate erythropoiesis. In fish, Epo and its receptor have low amino-acid sequence identity compared with mammalian species, and recombinant human Epo is inactive in fish. Even so, the critical functional residues and the physiological role in the regulation of erythropoiesis are well conserved.

The Zebrafish Model

Manipulation of animal models, such as mouse and zebrafish, has complemented and greatly extended studies of human hematopoiesis. The zebrafish (*Danio rerio*) has emerged in recent years as an exciting model for studying vertebrate organ development and heritable disorders. The combined genetic and embryologic advantages make zebrafish ideal for understanding the developmental biology of blood formation (hematopoiesis) in fish.

Although hematologic studies are being undertaken in other fish models, such as medaka (*Oryzias latipes*) and pufferfish (*Takifugu rubripes*), the genetic regulation of erythropoiesis is difficult to study in fish-model organisms other than zebrafish. However, several studies suggest that blood formation and its regulation are similarly widespread among fish.

Blood formation in the zebrafish is similar to that in mammals, but occurs more rapidly (**Figure 1(a)**). The zebrafish and most other fish do not form yolk-sac blood islands during early embryogenesis, but instead primitive hematopoiesis occurs at two intra-embryonic locations, that is, the anterior lateral mesoderm (ALM) and the posterior lateral mesoderm (PLM) that subsequently form the intermediate cell mass (ICM) of Oellacher (see below). The definitive hematopoiesis that contains long-term self-renewing HSC originates in the aorta-gonad mesonephros (AGM) region and caudal hematopoietic tissue (CHT). In zebrafish, the major site of definitive hematopoiesis in the larval period is the pronephros, whereas in the adult stage it takes place in the kidney as well as in the thymus, rather than in the bone marrow as in higher vertebrates.

Based on the expression patterns of blood-specific markers, erythroid, myeloid, and thromboid lineages are found in the CHT. Primitive myelopoiesis takes place in the ALM, whereas primitive erythropoiesis occurs in the ICM. The zebrafish spadetail mutant possesses regular myelopoiesis in the ALM, but fails to make erythroid cells in the ICM, suggesting distinct genetic regulations in these two regions. The ICM-forming fish have been regarded as exceptions to the general rule of yolk-sac hematopoiesis in vertebrates. However, some fish, such as killifish (*Fundulus* spp.), angelfish (*Pterophylum scalare*), and chondrichthyans, form blood predominantly in the yolk sac.

In the posterior ICM, some cells exhibit delayed maturation and may represent the HSCs or definitive progenitors. These may be analogous to definitive progenitors detected in cultures of mouse yolk sac. Hematopoiesis invokes a hierarchy in which HSCs give rise to multipotential progenitors, which, in turn, generate precursors committed to differentiation along selected lineages. HSCs are responsible for production of all mature blood cells, including red cells, myeloid cells (neutrophils and macrophages), mast cells, megakaryocytes, and B- and T-lymphocytes. HSCs may be considered as groups of cells with varying developmental potentials, dependent on intrinsic networks driven by transcription factors. All these networks confer great flexibility to the options of HSCs.

The first morphologically identifiable erythroid precursor cell is the proerythroblast, a cell characterized by a large nucleus and basophilic cytoplasm. The proerythroblast requires Epo for its survival and proliferation, and expresses small quantities of globin mRNA. The expansion, proliferation, and differentiation of erythroid progenitors into mature RBCs in response to exogenous Epo (Epo-injected zebrafish embryos) suggest that the signaling pathways regulated by Epo are functionally similar in mammals and fish. The regulation of Epo by O_2 concentration is functionally relevant to its role as modulator of erythroid cell mass for ensuring adequate O_2 supply. Activation of hypoxia-inducible factor (HIF-1), belonging to the family of basic helix–loop–helix transcription factors, increases the transcription rate of the *Epo* gene and Epo may influence RBC production in response to hypoxia, even if the role of hypoxia, HIF-1, and Epo in stimulating proliferation and differentiation of erythroid progenitor cells remain virtually unexplored in fish. However, evidence suggests that RBC production in fish is Epo sensitive.

Upon its differentiation, the proerythroblast matures progressively to the late normoblast stage, characterized by a condensed nucleus and hemoglobin-containing cytoplasm (**Figure 1(b)**). During primitive erythropoiesis in mammals, and throughout birds, amphibians, and fishes, the nucleus is retained, whereas in mammalian definitive erythropoiesis, mature RBCs become anucleate. The RBCs of all fish retain their nucleus and contain three filamentous systems: an actin/spectrin-based membrane cytoskeleton, intermediate filaments, and microtubules. In contrast, mammals RBCs lose intermediate filaments and microtubules during terminal differentiation and enucleate prior to entering the bloodstream. Thus, adult mammals RBCs are enucleated and contain only one filamentous system, a membrane cytoskeleton. Enucleation is a normal developmental/differentiation process in mammals. Together with the acquired biconcave shape, this lack allows maximal diffusion of O_2 and CO_2 within the cell. The number of molecules of hemoglobin is higher than that of a typical sphere containing a huge nucleus, conferring an important advantage for O_2 transport.

Once mature, RBCs in fish develop a characteristic flattened elliptical shape. Similar to humans, ectotherm RBCs exhibit high metabolic activity, due to the presence

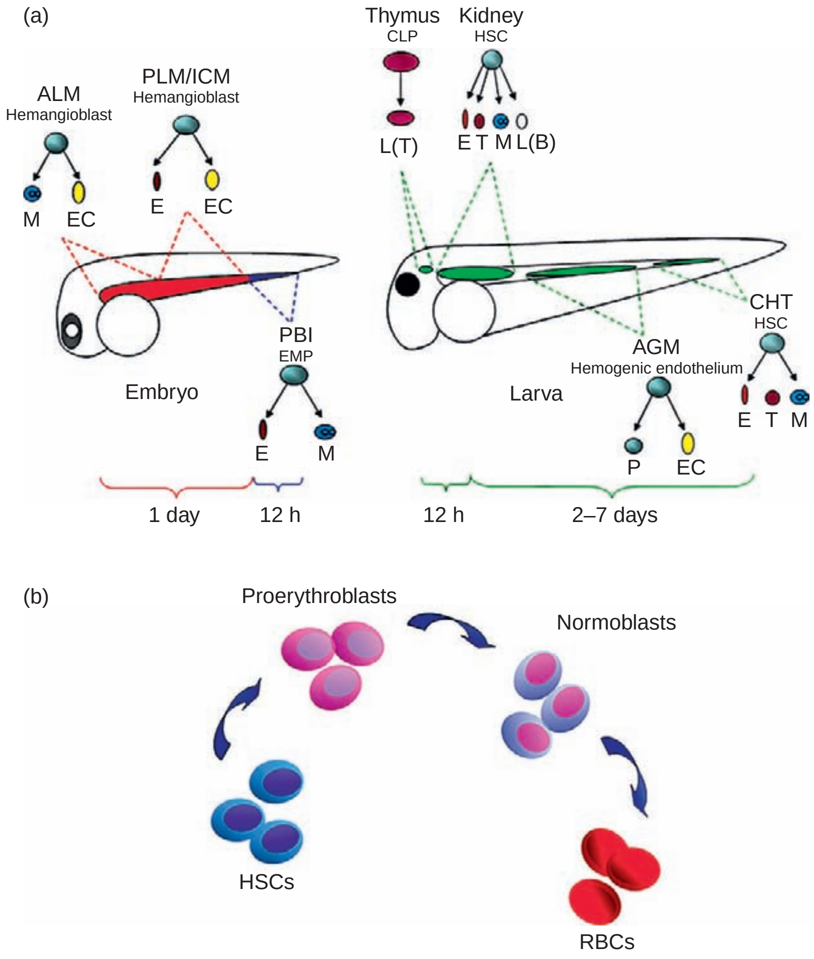


Figure 1 (a) Hematopoiesis in the zebrafish. The first step of hematopoiesis (in red) starts in the embryo with hemangioblasts. (Hemangioblast was hypothesized to be the common progenitor of blood and vascular cells on the basis of intimate association of these lineages in the blood islands of the yolk sac. The presence of hemangioblasts has also been addressed in zebrafish and current studies support the existence of these cells, although it is important to redefine the potential of these cells in the future. It is likely that, phylogenetically, hemangioblasts originated as bi- or pluripotent cells populating the coelomic epithelium of an ancestral invertebrate.) These hemangioblasts give rise to myeloid (M) and endothelial cells (EC) in the anterior lateral mesoderm (ALM), and to erythroid (E) and ECs in the posterior lateral mesoderm (PLM), which later become the intermediate cell mass (ICM). In the posterior blood island (PBI) (in blue), erythromyeloid progenitors (EMPs) originate erythroid and myeloid cells in a transient definitive wave. The next step of hematopoiesis (in green) occurs in the larva with the formation of hematopoietic stem cells (HSCs) in the aorta-gonad-mesonephros (AGM). In AGM, the hemogenic endothelium gives rise to ECs and hematopoietic progenitor cells (P) that migrate to and colonize other sites of definitive hematopoiesis. The AGM progenitor cells seed the caudal hematopoietic tissue (CHT), an intermediate site of definitive blood development, and the kidney that later becomes the primary site of hematopoiesis. The kidney contains all the different blood lineages, including the lymphoid B cell L(B) lineage. Erythroid, myeloid, and thromboid (T) lineages are found in the CHT. The common lymphoic progenitors (CLP) mature into lymphoid T cells L(T) in the thymus. The time of expression of blood-specific markers in different sites is indicated by curly brackets with different colors.. (b) Erythropoiesis during the progressive differentiation from the initial HSCs to the RBCs.

of a complex cellular apparatus (including nucleus, mitochondria, and endoplasmic reticulum). Mature RBCs play a key role in vertebrate and human metabolism. Besides their role in transporting O_2 to the tissues, RBCs interact with many factors (protons, chloride, and organic phosphates) for modulating tissue O_2 supply either by changing the equilibrium between the tense (low-affinity structure) and relaxed (high-affinity structure) states of hemoglobins or by regulating RBC rheology and tissue perfusion. Oxygenation–deoxygenation transitions appear to induce a cascade of tyrosine and serine/threonine phosphorylation reactions involved in O_2 signaling and may lead to the reorganization of the cytoskeleton in fish RBCs.

Definitive Erythropoiesis

Multipotent HSCs capable of self-renewing and producing all blood cells arise during definitive hematopoiesis. In zebrafish, these HSCs are initially found in the CHT and then in the kidney. A subset of HSCs also migrates to the thymus for lymphopoiesis. Definitive erythropoiesis begins at 3.5 days post-fertilization (dpf), as shown by expression of embryonic globins and GATA1 (see below). In zebrafish, the globin switch from embryonic to adult occurs between 15 and 30 dpf. As in other teleost fishes, the zebrafish globin loci are very complex, encoding many α and β chains for hemoglobin switching. The zebrafish embryonic and adult globins display high homology to the human globin genes early expressed during human embryogenesis. It may be postulated that embryonic globin genes in humans and fish are the most evolutionary conserved ones and that their differentiation in embryonic and adult subtypes, in relation to the developmental stage, might have occurred about 450 million years ago after the divergence between teleosts and the ancestors of mammals.

As in mammals, the zebrafish spleen functions as a reservoir for RBC storage and destruction. Erythroid maturation is reflected by changes in cytoplasmic shape, staining, nuclear size, and chromatin density. As in mammals, RBCs in fish are involved in metabolic regulations and blood rheology. Defects in enzymatic and/or metabolic activities produce RBCs abnormalities that, despite the presence of nuclei in fish, mirror human RBC-disease phenotype. Similar to other vertebrates, fish express hemoglobins displaying quaternary structure (α_2/β_2) that undergo globin switching from the embryonic stage to the adult one.

Genes Required in Erythropoiesis: The GATA-Family Example

Vertebrate embryonic hematopoiesis involves critical transcriptional regulation of coordinated gene expression, such as those encoding globins and cell-surface receptors. Several genes are required at the proerythroblast stage to permit or guide terminal erythroid differentiation. These include components of the Epo-signaling pathway and many transcription factors. For the sake of conciseness, only few examples are discussed here. For full description of all components involved in the process of hematopoiesis, the reader is addressed to the section Further reading at the end of the chapter.

The GATA family consists of six transcription factors, GATA1 to GATA6; they all bind to the DNA consensus sequence (A/T)GATA(A/G) by two characteristic (Cys-X2–Cys-X17–Cys-X2–Cys) zinc-finger motifs specific to the GATA family. GATA1 is fundamental for primitive erythropoiesis. It is expressed in primitive and definitive RBCs, megakaryocytes, esinophils, mast cells, and in the Sertoli cells of the testis. In mice, disruption of the GATAl or GATA2 gene leads to severe hematopoietic defects, demonstrating requirement of these factors for normal erythroid maturation. GATA1 and GATA2 are initially expressed during gastrulation, and GATA1 expression is restricted to hematopoietic cells during embryogenesis. GATA2 is expressed in early ventral ectoderm and hematopoietic cells, and is later expressed in the central nervous system. In each vertebrate species studied thus far, GATA1 levels increase and GATA2 levels decrease during erythroid differentiation (**Figure 2**). For instance, GATA1 is highly expressed in megakaryocytic/erythroid progenitors (MEPs) that give rise to megakaryocyte and RBC precursors. GATA2 is required at the level of multipotential progenitors for the proliferation/survival of multipotential progenitors.

In zebrafish, after 12 h post-fertilization (hpf), GATA1 is expressed in a subset of cells expressing the stem-cell leukemia (*scl*) gene encoding a basic helix-loop helix transcription factor. These cells give rise to the first circulating blood cells. Approximately 300 proerythroblasts (*GATA1+*) in the ICM enter circulation around 24 hpf and mature into RBCs. These primitive RBCs are morphologically different from adult zebrafish RBCs endowed with less cytoplasm and a large elongated nucleus. Blood cells of primitive hematopoiesis appear in circulation 1 week after fertilization.

The transcription factors critical for hematopoiesis virtually encompass all classes of DNA-binding proteins. Remarkably, the majority of transcription factors working in the hematopoietic system are also involved in chromosomal translocations or in somatic mutations in human hematopoietic malignancies. The coexistence of transcription factors of different lineages within a common cell (the HSC or immature progenitor) offers the potential for immediate information exchange at the molecular level. The requirements and functions of the principal transcriptional regulators are context dependent. The key lineage-specific factors may promote their own lineage differentiation, and may simultaneously act against factors favoring other choices in lineage programming. At each critical point, genes

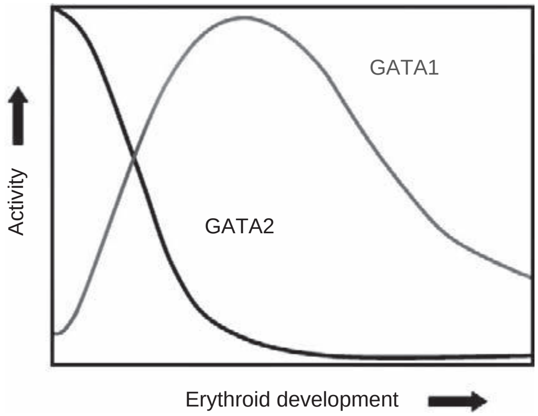


Figure 2 During RBC differentiation, GATA1 is downregulated by an autonomous cell signalling mechanism.

associated with the pertinent pathways are upregulated while the genes linked to lineages not chosen are silenced. The coordination of the silencing of some genes with the activation of others is the mechanism by which cells choose a differentiation pathway.

GATA1 and PU.1 (myeloid-specific transcription factor) promote erythroid/megakaryocytic/esinophil and myeloid differentiation, respectively. The balance between primitive erythroid and myeloid cell production is a delicate one, being mainly governed by the equilibrium between GATA1 and PU.1. Expression of PU.1 and GATA1 are mutually exclusive, suggesting antagonism between these two transcription factors via direct physical interaction. PU.1 is co-expressed with GATA1 in the ICM from 16 hpf to 20 hpf and the equilibrium between these two factors regulates production of primitive erythroid and myeloid cells. *In vivo* confirmation of the different roles of GATA1 and PU.1 exists for zebrafish. Inhibition of GATA1 expression shifts hematopoietic progenitors to a myeloid fate, whereas the opposite occurs upon inhibition of PU.1 expression. The involvement of multiprotein complexes in the action of hematopoietic transcription factors suggests that their relative concentrations influence the choice of lineage in the programming mechanisms. Experiments with zebrafish, in which the pattern of erythroid versus myeloid development is affected by inhibition of expression of GATA1 or PU.1, are consistent with this model. GATA1 also activates structural genes required in RBCs such as globins.

The Icefish Model

The variety of adaptations underlying the ability of modern Antarctic fish to survive at freezing temperatures represents the extreme of low-temperature adaptations in vertebrates. The Antarctic sea, perennially at or near freezing, has a higher O_2 concentration than other waters. In these environments, the selection pressure for O_2-binding hemoproteins was relaxed, and hemoglobin expression was lost in icefishes (family Channichthyidae) (**Figure 3**). Antarctic icefishes do not express the developmental program for RBC formation. The loss of hemoglobin exploits the high O_2 solubility and low metabolic rates in frigid water. The delivery of O_2 to tissues occurs by transport of the gas physically dissolved in the plasma.

The loss of hemoglobin is a derived character shared by the 16 species of the family. Fifteen out of 16 genomes lack most of the adult $\alpha\beta$-globin gene complex and retain only a fragment of the α gene. There is strong evidence that the $\alpha\beta$-globin complexes were also deleted in juveniles.

Therefore, they are a useful evolutionary mutant model for studying hemoglobinopathies and thalassemias found in humans.

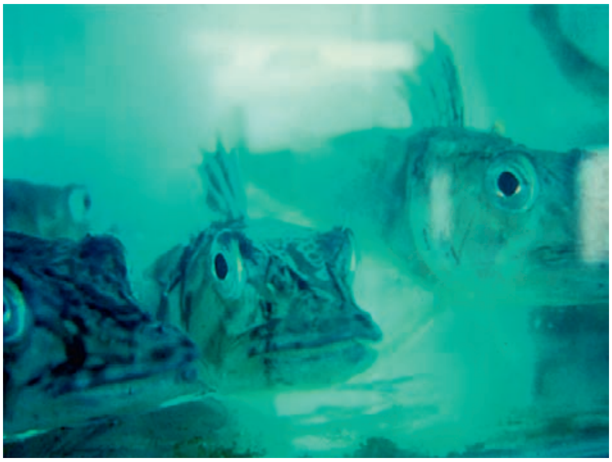


Figure 3 *Chionodraco hamatus* (icefish, family Channichthyidae, suborder Notothenioidei). Photograph by G. di Prisco.

Icefish are natural knockouts of the RBC lineage and therefore may be considered excellent models for erythroid gene discovery by comparative genomics. In fact, they could be paired with closely related, but red-blooded, notothenioids to discover novel erythropoietic genes by subtractive genomic strategies. How hemoglobin loss may influence the terminal erythroid differentiation from the proerythroblast progenitor remains a fascinating question yet to be explored.

See also: **The Reproductive Organs and Processes:** Regulation of Spermatogenesis.

Further Reading

Albertson RC, Cresko W, Detrich HW, III, and Postlethwait JH (2008) Evolutionary mutant models for human disease. *Trend in Genetics* 25: 74–81.

Carradice D and Lieschke GJ (2008) Zebrafish in hematology: Sushi or science? *Blood* 111: 3331–3342.

Cheng C-HC and Detrich III HW (2007) Molecular ecophysiology of Antarctic notothenioid fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362: 2215–2232.

De Jong JLO and Zon Li (2005) Use of the zebrafish system to study primitive and definitive hematopoiesis. *Annual Review of Genetics* 39: 481–501.

di Prisco G, Cocca E, Parker SK, and Detrich HW, III (2002) Tracking the evolutionary loss of hemoglobin expression by the white-blooded Antarctic icefishes. *Gene* 295: 185–191.

Farrell AP and Steffensen JF (eds.) (2005) *Fish Physiology, Volume 22: The Physiology of Polar Fishes,* 408pp. San Diego, CA: Academic Press.

Galloway JI, Wingert RA, Thisse C, Thisse B, and Zon LI (2005) Loss of gata 1 but not gata 2 converts erythropoiesis to myelopoiesis in zebrafish embryos. *Developmental Cell* 8: 109–116.

Hartenstein V (2006) Blood cells and blood cell development in the animal kingdom. *Annual Review of Cell and Developmental Biology* 22: 677–712.

Orchin HS and Zon LI (2008) Hematopoiesis: An evolving paradigm for stem cell biology. *Cell* 132: 631–644.

Sidell BD and O'Brien KM (2006) When bad things happen to good fish: The loss of hemoglobin and myoglobin expression in Antarctic icefishes. *Journal of Experimental Biology* 209: 1791–1802.

Song H-D, Sun X-J, Deng M, *et al.* (2004) Hematopoietic gene expression profile in zebrafish kidney marrow. *Proceedings of the National Academy of Sciences of the United States of America* 101: 16240–16245.

Warga RM, Kane DA, and Ho RK (2009) Fate mapping embryonic blood in zebrafish: Multi- and unipotential lineages are segregated at gastrulation. *Developmental Cell* 16: 744–755.

CIRCULATION

Design and Physiology of the Heart

Contents

Cardiac Anatomy in Fishes
Cellular Ultrastructure of Cardiac Cells in Fishes
The Outflow Tract from the Heart
Physiology of Cardiac Pumping
Action Potential of the Fish Heart
Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux
Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element
Cardiac Cellular Length-Tension Relationship
Intracardiac Neurons and Neurotransmitters in Fish
Accessory Hearts in Fishes
The Coronary Circulation

Cardiac Anatomy in Fishes

AP Farrell and S Pieperhoff, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
The General Anatomical Structure of a Fish Heart
Further Reading

Glossary

Atrium A thin-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.
Cardiac Pertaining to the heart.
Ductus Cuvier A pair of horn-shaped vessels that lie outside the pericardium, which are connected to the sinus venosus and collect venous blood from central veins.
Endocardium Inner part (infolding) of the pericardial sac, and is the inner lining of the cardiac chambers.
Ostial valves Flap-like connective tissues that extend one side of an opening (ostium), in this case the opening connecting cardiac chambers, which serve to prevent blood from flowing back through the opening when a chamber contracts.
Outflow tract A general term for the vessel through which blood leaves the ventricle. In fish, this is the bulbus arterious, the conus arteriosus, or the ventral aorta.
Pericardium A cellular membrane that creates a sac to surround and enclose the heart, and which contains a small amount of pericardial fluid. This sac is closed in teleost fishes, but in sharks there is a pericardio-peritoneal canal that allows regulated exchange of fluid between the pericardial and peritoneal cavities.
Sino-atrial node A region of the heart near the junction of the sinus venosus and atrium, where the specialized cardiac fibers that initiate the heartbeat (pacemaker cells) are located.
Sinus venosus A thin-walled chamber of the heart that contains little cardiac muscle but is the site of the cardiac pacemaker.
Trabecula(ae) A bundle of cardiac muscle fibers, which is the most common arrangement for the cardiac muscle in the walls of the atrium and ventricle.
Ventricle A thick-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Introduction

The heart is the powerhouse of the cardiovascular system. It propels blood (blood cells and plasma) around the circulatory system by imparting potential energy (blood pressure) and, to a lesser degree, kinetic energy (velocity) to the blood. This article considers the anatomy of the heart in fish.

Cardiac anatomy varies closely with cardiac function, which in turn varies considerably among fish. Nevertheless, some generalities apply to most fish. These generalities are considered first. Then, the anatomical diversity among fish is presented for each cardiac chamber. The cellular aspects of the fish heart are covered in **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish.

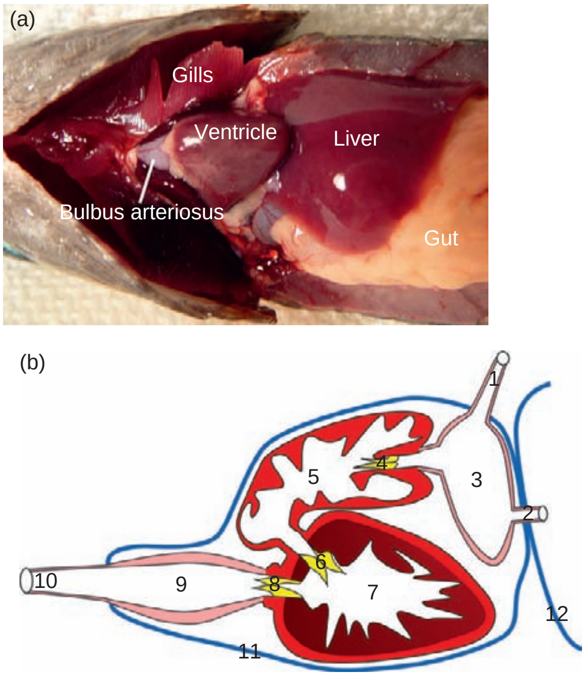
The General Anatomical Structure of a Fish Heart

Overview

The fish heart is located ventrally, in front of the pectoral fins and peritoneal (gut) cavity, but generally behind the gill structures (**Figure 1(a)**). The heart is also referred to as the branchial heart or systemic heart because it pumps blood first to the gill and then to the systemic circulations that lie in series. This arrangement is called a single circulatory system because a red blood cell that leaves the heart passes through both the respiratory and systemic circulations before returning to the heart. This nomenclature distinguishes the branchial heart from the accessory hearts also found in some fish (**see also Design and Physiology of the Heart:** Accessory Hearts in Fishes) and that assist in the pumping of fluid around the circulatory system.

The heart is positioned within a membranous sac, the pericardial cavity, which is created by a layer of mesothelial (epithelial-like) cells in addition to connective and fat tissue, called the pericardium. The epicardium represents the inner part (infolding) of the pericardium and is often directly attached to the cardiac chambers (see below). The pericardium separates the heart from the rest of the body. The pericardium is variably stiff and may be fused with other body tissues. For example, the peritoneum and pericardium are fused on the posterior wall of the pericardial cavity, and the dorsal wall is fused with esophageal tissue. The pericardial cavity contains a small amount of pericardial serous fluid that bathes the heart and provides lubrication as the heart moves with each heartbeat.

Within the pericardium, there are four distinct chambers that comprise the fish heart: the sinus venosus, the atrium, the ventricle, and an outflow tract (**Figure 1(b)**). Each chamber plays a key, but different role in the overall function of the heart. Consequently, fish are generally considered to have a four-chambered heart, with the chambers arranged in series to support a single circulation and so a red blood cell is pumped twice by the heart before reaching the systemic tissues, that is, a red blood cell leaving the heart normally returns to the heart after a single circuit through the body. In contrast, the mammalian heart, which is also considered to be four chambered, possesses two atria and two ventricles, with one pairing of an atrium and a ventricle being arranged in parallel with the other to form a double circulatory system, that is, a red blood cell leaving the heart would normally return to the heart after first passing through the pulmonary circulation. However, because only the atrium and the ventricle contribute appreciably to the generation of blood pressure, fish hearts are sometimes referred to as being two-chambered.



1 Ductus Cuvier
2 Hepatic vein
3 Sinus venosus
4 Sino-atrial valve (ostial valve)
5 Atrium
6 Atrio-ventricular valve
7 Ventricle
8 Bulbo-ventricular valve
9 Bulbus arteriosus
10 Ventral aorta
11 Pericardium
12 Peritoneum

Figure 1 (a) A ventral view of a rainbow trout with a mid-line incision to reveal the anatomical organization and shape of the cardiac chambers. The pericardial cavity was opened for this view. Note the coronary artery visible on the white surface of the bulbus arteriosus. (b) A schematic diagram of the cardiac chambers as seen from a lateral view.

From a lateral view, the chambers of the fish heart have an S-shaped appearance (**Figure 1(b)**). The sinus venosus is connected to the ductus Cuvier, a pair of horn-shaped collecting vessels lying outside the pericardium.

The outflow tract is connected to ventral aorta, which lies outside the pericardial cavity and takes blood to the gill vessels. Blood returning from the venous system collects in the ductus Cuvier and flows freely into the sinus venosus as no valves protect these connections. Similarly, there are no valves in the outflow tract and the ventral aorta except in elasmobranchs and other primitive fish lineages (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart).

The sino-atrial (SA) canal, which connects the sinus venosus to the atrium, has a one-way ostial valve. This valve prevents blood from back flowing into the sinus venosus when the atrium contracts. Similarly, the atrioventricular canal, which connects the atrium to the ventricle, has a one-way ostial valve serving a similar purpose. The ventricle then connects to an outflow tract (either the bulbus arteriosus (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart) or conus arteriosus via another one-way ostial valve (**Figure 2**)).

Cardiac muscle is the predominant tissue type in the walls of the atrium and ventricle and is mainly composed of connected muscle cells (the so-called cardiomyocytes). When this muscle contracts, the pressure of the blood contained with the lumen of these chambers rises appreciably. The ventricle contains more cardiac muscles than the atrium and therefore contributes most to the generation of blood pressure. Even so, relative ventricular mass varies over an order of magnitude, representing anywhere from 0.03% to 0.40% of the fish's body mass among fish species. Atrial mass is about one-tenth to one-fifth of the ventricular mass. The atrium has a thinner wall and a greater internal distended volume than the ventricle.

The single arterial outflow tract is also thick walled. Wall thickness, which is needed to contain and store blood pressure energy, varies among species. In teleosts, the wall is comprised mainly of vascular smooth muscle and connective tissues, containing for the most part fibroblastoid cells and extracellular matrix material such as elastin and collagen. It is called the bulbus arteriosus. In elasmobranchs and other primitive fish, the outflow tract has an additional outer layer of cardiac muscle and is called the conus arteriosus.

All cardiac chambers are lined with endothelial cells, the endocardium, similar to the remainder of the cardiovascular system in fish. The outside of the heart has a layer of epithelial cells and connective tissue called the epicardium, embryologically representing the inner part (infolding) of the pericardium. Therefore, the atrial and

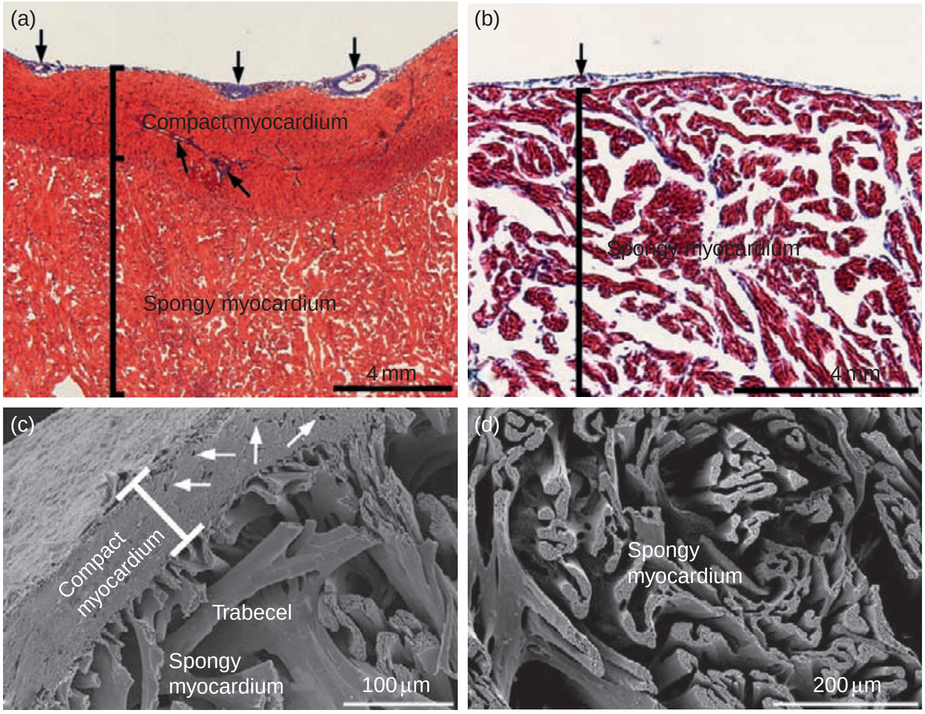


Figure 2 (a) A histological cross section from the heart of a salmonid fish to illustrate the composition of the ventricular wall and in particular the compact layer of myocardium that encases the spongy myocardium. (b) A histological cross section from the heart of *Tilapia* to illustrate the composition of the ventricular wall and in particular to illustrate the complete spongy myocardium. (c) A scanning electron micrograph of the wall of the salmon ventricle to illustrate the two types of myocardial architecture. The arrows point to coronary vessels in the compact myocardium. (d) A scanning electron micrograph of wall of the *Tilapia* ventricle heart to illustrate the two types of myocardial architecture.

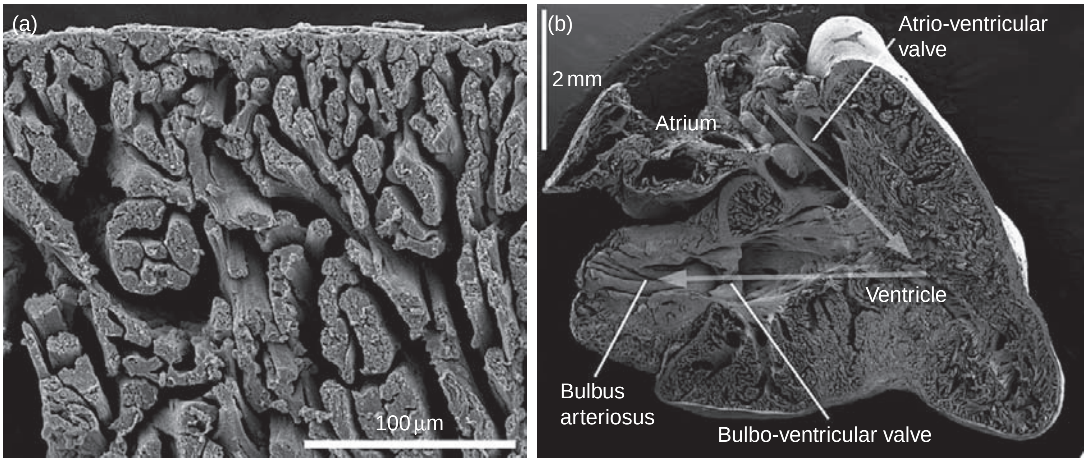


Figure 3 Scanning electron micrographs of (a) part of the atrium and (b) the ventricle and the outflow tract in a saggital section to reveal the interior architecture of the cardiac chambers of a sockeye salmon. The blood flow direction is indicated by the arrows.

ventricular cardiac muscle is sandwiched between endocardial and epicardial tissues. Interstitial cells and extracellular collagen and elastin fibers are also sparsely dispersed in between the interconnected cardiac muscle fibers.

The epicardium has a variable thickness (10 to more than 100 µm) depending on the age of the fish (**Figures 3(a)** and **3(b)**). It also contains blood vessels, nerve fibers, and adipocytes; in addition, a subepicardial layer of loose connective tissue contains predominantly collagen and elastin fibers. The blood vessels (part of the coronary circulation) either can be entirely epicardial (i.e., they do not penetrate the cardiac muscle) as found in the plaice (*Pleuronectes flesus*), or they can penetrate the cardiac muscle and form capillaries within the cardiac muscle, as found in rainbow trout (*Oncorhynchus mykiss*) (**Figure 2(c)**). Some of these superficial vessels are coronary veins and venules that coalesce toward the atrioventricular region, which is where the coronary vein drains into the lumen of the heart (**see also Design and Physiology of the Heart:** The Coronary Circulation).

Sinus venosus and ductus Cuvier

All systemic veins terminate in either the ductus Cuvier or the sinus venosus. There are no one-way valves associated with the termination of these veins. The thin-walled (5–100 µm thick) ductus Cuvier and sinus venosus contain smooth muscle, which can contract to alter their volume. The sinus venosus has the thinnest wall of all the cardiac chambers and its volume can be larger or smaller than that of the ventricle, depending on the species. The sinus venosus also contains a sparse and variable arrangement of bundles of cardiac muscle. An exception is the continuous, vascularized layer of cardiac muscle seen in the sinus of the common eel (*Anguilla anguilla*).

The cardiac muscle in the sinus venosus is spontaneously active and the electrical events associated with the contractions are seen as the V-wave preceding the P-wave of the electrocardiogram (ECG) (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). V-waves are not reported for all fish, perhaps because so few cardiomyocytes cannot produce a detectable ECG signal. Contraction of the sinus venosus, in the absence of a valve guarding the entrance of the sinus venosus, is just as likely to propel blood away from as well as toward the atrium.

Some of the cardiomyocytes in the basal region of the SA canal are specialized and spindle shaped. They act as pacemaker or conductive tissue. Electrophysiological recordings from cells in this region have revealed the typical pacemaker potentials that initiate the heartbeat (**see also Design and Physiology of the Heart:** Action Potential of the Fish Heart). This region is often called the SA nodal tissue and often forms a ring around the SA canal. It may be functionally analogous to the mammalian SA node in the right atrial wall. In contrast, the existence of a well-defined conduction system in fish is still controversial.

The cardiac branch of the vagus nerve, which is present in all fish except the hagfishes (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes), joins the wall of sinus venosus after following the tract of the ductus Cuvier. The SA node is richly innervated with myelinated and unmyelinated nerve fibers, and includes nerve ganglions (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes and **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish). The density of the innervation in this region is so high that it is often referred to as a plexus. Cardiac control of pacemaker activity is possible through the vagus nerve. The distribution of autonomic nerves and intracardiac nerves throughout the entire heart is detailed in **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish.

The density of collagen in the sinus venosus increases toward the SA canal. This is likely related to the structural support needed for the SA valve. The SA valve is typically comprised of cells that may be similar to the mammalian valvular interstitial cells, which build up huge amounts of extracellular matrix material (mainly collagen bundles). They act like the cuspid valve of other vertebrates in preventing blood from flowing backward when the atrium contracts.

Atrium

The SA canal connects to the middle of the posterior wall of the atrium. There are no layers to the atrial cardiac muscle. Instead, the cardiac muscle is arranged in bundles (trabeculae) of variable diameter (10–30 μm).

Although thin walled, the atrium is not simply a sac-like structure (**Figure 2**). Trabeculae crisscross the atrial wall, creating a mesh-like arrangement without generally traversing opposing walls. Instead, the trabeculae create shorter arcs that form small lobular or cava-like structures within the atrial chamber. Therefore, the atrium has a well-defined, tissue-free lumen.

The trabecular length (especially in the ventricle) greatly exceeds that of an individual cardiomyocyte, which rarely reaches 200 μm. This means that many cardiomyocytes must be longitudinally arranged to form a single trabecula. While mammalian cardiomyocytes are branched and connect end-on-end, the tapered shape of fish cardiomyocytes likely leads to an overlapping pattern to ensure that each cardiac cell is robustly connected with its neighbor. Since individual cardiomyocytes are typically 3–6 μm in diameter in fish (**see also Design and Physiology of the Heart:** Cellular Ultrastructure of Cardiac Cells in Fishes), each trabeculum likely contains no more than about 10 cardiomyocytes side by side.

Innervation of the atrial cardiac muscle is extensive (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes and **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish) with one study suggesting about one axon for every two cardiomyocytes.

Ventricle

The atrio-ventricular canal connects to the middle of the dorsal wall of the ventricle (**Figure 3**). For most fish, the ventricle has only one architectural arrangement of the cardiac muscle (**Figures 2(b)** and **2(d)**). Similar to the atrium, trabecular bundles crisscross the ventricle, but unlike the atrium the density is much higher and some trabeculae span to opposing walls of the ventricle. This is more clearly seen for trabeculae spanning almost the full length of the ventricle, from the apex to the base at the bulbo-ventricular junction. As a result of the extensive trabecular network, the lumen of the ventricle may be taken on an obscure, sponge-like appearance, more so in some species than others – hence the term spongy myocardium or spongiosa. However, scanning electron microscope images reveal a much more sophisticated structure to the trabeculae (**Figure 3**). The trabeculae can form sheet-like structures, which may assist the directional flow of blood within the ventricle. These sheet-like trabeculae also increase the cross-sectional area of a trabecula (the force that is generated by a muscle is proportional to its cross-sectional area) without greatly increasing the shortest diffusional distance to the center of a trabecula. In such cases, a trabecular muscle sheet can be over 100 μm wide, but only 30 μm deep. A narrow sheet like this has an advantage for myocardial oxygen delivery; venous blood can have a low oxygen partial pressure during exercise because the systemic tissues have already stripped blood of most of its oxygen (**see also Design and Physiology of the Heart:** The Coronary Circulation) and the diffusion rate for oxygen is determined principally by the venous oxygen partial pressure and the diffusion distance, which is set by the trabecular cross-sectional dimensions. A coronary vessel can be located inside of trabeculae of elasmobranchs and large pelagic teleosts, perhaps reducing the need for a sheet-like arrangement.

The ventricle has a second layer of cardiac tissue in those fish that have a coronary circulation that penetrates the ventricle (**see also Design and Physiology of the Heart:** The Coronary Circulation). The layer of cardiac muscle, which is richly invested with coronary capillaries (**Figures 2(a)** and **2(c)**), is sandwiched between the epicardium and the spongy myocardium, and envelops the spongy myocardium. This outer layer of cardiac muscle is referred to as the compact myocardium or compacta because the individual fibers are densely packed circumferentially around the ventricle. The compact myocardium can have 2–4 sublayers, each having a different fiber orientation. Depending on the fiber orientation, these sublayers can variously create an apically directed contraction, contraction that constricts the chamber and reduces the height and length of the ventricular walls. In contrast, when trabeculae contract, their crisscross arrangement across the ventricular walls tends to pull the walls of the ventricle inward. Also, the caudal apex toward the arterial outflow vessels if the trabeculae extend from the apex of the ventricle to the bulbo-ventricular junction can be pulled.

Innervation of ventricular muscle is far less extensive than in the atrium (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes and **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish), with one study suggesting about one axon for every 25 cardiomyocytes. While some of this innervation may reach the ventricle via the atrium, there is also a possibility that axons arrive along the coronary artery, which typically lies on the arterial outflow tract (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart). The presence of adipocytes in the epicardium is likely to be related to the fish's nutritional status. Over-fed farmed salmon have excessive cardiac fat deposits near the bulbo-ventricular region and around coronary vessels.

Arterial outflow vessels

The ventricle ejects blood into either a bulbus arteriosus or a conus arteriosus. The former is comprised mainly of vascular smooth muscle and connective tissue, whereas the latter has an outer coating of cardiac muscle. In addition, the valves are arranged differently. The anatomy and functions of the conus arteriosus and bulbus arteriosus are considered in detail in **Design and Physiology of the Heart:** The Outflow Tract from the Heart.

Comparative Cardiac Anatomy

With over 25 000 species of fish, it is impossible to do justice to the range of diversity that exists for cardiac anatomy. The following sections, therefore, simply discuss some of the more general differences and then discuss features that are unique to certain vertebrate groups.

Cardiac shapes and sizes

The shape and size of the cardiac chambers reflects two influences: fit and function. Foremost, the heart must fit within a fish, but body shape varies enormously among fish. Consequently, a fish with an elongated body shape tends to feature an elongated heart. Second, the heart must perform its job appropriately. Higher blood pressures demand a thicker ventricular wall (compact myocardium) and higher stroke volumes demand a larger chamber volume (**Figure 4**).

The atrium has a variable shape and size. One extreme is where the most anterior part of the atrium almost completely envelops the outflow vessels and laterally extends over the wall of the ventricle. The other extreme is where sides of a small atrium are barely in contact with the ventricle (**Figure 4**).

There are three primary ventricular shapes, in part, determined by that of the fish: tubular, sac-like, and pyramidal (**Figure 4**). However, tubular and sac-like shapes are not always associated with an elongated body shape. Anguillid eels have a pyramidal heart, as do salmonids and tunas. The pyramidal heart appears to be associated with a coronary circulation and compact myocardium.

The relative mass of the ventricle varies by almost an order of magnitude. At one extreme are sluggish fish that live on the bottom and invest only 0.05% of their body mass into a sac-like ventricle. These fish have spongy myocardium and a low-blood-pressure generating capability. At the other extreme are the tunas with up to 0.4% of their body mass invested in their ventricle. Their pyramidal heart can generate arterial blood pressures approaching those found in mammals. Relative ventricular mass is more typically 0.1% or less in most fish; a larger ventricle is associated with athletic fish, presumably to support a higher arterial blood pressure. An exception to this generality exists for certain cold-water species. These fish compensate for the weaker cardiac muscle contraction at cold temperature by enlarging their ventricle, either through an adaptation or by phenotypic modification during cold acclimation. As a result, they have a relatively larger ventricle. For example, rainbow trout acclimated to cold conditions can almost double relative ventricular mass compared to warm acclimation. An extreme example of ventricular enlargement

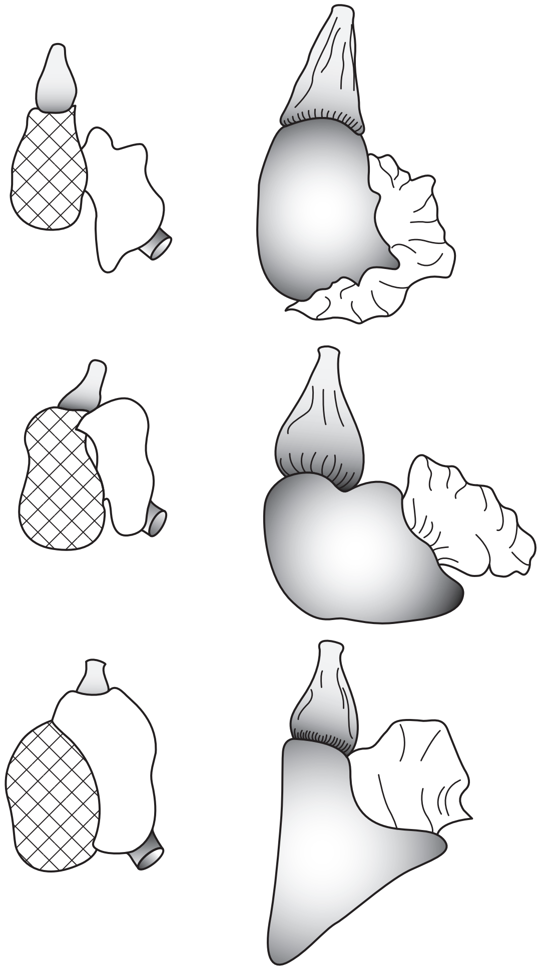


Figure 4 Illustrations of three different ventricular shapes (right column) and atrial arrangements relative to the ventricle (left column) in fish. These illustrate that the morphology of the heart is influenced by the fish's physiology (e.g., how hard does the heart work) and anatomy (e.g., into what body shape must the heart fit). Adapted from Santer RM (1985) Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology and Cell Biology* 89: 1–102.

has evolved in Antarctic icefish, which have no red blood cells and live in water typically below 0 °C. Their relative ventricular mass is 0.4%, which is as large as the ventricle in the more athletic tunas. However, unlike that of the tuna, the icefish ventricle is thin walled, has a large internal luminal volume, and lacks a coronary circulation. Sexual development in male rainbow trout as well as anemia in rainbow trout and zebrafish (*Danio rerio*) can produce ventricular remodeling and enlargement.

The proportion of compact myocardium also varies widely among fish. This can reach 75% in some tuna species, but is commonly less than 30%. Some elasmobranchs have 10% or less compact myocardium. Since the compact myocardium is always associated with a coronary circulation, it is not surprising to find a high percentage of compact myocardium in athletic fish with their high blood pressures and cardiac oxygen needs (**see also Design and Physiology of the Heart:** The Coronary Circulation). Habitat may also influence the development of compact myocardium. However, while the hypoxia-tolerant common eel has a compact myocardium, not all hypoxia-tolerant fish do so. Thus, phylogenetic considerations may override the roles of athleticism and hypoxia in terms of the presence and abundance of compact myocardium. For example, no cyclostome heart has compact myocardium; all elasmobranchs and only some teleosts have compact myocardium.

Cyclostomes

A number of anatomical features are specific to the cyclostome heart. Hagfish have a modified pericardial cavity, which communicates fully with the peritoneum via an enlarged pericardio-peritoneal canal (**see also Hagfishes and Lamprey:** Hagfishes). Further, the pericardium terminates anteriorly at the junction of the ventricle and the ventral aorta. As such, hagfish do not have an outflow tract inside of the pericardium and so there is no true conus or bulbus arteriosus. The ventral aorta is very thin walled, reflecting the low blood pressure within. In lampreys, the pericardium is closed, as in teleost species.

Adult cyclostomes possess a single ductus Cuvier. The left duct is retained during development in hagfish, whereas the right duct is retained in lampreys. Lampreys, but not hagfish, also have a high relative ventricular mass.

The hagfish heart lacks innervation, whereas the lamprey heart has rudimentary vagal innervation (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes and **Design and Physiology of the Heart:** Intracardiac Neurons and Neurotransmitters in Fish). Hagfish have specialized chromaffin cells that contain varying quantities of adrenaline and noradrenaline, and may have paracrine effects on the heart. The chromaffin cells predominate in the sinus venosus and decrease in abundance from the atrium to the ventricle.

Elasmobranchs

The pericardium of elamobranchs tends to be thicker, more fibrous, and more rigid than that of teleosts (**see also Chondrichthyes:** Physiology of Sharks, Skates, and Rays). The pericardium communicates with the peritoneum via a narrow pericardio-peritoneal canal, which allows pericardial fluid to move between the pericardium and the peritoneum. Adjusting the volume of pericardial fluid within a rigid pericardial cavity can greatly alter the pressure exerted on the outside of the heart. This transmural pressure then influences the ease with which the heart is filled by venous blood pressure. The intra-pericardial pressure is normally subambient, in part, because of the relatively stiff nature of the pericardium, but becomes more negative if pericardial fluid moves out of the pericardium.

All elasmobranches possess a conus arteriosus within the pericardium (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart). Branchial arteries are formed immediately anterior to the pericardium without there being a ventral aorta prior to these branches, as is the case for most teleosts. The conus arteriosus is invested with circumferentially arranged cardiac muscle and is contractile. Longitudinally arranged cardiac fibers have been found in the conus arteriosus of large sharks. Superficial coronary vessels, clearly visible on the surface of the conus arteriosus, deliver blood from the gills to the cardiac muscle of the conus arteriosus and ventricle. The longitudinal coronary vessels supply blood to the heart and the lateral branches supply blood to the conus arteriosus (**see also Design and Physiology of the Heart:** The Coronary Circulation). The conus arteriosus has multiple tiers of valves, which vary in number from 2 to 6 among species (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart) and its own ECG, as noted above.

Coronary arterioles and capillaries supply the ventricular trabeculae as well as the compact myocardium of elasmobranchs. The trabecular coronary vessels have been seen to communicate with, and therefore likely drain to, the lumen of the heart. Sometimes, the atrial trabeculae have capillaries. This means that the oxygen supply of the spongy myocardium of the elasmobranch heart is not restricted to the venous blood, as in most teleosts (**see also Design and Physiology of the Heart:** The Coronary Circulation). This anatomical feature may increase the hypoxia tolerance of elasmobranch heart. In contrast, capillaries in the ventricular trabeculae have been reported only for the highly athletic swordfish and tuna.

Air-breathing fish

Some of the most diverse cardiac anatomy is seen in air-breathing fish, but this is detailed in (**Air-Breathing Fishes:** Respiratory Adaptations for Air-Breathing Fishes.

See also: **Air-Breathing Fishes:** Respiratory Adaptations for Air-Breathing Fishes. **Brain and Nervous System:** Autonomic Nervous System of Fishes. **Chondrichthyes:** Physiology of Sharks, Skates, and Rays. **Design and Physiology of the Heart:** Cellular Ultrastructure of Cardiac Cells in Fishes; Intracardiac Neurons and Neurotransmitters in Fish; Physiology of Cardiac Pumping. **Design and Physiology of the Heart:** Accessory Hearts in Fishes; Action Potential of the Fish Heart; The Coronary Circulation; The Outflow Tract from the Heart. **Hagfishes and Lamprey:** Hagfishes.

Further Reading

Brill RW and Bushnell PG (2001) The cardiovascular system of tunas. In: Block BA and Stevens ED (eds.) *Tuna Physiology, Ecology and Evolution,* 1st edn., vol. 19, pp. 79–120. San Diego, CA: Academic Press.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac function in fish. *Physiological Zoology* 64: 1137–1164.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 1–73. San Diego, CA: Academic Press.

Gamperl AK and Farrell AP (2004) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Santer RM (1985) Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology and Cell Biology* 89: 1–102.

Satchell GH (1991) *Physiology and Form of Fish Circulation,* pp. 1–235. New York: Cambridge University Press.

Cellular Ultrastructure of Cardiac Cells in Fishes

GLJ Galli, University of Manchester, Manchester, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Fish Cardiomyocyte Ultrastructure
Summary
Further Reading

Glossary

A-band Dark region of the myofibril. It mainly consists of the protein myosin.

Acclimation Physiological and/or biochemistry adjustments made by an organism in response to a prolonged change in their environment, e.g., temperature.

Area composita Specialized very strong junction connecting adult mammalian cardiomyocytes. It consists of typical desmosomal and adherens junction components.

Cardiomyocyte Fundamental contractile cell of the working fish myocardium.

Caveolae Small (150 nm in diameter), flask-like invaginations of the cell (sarcolemmal) membrane which increases the cell's surface area-to-volume ratio and plays an important role in cell-signaling pathways.

Costamere Lateral attachment structure connecting the Z-bands of striated muscle cells to extracellular matrix material.

Cristae Invaginations of the inner membrane of a mitochondrion.

Desmosome A plaque-like structure on the surface of a cell that functions in adhesion with adjacent cells.

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Endotherm An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Fascia adheren junction Involved in cell-to-cell connections. It connects two membranes of adjacent cardiomyocytes and is usually attached to actin filaments of myofibrils.

Free sarcoplasmic reticulum Sarcoplasmic reticulum that is not closely associated with either the sarcolemmal membrane or the t-tubular network.

Gap junction A connection between two neurons that allows electrical signals to pass directly between them.

Hemoglobin An iron-containing oxygen-transport protein in the red blood cells of vertebrates and the tissues of some invertebrates.

Hyperplasia An increase in the number of cells of an organ or tissue. In this article, it refers to an increase in cardiomyocyte number.

Hypertrophy An increase in the cell size of an organ or tissue. In this article, it refers to an increase in cardiomyocyte size.

I-band Region of the myofibril that stains lightly. It mainly consists of the protein actin.

Invagination The folding of a cell membrane.

Junctional sarcoplasmic reticulum Sarcoplasmic reticulum that is closely associated with either the sarcolemmal membrane or the t-tubular network.

M-band Central region of the myofibril that forms a broad, dense band in the center of the A-band.

Mitochondrion A cellular organelle that acts as the 'powerhouse' of the cell and produces the chemical energy source adenosine triphosphate, using oxygen as the final electron acceptor.

Myofibrils Bundles of protein filaments that contain the contractile elements of the cardiomyocyte. They are the machinery, or motor, that drives contraction and relaxation.

Myoglobin An oxygen-binding protein in muscle that resembles hemoglobin in function, and enhances the rate of oxygen diffusion.

Organelle An intracellular component of a cell, for example, mitochondria, nucleus, and myofibril.

Ryanodine receptor It mediates the release of Ca^{2+} from the sarcoplasmic reticulum.

Sarcolemmal membrane The lipid bilayer that surrounds the cardiomyocyte, forming a barrier between the intra- and extracellular environments.

Sarcomere The functional contractile unit of the myofibril. The sarcomere is found between two Z-lines.

Sarcoplasmic reticulum A specialized form of endoplasmic reticulum that acts as a storage site for Ca^{2+}.

Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) An energy-consuming Ca^{2+}-pump located on the sarcoplasmic reticulum membrane that concentrates Ca^{2+} inside the sarcoplasmic reticulum.

Thick filament Protein filament in the myofibril that is made up of myosin.

Thin filament Protein filament in the myofibril that is made up of actin.

T-tubular network Deep invaginations of the sarcolemmal membrane that bring the external environment in close contact with the center of the cell.

Working myocardium The regions of the heart that contract and propel the blood when excited via excitation-contraction coupling. These muscular regions are made up of the contractile myocytes of the atrium and ventricle.

Z-line Narrow dark bands in the myofibril that define the length of a sarcomere.

Introduction

Contraction and relaxation of the working fish myocardium is a result of the complex interaction of many individual cells connected together by specialized adhesion structures. It is these cells that allow the heart to beat and generate pressure to pump blood around the body. The fundamental contractile cell of the working fish myocardium is called a cardiomyocyte. As with other cells that constitute organs, the structure of a cardiomyocyte is specially adapted for its function. In the case of fish cardiomyocytes, they are adapted to function under highly variable conditions, including fluctuating temperatures, oxygen, and pH. In fact, the ultrastructure of the fish cardiomyocyte is malleable and can both adjust (acclimate) and be adapted to its environment, making it an excellent example of how cellular structure can fit perfectly to its function.

Fish Cardiomyocyte Ultrastructure

Cardiomyocyte Dimensions

The dimensions of vertebrate cardiomyocytes differ greatly between vertebrate species (endotherms or ectotherms), tissue type (atria or ventricle), cell type (muscle or pacemaker), and even between different regions of the same tissue (outer or inner layer of the ventricle). **Table 1** shows the dimensions of cardiomyocytes from a range of different fish species and, for comparison, from a mammalian ventricle. Typical images of a fish and mammalian ventricular cardiomyocyte (**Figure 1**) reveal the obvious dimensional differences. Fish cardiomyocytes appear long, thin, and spindle shaped (**Figure 1**(**a**)), while mammalian cardiomyocytes are rectangular (**Figure 1**(**b**)). The width and depth of the fish cardiomyocyte is much smaller, leading to a lower volume, and greater surface area-to-volume ratio (S/A ratio), while lengths may be comparable (**Table 1**). Fish atrial myocytes are similar in length to ventricular myocytes, but are slightly thinner, leading to a smaller cell volume and S/A ratio (**Table 1**). The large S/A ratio in fish has important implications for Ca^{2+} movement into the cardiomyocyte during excitation–contraction coupling (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

Cardiomyocyte dimensions among different fish species are quite similar, even though body size and heart mass vary enormously (**Table 1**). Remarkably, cardio myocytes of a 0.55 g zebrafish (*Danio rerio*, heart mass = 0.001 g) are only marginally smaller than a 1400 g bluefin tuna (*Thunnus orientalis*, heart mass = 52 g) (**Table 1**). Thus, the fish heart grows larger primarily by increasing the number of cardiomyocytes (hyperplasia) in the myocardium, rather than the cardiomyocyte size (hypertrophy).

Cardiomyocyte hypertrophy does occur as fish grow, but most notably when a fish enters cold water for long periods of time (termed 'cold acclimation'). Under this circumstance, cardiomyocyte size is enlarged, mainly as an adaptation to the cold. Similarly, under pathological

Table 1 Cardiomyocyte dimensions in fishes compared with mammals

Dimensions	*Rat V*	*Trout V*	*Trout A*	*Carp V*	*Tuna V*	*Mackerel V*	*Zebrafish V*
Length (μm)	141	180	197	110	186	167	100
Width (μm)	32	8	5	6	8	5	5
Volume (pL)	34	3	2	1	3	2	2
Surface area-to-volume ratio (pF/pL)	8	18	15	19	14	22	12

Cell length, width and volume was measured by analysis of confocal or light microscopy images of cardiac myocytes. Rat, *Rattus norvegicus*; trout, *Oncorhynchus mykiss*; carp, *Carassius carassius*; mackerel, *Scomber japonicus*; zebrafish, *Danio rerio*. V = Ventricular myocytes, A = artrial myocytes.

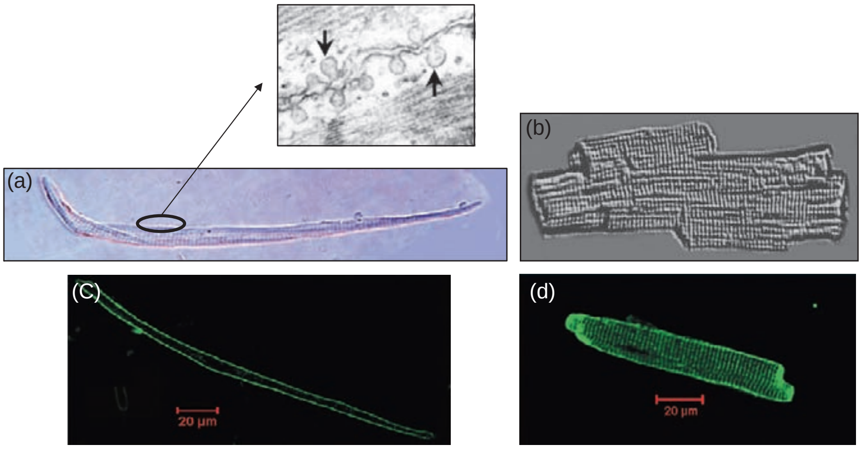


Figure 1 Morphology of live ventricular cardiomyocytes from adult Pacific bluefin tuna, *Thunnus orientalis* (a), adult rabbit, *Oryctolagus cuniculus* (b), trout, *Oncorhynchus mykiss* (c), and rat, *Rattus norvegicus* (d) Myocytes from (a) and (b) are not drawn to scale. The average dimensions of bluefin tuna, trout, and rat ventricular myocytes are given in **Table 1**. The average adult rabbit ventricular myocyte is approximately 100–150 µm in length and 20µ*m* in width. Light microscopy images are shown in (a) and (b). Inset of (a) = ultrastructure of the sarcolemmal membrane of a trout ventricular myocyte. Arrows point to flask-like structures, or invaginations of the membrane, known as caveolae. (c, d) Confocal microscopy images of ventricular myocytes labeled with di-8-ANEPPS (fluorescent dye). Images are from *x–y* planes taken near the center (*z–y*) of the myocyte. Notice the absence of T tubules in trout (c) and the presence in rat (d). Rat and trout images are adapted from Shiels HA and White E (2005) Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. *American Journal of Physiology* 288: R1756–R1766, with permission from American Physiological Society. Rabbit images are taken from Xu L (1999) Ontogeny of Myocardial Excitation–Contraction Coupling. MSc. Thesis, Simon Fraser University. Bluefin tuna image is taken from Shiels HA, Blank JM, Farrell AP, and Block BA (2004) Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from bluefin tuna and Pacific mackerel. *American Journal of Physiology* 286: R659–R658, with permission from American Physiological Society. Caveolae image adapted from Vornanen M, Shiels HA, and Farrell AP (2002). Plasticity of excitation–contraction coupling in fish cardiomyocytes. *Comparative Biochemistry and Physiology* 132: 827–846, with permission from Elsevier.

situations, such as anaemia, the *D. rerio* ventricle undergoes myocyte hypertrophy to counteract the extra demand placed on the heart. This is an example of how the fish cardiomyocyte responds to its environment.

The Cardiomyocyte Membrane

The cardiomyocyte is enclosed by a lipid membrane, called the sarcolemmal membrane, which provides a barrier between the inside (intracellular) and outside (extracellular) spaces of the cell. Embedded in the membrane are numerous ion channels, pumps, and exchangers that allow the movement of ions (e.g., Ca^{2+}) into and out of the cardiomyocyte.

Figures 1(**c**) and **1**(**d**) show the sarcolemmal membrane of a trout (*Oncorhynchus mykiss*) and rat cardiomyocyte that has been stained with a fluorescent red dye and visualized using confocal microscopy. The sarcolemmal membrane of the rat cardiomyocyte has folds (invaginations) into the cell interior – the ‘t-tubular network’ (**Figure 1**(**d**)). T-tubules allow the extracellular space to be bought into close contact with more central regions of the cardiomyocyte. Fish lack a t-tubular network (**Figure 1**(**c**)), likely because their small width makes this membrane system unnecessary. Instead, smaller (150 nm in diameter), flask-like invaginations of the membrane (caveolae) are present, which increase the cell S/A ratio (**Figure 1**(**a**) inset). Caveolae are more abundant in atrial versus ventricular cardiomyocytes and are thought to be important in cell signaling.

Myofibrils

Myofibrils are bundles of protein filaments that contain the contractile elements of the cardiomyocyte, that is, the machinery or motor that drives contraction and relaxation. Myofibrils have a characteristic banding pattern, refereed to as striations, where repeating light and dark transverse bands can be observed under a light microscope (**Figure 2**, labeled MF and **Figure 3**). The myofibrils are located peripherally, forming a single cylindrical shaped layer directly below the sarcolemmal membrane (**Figure 3**, labeled MF), a location that contrasts with mammals, which have numerous layers of myofibrils arranged longitudinally throughout the cell.

The contractile functional unit of the myofibril is called the sarcomere, which is approximately 1.6–2.0 µm in length. The different regions of the sarcomere are classified according to their ability to polarize light. A cross section of the fish sarcomere is shown in **Figure 3**.

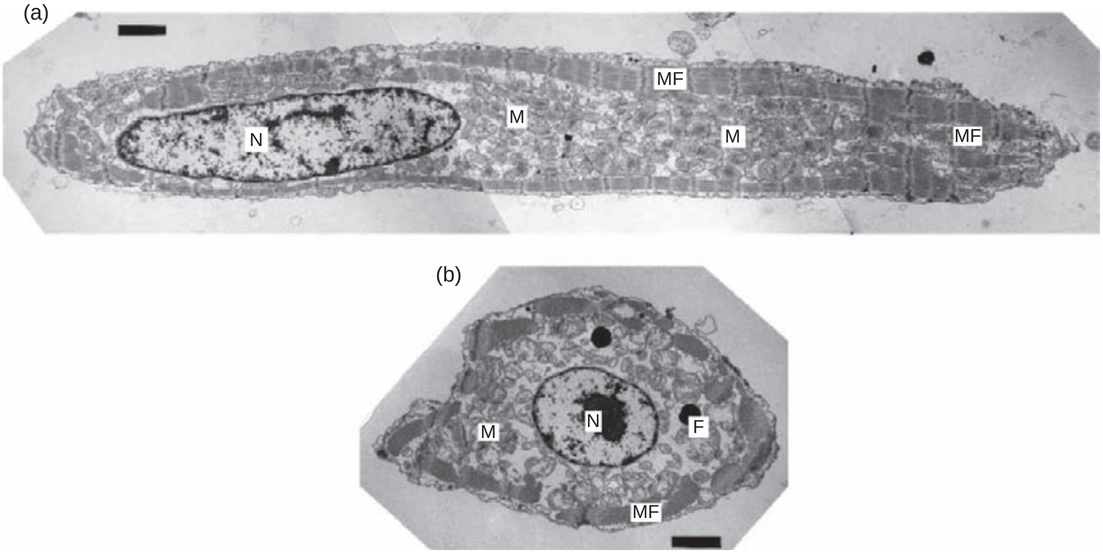


Figure 2 Electron micrographs of enzymatically isolated ventricular myocytes of rainbow trout (*Oncorhynchus mykiss*) heart. (a) A longitudinal section through a small part of a ventricular myocyte. (b) A cross section of a ventricular myocyte. M, mitochondria; MF, myofibrils; N, nucleus; F, fat. Scale bars, 2 μm. Reproduced from Vornanen M (1998) L-type Ca^{2+} current in fish cardiac myocytes: Effects of thermal acclimation and beta-adrenergic stimulation. *Journal of Experimental Biology* 201: 533–547, with permission from American Physiological Society.

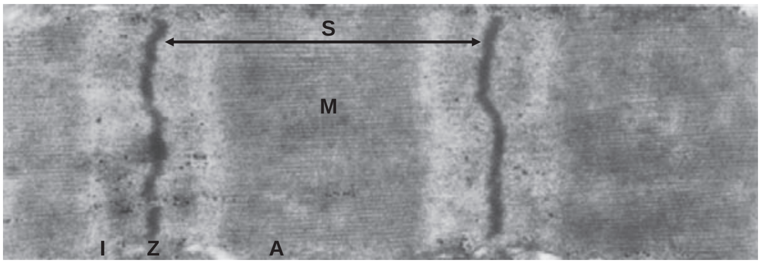


Figure 3 Longitudinal section of sturgeon atrium, showing the sarcomeric banding, Z-, I-, and A-band are clearly seen. The M-line (M) is more diffuse. Magnification = × 35 700. The sarcomere (S) is the distance between two Z-lines. Reproduced From Myklebust R and Kryvi H (1979) Ultrastructure of the heart of the sturgeon *Acipenser stellatus* (Chondrostei). *Cell Tissue and Research* 202: 431–438, with permission from Springer.

The more darkly staining bands are called A-bands (**Figure 3**, labeled A), while the lighter areas are called I-bands (**Figure 3**, labeled I). Each I-band is bisected by a narrow dark band, named the Z-line (**Figure 3**, labeled Z) and a broader dense M-band (**Figure 3**, labeled M) is found in the center of the A-band. The distance between two Z-lines is designated as one sarcomere.

A schematic diagram of a single sarcomere shows a relaxed state (**Figure 4**(**a**)) and a contracted state (**Figure 4**(**b**)), as well as the two types of filaments in sarcomeres, a thin filament (made of the protein actin) and a thick filament (made up of the protein myosin). During myocyte contraction, the thick and thin filaments slide across each other to form cross-bridges between the actin and myosin heads (**Figure 4**(**b**)).

The percentage of cell volume that myofibrils occupy in cardiomyocytes is rather uniform in most fish species, being approximately 40%. However, there are important

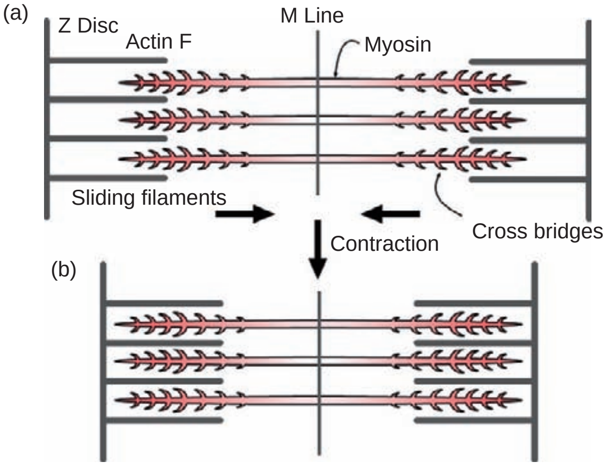


Figure 4 A schematic diagram of a single sarcomere during relaxation (a) and contraction (b). The illustration shows a shift in the amount of cross-bridges formed by actin and myosin during contraction. Illustration drawn by Gina Galli and Jake Rigby.

exceptions to this rule. The burbot (*Lota lota*), which usually inhabits very cold environments during the winter (1–4 °C), has an exceptionally high myofibrilar density (65%), and possesses very long sarcomeres (2.6 μm). These myofibrillar adaptations are characteristic of a slow contraction rate and large force production. The adaptation likely relates to the cold-dependent increase in blood viscosity and decrease in cardiac contractility.

At the other end of the spectrum, certain species of icefish have very low volumes of myofibrils (29%). Although this may seem disadvantageous for the icefish (which also has to tolerate exceptionally cold temperatures), as we shall see in later sections, the icefish sacrifices myofibrillar volume to increase the density of another important cellular constituent, the mitochondria, and correspondingly has a very low cardiac power output.

The Mitochondria

Mitochondria are centrally located just under the myofibrils (**Figure 2**, labeled M), which differs from the uniform distribution throughout the cell between the myofibrils in mammals. As with other cell types, the cardiomyocyte mitochondrion contains an outer and inner membrane (**Figure 5**(**a**)), with invaginations (cristae) that greatly increase the surface area of the inner membrane. The inside of the mitochondria, or matrix, contains all of the enzymes and proteins necessary for cellular respiration (**see also Temperature:** Mitochondria and Temperature).

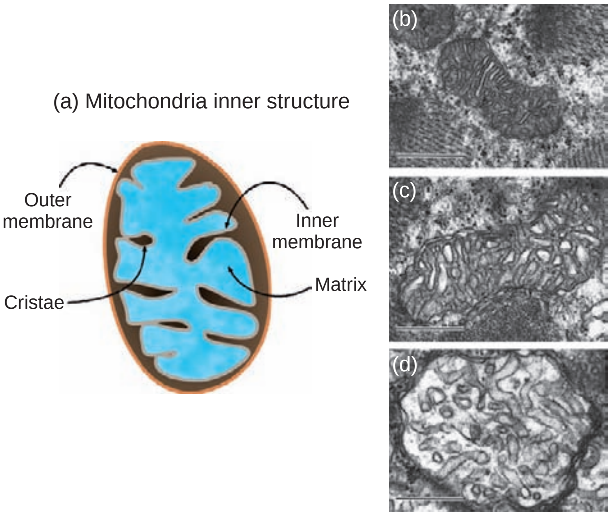


Figure 5 (a) Schematic diagram of the inner structure of mitochondria. (b–d) Electron micrographs of mitochondria from cardiac muscle of three species of Antarctic fishes. Mitochondria differ in both the density of the inner mitochondrial membrane and the surface-to-volume ratio according to the expression of oxygen-binding proteins, myoglobin, and hemoglobin. (b) *Gobionotothen gibberifrons* (has both myoglobin and hemoglobin); (c) *Chionodraco rastrospinosus* (has just myoglobin); (d) *Chaenocephalus aceratus* (neither myoglobin nor hemoglobin). Scale bar = 0.5 μm. Schematic illustration drawn by Gina Galli and Jake Rigby. (b–d) Reproduced from O′Brien KM and Sidell BD (2000) The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *Journal of Experimental Biology* 203: 1287–1297, with permission from Company of Biologists.

Mitochondrial volume (compared to total cardiomyocyte volume) ranges between 15% and 45% in fish. The enormous variation between species is a good example of how the cell is modified to fit its need. In general, active species that generate high heart rates and blood pressures tend to contain more mitochondria to support a higher rate of energy utilisation. For example, the *T. orientalis* contains 10–15% more mitochondria than a more sedentary fish species, such as the cichlid, and generates heart rates and blood pressures that greatly exceed those in most teleosts. An important exception to this rule is the Antarctic icefish. Mitochondrial volume can be exceptionally high (41%) in some icefish species, or exceptionally low (15%) in others, with this variability apparently directly proportional to the expression of oxygen binding proteins. Mitochondrial volume is higher in species that lack hemoglobin or myoglobin (**Figures 5**(**b**)–**5**(**d**)). Interestingly, the high mitochondrial density is not related to cellular aerobic capacity, but may reduce the diffusion distance for oxygen to reach mitochondria, thereby compensating for the lack of intracellular oxygen-binding proteins.

Mitochondrial volume is altered by the environment. Cold acclimation causes similar increases in both mitochondrial volume and myofibril density in the stripped bass as well as a hypertrophy of the cardiomyocyte. These changes are thought to compensate for the kinetic constraint of cold temperature.

The Nucleus

The nucleus of the fish cardiomyocyte (**Figure 2**, labeled N) is also centrally located near the mitochondria, taking up approximately 25% of cell volume in the trout ventricular myocyte. Most fish (90%) possess only one nucleus, but on rare occasions fish cardiomyocytes with two nuclei have been observed. As with other cells, the nucleus contains most of the cells genetic material, organized as DNA to form chromosomes. Triploid fish are genetically engineered to have an additional copy of their DNA (3N) and the nucleus is correspondingly one-third large, but the cell size is not.

Fat and Glycogen Deposits

Glycogen clusters, in the form of β-particles, and fat droplets are found in the spaces between the myofibrils and mitochondria. They vary in density between species, according to feeding status, energy stores, and season. For example, *O. mykiss* have sparse glycogen reserves but large fat droplets, while crucian carp (*Carassius carassius*) and *T. orientalis* contain huge glycogen clusters, but no fat droplets.

The Sarcoplasmic Reticulum

The sarcoplasmic reticulum (SR) is a specialized form of endoplasmic reticulum (ER) found in most cells, which forms a system of intracellular membranes. The SR is separate from the sarcolemma and T-tubules. Unlike the ER, which is primarily involved in protein synthesis, the SR lacks ribosomes (the machinery required to make proteins) and is primarily involved in controlling Ca^{2+} concentrations inside the cardiomyocyte. There are two proteins embedded in the SR membrane that permit this function – a Ca^{2+} pump (termed the SR Ca^{2+} ATPase, or SERCA) and a Ca^{2+}-release channels (termed ryanodine receptors, or RyRs). Ca^{2+} is stored in the SR, moving into the cytoplasm via the RyR and being removed from the cytoplasm by SERCA. Therefore, Ca^{2+} is moved back and forth from the inside of the cell to the SR as part of E–C coupling.

There are two types of SR in fish cardiomyocytes: junctional SR (jSR) and free SR (fSR) (see **Figure 6**). jSR forms peripheral couplings with either the sarcolemmal or t-tubular network (**Figure 6**(**a**), double arrows). In these peripheral couplings where the jSR profile aligns with the sarcolemma or t-tubule, rows of proteins known as 'feet' are visible between the two membranes (**Figure 6**(**b**), inset). These proteins are the Ca^{2+}-release ryanodine receptor channels. fSR is not closely associated with any other membrane system, and can be found throughout the cardiomyocyte (**Figure 6**(**a**), arrows).

Quantitative measurements of SR volume (relative to total volume) have only been made for two fish species: the perch (*Perca fluviatilis*; 4.5–6.5%) and the *T. orientalis* (2–3%). In mammals, SR density is on average much greater, but ranges between 1% and 12%. The highly active *T. orientalis* possesses both fSR and jSR that contribute significantly to overall Ca^{2+} regulation. In this species, atrial cardiomyocytes have a greater proportion of SR (3.2%) in comparision to ventricular cardiomyocytes (2.2%). Lastly, fish species that inhabit cold environments and those that acclimate to the cold seasonally enhance SR density. The warm-acclimated *P. fluviatilis*, for example, increase SR density from 4.5% to 6.5% when acclimated to the cold. Presumably, this adaptation allows Ca^{2+} levels within the cell to remain normal in the cold, which would usually slow down the rates of protein and ion channel activities.

Balancing the Volumes of Cellular Constituents

One may ask, if increasing mitochondrial or myofibrillar volume has the potential to dramatically increase energy and force production, respectively, in a fish cardiomyocyte, then why don't they all do it? The fact is that an alteration in the volume of one cardiomyocyte constituent or organelle must come at a cost to another. For example, if cardiomyocyte volume remains constant, then an increase in mitochondrial density will require a decrease in some other component of the cell. In most cases, myofibril density will suffer. This is shown in **Table 2**, where the volume of various fish cardiomyocytes that is occupied by mitochondria is generally inversely related to myofibrillar volume. Thus, although aerobic capacity may increase with a higher mitochondrial density, contractile force may suffer, or vice versa. This trade-off is delicately

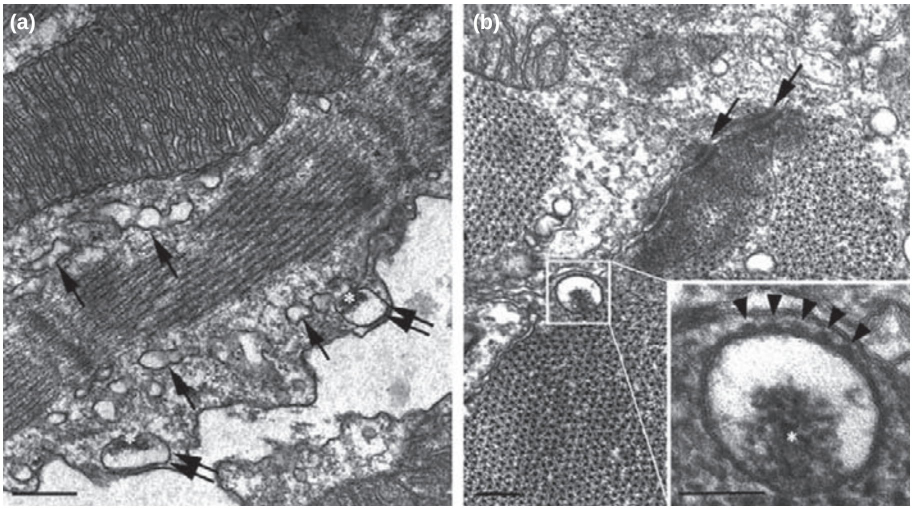


Figure 6 Distribution of the junctional SR (jSR) in peripheral couplings in the ventricle of the Pacific bluefin tuna (*Thunnus orientalis*). (a) Longitudinal thin section. The jSR appears as wide cisternae containing calsequestrin (white asterisk) and is located only at the periphery of the cells (double arrows). The extensive internal fSR network (arrows) is also visible throughout the cytoplasm. (b) Transverse section. The form and shape of the jSR do not change. Note that in peripheral couplings ((b), inset) formed by the apposition of the jSR profile with the plasmalemma, rows of feet (ryanodine receptors, RyRs) are visible between the two membranes ((b), arrowheads). Bars = 0.3 μm (a), 0.2 μm (b), 0.1 μm (inset). Reproduced with permission from Di Maio A and Block BA (2008) Ultrastructure of the sarcoplasmic reticulum in cardiac myocytes from Pacific bluefin tuna. *Cell and Tissue Research* 334: 121–134.

Table 2 Percentage of cell volume that mitochondria and myofibrils occupy in ventricular muscle from various fish species

Volume	*Trout*	*Carp*	*Icefish (CA)*	*Icefish (NN)*	*Icefish (GG)*	*Burbot*
Mitochondrial (%)	45	22	41	25	15	27
Myofibrils (%)	40	40	29	47	40	65

Percentage of volume (compared to total cell volume) that the mitochondria and myofibrils occupy in ventricular muscle from various fish species. Three species of Antarctic icefish are given; CA = *Chaenocephalus aceratus*, NN = *Neglecta nototheria*, GG = *Globionotothen gibbenifons*. Trout, *Oncorhynchus mykiss*; carp, *Carassius carassius*; burbot, *Lota lota*.

balanced in many fish species according to their environment, highlighting another example of the cardiomyocyte structure–function relationship.

Cell–Cell Connections

Cardiomyocytes are connected to each other by three main structures found at the sarcolemma: (1) fascia adherens, (2) desmosomes, and (3) gap junctions. These specialized structures provide strong mechanical linkages between cardiomyocytes as well as a low electrical resistance pathway that facilitates conduction of the cardiac impulse and electrophysiological coupling.

Fascia adherens and desmosomes are composed of the two sarcolemmal membranes of adjacent cardiomyocytes. The thin actin filaments and intermediate filaments of the myofibrils protrude to the sarcolemmal membrane and bind tightly to either side of the fascia adherens and desmosomes, respectively. In contrast to adherens and desmosomes, gap junctions do not seal membranes together, but actually link the interior of cardiomyocytes, allowing the movement of ions between them and provide a low resistance electrical connection. Proteins called connexins form the gaps between two neighboring sarcolemmal membranes, and can be in an open or closed state, thereby coupling or uncoupling adjacent cardiomyocytes. This property allows cardiomyocytes to be in electrical contact, thereby propagating the cardiac signal and making sure cardiomyocytes contract and relax in synchrony.

In fish, all three junction types are known to be distributed all around and at the polar tips of the spindle-shaped cardiomyocytes (**Figure 7**(**a**)). The actin-binding

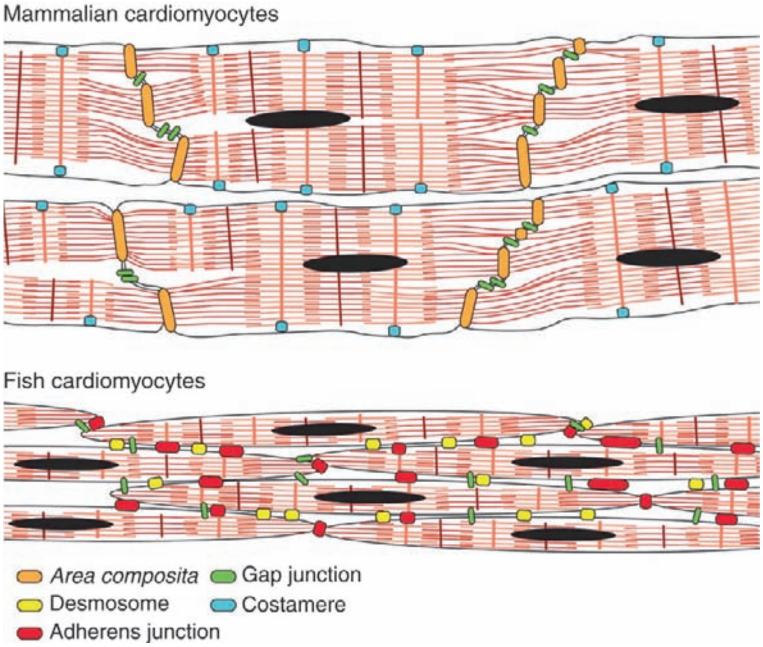


Figure 7 Schematic representation indicating adhering junction localizations and cardiomyocyte arrangements in the mammalian and fish ventricle. Note that mammalian cardiomyocytes are more rectangular shaped and often connected at the two poles by specialized adhering junctions (area composita; plural areae compositae). The Z-bands are attached to the lateral costameres. By contrast, fish cardiomyocytes are elongated and spindle shaped and, for the most part, laterally connected by desmosomes and adherens junctions. The Z-bands of two neighboring cardiomyocytes are often directly connected by adherens junctions. Illustration drawn by Sebastian Piepenhoff.

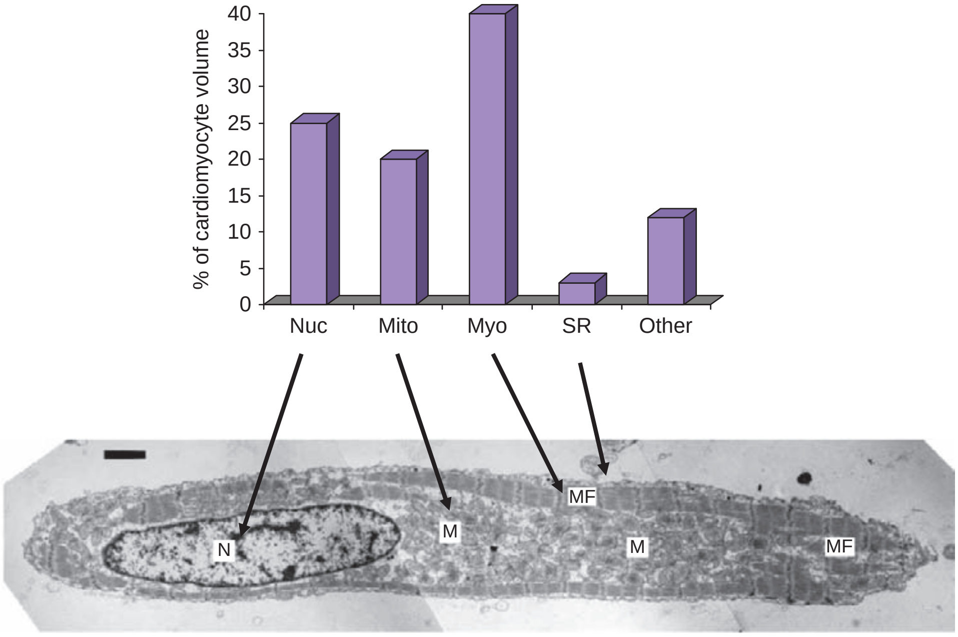


Figure 8 Percentage volume that each cardiomyocyte component occupies compared with total cell volume. Nuc = nucleus, Mito, mitochondria; Myo, myofibrils; SR, sarcoplasmic reticulum. Reproduced from Vornanen M (1998) L-type Ca^{2+} current in fish cardiac myocytes: Effects of thermal acclimation and beta-adrenergic stimulation. *Journal of Experimental Biology* 201: 533–547, with permission from Company of Biologists.

adherens junction can often be found at the lateral sides, directly attached to the Z-band material. By contrast, adult mammalian cardiomyocytes are usually connected at two poles via structures called intercalated disks (**Figure 7**(**b**)). The majority of the junctional area (≥90%) between mammalian cardiomyocytes is occupied by structures called area composita located together with gap junctions within the intercalated disks. Area composita structures represent mammalian-specific mixed-type adhering junctions. These junctions consist of typical desmosomal and adherens junctional components. The Z-bands are for the most part connected laterally at structures called costameres.

Summary

We can now piece together the entire fish cardiomyocyte and the percentage volume (on average) that each component occupies (**Figure 8**). Fish cardiomyocytes are long, thin, and spindle shaped, with a large S/A ratio and no t-tubular system. The external and internal environments of the cardiomyocyte are separated by the sarcolemmal membrane. The nucleus (or nuclei) has a central location, occupying approximately 25% of total cell volume. The myofibrils lie directly under the sarcolemmal membrane and occupy between 30% and 65% of cardiomyocyte volume. The percentage of space that mitochondria take up is generally inversely related to myofibrillar volume, being approximately 15–45%. The mitochondria are found centrally within the cell, with a variable amount of glycogen and fat droplets surrounding it. The SR is located at the periphery of the cell, either in close association with (jSR) or free from (fSR) the sarcolemmal membrane. SR volume is 2–6% of total cardiomyocyte volume. We must remember, however, that fish cardiomyocyte ultrastructure is neither uniform nor permanent; it is malleable and several factors (including species, activity level, and temperature) can impact the percentage volume occupied by each component within the cardiomyocyte. Thus, the fish cardiomyocyte is an excellent example of how cellular structure can fit perfectly to its function.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches:** Physiology of Triploid Fish. **Design and Physiology of the Heart:** Action Potential of the Fish Heart; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux. **Fish Migrations:** Tracking Oceanic Fish.

Further Reading

Cobb JL (1974) Gap junctions in the heart of teleost fish. *Cell and Tissue Research* 154: 131–134.

Di Maio A and Block BA (2008) Ultrastructure of the sarcoplasmic reticulum in cardiac myocytes from Pacific bluefin tuna. *Cell and Tissue Research* 334: 121–134.

Farrell AP, Hammons AM, Graham MS, and Tibbits GF (1988) Cardiac growth in rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* 66: 2368–2373.

Franke WW, Borrmann CM, Grund C, and Pieperhoff S (2006) The area composita of adhering junctions connecting heart muscle cells of vertebrates. I. Molecular definition in intercalated disks of cardiomyocytes by immunoelectron microscopy of desmosomal proteins. *European Journal of Cell Biology* 85: 69–82.

Holthöfer B, Windoffer R, Troyanovsky S, and Leube RE (2007) Structure and function of desmosomes. *International Review of Cytology* 264: 65–163.

Myklebust R and Harald K (1979) Ultrastructure of the heart of the sturgeon *Acipenser stellatus* (Chondrostei). *Cell and Tissue Research* 202: 431–438.

Myklebust R and Kryvi H (1979) Ultrastructure of the heart of the sturgeon *Acipenser stellatus* (Chondrostei). *Cell Tissue and Research* 202: 431–438.

O′Brien KM and Sidell BD (2000) The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *Journal of Experimental Biology* 203: 1287–1297.

Pieperhoff S, Bennett W, and Farrell AP (2009) The intercellular organization of the two muscular systems in the adult salmonid heart, the compact and the spongy myocardium. *Journal of Anatomy* 215: 536–547.

Pieperhoff S and Franke WW (2008) The area composita of adhering junctions connecting heart muscle cells of vertebrates. VI. Different precursor structures in non-mammalian species. *European Journal of Cell Biology* 87: 413–430.

Severs NJ (1989) Gap junction shape and orientation at the cardiac intercalated disk. *Circulation Research* 65: 1458–1462.

Shiels HA and Farrell AP (2002) Plasticity of excitation–contraction coupling in fish cardiomyocytes. *Comparative Biochemistry and Physiology* 132: 827–846.

Shiels HA and White E (2005) Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. *American Journal of Physiology* 288: R1756–R1766.

Shiels HA, Blank JM, Farrell AP, and Block BA (2004) Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from bluefin tuna and Pacific mackerel. *American Journal of Physiology* 286: R659–R658.

Tiitu V and Vornanen M (2002) Morphology and fine structure of the heart of the burbot (*Lota lota)*, a cold stenothermal fish. *Journal of Fish Biology* 61: 106–121.

Vornanen M (1998) L-type Ca^{2+} current in fish cardiac myocytes: Effects of thermal acclimation and beta-adrenergic stimulation. *Journal of Experimental Biology* 201: 533–547.

Vornanen M, Shiels HA, and Farrell AP (2002) Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology A* 132: 827–846.

Xu L (1999) Ontogeny of Myocardial Excitation–Contraction Coupling. MSc. Thesis, Simon Fraser University.

The Outflow Tract from the Heart

DR Jones[†], University of British Columbia, Vancouver, BC, Canada

MH Braun, University of Calgary, Calgary, AB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Terminology, Phylogeny, and Development
Form and Function of the Bulbus Arteriosus
Form and Function of the Conus Arteriosus
Acknowledgment
Further Reading

Glossary

Bulbus arteriosus The term given to the outflow tract found in most teleost fishes.

Collagen An extracellular protein, typically found in large blood vessels, that has less elastic properties than elastin.

Conus arteriosus The term given to the outflow tract found in all elasmobranch fishes and some other primitive fishes.

Elastin An extracellular protein, typically found in large blood vessels that is characterized by its elastic properties.

Glycosaminoglycans Long unbranched sugar molecules surrounding a protein core which are an important component of connective tissues due to their effectiveness in resisting pressure changes.

Truncus arteriosius The term given to the section of vessel invested with cardiac epithelium but devoid of cardiac muscle that follows the conus in the outflow tract of lungfishes and coelacanths.

Introduction

The outflow tract of the heart is the name given to that part of the vasculature, within the pericardium, which connects the ventricle to the ventral aorta in most fishes (**Figures 1** and **2**; **see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). This terminology is factual and implies no homology except that the tract is derived from the subintestinal blood vessel in embryological development. The actual junction between the connecting vessel and the ventral aorta defines the arterial pole of the heart, implying that all structures behind the arterial pole are derived from cardiac mesoderm although this has been the subject of considerable debate over the last century – a debate that continues today.

Terminology, Phylogeny, and Development

All jawed fishes (Gnathostomes) have an outflow tract (**Figure 1**). In sharks and rays (elasmobranchs), the predominant connecting vessel (*conus arteriosus*) is tubular and largely composed of cardiac muscle, whereas in bony fishes (teleosts), the outflow tract (bulbus arteriosus) resembles a greatly swollen blood vessel and is largely made up of elastin, collagen, and smooth muscle (**Figures 1** and **2**). The conus is also well developed in the more phylogenetically ancient teleosts and is often regarded as the ancestral condition, whereas the bulbus represents the apogee of teleost evolution. Developmental and histochemical studies suggest that all structures within the pericardium are of cardiac origin and should be recognized as such in the nomenclature of the regions of the wall. Nevertheless, in the present discussion, generic nomenclature for bulbus and conus will be used: middle, inner (facing the blood vessel lumen), and outer (facing the pericardium).

The predominant view for a century and a half has been that, during the evolution of fishes, the conus was replaced by the ventral aorta growing backward into the pericardium. Perhaps due to stresses imposed by the ejection of blood from the ventricle, this region of the ventral aorta was then remodeled to form a distinct bulbus (**Figure 3**). Recently, however, this view has been challenged as a bulbar vessel has been identified between the conus and the ventral aorta in sharks (**Figure 4**) and a conal remnant has been identified at the junction between the ventricle and bulbus in numerous species of advanced teleosts (**Figure 5**).

The jawless fishes (Agnathans), as a potential stem group for the craniates, could provide answers regarding the ancestral circulatory design. However, the strangeness

---

[†] Deceased

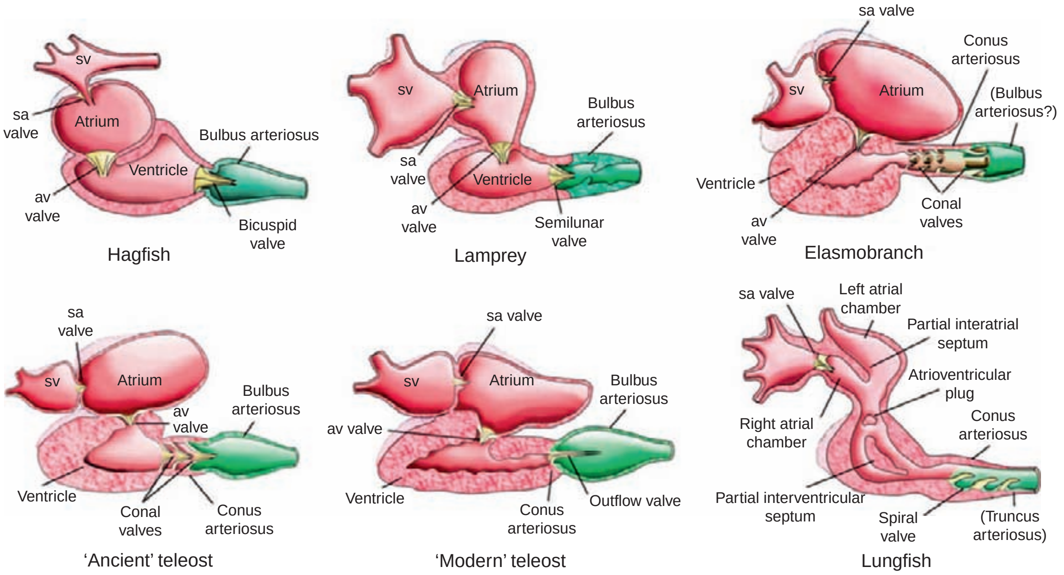


Figure 1 Generalized, diagrammatic representation of the phylogenetic range of hearts in extant fishes. In each case, dorsal is to the top and cranial to the right (not drawn to scale). Myocardium-derived chambers have been shaded in red. Green-shaded regions are nonmyocardial and contain primarily smooth muscle and, or fibrous proteins. Endocardium derived valve structures are colored yellow. av, atrioventricular; sa, sinoatrial; sv, sinus venosus. From Grimes AC and Kirby ML (2009) The outflow tract of the heart of fishes: Anatomy, genes and evolution. *Journal of Fish Biology* 74: 983–1036.

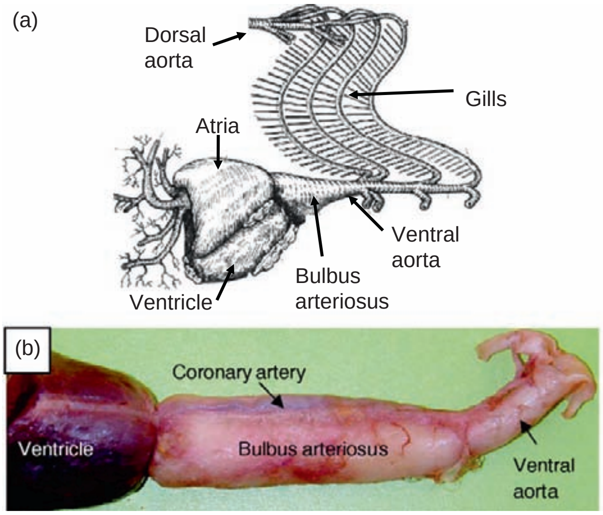


Figure 2 The cardiac outflow tract separates the ventricle from the ventral aorta. (a) An illustration of an adult zebrafish heart and the major vasculature in the cardiac region. The heart pumps the blood from the atrium and ventricle into the bulbus arteriosus before entering the ventral aorta. (b) The ventricle, bulbus arteriosus and ventral aorta of a blue marlin. Scale = 10 cm. (a) Modified from Hu N, Yost HJ, and Clark EB (2001) Cardiac morphology and blood pressure in adult zebrafish. *Anatomical Record* 264: 1–12. (b) From Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores*), bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): Static properties. *Journal of Experimental Biology* 206: 3311–3326.

and variety of their circulatory systems preclude them from contributing much to this debate. As opposed to higher vertebrates, Agnathans have a partially open circulation lacking capillary beds. Hagfishes have large sinuses on the venous side and up to five accessory pumps to assist the heart in boosting the low-pressure circulation. Centrally, they lack an intrapericardial outflow tract as the ventral aorta, lying outside the pericardium, connects directly to the ventricle (**see also Hagfishes and Lamprey:** Hagfishes and **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). However, although there is no evidence of a bulbus or conus within the pericardium, a slight thickening of the ventral aorta outside the pericardium has been referred to as a bulbus. On the other hand, lampreys, which are better candidates for the stem group of the fishes and have a more robust circulatory system, do have a bulbus downstream of the ventricle that bears resemblances to the teleost bulbus in structure but lacks elastin. However, the lamprey bulbus may be a necessary rather than ancestral condition as blood is incompressible and the heart output has to be accommodated somewhere.

The situations in lungfishes and lobed-fin fishes are also unique. In coelacanths (*Latimeria chalumnae*, the surviving member of the lobe-finned fishes), a conus, containing cardiac muscle, is followed by a section of vessel invested with cardiac epithelium but devoid of cardiac muscle, which is called the truncus arteriosus (**Figure 6**). It is surprising that the recently described

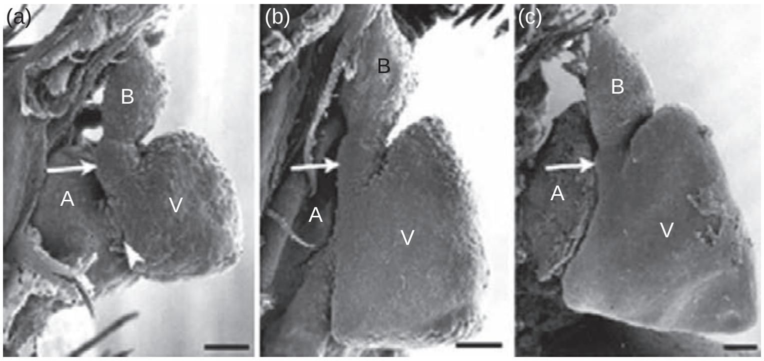


Figure 3 Changes in the conus during development. This sequence of figures shows selected SEM views of the early development of *Sparus auratus* (Gilthead seabream) heart. Right lateral views. (a) Sixteen days postfertilization (dpf); (b) 34 dpf; (c) 50 dpf. In all images, the conus arteriosus (arrow) appears as a distinct heart segment interposed between the bulbus and the ventricle. The conus occupies a dorsal position in (c) Of note is the relative reduction in size of the conus relative to the bulbus. Arrowhead in (a) indicates the atrioventricular segment. A, atrium; B, bulbus arteriosus; V, ventricle. Scale = 25 μm. Modified from Icardo JM (2006) Conus arteriosus of the teleost heart: Dismissed, but not missed. *Anatomical Record Part A* 288A: 900–908.

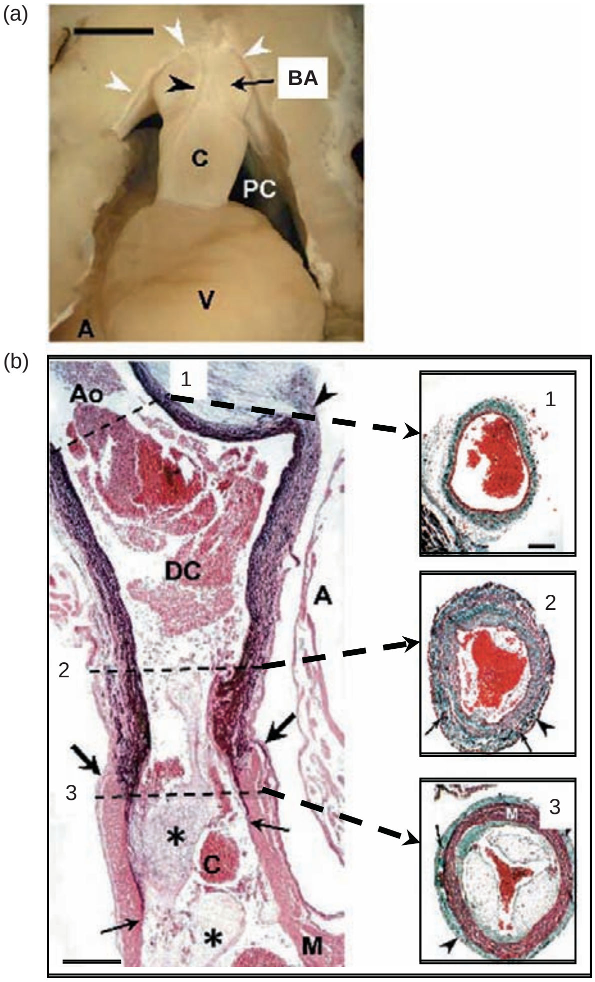


Figure 4 Bulbus arteriosus in an elasmobranch. (a) Ventral view of the heart of an adult *Galeus atlanticus* (catshark). A, atrium; C, conus arteriosus; BA, bulbus arteriosus. The white arrowheads point to the pericardium, black arrowhead indicates a coronary artery. Scale = 0.2 cm. (b) Sagittal section of the ventral aorta (VAo) and cardiac outflow tract of an adult catshark. The separation between the two sections of the cardiac outflow tract (conus arteriosus (C)) and bulbus arterious (BA)) is marked with large arrows. The conus contains myocardium (M) in its walls and has two rows of valves (asterisks). The bulbus has no myocardium and is rich in elastin (purple staining). Small arrows indicate the beginning of elastin staining. Arrowhead = distal boundary of pericardial cavity. Scale = 500 μm. Sections 1–3. Transverse sections of the (1) ventral aorta, (2) bulbus arteriosus, and (3) conus arteriosus. At sections 2 and 3, the outflow tract is lined with epicardium (arrows) and crossed by coronary arteries (arrowheads). Scale = 300 μm. Modified from Duran AC, Fernandez B, Grimes AC, *et al.* (2008) Condrichthyans have a bulbus arteriosus at the arterial pole of the heart: Morphological and evolutionary implications. *Journal of Anatomy* 21: 597–606.

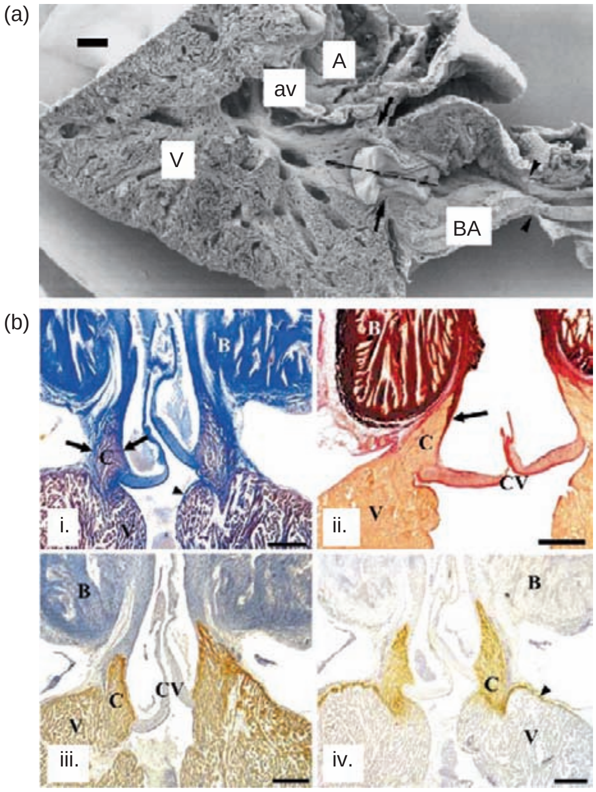


Figure 5 Conus arteriosus in a teleost (a) Mid-sagittal section of the left side of an adult gilthead seabream heart. V, ventricle; A, atrium; av, atrioventricular valve; BA, bulbus arteriosus. Arrows indicate the conus region and the left conus valve. Arrowheads mark the bulbus–ventral aorta junction and the insertion of the pericardium. Scale = 50 μm. The broken line through the conus valve indicates the approximate planes of section in (b) Longitudinal sections of the heart through the ventricle–bulbus junction. A ring of compact myocardium is interposed between the ventricle and the bulbus arteriosus. This myocardial segment has a conus shape which tapers at its distal end. Its base blends with the ventricular myocardium. (i) Mallory's trichrome stain for fibrous connective tissue. The conus myocardium is surrounded on its inner and outer sides by collagenous fibrous layers (arrows) which merge at the tapered end. The outer fibrous layer separates the conus from the bulbus distally. The inner fibrous layer continues proximally with the ventricular subendocardium (arrowhead). (ii) Weigert–van Gieson stain. Muscle = yellow, Collagen and elastin = pink and red. The inner fibrous layer (arrow) is very rich in elastin. Note the differences in staining with the outer fibrous layer. The bulbus also stains intensely. (iii) MF20 immunostaining for myosin heavy chains (muscle). Both the conus and the ventricular myocardium stains intensely. (iv) L9393 immunostaining for laminin. Intense staining for laminin is specific to the conus. Laminin is barely detectable in the ventricular myocardium, except at the outer ventricular surface (arrowhead). Scale = i–iii, 250 μm; iv, 200 μm. Modified from Schib JL, Icardo JM, Duran AC, *et al.* (2002) The conus arteriosus of the adult gilthead seabream (*Sparus auratus*). *Journal of Anatomy* 201: 395–404.

bulbus at the termination of the outflow tract in elasmobranches was not referred to as a truncus. In lungfishes, there is a complex spiral valve within the conus (**Figure 7**) and a somewhat simpler division within the truncus, strongly suggesting that a premium is placed on separation of the oxygenated and deoxygenated blood as it flows from the ventricle where the two streams are effectively divided during ventricular contraction.

Form and Function of the Bulbus Arteriosus

Form

The bulbus arteriosus comes in a myriad of shapes, from bulbous to tubular (**Figure 8**). However, the functional significance of these shapes is unknown. In the bulbous types, the major swelling is proximal and the bulbus tapers distally to meet the ventral aorta. There are no valves within or at either end of the bulbus in the vast majority of fishes and the few contrary reports may be erroneous. When the bulbus is collapsed, the volume of the chamber is reduced by ridges or even discrete longitudinal and radial elements in less phylogenetically advanced teleosts such as rainbow trout, tuna, and marlin (**Figure 9**). The ridges and elements, with very little mechanical strength, do not make any contribution to the structure of the wall. Another interesting modification in deep-sea fishes (**see also Deep-Sea Fishes**) is an inner tube that extends from the ventricle for about one-third of the bulb. The inner tube is made up of gelatinous and collagenous tissue.

The middle layer makes up the majority of the wall and smooth muscle is the predominant cell type, often being arranged helically or circumferentially along the length of the bulbus (**Figure 10**). The smooth muscle of the middle layer is embedded in an extracellular filamentous matrix of thin and thick filaments. The thick filaments are microfibrillar elastin, which also occurs in the external and internal layers of the bulbus. Microfibrillar elastin is much more compliant than the amorphous type of elastin found in many fish blood vessels. Support for the histology has come from an examination of the zebrafish (*Danio rerio*) genome, which has revealed the existence of two elastin genes, presumably one for the fibrillar and one for the amorphous type. The two structural proteins, collagen (stiff) and elastin (compliant), as well as polysaccharide chains and glycoproteins, make up the extracellular matrix and are secreted by smooth muscle cells. These muscle cells undergo a process of maturation from a secretory to a muscle phenotype as they migrate from the inner toward the middle layer. Collagen occurs throughout the layers of the bulbus, although it is more compact and ordered in the outer layer.

Blood vessels, derived from the coronary circulation (**see also Design and Physiology of the Heart:** The Coronary Circulation) which runs along the bulbus (**Figures 1** and **2**),

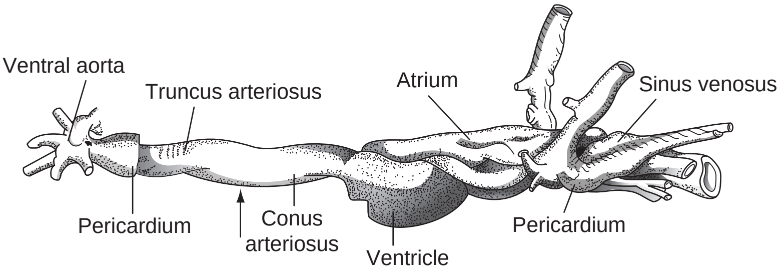


Figure 6 Sketch of the excised heart from *Latimeria chalunnae* (Coelocanth). Lateral view, dorsal to the top, cranial to the left. The black arrow marks the junction of the truncus arteriosus with the conus arteriosus. Modified from Grimes AC and Kirby ML (2009) The outflow tract of the heart of fishes: Anatomy, genes and evolution. *Journal of Fish Biology* 74: 983–1036.

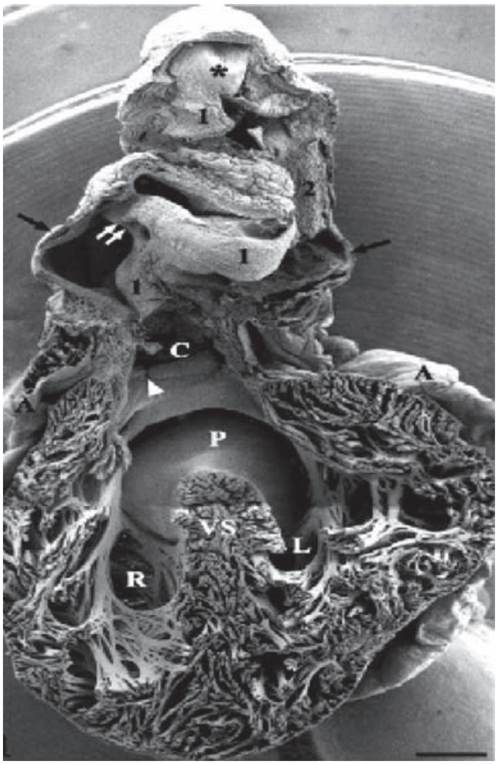


Figure 7 *Protopterus dolloi* (African lungfish). (a) Panoramic view of the heart. The heart has been sectioned frontally (dorsal segment, anterior view). The outflow tract has been dissected further to show the location of the intraluminal folds (1 (spiral) and 2 (bulbar) folds). The ventricle and the ventricular septum (VS) (L, R, left and right ventricle, respectively). The atrioventricular plug (P) sits on top of the ventricular septum, nearly occluding the atrioventricular communication. The outflow tract arises from above the ventricular septum. It contains three portions separated by proximal and distal flexures (large arrows). The proximal portion, the conus arteriosus (C), is almost vertical and contains the outflow valves (arrowheads indicate vertical indentation). The spiral fold (1) follows a spiraling course and runs the entire length of the outflow tract. This fold is connected to an additional mass of mesenchymal tissue (double arrow) located near the proximal flexure. Note the presence of a hole in the connecting area. The bulbar fold (2) occupies the middle and distal outflow tract, upward from the distal flexure. The two folds fuse at the distal outflow tract (asterisk). A, atrium. Scale = 500 μm. Modified from Icardo JM, Brunelli E, Perrotta I, *et al*. (2005) Ventricle and outflow tract of the African lungfish *Protopterus dolloi*. *Journal of Morphology* 265: 43–51.

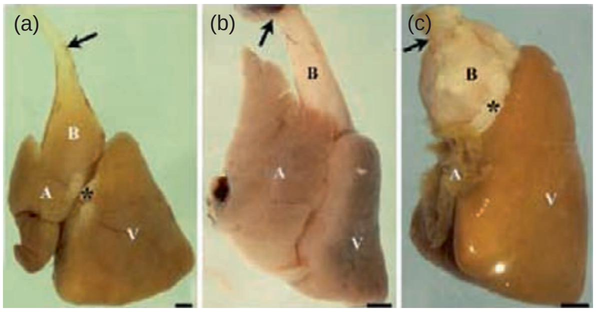


Figure 8 Examples of the variety of bulbus shapes.
(a) *Trachurus trachurus* (Atlantic horse mackerel): Pear-shaped
(b) *Trigla lucerna* (tub gurnard): Cylindrical and elongated.
(C.) *Sparus pagrus* (Seabream): short and thick. V = ventricle, A= atrium, B = bulbus arteriosus. The arrows mark the upper insertion of the pericardium and the boundary between the bulbus and the ventral aorta. Fat tissue (asterisks in a and c) accumulates in the subepicardium. Scale = 0.1 cm. Modified from Icardo JM (2006) Conus arteriosus of the teleost heart: Dismissed, but not missed. *Anatomical Record Part A* 288A: 900–908.

are usually accompanied by autonomic nerve fibers (lacking a myelin sheath). Both blood vessels and nerves are restricted to the outer layer of the bulbus and, as a result, these nerves innervate smooth muscle fibers at their ends, not along their length. However, as blood vessel penetration was long thought to be essential for nerve growth, fishes lacking a well-developed coronary circulation have little or no direct nerve connections with smooth muscle. Regardless, these nerves can release transmitters that diffuse through the wall to cause smooth muscle contraction or relaxation. A coordinated contraction can be developed between smooth muscle cells if both chemical and electrical connectivity can be established between them. This may be ensured by specialized regions of their walls that are very closely apposed (**Figure 10**(**c**)), but such regions have not been confirmed in many species. Of note is the fact that the bulbi of most Antarctic teleosts, despite lacking blood vessels, are innervated, implying that blood vessels are not necessary for innervation.

The internal epithelium of endocardial cells, thought to align with the direction of blood flow, contains large

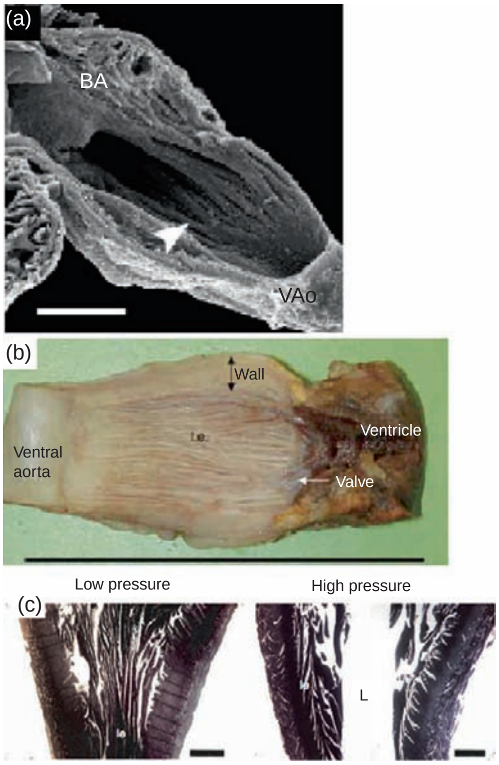


Figure 9 Internal elements of the bulbus arteriosus. (a) Scanning electron microscopy of the sagittal section of the bulbus arteriosus of a 3 months postfertilization zebrafish heart. BA, bulbus arteriosus; VAo, ventral aorta. The asterisk indicates the bulboventricular valve. The arrowhead identifies one of the elevated ridges along the inner surface of the bulbar wall. Scale = 100 μm. (b) The bulbus of a blue marlin dissected open to reveal the internal longitudinal elements (l.e.). Scale = 10 cm. (c) Longitudinal sections through a yellowfin tuna bulbus demonstrating what occurs with the longitudinal and radial elements at low and high pressures. Scale = 1 mm. (a) Modified from Hu N, Yost HJ, and Clark EB (2001) Cardiac morphology and blood pressure in adult zebrafish. *Anatomical Record* 264: 1–12. (b, c) Modified from Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores)*, bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): Static properties. *Journal of Experimental Biology* 206: 3311–3326.

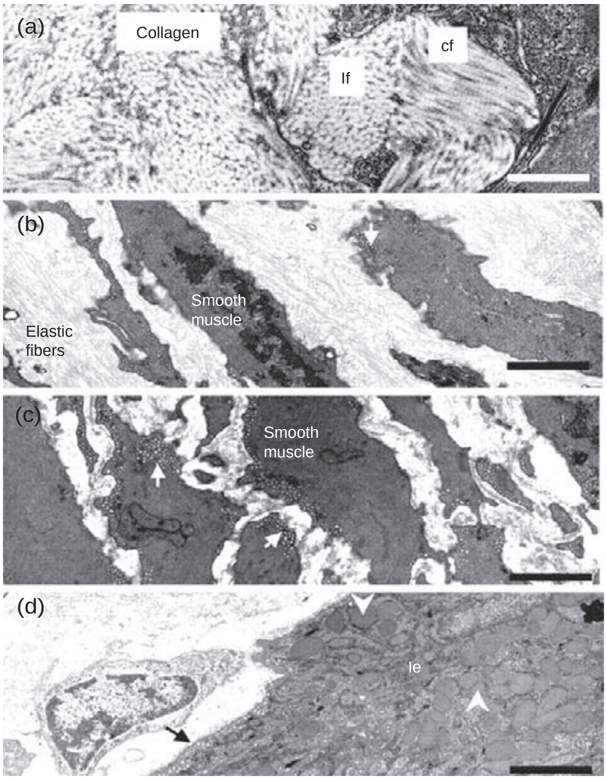


Figure 10 Transmission electron micrographs demonstrating the structure of the layers of the bulbus. From yellowfin tuna. All sections are transverse. (a) The collagenous outer layer. The collagen fibers are not arranged in a single direction. The majority of the fibers is arranged longitudinally (lf) but there are also bundles of circumferential fibers (cf). Scale = 300·nm. (b) The middle layer. The long axes of the elastin fibrils are aligned with the long axes of the smooth muscle cells, indicating a circumferential arrangement. The smooth muscle cells possess a number of plasmalemmal vesicles along the edge of the membrane (arrow). Scale = 1.45 μm. (c) Smooth muscle within the middle layer of the bulbus. This section reveals that smooth muscle cells are not always sparsely distributed within the elastin and can occur in close proximity. The smooth muscle cells have plasmalemmal vesicles under the edge of the membrane (arrows). Scale = 1.55 = μm. (d) Electron micrograph of a longitudinal element (le). The endothelial cells contain plasmalemmal vesicles underneath the membrane (arrow) as well as larger, electron-dense vesicles scattered throughout the cell (arrowheads). Scale = 1.55 μm. Stained with uranyl acetate and lead. From Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores)*, bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): Static properties. *Journal of Experimental Biology* 206: 3311–3326.

numbers of moderately dense bodies (MDBs; **Figures 10(d)** and **11**) that are released from the inner layer into the blood stream. In various species, MDBs have been suggested as storage sites for catecholamines, histamines, and mucopolysaccharides (long sugar chains), but it may be that both the population of cells containing the MDB and the MDB themselves are not uniform and contain a variety of substances, each with different functions. Endocardial cells invest the ridges as well as the free cord-like structures that lie along the inner bulbus wall. The outer, pericardial, epithelium is formed by flattened cells which are tightly linked to each other and contain large number of vesicles, which suggests exchange of materials with the pericardial cavity. There can be no doubt that one of the major roles of the bulbus is secretory, affecting both proximal and distal targets.

The Antarctic ice fishes, both those with and without red blood cells, are unique among teleosts because there is no elastin in the wall of the bulbus. Smooth muscle of the middle layers is embedded in a filamentous meshwork, which may confer elastic properties to the bulbar wall. The filamentous meshwork structurally resembles

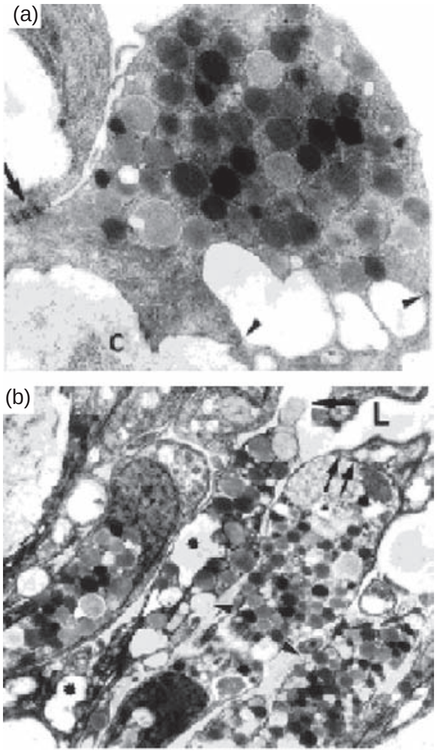


Figure 11 (a) Transmission electron micrographs of bulbar endocardial cells. They contain rounded, membrane-bound vacuoles filled with a homogeneous material of low-to-high electron density. Cells are joined by desmosome-like plaques (arrows) and gap junctions (arrows). Some collagen fibrils (C) appear under the endocardium. Magnification ×7075. (b) Detail of one endocardial element in contact with the bulbar lumen (L). Cells appear filled with rounded secretory vacuoles, many of which appear to be discharging their content directly into the blood (arrow) and into the extracellular area of the element (arrowheads). Some cells show a peripheral band of microfilaments (double arrow) which is accompanied by the formation of irregular cell surface extensions. Asterisks = fused vesicles. Magnification ×4550. Modified from Icardo JM, Colvee E, Cerra MC, and Tota B (1999) Bulbus arteriosus of the Antarctic teleosts. I. The white-blooded *Chrionodraco hamatus. Anatomical Record* 254: 396–407.

glycosaminoglycans (GAGS, long sugar chains) and indicates that a change in synthetic activity, from myofibrillar to structural polysaccharide chains, occurs at extremely low temperatures. Collagen is restricted in the outer layer but never associated with the smooth muscle of the middle layer.

Function

For efficient gas exchange, water flow over and blood flow through the gills should be continuous (**see also Ventilation and Animal Respiration:** Efficiency of Gas Exchange Organs). However, unlike in air-breathing vertebrates, which use the summed compliance of a long arterial tree to smooth pulsatile output from the heart, the juxtaposition of the heart and gills in fish requires the same job to be subserved by a relatively short ventral aorta and bulbus arteriosus. To this end, the extreme compliance of the bulbus allows it to expand during contraction, absorbing some of the energy of ventricular contraction. Then, during relaxation, and contrary to the situation in normal blood vessels, the bulbus maintains an elevated blood pressure even as internal diameter and volume decrease. An insight into this phenomenon can be gained by inflating the bulbus using a syringe and measuring the pressure generated. Plotting pressure against volume yields an r-shaped curve, showing that initially the bulbus is quite stiff but that it grows more compliant as more volume is injected (**Figure 12**(**a**)). Conversely, as the bulbus empties, it effectively becomes stiffer as it shrinks, resulting in a higher-than-expected pressure at small volumes.

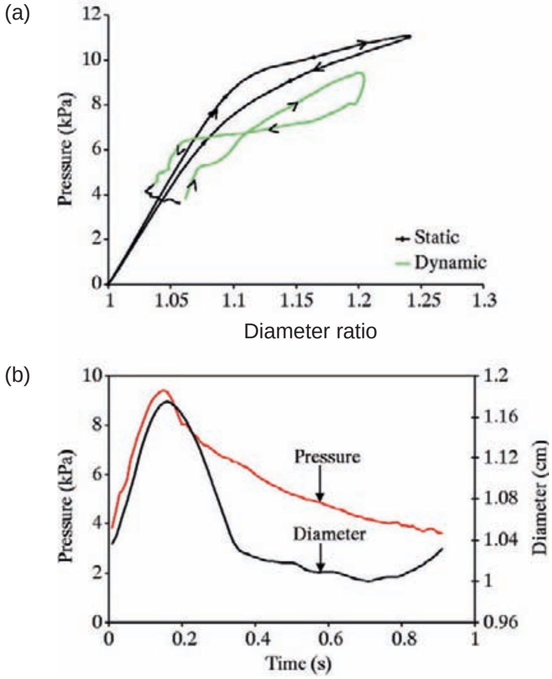


Figure 12 Recordings from a yellowfin tuna. (a) Comparison of static and dynamic pressure–diameter (P–D) loops. The dynamic trace (green) was created by plotting pressure against diameter for a single cardiac cycle. The static P–D curve (black) was created by a bulbar inflation from a syringe, post mortem. Arrows indicate the clockwise or anticlockwise cycling of the loop. (Diameter ratio is the diameter divided by the initial diameter.) (b) Recordings of bulbar blood pressure and diameter changes through a single cardiac cycle show that pressure remains significantly elevated despite large decreases in bulbar diameter. Elevated pressure = continued flow. Modified from Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores)*: Dynamic properties. *Journal of Experimental Biology* 206: 3327–3335.

Along with this specialized inflation behavior, the bulbus is compliant enough to hold several times the output of each heartbeat. A large compliance plus maintenance of positive pressure results in continuous blood flow throughout the cardiac cycle (**Figure 12**(**b**)). *In vivo* recordings of bulbar dimensions and pressures in a living tuna show that the bulbus in living fish does indeed act as predicted by mechanical inflation of the isolated bulbus: it helps convert the discontinuous blood flow from the ventricle into continuous blood flow in the ventral aorta (**Figures 12**(**a**) and **13**).

These properties of the bulbus are mostly passive, due primarily to both the shape and the material properties of the bulbar wall. Therefore, it is expected that the costs for maintenance of blood flow will be relatively low. Indeed, the difference between the inflation and deflation curves, or the amount of energy lost in a single inflation cycle, is relatively small (**Figure 13**) and, as a result, the bulbus is very efficient at returning energy to the circulation.

The plateau of the r-shaped curve represents the range over which the bulbus is most effective at storing large volumes of blood. The extreme compliance of the plateau is due to a number of factors, but most important is the high content of elastin in the walls of the bulbus. For example, 70% of the bulbus in salmonids is elastin, while carp bulbus is made up of nearly 90% elastin. The amount of stiff collagen in most bulbi is relatively low, for example, in rainbow trout (*Oncorhynchus mykiss*) the elastin:collagen ratio is 14. Despite this, the high stiffness and strength of collagen mean that this amount should be sufficient to significantly limit the expansion of the bulbus. However, unlike in normal blood vessels, the collagen fibers are usually arranged in such a manner that they are not significantly recruited until the bulbus undergoes a very large, possibly nonphysiological inflation. Over the operational range of the bulbus, it is unlikely that the collagen fibers play much of a role in limiting bulbar expansion. Some of the collagen may also be involved in setting the plateau of the r-shaped pressure–volume curve, as removing the contribution of either collagen or smooth muscle to the inflation results in a decrease in the height of the plateau (**Figure 14**). However, it is the shape and internal anatomy of the bulbus that play the largest role in setting the height of the plateau.

Despite the amount of elastin and limited effect of collagen, the bulbus is quite stiff at low filling pressures, resulting in the steep initial rise of the r-shaped curve. It is an interaction between radius, pressure, and tension, as described by the law of Laplace that explains this. The force in the wall (tension, T) that must be overcome for expansion to occur is equal to the pressure (P) in the vessel multiplied by the radius ($T = P \times R$). Therefore, a small lumen requires high pressure to open. The lumen of the relaxed bulbus is extremely small and, thus, a high pressure is required to open the lumen for flow (**Figure 9**(**c**)).

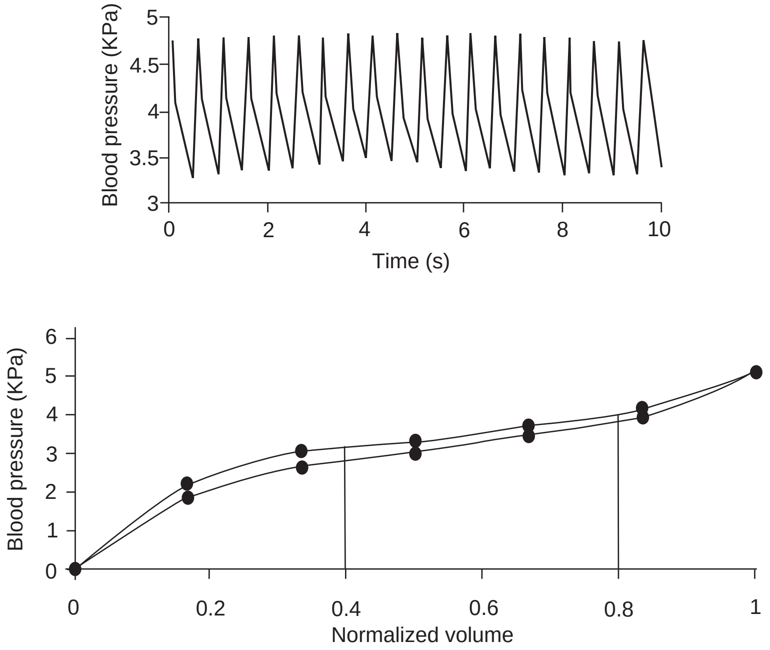


Figure 13 Pressure–volume loop for a 2.17-g stickleback showing that the blood pressure recorded from an anesthetized 5.5-g stickleback by bulbar puncture (inset) corresponds to the plateau of the curve created via static inflation of the bulbus with a syringe. Note that the blood pressure in the fish is always maintained above zero throughout the cardiac cycle. Modified from Jones DR, Perbhoo K, Braun MH (2005) Necrophysiological determination of blood pressure in fishes. *Naturwissenschaften* 92: 582–585.

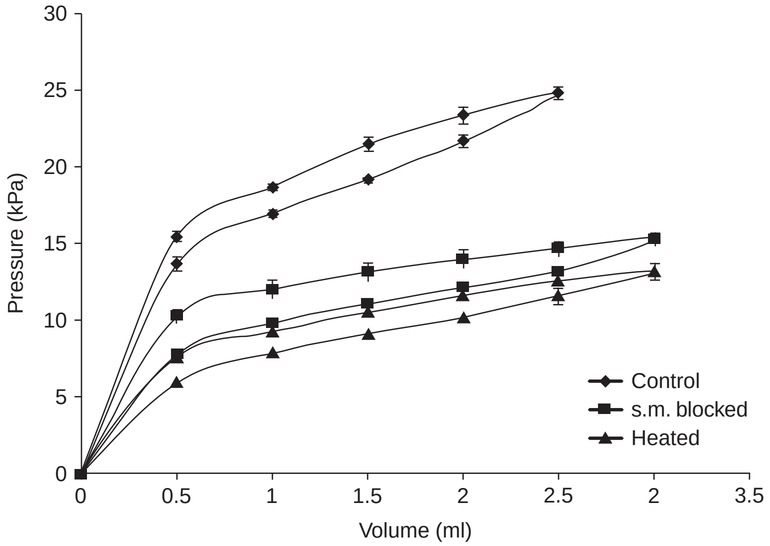


Figure 14 Yellowfin tuna (*N* = 4). Mean *P–V* loops from fresh bulbi before and after undergoing two treatments: a 10^{-5}·mol l^{-1} solution of nicardipine to inactivate the smooth muscle (s.m. blocked) and a high-temperature tissue bath to denature the collagen (heated). As the mechanical contributions of smooth muscle and collagen are effectively removed, the level of the plateau decreases. Values are means ± SEM. From Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores)*, bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): Static properties. *Journal of Experimental Biology* 206: 3311–3326.

Variations in the internal anatomy (ridges, longitudinal and radial elements, and tubes) result not only in variation in the effective internal radius but also in differences in the pressures required to open the bulbus, and, consequently, differences in the levels of the plateau.

The plateau portion of the pressure–volume loop has a strong correlation with the blood pressure in the living animal (**Figure** 15(**a**)). Moreover, central arterial blood pressure varies considerably among fishes (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). Thus, the material properties and shape must be matched to the physiological needs and arterial blood pressure of fishes. Therefore, in teleosts, the bulbus is tuned to the arterial blood pressure through variations in the shape and major constituents of the wall in the long term and smooth muscle contraction or relaxation in the short term. The short-term tuning changes allow blood pressure to increase during exercise and fall at rest. The tuning is so precise that it is possible to obtain a value for blood pressure in the living animal from inflating the isolated bulbus because the plateau of the r-shaped pressure–volume curve corresponds to the operational blood pressure range of the fish (**Figure** 15(**b**)).

In any cardiac system, a large elastic chamber just outside the heart smoothes the pressure fluctuations generated by the ventricle, reducing the peak and raising the trough. In fishes, the claim is frequently made that the reduction in peak pressure will protect the delicate gill capillaries from high blood pressure. However, there is some doubt whether the capillaries are ever in danger. In fish, gill capillaries are able to withstand considerable pressure due to their small size. For instance, the wall tension in a 10-μm diameter gill capillary at an extreme pressure (for a fish) of 80 mmHg is 0.1 g cm^{-1}, a very small force. For comparison, the tearing strength of 1 cm of the finest tissue paper requires a force 500-times larger. The very small size of capillaries gives the wall tension a considerable mechanical advantage over the pressure. Obviously, systolic pressure reduction to protect the gill capillaries is not a necessary function of the bulbus.

Form and Function of the Conus Arteriosus

Form

The conus is a tubular, muscular structure of variable length with many pocket valves either in rows running along its length or in discrete tiers running around the inner circumference. The conus of most sharks has between two and six transverse rows with three to six valves in each row, although even in a single species (dogfish) there may be two or three rows of valves depending on the length of the conus (**Figure 16**). When the rows are well defined, they divide the conus into separate compartments. Primitive actinopterygians can have nine transverse rows with four to eight valves in each row (**Figure** 17). Embryologically, the conus has

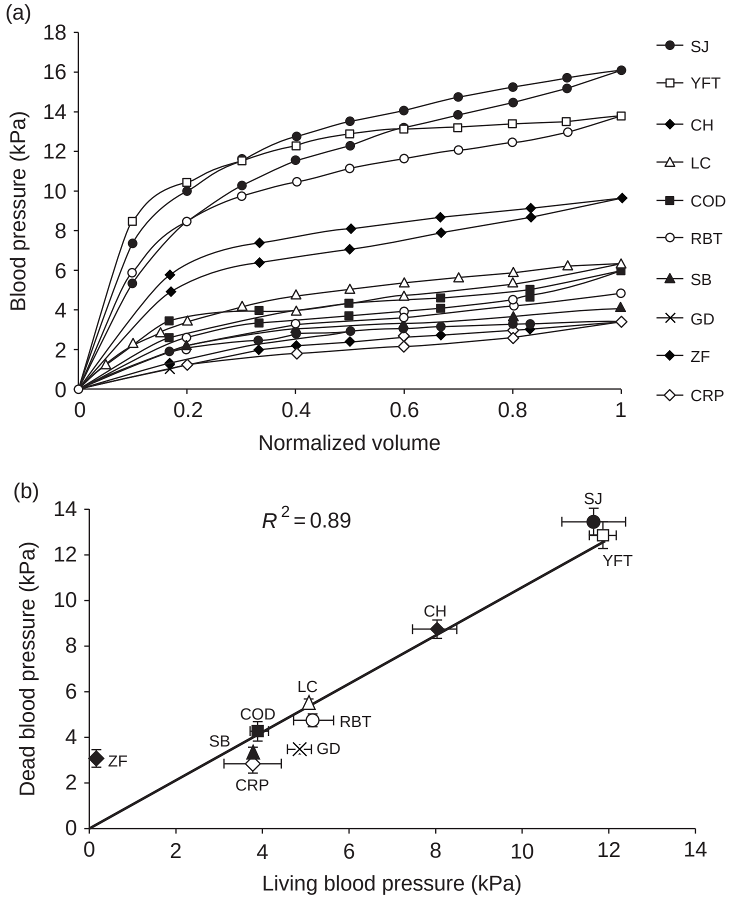


Figure 15 (a) Pressure–volume loops for the bulbus arteriosus of ten species of teleost fish showing how the level of the plateau varies between species. Species are as follows: SJ, skipjack tuna, *Katsuwonus pelamis* (<5 kg); YF, yellowfin tuna, *Thunnus albacares* (1.9 ± 0.1 kg); CH, coho salmon, *Oncorhynchus kisutch* (3.1 ± 0.4 kg); LC, lingcod, *Ophiodon elongates* (<2 kg); COD, cod, *Gadus morhua* (>0.5 kg); RBT, rainbow trout, *Oncorhynchus mykiss* (0.6 kg); SB, stickleback, *Gasterosteus aculeatus* (0.003 ± 0.001 kg); GD, giant danio, *D. aequipinnatus* (0.0037 ± 0.0003 kg); ZF, zebrafish, *Danio rerio* (0.0006 ± 0.0001 kg); CRP, carp, *Cyprinus carpio carpio* (0.5 ± 0.1 kg). (b) The relationship between the *in vivo* blood pressures recorded from fish in (a) and the blood pressures calculated by integration of the area between 40% and 80% of the r-shaped inflation curves. Standard error of the mean for *in vivo* determinations and bulbar inflations are shown for each species. R^2 = coefficient of determination. The relationship is highly significant and indicates that not only can *in vivo* blood pressure be calculated from static *in vitro* inflations, but that each bulbus is tuned to work at an optimal pressure range. Modified from Jones DR, Perbhoo K, Braun MH (2005) Necrophysiological determination of blood pressure in fishes. *Naturwissenchaften* 92: 582–585.

four endocardial ridges which, even in the absence of valve formation, are capable of coming together and occluding the conus during contraction. Pocket valves, with the cavity oriented toward the ventral aorta, develop from the dorsal and lateral ridges, the ventral ridge usually being vestigial. Up to six tiers can be present in selachians, but in more advanced representatives the number is usually reduced. In contrast, in the ancient teleosts (e.g., *Polypterus* (Bichirs and ropefish) and *Lepidosteous* (gar pike)), many rows of valves occur in the conus and their claimed orientation into tiers seems to be dependent on the 'eye of the beholder'. The valvular system is extremely complex; valves vary in shape from square to triangular to circular and in size from really elongated to just a small prominence (**Figure 16**). Nevertheless, the mechanism of valve formation has been modeled on a computer using the interaction of only two substances, I (inhibitor) and A (activator). I inhibits the synthesis of the A, which in turn activates the production of both I and A. All types of valve formations observed in nature can be generated as long as the conus is allowed to lengthen, which mirrors the natural situation because the longer the conus the more valves there are in it.

The outer layer of the conus consists of cardiac epithelial cells, which are continuous with those covering the ventricle. The region between the epithelium and the middle layer contains connective tissue abundant in collagen fibers and many blood vessels that arise from the coronary vasculature. The middle layer consists of

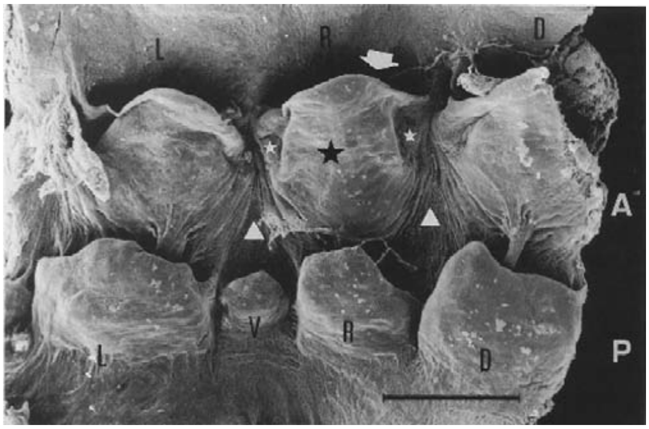


Figure 16 Scanning electron micrograph of the conus arteriosus from the dogfish (*Scyliorhynus canicula*) displayed in the open cut view to show the anterior (A) and posterior (P) transverse rows of valves. In the anterior row, there are three rows of valves: left (L), right (R), and dorsal (D). In the posterior row, there are four rows: left (L), ventral (V), right (R), and dorsal (D). As indicated in the right anterior valve, each valve has two components, namely, the leaflet (black asterisk) and the sinus (arrow). Note the difference in thickness between the central body and the lateral parts (white asterisks) of the leaflet. The triangle indicates the triangular spaces between adjacent leaflets in the anterior rows of valves. Scale = 1 mm. Modified from Sans-Coma V, Gallego A, Munoz-Chapuli R, et al. (1995) Anatomy and histology of the cardiac conal valves of the adult dogfish (*Scyliorhinus canicula*). *Anatomical Record* 241: 496–504.

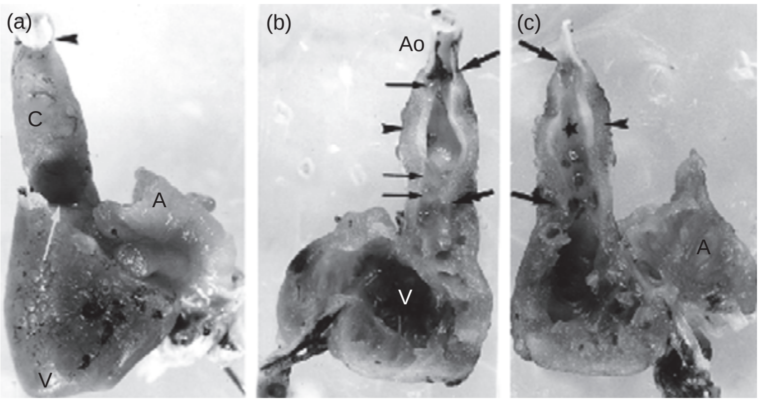


Figure 17 (a) Panoramic view of the sturgeon heart. Left-sided view. The conus (C) is displaced toward the right side of the ventricle (V). White arrow indicates the constriction at the conus origin. A, atrium. Magnification ×4. (b)This sturgeon heart was opened longitudinally following the conus axis. The (b) left and (c) right halves are presented. Note that the left side of the ventricular chamber is larger than the right side due to the location of the ventricle–conus junction. (b, c) Thick arrows indicate the conus limits. The lower thick arrow also indicates the ventricle-conus constriction. Thin arrows in (b) indicate the location of the conus valves. Note that a long segment of the conus is devoid of valves (star in (c). The subendocardial layer in this segment is very thick and resembles the aortic wall. Arrowheads point to the myocardial coat. A, atrium; Ao, intrapericardial portion of the ventral aorta; V, ventricle. Magnification ×4. Modified from Icardo JM, Colvee E, Cerra MC, and Tota B (2002) Structure of the conus arteriosus of the sturgeon (*Acipenser naccrii*) heart. I. The conus valves and the subendocardium. *Anatomical Record* 267: 17–27.

myocytes, which are generally smaller in diameter than those of the atrium and ventricle. The myocytes are separated by capillaries as well as by collagen and some elastin fibers, resembling the compact myocardium of the ventricle (**Figure 18**). Even if the ventricle is predominantly spongy with many trabeculae, the conus is always made up of compact myocardium. Complex connections, which greatly increase the surface area for electrical and chemical contact, join the ends of adjacent myocytes, while junctional complexes along their length hold the myocytes in register for synchronous contraction. In elasmobranchs, the myocytes are arranged circularly, while in the sturgeon (*Acipenser naccarii*), the arrangement is considerably more complex which may allow better emptying of the conus. Collagen fibers occur in the subendocardial space along with nerve fibers and terminals (**Figure 19**). In close contact with these terminals, granulated cells containing dense core vesicles occur. These granulated cells occur near axonal endings in the subendocardium, which have been interpreted as endings involved in the liberation of catecholamines. The granulated cells are made of chromaffin tissue that secretes

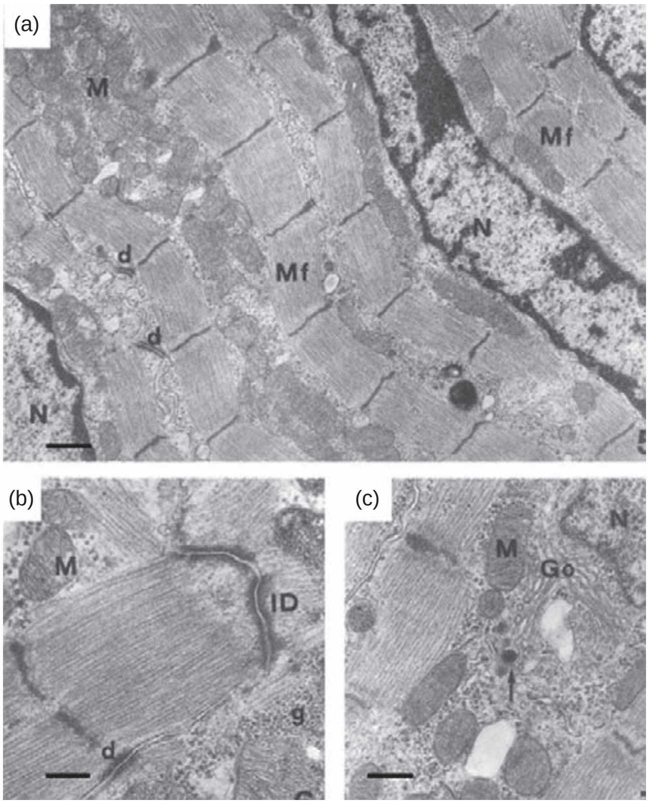


Figure 18 Transmission electron micrograph of the conus arteriosus from the dogfish (*Scyliorhynus sellaris*). (a) Myocardial cells in the middle layer of conus arteriosus. Myocytes, joined by desmosomes (d) at the lateral surface of the sarcolemma, contain a centrally located nucleus (N) and myofibrils (Mf) peripherally located. Mitochondria (M) in the perinuclear and in the interfibrillar regions. Scale = 0.595 μm. (b) Detail of the fine structure of the junctional complexes between the myocytes. Intercalated disks (ID) at the terminal end desmosome (d) at the lateral surface of the myocyte. M, mitochondria, g, glycogen granules. Scale = 0.3 μm. (c) Myocyte of the dorsal surface, perinuclear region. N, nucleus, M, mitochondria. In the perinuclear region is the Golgi complex (Go) from which originate central core granules (arrow). Scale = 0.38 μm. Modified from Zummo G and Farina F (1989) Ultrastructure of the conus arteriosus of *Scyliorhinus stellaris. Journal of Experimental Zoology Supplement* 2: 158–164.

amines into the bloodstream of the conus. It has been suggested that the chromaffin tissue could hemodynamically couple ventricular and conal activity (**Figure 19**).

Conal valves vary in complexity not only between species but also depending on their position within the conus. Typically, each valve has two parts. An outer leaflet which is free of attachment to the wall along its anterior border, containing and surrounding a sinus, which is probably sculpted from the endocardial ridges by the force of the blood **Figures 15** and **20**). The leaflets are thicker in the center than at their attachment to the walls and are stratified into two or three main layers: an outer and inner fibrosa, mainly composed of collagenous fibers oriented circumferentially sandwiching a layer of loose connective tissue rich in GAGs mixed up with collagenous and elastic fibers (**Figure 21**). Valves in the lower tier may also be attached to the ventricle by way of tendinous cords. In dogfish (*Scyliorhinus canicula*) with 2 sets of valves, those at the junction of the conus and ventral aorta receive contributions to their structure from the ventral aorta and are very large and can close against one another, and resist considerable back pressure from the blood without opening, even when the conus is relaxed. In contrast, the leaflets at the junction of the ventricle and conus are smaller and anchored to the conus wall by tendinous cords and cannot close in the absence of conal contraction. The ineffectiveness of the lower valve group in stopping flow in the absence of conus contraction is compounded by the fact that the ventral valve is much reduced. In the dogfish, each conal-aortic valve is tied to its corresponding ventricular-conal valve by a stout tendinous cord, made up of collagenous and elastic fibers covered by endocardium, suggesting that closing of the anterior valves begins as the posterior valves close. Embellishments in other sharks include increases in the complexity and branching of the cords, which act as guide ropes and prevent the individual valves being

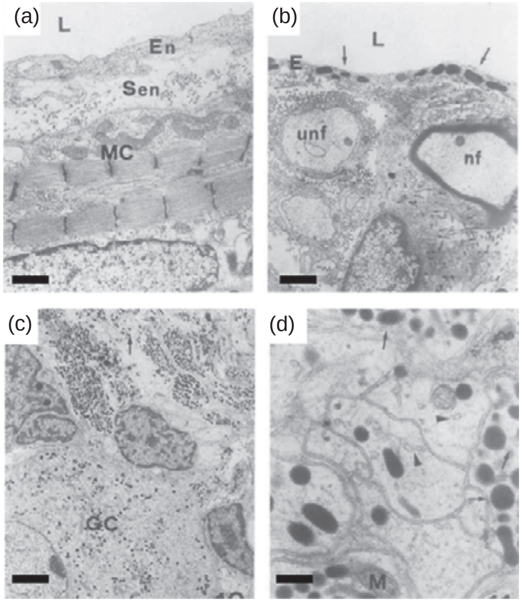


Figure 19 Transmission electron micrograph of the conus arteriosus from the dogfish (*Scyliorhynus sellaris*). (a) The endocardium covering the luminal surface. L, conal cavity. The lining consists of endocardial cells (En). Sen, subendocardium, MC, myocyte. Scale = 0.97 μm. (b) The inner endocardial layer near the ventricle shows endocardial cells (E) containing electron-dense granules (arrows). In the subendocardium, myelinated (nf) and unmyelinated (unf) axons are seen. Scale = 1.13 μm. (c) In the same region as (b) in the subendocardium, granulated, noncontractile cells (GCs) and axonal endings (arrow) occur. Scale = 2.1 μm. (d) Higher magnification showing many axonal ends in the subepicardium. M, mitochondria; arrows, electron-dense granules; arrowheads, vesicles. Scale = 0.23 μm. Modified from Zummo G and Farina F (1989) Ultrastructure of the conus arteriosus of *Scyliorhinus stellaris. Journal of Experimental Zoology Supplement* 2: 158–164.

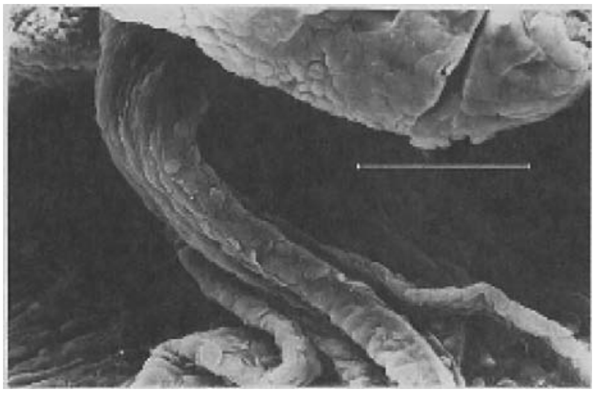


Figure 20 Scanning electron micrograph from the conus arteriosus of the dogfish (*Scyliorhynus canicula*). The commissure between two anterior valves viewed from the aortic aspect. The thin lateral parts (white asterisk) of the leaflets, which join to form the commissure, are attached to the walls of the ventral aorta. The black asterisk indicates the stout central body of one of the leaflets at the level of its free margin, whereas the white arrows point to the sinuses. Scale = 100 μm. From Sans-Coma V, Gallego A, Munoz-Chapuli R, et al. (1995) Anatomy and histology of the cardiac conal valves of the adult dogfish (*Scyliorhinus canicula*). *Anatomical Record* 241: 496–504.

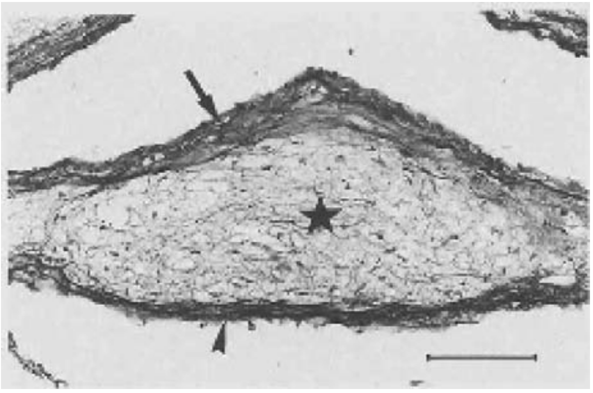


Figure 21 Light micrograph from the conus arteriosus of the dogfish (*Scyliorhynus canicula*). Transverse section of the leaflet of an anterior valve. The connective tissue of the central body is stratified in three layers: outer fibrosa (arrow), spongiosa (asterisk), and inner fibrosa (arrowhead). Scale = 100 μm. From Sans-Coma V, Gallego A, Munoz-Chapuli R, et al. (1995) Anatomy and histology of the cardiac conal valves of the adult dogfish (*Scyliorhinus canicula*). *Anatomical Record* 241: 496–504.

turned inside out as well as in the number of cords linking corresponding valves in different rows together (**Figure 22**). Included in the variability mix are a couple of sharks (*Pristiophorus japonicus* and *Discobatis sinensis*) which have the smallest valves bordering the aorta and the largest closest to the ventricle!

The variability in valve formations among elasmobranchs seems rather minor when other species are investigated. For example, in the ancient living fossil, the sturgeon, the conus has three rows of valves. The distal row has four leaflets, whereas the two proximal rows exhibit great variation in leaflet number, size, and shape. The three rows of valves are joined by tendinous cords. The conus has two restrictions at the conus-aorta and ventricular-conus junctions which, due to the ineffective nature of the valves and their arrangement, may form sphincters which arrest blood flow during conus contraction.

In sturgeon, the conus does not run right to the edge of the pericardium to connect to the ventral aorta. A distinct transitional region exists between the conus and ventral aorta, which differs from the conus behind and ventral aorta ahead. This could be an equivalent to the truncus of higher vertebrates, although recent investigations have questioned this. During early development, the entire conus is myocardial, but around the fourth day the distal portion takes on smooth muscle characteristics. Elastin appears somewhat later and the distal segment comes to look more and more like a blood vessel. This vessel is undoubtedly of cardiac origin. The distal segment has been named the bulbus arteriosus, which implies that the outflow tract came to be formed by a bulbus arteriosus

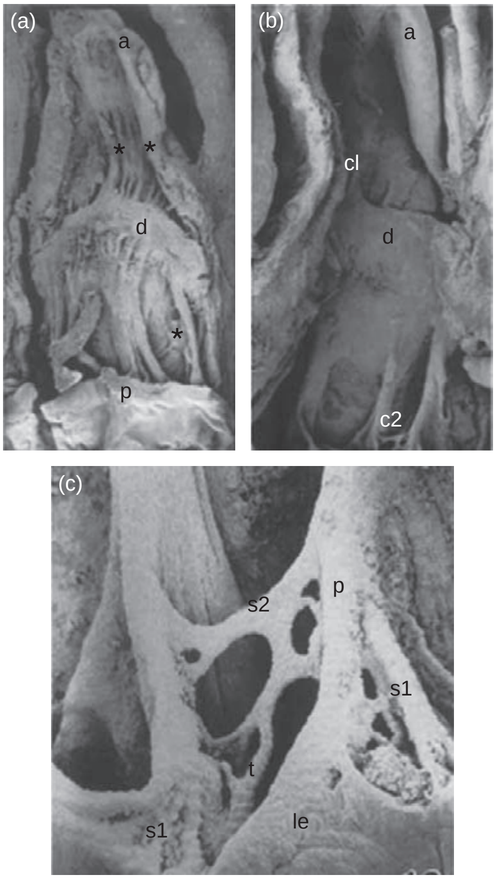


Figure 22 Images from the conus arteriosus of Rhizoprionodon terraenovae (Atlantic sharpnose shark). (a) Conal valves from 50 cm R. terraenovae. Valves of the anterior tier (a) lack chordae tendinae, while the proximal (p) and distal (d) tiers are supported by chordae (asterisks). X33. (b) Conal valves from adult R. terraenova. Chordae (c1) that support the distal (d) tier of valves branch less than the chordae (c2) that support the proximal (p) tier. a = anterior valve. X40. (c) Conal valve from adult R. terraenovae. Primary (p) chordae may attach directly to the valve leaflet (le) or branch to form secondary chordae that may attach to the leaflet (s1) or to adjacent chordae (s2). Secondary chordae may then branch to form tertiary (t) chordae, which then attach to the leaflet. X 200. Modified from Hamlett WC, Schwartz FJ, and Schmeinda CE (1996) Anatomy, histology and development of the cardiac valvular system in elasmobranchs. *Journal of Experimental Biology* 275: 83–94.

and conus arteriosus in early evolutionary history. If the bulbus truly is a bulbus and not a truncus, then this seems to place the final nail in the coffin of the theory that the ventral aorta grows backward into the pericardium to obliterate the conus and form the bulbus.

In fact, if the ventral aorta actually gobbles up the conus arteriosus in the course of its evolutionary advance, how is it that a conus has been identified in a wide range of advanced teleosts? Initial studies on the gilthead seabream (*Sparus auratus*) showed a distinct muscular segment between the ventricle and bulbus, organized very differently from the completely trabeculated (spongy) ventricle (**Figure 5**). Criteria used to identify this muscular segment as a conus were its shape, its inner wall of well-vascularized compact myocardium, the presence of elastin and collagen in the inner and outer layers of the wall, and support of the ventricular-bulbar valves. The presence of a conus or conal segment has been confirmed in a large number of other species recently. The conus is most obvious in the 80% or so of species with trabeculated hearts because the conus is made up of compact myocardium and readily distinguished from the heart muscle. In species with compact hearts, if the conus is not a discrete chamber then it generally has the form of a fibrous ring at the base of the ventricle. The valve leaflets are inserted into the lower end of the conus forming a fibrous hinge, facilitating valve movement. The conus is furnished with two pocket-like valves, although there is considerable variation between species. The fact that the valves are attached to the conus wall means that they should properly be called conal valves.

Function

What function(s) does the conus perform? One role it certainly shares with the bulbus is secretory. Amines are released from glomus cells in the inner portion of the wall in elasmobranchs. Other substances are released from the outer layer of the conal wall. The presence of many vesicles in the superficial epithelium suggests very active exchange with the pericardial fluid. In addition, the outer layer may contribute to the formation and maintenance of the immune system in sturgeons as it has the appearance of a complex lympho-hemopoietic tissue. It would be interesting to investigate this tissue in other fishes.

From a hemodynamic perspective, the view of many authors is that the conus is just a muscular bulbus and performs similar roles as the elastic bulbus. Can the conus store and release some of the ventricular pulse and prolong outflow from the heart? This seems unlikely because cardiac muscle is much stiffer than the smooth muscle that predominates in the wall of the bulbus. Furthermore, the conus is contracting during cardiac outflow, which will further stiffen the wall and reduce its volume. Perhaps by its contraction the conus adds to the ejection fraction from the heart, although virtually all studies show this contribution to be minor with blood flow slowing or even stopping before or by the end of the each cardiac cycle.

Conal contraction is delayed with respect to that of the ventricle by the presence of nodal tissue between the ventricle and conus by 250–500 ms. The wave of electrical activation spreads across the conus causing it to contract from the bottom to the top. The first thing that occurs is that the ventricular-conal valves, which are too small to bridge the opening in the relaxed conus, will be brought together and stop flow from the heart. The same will take place in other tiers of valves as the contraction wave passes. The conal-aortic valves are large and can close passively due to backflow of blood from the ventral aorta. When the heart refills, it is probable that the conus is filled along with the ventricle. Hence, there will be a volume of blood contained within the conus for the ventricle to push against. As noted previously, blood is incompressible and this contained blood places an extra burden on the ventricle and accommodating this extra load may be an added bonus of the presence of a bulbar structure between the conus and ventral aorta. Nevertheless, the whole stroke volume has to be accommodated and this function falls on the ventral aorta and other elastic structures such as cavernous bodies, which are present in the gill vasculature.

The conus provides us with snapshots in development of form and function that stretches from the most ancient to most advanced jawed fishes. The early conal design of a contractile conus with four endocardial ridges is capable of arresting blood flow in the low-pressure embryonic circulation and embellishments such as valves and sphincters improve the capacity to hold against higher pressures, both above and below atmospheric in sharks and rays. Reductions in both the size of the conus and number of valves finally give us a superb design that is able to hold against pressures that in some teleosts can be among the highest in the animal kingdom and also closes actively. This is evolution by natural selection painted with a broad brush and, as Stephen J. Gould pointed out, the character of imperfections and improvisations sheds light on evolutionary origins, relationships, and mechanisms.

Acknowledgment

The author was supported by a Discovery grant from NSERC of Canada.

See also: **Deep-Sea Fishes. Design and Physiology of the Heart:** Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping; The Coronary Circulation. **Hagfishes and Lamprey:** Hagfishes. **Ventilation and Animal Respiration:** Efficiency of Gas Exchange Organs.

Further Reading

Benjamin M, Norman D, Santer RM, and Scarborough D (1983) Histological, histochemical and ultrastructural studies on the bulbus arteriosus in the sticklebacks, Gasterosteus and Punggitius pungitius (Pisces:Teleostei). *Zoology* 200: 325–346.

Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores*): Dynamic properties. *Journal of Experimental Biology* 206: 3327–3335.

Braun MH, Brill RW, Gosline JM, and Jones DR (2003) Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacores*), bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): Static properties. *Journal of Experimental Biology* 206: 3311–3326.

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A Cardiovascular System*, pp. 89–139. San Diego, CA: Academic Press.

Duran AC, Fernandez B, Grimes AC, *et al.* (2008) Condrichthyans have a bulbus arteriosus at the arterial pole of the heart: Morphological and evolutionary implications. *Journal of Anatomy* 21: 597–606.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A: The Cardiovascular System*, pp. 1–88. San Diego, CA: Academic Press.

Grimes AC and Kirby ML (2009) The outflow tract of the heart of fishes: Anatomy, genes and evolution. *Journal of Fish Biology* 74: 983–1036.

Hamlett WC, Schwartz FJ, and Schmeinda CE (1996) Anatomy, histology and development of the cardiac valvular system in elasmobranchs. *Journal of Experimental Biology* 275: 83–94.

Hu N, Yost HJ, and Clark EB (2001) Cardiac morphology and blood pressure in adult zebrafish. *Anatomical Record* 264: 1–12.

Icardo JM (2006) Conus arteriosus of the teleost heart: Dismissed, but not missed. *Anatomical Record Part A* 288A: 900–908.

Icardo JM, Brunelli E, Perrotta I, *et al.* (2005) Ventricle and outflow tract of the African lungfish Protopterus dolloi. *Journal of Morphology* 265: 43–51.

Icardo JM, Colvee E, Cerra MC, and Tota B (1999) Bulbus arteriosus of the Antarctic teleosts. I. The white-blooded Chrionodraco hamatus. *Anatomical Record* 254: 396–407.

Icardo JM, Colvee E, Cerra MC, and Tota B (2000) The bulbus arteriosus of stenothermal and temperate teleosts: A morphological approach. *Journal of Fish Biology* 57(supplement A): 121–135.

Icardo JM, Colvee E, Cerra MC, and Tota B (2002) Structure of the conus arteriosus of the sturgeon (*Acipenser naccrii*) heart. I. The conus valves and the subendocardium. *Anatomical Record* 267: 17–27.

Icardo JM, Colvee E, Cerra MC, and Tota B (2002) Structure of the conus arteriosus of the sturgeon (*Acipenser naccrii*) heart. II. The myocardium, the subepicardium, and the conus-aorta transition. *Anatomical Record* 268: 388–398.

Jones DR, Perbhoo K, Braun MH (2005) Necrophysiological determination of blood pressure in fishes. *Naturwissenchaften* 92: 582–585.

Munoz-Chapuli R, Gallgo A, and Perez-Pomares J (1997) A reaction–diffusion model can account for the anatomical pattern of the cardiac conal valves in fish. *Journal of Theoretical Biology* 185: 233–240.

Pelouch V and Vornanen M (1996) Effects of thermal acclimation on ventricle size, protein composition, and contractile properties of crucian carp heart. *Journal of Thermal Biology* 21(1): 1–9.

Sans-Coma V, Gallego A, Munoz-Chapuli R, *et al.* (1995) Anatomy and histology of the cardiac conal valves of the adult dogfish (*Scyliorhinus canicula*). *Anatomical Record* 241: 496–504.

Schib JL, Icardo JM, Duran AC, *et al.* (2002) The conus arteriosus of the adult gilthead seabream (*Sparus auratus*). *Journal of Anatomy* 201: 395–404.

Watson AD and Cobb JLS (1979) A comparative study on the innervation and the vascularization of the bulbus arteriosus in teleost fish. *Cell and Tissue Research* 196: 337–346.

Zummo G and Farina F (1989) Ultrastructure of the conus arteriosus of *Scyliorhinus stellaris*. *Journal of Experimental Zoology Supplement* 2: 158–164.

Relevant Website

http://www.bishops.k12.nf.ca – Fish Circulatory System: Heart.

Physiology of Cardiac Pumping

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Cardiac Cycle
Pumping Assistance
Cardiac Output
Cardiac Stroke Volume
Heart Rate
Central Arterial Blood Pressure
Cardiac Work
Summary
Further Reading

Glossary

Action potential A small and transient change of voltage across the cell membrane when the negative resting potential momentarily becomes nearly zero volts and quickly returns to the original level in a few milliseconds. The action potential is possible because of the activity of ion channels, proteins of the cell membrane selectively allowing certain inorganic ions to pass through their pore.

Atrium A thin-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Cardiac cycle The changes in blood pressure, blood flow, and electrical activity that accompany cardiac filling and emptying associated with a single heartbeat.

Cardiac output (V_b) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Cardiac stroke volume (V_s) Volume of blood pumped out of the ventricle in a single heartbeat; the difference between ventricular end-diastolic volume and end-systolic volume.

Chronotropy Pertaining to heart rate.

Diastole A period of the cardiac cycle when cardiac muscle is relaxed or relaxing. It is associated with filling the heart with blood.

Diastolic Pertaining to diastole, it is the lowest pressure in a pressure pulse.

Electrocardiogram A recording of the waves of electrical activity associated with each heartbeat.

End-diastolic volume Volume of blood in a cardiac chamber at the end of filling (diastole).

End-systolic volume Volume of blood in a cardiac chamber at the end of emptying (systole).

Heart A valved, muscular pump that propels fluid, usually blood or lymph, around the circulatory system.

Inotropy Pertaining to force or vigor of cardiac contraction.

Isometric A contraction with the same length.

Isotonic A contraction with the same force or tension.

Myocardial power output The external work performed by the heart per unit time.

Systole A period of the cardiac cycle when cardiac muscle is contracted or contracting. It is associated with the ejection of blood from the heart.

Systolic Pertaining to systole, it is the highest pressure in a pressure pulse.

Ventricle A thick-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Vis-a-fronte A term for a supplementary cardiac filling mechanism that uses energy directly from ventricular contraction; literally, the force from in front.

Vis-a-tergo A term for a cardiac filling mechanism that uses energy contained in venous blood; literally, the force from behind.

Introduction

The fish's heart, like that of other vertebrates, is a muscular pump that contracts rhythmically (the heartbeat). Each cardiac contraction generally produces sufficient energy to eject blood into an artery, propelling it in a continuous motion through blood vessels and back to the heart, usually without further pumping. Therefore, the heart is the powerhouse of the circulatory system.

The cardiac chambers within the pericardial cavity of fish are the sinus venosus, atrium, ventricle, and an outflow tract. Each chamber plays an important role in the integrated function of the heart. The main role of the sinus venosus is to generate and propagate the action potential, which initiates the heartbeat (**see also Design and Physiology of the Heart:** Action Potential of the Fish Heart). The main role of the outflow tract is to help depulse arterial pressure and flow before blood reaches the delicate gill capillaries (**see also Design and Physiology of Arteries and Veins:** Branchial Anatomy). This article delineates the primary role of the atrium and ventricle, which is to rhythmically pump blood and eject a specific volume of blood at a specific blood pressure with each heartbeat.

The rhythmic activity associated with each heartbeat is called a cardiac cycle. The cardiac cycle can be described from three perspectives: the emptying and filling of cardiac chambers; the patterns of pressure generation; and the electrical events associated with this activity (the electrocardiogram (ECG)). Cardiac anatomy and cellular structure, as well as the mechanistic basis for the action potential and cardiac contraction, are discussed elsewhere in this encyclopedia (**see also Design and Physiology of the Heart:** Cardiac Anatomy in Fishes, Action Potential of the Fish Heart, Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux, and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

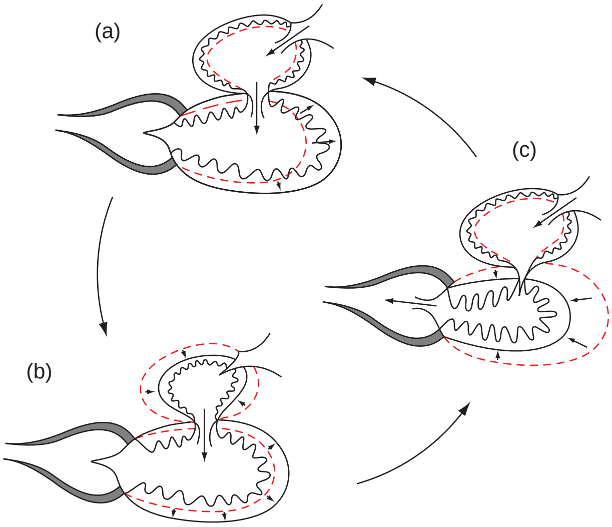


Figure 1 A schematic diagram of the three main phases of filling and emptying of four cardiac chambers of the fish heart. (a) Both the atrium and ventricle are filling due the venous blood pressure. (b) The atrium contracts (as shown by the small arrows), further expanding and completing the filling of the ventricle. (c) The ventricle contracts, ejecting fluid into the bulbus arteriosus. At the same time, the atrium begins to fill.

The Cardiac Cycle

The cardiac cycle can be divided into periods of systole and diastole, which correspond to cardiac muscle contraction and relaxation, respectively. During systole, cardiac muscle generates blood pressure and ejects blood by decreasing the volume of the ventricle or atrium. When the cardiac muscle relaxes during diastole, the atrium and ventricle can fill with blood and increase their volume. For example, in a spontaneously beating isolated heart from a rainbow trout, the atrial contraction can be seen to precede ventricular contraction and how both chambers alternate between diastolic and systolic states (see **Video Clip 1**). The net result of these synchronized contractions is a unidirectional flow of fluid through the cardiac chambers.

The Flow Events: Cardiac Chamber Filling and Emptying

Figure 1 illustrates the sequential filling and emptying of the cardiac chambers during a cardiac cycle.

The cardiac cycle begins with the atrium and ventricle in a relaxed state. During diastole, blood flowing from central veins fills the atrium and partially fills the ventricle, passing through the sinus venosus, the sino-atrial (SA) canal and the atrio-ventricular (AV) canal. Atrial contraction (atrial systole) then ejects blood from the atrium and into the ventricle, which is still in diastole, and this completes ventricular filling. The contributions of these two phases of ventricular filling vary among species, with sometimes only ~50% occurring via atrial contraction. In addition, the contribution of passive ventricular filling may decrease at high heart rates when the diastole is shorter.

Atrial relaxation (atrial diastole) occurs while the ventricle is contracting (ventricular systole). However, before ventricular contraction starts, the propagation of the action potential from the atrium is briefly delayed in the AV canal. This AV delay ensures that blood has sufficient time to move from the atrium and complete ventricular filling. Ventricular contraction ejects blood into the outflow tract past an opened bulbo-ventricular (BV) valve. The cardiac cycle is completed when the ventricle relaxes (ventricular diastole).

The Pressure Events

Blood flow follows an energy gradient, which is usually a difference in blood pressure (potential energy) between two points in the circulatory system. In general, the pressures developed by the fish heart are similar in form but lower in magnitude to those of the mammalian left ventricle. There remain, however, important differences among fishes in both the magnitude and the form of the pressure waves during the cardiac cycle, as shown by the three examples in **Figure 2**.

The heart imparts energy to the blood when it starts its circuit of the body. Most of this energy is lost to vascular resistance. Cardiac filling uses any remaining energy in venous blood. Venous blood in the central veins normally has sufficient kinetic and potential energy to completely fill the atrium and start filling the ventricle during atrial

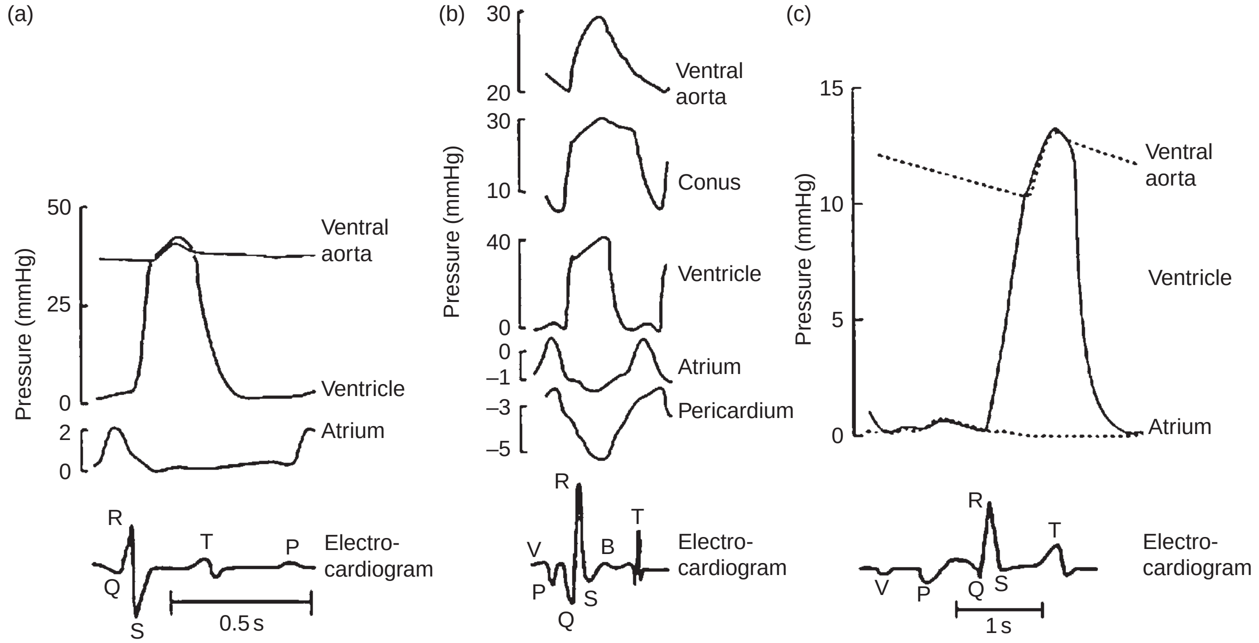


Figure 2 Features of the cardiac cycle in three different fishes. Representative examples of the changes in blood pressure and electrical activity (the electrocardiogram, ECG) associated with the cardiac cycle are illustrated for (a) the trout heart, (b) the shark heart, and (c) the hagfish heart. The ECG is shown in the lower panel, with each of the major electrical waves labeled (see text for details). The coincidence of these electrical waves with the contractions and relaxations of the cardiac chambers can be seen by a comparison with the blood pressure events for the atrium, ventricle, and main outflow vessels shown above. From Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, ch. 1, pp. 1–88. San Diego, CA: Academic Press.

diastole (**Figure 2**). This type of cardiac filling is termed *vis-a-tergo* (literally, the force from behind) because the energy comes from venous blood.

Atrial and ventricular blood pressures rise slightly during diastolic filling of these chambers. Atrial contraction (systole) then increases atrial blood pressure and completes ventricular filling. Atrial systole consists of an isometric (same length) muscle contraction, which builds up blood pressure and closes the SA valve. Once the SA valve is closed the muscle contraction becomes isometric, resulting in a rise in atrial pressure to its systolic value, which forces blood into the ventricle through the AV canal. When the atrium relaxes, atrial blood pressure returns to its diastolic level.

The AV canal and its valves offer minimal resistance to blood flow between the atrium and ventricle; consequently, there is never a very large difference between atrial and ventricular blood pressures during ventricular diastole.

Although the thin-walled atrium generates a much smaller systolic pressure compared with the thick-walled ventricle, there is sufficient pressure to stretch the wall of the ventricle and achieve a faster rate of ventricular filling. Conversely, the more compliant atrium allows atrial filling to be accomplished using just the low venous blood pressure.

Before blood can leave the ventricle, ventricular contraction must generate a blood pressure that can overcome the arterial diastolic blood pressure, which is considerably higher than the ventricular end-diastolic blood pressure. Therefore, ventricular systole involves a vigorous isometric contraction that rapidly and substantially increases ventricular pressure (**Figure 2**). This pressure rise first closes the AV valve and then opens the BV valve when ventricular pressure slightly exceeds arterial diastolic blood pressure. Ventricular contraction then becomes progressively isotonic, emptying the ventricle and forcefully ejecting blood into the outflow tract. This isotonic contraction results in a parallel rise of arterial blood pressure until systolic arterial blood pressure is reached. Ventricular relaxation follows, causing ventricular pressure to fall below arterial blood pressure, which closes the BV valve. Arterial pressure falls slowly toward its diastolic pressure until the next heartbeat, as blood moves through the circulatory system. As a result of the cardiac cycle, arterial blood pressure has a pulse (peak = systolic; trough = diastolic).

Cardiac Filling

Atrial filling (end-diastolic volume) is determined by various factors, including: end-systolic volume (which sets how much blood was present before filling started), the pressure gradient (which sets the rate of filling and the degree of cardiac stretch), wall compliance (which sets the amount of pressure needed to stretch the wall), and heart rate (which sets the time available for filling). The same factors, as well as atrial contraction, set end-diastolic volume of the ventricle. Atrial and ventricular end-systolic volumes are set by the strength of their respective contractions, which are determined in large part by cardiac stretch (see Frank–Starling effect below). All of the above factors can vary, altering the volume and pressure outputs of the heart.

The Special Case of *vis-a-fronte* Cardiac Filling in Fishes

A special form of supplementary atrial filling has been recognized in sharks, lungfishes, salmonids, and tunas, suggesting an early appearance in the evolution of the chambered vertebrate heart. These fishes have a pericardial cavity that acts like a rigid structure, largely as a result of stiff surrounding tissues adhering to the pericardium. Consequently, whenever the contracting ventricle decreases its volume, another chamber must correspondingly expand to conserve volume within the rigid pericardial cavity (**Figure 3**). Thus, the more compliant atrium expands, assisting atrial filling, courtesy of the energy imparted by the powerful ventricular contraction. Mechanistically, ventricular contraction actually creates a subambient intrapericardial pressure pulse, which is transmitted across the atrial wall, lowering its diastolic blood pressure and creating a more favorable blood pressure gradient for atrial filling from central veins. This supplementary atrial filling mechanism is termed *vis-a-fronte* (literally, a force from in front) because some of the energy of a ventricular contraction is used directly to fill the heart rather than have the energy travel all the way through the circulatory system.

Vis-a-fronte filling may be particularly important in fish with subambient venous blood pressures (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). However, *vis-a-fronte* filling gives way to *vis-a-tergo* filling when fish become more active, likely because venous blood pressure increases with activity.

Another type of *vis-a-fronte* cardiac filling probably plays a minor role in the filling of the atrium and ventricle of all chambered hearts. It involves the recoil of the elastic elements of the heart. Some of the energy of contraction is stored in the wall of the heart until diastole. Elastic recoil during relaxation then sucks blood into the chamber.

The Electrical Events

Waves of electrical activity accompany each heartbeat. This electrical activity has a magnitude that can be recorded on or near the surface of the skin as the ECG. Because the ECG is the sum of the electrical changes (depolarizations and repolarizations) during the cardiac cycle, its pattern closely corresponds to and precedes contractions and relaxations (**Figure 2**). The ECG pattern seen in fishes is very similar to that of mammals, but with additional events.

The principal electrical components are the P-wave, the QRS-wave, and the T-wave. In some fish, the ECG starts with a small V-wave, which represents the contraction of bundles of cardiac muscle in the sinus venosus; a V-wave is absent in the mammalian ECG. Shortly afterward, a larger P-wave represents the syncytial contraction of atrial muscle (**Figure 2**). The interval between the V- and P-waves is indicative of the time taken for the action potential to travel

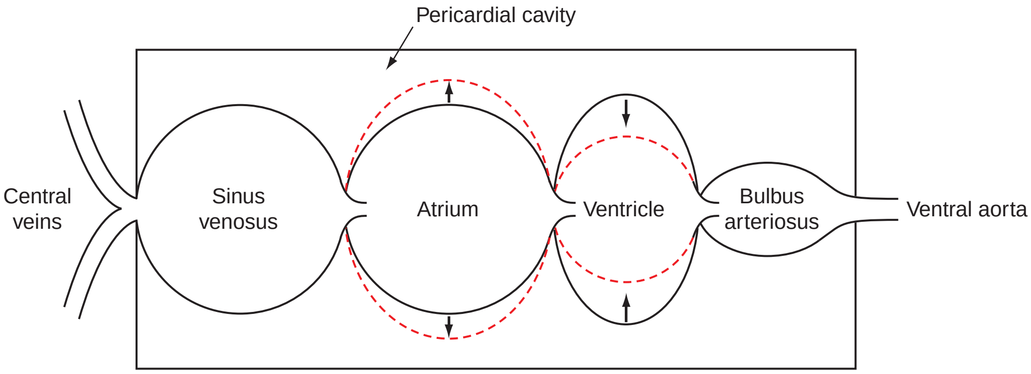


Figure 3 A schematic diagram of the synchrony between ventricular contraction and atrial expansion as produced with *vis-a-fronte* atrial filling when a rigid pericardium creates a pericardial cavity with a near constant volume. As the volume of the ventricle decreases (direction of arrows to the broken line), the volume of the atrium expands (direction of arrows to the broken line).

from the sinus to the atrium. A considerably larger QRS-wave represents the syncytial contraction of the thicker ventricular muscle. The interval between the P- and R-waves is indicative of the AV delay, the time taken for the action potential to travel through the AV canal, the connection channel between the atrium and ventricle.

The electrical activity associated with the ventricular relaxation is represented by the T-wave (**Figure 2**). However, atrial repolarization is usually overwhelmed by the simultaneous ventricular contraction because the ECG only integrates and does not differentiate between simultaneous electrical events. In hagfishes, however, a protracted AV delay of the action potential along its long AV (**see also Design and Physiology of the Heart:** Action Potential of the Fish Heart) canal means that atrial repolarization can be visualized as a T-wave preceding the QRS-wave.

Pumping Assistance

In some fishes, the ventricle does not generate sufficient energy for blood to complete the circuit of the entire circulatory system. There are several assistance mechanisms.

Contractions by Other Cardiac Chambers

The multivalved conus arteriosus of elasmobranchs assists arterial blood flow with rhythmic contractions of its cardiac muscle (**see also Design and Physiology of the Heart:** The Outflow Tract from the Heart). These contractions result in a small B-wave in the ECG, in between the S- and T-waves. A Br-wave, following the T-wave, can sometimes signal conal repolarization.

While the V-wave in the ECG corresponds to a coordinated contraction of cardiac muscle, the contribution of sinus contraction to cardiac filling is uncertain. Without ostial valves between the sinus venosus and central veins, a sinus contraction is just as likely to propel blood backward rather than forward into the atrium. Thus, rhythmic contractions of the sinus venosus may be more important in preventing the *vis-a-fronte* effect of ventricular contraction from distending the very thin-walled sinus venosus, which would slow down atrial filling.

Other Mechanisms

In mammals, venous blood flow is assisted by the massaging effect of skeletal muscle contractions, which is all too apparent when humans collapse after standing still for too long because venous return from the lower limbs becomes insufficient. The importance of this mechanism is unknown for fish because they lack parietal venous valves, which prevent backflow of blood during vein compression in mammals.

Some fishes also have accessory hearts that assist venous blood flow (**see also Design and Physiology of the Heart:** Accessory Hearts in Fishes). The volumes of the sinus venosus and ductus Cuvier may be regulated by tonic activity of the muscle in their walls.

Cardiac Output

Cardiac output ($\dot{V}_b$) is the volume of blood pumped per unit time out of the ventricle. $\dot{V}_b$ varies by an order of magnitude among fishes ($10\text{–}100\ \mathrm{ml\ min^{-1}\ kg^{-1}}$) (**Table 1**). Because the main purpose of blood flow is to deliver oxygen to tissues, $\dot{V}_b$ is tightly coupled with a fish's metabolic rate. $\dot{V}_b$ is determined by how fast the heart beats (heart rate, f_H) and the volume of blood ejected from the ventricle with each heartbeat (cardiac stroke volume, V_s).

Cardiac Stroke Volume

V_s is typically in the range of $0.3\text{–}0.6\ \mathrm{ml\ kg^{-1}}$, but varies by an order of magnitude among fishes. It is exceptionally large in Antarctic ice fishes ($10\ \mathrm{ml\ kg^{-1}}$) (**Table 1**). V_s is the difference between ventricular end-diastolic volume and end-systolic volume. However, end-systolic volume cannot decrease very much because the heart normally ejects 80–95% of its volume. Consequently, altering end-diastolic volume is the main mechanism for changing V_s with environmental change.

Over the long term, the heart ejects all the blood it receives. This occurs automatically because cardiac muscle cells have a special intrinsic property, the Frank–Starling mechanism: they can automatically increase their mechanical work when muscle fibers are stretched. (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and Cardiac Cellular Length-Tension Relationship). Consequently, whenever end-diastolic volume increases with additional filling, cardiac stretch enhances the force of the subsequent contraction and end-systolic volume remains unchanged. In essence, hearts automatically pump harder when stretched so that additional filling is automatically matched by appropriate cardiac emptying. A consequence of the Frank–Starling mechanism is that V_s is directly related to venous filling pressure (**Figure 4**).

Fishes increase V_s up to threefold (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping and **Integrated Control and Response of the Circulatory System:** Integrated Responses of the Circulatory System to Hypoxia). Much of this range is

Table 1 Cardiovascular variables in resting fish

Species	°*C*	*Body mass (kg)*	*Cardiac output (ml* min^{-1} kg^{-1}*)*	*Heart rate* $(min)^{-1}$	*Cardiac stroke volume* $(ml\ kg)^{-1}$	*Ventral aortic blood pressure (kPa)*	*Myocardial power output* $(mW\ kg)^{-1}$
Hagfishes							
Myxine glutinosa	11	0.6–0.9	8.7	22	0.41	1.03	0.15
Eptatretus cirratus	17	1.4	15.8	25	0.67	1.60	0.42
Elasmobranchs							
Scyliorhinus stellaris	19	2.8	52.5	43	1.21	3.35	2.93
Scyliorhinus canicula	14	0.6–1.1	23.2	30	0.81	4.18	1.62
Teleosts							
Anguilla anguilla	15	0.51	11.5	37	0.29	5.05	0.97
Cyprinus carpio	5	1.3	4.1	9	0.44	2.39	0.16
Cyprinus carpio	10	1.3	10.5	14	0.77	3.26	0.57
Cyprinus carpio	15	1.3	13.5	21	0.60	3.70	0.81
Gadus morhua	11	0.4–0.8	17.3	43	0.39	4.89	1.41
Hemitripterus americanus	11	0.7–1.4	18.8	37	0.51	3.79	1.19
Oncorhynchus mykiss	11	0.7–1.5	17.6	38	0.46	5.19	1.52
Ophiodon elongatus	10	3.8–8.5	11.2	29	0.38	5.05	0.94
Pagothenia borchgrevinki	0	0.06	29.6	11	2.16	3.73	1.84
Thunnus albacares[a]	26	1.4	115	97	1.30	11.9	22.9
Katsuwanus pelamis[a]	26	1.6	132	126	1.08	11.6	25.5

[a]spinalectomized fish.

Adapted from Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology*, 3rd edn., pp. 119–152. Boca Raton, FL: CRC Press.

possible through the intrinsic response to cardiac filling (**Figure 4**) and so automatic regulation of V_s is particularly important for fishes, even hagfish. (In contrast, mammals rarely increase V_s by 50%.) As a result, venous blood pressure is directly related to V_s. Even so, with end-systolic volume near zero, any substantial increase in V_s requires more blood to be returned to the heart. Whenever a portion of the Frank–Starling curve occurs at subambient filling pressures (**Figure 4**), this is usually taken as an indication of *vis-a-fronte* atrial filling. In fact, opening the pericardium shifts the Frank–Starling curve toward higher filling pressures (**Figure 4**).

Extrinsic factors alter the sensitivity of the heart to a given filling pressure (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). For example, increasing cardiac contractility (positive inotropy) with adrenergic stimulation (of neural or humoral origin) shifts the Frank–Starling curve to the left, increasing cardiac sensitivity to venous blood pressure (**Figure 4**). Conversely, weakening cardiac contraction (negative inotropy) with cholinergic stimulation (of vagal origin) lowers its sensitivity to venous blood pressure. Cardiac contractions are also weakened by hypoxia and reducing blood pH (acidosis). These adverse conditions are tolerated better by some fish (e.g., hagfish, carp, and eels) than others (e.g., salmon). Temperature also directly affects the force of cardiac contraction. To compensate for the negative inotropic effect of cold temperature, rainbow trout can increase in ventricular mass over several weeks; a larger mass compensates for the lower force per unit mass.

Heart Rate

The intrinsic activity of pacemaker cells sets the basal rate at which a fish heart beats (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping and Action Potential of the Fish Heart). Heart rate varies by an order of magnitude among fishes (**Table 1**). Active fishes at warmer temperatures tend to have a high f_H in association with their high metabolic rate. In addition, many extrinsic factors modulate this basal rate, making f_H either faster (positive chronotropy) or slower (negative chronotropy) than the pacemaker rate.

Autonomic nerves can slow and accelerate f_H. Parasympathetic vagal stimulation slows down f_H in most fishes. Vagal nerve endings (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes) release acetylcholine, which binds to muscarinic receptors, causing cardiac cells to hyperpolarize and take longer to reach the threshold for an action potential (**see also Design and Physiology of the Heart:** Action Potential of the Fish Heart). In addition, acetylcholine slows the rate of rise of the pacemaker potential and increases the AV delay. Tonic vagal activity normally exists in resting fish, which depresses f_H below the intrinsic

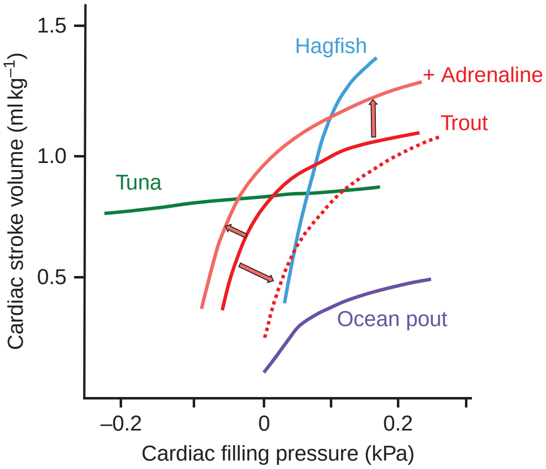


Figure 4 Starling curves for fish hearts *in vitro*. The Starling curve is the relationship between the cardiac filling pressure and the resulting volume of fluid ejected with each heartbeat. When the heart is distended with a higher filling pressure, there is greater filling and it responds by increasing the force of contraction to ensure that the additional volume of fluid entering the heart is ejected. Consequently, the heart automatically ejects the volume of fluid it receives over the range of the Starling curve. Starling curves vary among species, however, as shown by the lines for the different species. Stroke volume is lower in the sluggish ocean pout (purple solid line), whereas tuna (green solid line) operate at subambient filling pressure and are relatively insensitive to filling pressure. In addition, both the strength of contraction, as influenced by hormones and nerves, and the pericardium can influence the position of the curve. In trout (red lines), opening the pericardium (red broken line) shifts the curve to the right of the closed pericardium preparation (red solid line) by reducing *vis-a-fronte* filling. Adrenaline increases contractility and shifts the trout curve to the left and upward (pink solid line). Adapted from Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology*, 3rd edn., pp. 119–152. Boca Raton, FL: CRC Press.

pacemaker rate. In contrast, sympathetic neural stimulation typically increases f_H. Sympathetic nerve endings release noradrenaline (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes), which typically binds to cardiac β-adrenoceptors, increasing the pacemaker frequency, improving conduction velocity, and increasing the rate of relaxation of cardiac muscle. Likewise, hormones, such as adrenaline and noradrenaline, which are released from chromaffin cells in fishes (**see also Hormonal Responses to Stress:** Catecholamines), also stimulate β-adrenoceptors. Tonic adrenergic tonus can also exist in resting fish, which increases f_H above the intrinsic pacemaker rate. Thus, simultaneous adrenergic tonus and cholinergic tonus can have opposing effects on f_H in a resting fish, and their relative importance can vary considerably among species and with changes in activity and environments.

Important exceptions to the above scheme are hagfishes and lampreys. Hagfishes have no cardiac innervation, but have chromaffin cells within the heart (**see also Hagfishes and Lamprey:** Hagfishes). Lamprey hearts have nicotinic–cholinergic receptors, which increase f_H when acetylcholine is released from the vagus nerve. Many fishes, including lampreys, elasmobranchs, and some teleosts, lack sympathetic cardiac innervation (**see also Brain and Nervous System:** Autonomic Nervous System of Fishes).

Temperature is the most potent environmental factor affecting f_H, which typically doubles with an acute increase of 10 °C. Temperature directly affects the pacemaker potential and the duration of the action potential (**see also Integrated Control and Response of the Circulatory System:** Integrated Responses of the Circulatory System to Temperature and **Design and Physiology of the Heart**: Action Potential of the Fish Heart). To compensate for the direct effect of temperature, fishes can reset their pacemaker rate over time at a new temperature, for example, warm acclimation lowers the pacemaker rate. Temperature compensation can also involve changes in cholinergic and adrenergic tonus.

Very few fish have a maximum f_H much greater than $\sim$120 beats per minute. Recognized exceptions are tunas, zebrafish, and fish larvae. This upper limit to f_H may reflect cellular limitations related to excitation–contraction coupling events (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux).

Central Arterial Blood Pressure

The heart generates blood pressure to overcome the vascular resistance to blood flow. Similar to a mechanical pump, the heart operates over a certain range of arterial blood pressures without compromising cardiac stroke volume, that is, end-systolic volume is unchanged by increased work. This intrinsic property of the heart is termed 'homeometric regulation'. Species differences in the homeoregulatory ability of fish hearts are reflected in the almost order of magnitude difference in central arterial blood pressure among fishes (**Table 1**). These species differences in central arterial blood pressure reflect differences in ventricular wall thickness, shape and composition, as well as vascular resistance. The number of myofibrils per cardiac cell and the percentage compact myocardium also vary among fishes (**see also Design and Physiology of the Heart:** Cellular Ultrastructure of Cardiac Cells in Fishes).

Cardiac Work

The heart's overall performance, like any pump, is assessed by its ability to generate flow and pressure. The external work performed by the heart per unit time (myocardial power output, mJ s^{-1} or mW) is estimated from the product of V_b and arterial blood pressure (an estimate that neglects the small contribution of venous blood pressure). The range for power output per kilogram of fish varies by 100-fold among fishes. Hagfishes have the weakest heart and only tuna hearts are able to routinely generate central arterial blood pressures that approach those found in mammals (**Table 1**).

The cardiac muscle normally works aerobically and myocardial oxygen consumption is linearly related to myocardial power output. Therefore, the estimates of power output weighted to body mass (**Table 1**) provide an indirect index of the cost to the fish of cardiac pumping. This cost is typically less than 1% of the fish's overall oxygen uptake. Even so, only a small portion of the oxygen consumed by the heart is converted to mechanical work such that mechanical efficiency is generally of 10–20%. This estimate power output, however, does not accurately reflect the mass-specific capability of the heart since ventricular mass varies eightfold among fishes. Species comparisons are also made using power output weighted to ventricular mass to take into account the eight-fold variation in ventricular mass among fish species. The result is a much lower range for power output values among species because the power capability of a gram of fish heart does not vary by 100-fold. Thus, variation in ventricular mass is important in determining the power output developed by the heart of a fish.

Summary

The job of the heart is to generate blood flow and blood pressure. This task falls primarily to the ventricle and secondarily, the atrium. V_b and central arterial blood pressure vary considerably not only among fishes but also in response to metabolic needs and environmental change.

See also: **Brain and Nervous System:** Autonomic Nervous System of Fishes. **Design and Physiology of Arteries and Veins:** Branchial Anatomy. **Design and Physiology of the Heart:** Accessory Hearts in Fishes; Action Potential of the Fish Heart; Cardiac Anatomy in Fishes; Cardiac Cellular Length-Tension Relationship; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Cellular Ultrastructure of Cardiac Cells in Fishes; Physiology of Cardiac Pumping; The Outflow Tract from the Heart. **Hagfishes and Lamprey:** Hagfishes. **Hormonal Responses to Stress:** Catecholamines. **Integrated Control and Response of the Circulatory System:** Central Control of Cardiorespiratory Interactions in Fish. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia; Integrated Responses of the Circulatory System to Temperature.

Further Reading

Brill RW and Bushnell PG (2001) The cardiovascular system of tunas. In: Block BA and Stevens ED (eds.) *Tuna Physiology, Ecology and Evolution*, 1st edn., vol. 19, pp. 79–120. San Diego, CA: Academic Press.

Burggren WW, Farrell AP, and Lillywhite H (1997) Vertebrate cardiovascular physiology. In: Dantzler WH (ed.) *The Handbook of Physiology*, pp. 215–308. New York: American Physiological Society.

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, ch. 2, pp. 89–140. San Diego, CA: Academic Press.

Farrell AP (1984) A review of cardiac performance in the teleost heart: Intrinsic and humoral regulation. *Canadian Journal of Zoology* 62: 523–536.

Farrell AP (1991) Circulation of body fluids. In: Prosser CL (ed.) *Comparative Animal Physiology*, pp. 509–558. New York: Wiley.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac function in fish. *Physiological Zoology* 64: 1137–1164.

Farrell AP (1996) Features heightening cardiovascular performance in fishes with special reference to tunas. *Comparative Biochemistry and Physiology* 113A: 61–67.

Farrell AP (2002) Cardiorespiratory performance in salmonids during exercise at high temperature: Insights into cardiovascular design limitations in fishes. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 132: 797–810.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, ch. 1, pp. 1–88. San Diego, CA: Academic Press.

Gamperl AK and Farrell AP (2004) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Hoar WS, Randall DJ, and Farrell AP (eds.) (1992) *Fish Physiology: The Cardiovascular System,* vols. 12A and 12B. San Diego, CA: Academic Press.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology*, 3rd edn., pp. 119–152. Boca Raton, FL: CRC Press.

Santer RM (1985) Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology and Cell Biology* 89: 1–102.

Satchell GH (1991) *Physiology and Form of Fish Circulation*, pp. 1–235. New York: Cambridge University Press.

Stecyk JAW, Stensløkken K-O, Farrell AP, and Nilsson GE (2004) Maintained cardiac pumping in anoxic crucian carp. *Science* 306: 77.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Action Potential of the Fish Heart

M Vornanen, University of Eastern Finland, Joensuu, Finland

© 2011 Elsevier Inc. All rights reserved.

What Is the Cardiac Action Potential?
Different Phases of the AP
Species-Specific Differences in AP
Further Reading

Glossary

Action potential A small and transient change of voltage across the cell membrane. The action potential is possible because of the activity of ion channels, proteins of the cell membrane selectively allowing certain inorganic ions to pass through their pore.

Delayed rectifier A potassium ion channel that activates relatively late (delayed opening) after the onset of cardiac action potential, that is, its opening is time dependent. A rapid and a slow component are separated according to their relative rates of opening. It allows efflux of potassium ions from the cell and thereby makes the membrane potential more negative.

Depolarization The reduction in electrical potential difference (voltage) measured across a cell membrane. This means the inside of the cell becomes less negative with respect to the outside of the cell. Depolarization occurs during the upsweep of the action potential (opposite of repolarization).

Diastolic depolarization Slow depolarization of membrane potential between two successive action potentials in the pacemaker cells of the heart. It forms the basis for automaticity of the heart and determines heart rate. It is also called pacemaker potential.

Electrocardiogram A voltage waveform generated by the electrical activity of the whole heart. Action potentials of cardiac muscle cells sum and cause voltage changes which are conducted by body fluids and can be recorded from a distance of the heart.

Equilibrium potential The membrane potential (voltage) at which there is no net flow of current for a particular ion. This value can be calculated for any ion using the Nernst equation and is based on the intracellular and extracellular concentrations of that ion.

Inward current A flow of positively charged ions across the membrane into the cell.

Inward rectifier A channel that passes potassium ions much better into the cell than out of the cell. Passive flow of ions through ion channels can principally occur in either direction (in or out) depending on the electrochemical gradient (driving force) of the ion across the membrane. However, the small outward current is physiologically more important.

Ion channel A protein or a group of proteins that form water-filled pores that span the cell membrane. These pores, when open, allow a rapid flow of ions through the cell membrane. Each channel type is specific for one inorganic ion (sodium, calcium, potassium, or chloride).

Outward current A flow of positively charged ions across the membrane out of the cell.

Repolarization A change of membrane voltage back to the polarized state that prevailed before the action potential was generated, that is, increase in the negativity of membrane potential.

Resting membrane potential The stable negative membrane potential (from –70 to –90 mV) of the resting myocyte.

Threshold potential The value of membrane voltage needed to excite a full-blown action potential, that is, the potential (about –60 mV) at which sodium influx exceeds potassium efflux across the cell membrane and results in depolarization of the cell membrane.

Voltage-gated channel An ion channel whose opening and closing is governed by changes in membrane voltage. Opening and closing of the channel takes some time, that is, its operation is voltage and time dependent.

What Is the Cardiac Action Potential?

Contraction of each cardiac myocyte is initiated by an electric excitation of the cell membrane (sarcolemma) in the form of a small voltage change ($\sim$0.1 V) called the cardiac action potential (AP). The rate and rhythm of the heart beat, and an orderly spread of contraction through atrial and ventricular myocardium of the fish heart are determined by the conduction of AP over the heart.

The AP excites all cardiac myocytes in each heartbeat because cardiac cells are electrically connected. The rapid and orderly spread of the AP ensures that the cardiac muscle contracts in an effective manner to reduce the volume of the lumen of the cardiac chamber and ejects blood into either another cardiac chamber or the arterial system. Without an AP, there would be no cardiac pumping. If all cardiac cells were not synchronized in their activity, the pumping would become inefficient. With all cardiac cells contracting, the small voltages in different parts of the heart are summed and can be observed from the surface of the heart or from the surface of the fish as an electrocardiogram (ECG) (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping).

The cardiac AP is necessary to maintain a constant and undisturbed pumping function of the heart in exercise and under various environmental stresses (hypoxia, anoxia, acidosis, and temperature changes) that fish may experience in their habitat. However, the shape, amplitude, and duration of the cardiac AP vary depending on environmental conditions and exercise level of the fish. In fact, the functionally different myocyte types of the fish heart (the pacemaker cells, atrial myocytes, ventricular myocytes, and conducting cells) generate an AP with different shapes that are appropriate for their special functions.

The cardiac AP is produced by orderly opening and closing of sarcolemmal ion channels, ion-specific pores of the cell membrane, which allow carriage of electric charges mainly by sodium, potassium, and calcium ions.

Different Phases of the AP

In the vertebrate heart, the shape of AP is complex and heterogeneous in different cardiac compartments. Five functionally different phases (0–4) can be separated from the vertebrate cardiac AP, as exemplified by the AP of a rainbow trout (*Oncorhynchus mykiss*) ventricular myocyte (**Figure 1**).

A noncontracting cardiomyocyte has a stable resting membrane potential (RMP) of −70 to −90 mV (phase 4) relative to the extracellular compartment. It is characteristic for atrial and ventricular myocytes of the heart, and maintains the relaxed and unexcited state (diastole) of atrial and ventricular muscle.

When depolarizing current hits the myocyte, membrane voltage abruptly swings from the RMP toward positive voltages generating an upstroke (depolarization) of the AP (phase 0). As a consequence, membrane potential attains a value close to the zero membrane potential or slightly above it. This is the initiation signal for the contraction phase (systole) of the heart, since it activates calcium influx from extracellular space into the cytoplasm of the cardiac myocyte. The rate and extent of AP upstroke also determine the velocity at which the excitation spreads over the different parts of the heart.

The upstroke of the cardiac AP is followed by a small negative deflection (phase 1) that is often inconspicuous or even absent in fish cardiac myocytes. Phase 2 is the longest stage of the AP and is called the plateau phase because the membrane potential remains relatively constant near the zero membrane potential or slowly creeps toward more negative voltages. This phase is crucial for shortening and force production of cardiac myocytes since it determines the time which is available for diffusion of calcium from extracellular space into the cytosol of myocytes to activate contraction of the myofilaments (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac

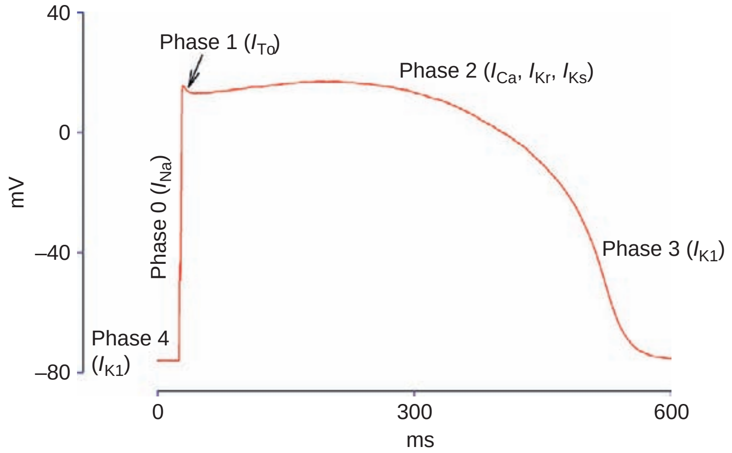


Figure 1 Different phases of cardiac action potential in a ventricular myocyte of the rainbow trout. Five phases (phase 0–phase 4) and ion currents responsible for those voltage changes are indicated. Transient outward current (I_{To}) responsible for phase 1 has not been examined in fish.

Excitation-Contraction Coupling: Calcium and the Contractile Element).

The last phase of cardiac AP (phase 3) is the fast restoration of the negative RMP or final repolarization, which initiates a decrease of cytosolic calcium concentration by turning a net sarcolemmal calcium influx into a net calcium efflux and thereby results in cessation of the systole. Restoration of the RMP is essential for two reasons: it moves the heart into diastole and allows refilling of the atrium and ventricle with blood. Further, it restores the excitability of the cell membrane. Recovery of voltage-gated ion channels from the inactivated state requires a certain time at negative membrane potentials to restore the three-dimensional conformation which is necessary for their opening.

The Ionic Basis of AP

The changes in membrane voltage associated with the AP require electrical charge to be moved across the cell membrane. These electrical charges are mainly carried by sodium, calcium, and potassium ions that pass through ion-specific channels of the sarcolemma (**Figure 1**). Ion channels are integral membrane proteins that form waterfilled pores in the lipid membrane. There are several families of ion channels, each including several channels with distinct ion specificities, voltage dependences, and opening and closing rates.

The stable RMP of atrial and ventricular myocytes is maintained by a diffusion of potassium through potassium-selective pores called the inward rectifier (Kir2) channels. In resting myocytes, the Kir2 channels are constantly open and allow a small efflux of potassium out of the cell (I_{K1}, an outward current) that balances any inward leakage of positively charged ions into to the cell. Potassium efflux through the Kir2 channels locks the membrane potential to the equilibrium potential of potassium ions, until depolarization from the neighboring myocytes will distort the membrane potential from its resting value.

The phase 0 upstroke of AP is produced by sodium influx into the myocyte via sodium-selective ion channels. Sodium channels open and close rapidly generating very fast and transient sodium current (I_{Na}, an inward current) that quickly depolarizes the membrane potential. The larger the I_{Na}, the faster the rate of AP upstroke, that is, the voltage change at the site of AP generation that then excites the next site. The size of I_{Na} is dependent on the density of sodium channels in the cell membrane, and therefore conduction velocity of the AP is determined by the number of sodium channels per unit of membrane area.

Depolarization of the sarcolemma causes closing of the Kir2 channels as the changing membrane voltage drives intracellular magnesium ions and free polyamines (spermine and spermidine) into the pore of these channels thereby clogging them. On the other hand, sodium-dependent depolarization of cell membrane triggers opening of the voltage-dependent calcium channels, which mediate calcium influx (I_{Ca}, an inward current) into the cell and contribute to the long phase 2 plateau of the cardiac AP. During the plateau, changes in membrane potential are small, since a small efflux of potassium through various potassium channels and calcium influx through L-type calcium channels almost cancel each other and practically prevent changes of membrane potential.

Toward the end of the plateau, depolarization of cell membrane causes opening of various voltage- and time-dependent potassium channels, thereby increasing potassium efflux across the membrane. Enhanced potassium efflux together with decreasing calcium influx initiates repolarization of cell membrane at the end of phase 2. There are two main voltage-dependent potassium currents, the rapid and slow component of the delayed rectifier (I_{Kr} and I_{Ks}, respectively), which contribute to the initiation of repolarization. When these channels start to repolarize the cell membrane, the increasing polarization of membrane potential unplugs the Kir2 channels (by forcing magnesium and polyamines out of the channel pore), which then terminates AP by the fast repolarization of phase 3 and restores the RMP (phase 4).

The Shape of AP Varies in Different Sites of the Heart

The basic shape of the cardiac AP, as exemplified by the ventricular AP, is modified according to the varying requirements of different cardiac compartments (**see also Design and Physiology of the Heart:** Cardiac Anatomy in Fishes), producing an ordered propagation of electrical excitation (**Figure 2**) and contraction. The electrical excitation of the fish hearts originates from a small aggregation of specialized myocytes located in the junction between the sinus venosus and the atrium as a ring-formed structure. The cells of the sinoatrial tissue generate APs, which first spread to and among atrial myocytes, triggering atrial contraction, and then to and among the ventricular muscle. Between the atrium and the ventricle, the spread of the electrical impulse is slowed in the myocytes of the atrio-ventricular canal, creating a sufficient delay that allows enough time to fill the ventricle with blood before the contraction of ventricular wall begins. From the myocytes of the atrio-ventricular canal AP is rapidly conducted to the apex of the ventricle and, from there, toward the base of the ventricle triggering a squeezing contraction of ventricular muscle and forcing blood toward and into the bulbus arteriosus.

Pacemaker APs

Pacemaker cells of the sino-atrial tissue usually lack the I_{K1} described above and therefore do not have a stable

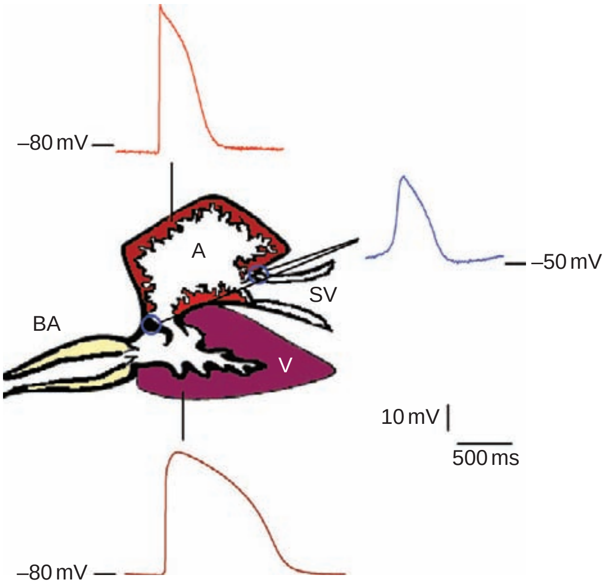


Figure 2 Schematic presentation of action potential shapes in different parts of the fish heart. Spontaneously firing nodal tissues of the sino-atrial junction and the atrio-ventricular canal are indicated with blue rings. SV, sinus venosus; A, atrium; V, ventricle; BA, bulbus arteriosus.

RMP. Instead, phase 4 is characterized by a slowly rising diastolic depolarization, also called pacemaker potential that results in spontaneous firing of pacemaker APs. Several ion channels/transporters of the cell membrane and calcium released from the sarcoplasmic reticulum are involved in the generation of the pacemaker AP, but the mechanism of cardiac pacemaking is still heavily disputed.

Steepness of diastolic depolarization dictates the firing rate of the pacemaker cell: the steeper the rate of rise, the sooner the threshold voltage for the firing of the upstroke is achieved. In pacemaker cells, the AP upstroke is generated by calcium currents, and not by sodium channels as in the nonpacemaker tissues. Since each pacemaker firing triggers a heartbeat, the steeper the pacemaker potential, the higher the heart rate.

In rainbow trout of 200–300 g in body mass, the circular nodal tissue is about 0.2 mm in width (diameter) and 3 mm in height and contains approximately 30 000 myocytes. However, there are probably only a few number of small primary pacemaker cells that have fast diastolic depolarization and which determine the heart rate. The nodal tissue also contains secondary pacemaker cells with slightly shallower diastolic depolarization and therefore lower firing rate. In these cells, the rate of final upstroke is faster and they conduct impulse faster than the primary pacemaker cells. The cells of the atrio-ventricular canal also produce slowly rising pacemaker APs.

Atrial AP

Atrial and ventricular myocytes have a stable negative RMP of about –70 to –90 mV, maintained by the I_{K1} current, and therefore need an inward current flow (depolarization) from a neighboring myocyte to elicit their own AP. It is the current flow from the pacemaker cells (pacemaker AP) that elicits AP in atrial myocytes. Primary and secondary pacemaker cells are electrically coupled to atrial cells, and when electric current from pacemaker cells depolarizes the RMP of atrial myocytes to the threshold value of about –60 mV, that is, to voltage where inward I_{Na} exceeds outward currents through potassium channels, a fast depolarization is blown out. Electrical coupling among atrial cells enables a fast spread of AP throughout the atrial myocardium.

The fast upstroke and short duration of the atrial AP compared with that of ventricular AP are functionally important as they enable fast atrial emptying into the ventricle. The faster upstroke of atrial AP results in a faster impulse conduction through atrial muscle. The shorter duration of the atrial AP results in a shorter duration of atrial contraction.

These distinctive features of the atrial AP are due to specific ion channel composition of atrial myocytes. The density of the I_{K1} is usually much smaller in atrial than ventricular myocytes, which makes them more easily excitable by current flow from the pacemaker tissue. On the other hand, the density of the I_{Kr} is much larger than in ventricular myocytes, which helps to limit the duration of atrial AP. At least in some fish species (e.g., crucian carp, *Carassius carassius*), the slow component of the delayed rectifier potassium current (I_{Ks}) is also present.

AP of the atrio-ventricular canal

The atrial muscle AP is conducted to the ventricle via the atrio-ventricular canal along the myocytes in the inner layer of the canal. Here, the AP has a similar shape as in the primary pacemaker tissue of the sinoatrial ring. This has several important implications: (1) the rate of AP upstroke is slower, delaying the conduction of the impulse and allowing adequate filling of the ventricle with blood; (2) the AP does not have stable RMP and therefore myocytes of the atrio-ventricular canal can function as an ancillary pacemaker – this would only become important if the sino-atrial pacemaker fails or if atrial conduction is blocked for some reason because the pacemaker potential is not as steep as that for the primary pacemaker cells; and (3) atrial tissue is prone to arrhythmias, which could easily propagate to the ventricle and generate detrimental ventricular arrhythmias without the slow conduction of the atrio-ventricular canal.

Ventricular AP

Ventricular APs are characterized by fast upstroke and long plateau duration. They are elicited by impulses from the atrial tissue by the myocytes of atrio-ventricular canal. Long AP duration is correlated with longer duration of contraction in comparison to atrial muscle. The long plateau phase makes the ventricular myocardium less vulnerable to arrhythmias than the atrial muscle. The ventricular contraction proceeds from the apex of ventricle to the base of the ventricle, thereby giving forward direction to the blood flow toward the bulbus arteriosus. In order to do this, the AP must be quickly conducted from the atrio-ventricular canal to the apex of the ventricle. It is not known how this happens, since corresponding histological structure to the mammalian His–Purkinje conduction pathway has not been found in the fish ventricle.

Species-Specific Differences in AP

There are large species-specific differences in shape and duration of cardiac AP among teleost fishes (**Figure 3**). In general, the more active or athletic species, which tend to have higher heart rates, also have shorter atrial and ventricular APs than more sluggish species. Relatively short cardiac APs are found, for example, in rainbow trout and perch (*Perca fluviatilis*), while long APs are typical, for example, in the less athletic swimmers such as the crucian carp. At 18 °C, the duration of atrial AP ranges from about 150 ms (roach, *Rutilus rutilus*) to 350 ms (crucian carp), while the duration of ventricular AP varies from approximately 315 ms (rainbow trout) to 740 ms (crucian carp and burbot, *Lota lota*). At 4 °C, the duration of fish cardiac AP is 2–4 times longer than at 18 °C. Species-specific differences in AP duration are due to variation in ion channel densities and differences in ion channel isoforms among the fish.

Effects of Autonomic Nervous Transmitters

The rate and force of cardiac contraction are modulated by the autonomous nervous system through the parasympathetic cholinergic and the sympathetic adrenergic nervous systems via release of acetylcholine and adrenaline, respectively. These changes must, in the first instance, involve alterations to the AP shape.

Pacemaker cells, atrio-ventricular canal myocytes, and atrial myocytes are particularly densely innervated by the autonomic nerves (**see also Design and Physiology of the Heart:** Cardiac Anatomy in Fishes). Acetylcholine, the neurotransmitter released from the parasympathetic nervous system, has a strong effect on APs of pacemaker cells and atrial myocytes. Acetylcholine release slows heart rate by strongly decreasing the rate of diastolic depolarization of the primary pacemaker cells. Low concentrations of acetylcholine markedly shorten the AP duration of atrial myocytes, greatly weakening atrial contraction. In some fish species (tilapia *Oreochromis nilotica* and *O. aureus*, crucian carp) shortening of AP occurs at lower concentrations than its pacemaker effect, causing a depression of atrial contraction before any changes appear in the heart rate. The effects of acetylcholine are mediated by a specific kind of inward rectifier potassium channels, which are activated by binding of acetylcholine to the muscarinic cholinergic receptors of the myocyte plasma membrane and by the inhibition of I_{Ca}.

The effects of the sympathetic nervous system are much weaker in comparison to those of the parasympathetic nervous system in fish. The effects of adrenergic neurotransmitters on cardiac AP are relatively poorly elucidated in fish. In the goldfish (*Carassius auratus*) adrenaline increases the duration of atrial and ventricular AP, while in the winter-acclimatized eel (*Anguilla anguilla*) adrenaline prolongs ventricular AP without effect on atrial AP. Lengthening of AP duration is produced by the increased density of I_{Ca} and results in stronger heartbeats.

Effects of Oxygen Shortage

In mammalian hearts, hypoxia directly and greatly shortens the cardiac AP, which helps to reduce the energetic adenosine triphosphate (ATP) demand of the myocyte and aids survival of the hypoxic cell. AP duration is shortened due to the opening of sarcolemmal ATP-sensitive potassium channels. In the fish heart, the effects of hypoxia on the duration of cardiac AP are

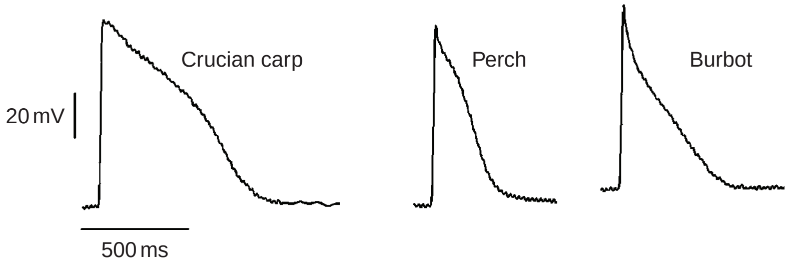


Figure 3 Comparison of atrial action potentials (APs) from crucian carp, perch, and burbot hearts. Note the triangular shape of burbot AP and large differences in AP duration between crucian carp and perch atria.

apparently much milder or even opposite to that of the mammalian heart, despite ATP-sensitive potassium channels also being present in fish heart. A small shortening of ventricular AP has been noted in the hypoxic goldfish heart, while a modest prolongation of cardiac AP is present in the cyanide poisoned (anoxic) ventricle of the crucian carp, a sibling species to the goldfish. Similar effects can be deduced from the QT segment of the ECG in other fish species during hypoxia. These findings suggest that hypoxic shortening of cardiac AP duration may be superseded by vagally mediated bradycardia, which is a reflex unique to fishes (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia).

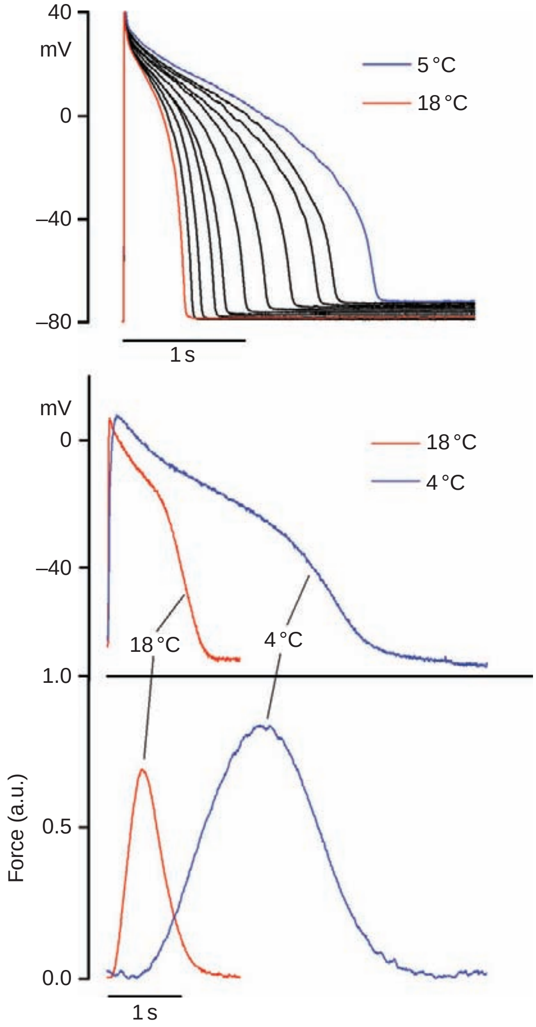


Figure 4 Temperature has strong effect on the duration of fish cardiac AP and contraction. (Top) Continuous recording of AP from a ventricular myocyte of crucian carp when temperature was gradually elevated from 5 to 18 °C. Note the shortening of AP duration and increase in RMP. (Middle and bottom) Effects of temperature change on ventricular AP and associated contractions in crucian carp heart at 4 and 18 °C. Modified from Paajanen V and Vornanen M (2004) Regulation of action potential duration under acute heat stress by $I_{K,ATP}$ and I_{K1} in fish cardiac myocytes. *American Journal of Physiology* 286: R405–R415.

Effects of Temperature Changes

The cardiac AP is strongly changed by temperature (**Figure 4**). Lowering of temperature increases the duration and reduces the rate of upstroke of cardiac AP (**see also Temperature:** Temperature and Excitable Membranes). As a consequence, heart rate and velocity of impulse conduction over the heart are depressed, and the duration of contraction of each cardiac myocyte lasts longer. The longer duration of AP allows more time for calcium influx through the sarcolemma and at least partly compensates for the temperature-dependent decrease in calcium influx via I_{Ca}. Prolonged depolarization also allows more time for the myofilaments to generate force. On the other hand, due to the prolonged AP contraction–relaxation cycle gets longer and may limit maximum heart rates at low temperatures. Due to these changes in electrical activity of cardiac myocytes, the fish heart becomes sluggish in the cold but may contract more forcefully. However, in several fish species chronic exposure to low temperatures results in compensatory changes in cardiac AP. Cold-induced increase in the density of I_{Na} partly alleviates the temperature-dependent depression in the rate of AP upstroke and velocity of impulse conduction, while upregulation of sarcolemmal potassium currents counteracts temperature-dependent prolongation of AP. These cold-induced changes in ion channel function make room for positive compensation in heart rate and hence cardiac output, which may be adaptive at seasonally changing temperature conditions at north-temperature latitudes.

See also: **Design and Physiology of the Heart:** Cardiac Anatomy in Fishes; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping. **Integrated Response of the Circulatory System:** Integrated Responses of the Circulatory System to Hypoxia. **Temperature:** Temperature and Excitable Membranes.

Further Reading

Cameron JS, Hoffmann KE, Zia C, *et al.* (2003) A role for nitric oxide in hypoxia-induced activation of cardiac K_{ATP} channels in goldfish (*Carassius auratus*). *Journal of Experimental Biology* 206: 4057–4065.

Chi NC, Shaw RM, Jungblut B, *et al.* (2008) Genetic and physiologic dissection of the vertebrate cardiac conduction system. *PLoS Biology* 6: e109.

Galli GLJ, Lipnick MS, and Block BA (2009) Effect of thermal acclimation on action potentials and sarcolemmal K^+ channels from Pacific bluefin tuna cardiomyocytes. *American Journal of Physiology* 297: R502–R509.

Haverinen J and Vornanen M (2006) Significance of Na^+ current in the excitability of atrial and ventricular myocardium of the fish heart. *Journal of Experimental Biology* 209: 549–559.

Haverinen J and Vornanen M (2007) Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *American Journal of Physiology* 292: R1023–R1032.

Haverinen J and Vornanen M (2009) Responses of action potential and K^+ currents to chronic thermal stress in fish hearts. Phylogeny or thermal preferences? *Physiological and Biochemical Zoology* 82, doi:10.1086/590223.

Huang TF (1973) The action potential of the myocardial cells of the golden carp. *Japanese Journal of Physiology* 23: 529–540.

Irisawa H (1978) Comparative physiology of the cardiac pacemaker mechanism. *Physiological Reviews* 58: 461–498.

Molina CE, Gesser H, Llach A, Tort L, and Hove-Madsen L (2007) Modulation of membrane potential by an acetylcholine-activated potassium current in trout atrial myocytes. *American Journal of Physiology* 292: R388–R395.

Paajanen V and Vornanen M (2004) Regulation of action potential duration under acute heat stress by $I_{K,ATP}$ and I_{K1} in fish cardiac myocytes. *American Journal of Physiology* 286: R405–R415.

Pennec JP and Peyraud C (1983) Effects of adrenaline on isolated heart of the eel (*Anguilla anguilla* L.) during winter. *Comparative Biochemistry and Physiology* 74C: 477–480.

Rantin FT, Glass ML, Kalinin AL, Verzola RMM, and Fernandes MN (1993) Cardiorespiratory responses in two ecologically distinct erythrinides (*Hoplias malabaricus* and *Hoplias lacerdae*) exposed to graded environmental hypoxia. *Environmental Biology of Fishes* 36: 93–97.

Santer RM (1985) Morphology and innervation of the fish heart. *Advances in Anatomy Embryology and Cell Biology* 89: 1–102.

Sedmera D, Reckova M, deAlmeida A, *et al.* (2003) Functional and morphological evidence for a ventricular conduction system in zebrafish and *Xenopus* hearts. *American Journal of Physiology* 284: H1152–H1160.

Shiels HA, Vornanen M, and Farrell AP (2000) Temperature-dependence of L-type Ca^{2+} channel current in atrial myocytes from rainbow trout. *Journal of Experimental Biology* 203: 2771–2880.

Talo A and Tirri R (1991) Temperature acclimation of the perch (*Perca fluviatilis* L.): Changes in duration of cardiac action potential. *Journal of Thermal Biology* 16: 89–92.

Vornanen M, Shiels HA, and Farrell AP (2002) Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology* 132: 827–846.

Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux

HA Shiels, University of Manchester, Manchester, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Ca Transient
Excitation–Contraction Coupling
The Rising Phase of the Ca Transient
The Falling Phase of the Ca Transient
Summary
Further Reading

Glossary

ATPase A protein whose activity is fueled by the hydrolysis of adenosine triphosphate (ATP). Key ATPases involved in excitation–contraction coupling are Ca-pumps and myosin ATPases.

Buffers Proteins that bind and hold other molecules. They can bind a given molecule strongly (high affinity) or bind a large number of a given molecule (high capacity). The affinity and capacity are determined by the type of buffer and by physical variables such as temperature and pH.

Ca transient The term used to describe the transient rise and fall of cytosolic Ca with each contraction–relaxation cycle.

Ca-pump Proteins (such as sarco(endo)plasmic reticulum Ca-ATPase or plasma membrane Ca-ATPase) that utilize the energy of adenosine triphosphate to pump Ca across a membrane, usually against a concentration gradient. These are often called Ca-ATPases.

Calcium-induced calcium release (CICR) The term that describes how extracellular Ca influx across the cell membrane induces the release of Ca from the intracellular stores of the sarcoplasmic reticulum.

Cell-surface-area-to-volume ratio A mathematical expression of the amount of cell membrane (area) divided by the amount of intracellular space (cytosolic volume).

Contractility A measure of the power and/or the capacity of myofilament contraction.

Cytosol The fluid part of the intracellular space, which exists between the cell-surface membrane and the membrane-bound organelles.

Depolarization The reduction in electrical potential difference (voltage) measured across a cell membrane. This means the inside of the cell becomes less negative with respect to the outside of the cell. Depolarization occurs during the upsweep of the action potential (opposite of repolarization).

Excitability The capability of a living cell to respond to stimuli and is often related to membrane voltage.

Excitation–contraction coupling The term used to describe the progression from membrane excitation with an AP to the rise of the Ca transient to cell contraction.

Force–frequency relationship The term which describes how the strength of cardiac contraction varies with the frequency of cardiac contraction. If force increases as contraction frequency increases, it is called a positive force–frequency relationship. If force decreases with increased contraction frequency, it is called a negative force–frequency relationship. Most fish show a negative force–frequency relationship across their normal range of heart rates.

Hormones Substances produced by one tissue that are transported to another tissue to induce a physiological response.

Myofilaments The contractile apparatus of the myocyte (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

Plasma membrane Ca-ATPase (PMCA) The Ca pump on the sarcolemmal membrane that transports Ca out of the cell.

Repolarization A change of membrane voltage back to the polarized state that prevailed before the action potential was generated, i.e., increase in the negativity of membrane potential.

Rest (resting) state When the membrane of an excitable cell is not excited. The membrane is held in a polarized state called 'the resting membrane potential' awaiting a depolarizing action potential (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart).

Sarco(endo)plasmic reticulum Ca-ATPase (SERCA) The Ca pump on the sarco plasmic reticulum (SR) membrane that is responsible for pumping Ca out of the cytosol and into the SR.

Steady state This is achieved when Ca flux routes are balanced so that the amplitude and rate of the Ca transient is the same from beat to beat.

Working myocardium The regions of the heart that contract and propel the blood when excited via excitation–contraction coupling. These muscular regions are made up of the contractile myocytes of the atrium and ventricle.

Introduction

The cardiac action potential (AP) and the ion channels that open and close to excite the cardiac myocyte are discussed in **Design and Physiology of the Heart**: Action Potential of the Fish Heart; this article focuses on the cellular Ca fluxes that result from the cardiac AP and lead to myocyte contraction. Specifically, this article explores the sources and routes of Ca movement during each contraction–relaxation cycle. The contraction–relaxation cycle is the cellular equivalent of the heartbeat. Routes of cellular Ca flux associated with the contraction–relaxation cycle are very important for understanding heart contractility because the rate (how fast) and magnitude (how much) Ca movement directly determines the rate and strength heart contraction. How Ca actually causes contraction the myofilaments in fish cardiac cells is explained in **Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element.

The Ca Transient

The most important term in this article is the 'Ca transient'. It is often abbreviated as $\Delta[\mathrm{Ca}^{2+}]_i$, where Δ is a change in intracellular ('$_i$') Ca concentration ($[\mathrm{Ca}^{2+}]$) during a single contraction–relaxation cycle. A typical Ca transient from a mammalian cardiac myocyte is shown in **Figure 1**. One can see that Ca (blue line) rises quickly (~100 ms) and then decays more slowly (~400 ms), thus causing the characteristic shape of the transient change in intracellular Ca concentration.

The Ca transient actually refers to the change in 'free' Ca inside the cytosol. A large amount of Ca is not free inside the cell but rather is bound to buffers. Buffers are proteins that bind other molecules. For example, a Ca buffer binds Ca. The amount of Ca that a buffer can bind and the strength of the binding greatly vary. Very little is known about the buffering power of the fish cardiac myocyte; hence, for the purpose of this article we will assume it does not differ sizably from mammals. The important point to remember is that because the Ca transient is a measure of free Ca, much larger amounts of total Ca are required to activate a free Ca transient of a given magnitude.

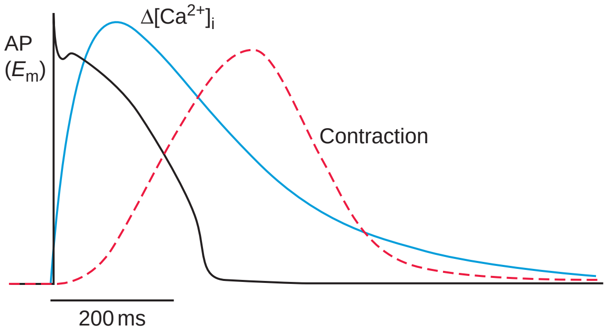


Figure 1 The three key events in the process of excitation–contraction coupling. The figure shows the time course of an action potential (black line), Ca transient (blue line), and myocyte contraction (red dashed line). Data are from a rabbit ventricular myocyte at 37 °C. Adapted from figure 1 inset in Bers DM (2002) Cardiac excitation–contraction coupling. *Nature* 415: 198–205.

Excitation–Contraction Coupling

Excitation–contraction (e–c) coupling is the term used to describe the progression from membrane excitation with an AP to the rise of the Ca transient to myocyte contraction. The time course of e–c coupling can be observed for a mammalian myocyte in **Figure 1**. Notice the central role of the Ca transient (blue line). It is the link between cardiac excitation (black line showing the AP) and cardiac contraction (dotted red line).

As discussed in **Design and Physiology of the Heart**: Action Potential of the Fish Heart, there are a number of different types of myocytes that make up the fish heart and each are specialized for different functions. For example, some cells do not contract very much but are important in electrical conduction (i.e., pacemaker cells). The process of e–c coupling usually refers to the cardiac myocytes that are powering cardiac contraction. These are the myocytes of the atrium and the ventricle. Thus, in this article, the term cardiac myocyte refers to an atrial or ventricular myocyte that is part of the working myocardium.

When thinking about routes of cellular Ca flux during e–c coupling, it is perhaps best to visualize a schematic model through which one can track Ca movement. A schematic model of a fish cardiac myocyte is provided in **Figure 2**. The key components involved in cellular Ca flux are labeled and briefly described in the legend, but each is discussed in detail later.

The most important aspect of myocyte morphology for e–c coupling is the large surface area-to-volume ratio found in fish myocytes, which is on average fourfold greater than in mammals. Thus, for any given volume of cytosol, there is four times as much area of membrane through which Ca can enter in the fish myocyte than in the mammalian myocyte. Consequently, this increase in surface area-to-volume ratio increases the effectiveness of extracellular ion flux pathways in changing the ionic makeup of the cytosol.

Figure 2 shows how the sarcolemma (SL) is the outer cell membrane and the interface between the extracellular and intracellular space (cytosol). Embedded in that membrane are a number of ion channels (**see also Design and Physiology of the Heart**: Action Potential

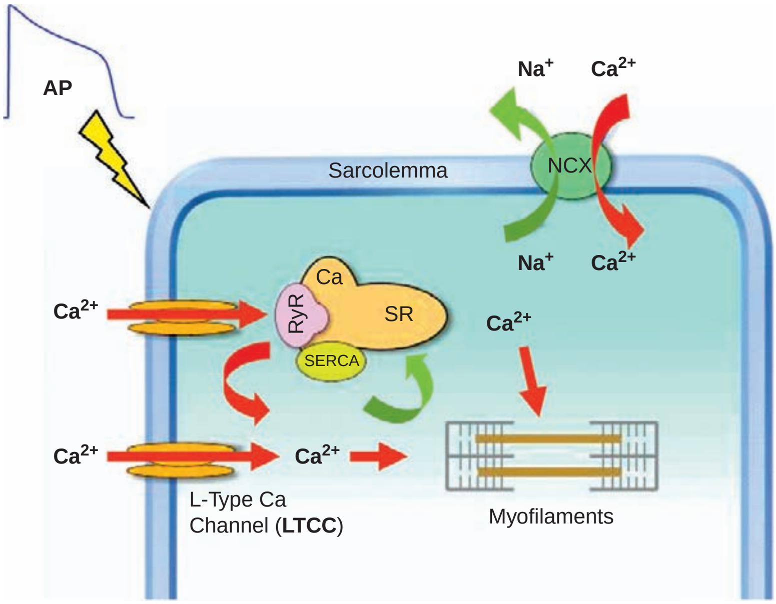


Figure 2 Schema for excitation–contraction coupling in the fish cardiac myocyte. The figure shows a portion of a fish cardiac myocyte. The sarcolemma (SL) is the cell membrane separating the intra- and extracellular space. The figure shows the SL being excited by an action potential (AP) which opens L-type Ca channels (LTCC) in the cell membrane allowing Ca influx (red arrows) down its concentration gradient into the cell. Ca can also enter the cell via reverse-mode Na–Ca exchange (NCX). Ca influx can trigger Ca release from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). Together, these Ca influxes cause a transient rise in Ca that initiates contraction at the myofilaments. Relaxation occurs when Ca is removed from the cytosol (green arrows) either back across the SL via forward-mode NCX or back into the SR via the SR Ca-pump (SERCA).

of the Fish Heart that allow ions to pass from outside to inside or inside to outside. The key SL membrane proteins involved in the Ca transient are the L-type Ca channels (LTCC) and the Na–Ca exchanger (NCX). These proteins are responsible for the movement of Ca and Na across the SL membrane.

The key intracellular structure involved in generating the Ca transient is the sarcoplasmic reticulum (SR). This organelle can store large amounts of Ca (~1 mM) and can release a portion of it into the cytosol during e–c coupling. Ca release from the SR occurs primarily through proteins called ryanodine receptors. Ca can be removed from the cytosol by reuptake into the SR via a Ca pump called sarco(endo)plasmic reticulum Ca-ATPase (SERCA). Ca is buffered inside of the SR with a protein called calsequestrin. There are a number of other intracellular organelles, such as the mitochondria and the nucleus, that can accumulate Ca, but this Ca is not directly involved in e–c coupling; therefore, it is not dealt with in this article.

The myofilaments are also illustrated in **Figure 2**. Ca must bind to the myofilaments for contraction to occur and be removed from the myofilaments for relaxation to proceed. The rise and fall of cytosolic Ca determines the interaction between Ca and the myofilaments, and hence myofilament contraction, as discussed in **Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element.

Finally, there are a number of other buffers in the cytosol that bind Ca with different affinities and rates. Although these will impact on the shape and rate of the Ca transient, no information exists for these buffers in the fish heart and therefore, they are not further discussed.

The Rising Phase of the Ca Transient

The rising phase of the Ca transient in **Figure 1** is due to Ca influx into the cytosol. This Ca can be of extracellular origin (coming in across the SL) or of intracellular origin (released from the SR). The relative contribution of each Ca source can vary between fish species. Similarly, the source of Ca contributing to the mammalian Ca transient varies. The relative contribution of SR versus SL Ca flux is dependent upon species, tissue type (atrium or ventricle), and development stage. In fish, in general, the extracellular Ca route is by far the most important for contributing Ca to the Ca transient. The SR plays a minor role (<15%). However, in certain species, such as rainbow trout and tuna, and under certain conditions, Ca release from the SR can play a greater (up to 60%) role. Important modulators of the relative importance of SR Ca release are temperature and heart rate. These are discussed later on.

Extracellular Ca Influx – the LTCC

The main Ca influx pathway across the SL is the LTCC. This is a voltage-gated ion channel that is closed when the membrane is at rest but opens during depolarization (e.g., during an AP) allowing Ca to flow into the cell. There is a large driving force for Ca influx when these channels open because the concentration of extracellular Ca (millimolar range) is more than 100-fold greater than the concentration of intracellular Ca (nanomolar range). This large driving force, the high number of LTCCs in the cell membrane, and the large surface area-to-volume ratio are responsible for the rapid rising phase of the Ca transient. This rapid rising phase is evident in the Ca transients from a bluefin tuna (*Thunnus orientalis*) cardiac myocyte (**Figure 3**). One can measure the amount of Ca entering the cell via LTCC during an AP in fish myocytes using electrophysiological techniques. Studies show that between 20 and 80 μmol l^{-1} of Ca can enter the fish myocyte through LTCCs (with the exact amount depending on fish species and method of investigation). This Ca influx generates a Ca transient that is alone of sufficient concentration to initiate myofilament interactions and myocyte contraction.

It is important to notice that the rising phase of the Ca transient, though rapid, is short. This is because LTCCs stop conducting Ca soon after opening, which is termed 'ion-channel inactivation'. This inactivation depends on two features: the repolarization of the membrane during the AP (voltage-dependent inactivation), and the fact that Ca itself can stop the channel from conducting more Ca (Ca-dependent inactivation). The cessation of Ca conductance is very important for the heart. Without it, cytosolic Ca would stay elevated and the heart would not be able to relax and thus not be able to fill with blood between beats.

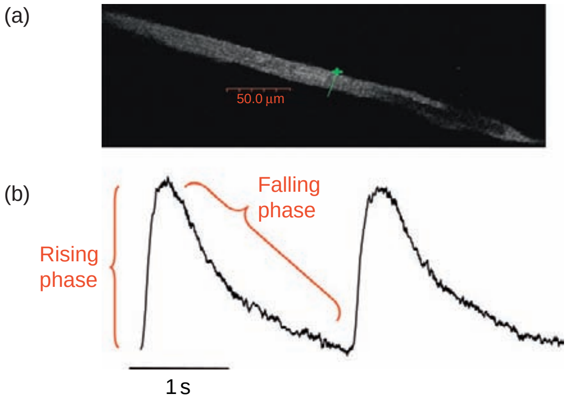


Figure 3 Cellular Ca transient in a ventricular myocyte of the bluefin tuna (*Thunnus orientalis*). (a) A tuna myocyte filled with a fluorescent dye that images Ca. The green line shows where in the myocyte the Ca transient (b) is measured from. (b) The time course of two consecutive Ca transients from the above myocyte. The fast rising phase and the slow decaying phase of the transient are clearly visible. Adapted from figure 2 in Shiels HA, Di Maio A, Thompson S, and Block BA (2011) Warm fish with cold hearts: Thermal plasticity of excitation–contraction coupling in bluefin tuna. *Proceeding of the Royal Society B* 278: 18–27

Extracellular Ca Influx – the NCX

The primary role of the NCX is to remove Ca from the cytosol. The NCX transfers three Na^{+} and one Ca^{2+} in opposite directions across the SL membrane. Because of the unequal charge transfer (Na movement carries a net charge of 3 and Ca carries a net charge of 2), there is a net movement of positive charge in the direction of Na transport. When the myocyte is at rest, the exchanger moves Ca out of the cell and brings Na in. This is called 'forward-mode exchange' and is important for the decaying phase of the Ca transient as dicussed later on.

The NCX can also bring Ca in across the SL membrane and can thus contribute to the rising phase of the Ca transient. This is called 'reverse-mode exchange'. This Ca influx mechanism is dependent on the membrane potential. During the upsweep (i.e., phase 0; **see also Design and Physiology of the Heart**: Action Potential of the Fish Heart) of the AP when the membrane is depolarized, the exchanger reverses direction and brings Ca into the cell and moves Na out. The direction of the exchanger is secondarily dependent on the concentrations of Ca and Na on either side of the membrane. Thus, the direction of ion transport on the exchanger is dependent on both the membrane potential and ion concentrations.

There are only a small range of membrane voltages (~0–10 mV during a normal AP) that favor reverse-mode Ca influx via the NCX. In most mammalian myocytes, this Ca-influx pathway is not very important to the rising phase of the Ca transient. However, the high surface area-to-volume ratio enhances the effectiveness of this pathway in fish myocytes. Further, fish tend to have a higher intracellular Na concentration, which again enhances the efficacy of reverse-mode NCX. As a result, Ca influx via this pathway can be a significant contributor to the rise of the Ca transient in fish compared with mammals. Indeed, the Ca transient resulting from Ca influx on the NCX is alone sufficient to cause partial myofilament contraction in most fish species studied to date. In rainbow trout, inhibiting Ca influx on the NCX reduces the contractility of the myocytes by 30–50%. In crucian carp (*Carassius carassius*), ~70 μmoll $^{-1}$ can be brought into the cell during the upsweep of an AP via reverse-mode NCX. Together, the NCX and the LTCC therefore can bring in ~150 μmoll $^{-1}$ Ca from the extracellular space, which generates a Ca transient of sufficient magnitude to activate a full-scale contraction in crucian carp. Of this amount, almost 50% of the total SL Ca influx occurs via reverse-mode NCX.

Similar to the LTCC, there are processes that stop Ca influx on the NCX, thereby limiting the size and duration of the rising phase of the Ca transient. The first is repolarization of the membrane potential during phase 3 of the AP (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart), which removes the permissive membrane voltage window for Ca influx. Second, as the Ca transient rises, the concentration of Ca in the cytosol rises reducing the favorable concentration gradient for Ca influx on the NCX. In fact, together, the repolarization of the membrane and the rising phase of the Ca transient itself cause the NCX to change direction and start moving Ca out of the cell. As discussed more in the following sections, this switch to forward-mode NCX activity (Ca pumped out) is important for the decay of the Ca transient and myocyte relaxation.

Thus, the inactivation of the LTCCs and the change in direction of the NCX brings the contribution of extracellular Ca influx to the rising phase of the Ca transient to an end.

Intracellular Ca Flux – SR

The SR is a large intracellular membranous network-like organelle that forms intimate contacts with the SL and the myofilaments. The primary role of the SR in cardiac myocytes is to act as a site for regulated storage and release of Ca. The amount of SR in the fish myocyte varies between species and although there are exceptions to this rule, it appears that active fish, such as salmonids (e.g., salmon and trout) and scombrids (e.g., tuna and mackerel), have a greater amount of SR than less active species (e.g., carp).

In general, the fish SR plays a minor role in e–c coupling compared with adult mammals. For example, in rainbow trout, release of Ca from the SR contributes ~15–20% of the Ca in the Ca transient; the remainder comes across the SL via LTCCs or reverse-mode NCX. In contrast, in the adult rat, ~15–20% comes in across the SL and the rest is released from the SR. Thus, e–c coupling in fish differs from mammals primarily with respect to the role of the SR in Ca transient formation. In mammals, extracellular Ca influx is small and contributes only a small amount of Ca to the rising Ca transient. Even so, this small influx is vital as it acts as an ionic trigger to cause the ryanodine receptors to open and the SR to release its Ca into the cytosol. This process is called Ca-induced Ca release (CICR) and is discussed subsequently. This SR Ca release is far larger than the Ca influx across the SL and thus, the rate and magnitude of SR Ca release is the key determinant of the rising phase of the Ca transient in mammals.

Ca-Induced Ca Release

The process by which Ca influx across the SL causes Ca release from the SR is called CICR. A role for CICR is hotly debated for fish cardiac myocytes. It is completely absent in some fish species, usually (but not always) in slow and sluggish fish such as carp. Athletic fish with fast heart rates and high blood pressures (such as salmon and tuna) often show more CICR. Ca release from the SR therefore plays a larger role in the rise of the Ca transient in these species. This is illustrated for bluefin tuna myocytes in **Figure 4**. Clearly, inhibiting the SR (and thus CICR) reduces the overall Ca transient amplitude and slows the rising phase of the Ca transient in this species. Also apparent is how inhibiting the SR slows the falling phase of the Ca transient. This is discussed subsequently.

Under supra-physiological conditions (10 μM isoprenaline – a pharmacological opener of LTCCs), SL Ca influx can result in significant CICR in the rainbow trout myocyte, but its physiological role is still unclear. Current research suggests that physiological stimuli, such as rapid changes in temperature and *in vivo* levels of adrenergic stimulation, can increase the importance of CICR in rainbow trout cardiac myocytes. Nevertheless, only a small percentage (<10%) of total SR stores appears to be released via CICR in fish myocytes.

Theoretically, the Ca influx across the SL that causes CICR can be through either or both the LTCC and the NCX. In mammals, it is almost exclusively via the LTCC. However, due to the larger role for Ca influx on the NCX

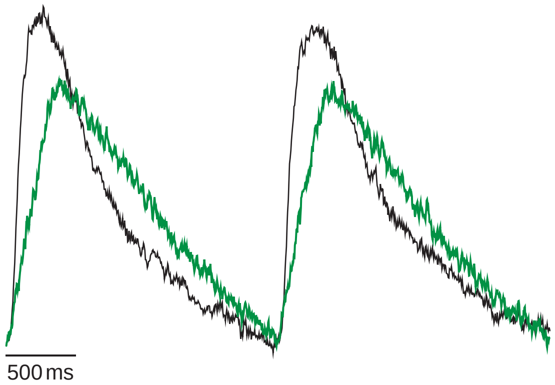


Figure 4 Role of the SR in the Ca transient in the bluefin tuna (*Thunnus orientalis*) ventricular myocyte. The black line shows the Ca transient under normal conditions. The green line shows the effect of inhibiting SR Ca release and SR Ca uptake on the shape of the Ca transient. In this species, inhibiting SR Ca release decreases Ca transient amplitude and slows the rising phase of the Ca transient. Inhibiting SR Ca uptake slows the decay of the Ca transient. These experiments were conducted at 14 °C. Adapted from figure 3 in Shiels HA, Di Maio A, Thompson S, and Block BA (2011) Warm fish with cold hearts: Thermal plasticity of excitation–contraction coupling in bluefin tuna. *Proceeding of the Royal Society B* 278: 18–27.

in fishes, studies show that NCX can induce CICR in the fish myocyte.

In general, the contribution of the intracellular Ca stores of the SR to the rise in the Ca transient is small for most fish studied to date. However, due to the large surface area-to-volume ratio of the fish myocyte, the peripheral location of myofilaments and the high efficacy of SL Ca flux pathways, the SR's Ca store may not be necessary for e–c coupling under normal conditions. The fact that large levels of adrenergic stimulation can induce greater CICR may suggest that under periods of stress or intense activity, the SR stores are released to augment SL Ca fluxes and generate a larger and faster Ca transient. This could create a stronger faster heart beat, enabling the fish to escape/survive adversity.

The Effect of Temperature on the Rising Phase of the Ca Transient

Fish body temperature is dependent on the temperature of their environment. Thus, when water temperature changes, cardiac temperature changes. The rate and amplitude of the rising phase of the Ca transient is directly affected by temperature. This is illustrated for a rainbow trout atrial myocyte in **Figure 5**. **Figure 5(a)** shows the effect of rapid (seconds) temperature change on the Ca transients, and **Figure 5(b)** shows the effect on Ca influx through LTCCs. Warm temperatures tend to speed up the rate of rise of the Ca transient. This is partially because of the effect of temperature on Ca influx via LTCCs, which is faster and greater in amplitude at warm temperatures (larger downward deflection in **Figure 5(b)**). Cool temperatures tend to slow down the rate of rise and also increase the amplitude of the Ca transient. This is because slower kinetics allow more time for Ca influx across the membrane through LTCC.

Fish cannot avoid temperature effects on their heart cells when they swim through water of different temperatures. However, certain hormones can compensate for the effects of temperature on cellular Ca flux. For example, adrenaline is released when a fish moves rapidly into water of a new temperature and it stimulates Ca flux through the LTCC.

Interestingly, unlike the LTCC, Ca flux via NCX is not dramatically affected by either rapid temperature change or adrenergic stimulation in fish. This means that this pathway operates independent of rapid changes in environmental temperature, certainly a beneficial feature for maintaining heart function of fish living in fluctuating environments.

Rapid temperature changes can also affect the Ca transient by changing the rate at which the heart beats. Warm temperatures increase heart rate through direct action on the pacemaker rate (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart). Increased frequency of contraction causes a decrease in the amplitude of the intracellular Ca transient. This reduces the force of contraction and is termed the negative force–frequency relationship. The negative force–frequency relationship impacts the ability of the heart to beat forcefully at fast contraction rates. Most fish do not have fast heart rates; they are typically below 120 beat per minute (2 Hz) even at warm temperatures. Fish compensate for this limitation in maximum heart rate by having stretchy hearts that can pump large volumes of blood (albeit slowly). This works because cardiac output is the product of heart rate and the volume of blood pumped by the heart. If heart rate is limiting, then cardiac output can be modulated by changing the volume of blood pumped at a given rate. These ideas are discussed in more detail in **Design and Physiology of the Heart**: Cardiac Cellular Length-Tension Relationship.

Seasonal temperature changes also impact cellular Ca flux in fish hearts. When fish are chronically exposed to the cold (e.g., during winter) they compensate by building more SR in their myocytes and the SR

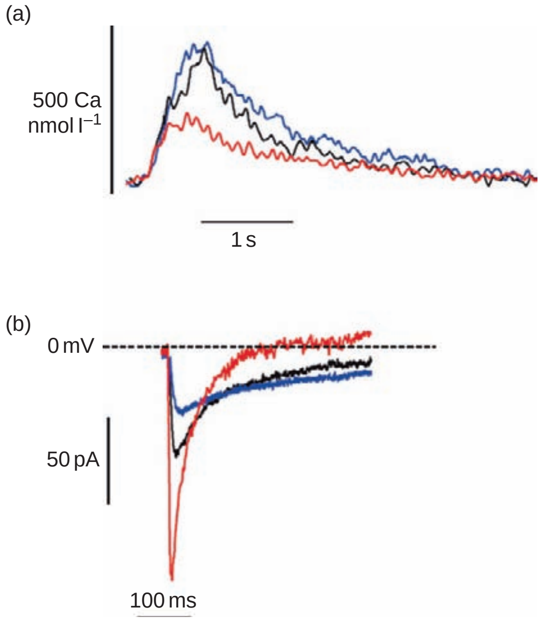


Figure 5 The effect of acute temperature change on the cellular Ca transient (a) and LTCC currents (b) from an atrial myocyte from the rainbow trout (*Oncorhynchus mykiss*) at 7 (blue), 14 (black), and 21 (red)°C. (a) The effect of temperature on the Ca transient in this species. Warm temperatures result in smaller Ca transients than cold temperatures. (b) Ca influx as an ionic current (measured in pico-amps, pA) across the SL membrane through the LTCCs. Warm temperature gives a faster current with a larger peak amplitude than colder temperatures. However, at colder temperatures, the ion channels stay open longer so that more Ca crosses the SL and the Ca transient is bigger. Data adapted from Shiels HA, Vornanen M, and Farrell AP (2002) Effects of temperature on intracellular [Ca^{2+}] in trout atrial myocytes. *Journal of Experimental Biology* 205: 3641–3650.

plays a greater role in delivering and removing Ca during the Ca transient. Interestingly, species that live at cold temperatures all year round (such as the cold-active burbot (*Lota lota*) and salmon shark (*Lamna ditropis*)) have a steady-state large complement of SR in their myocytes, which appears to be greatly involved in the cellular Ca transient during e–c coupling. Furthermore, cold-tolerant mammals, such as hibernators, also increase their SR content during the cold. Thus, intracellular Ca flux through the SR may be an evolutionarily conserved adaptation for cold tolerance.

The Falling Phase of the Ca Transient

Relaxation of the cardiac myocyte is dependent on Ca removal from the cytosol. This Ca efflux leads to the falling (decay) phase of the Ca transient, which can be seen for both the mammalian myocyte in **Figure 1** and the fish myocyte in **Figure 3**. This Ca removal can occur via either of the two main efflux pathways illustrated in **Figure 2**. Ca can either be moved back across the SL membrane via the NCX, or pumped into the SR via the SERCA pump. As the majority of Ca enters the fish cardiac myocyte across the SL membrane, in a steady state, the majority of Ca must also leave the cell via this route. Similar to Ca influx, the large surface area-to-volume ratio of the fish myocyte will aid in the efficacy of SL Ca efflux. However, the SR of active fish species is able to store large quantities of Ca and thus, in these animals, the SR may also play a role in the decay of the Ca transient. This is illustrated in **Figure 4** by the significant slowing of the Ca transient decay when SR uptake is inhibited in the bluefin tuna ventricular myocyte.

Extracellular Ca Efflux

The NCX operating in forward mode transports Ca out of the cell. This is the primary pathway to remove Ca from the cytosol and is the main cause of the decay in the Ca transient in fish cardiac myocytes. Forward-mode NCX is favorable during the onset of relaxation because of the initially high intracellular Ca concentration and the progressive repolarization of the membrane potential. An alternative route for Ca efflux across the SL is the plasma membrane Ca-ATPase (PMCA) Ca pump. However, based on mammalian studies, this pathway is expected to make a very minor contribution (if any) to the decay of the Ca transient, although its activity has not been directly measured in fish myocytes.

The prominent role of the NCX in relaxation is evidenced by the fact that NCX activity alone (SR function inhibited) can clear the cytosol of Ca and allow relaxation in rainbow trout myocytes. In contrast, inhibiting the rainbow trout NCX severely retards relaxation. Inhibiting both the NCX and the SR completely abolishes relaxation. This is because Ca levels remain elevated inside the cytosol and the myofilaments stay in a contracted state.

Ca Storage in the SR

The SR contributes to the falling phase of the Ca transient by pumping Ca out of the cytosol and into the SR. This pumping action is achieved through the efforts of the SR Ca-pump or SERCA. Pumping Ca into the SR takes energy, which is generated by breaking down ATP.

A surprising finding is that despite its limited role in e–c coupling, the fish SR has a massive ability to store Ca. The steady-state SR Ca content of the rainbow trout atrial myocyte is $>1000\,\mu\text{mol}\,\text{l}^{-1}$ (measured via application of caffeine, which opens ryanodine receptors and lets Ca out of the SR and into the cytoplasm). Mammalian SR Ca content is in the range of $\sim 50–250\,\mu\text{mol}\,\text{l}^{-1}$. Furthermore, the mammalian SR spontaneously releases Ca when the content reaches much above $150\,\mu\text{mol}\,\text{l}^{-1}$. Thus, fish SR can hold much greater amounts of Ca without spontaneously releasing it. How the fish SR is able to store this level of Ca is unclear. Differences in intra-SR buffering via calsequestrin may be involved. Other Ca buffers inside the SR or accessory proteins (triadin and junctin) that regulate ryanodine receptor function may be involved but this awaits future study. Certainly, for the rainbow trout at least, the SR contains more than enough Ca to support contraction. In fact, if the SR released all of its Ca into the cytosol in one go, levels could approach those associated with Ca toxicity.

A separate and fascinating question is: Why is the fish SR capable of accumulating large amounts of Ca? Again, the reasons are unclear. Because SR Ca content is only high in active fish species (or cold fish species), it has been speculated that the SR may act as a Ca reservoir that can be tapped into if physiology demands it. For example, if a faster or greater Ca transient is required (because the fish is chasing or being chased), the SR may provide a Ca reserve that can be added to SL Ca influx to bolster the size and rate of the Ca transient. However, so far this hypothesis has been difficult to prove.

The Effect of Temperature on the Decay of the Ca Transient

The low temperature sensitivity of the NCX was described above. The NCX is the primary way Ca is removed from the cytosol during relaxation and thus Ca removal via this pathway is not overly temperature dependent. However, the activity of SERCA is temperature dependent in fish. Thus, the role of SR Ca uptake in the decay of the Ca

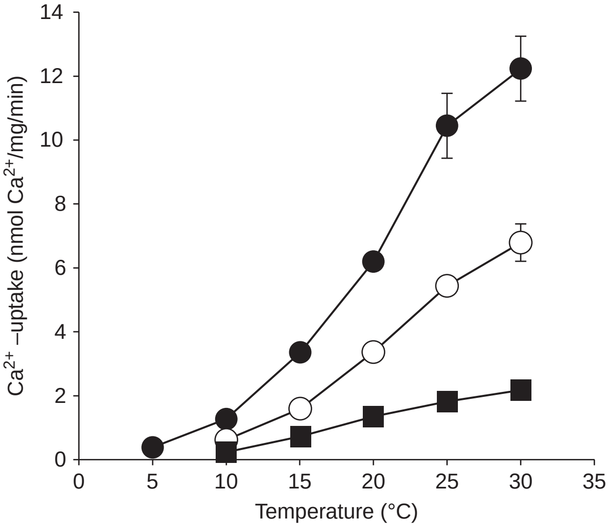


Figure 6 The temperature dependence of SR Ca uptake in isolated SR membrane preparations from different tuna species. ●, Pacific bluefin (*Thunnus orientalis)*; ○, albacore (*Thunnus alalunga)*; ■, yellowfin (*Thunnus albacares)*. The figure clearly shows how SERCA function depends on species and also how cold temperature reduces SERCA activity. Values are means ± SE of three to four experiments. Adapted from figure 2a in Landeira-Fernandez AM, Morrissette JM, Blank JM, and Block BA (2004) Temperature dependence of the Ca^{2+}-ATPase (SERCA2) in the ventricles of tuna and mackerel. *American Journal of Physiology* 286: R398–R404.

transient varies with temperature in fish hearts. Ca pumping by SERCA slows down as temperatures dip. This can be seen for a number of tuna species in **Figure 6**. It is important to note that tuna are athletic fish which actively utilize the SR during e–c coupling. Furthermore, all of the tuna species in **Figure 6** are subject to temperatures between 10 and 25 °C during normal foraging behavior in the oceans. Thus, temperature can severely affect the ability of the SR to contribute to the decay of the Ca transient in this group of fish.

Adrenergic stimulation can help to combat this depressive effect of the cold. There is an accessory protein attached to SERCA called 'phospholamban', which is sensitive to the signaling cascades activated by adrenaline. When the fish gets cold and releases adrenaline, this adrenaline activates phospholamban, which increases SERCA activity and results in more Ca being pumped into the SR. Interestingly, long-term cold exposure (overwintering; cold acclimation) increases the amount of SERCA protein in the fish SR too. Whether this is just because there is more SR in chronic cold species as discussed earlier, or if there is also specifically more SERCA in these animals' SR, remains unclear.

Ultimately, the proteins involved in removing Ca from the cytosol between heartbeats must keep up as heartbeat frequency increases, otherwise the decay of the Ca transient is severely compromised. As warm temperatures increase heart rate, relaxation must occur more quickly to return Ca to resting levels (i.e., pre-AP levels) before the next contraction begins. Failure to do so increases resting-state (diastolic) Ca, a response that occurs at fast contraction frequencies in fish and compounds the negative force–frequency response. The inability of the myocyte to relax between beats disrupts cardiac filling, which negatively impacts cardiac pumping.

Summary

The contraction–relaxation cycle of the cardiac myocyte is fueled by the cycling of Ca to and from the myofilaments. This Ca can be of intra- or extracellular origin with the relative proportion changing with species, temperature, and heart rate. The transient rise and fall of cytosolic Ca is called the Ca transient and its rate and amplitude determine the rate and strength of myocyte contraction. The collective rate and strength of myocyte contraction throughout the working myocardium force blood from the heart and around the body of the fish.

See also: **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Cardiac Cellular Length-Tension Relationship; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element.

Further Reading

Aho E and Vornanen M (1998) Ca^{2+}-ATPase activity and Ca^{2+} uptake by sarcoplasmic reticulum in fish heart: Effects of thermal acclimation. *Journal of Experimental Biology* 201: 525–532.

Bers DM (2002) Cardiac excitation–contraction coupling. *Nature* 415: 198–205.

Elias CL, Xue XH, Marshall CR, *et al.* (2001) Temperature dependence of cloned mammalian and salmonid cardiac Na^{+}/Ca^{2+} exchanger isoforms. *American Journal of Physiology* 281: C993–C1000.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac-function in fish. *Physiological Zoology* 64: 1137–1164.

Hove-Madsen L, Llach A, and Tort L (2000) Na^{+}/Ca^{2+}-exchange activity regulates contraction and SR Ca^{2+} content in rainbow trout atrial myocytes. *American Journal of Physiology* 279: R1856–R1864.

Hove-Madsen L and Tort L (2001) Characterization of the relationship between Na^{+}–Ca^{2+} exchange rate and cytosolic calcium in trout cardiac myocytes. *Pflugers Archives* 441: 701–708.

Landeira-Fernandez AM, Morrissette JM, Blank JM, and Block BA (2004) Temperature dependence of the Ca^{2+}-ATPase (SERCA2) in the ventricles of tuna and mackerel. *American Journal of Physiology* 286: R398–R404.

Shiels HA, Di Maio A, Thompson S, and Block DA (2011) Warm fish with cold hearts: Thermal plasticity of excitation–contraction coupling in bluefin tuna. *Proceeding of the Royal Society B* 278: 18–27.

Shiels HA, Vornanen M, and Farrell AP (2000) Temperature-dependence of L-type Ca^{2+} channel current in atrial myocytes from rainbow trout. *Journal of Experimental Biology* 203: 2771–2780.

Shiels HA, Vornanen M, and Farrell AP (2002) Effects of temperature on intracellular [Ca^{2+}] in trout atrial myocytes. *Journal of Experimental Biology* 205: 3641–3650.

Shiels HA, Vornanen M, and Farrell AP (2002) Temperature dependence of cardiac sarcoplasmic reticulum function in rainbow trout myocytes. *Journal of Experimental Biology* 205: 3631–3639.

Shiels HA, Vornanen M, and Farrell AP (2002) The force–frequency relationship in fish hearts: A review. *Comparative Biochemistry and Physiology A* 132: 811–826.

Shiels HA, Vornanen M, and Farrell AP (2003) Acute temperature change modulates the response of I_{Ca} to adrenergic stimulation in fish cardiomyocytes. *Physiological and Biochemical Zoology* 76: 816–824.

Shiels HA and White E (2005) Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. *American Journal of Physiology* 288: R1756–R1766.

Shiels HA, Paajanen V, and Vornanen M (2006) Sarcolemmal ion currents and sarcoplasmic reticulum Ca^{2+} content in ventricular myocytes from the cold stenothermic fish, the burbot (*Lota lota*). *Journal of Experimental Biology* 209: 3091–3100.

Tibbits GF, Moyes CD, and Hove-Madsen L (1992) Excitation–contraction coupling in the teleost heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 267–304. San Diego, CA: Academic Press.

Vornanen M (1997) Sarcolemmal Ca influx through L-type Ca channels in ventricular myocytes of a teleost fish. *American Journal of Physiology* 41: R1432–R1440.

Vornanen M (1998) L-type Ca^{2+} current in fish cardiac myocytes: Effects of thermal acclimation and beta-adrenergic stimulation. *Journal of Experimental Biology* 201: 533–547.

Vornanen M (1999) Na^{+}/Ca^{2+} exchange current in ventricular myocytes of fish heart: Contribution to sarcolemmal Ca^{2+} influx. *Journal of Experimental Biology* 202: 1763–1775.

Vornanen M, Shiels HA, and Farrell AP (2002) Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology A* 132: 827–846.

Weng KC, Castilho PC, Morrissette JM, *et al.* (2005) Satellite tagging and cardiac physiology reveal niche expansion in salmon sharks. *Science* 310: 104–106.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element

TE Gillis, University of Guelph, Guelph, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Cardiomyocyte Contraction: An Overview
The Activation of a Cardiomyocyte Contraction by Ca^{2+}
The Influence of Temperature on Ca^{2+} Binding by the Contractile Element
Adaptation of the Trout Contractile Element to Low Temperature
Phylogenetic Analysis of cTnC from a Variety of Vertebrate Species
Cardiac Contractility in Changing Environmental Temperatures
Future Directions
Further Reading

Glossary

Ca^{2+} activation When a muscle contraction is stimulated by the binding of Ca^{2+} to the cardiac troponin (cTn) complex.

Ca^{2+} affinity The measure of the ability of a protein to bind Ca^{2+}.

Ca^{2+} sensitivity The measure of the ability of a myocyte to contract in response to Ca^{2+}.

Cardiac troponin complex A group of three proteins, cardiac troponin I (cTnI), cardiac troponin C (cTnC), and cardiac troponin T (cTnT), that is attached to the actin thin filament that triggers the formation of cross-bridges during contraction.

Contractile element The components of the sarcomere responsible for the contractile reaction.

Contractile reaction The series of biochemical and biophysical reactions that result in the formation of cross-bridges and the generation of force by the cardiomyocyte.

Cross-bridge A physical connection that forms between the myosin thick filament and the actin thin filament. The formation of cross-bridges causes the sarcomere to shorten during a contraction.

Ectotherm (adj ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioural mechanisms to regulate body temperature.

Endotherm An animal that maintains a set body temperature, regardless of a change in external temperature.

Muscle contraction When a muscle cell shortens and generates force.

Protein conformation The three-dimensional shape of a protein in space.

Protein sequence The specific sequence of amino acids that compose a protein.

Sarcomeres The functional elements of a muscle cell responsible for shortening during a contraction.

Thick filament The structural element of a sarcomere that is composed of the protein myosin.

Thin filament The structural element of a sarcomere that is composed of the protein actin.

α-stat The change in blood and cellular pH that occurs in ectotherms when their body temperature changes.

Cardiomyocyte Contraction: An Overview

The contraction of all vertebrate hearts is powered by the generation of cross-bridges between two very abundant proteins termed thick and thin filaments, which are found in all muscle cells. The thick filaments are composed of the protein myosin and the thin filaments are primarily composed of the protein actin. Globular actin monomers are aligned in a chain, like beads on a string, to create the thin filament (**Figure 1**). The myosin molecule has a head and a tail. The tails are aligned side by side but staggered so that the myosin heads protrude out from the assemblage called the thick filament. Within the cardiomyocytes, the many such myosin thick filaments and actin thin filaments are arranged in a parallel overlapping pattern within structures called 'sarcomeres' (**Figure 1**). During a contraction, the myosin heads rotate out from the thick filament and form temporary connections with the actin thin filament. These physical connections are called cross-bridges. Once attached, the myosin head flexes causing the thin filament (actin) to slide past the thick filament (**Video Clip 1**). This causes

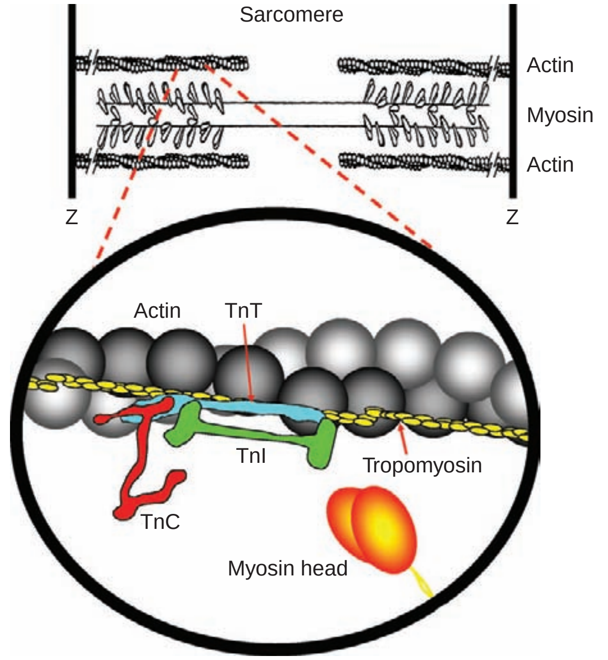


Figure 1 Schematic diagram of the cardiac sarcomere (a) and of the regulatory complex (b) associated with the actin filament responsible for making the contractile reaction activated by Ca^{2+}. Adapted part of figure 1 from Gordon AM, Regnier M, and Homsher E (2001) Skeletal and cardiac muscle contractile activation: Tropomyosin "rocks and rolls". *News in Physiological Science* 16, 49–55, used with permission from the American Physiological Society.

the length of the sarcomere to shorten by about 10%, which is termed a 'contraction'. During relaxation, the cross-bridges release and the myosin and actin filaments return to their original positions. This mechanism of muscle contraction is described as the 'sliding-filament model'. Thus, each time the heart contracts, every cardiomyocyte undergoes this contractile reaction almost in unison for each cardiac chamber. Therefore, for a heart that is beating 20 times per minute, it repeats every 3 s. The number of cross bridges that are formed is proportional to the amount of force that is developed and the rate of cross-bridge cycling is related to the rate at which force is developed. The number of cross-bridges that can be formed is directly related to the amount of calcium released into the cytoplasm after the cell is excited by the action potential. In this manner, calcium is not only critical in determining the force of contraction, but it also initiates the contraction–relaxation cycle of the cardiomyocyte.

The Activation of a Cardiomyocyte Contraction by Ca^{2+}

When a muscle is relaxed, actin and myosin are not interacting and force cannot be generated. This is because the sites where myosin binds to actin are blocked by a rod-shaped protein called 'tropomyosin' (TM). This protein lies along the actin filament (**Figure 1**) and is locked in place by a group of proteins called 'the cardiac troponin (cTn) complex'. The cTn complex is made up of three different proteins: cardiac troponin I (cTnI) (I for inhibitory), which binds directly to actin; cardiac troponin T (cTnT) (T for tropomyosin), which binds the cTn complex to TM; and cardiac troponin C (cTnC) (C for Ca^{2+}), which binds Ca^{2+} to trigger cardiomyocyte contraction. Thus, the cTn complex is bound directly to the thin filament but acts as a latch mechanism to initiate the binding of actin and myosin.

Muscle contraction is initiated when intracellular Ca^{2+} rises as a result of the action potential and binds to cTnC. The cTnC protein then changes its shape (a conformational change), which exposes a number of hydrophobic, charged amino acids within the core of cTnC. These amino acids then attract and pull cTnI away from the actin filament (the latch mechanism), allowing TM to move freely across the surface of actin, which uncovers the myosin-binding sites on the actin molecules. Myosin heads can now attach to actin, which is termed a cross-bridge (**Video Clip 1**).

The cross-bridges release when the intracellular Ca^{2+} decreases (pumped back into the sarcoplasmic reticulum (SR) or out of the cell), causing Ca^{2+} to be released from cTnC. As a result, cTnC returns to its original conformational shape and releases cTnI, which reattaches to the actin filament. Thus, the latch mechanism that locks TM in a blocking position over the myosin binding sites is reestablished. As no new cross-bridges can form, the myocyte relaxes.

The Influence of Temperature on Ca^{2+} Binding by the Contractile Element

As is the case with most physiological systems, a change in temperature alters the ability of the heart to contract. Reducing temperature a few degrees causes the human heart to beat slower and with weaker contractions. This compromises the heart's ability to circulate blood. One reason for the weaker contraction is that the heart has become less sensitive to Ca^{2+} as temperature decreases. As a result, more Ca^{2+} is required to generate the same strength of contraction. However, the maximum amount of Ca^{2+} that can be released to trigger a myocyte contraction is limited. Even so, animals that routinely function at a lower temperature than humans cannot survive such a loss of Ca^{2+} sensitivity and cardiac function. Something must be different in the fish heart because the human heart would literally stop beating at 5 °C, whereas rainbow trout (*Oncorhynchus mykiss*) heart functions fine at 5 °C. Hearts in polar fishes operate at even colder temperatures. So, the fish heart must compensate for the effect of low temperature on Ca^{2+} sensitivity.

One way the rainbow trout heart does this is by being more sensitive to Ca^{2+} than the mammalian heart. When compared at the same temperature, the trout heart is approximately 10 times more sensitive to Ca^{2+} than the mammalian heart. This means that it takes 1/10th the amount of Ca^{2+} to generate a contraction in the rainbow trout heart when compared with a mammalian heart at the same temperature. The ability for cardiac tissue from rainbow trout, frog (*Rana pipiens*), and rat (*Rattus norvegicus*) to generate force in response to Ca^{2+} over a range of temperatures is shown in **Figure 2**. The Ca^{2+} concentration (μM) required to generate half-maximal force ($K_{F½}Ca$) is plotted on a logarithmic scale. This figure clearly shows, for all three species, the inverse relationship between temperature and the Ca^{2+} concentration required to generate half-maximum force. The difference among species is evident when a comparison is made at the same temperature. For example, at 7 °C, it takes less Ca^{2+} to generate half-maximal force in the rainbow trout heart (2.8 μM) compared with either the frog (5.6 μM) or the rat (28.2 μM) – a 10-times difference between rainbow trout and rat.

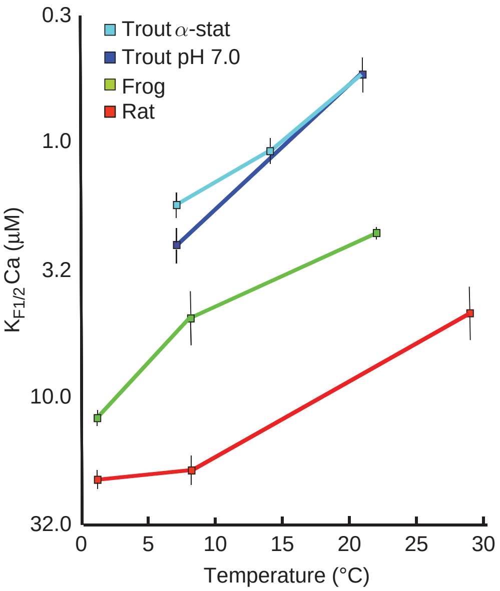


Figure 2 Comparison of the Ca^{2+} sensitivity of force generation by ventricular fibers isolated from hearts of rainbow trout, frog, and over a range of temperatures. Ca^{2+} sensitivity was measured as $K_{F1/2}Ca$, which is the Ca^{2+} concentration required to generate half-maximum force. When compared at the same temperature, the trout preparations required 10-fold less Ca^{2+} to generate the same measure of force than those from the mammalian species. Adapted from Churcott CS, Moyes CD, Bressler BH, Baldwin KM, and Tibbits GF (1994) Temperature and pH effects on Ca^{2+} sensitivity of cardiac myofibrils: A comparison of trout with mammals. *American Journal of Physiology* 267: R62–R70, used with permission from the American Physiological Society.

An equally important observation is possible, if the line for the rat is extrapolated to 37 °C. The $K_{F1/2}Ca$ for rat is similar to that of the rainbow trout at 7 °C. Thus, when compared at their respective physiological temperatures, the Ca^{2+} sensitivities of the rat and rainbow trout hearts are approximately the same. This suggests that the comparatively high Ca^{2+} sensitivity of the rainbow trout heart acts to compensate for the desensitizing effect of low temperature on Ca^{2+} sensitivity.

Adaptation of the Trout Contractile Element to Low Temperature

What is responsible for the comparatively high Ca^{2+} sensitivity of the rainbow trout heart? To begin answering this question, a series of studies were undertaken to compare the structure and function of rainbow trout and human cTnC. The logical place to start with is cTnC because it is a Ca^{2+}-binding protein. The comparison of rainbow trout and human cTnC structure and function is revealing in this regard.

Of the 161 amino acids that comprise cTnC, rainbow trout cTnC has 13 unique residues compared with the amino acid sequence of human cTnC (**Figure 3**). Since the amino acid sequence determines protein function, such differences in amino acid sequence could be important to Ca^{2+}-binding ability. Thus, the ability of these two proteins to bind Ca^{2+} was compared by monitoring the protein as it bound Ca^{2+} at 37 °C, 21 °C, and 7 °C. At all temperatures, Ca^{2+} affinity of rainbow trout cTnC is ~2.3 times that of human cTnC (**Figure 4**). In addition, the Ca^{2+} affinity of both proteins decreased as temperature decreased. Therefore, rainbow trout cTnC activates the contractile reaction at a lower Ca^{2+} concentration and the Ca^{2+} affinity of cTnC is directly related to temperature. Thus, the effect of low temperature on the Ca^{2+} affinity of cTnC is at least partially responsible for desensitizing the heart to Ca^{2+}.

Mutant human cTnC proteins have been used to identify the specific amino acids responsible for the comparatively high Ca^{2+} affinity of trout cTnC. These proteins contained different combinations of the amino acids found in rainbow trout cTnC. Four amino acid residues in rainbow trout cTnC were responsible for its comparatively high Ca^{2+} affinity: asparagine (N), isoleucine (I), glutamine (Q), and aspartate (D) at positions 2, 28, 29, and 30, respectively, in cTnC (NIQD HcTnC). Furthermore, when NIQD HcTnC was incorporated into rabbit cardiomyocytes, the Ca^{2+} sensitivity of force generation by these cardiomyocytes was increased by around twofold (**Figure 5**). This confirms that a cTnC molecule with a higher Ca^{2+} affinity does increase the Ca^{2+} sensitivity of cardiac

```
Salmonid   mndiykaaveqltdeqknefkaafdifiqdaedgcistkelgk
Mammalian  ,d,,,,,,,,,,,e,,,,,,,,,,,,,vlg,,,,,,,,,,,,,

           vmrmlgqnptpeelqemidevdedgsgtvdfdeflvmmvrcmkddskgk
           ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,


           teeeladlfcmfdknadgyidlqelkvmleatgeaiteddieel
           s,,,,s,,,r,,,,,,,,,,,,e,,,i,,q,,,,t,,,,,,,,,

           mkdgdknndgkidydeflefmkgve
           ,,,,,,,,,,r,,,,,,,,,,,,,,
```

Figure 3 Alignment of rainbow trout cardiac troponin C and human cardiac troponin C amino acid sequences. The differences in amino acid sequence between the two proteins are shown in bold print within the human sequence.

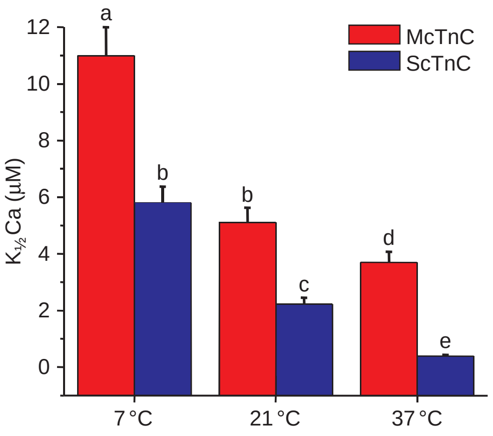


Figure 4 Comparison of the Ca^{2+} affinity of human cardiac troponin C and rainbow trout cardiac troponin C at different temperatures. Ca^{2+} affinity is shown as the $K_{1/2}Ca$, which is the Ca^{2+} concentration (in μM) required to half-saturate the molecule. Ca^{2+} affinity was measured at 37 °C, 21 °C, and 7 °C. All values are shown as mean ± SE. The Ca^{2+} affinity of rainbow trout cTnC was greater than that of human cTnC at each temperature. Adapted from Gillis TE and Tibbits GF (2002) Beating the cold: The functional evolution of troponin C in teleost fish. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 132: 763–772, with permission from Elsevier.

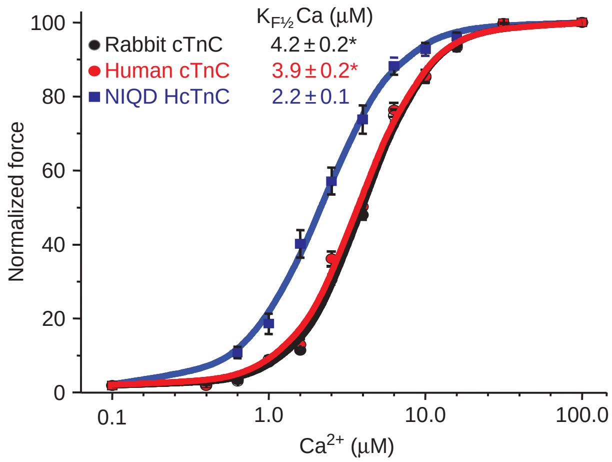
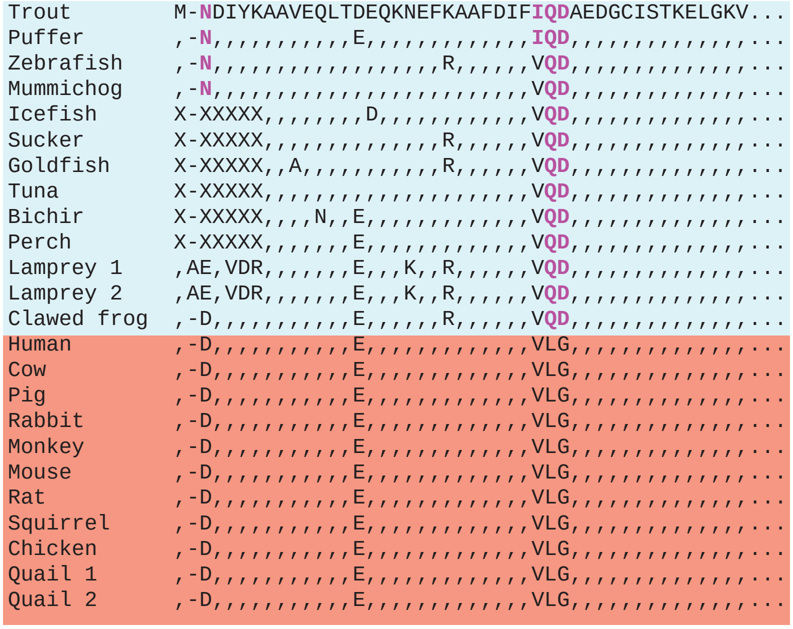


Figure 5 Ca^{2+} titration of force generation in rabbit cardiac cardiomyocytes containing either native rabbit cTnC ($n = 19$); human cardiac troponin C ($n = 12$); or NIQD human cardiac troponin C ($n = 7$), at 15 °C, pH 7.0. Data are normalized with respect to the maximal force generated during each Ca^{2+} titration and presented as mean ± SE. The Ca^{2+} concentration required to generate half-maximum force ($K_{F1/2}Ca$) are shown as mean ± SE. Means indicated with the same superscript are not significantly different from each other ($p > 0.05$). Adapted from Gillis TE, Marshall CR, and Tibbits GF (2007) Functional and evolutionary relationships of troponin C. *Physiological Genomics* 32: 16–27, used with permission from the American Physiological Socity.

tissue. Together, these results demonstrated that trout cTnC is partially responsible for the high Ca^{2+} sensitivity of the rainbow trout heart. However, further work is needed to explain why the rainbow trout heart is ~10-fold more sensitive to Ca^{2+} than the mammalian heart and this may involve other proteins such as cTnI and cTnT.

Phylogenetic Analysis of cTnC from a Variety of Vertebrate Species

Clearly, the sequence and function of cTnC have changed during the evolution of the vertebrate heart. By compiling all known amino acid sequences of cTnC, a phylogenetic tree can be created, based on how similar the proteins are to each other. This type of analysis aligns the protein sequences to identify amino acid substitutions. The more similar two proteins are, the closer they are on a phylogenetic tree. This type of analysis also helps to identify specific amino acids that are critical to the function of a protein – for example, if a specific amino acid is always at the same position in the protein from a variety of unrelated animals. In addition, if there are specific amino acids that are unique to animals with similar physiological conditions – for example, body temperature – this helps to identify which amino acids have evolved for that specific condition.



```
             Trout        M-NDIYKAAVEQLTDEQKNEFKAAFDIFIQDAEDGCISTKELGKV...
             Puffer       ,-N,,,,,,,,,,,E,,,,,,,,,,,,,IQD,,,,,,,,,,,,,,...
             Zebrafish    ,-N,,,,,,,,,,,,,,,,,,R,,,,,,VQD,,,,,,,,,,,,,,...
             Mummichog    ,-N,,,,,,,,,,,,,,,,,,,,,,,,,VQD,,,,,,,,,,,,,,...
             Icefish      X-XXXXX,,,,,,,,D,,,,,,,,,,,,VQD,,,,,,,,,,,,,,...
             Sucker       X-XXXXX,,,,,,,,,,,,,,R,,,,,,VQD,,,,,,,,,,,,,,...
Ectothermic  Goldfish     X-XXXXX,,A,,,,,,,,,,,R,,,,,,VQD,,,,,,,,,,,,,,...
             Tuna         X-XXXXX,,,,,,,,,,,,,,,,,,,,,VQD,,,,,,,,,,,,,,...
             Bichir       X-XXXXX,,,,N,,E,,,,,,,,,,,,,VQD,,,,,,,,,,,,,,...
             Perch        X-XXXXX,,,,,,,E,,,,,,,,,,,,,VQD,,,,,,,,,,,,,,...
             Lamprey 1    ,AE,VDR,,,,,,,E,,,K,,R,,,,,,VQD,,,,,,,,,,,,,,...
             Lamprey 2    ,AE,VDR,,,,,,,E,,,K,,R,,,,,,VQD,,,,,,,,,,,,,,...
             Clawed frog  ,-D,,,,,,,,,,,E,,,,,,R,,,,,,VQD,,,,,,,,,,,,,,...
             Human        ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Cow          ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Pig          ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Rabbit       ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Monkey       ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
Endothermic  Mouse        ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Rat          ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Squirrel     ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Chicken      ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Quail 1      ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
             Quail 2      ,-D,,,,,,,,,,,E,,,,,,,,,,,,,VLG,,,,,,,,,,,,,,...
```

Figure 6 Comparison of the N-terminus of all known cardiac troponin C homologs. Residues identified as being responsible for the comparatively high Ca^{2+} affinity of trout cTnC are indicated in mauve. Sequences are organized to indicate if they were cloned from ectothermic or endothermic species. The X in place of an amino acid indicates that the residue is not known. Adapted from Gillis TE, Marshall CR, Tibbits GF (2007) Functional and evolutionary relationships of troponin C. *Physiological Genomics* 32: 16–27, used with premission from the American Physiolocical Society.

Interestingly, all ectothermic species (this includes teleost fish, lamprey, and frogs) contain at least two of the four amino acids identified in rainbow trout as being responsible for high Ca^{2+} affinity of cTnC (**Figure 6**). In contrast, cTnC from endothermic species (birds and mammals) do not have these four amino acids at these same positions. As lampreys are the earliest known vertebrates, and fish and amphibians evolved earlier than either mammals or birds, it is suggested that these identified residues (N, I, Q, and D at positions 2, 28, 29, and 30) were present in the earliest forms of cTnC. These residues were then lost through natural selection as the body temperature of the ancestor species of mammals and birds started to increase and become constant.

This suggests that the lower Ca^{2+} affinity of mammalian cTnC is an adaptation to high temperature (37 °C) and high heart rate. Consider a human heart at 37 °C; if it contained a cTnC with the same Ca^{2+} affinity as rainbow trout cTnC, it would likely remain in contracture or beat very slowly as Ca^{2+} would be released at a very slow rate. Remember that rainbow trout cTnC has twice the Ca^{2+} affinity as human cTnC and that as temperature increases the Ca^{2+} affinity increases (**Figure 4**). The heart would remain in contracture as the Ca^{2+} affinity of the protein would be so high that Ca^{2+} would remain bound to the protein. This would result in the continuous formation of cross-bridges between actin and myosin. If a heart is in contracture, the animal dies. It is no surprise, therefore, that the four residues identified as being responsible for the high Ca^{2+} affinity of rainbow trout cTnC are not found in human cTnC.

Cardiac Contractility in Changing Environmental Temperatures

Fish, unlike mammals, expose their heart to a highly variable temperature. Rapid temperature changes can be >10 °C. In addition to Ca^{2+} sensitivity, temperature changes blood and tissue pH. This change in pH is automatic and acts to keep the relative alkalinity ($[OH_2]/[H]$) approximately constant. This relationship, called α-stat regulation, is a change of –0.016 to –0.019 pH units $°C^{-1}$. Therefore, a 10 °C decrease in temperature would cause a ~0.2-unit increase in cellular pH.

This pH change is relevant to cardiac function because an increase in pH increases the Ca^{2+} sensitivity of the heart. **Figure 1** compares the Ca^{2+} sensitivity of rainbow trout cardiac tissue over a range of temperatures when pH is kept constant at 7.0 versus when pH is allowed to change according to α-stat regulation. At 7 °C, the pH difference between the two treatments is 0.2 pH units. The $K_{F1/2}Ca$ of the rainbow trout cardiac tissue at pH 7.2 is 1.6 μM versus 2.8 μM at pH 7.0, which corresponds to a ~1.6

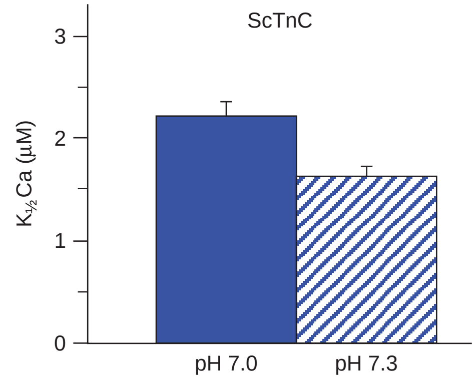


Figure 7 Effect of a 0.3 pH unit increase on the Ca^{2+} affinity of trout cTnC. Ca^{2+} affinity is shown as the $K_{1/2}Ca$, which is the Ca^{2+} concentration (in μM) required to half-saturate the molecule. Measurements were made at 21 °C. The Ca^{2+} affinity of trout cTnC was greater at pH 7.3 than at pH 7.0. Adapted from Gillis TE, Marshall CR, Xue XH, Borgford TJ, and Tibbits GF (2000) Ca^{2+} binding to cardiac troponin C: Effects of temperature and pH on mammalian and salmonid isoforms. *American Journal of Physiology* 279: R1707–R1715.

difference. Therefore, as pH increases with decreasing temperature, the Ca^{2+} sensitivity of the contractile element increases as a result of the pH change, allowing the heart to maintain contractile ability despite the decrease in temperature.

The effect of pH on the Ca^{2+} sensitivity of cardiac tissue is due, at least in part, to a pH effect on the Ca^{2+} affinity of cTnC. A 0.3-pH increase significantly increases the Ca^{2+} affinity of rainbow trout cTnC by ~1.4-fold (**Figure 7**). This increase in Ca^{2+} affinity would make it possible for cTnC to be activated by Ca^{2+} and help the heart to continue beating if a fish swims from warm surface water into colder waters after prey.

Future Directions

There is much to be learned about the molecular mechanisms that regulate cardiac contraction in fish and how these have been adapted to enable cardiac function in the variety of environments in which different fish are found. One current area of interest is the signaling pathways that are used to rapidly change the rate and strength of cardiac contraction. This includes the α- and β-adrenergic pathways. These pathways turned on by the stress response (also called the fight-or-flight response) activate functional proteins called 'kinases' that phosphorylate a number of the cardiac proteins, including cTnI and cTnT. Phosphorylation takes place when a phosphate group is attached to a specific amino acid, usually a serine or tyrosine, thereby adding an additional positive charge to the protein. This acts to change how the amino acids around the phosphate group interact with each other and, as a result, affect the function of the protein. The phosphorylation of cTnI and cTnT in the mammalian heart acts to increase the rate of cardiac contraction. Little is known of how phosphorylation of fish contractile proteins regulates the function of the fish heart.

See also: **Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping. **Integrated Response of the Circulatory System:** Integrated Responses of the Circulatory System to Temperature.

Further Reading

Bers D (2001) *Excitation–Contraction Coupling and Cardiac Contractile Force*. London: Kluwer.

Churcott CS, Moyes CD, Bressler BH, Baldwin KM, and Tibbits GF (1994) Temperature and pH effects on Ca^{2+} sensitivity of cardiac myofibrils: A comparison of trout with mammals. *American Journal of Physiology* 267: R62–R70.

Gillis TE, Liang B, Chung F, and Tibbits GF (2005) Increasing mammalian cardiomyocyte contractility with residues identified in trout troponin C. *Physiological Genomics* 22: 1–7.

Gillis TE, Marshall CR, and Tibbits GF (2007) Functional and evolutionary relationships of troponin C. *Physiological Genomics* 32: 16–27.

Gillis TE, Marshall CR, Xue XH, Borgford TJ, and Tibbits GF (2000) Ca^{2+} binding to cardiac troponin C: Effects of temperature and pH on mammalian and salmonid isoforms. *American Journal of Physiology* 279: R1707–R1715.

Gillis TE and Tibbits GF (2002) Beating the cold: The functional evolution of troponin C in teleost fish. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 132: 763–772.

Gordon AM, Regnier M, and Homsher E (2001) Skeletal and cardiac muscle contractile activation: Tropomyosin "rocks and rolls". *News in Physiological Science* 16: 49–55.

Moyes CD, Borgford T, LeBlanc L, and Tibbits GF (1996) Cloning and expression of salmon cardiac troponin C: Titration of the low-affinity Ca^{2+}-binding site using a tryptophan mutant. *Biochemistry* 35: 11756–11762.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Cardiac Cellular Length–Tension Relationship

HA Shiels, University of Manchester, Manchester, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Frank–Starling Relationship
The SL–Tension Relationship
Myofilament Ca Sensitivity
The SL–Tension Relationship in the Fish Heart
Conclusions
Further Reading

Glossary

Active tension Increase in muscle tension during activation (during systole).

$[Ca^{2+}]_i$ Free intracellular calcium concentration.

Ca transient ($\Delta[Ca^{2+}]_i$) The term used to describe the transient rise and fall of cytosolic Ca with each contraction–relaxation cycle.

Cardiac output The volume of blood pumped by the heart per unit time; the product of heart rate and stroke volume.

Ejection fraction The end-systolic volume as a fraction of end-diastolic volume, that is, the fraction of blood at the end of ventricular filling that is pumped out during the subsequent contraction. A value of 1 means complete emptying.

Endocardium Inner part (infolding) of the pericardial sac, and is the inner lining of the cardiac chambers.

Epicardium The compact myocardium on the outer side of the ventricle(s).

Intrinsic Inherent, built in.

Myocyte An individual cardiac muscle cell.

Myofilament Either a thick filament made up principally of myosin molecules or a thin filament made up of actin molecules and associated troponin and tropomyosin complexes.

Passive stiffness The resistance of the resting muscle to deformation by an applied force; the inverse of compliance.

Passive tension Muscle tension at rest (during diastole).

pCa_{50} $\log_{10}$ Ca^{2+} concentration that activates 50% of maximal force, used as an index of changes in myofilament Ca^{2+} sensitivity in response to, for example, a change in sarcomere length.

Stroke volume The volume of blood ejected into the aorta by a single contraction of the ventricle(s).

Venous return The return of blood to the heart via the venous system.

Z-Line A dark line which separates the sarcomeres of striated muscle. It is the anchor site of actin filaments from neighboring sarcomeres.

Introduction

How Ca causes the myofilaments (contractile elements) to contract and generate force (or tension) within the cardiac myocyte is discussed in **Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element. It was explained that Ca binds to troponin C (TnC), which initiates a sequence of events that culminate in the myosin (thick filament) heads interacting with actin (thin filament) and generating force via the cross-bridge. From this discussion, it is apparent that there is a direct link between Ca and contractility. Indeed, there is a direct relationship between the amplitude of the Ca transient (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux) and the number of cross-bridges generated between actin and myosin. Because of this, altering the amplitude and rate of the Ca transient effectively regulates the strength and rate of cardiac contraction. This is clearly seen during exercise when adrenergic stimulation increases the amplitude of the Ca transient increasing, in turn, contractile strength as well as cardiac output to meet the elevated demands of the body.

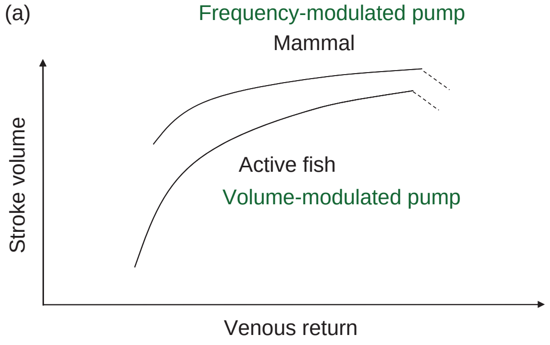
There is, however, another way to increase the strength of contraction, one that does not rely on an increase in Ca. This comes about by increasing the sensitivity of the myofilaments to Ca. Thus, the term ‘myofilament Ca sensitivity’ describes how effective a given amount of Ca is at generating contractile force. If myofilament Ca sensitivity is increased, more force is produced at the myofilaments for the same amount of Ca. Myofilament Ca sensitivity is already higher in fish hearts than mammalian hearts. The mechanisms underlying this inherently high Ca sensitivity in relation to temperature and pH are discussed in **Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element. The focus of this article is on how changes in muscle length (i.e., stretching cardiac muscle) also affect myofilament Ca sensitivity. It is universally accepted that increasing muscle length (up to a point) increases myofilament Ca sensitivity and thus contractile strength. However, the exact mechanisms by which length increases cardiac myofilament Ca sensitivity are still unknown. Elucidating these mechanisms is an active area of research for muscle biomechanics and cardiologists alike.

At the cellular level, the relationship between muscle length and contractile strength is called the 'length–tension (L–T) relationship' or the 'sarcomere length (SL)–tension relationship'. At the level of the whole heart, it is called the Frank–Starling relationship (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping). This article discusses what is known about these relationships in the fish heart and how they regulate fish heart function.

The Frank–Starling Relationship

The relationship between muscle length and muscle contractile strength was first recognized by Otto Frank and Ernest Starling at the turn of the twentieth century. They noticed that the greater the amount of blood in the ventricles at the moment they began to contract (end diastolic volume), the greater the pressure they developed during the contraction (systolic pressure). Thus, the Frank–Starling relationship describes how stretching the heart with increased volumes of blood causes it to contract more forcefully. This mechanism is vital for linking changes in the volume of blood returning to the heart (venous return), with changes in the volume of blood pumped per beat by the ventricle(s) (stroke volume).

The coupling of stroke volume and venous return via the Frank–Starling mechanism is illustrated graphically in **Figure 1(a)**. The figure shows two hypothetical Starling curves: one for an active fish and the other for a mammal. For both curves, as venous return increases, stroke volume increases. It is important to notice two features from **Figure 1(a)**. First, stroke volume increases with increased venous return up until a point and then plateaus. Eventually, stroke volume will drop if venous return continues to increase (dashed line). Second, the fish heart demonstrates a greater response in stroke volume than the mammalian heart over a similar change in venous return. This indicates that fish hearts are more sensitive to the Frank–Starling mechanism than mammalian hearts. This feature of the fish heart has been elegantly demonstrated through the use of an *in situ* heart preparation (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping), which shows how very small increments (0.2–0.3 kPa) in filling pressure (caused by an increased venous return) result in very large (threefold) increases in stroke volume in fish. Much larger increases in filling pressure are required for smaller increases in stroke volume in mammalian hearts.



(b)

$$\begin{array}{ccccc}\text{Cardiac output} & = & \text{Frequency} & \times & \text{Stroke volume}\\ \text{ml min}^{-1} & & \text{min}^{-1} & & \text{ml}\end{array}$$

Figure 1 The Frank–Starling relationship. (a) A schematic representation of the Frank–Starling relationship. (b) The formula for cardiac output showing the roles of heart rate and stroke volume.

Thus, the fish heart is very sensitive to stretch and can operate *in vivo* over much of their Starling curve.

The Importance of the Frank–Starling Relationship to Fish Hearts

The Frank–Starling relationship is important for regulating cardiac function in fish. The reasons for this are at least twofold. First, fish have less neural and hormonal control over their hearts than other vertebrates, such as birds and mammals. This means that intrinsic mechanical regulation can exert a greater influence on heart function. Second, and more importantly, the Frank–Starling relationship is important for fish because they regulate cardiac output via stroke volume. Cardiac output is the product of heart rate and stroke volume (**Figure 1(b)**). Maintaining and adjusting cardiac output to meet changing circulatory demands can be achieved by altering either heart rate or stroke volume (or both). Fish tend to alter stroke volume to a larger degree than heart rate. For example, in a maximally exercising rainbow trout, heart rate increases by ~50%, whereas stroke volume can increase by as much as 300% (i.e., threefold). Increases in stroke volume stretch the cardiac chambers and invoke the Frank–Starling mechanism (i.e., increased contractile force).

The reasons for the greater reliance on volume modulation of cardiac output in fish compared with other vertebrate hearts are not clear. One idea centers around the fact that as ectotherms, body (and thus heart) temperature changes with that of the surrounding aquatic environment. Fish heart rate is profoundly influenced by temperature through direct effects on the pacemaker myocytes (**see also Design and Physiology of the Heart:** Action Potential of the Fish Heart). Cooler temperatures slow down heart rate and warmer temperatures increase heart rate. Thus, controlling cardiac output via heart rate could be difficult for rainbow trout living in, and moving through, a changing thermal environment. With control of cardiac output via heart rate limited by the direct effect of temperature on pacemaker frequency, stroke volume modulation of cardiac output emerges as an important strategy for fish.

It should be noted that the reliance on stoke volume regulation of cardiac output varies with fish species and with activity within one species. Moreover, there are fish that do not modulate stroke volume and rely entirely on varying heart rate to change cardiac output. For example, very active species, such as tuna, have a limited capacity to increase stroke volume during exercise, and often actually decrease stroke volume coincident with an increase in heart rate. The hearts of Antarctic fish (who live in one of the most constant thermal environments on the planet) are known to increase heart rate during exercise with little changes in stroke volume. Nevertheless, in a broad sense, as endothermy emerged during vertebrate evolution, so did the reliance on frequency modulation of cardiac output.

The SL–Tension Relationship

Stretch of the whole heart will result in stretch of the myocytes that make up that heart. For a fish, such as the rainbow trout, a threefold increase in stroke volume (e.g., during maximum exercise) would require a stretch of the underlying cardiac myocytes by ~40%. These changes in myocyte length will affect myocyte contractile force in a manner akin to the Frank–Starling relationship at the whole heart.

The myocyte equivalent of the Frank–Starling relationship is the SL–tension relationship. It states that the force of contraction increases with SL up to an optimum length, beyond which force of contraction drops. The SL–tension relationship from the mammalian heart is illustrated in **Figure 2**. The *x*-axis shows SL and is a cellular correlate to the *x*-axis from **Figure 1**(**a**) showing venous return. The *y*-axis in **Figure 2** is normalized tension and is a cellular correlate of whole heart stroke volume from **Figure 1**(**a**).

Importance of the Sarcomere

To understand the SL–tension relationship, it is important to understand the sarcomere. The sarcomere is the fundamental unit of myocyte contraction. Sarcomeres are recognizable as the familiar banding pattern observed when striated muscle is seen through the light microscope. **Figure 3**(**a**) shows a portion of a ventricular myocyte from a bluefin tuna where the regular banding pattern of the sarcomeres is clearly visible. A schematic of a mammalian sarcomere and its composite proteins is provided in **Figure 3**(**b**). The morphology of the rainbow trout sarcomere is similar to that of the mammalian sarcomere and thin-filament length is approximately 0.95 μm in both rat and rainbow trout ventricular myocytes. A sarcomere is defined as the distance between the Z-lines. The Z-lines are pulled closer together during contraction and move further apart during relaxation. The Z-lines are closer during contraction because actin and myosin interaction generates cross-bridges, which

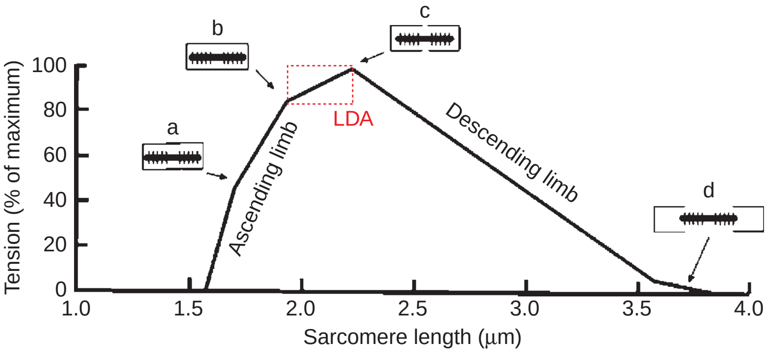


Figure 2 A schematic diagram of the sarcomere length (SL)–tension relationship for mammalian cardiac muscle. The insets (a–d) show the role of myofilament overlap in the length-dependent increase in force. Inset (a) shows the position of actin and myosin at short SLs when myosin comes in contact with the Z-line and there is a rapid decline in tension as SLs decrease (to the left of the arrow). SLs to the left of inset (b) show how tension decreases when the thin filaments from opposite ends of the sarcomere overlap at the M-line. The region between (b) and (c) is the range of SLs where the potential availability of cross-bridges remains constant during sarcomere stretch because the central cross-bridge head-free zone of the myosin filament (M-line) is progressively uncovered. Over this range of lengths, length-dependent activation (LDA) occurs as indicated by the red box. Inset (d) shows how tension declines toward zero when the sarcomere is stretched such that there is no overlap between thick and thin filaments. Adapted from Bers DM (2001) *Excitation–Contraction Coupling and Contractile Force*, p. 27. Dordecht: Kluwer.

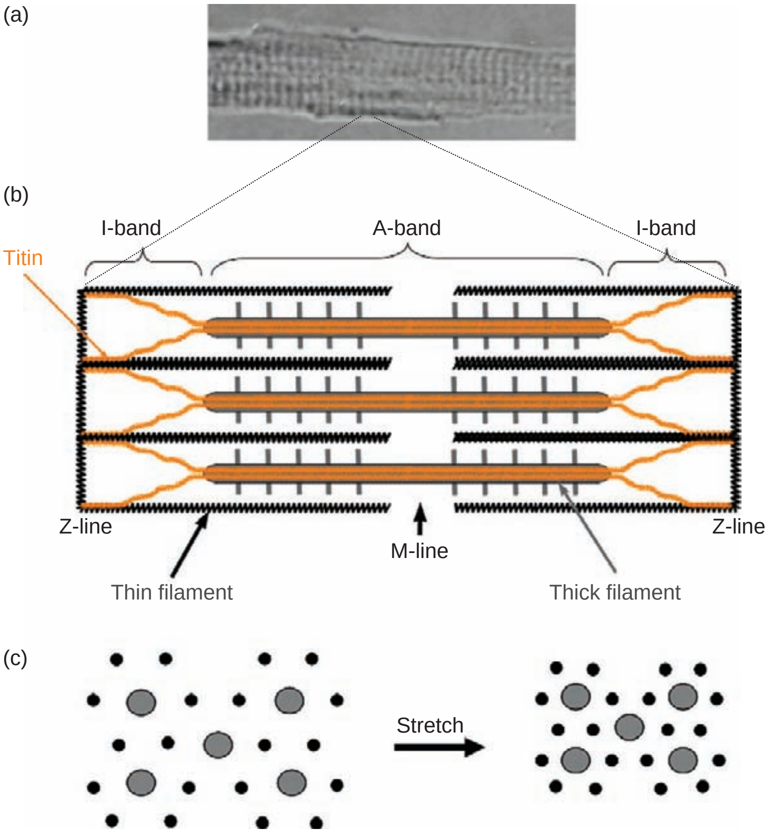


Figure 3 (a) A portion of a ventricular myocyte from a bluefin tuna taken with a light microscope showing the striation pattern of the sarcomeres. The myocyte is ~15 μm in diameter and the images shows 50 μm of its length. (b) Schematic diagram of a cardiac sarcomere. The sarcomere is the fundamental unit of contraction and is defined as the region between two Z-lines. Each sarcomere consists of a central A-band (thick filaments) and two halves of the I-band (thin filaments). The I-band from two adjacent sarcomeres meets at the Z-line. The central portion of the A-band is the M-line, which does not contain actin. The figure shows the positioning of the major filament systems that compose the sarcomere: titin, actin (thin), and myosin (thick) filaments. (c) Pictorial description of the cross section through striated muscle showing the effect of stretch on myofilament lattice spacing. Light gray circles demark thick (myosin) filaments and black circles demark thin (actin) filaments. Adapted from Shiels HA and White E (2008) The Frank–Starling mechanism in vertebrate cardiac myocytes. *Journal of Experimental Biology* 211: 2005–2013.

slide the myofilaments over each other. During relaxation, myosin and actin detach and the Z-lines slide back apart. The role of myofilament overlap in sarcomere shortening is expanded upon in the following section (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

Myofilament Overlap

The distance between Z-lines determines the degree of overlap between thick and thin filaments and thus the potential availability of cross-bridges to generate tension. The insets in **Figure 2** show how changes in SL affect myofilament overlap and thus tension in cardiac muscle. The sarcomeric filaments are drawn schematically in the insets (a–d), where the thick lines with cross-hatching are myosin and the thin lines are actin. Insets (a and b) illustrate how overlap between thick and thin filaments increases during stretch which results in a greater number of potential cross-bridges and thus greater tension development. This phase of the SL–tension relationship is called the ‘ascending limb’. Beyond the length for optimal overlap, additional stretching reduces myofilament overlap, decreasing the probability of cross-bridge formation and decreasing tension (inset d). This region of the SL–tension relationship is called the ‘descending limb’.

A large proportion of the increase in force with increased muscle length on the ascending limb of the SL–tension relationship can be explained by myofilament overlap. Indeed, in skeletal muscle, the majority of the SL–tension relationship is explained in this way. However, in cardiac muscle, other mechanisms contribute to the length-dependent increase in force. A significant increase in active tension occurs over the region of SLs where the potential availability of cross-bridges remains constant. This is shown in **Figure 2** by the increase in tension between insets (b) and (c). Over this SL range, an

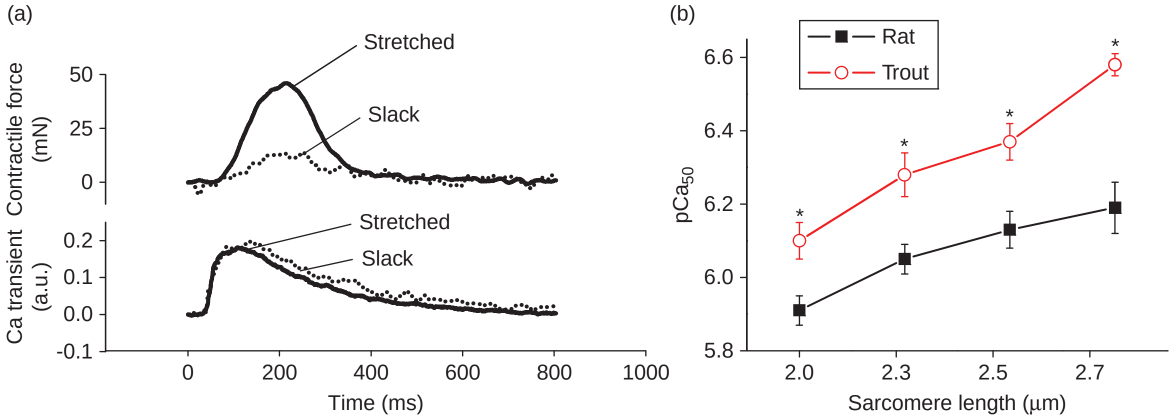


Figure 4 Length-dependent increases in myofilament Ca sensitivity in the trout heart. (a) Simultaneous measurement of changes in force (upper panel) and the Ca transient measured with fura-2 (lower panel) at slack length and after a stretch show how stretch increases force but not the intracellular Ca transient in single trout cardiac myocytes. (b) The sarcomere-length (SL) dependency of myofilament Ca sensitivity in rat and trout permeabilized ventricular myocytes. The pCa for half-maximal activation (pCa_{50}) is an index of myofilament Ca sensitivity of the contractile machinery. The asterisks show the data are significantly different. (a) Shiels HA, Calaghan SC, and White E (2006) The cellular basis for enhanced volume-modulated cardiac output in fish hearts. *Journal of General Physiology* 128: 37–44. (b) Patrick SM, Hoskins A, Kentish J, *et al*. (2010) Enhanced length-dependent Ca^{2+} activation in fish cardiomyocytes permits a large operating range of sarcomere lengths. *Journal of Molecular and Cellular Cardiology* 48: 917–924.

increase in length does not equate to an increase in potential cross-bridge sites, as the central cross-bridge head-free zone of the myosin filament (near the M-line in **Figure 4(a)**) is progressively uncovered. Hence, myofilament overlap cannot fully account for the SL–tension relationship in cardiac muscle: the remainder is due to increased myofilament Ca sensitivity and is referred to as length-dependent activation (LDA).

Myofilament Ca Sensitivity

Stretch of the myocyte increases myofilament Ca sensitivity, which increases contractile force. This is illustrated for the rainbow trout in **Figure 4(a)**. The figure shows simultaneous measurements of the intracellular Ca transient (top panel and **see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux) and force production (bottom panel) in a single rainbow trout myocyte at slack length and after a stretch. When the myocyte is stretched, there is an immediate increase in the size of the contraction but no change in the Ca transient. An increase in contractile force in the absence of a rise in $[Ca^{2+}]_i$ indicates that the myofilament sensitivity for Ca has increased. Similar observations occur in amphibian and mammalian cardiac myocytes. Surprisingly, the mechanisms by which stretch increases myofilament Ca sensitivity are still poorly understood.

The stretch-induced increase in myofilament Ca sensitivity is frequently quantified by the $[Ca^{2+}]_i$ required for half-maximal activation of tension (pCa_{50}) at different lengths. pCa_{50} as an index of myofilament Ca sensitivity is explained in **Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element. **Figure 4(b)** compares the pCa_{50} values of rainbow trout and rat cardiac myocytes at a number of SLs. What is important here is that as SL increases in **Figure 4(a)**, so does the Ca sensitivity of the myofilaments as illustrated by the increase in pCa_{50}. It is immediately apparent that the rainbow trout myofilaments are more sensitive to Ca than the rat myofilament at any given SL. This is partially due to differences in TnC between fish and mammals (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element) and may be required to offset the negative effects of cold temperature on myofilament Ca sensitivity in fish (**see also Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

The SL–Tension Relationship in the Fish Heart

The SL–tension relationship in the fish myocyte is illustrated in **Figure 5**. **Figure 5(a)** shows a rainbow trout ventricular myocyte held between two carbon fibers. Images show the myocyte at slack length (top, SL 1.80 μm), and following stretches to SLs 2.16 μm (middle) and 2.50 μm (bottom). **Figure 5(b)** shows data from a different rainbow trout myocyte illustrating how stretching a myocyte to SLs, shown by the numbers above each stretch (bottom trace), increases passive and active tension (top trace).

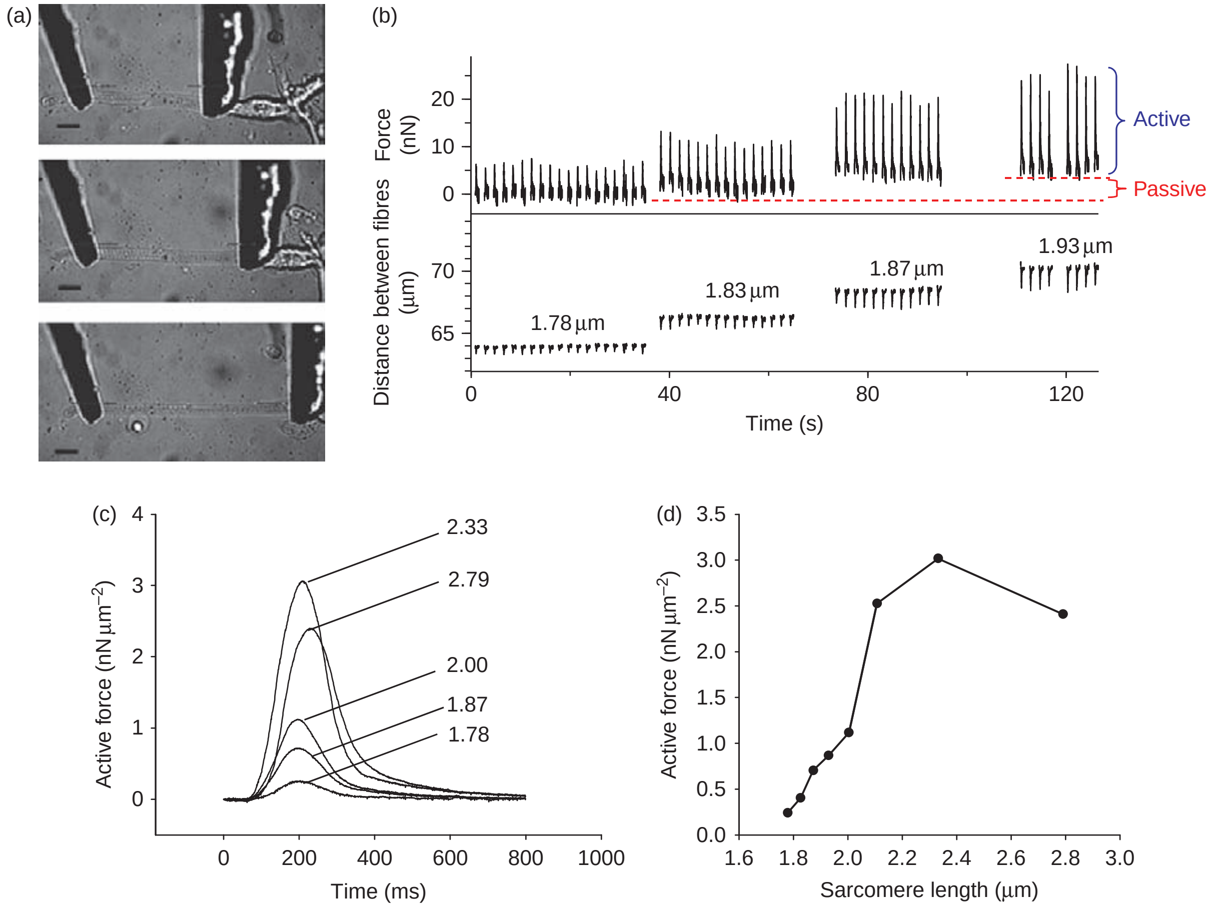


Figure 5 The SL–tension relationship in the trout ventricular myocyte. (a) Stretch of a single rainbow trout ventricular myocyte held between two carbon fibers. Images show the myocyte at slack length (top; SL 1.80 μm), and following stretches to SL 2.16 μm (middle) and 2.50 μm (bottom). Scale = 10 μm (**Video Clip 1**). (b) Data from a different cell showing how progressive stretching increases passive and active tension (upper trace), when the distance between the fibers is increased causing stretch of the sarcomere (lower trace; numbers show SL at each stretch). (c) Data from a different cell which shows contractile force produced by a single rainbow trout myocyte as it is stretched. The figure shows the contractions at each SL (indicated by numbers in micrometers). (d) SL–tension relationship from the same cell. Notice the similarity between the SL–tension relationship in the single trout myocyte and the schematic diagram in **Figure 2**. (a–d) From Shiels HA, Calaghan SC, and White E (2006) The cellular basis for enhanced volume-modulated cardiac output in fish hearts. *Journal of General Physiology* 128: 37–44.

The contraction curves from a different cardiac myocyte at increasing SL are shown in **Figure 5(c)**. Each curve shows the cell contracting and developing force at a given SL, and then relaxing. In **Figure 5(d)**, the amplitude of the contraction is plotted against SL, providing a single rainbow trout ventricular myocyte SL–tension relationship. Notice the similarity to the schematic curve based on mammalian hearts in **Figure 2**. The rainbow trout SL–tension relationship shows an ascending limb where increased SLs increase the force of contraction, a peak, and the beginning of a descending limb.

As the morphology of the rainbow trout sarcomere is similar to that of the mammalian sarcomere (**Figures 3(a)** and **3(b)**), we can assume that similar to mammals, some of the SL–tension relationship is due to changes in myofilament overlap with stretch. However, also similar to mammals, other mechanisms are at work in the fish sarcomere, which enhance the increase in force with increase in length beyond that predicted from myofilament overlap alone. These mechanisms have not been investigated in fish hearts but are discussed in a general way in the following section.

Lattice Compression

When muscle is stretched, in addition to an increase in length, there is a decrease in cross-sectional area (**Figure 3(c)**). This results in compression of the lattice spacing of the muscle (the ordered structure of thick and thin filaments). This compression is thought to encourage the recruitment of strong force producing cross-bridges between the thick and thin filaments. However, the precise role of filament spacing remains controversial in mammalian studies and has not been investigated in fish.

Thin-Filament Activation

Thin-filament activation or cooperativity has also been put forward as a contributor to length-dependent changes in myofilament Ca sensitivity in mammals but has not been explored in fish hearts.

Titin and Passive Tension

Passive tension of the sarcomere offers resistance to stretch. The main determinant of passive tension at SLs on the ascending limb of the SL–tension relationship is the giant elastic protein titin (**Figure 3(b)**). Titin develops passive forces which act to draw the Z-lines together as the sarcomere is stretched. In addition to these longitudinal forces, titin can also produce radial force during stretch as it pulls the thin filament closer to the thick filament, which results in reduced myofilament lattice spacing (**Figure 3(c)**). This may help to explain why passive tension is linked to the length sensitivity of the preparation in both fish and mammals. Titin strain may also increase the likelihood of actin and myosin interaction by increasing the disorder of the myosin heads.

LDA of fish myofilaments has been observed and can be explained, at least in part, by length-dependent increases in myofilament Ca sensitivity. For example, the slope of the relationship between length and pCa_{50}, shown in **Figure 3(b)**, is steeper for fish than for mammals, suggesting that the fish heart does show greater length-dependent increases in myofilament Ca sensitivity than mammals, especially at long SL. This may be important for fish heart function because larger stretches are predicted for the fish heart due to the large volume changes inherent in stroke volume modulation of cardiac output.

Conclusions

During the evolution of the vertebrate heart, the main modulator of cardiac output has shifted from stroke volume in fish, to heart rate in mammals. Thus, in fish, cardiac output is largely volume regulated, meaning that the fish heart is specialized for large distensions during its normal filling-and-emptying cycle. To be able to contract at long lengths, the contractile apparatus in the fish myocytes show greater length-dependent Ca sensitivity than mammalian hearts. This property allows fish myocytes to beat forcefully at the long SLs required to volume-modulate cardiac output.

See also: **Design and Physiology of the Heart:** Action Potential of the Fish Heart; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping.

Further Reading

Allen D and Kentish J (1985) The cellular basis of the length–tension relation in cardiac muscle. *Journal of Molecular and Cellular Cardiology* 9: 821–840.

Axelsson M, Davison W, Forster ME, and Farrell AP (1992) Cardiovascular responses of the red-blooded Antarctic fishes *Pagothenia bernacchii* and *P. borchgrevinki*. *Journal of Experimental Biology* 167: 179–201.

Bers DM (2001) *Excitation–Contraction Coupling and Contractile Force,* p. 27. Dordecht: Kluwer.

Burggren W, Farrell AP, and Lillywhite H (1997) Vertebrate cardiovascular systems. In: Dantzler WH (ed.) *The Handbook of Physiology*, pp. 215–308. New York: Oxford University Press.

Cazorla O, Freiburg A, Helmes M, *et al.* (2000) Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circulation Research* 86: 59–67.

Cazorla O, Pascarel C, Garnier D, and Le Guennec J (1997) Resting tension participates in the modulation of active tension in isolated guinea pig ventricular myocytes. *Journal of Molecular and Cellular Cardiology* 29: 1629–1637.

Cazorla O, Wu Y, Irving TC, and Granzier H (2001) Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circulation Research* 88: 1028–1035.

Fabiato A and Fabiato F (1975) Dependence of the contractile activation of skinned cardiac cells on the sarcomere length. *Nature* 256: 54–56.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac-function in fish. *Physiological Zoology* 64: 1137–1164.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *The Cardiovascular System*, pp. 1–88. San Diego, CA: Academic Press.

Franklin CE and Davie PS (1992) Dimensional analysis of the ventricle of an *in situ* perfused trout heart using echocardiography. *Journal of Experimental Biology* 166: 47–60.

Gillis TE, Marshall CR, Xue XH, Borgford TJ, and Tibbits GF (2000) Ca^{2+} binding to cardiac troponin C: Effects of temperature and pH on mammalian and salmonid isoforms. *American Journal of Physiology* 279: R1707–R1715.

Gillis TE and Tibbits GF (2002) Beating the cold: The functional evolution of troponin C in teleost fish. *Comparative Biochemistry and Physiology A* 132: 763–772.

Korsmeyer KE, Lai NC, Shadwick RE, and Graham JB (1997) Heart rate and stroke volume contribution to cardiac output in swimming yellowfin tuna: Response to exercise and temperature. *Journal of Experimental Biology* 200: 1975–1986.

Patrick SM, Hoskins A, Kentish J, *et al.* (2010) Enhanced length-dependent Ca^{2+} activation in fish cardiomyocytes permits a large operating range of sarcomere lengths. *Journal of Molecular and Cellular Cardiology* 48: 917–924

Shiels H and White E (2007) The effect of mechanical stimulation on vertebrate hearts: A question of class. In: Kamkin A and Kiseleva I (eds.) *Mechanosensitive Ion Channels*, pp. 331–350. New York: Springer.

Shiels HA, Calaghan SC, and White E (2006) The cellular basis for enhanced volume-modulated cardiac output in fish hearts. *Journal of General Physiology* 128: 37–44.

Shiels HA and White E (2008) The Frank–Starling mechanism in vertebrate cardiac myocytes. *Journal of Experimental Biology* 211: 2005–2013.

Wu Y, Cazorla O, Labeit D, and Granzier H (2000) Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. *Journal of Molecular and Cellular Cardiology* 32: 2151–2162.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Intracardiac Neurons and Neurotransmitters in Fish

G Zaccone, F Marino, and D Zaccone, University of Messina, Messina, Italy

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Gross Morphology and Anatomy of the Heart
Extrinsic Innervation and Chemical Codes of Autonomic Neurons
Postganglionic Neurons and Neurotransmitter Localization
The Cardiac Pacemaker and Conducting System
Conclusions
Further Reading

Glossary

Arterial Adjective pertaining to blood that has been oxygenated.

Arteriosus Pertaining to the outflow tract of the heart leading to capillaries.

Autonomic nervous system A collection of motor neurons (ganglia) ordered segmentally from cranial to caudal levels. The system controls the inner organs (viscera) through reflexes without conscious control.

Autonomic neurotransmitters A group of chemicals released by sympathetic and parasympathetic preganglionic neurons that transmit information to neighboring cells.

Chemical code A precise combination of both peptide and nonpeptide transmitters that identifies different populations of neurons.

Cholinergic system Motor neurons that ramify throughout their target tissues, including cardiac muscle, and release the neurotransmitter acetylcholine.

Coronary circulation A specialized system of blood vessels dedicated to the heart, which normally delivers fully oxygenated blood to at least a portion of the cardiac muscle. A coronary blood vessel refers loosely to any vessel in this specialized circulation, from the coronary artery, through the coronary capillaries to the coronary veins.

Neuropeptides A group of chemicals (co-transmitters) that facilitate cholinergic transmission by mediating slow excitatory postsynaptic potentials.

Nitric oxide synthase/NO system It refers to a gaseous molecule, NO, which is generated by the enzyme NOS, and acts as a neurotransmitter. It is released by cardiomyocytes and myocardial neurons.

Trabeculae A bundle of cardiac muscle fibers that is a characteristic of the spongy myocardium of fishes and other lower vertebrates.

Venous Adjective pertaining to blood that has passed through tissues and has had some of its oxygen removed for metabolism.

Introduction

This article concerns a specialized system of neurons that populate the heart – the intracardiac neurons. These neurons are distinct from the adrenergic and cholinergic neurons that reach cardiac tissues as part of the sympathetic nervous system (**see** section Extrinsic Innervation and Chemical Codes of Autonomic Neurons). Intracardiac neurons recognized in the sino-atrial area suggest the possibility of the heart containing intrinsic circuits that modulate local activities.

Fishes are the most prolific vertebrate group in terms of biomass and number of species, occupying widely differing aquatic environments, which results in different lifestyles and different demands on the heart. Some fishes even breathe air, and this has resulted in the evolution of circulatory changes as extreme as a double circulatory loop in obligate air-breathing lungfish (Dipnoi) and a partial structural division of the ventricle, by a vertical septum and an atrio-ventricular plug, into a larger right and a smaller left side (**see** section The Gross Morphology and Anatomy of the Heart). Consequently, these evolutionary changes are expected to have resulted in alterations in the intracardiac innervation among fishes, as is the case for the sympathetic and parasympathetic cardiac innervation patterns (**see** section Extrinsic Innervation and Chemical Codes of Autonomic Neurons). While the discoveries concerning intracardiac neurons are still in an early stage, certain patterns have already begun to emerge.

The Gross Morphology and Anatomy of the Heart

The piscine circulation features a single ventricle that pumps blood into the gas exchanger (the gill) and then the systemic circulation (the tissues) without further pumping, unlike other vertebrates. The chambered fish heart is enclosed within a pericardial sac and possesses three layers: endocardium, myocardium, and epicardium. It is often called a 'venous heart' because it only pumps venous blood.

Based on the myocardial architecture, the myogenic heart of fish may be divided into four principal chambers arranged in series: the sinus venosus, the atrium, the ventricle, and the bulbus cordis (cous arteriosus) in elasmobranches and dipnoans, and in teleosts, the bulbus arteriosus (see also **Design and Physiology of the Heart:** Cardiac Anatomy in Fishes).

The sinus venosus is the site of a specialized group of autonomous pacemaker cells in many fish and its major functional role is related to the initiation and control of the heartbeat. The excitation of the heart initiates from these cells, which generate spontaneous action potentials (APs). The AP of the pacemaker cells is modulated by temperature, the autonomic nerves, the purinergic system, and possibly by intracardiac neurons.

The atrium is more richly supplied with cardiac muscle when compared with the sinus venosus. It is an irregularly shaped, large muscular, contractile chamber that is situated dorsal to the ventricle in most of the fish. The atrium consists of two parts: a sinoatrial canal and an atrium proper. The atrium communicates with the ventricle through a tubular structure called the atrio-ventricular funnel. The ventricle is a thick-walled chamber of tubular, sac-like and pyramidal shapes (teleosts), or rounded, sac-like (elasmobranchs) contours. The bulbus arteriosus in teleosts and the bulbus cordis (cartilaginous fish) represent the ventricular-outflow tract, but have important auxiliary functions (**see** section Postganglionic Neurons and Neurotransmitter Localization).

The central circulatory system of ordinary fish is essentially symmetrical in sharp contrast to the asymmetrical vascular arrangements seen in the remainder of the vertebrate groups, including lungfish. With the advent of separate pulmonary drainage vessels as seen in lungfish, the heart becomes polarized such that the left side accommodates pulmonary venous blood, either completely or incompletely separated from the right side which pumps the systemic venous blood to the lungs. In the lungfish (Dipnoi), a single ventricle pumps blood, but the heart is quite different from the usual piscine pattern due to the partial separation of the heart for lung-oxygenated and systemic-deoxygenated blood. The development of the divided heart in lungfish is associated with the special pulmonary vascular circuit having a separate return to the left side of the heart, but coupled in parallel with the systemic or the internal gas-exchange circuit on the arterial side.

In the Polypterid heart, the sinus venosus receives blood from the duct of Cuvier, where blood is collected from the inferior hepatopulmonary vein. The wall of the sinus venosus is rather smooth and a scattering of muscular strands is seen in the myocardial tissue and at the junction between the sinus and the atrium.

Extrinsic Innervation and Chemical Codes of Autonomic Neurons

The autonomic nervous system (ANS) pathways are divided into sympathetic and parasympathetic divisions (see also **Brain and Nervous System:** Autonomic Nervous System of Fishes). The ANS in fish is defined simply as that which originates from the cranium, called ' parasympathetic outflow', and that which originates from the spinal cord, termed ' sympathetic outflow'. Preganglionic cell bodies for the parasympathetic outflow are in the brainstem and those for the sympathetic outflow are in the trunk of the spinal cord. Each system contains neurons that have structural similarity, but differ functionally based on their neurotransmitter complement.

The heart of fishes is innervated by the paired cardiac branches of the vagosympathetic trunk. Like other vertebrates, the heart is under autonomic control. The vagus, at its origin, is parasympathetic but receives postganglionic autonomic fibers from the sympathetic chain in the head region, creating the vagosympathetic trunk. The vagus of all groups of fish has cardiac and visceral rami innervating the heart and gastrointestinal tract, respectively. The paired cardiac rami of the vagus arise from the ventral visceral rami, and, in cartilaginous fish and at least some teleost species, they also receive contributions from the most posterior branchial rami. In both teleosts and cartilaginous fish, the cardiac nerves run in the dorsal wall of the ducts of Cuvier to the sinus venosus, where they break up into a ganglionated plexus, extending only as far as the proximal region of the atrium.

The physiological regulation of the cardiovascular system is remote and local. The remote system maintains a constant blood pressure that allows each tissue to adjust its own perfusion through a local regulation of vascular resistance. Various molecules coming from the vasculature (such as the gases CO, NO, and H_2S) provide a variety of signaling pathways in the homeostatic regulation of the cardiovascular function thereby producing vasoactive paracrine signals (see below).

In fish, peripheral autonomic neurons are often globular or ellipsoid in shape and are generally considered unipolar, bearing a single axon but no dendrites. However, recent studies have demonstrated that most sympathetic neurons in goldfish have dendrites and display a complex morphology.

Over the last 25 years, an increasing number of biologically active substances have been identified as transmitters of autonomic neurons. However, comparative studies on the identity of neurotransmitters are often fragmentary, and available information indicates a similarity in the chemical nature among the transmitters in different vertebrate classes. The classical transmitter substances are adrenaline/noradrenaline (A/NA) and acetylcholine (ACh). All preganglionic autonomic neurons, both sympathetic and parasympathetic, use ACh as their fast excitatory transmitter. The most prominent among other transmitters are the neuropeptides. A distinctive combination of these substances may occur in homologous populations of neurons in different species and form the chemical codes. Autonomic neurons may also contain purines, indoleamines, and nitric oxide synthase (NOS), the enzyme that forms nitric oxide (NO) from L-arginine. Thus, neurons can release more than one transmitter, giving rise to co-transmission which accounts for the type of the target tissue that is innervated. These co-transmitters mediate slow excitatory postsynaptic potentials, facilitating cholinergic transmission.

In many fish tissues, blockade of adrenergic and cholinergic transmission results in residual nonadrenergic noncholinergic (NANC) responses. The majority of NANC neurotransmission is due to the release of neuropeptides, adenosine triphosphate (ATP), and NO. However, NANC transmission is apparently absent in the teleost heart with a vagal stimulation after cholinergic and adrenergic blockade. In contrast, NO may be present. Parasympathetic motor neurons utilize ACh or NO, or both, as nonpeptide transmitters, as well as a wide range of co-transmitter peptides, including vasoactive intestinal peptide (VIP).

Cholinergic neurons are present in numerous autonomic pathways as demonstrated by a vast number of pharmacological, histochemical, immunohistochemical, and physiological studies. Immunostaining of choline acetyltransferase (ChAT) with specific antisera is useful to identify cholinergic neurons. Adrenergic neurons are identified using antibodies to tyrosine hydroxylase (TH).

Postganglionic Neurons and Neurotransmitter Localization

Cardiac nervous tissue is most concentrated in a sino-atrial plexus, corresponding to the pacemaker. This plexus consists of a developed network of nerve fibers and nerve cell bodies. These cells are often isolated or clustered into small groups to form microganglia and are not only distributed in the sino-atrial area, but are scattered along the entire atrial canal up to the atrio-ventricular junction. Synaptic contacts and synaptic specializations of these cells with preganglionic vagal fibers have been seen in several fish species (as detailed below). Besides the axo-somatic relationships between the nerve terminals and the somata of the cardiac neurons, a large component of the nervous tissue in the sino-atrial plexus is represented by the postganglionic nonmyelinated outflow originating from the axons of the intramural cardiac neurons, giving rise to numerous nerve terminals mostly concentrated in the sino-atrial junction, the atrial canal, and the atrio-ventricular funnel.

Ray-Finned Fish, Bichirs

Nerve cell bodies are present in the sinus venosus and the sino-atrial junction in the heart of the bichirs. Single and immunolabeling methods have enabled a mapping of these cells in the heart of *Polypterus bichir bichir*. Several populations of neuronal cell bodies have been distinguished using neurotransmitter immunoreactivity. Acetylcholine esterase (AChE), TH, galanin (GA), substance P (SP), vasoactive intestinal polypeptide (VIP), adenylate cyclase-activating peptide (PACAP), and neuronal nitric oxide synthase (nNOS) all occur in nearly every intracardiac neuronal cell body (**Figure 1**). Nerve varicosities showing nNOS and/or TH immunoreactivity are found in close proximity to some neurons or boutons on the nerve cell soma. The AchE and SP double-labeling method shows that the majority of the nerve cells are labeled with SP antibody. Immunoreactivity to SP is found in the preganglionic axons or boutons associated with these neurons (**Figure 1**). Preganglionic axons showing VIP and PACAP (**Figure 1**) immunoreactivity lie on the soma of numerous nerve cell bodies in the sino-atrial ostium.

Lungfish

Immunoreactivity to GA and SP occurs in the intracardiac neuronal cell bodies of the sinus venosus in *Neoceratodus*. These neurons have immunoreactivity for these neuropeptides in their axons. Some neuronal cell bodies seem to receive GA-positive collaterals from other neurons in the sinus. SP does not coexist with TH. The intracardiac neurons do not contain immunoreactivity to ChAT and nNOS. Therefore, GA should be a candidate for mediating the noncholinergic vagal inhibition of the heart of lungfish.

Teleosts

Distribution of specific types of autonomic nerves and the presence of various transmitter substances have been examined in mullet (*Mugil cephalus*) and Nile catfish

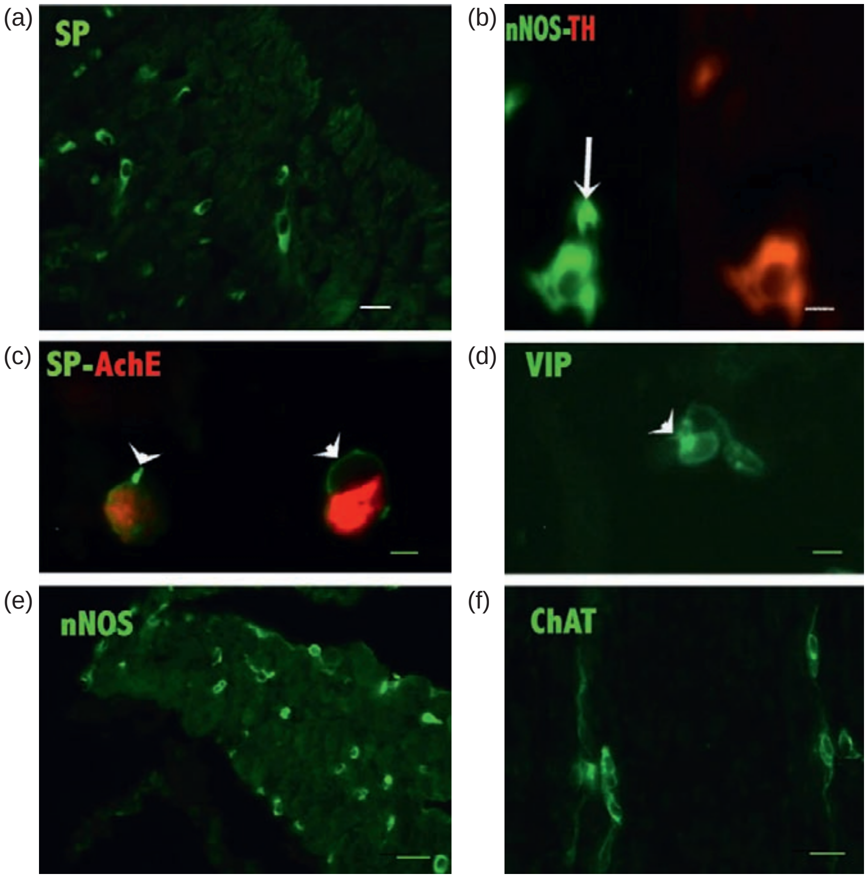


Figure 1 (a) A group of substance P (SP) immunoreactive nerve cell bodies in the sino-atrial tissue of *Polypterus bichir bichir*. (b) Double immunolabeling of neuronal nitric oxide synthase (nNOS) and tyrosine hydroxylase (TH) in the sino-atrial region. Combination of the green and red channel showing the presence of nNOS positive axon (white arrow) lying near the soma of an nNOS positive nerve cell. (c) Double immunolabeling of acetylcholinesterase (AChE) and SP in the sinus venosus showing the presence of SP positive axon (arrowheads) associated with the soma of two nerve cells. (d) VIP immunoreactivity in a nerve cell and axon bearing varicosities near the nerve cell soma. (e) nNOS immunoreactivity of several nerve cell bodies in the sinus venosus of the trout (*O. mykiss*). (f) Unipolar neurons containing immunoreactivity to ChAT in a section of the sinus venosus of the trout (*O. mykiss*), Scale (a–f) = 20 μm.

(*Synodontis nigriventris*). Most intracardiac nerve cell bodies are located in the sinus venosus and in the sino-atrial junction. These are immunoreactive to SP, GA, nNOS, VIP, and PACAP. The presence of a large population of postganglionic nerve cell bodies is demonstrated in sinus venosus using immunolabeling methods with antibodies to nNOS, SP, and ChAT (**Figure 1**). These cells are often intermingled in the nerve plexuses. A nerve plexus consisting of a well-developed network of nerve fibers and nerve cell bodies may possibly correspond to a cardiac pacemaker in the teleosts, but its function in fish cardiac regulation as well as the characterization of the pacemaker tissue are not known.

In view of the antibody reactivity to ChAT and nNOS, cholinergic and nitrergic cell bodies of vagal postganglionic neurons, which are located in an extensive cardiac nerve plexus, may represent a component of the vagal inhibition of the sinus venosus and atria of the fish in general, and should be mediated by noncholinergic mechanisms. However, there have been no pharmacological studies that have clearly demonstrated the presence of inhibitory responses in fish cardiac muscle cells.

In the mullet, the intracardiac neurons form a ganglion consisting of adrenergic and nNOS-immunopositive (nitrergic) nerve cell bodies (**Figure 2**). Double immunolabeling with antibodies against TH and nNOS show immunoreactivity co-localized in these cells. Nerve branches showing TH immunoreactivity enter the sinus venosus. Muscle layers are also innervated by GA and nNOS-immunopositive nerve fibers. Fine varicose substance P-immunopositive fibers are present in the wall of the sinus venosus and the atrium. Bundles of GA-immunopositive nerves are present in the outer compact layer and the spongy layer of ventricular myocardium. Few nerve bundles showing SP-immunoreactivity are found running in the adventitia of the bulbus into the peripheral compact and inner layers of the ventricle.

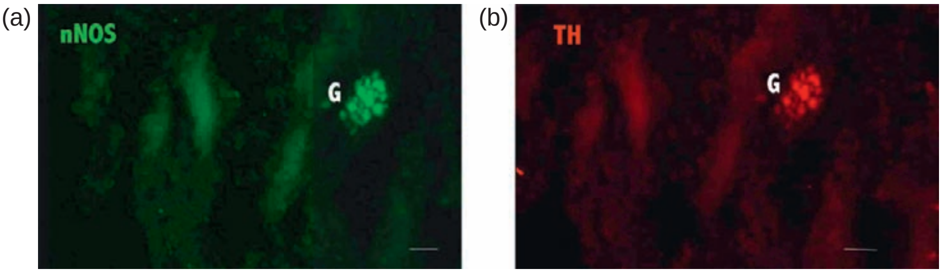


Figure 2 (a, b) Double labeling of nNOS and TH of neurons in a ganglion (G) of sinus venosus in the mullet (*M. cephalus*). Scale = 20 μm.

The compact layer of the myocardium contains coronary blood vessels, which bear a perivascular plexus of TH-immunopositive varicose nerve fibers. In the catfish species (*S. nigriventris*) TH-immunopositive nerve fibers are present in the epicardial coronary vessels.

The Cardiac Pacemaker and Conducting System

A question concerning the lower vertebrate heart is whether it is the site of specialized cardiac pacemaker tissue comparable with that of higher vertebrates. In Gnathostomatous fish, the major functional role of the sinus venosus is related to the initiation and control of the heartbeat. A ring of specialized myocardial cells (sino-atrial node) is situated in the right atrium. An atrio-ventricular node is also placed at the caudal end of the interatrial septum near the coronary sinus and the atrio-ventricular bundle (bundle of His) placed above the interventricular septum. This system is the so-called 'Purkinje system' of birds and mammals. It consists of specialized tissues and their muscle fibers (Purkinje fibers) which are situated sub-endocardially both in the atria and in the ventricle.

There is a general indication that Purkinje fibers similar to those of higher vertebrates are absent in fish hearts. While fish hearts beat in isolation and without neurogenic input (a myogenic heartbeat) and pacemaker potentials have been recorded from cells in the sinus venosus of fish (**see** section The Gross Morphology and Anatomy of the Heart), there is no unanimity among investigators regarding the occurrence of nodal tissues in fish. They have also found an intimate nerve connection at the sino-atrial junction. A muscular continuity in the various chambers of the heart without an interruption by nodes is also a key characteristic. The presence of histologically specialized muscles is also reported. In the eel (*Anguilla vulgaris*) and lungfish (*Protopterus aethiopicus*), specialized pacemaker cells appear at the junction of the sinus venosus and Cuverian ducts. These cells are also located in the atrium of the plaice and the brown bullhead (*Ictalurus nebulosus*), as well as in the atrio-ventricular region. Fish studies have produced evidence that the contraction wave arising from the sino-atrial region successively invades the atrium, the atrio-ventricular funnel, and the ventricular myocardium. A functional equivalent of the Purkinje system has been postulated for the ventricular trabeculae in the zebrafish (*Danio rerio*). The cardiac conducting system of the heart in fish is thought to be neither purely myogenic nor entirely neurogenic, but a complex combination of the two.

Histological studies have concluded that in the sino-atrial region of fish heart, every muscle cell is supplied by at least one nerve profile, thus confirming the role of this part of the heart in initiating and controlling the heartbeats. The innervation of the muscle cells also displays a peculiar arrangement. In addition to the direct neuromuscular contacts, transmission occurs via an intermediate cell interposed between the postganglionic axon and the muscle cell. This type of cell, the so-called 'internucial cell,' plays a key role in the distribution and modulation of the autonomic control of the myocardial cells. The internucial cell is only reported in the nodal tissue of the loach, but not described in other fish species.

Besides the clear recognition of nodal tissue and a specialized network of conduction cells in the fish heart, neurohumoral regulation of such tissues is not clearly understood. Future studies must be addressed to verify whether paracrine substances contained in the ganglion cells contribute to the intrinsic regulation of the heart.

Conclusions

To date, there is very little information on the functions of transmitters and co-transmitters in autonomic cardiac neurotransmission in fish. Besides the inhibitory vagal control and the adrenergic control of the heart, cardiac NANC innervation could be a feature of fish heart. Future studies are needed in order to investigate additional types of neurons involved in the cardiac control as well as the roles of neuropeptides and other putative transmitters in the intracardiac neurons and nerves that directly innervate the pacemaker region.

See also: **Air-Breathing Fishes:** Respiratory Adaptations for Air-Breathing Fishes. **Brain and Nervous System:** Autonomic Nervous System of Fishes; Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern. **Design and Physiology of the Heart:** Cardiac Anatomy in Fishes; Cellular Ultrastructure of Cardiac Cells in Fishes.

Further Reading

Blessing B and Gibbins A (2008) Autonomic nervous system. *Scholarpedia* 3(7): 2787.

Davie PS and Farrell AP (1991) The coronary and luminal circulations of the myocardium of fishes. *Canadian Journal of Zoology* 69: 1993–2001.

Davies PJ, Donald JA, and Campbell G (1994) The distribution and colocalization of neuropeptides in fish cardiac neurons. *Journal of the Autonomic Nervous System* 46: 261–272.

Donald J (1998) Autonomic nervous system. In: Evans DH (ed.) *The Physiology of Fishes*, 2nd edn., pp. 407–439. Boca Raton, FL: CRC Press.

Farrell AP (2007) Cardiovascular systems in primitive fishes. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Primitive Fishes. Fish Physiology*, vol. 26, pp. 53–120. San Diego, CA: Academic Press.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds) *The Cardiovascular System. Fish Physiology,* vol. 12, part A, pp. 1–73. San Diego, CA: Academic Press.

Funakoshi K and Nakano M (2007) The sympathetic nervous system of anamniotes. *Brain, Behavior and Evolution* 69: 105–113.

Gibbins I (1994) Comparative anatomy and evolution of the autonomic nervous system. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, vol. 4, pp. 1–67. Chur: Harwood Academic Publishers.

Grimes AC and Kirby ML (2009) The outflow tract of the heart in fishes: Anatomy, genes and evolution. *Journal of Fish Biology* 74: 983–1036.

Holmgren S and Jensen J (1994) Comparative aspects of the biochemical identity of neurotransmitters of autonomic neurons. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, vol. 4, pp. 69–95. Chur: Harwood Academic Publishers.

Kapoor BG and Khanna B (2004) *Ichthyology Handbook*. Berlin, Heidelberg, New York: Springer.

Kumar S (1997) The structure, function and autonomic innervation of the fish heart. In: Singh BR (ed.) *Advances in Fish Research*, vol. 2, pp pp. 9–27. Delhi: Narosa Publishing House.

Laurent P, Holmgren S, and Nilsson S (1983) Nervous and humoral control of the fish heart: Structure and function. *Comparative Biochemistry and Physiology* 76A: 525–542.

Morris JL and Nilsson S (1994) The circulatory system. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, vol. 4, pp. 193–246. Chur: Harwood Academic Publishers.

Nilsson S and Holmgren S (1992) Autonomic nerve function and cardiovascular control in lungfish. In: Wood SC, Weber RE, Hargens AR, and Millard RW (eds.) *Physiological Adaptations in Vertebrates*, pp. 377–395. New York: Marcel Dekker.

Nilsson S and Holmgren S (1992) Cardiovascular control by purines, 5-hydroxytryptamine and neuropeptides. In: Randall DJ and Farrell AP (eds.) *The Cardiovascular System. Fish Physiology*, vol. 12B, pp. 301–341. New York: Academic Press.

Olson K (1998) The cardiovascular system. In: Evans DH (ed.) *The Physiology of Fishes*, 2nd edn., pp. 129–154. Boca Raton: FL: CRC Press.

Sedmera D, Reckova M, DeAmeida A, *et al.* (2003) Functional and morphological evidence for a ventricular conduction system in zebrafish and *Xenopus* hearts. *American Journal of Physiology – Heart and Circulatory Physiology* 284: H1152–H1160.

Vornanen M, Shiels HA, and Farrell AP (2002) Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology Part A* 132: 827–846.

Zaccone G, Mauceri A, Maisano M, and Fasulo S (2009) Innervation of lung and heart in the ray-finned fish, bichirs. *Acta Histochemica* 111(3): 217–229.

Accessory Hearts in Fishes

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Portal Heart of Hagfishes
The Branchial Pumps
Caudal Pumps
Summary
Further Reading

Glossary

Blood pressure The potential energy of blood within the circulatory system. Blood pressure varies from location to location with the circulation (highest in the central arteries and lowest in the veins), as well as within the cardiac cycle (highest during contraction and lowest during relaxation).

Branchial heart The heart that generates the arterial blood pressure in fishes, and so termed because arterial blood flows first to the gills.

Kinetic energy Energy associated with movement; it is the product of mass and velocity of an object.

Myogenic Originating in the muscle. In the case of a heart, a muscle contraction that does not require outside stimulation.

Potential energy Energy associated with height above a reference point; it is the product of mass, height, and the gravitational constant, *g*.

Vis-a-fronte A term for a supplementary cardiac filling mechanism that uses energy directly from ventricular contraction; literally the force from the front.

Vis-a-tergo A term for a cardiac filling mechanism that uses energy contained in venous blood; literally the force from behind.

Introduction

Other articles have dealt with the return of blood from the venous system and from the secondary circulation back to the branchial heart, where blood is repressurized to create a continuous circulation of blood (**see also Circulation:** Circulatory System Design: Roles and Principles, **Design and Physiology of the Heart:** Physiology of Cardiac Pumping, **Design and Physiology of Arteries and Veins:** Physiology of Capacitance Vessels, and **Design and Physiology of Capillaries and Secondary Circulation:** Secondary Circulation and Lymphatic Anatomy). These articles also acknowledged that fishes can possess an accessory heart, which helps blood return toward the branchial heart. The need for accessory hearts in fishes is in part due to low arterial blood pressures, which leave little potential energy in venous blood once blood has passed through the capillaries (i.e., the energy responsible for *vis-a-tergo* cardiac filling). Indeed, some fishes under certain physiological states have a subambient venous blood (negative potential energy relative to the heart). In these situations, the kinetic energy in the blood and suction by the heart (i.e., *vis-a-fronte* cardiac filling; **see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping) ensure that blood actually flows into the heart.

This status quo does not mean that the venous blood cannot benefit from a boost in its for any toxicants that may have been taken potential energy, which can be provided by an accessory heart. This is especially true for the secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation:** Secondary Circulation and Lymphatic Anatomy), which has low arterial pressures in the first place. Therefore, it is hardly surprising that fishes such as eels, with their long tails, and selachian sharks, with their long fins, and hagfish, with their inherently low arterial blood pressure, provide good examples of the accessory hearts found among fishes.

Fishes evolved a variety of structures that act as accessory hearts. In this regard, a heart can be generally defined as a structure that pumps (adds energy to) blood. Accessory hearts are always found on the venous side of the circulation, which is where the lowest blood pressures exist (**see also Circulation:** Circulatory System Design: Roles and Principles). Therefore, accessory hearts could easily be described as auxillary venous pumps that act to steepen the venous blood pressure gradient between capillaries and the branchial heart. A common theme in their design is the presence of valved chambers that are compressed by skeletal muscle, sometimes against skeletal elements.

Among the accessory hearts discovered to date, the portal heart and caudal heart of hagfishes are true hearts in that their associated muscles only power the pump. The other accessory hearts might be better termed as propulsors, because they are powered incidentally by muscles that serve another function such as locomotion and gill ventilation: these include the branchial pump, caudal hearts in other fishes, and the cardinal heart of hagfishes. This article describes five types of accessory hearts. Others may still be discovered.

Portal Heart of Hagfishes

All blood sent to the vertebrate intestine returns to the heart through the liver (**see also Design and Physiology of Arteries and Veins:** Anatomical Pathways and Patterns). This arrangement permits absorbed nutrients to be immediately processed by the liver before entering the general circulation for distribution. Likewise, the liver can act as a critical metabolic barrier for any toxicants that may have been taken by the gut along with the nutrients.

The blood vessels connecting the intestinal circulation to the liver are collectively termed the hepatic portal system, with the major blood vessel being the hepatic portal vein. This vascular arrangement represents one of the few exceptions where two capillary beds are arranged in series without an intervening heart (**see also Circulation:** Circulatory System Design: Roles and Principles). Thus, the pressure remaining in blood after it passes through the intestinal capillaries must carry it through a second, hepatic, capillary bed. The only known exception to this rule is the hepatic portal vein of hagfishes. This situation may not come as a surprise since the arterial blood pressure in hagfishes approaches those that are found in the veins of some animals! The portal heart of hagfish is exceptional in another regard; it is the only known example of cardiac muscle being located outside of the pericardium of any vertebrate.

The portal heart has the shape and structure similar to that of the atrium of the branchial heart (**see also Design and Physiology of the Heart:** Cardiac Anatomy in Fishes). It is comprised of true cardiac muscle that has a trabeculated form and is valved on its inflow and outflow vessels. Being composed of cardiac muscle, it is myogenic and generates its own electrocardiogram when the heart beats (**see also Design and Physiology of the Heart:** Physiology of Cardiac Pumping and Action Potential of the Fish Heart). The electric waves are termed P-wave and T-wave to reflect the contraction and relaxation, respectively. The blood pressure generated by the portal heart is not large (0.10–0.15 kPa), but then similar pressures are generated by the atrium. The ventricle of hagfishes generates arterial pressures that are only 10-times higher.

The Branchial Pumps

The muscles used for gill ventilation can also benefit venous blood flow, that is, that act as a branchial pump.

The Cardinal Heart of Hagfishes

The cardinal heart of hagfishes is not a true heart, as is the case for the portal heart. Instead, the cardinal heart relies on supplementary use of muscles used for gill ventilation and is therefore termed a branchial pump.

Water is propelled through the gill pouches of hagfishes by a velum (**see also Hagfishes and Lamprey:** Hagfishes). Immediately above the velar chamber is the hyoid plate, with an extensive blood sinus (the hypophysio-velar sinus) that is sandwiched between the plate and the more dorsal olfactory organ. Red skeletal muscles inserted onto the hyoid plate move the plate up and down, thereby moving the velum. These ventilatory movements also compress the sinus during each ventilation cycle.

The sinus receives blood from the subcutaneous sinus through a valved vessel. The sinus empties into the anterior cardinal sinus through valved ostium. Therefore, compressions of the sinus result in a one-way flow of blood from the subcutaneous sinus toward the heart.

Branchial Pumps in Other Fishes

The anterior cardinal sinus receives blood from the valved branchial veins and opens into the ductus Cuvier, where there is an ostial valve. These vessels are easily compressed by external water pressure, as generated during each gill ventilation cycle. Furthermore, the valve arrangement is such that compressions direct the resultant blood flow toward the heart. Indeed, ventilation has been observed to expel blood passed these valves. Thus, each ventilation cycle has the potential to propel some of the venous blood from the head region back to the heart. Compression forces would presumably increase with more forceful ventilations when stroke volume increases, say during hypoxia. The synchrony known to occur between the cardiac and ventilation cycles of fishes (**see also Control of Respiration:** Generation of the Respiratory Rhythm in Fish) could be useful in synchronizing venous return with cardiac ejection.

Caudal Pumps

There are three evolutionarily distinct and anatomically different caudal pumps that have been discovered so far in fishes. Their role as an auxillary pump is similar and all are powered by the skeletal muscle.

Caudal Heart of the Hagfish

Within the tail region of hagfish are two, thin-walled, lateral chambers. Each one drains into the caudal vein through a valved ostium. Input into both chambers is through several valved ostia, which provide bilateral connections to the marginal vein and veins from the subcutaneous sinus. This accessory heart, therefore, helps propel blood from both the extensive subcutaneous sinuses and the venous system at the end of the tail.

Two thin sheets of skeletal muscle are arranged on either side of the chambers, separated by part of a median fin skeleton and inserted onto vertebrae. These muscles alternately contract, compressing one chamber and then the other. This results in two pressure pulses in the caudal vein. Since the muscles serve no other function, it is reasonable to refer to the caudal heart of hagfish as a true heart, but one that is powered by skeletal muscle rather than cardiac muscle in the case of the portal heart.

The caudal heart is not continuously active. For example, it starts beating after swimming activity, but contractions gradually fade away with time. Spinal nerves activate the skeletal muscle associated with the caudal heart, which suggests central control. However, reflex loops have not been eliminated as a possible source of activation.

The caudal heart and the cardinal heart of hagfishes are clearly not lymph hearts (like those that are prominent in amphibians). They are connected to the subcutaneous sinuses, which contain plenty of red blood cells (**see also Hagfishes and Lamprey:** Hagfishes). The question of whether they are part of the secondary circulation is still being debated. Some consider the subcutaneous sinuses in hagfishes as a functional forerunner to the secondary circulation of modern fishes (**see also Design and Physiology of Capillaries and Secondary Circulation:** Secondary Circulation and Lymphatic Anatomy). Although the input to the cardinal heart makes such a distinction possible, the situation for the caudal heart is a little more complex.

Caudal Heart in Sharks

The dorsal, lateral, and ventral cutaneous veins of elasmobranchs and teleosts may extend into the tail fin to supply a marginal vein. In the case of sharks, such as the Port Jackson shark (*Heterodontus portusjacksoni*), the carpet shark (*Cephaloscyllium isabella*), and the smooth hound (*Mustelus antarcticus*), the marginal vein passes around the tail fin and collects into threadlike, sinuses that lie alongside the ventral fin skeleton and beneath the caudal vein. Although the exact structure of the sinus varies among species, it always contains numerous, short, valved, ventral vessels that connect to the caudal vein. These sinuses act as caudal hearts (also termed a haemal arch pump).

The caudal heart is compressed by contractions of radial skeletal muscles that insert on the fin spines and, in doing so, extend over the sinus. While activation of these radial muscles by spinal nerves is similar to that in the caudal heart of hagfish, the difference is that these muscles, depending on the species, also produce tail movements during swimming or rippling waves along the fins of stationary sharks. Therefore, compression of the caudal heart is an ancillary use of these muscles.

Caudal Heart in Eels

The last vertebra of an eel has two thin-walled chambers that communicate centrally through a valved ostium and lie ventral between fin rays. The right chamber receives the inflow through a valved connection with the lateral cutaneous vein. The left chamber provides the only outflow through a valved ostium to the caudal vein.

Contraction of skeletal muscles associated with fin spines alternately compresses each chamber against the skeleton, with the right chamber emptying into the left chamber and the left chamber emptying into the caudal vein. These muscle contractions are controlled by spinal nerves, but the muscles are so tiny that fin movements are not always visible. Furthermore, in the case of eels, the caudal heart lies directly above the urophysis, an endocrine gland in the tail of fishes. Urotensin 2, a hormone released by the urophysis, increases the beating rate of the caudal heart in eels.

Caudal heart structures, with a slightly different anatomy to that of eels, are reported for other fishes such as tench (*Tinca tinca*) but without any study of their physiology. Caudal hearts are absent among the Acanthopterygii, the spiny-finned fishes that include the wrasses, the perch, and the flounders.

Summary

Fishes from hagfishes through to eels have evolved structures that act as accessory hearts. These auxillary venous pumps help move blood from the venous system and the secondary circulation toward the branchial heart. Beyond the unique and myogenic portal heart of hagfishes, a valved chamber that is compressed against skeletal elements by the skeletal muscle is a common feature across fishes. However, not all fishes need an accessory heart.

See also: **Circulation:** Circulatory System Design: Roles and Principles. **Control of Respiration:** Generation of the Respiratory Rhythm in Fish. **Design and Physiology of Arteries and Veins:** Anatomical Pathways and Patterns;

Physiology of Capacitance Vessels. **Design and Physiology of Capillaries and Secondary Circulation:** Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of the Heart:** Action Potential of the Fish Heart; Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping. **Hagfishes and Lamprey:** Hagfishes.

Further Reading

Birch MP, Carre CG, and Satchell GH (1969) Venous return in the trunk of the Port Jackson shark, *Heterodontus portusjacksoni*. *Journal of Zoology London* 159: 31–49.

Bushnell PG, Jones DR, and Farrell AP (1992) The arterials system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A Cardiovascular System*, pp. 89–139. San Diego, CA: Academic Press.

Davie PS (1982) Changes in the vascular and extravascular volumes of eel muscle in response to catecholamines: The function of the caudal lymphatic heart. *Journal of Experimental Biology* 96: 195–208.

Davie PS, Forster ME, Davison W, and Satchell GH (1987) Cardiac function in the New Zealand hagfish, *Epatretus cirrhatus*. *Physiological Zoology* 60: 233–240.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A The Cardiovascular System*, pp. 1–88. San Diego, CA: Academic Press.

Mayer P (1888) Uber Eigentumlichkeiten in den Kreislauforganen Selachiern. Mitt. *Zoology Station Neapel* 8: 307–373.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology*, 3rd edn., pp.119–152. Boca Raton, FL: CRC Press.

Satchell GH (1971) *Circulation in Fish,* pp. 1–131. Cambridge: Cambridge University Press.

Satchell GH (1984) On the caudal heart of Myxine (Myxinoidea: Cyclostomata). *Acta Zoologica Stockholm* 65: 125–133.

Satchell GH (1991) *Physiology and Form of Fish Circulation,* pp. 1–235. New York: Cambridge University Press.

Vogel WOP (1985) The caudal heart of fish: Not a lymph heart. *Acta Anatomica* 121: 41–45.

The Coronary Circulation

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Anatomy
Physiology
Further Reading

Glossary

Cardiac circulation The venous blood pumped by the heart and contained in the lumen of the cardiac chambers. The cardiac circulation, being venous blood, has variable levels of oxygenation, unlike the well-oxygenated blood of the coronary circulation. In the absence of a coronary circulation, the muscular trabeculae of the spongy myocardium derive their oxygen from the venous blood contained in the lumen of the cardiac chambers. The terms luminal and lacunary oxygen supply are also used in this regard.

Cardiac oxygen demand The amount of oxygen consumed by the heart per unit time. The cardiac oxygen consumption rate may be scaled to either cardiac mass or body mass to permit comparisons among hearts or animals of different size. Cardiac oxygen demand is primarily determined by the amount of work performed by the heart per unit time, which is termed myocardial power output.

Coronary blood flow The blood flowing through the main coronary artery. The flow rate per unit time may be scaled to either cardiac mass or body mass to permit comparisons among hearts or animals of different size.

Coronary circulation A specialized system of blood vessels dedicated to the heart, which normally delivers fully oxygenated blood to at least a portion of the cardiac muscle. A coronary blood vessel refers loosely to any vessel in this specialized circulation, from the coronary artery, through the coronary capillaries to the coronary veins.

Coronary vascular tone The contractile state of coronary vascular smooth muscle. Vascular tone is influenced by various vasoactive hormones, neurotransmitters, and paracrine substances that contract and relax vascular smooth muscle cells. This tonus determines the resistance to blood flow in the coronary circulation. A vasodilatory reserve would mean that the resistance vessels are at least partially constricted and that they can then relax to decrease coronary vascular resistance, thus increasing coronary blood flow.

Myocardium A general term for the cardiac muscle of the heart, which comprises several subtypes, including the compact myocardium and the spongy myocardium of fishes.

Threshold P_{vO_2} A threshold level for venous oxygen partial pressure (P_{vO_2}) below which the trabeculae of the spongy myocardium become hypoxic. Since oxygen diffuses into the trabeculae from the venous blood in the lumen of the heart, P_{vO_2} sets the driving force for oxygen diffusion, whereas the thickness of a trabecula sets the diffusion distance, and the workload of the heart sets the oxygen demand.

Trabeculae A bundle of cardiac muscle fibers that is characteristic of the spongy myocardium of fishes and other lower vertebrates.

Introduction

All muscles, including those of the heart (the myocardium), generate external mechanical work using adenosine triphosphate (ATP) when the muscles contract. Hearts, therefore, need a reliable and sustained oxygen supply to regenerate ATP. The adult mammalian heart has a specialized and dedicated system of blood vessels that deliver fully oxygenated blood to almost the entire myocardium (i.e., a coronary circulation). This circulation is essential for sustained cardiac function because without its coronary oxygen supply the mammalian myocardium dies within minutes. However, fishes have various different arrangements to supply oxygen to the heart that are more analogous to the neonatal mammalian heart. Thus, these various arrangements can be appreciated by considering when and why the coronary circulation develops during mammalian fetal growth.

Early in fetal development, the heart is small and thin-walled; hence, a complex coronary capillary network is not needed for a heart that has a low oxygen demand. Instead, the heart derives oxygen from the venous blood that is being pumped by the heart (oxygen that is contained in the lumen of the heart). The rate of oxygen diffusion from luminal blood into the myocardium is enhanced because the fetal blood is oxygenated by the placenta. As the neonatal heart grows, its walls thicken and a coronary capillary network develops to avoid large diffusion distances for oxygen. Initially, the coronary circulation is associated with only the outer layers of myocardium, but over time it expands to almost the entire heart. Thus, during fetal development, there is a transition from reliance on a luminal oxygen supply to dependence on a coronary oxygen supply.

The hearts of most fish species do not have a coronary circulation and, like in neonates, derive oxygen from the venous blood contained in the lumen of the heart. For those fishes that have a coronary circulation, only a portion of the cardiac myocardium is invested with coronary capillaries. Therefore, fish hearts have either a single (luminal) (**Figure 1(a)**) or a dual (coronary and luminal) myocardial oxygen supply (**Figure 1(b)**). The analogy between fish and fetal hearts breaks down in two important respects. First, the dependence of fish on the coronary circulation is never as great as that seen in mammals. Second, the fish heart pumps oxygen-poor venous blood (**Figure 1(a)**). The term cardiac circulation applies to the venous blood that is pumped by the heart and this venous blood provides oxygen to some or all of the cardiac muscle.

Anatomy

Coronary Circulation in Relation to Ventricular Architecture

As noted in the article on cardiac anatomy, (**see Design and Physiology of the Heart**: Cardiac Anatomy in Fishes) the architecture of ventricular muscle has two morphological arrangements: compact myocardium and spongy myocardium. The outer compact myocardium (also termed compacta) depends on the coronary circulation for its oxygen supply. The inner spongy myocardium (also termed spongiosa) depends primarily on the cardiac circulation for its oxygen supply. The mesh-like arrangement of muscular trabeculae for the spongy myocardium (**Figure 2(a)**) creates a large surface area and small diffusion distances for oxygen exchange.

The compact myocardium varies in thickness among species and with fish development. Consequently, the proportion of compact myocardium acts as an index of the heart's dependence on its coronary circulation. The proportion of compact myocardium is estimated either from histological analysis, or by peeling off and weighing the compact myocardium (**Figure 2(b)**). The compact myocardium is usually expressed as a percentage of ventricular mass.

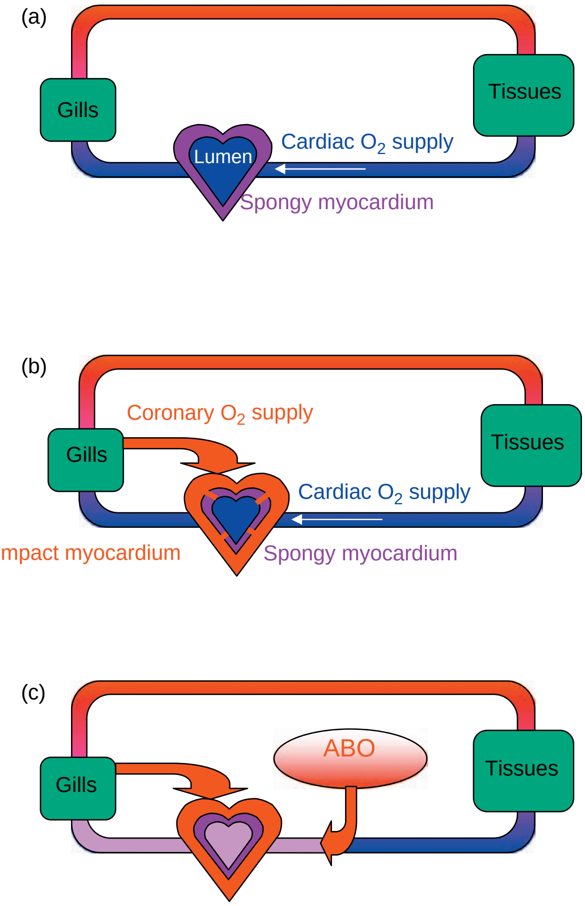


Figure 1 Schematic representation of the circulatory systems, blood oxygenation status (red = fully oxygenated blood; blue = deoxygenated (venous) blood), and composition of the ventricle for (a) fishes with only spongy myocardium, a cardiac circulation, and no coronary circulation, (b) fishes with a coronary circulation to an outer compact myocardium, and (c) air-breathing fishes. Reproduced from Farrell AP, Eliason E, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835-851.

As in mammals, the percentage of compact myocardium in fishes also increases with cardiac growth (**Figure 3**), but never to the same extent. For most fish species, the proportion of compact myocardium is typically <50%, but can reach a high of over 60% in athletic tunas and a low of 5% in primitive ratfishes. Therefore, for the majority of fishes, the coronary circulation is probably best described as a supplement to the cardiac circulation in terms of overall cardiac oxygen supply.

Fishes That Have a Coronary Circulation

Phylogeny, physiology, and environment all seem to have played prominent roles in the evolution of coronary circulation in fishes. The coronary circulation was

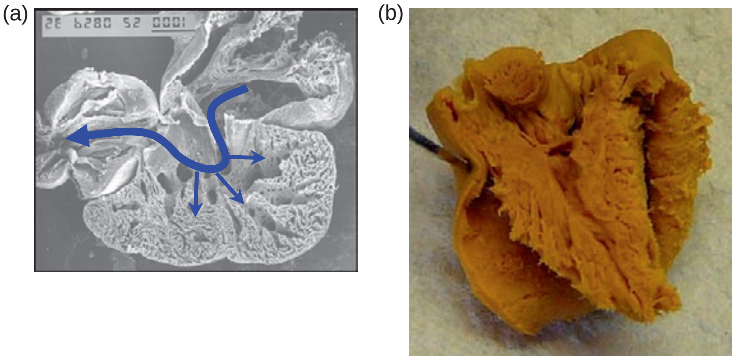


Figure 2 Photographs of preserved ventricles longitudinally sectioned to reveal the wall architecture. (a) A longitudinal section through the heart of the air-breathing catfish, *Heteropneustes fossilis*, to illustrate the flow of blood from the atrium through the ventricle to the bulbus arteriosus and the trabeculae of a type I ventricle. (b) A section through the outer compact and inner spongy myocardium of a salmon, with the compact layer partially peeled away from the spongy layer. (a) Adapted from Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp.119–152. Baton Rouge, LA: CRC Press. (b) Reproduced from Farrell AP, Eliason E, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835–851.

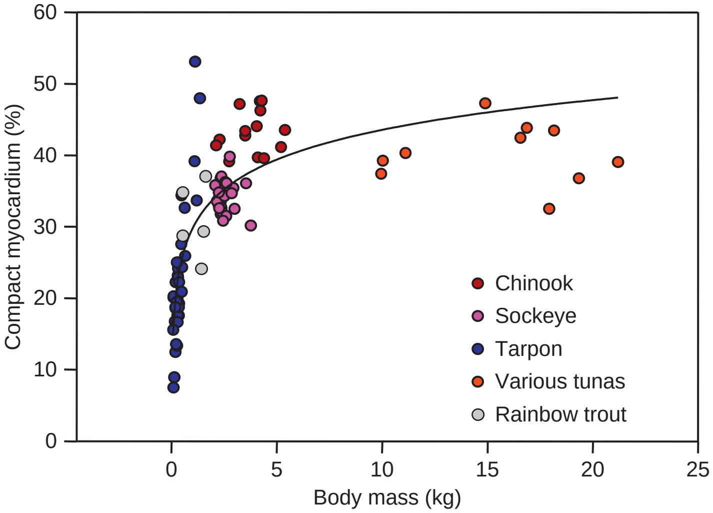


Figure 3 The percentage of compact myocardium in a variety of fish species to illustrate the increase in compact myocardium with fish growth and the upper limit for the proportion of compact myocardium even in large tunas. Adapted from Farrell AP, Eliason E, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835-851.

likely not present when the chambered heart first appeared in vertebrates because extant representatives of the most primitive fishes (hagfishes and lampreys) do not possess a coronary circulation. However, all elasmobranchs examined to date, as well as most other primitive fishes, including sturgeon, holosteans, and lungfishes, possess a coronary circulation. This phylogenetic distribution of the coronary circulation firmly entrenches the idea that the coronary circulation is an old morphological adaptation associated with the vertebrate heart and that the advent of a coronary circulation likely postdates the cyclostomes.

The coronary circulation disappeared repeatedly among teleost fish lineages. Indeed, potentially 50% of all extant vertebrate species may lack a coronary circulation, since ~50% of all species of vertebrates belonging to the Teleostei, and as many as 80% of teleost species lack a coronary circulation. However, an in-depth understanding of the exact phylogenetic distribution of the coronary circulation in teleosts is still lacking.

Across all teleost fishes, it appears that those that have a coronary circulation are either highly athletic (e.g., salmonids and tunas) or tolerant of hypoxic environments (e.g., freshwater eels). The most athletic fishes have the greatest dependence (highest percentage of compact myocardium) on the coronary circulation. Possible explanations are provided below.

What Percentage of the Fish Heart Is Supplied by the Coronary Circulation?

From the perspective of myocardial oxygen supply, fish hearts have four major architectural arrangements. The classification system is as follows:

The 'type I' fish heart has entirely spongy myocardium and a cardiac circulation. Atlantic cod (*Gadus morhua*) and members of the sculpins family are examples of fish with type I hearts. A subtype, 'type Ib', differs in that epicardial coronary vessels are present, but these vessels do not penetrate the myocardium. Plaice (*Pleuronectes family*) is an example of a fish with type Ib heart.

The 'type II' heart has an outer compact myocardium separated from the spongy myocardium by a layer of connective tissue. The coronary vessels in the compact myocardium do not penetrate the spongy myocardium. The coronary veins drain into the atrium near the atrioventricular region (**Figure 4**(**c**)). Rainbow trout (*Oncohynchus mykiss*) and other salmonids are examples of fish with type II hearts.

The 'type III' and 'type IV' fish hearts differ from the type II hearts in that the connective tissue layer is lacking and coronary vessels penetrate the spongy myocardium. Trabeculae appear to have a single, centrally located coronary vessel that empties into the ventricle, suggesting an arrangement analogous to the Thebesian vessels of mammalian hearts. Type III hearts have a lower percentage of compact myocardium (an arbitrary value of <30% of ventricular mass) than type IV hearts. Most sharks and rays are examples of fish with type III hearts. The type IV heart has >30% compact myocardium and known examples are limited to tunas and lamnid sharks. The atrium of type IV hearts of tunas and lamnid sharks also has a coronary circulation, possibly related to the increased atrial wall thickness and diffusion distances of these large fishes.

The coronary circulation also supplies the cardiac muscle associated with the conus arteriosus of primitive fishes including elasmobranchs. The well-developed conal circulation of sharks is less obvious for primitive fishes because the conus arteriosus is often reduced in size.

The coronary vasculature has a dense arrangement, as might be expected for a working muscle, but the regional distribution of coronary vessels within the fish ventricle has received little attention. In rainbow trout, it is suggested that there is one capillary per myocyte fiber.

To summarize, the ventricle of all fish species contains spongy myocardium and this dominant muscle type lacks a coronary circulation in most fish species. Coronary capillaries are always found in compact myocardium. However, complicating these simple arrangements are a limited number of fish species with coronaries in the spongy myocardium and those with epicardial, nonpenetrating coronary vessels.

Origin and Routing of the Coronary Arteries

The coronary artery represents the first branch of the aorta; hence, in mammals the heart is the first organ that receives oxygenated blood. The coronary circulation in fish has an analogous origin in that it represents the first opportunity to route oxygenated blood back to the heart. However, this necessitates a post-gill origin, which makes the origin a little more distant from that of the mammalian heart (**Figure 1**(**b**)) Of course in fish without a coronary circulation, the heart then becomes the last organ to receive oxygen from the circulatory system!

For many fishes, the coronary circulation originates from the efferent branchial arteries in the gills, and it is termed either the cranial hypobranchial or anterior coronary supply. Bilateral vessels carry the oxygenated blood retrograde toward the ventral aorta in gill arches 1, 2, or 3, depending on the species. These vessels unite on the midline of the isthmus often to form a single vessel, commonly termed the hypobranchial artery. The coronary artery forms as a branch of the hypobranchial artery, anterior to the pericardium, which goes on to supply the pectoral musculature.

Within the pericardium, the coronary artery joins the surface of the bulbus arteriosus and may bifurcate caudally to form several arteries en route toward the ventricle (**Figure 4**(**a**)). Alternatively, several coronary arteries may join the conus arteriosus of elasmobranchs, where circumferentially arteries supply the compact myocardium of the conus arteriosus en route toward the ventricle.

A few fish groups (e.g., rays, eels, and marlin) have an additional posterior or caudal coronary supply. This supply is via the coracoid artery, which has its origin on the dorsal aorta posterior to the heart and then passes through the pericardio-peritoneal wall to reach the ventricle. The relative roles of the anterior and posterior coronary supply routes have not been studied.

Superficial coronary arteries are usually visible on the surface of the ventricle (**Figure 4**(**b**)). Similarly, superficial coronary veins are also visible draining toward the atrioventricular junction, where the coronary circulation drains into the atrium. These large distribution vessels, as well as the extensive capillary network in the compact myocardium of rainbow trout, are easily visualized with plastic corrosion casts of the coronary circulation (**Figure 4**(**c**)).

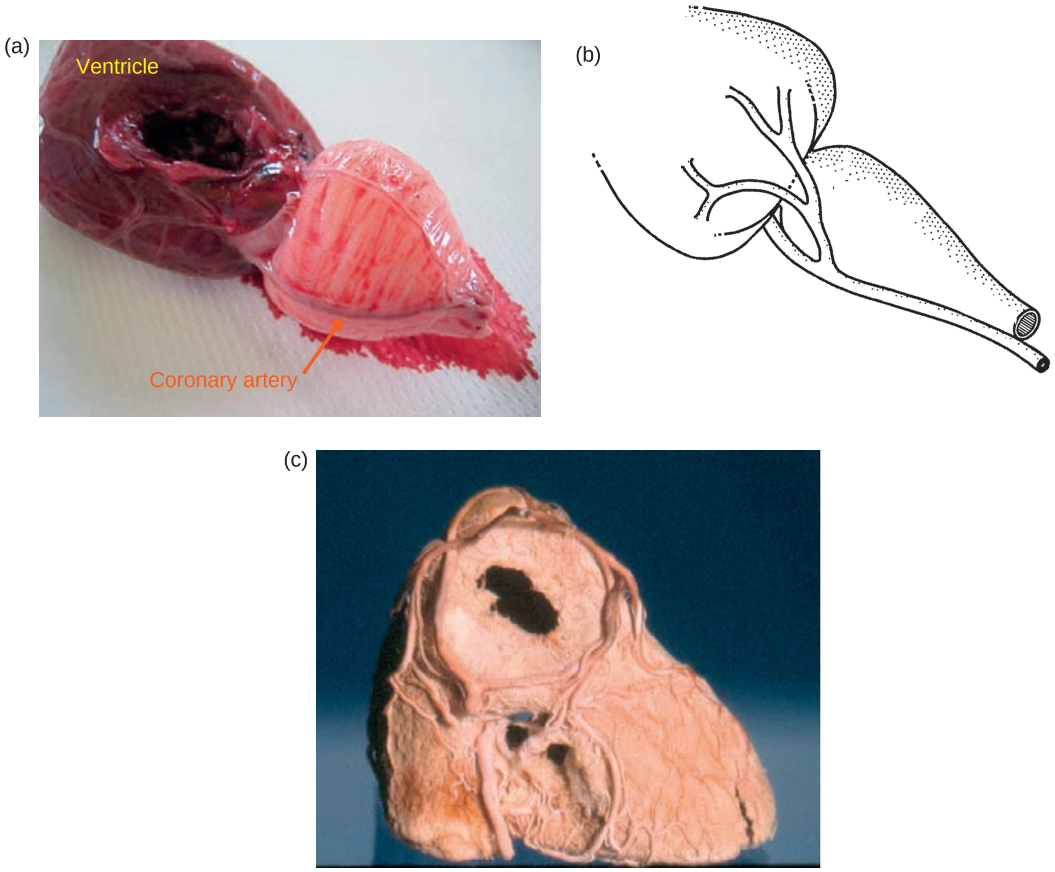


Figure 4 (a) Photograph of the superficial coronary vessels on the ventral aorta and the ventricle of a tarpon. (b) A schematic representation of the arrangement of the coronary artery on the bulbus arteriosus and ventricle. (c) A vascular cast of the coronary vessels of a salmon with all tissue digested away leaving only the vessels visible. The upper opening is the ventricular–bulbar canal and the lower opening is the atrioventricular canal. (a) Courtesy of Dr. R. Seymour. (b) Courtesy of Dr. M. Axelsson.

Histology of the Coronary Artery

The histological structure of the fish coronary artery is similar to that of the mammalian coronary artery and other arteries. The main tissue layers, from inside to outside, are endothelium, internal elastic membrane or lamina, the media consisting of circular or spirally arrayed vascular smooth muscle and elastic fibers in the interstitium, and an outer adventitial layer consisting largely of connective tissue. The media of the fish coronary artery is considerably thinner than in mammals. This reduced amount of vascular smooth muscle likely reflects the lower coronary arterial blood pressure (and hence vessel wall tension) in fish.

Coronary arteriosclerotic lesions develop in the main artery of salmon. These lesions increase appreciably in their prevalence and severity as salmon grow and mature. The coronary lesions in salmon are primarily composed of vascular smooth muscle and a disrupted elastic lamina, and lack the fatty deposits that are typical of atherosclerotic plaques in human coronaries (**Figure 5**). The exact etiology and consequences of coronary arterial lesions in fishes are unknown. Vascular damage may initiate coronary lesions in salmonids – this is in line with the fact that there is a particularly high prevalence and severity of lesions in adult migratory salmon that often undergo long, stressful, and energetic upstream river migrations to reach their spawning grounds. Elasmobranchs appear to be free of these coronary lesions in the main coronary artery, but lesions are reported in the arterial branches on the surface of the ventricle.

Physiology

Regulation of Coronary Blood Flow

The primary function of the coronary circulation is to supply oxygenated blood to and remove wastes from cardiac muscle. Consequently, coronary blood flow is

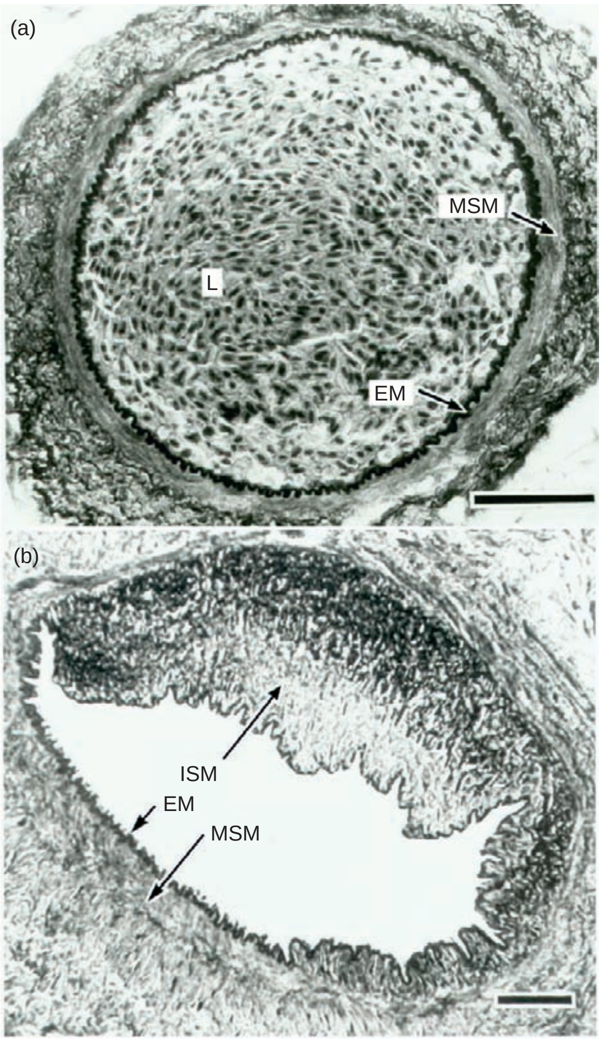


Figure 5 Histological cross sections of the main coronary artery of an Atlantic salmon to illustrate (a) the normal structure of the arterial wall and (b) a large arteriosclerotic lesion composed of intimal smooth muscle (ISM) that obstructs the lumen of the coronary artery. Scale = 50 μm. EM, elastic membrane; L, lumen of the vessel filled with nucleated red blood cells; MSM, medial smooth muscle. Adapted from Saunders RL, Farrell AP, and Knox DE (1992) Progression of coronary arterial lesions in Atlantic salmon (*Salmo salar*) as a function of growth rate. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 878–884.

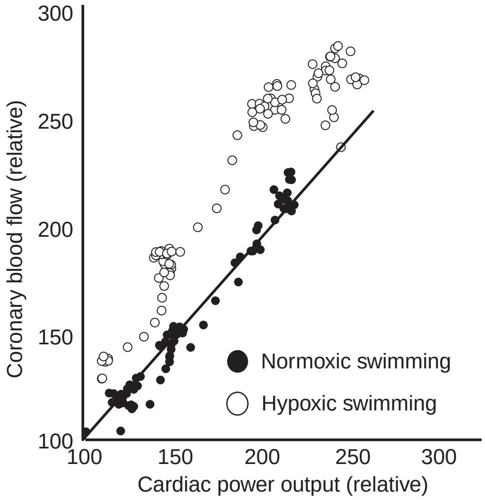


Figure 6 The relationship between cardiac power output and coronary blood flow in resting and exercising salmon. Adapted from Gamperl AK, Axelsson M, and Farrell AP (1995) Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. *The American Journal of Physiology* 269: R1258–R1266; Figure 3.

closely matched with the work performed by the heart to pump blood through the circulatory system. Coronary blood flow has been estimated as 0.6–3.0% of cardiac power output, and 0.2–0.4 ml $\min^{-1} g^{-1}$ ventricular mass. As the heart works harder during exercise, it requires more oxygen to pump a greater quantity of blood at a faster rate and at a higher blood pressure. Thus, coronary blood flow increases by almost threefold when salmon swim fast (**Figure 6**). However, this increase in coronary blood flow during exercise does not quite match the increase in cardiac oxygen demand as measured by the increase in cardiac power output. Therefore, it is likely that either oxygen extraction from the coronary blood supply increases or the heart becomes mechanically more efficient, or some combination.

Compared with the cardiac circulation, the coronary circulation of athletic fishes provides a much more reliable myocardial oxygen supply during exercise. This is because when locomotory muscles work harder during exercise, they extract more oxygen from arterial blood, leaving less oxygen in the venous blood returning to the heart (i.e., in the cardiac circulation). It is the partial pressure of oxygen in venous blood (P_{vO_2}) that drives oxygen diffusion into the trabecular muscle. Therefore, the decrease in P_{vO_2} during exercise occurs precisely when there is an appreciable increase in myocardial oxygen demand; any imbalance between oxygen supply and demand would render the trabecular muscle hypoxic. In addition, the heart rate increases with exercise, which means that venous blood is contained in the lumen of the heart for a shorter time, leaving less time for diffusion of oxygen into the trabeculae.

Coronary blood flow also increases appreciably when salmon are exposed to hypoxic water (**Figure 6**), but in this case the workload of the heart remains unchanged. However, the oxygen supply to both the compact and the spongy myocardium is potentially compromised because arterial and venous oxygen partial pressures are reduced by hypoxia. The fish heart beats slower during hypoxia (hypoxic bradycardia), allowing more time for diffusion of oxygen from the lumen into the trabeculae.

The primary determinants of coronary blood flow are dorsal aortic blood pressure (the driving force) and coronary vascular resistance (which is determined by coronary arterial vascular tone). While arterial blood pressure is under reflex control in fishes (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System and Central Control of Cardiorespiratory Interactions in Fish), dorsal aortic blood pressure can still increase by 50% under stressful situations. However, even an increase in arterial blood pressure of this magnitude cannot account for a two- to threefold increase in coronary blood flow. Therefore, the large increases in coronary blood flow seen during exercise and hypoxia must involve corresponding decreases in coronary vascular resistance.

Coronary vascular resistance is determined by vascular tone (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). Coronary vascular tone is decreased by stimulation of beta-adrenergic receptors and increased by stimulation of alpha-adrenergic and muscarinic cholinergic receptors. These adrenergic effects might be mediated by the sympathetic innervation present in the fish ventricle (via release of the sympathetic neurotransmitter noradrenaline) and by catecholamines (the hormones adrenaline and noradrenaline) released into the circulation during stressful hypoxia and exercise. However, when catecholamines are injected into the fish coronary circulation, an alpha-adrenergic vasoconstriction dominates and this leads to an increase, and not a decrease, in coronary vascular resistance. Thus, the decreases in coronary vascular resistance observed during exercise and hypoxia must involve either other vasoactive compounds or a large coronary vasodilatory reserve.

A vasodilatory reserve would come about because the coronary vasculature is under tonic vasoconstriction, possibly involving a sympathetic tonus. Many compounds affect the vasoactivity of the main coronary artery (e.g., endothelin and prostaglandin $F_{2\alpha}$ are particularly potent vasoconstrictors), but their roles in controlling coronary vascular resistance are unclear. Acetylcholine is unlikely to be involved because it is released only as a neurotransmitter from the parasympathetic vagus nerve and cardiac vagal innervation is restricted to the sinus venosus and atrium in fish.

Coronary blood flow is in phase with the cardiac cycle, being lowest during ventricular systole and highest during diastole, as in the mammalian heart. This is because muscles such as myocardium physically compress capillaries during contraction. Accordingly, each ventricular contraction slows down coronary blood flow.

The amount of coronary blood flow to the spongy myocardium has never been measured, but is presumed to be small. Whether vasoactive agents regulate the relative distribution of coronary blood flow between the compact and the spongy myocardium in type III and IV hearts is unknown.

Limitations on the Myocardinal Oxygen Supply

The coronary circulation clearly evolved in fishes as a supplement to the cardiac circulation, which implies that myocardial oxygen supply may become limited under certain situations. As noted earlier, exercise is a potential situation where oxygen supply from the cardiac circulation to the trabeculae may become limited. Thus, a coronary circulation represents a more dependable cardiac oxygen supply for athletic fishes, which generate high blood flow and blood pressure with a resulting higher cardiac oxygen demand compared with less-active fishes. Even so, environmental hypoxia compromises cardiac oxygen supply to the compact and the spongy myocardium. Consequently, while environmental hypoxia and exercise are clearly implicated in the evolution of coronary circulation, identifying the exact conditions when oxygen supply to the fish myocardium might be limited will require more information than is currently available on cardiac oxygen supply and demand.

Since it has been calculated that venous oxygen content is more than sufficient to meet the needs of the fish heart, even during maximum cardiac effort of a swimming salmon, there is presumably a threshold P_{vO_2} at which oxygen cannot diffuse fast enough into the trabeculae to meet demands. The threshold P_{vO_2} will then depend on the oxygen demand of the heart (determined by myocardial power output), the thickness of the trabeculae, the heart rate (the inverse of blood residence time in the lumen of the heart), the environmental oxygen conditions, and the shape of the hemoglobin dissociation curve. All these parameters vary considerably among fish species; hence, it is impossible to generalize as to an exact P_{vO_2} at which oxygen diffusion from the cardiac circulation might limit trabecular function.

Given that cardiac oxygen demand is about 3 times higher during exercise than that during hypoxia (based on a threefold increase in cardiac power output when salmon swim), one might expect the difference in threshold P_{vO_2} driving diffusion to be 3 times higher during exercise than that during hypoxia. (A caveat here is that the heart rate slows during hypoxia (potentially favoring oxygen diffusion) but accelerates during exercise (potentially hampering oxygen diffusion).) In salmonids, P_{vO_2} decreases to as low as 0.8 kPa during environmental hypoxia and P_{vO_2} usually remains >2.0 kPa during maximum prolonged swimming. These P_{vO_2} values may turn out to be the threshold P_{vO_2} values for these two different cardiac work conditions.

An Alternate Way of Increasing Oxygen Delivery to the Fish Heart

The physiology of air-breathing fishes is detailed in other articles (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; Respiratory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) and so this article considers only the cardiac benefit of air breathing. Oxygenated blood from an air-breathing organ (ABO) generally returns directly to the heart, where it mixes fully or partially with systemic venous blood (**Figure 1**(**c**)), thereby raising the overall P_{vO_2} in the lumen of the heart. This has a clear benefit in terms of the myocardial oxygen supply via the cardiac circulation.

Despite such an obvious benefit, many air-breathing fishes possess a coronary circulation. This suggests that the benefit of air breathing was insufficient to select for the loss of a coronary circulation, an event that neverthe less has occurred in water-breathing teleosts.

It has been proposed that air breathing evolved in fishes as a means of supplementing the heart with oxygen during exercise rather than hypoxia. Air breathing would certainly moderate the decrease in P_{vO_2} that occurs when fish swim, especially because exercise can increase the air-breathing frequency. However, not all air-breathing fishes increase the air-breathing frequency during exercise. Given that most air-breathing fishes increase the air-breathing frequency during hypoxia, both environmental hypoxia and exercise were likely important driving forces for the evolution of air breathing, at least from the perspective of the heart.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; Respiratory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels. **Design and Physiology of the Heart**: Accessory Hearts in Fishes; Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish; Integrated Control of the Circulatory System.

Further Reading

Axelsson M and Farrell AP (1993) Coronary blood flow *in vivo* in the coho salmon (*Oncorhynchus kisutch*). *American Journal of Physiology* 264: R963–R971.

Clark JJ, Clark RJ, McMinn JT, and Rodnick KJ (2004) Microvascular and biochemical compensation during ventricular hypertrophy in male rainbow trout. *Comparative Biochemistry and Physiology B* 139: 695–703.

Davie PS and Farrell AP (1991) The coronary and luminal circulations of the myocardium of fishes. *Canadian Journal of Zoology* 69: 1993–2001.

Farmer C (1997) Did lungs and the intracardiac shunt evolve to oxygenate the heart in vertebrates? *Paleobiology* 23: 358–372.

Farrell AP (1987) Coronary flow in a perfused rainbow trout heart. *Journal of Experimental Biology* 129: 107–123.

Farrell AP (2002) Coronary arteriosclerosis in salmon: Growing old or growing fast? *Comparative Biochemistry and Physiology A* 132: 723–735.

Farrell AP (2007) Cardiovascular systems in primitive fishes. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Primitive Fishes, Vol. 26: Fish Physiology*, pp. 53–120. San Diego, CA: Academic Press.

Farrell AP, Eliason E, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835–851.

Farrell AP, Davie PS, and Sparksman R (1992) The absence of coronary arterial lesions in five species of elasmobranchs, *Raja nasuta, Squalus acanthias* L., *Isurus oxyrinchus* Rafinesque, *Prionace glauca* (L.) and *Lamna nasus* (Bonnaterre). *Journal of Fish Diseases* 15: 537–540.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology*, vol. XIIA, pp. 1–88. San Diego, CA: Academic Press.

Farrell AP, Simonot DL, Seymour RS, and Clark TD (2007) A novel technique for estimating the compact myocardium in fishes reveals surprising results for an athletic air-breathing fish, the Pacific tarpon. *Journal of Fish Biology* 71: 389–398.

Gamperl AK, Axelsson M, and Farrell AP (1995) Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. *American Journal of Physiology* 269: R1258–R1266.

Grant RT and Regnier M (1926) The comparative anatomy of the cardiac coronary vessels. *Heart Journal* 13: 285–316.

Halpern MH and May MM (1958) Phylogenetic status of extracardiac arteries to the heart. *American Journal of Anatomy* 102: 469–480.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes* 3rd edn., pp. 119–152. Baton Rouge, LA: CRC Press.

Santer R (1985) Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology and Cell Biology* 89: 1–102.

Saunders RL, Farrell AP, and Knox DE (1992) Progression of coronary arterial lesions in Atlantic salmon (*Salmo salar*) as a function of growth rate. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 878–884.

Seymour RS, Farrell AP, Christian K, *et al.* (2007) Continuous measurements of oxygen tensions in the air-breathing organ of Pacific tarpon (*Megalops cyprinoides*) in relation to aquatic hypoxia and exercise. *Journal of Comparative Physiology B* 177: 579–587.

Small SA and Farrell AP (1990) Vascular reactivity of the coronary artery in steelhead trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology C* 97: 59–63.

Tota B (1983) Vascular and metabolic zonation in the ventricular myocardium of mammals and fishes. *Comparative Biochemistry and Physiology A* 76: 423–427.

Tota B, Cimini V, Salvatore G, and Zummo G (1983) Comparative study of the arterial and lacunary systems of the ventricular myocardium of elasmobranch and teleost fishes. *American Journal of Anatomy* 167: 15–32.

CIRCULATION

Design and Physiology of Arteries and Veins

Contents

Anatomical Pathways and Patterns
Branchial Anatomy
Physiology of Resistance Vessels
Physiology of Capacitance Vessels
The Retia
The Gastrointestinal Circulation

Anatomical Pathways and Patterns

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Fish Vascular Anatomy
Arterial System
Venous System
Hagfish
Elasmobranchs
Further Reading

Glossary

Anastomose To join, plural anastomoses. An anastomosis is a connection joining two blood vessels. This can result from two or more vessels coalescing to form a single vessel, or it can be a shunt created when an artery connects directly to a vein (arteriovenous anastomosis).

Bifurcate To branch evenly. Commonly used to describe the branching of an artery or arteriole into two smaller arteries or arterioles of equal diameter.

Caudal peduncle The fleshy part of the tail anterior to the tail fin.

Cyclostomes Jawless fish including hagfish and lamprey.

Elasmobranch The subclass of fishes (Elasmobranchii) found within the class Chondricthyes that includes sharks, skates, and rays, making up the Chondricthyes or cartilaginous fishes. Their bones are made up of cartilage rather than calcium found in all other vertebrates.

Lentiform body A countercurrent vascular network in the eye of some fish that is used to concentrate oxygen to supply the retina.

Myotome Blocks of white muscle in fish used for burst swimming. When cooked, they separate as flakes.

Pectoral girdle Structures associated with the paired pectoral fins in the anterioventral body, usually just posterior to the operculum of a fish. The pectoral fins are homologous to the forelimbs of tetrapods.

Pelvic girdle Structures associated with the paired pelvic fins. These are located posterior, and generally ventral, to the pectoral fins. Pelvic fins are homologous to the hindlimbs of tetrapods.

Perfuse To pump blood or fluid through a tube or blood vessel.

Pericardial cavity The cavity containing the heart. It is usually found dorsal to the pectoral girdle.

Peritoneal cavity The body cavity containing visceral organs such as the liver, kidney, spleen, and stomach and intestines.

Physoclist A fish with a completely enclosed swimbladder.

Phystostome A fish with the swimbladder connected to the esophagus via a pneumatic duct.

Systemic All tissues of the body except the respiratory tissues.

Urophysis A small neuroendocrine gland at the caudal end of the spinal cord.

Fish Vascular Anatomy

The fish circulatory system consists of two major vascular beds, the respiratory gill (or branchial) and the systemic circulations. The gill and systemic vascular beds are in series. A four-chambered heart pumps blood through a brief arterial network into the gills for oxygenation. From there, a second, more complex arterial network delivers oxygenated blood from the gills to systemic tissues. The venous system returns the deoxygenated blood from systemic tissues and nonrespiratory gill tissue back to the heart. This basic design is common to all fish with surprisingly few, and generally minor, exceptions. Therefore, in this article, the general vascular architecture of a bony fish, the rainbow trout (*Oncorhynchus mykiss*), is described in detail as the general vascular model. A few phylogenetic variations in cyclostomes and elasmobranchs are also described. The most significant exceptions to the general fish model are found in the form of the varied anatomical pathways in air-breathing fish (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction), the presence of rete mirable in tunas and some sharks (**see also Design and Physiology of Arteries and Veins**: The Retia), and the enigmatic secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy).

The pre-gill and post-gill arterial vasculature in the head is illustrated in **Figure 1**. The systemic arterial vessels are illustrated in **Figure 2**. The trunk segmental arterial and venous vessels are shown in **Figure 3**. Systemic venous vessels are illustrated in **Figure 4** and major venous vessels in the head in **Figure 5**.

Arterial System

Pre-Gill Arteries

The muscular ventricle (V) pumps blood through the bulboventricular valve (BV) and into the bulbus arteriosus

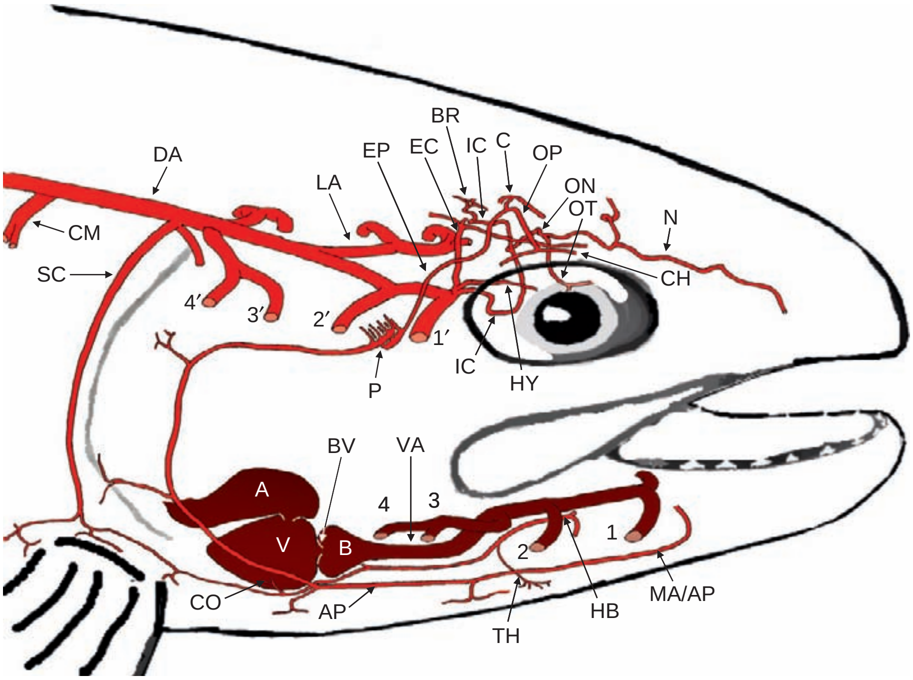


Figure 1 Pre-branchial and post-branchial arteries in the head of the rainbow trout. A, atrium; AP, afferent pseudobranch artery; B, bulbus arteriosus; BR, brain; BV, bulboventricular valve; C, commisure vessel; CH, choroid artery; CM, celiacomesenteric artery; CO, coronary artery; DA, dorsal aorta; EC, external carotid artery; EP, efferent pseudobranch artery; HB, hypobranchial artery; HY, hyoidean artery; IC, internal carotid artery; LA, lateral aorta; MA, mandibular artery; N, nasal artery; ON, orbito-nasal artery; OP, ophthalmic artery; OT, optic artery; SC, subclavian artery; TH, thyroidean artery; V, ventricle; VA, ventral aorta; 1–4 afferent branchial arteries to gill arches 1–4; 1‘–4’ efferent branchial arteries from gill arches 1–4. AB v., abdominal vein; AC v., anterior cardinal vein; CA v., caudal vein; CAP capillary network; CHT caudal heart; DC ductus Cuvier; DI dorsal intestinal artery; DI v., dorsal intestinal vein; DISeg v., dorsal intersegmental vein; DS, duodenosplenic artery; DSeg, dorsal segmental artery; Dseg v., dorsal segmental vein; EG, epigastric artery; EG v., epigastric vein; GA, gastric artery; GI, gastrointestinal artery; GO, gonadal artery; GS, gastrosplenic artery; H, hepatic artery; H v., hepatic vein; HP v., hepatic portal vein; IA, intercostal artery; ICos, intercostal artery; ICos v., intercostal vein; IN, intestinal artery; J v., jugular vein; K, kidney; L, liver; LC v., lateral cutaneous vein; LISeg, lateral intersegmental artery; LISeg v. lateral intersegmental vein; LSeg lateral segmental artery; LSeg v., lateral segmental vein; P, pseudobranch; PC v., posterior cardinal vein; RA, renal artery; RP, renal portal; RP v., renal portal vein; S, spleen; SA, swimbladder artery; SC v., subclavian vein; SM, secondary muscular artery; SM v., secondary muscular vein; ST, stomach; SV, sinus venosus; TM, tertiary muscular artery; TM v., tertiary muscular vein; Vb, vertebra; VI, ventral intestinal artery; VI v., ventral intestinal vein; VISeg, ventral intersegmental artery; VISeg v., ventral intersegmental vein. Abbreviations used in all the figures are given here.

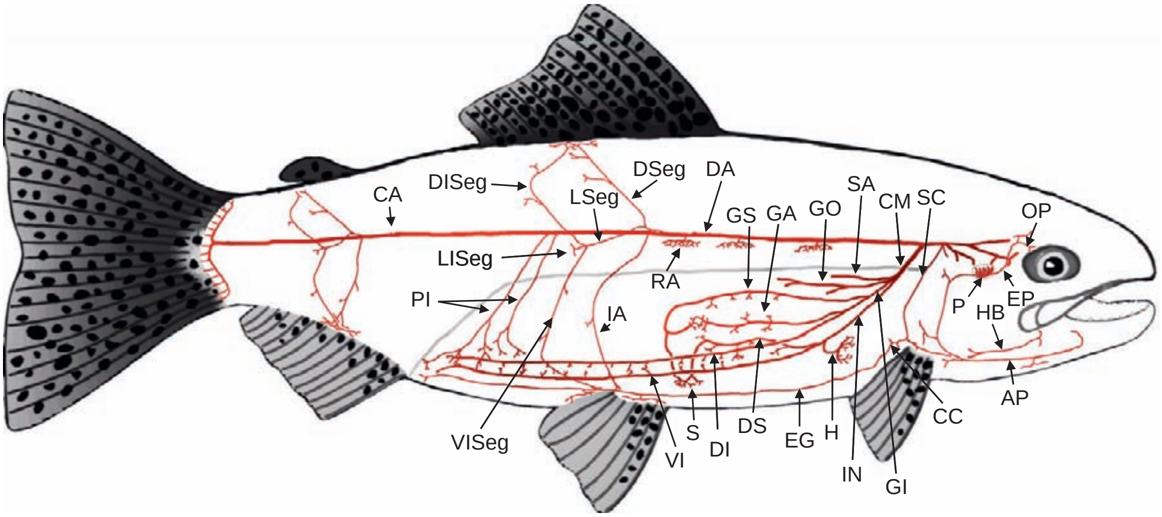


Figure 2 Major systemic arteries in the rainbow trout. The hypobranchial (HB) and afferent pseudobranch (AP) arteries arise from ventral extensions of the efferent branchial arteries. All other systemic arteries shown are supplied from the dorsal efferent branchial arteries. See **Figure 1** for abbreviations.

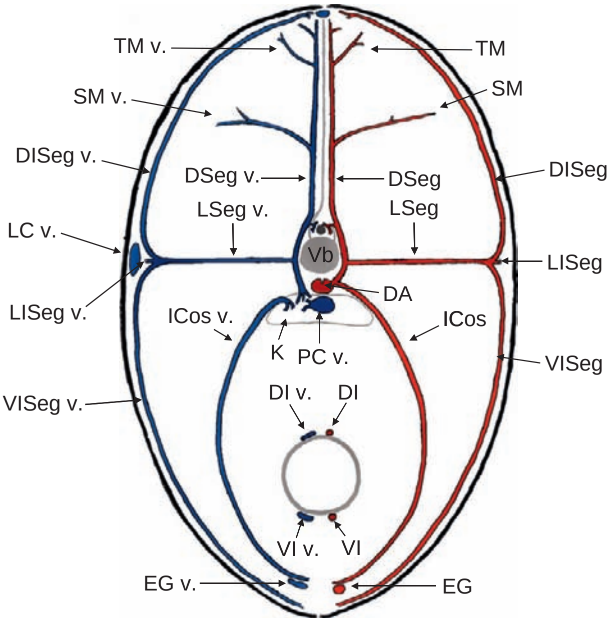


Figure 3 Segmental vessels to the trunk of the rainbow trout. Arterial vessels are drawn on the right and venous vessels on the left. The venous system essentially mirrors the arterial except that segmental veins drain into the renal portal sinusoids and blood re-enters the posterior cardinal vein before returning to the heart. **See Figure 1** for abbreviations. Modified from Gorkiewicz G (1947) Les vaisseauz sanguins des muscles du tronc de la truite (*Salmo irideus* Gibb.). *Bulletin International De L'Academie Polonaise des Sciences et des Lettres. Classe des Sciences Mathematiques et Naturelles Serie B: Sciences Naturelles* III: 241–261.

(B) in teleosts or conus arteriosus in most other fish (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). The bulbus is typically a pear-shaped vessel with thick elastic walls and may be lined with a dozen or more longitudinal septa. The bulbus is over 30 times more compliant than the mammalian aorta and because it may be able to store up to 100% of the stroke volume, it serves an important role in dampening ventricular pulse pressure. The bulbus is well endowed with vascular smooth muscle and connective tissue (elastin and collagen) and it has a physiological role in storing the energy of cardiac contraction and thereby dampening the arterial pulse pressure (**see also Design and Physiology of the Heart**: The Outflow Tract from the Heart and Physiology of Cardiac Pumping). It remains to be determined if this has a physiological role in affecting compliance and therefore arterial pulse pressure. The septa, however, clearly add structural integrity to the bulbus. The conus arteriosus of elasmobranchs and other subteleostean fish, such as bowfin (*Amia calva*) and gar (*Lepisosteus* sp.), occupies a similar position within the pericardial cavity and presumably has a similar function. However, the conus has true cardiac muscle surrounding the connective tissue (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). The conus has its own pacemaker and, in some fish, it initiates as well as conducts an action potential, typically down the length of the conus away from the heart. The conus usually has numerous valves. After passing through the bulbus or conus, the blood leaving the heart enters the ventral aorta (VA), which at this point is still within the pericardial cavity.

The ventral aorta passes anteriorly out of the pericardial cavity and branches to give rise to the afferent branchial arteries that deliver deoxygenated blood to the gill arches. There are four pairs of gill arches in bony fish, and, in some fish, pairs of independent afferent branchial

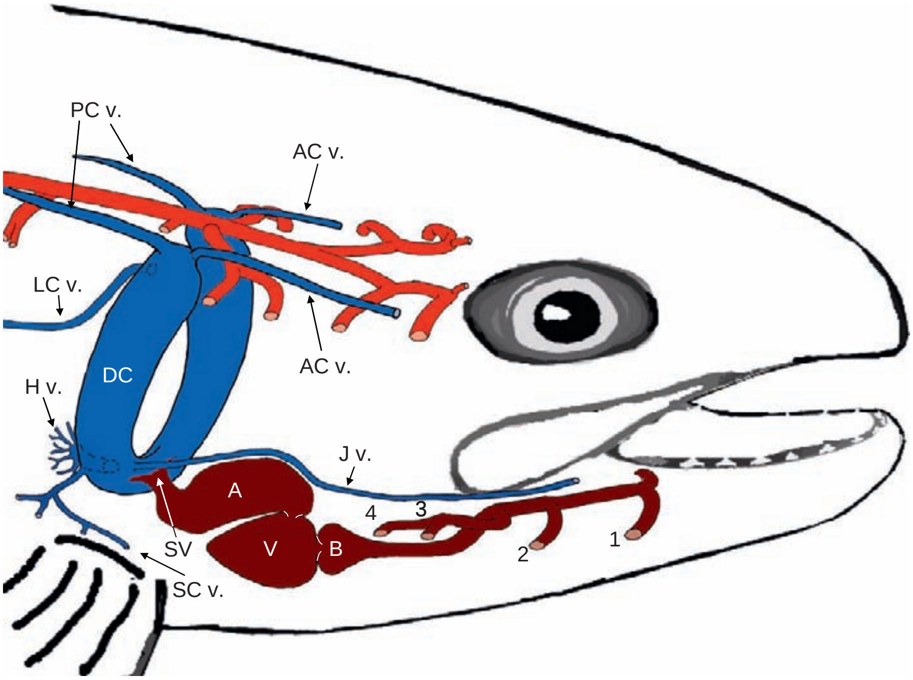


Figure 4 Central venous system in the rainbow trout head. **See Figure 1** for abbreviations.

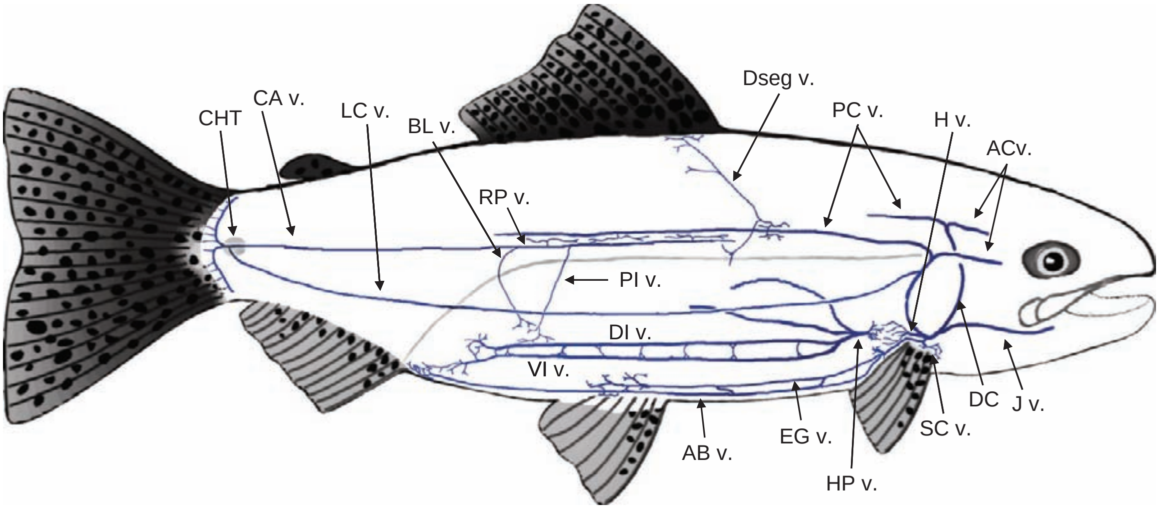


Figure 5 Major systemic veins in the rainbow trout. **See Figure 1** for abbreviations.

arteries successively supply the fourth through the first pair of gills. In other fish, such as the rainbow trout, the first pair of vessels to arise from the ventral aorta turn posteriorly and then bifurcate, with the most posterior branch forming the fourth afferent branchial artery (4) and the other the third (3). The main trunk of the ventral aorta continues anteriorly, and, after giving rise to a pair of afferent branchial arteries that supply the second gill arches (2), bifurcates to form the first pair of afferent branchial arteries (1). The afferent branchial arteries enter the gill arches and ultimately form the microcirculation of the gill (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy and **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics).

Post-Gill (Systemic) Arteries

Post-gill arterial vessels supply the systemic (body) circulation. Most of the gill blood supply exits via the efferent branchial arteries that emanate from the posterio-dorsal aspect of the gill arches. A comparatively minor circulation

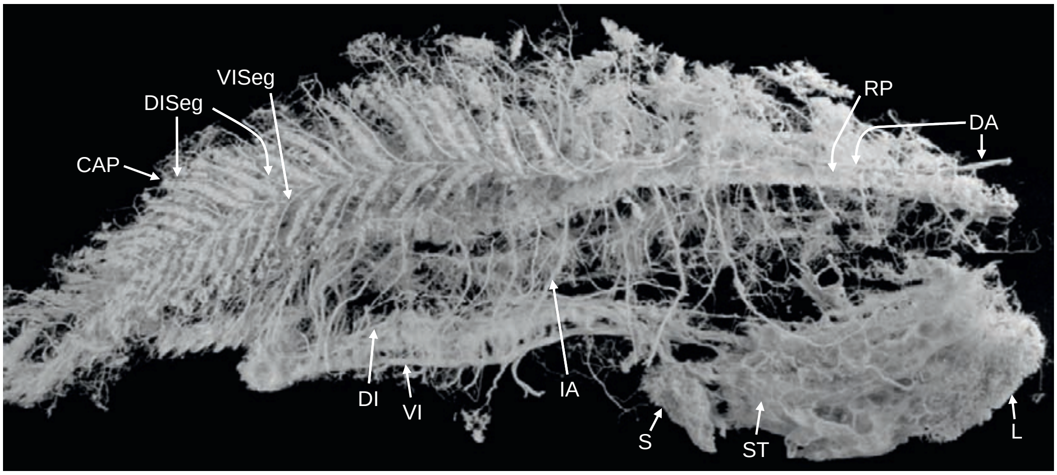


Figure 6 Vascular corrosion replica of the systemic circulation of rainbow trout. The dorsal aorta (DA) and celiacomesenteric artery (not visible) were perfused with liquid methyl methacrylate. After polymerization, the tissue was digested away leaving a vascular replica. This figure shows the dorsal and ventral intersegmental (DISeg and VISeg) and intercostal (IA) arteries and the extensive capillary networks (CAP) they supply. Also visible is the renal portal (RP), liver (L), stomach (S), spleen (SP), and dorsal and ventral intestinal (DI and VI) circulations. **See Figure 1** for abbreviations.

also exits via the anterio-ventral and ends of the gill arches through extensions of efferent branchial arteries. This latter circulation is usually limited to supplying the ventral jaw and, if present, the coronary circulation. The gill also has its own systemic circulation (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy and **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics).

Posterio-dorsal post-gill arteries

Arteries in the head

The efferent branchial arteries that arise from the posterio-dorsal regions of the gill arch supply the bulk of the systemic circulation. Although efferent branchial arteries from all four arches are interconnected into a common manifold, additional vessels, the carotids and hyoidean, typically emanate from the first arch efferent branchial artery (1′) and form the major supply network to the anterior head region. The main portion of the efferent branchial artery from the first gill arch turns posteriorly and becomes the lateral dorsal aorta (LA) and is soon joined by the efferent branchial artery from the second gill arch (2′). The two lateral aortas then anastomose to form the anterior segment of the dorsal aorta (DA). Efferent branchial arteries from the third (3′) and fourth (4′) gill arches on either side of the fish frequently anastomose to form a single vessel, often called the epibranchial artery, prior to joining the dorsal aorta. Efferent branchial arteries that arise from the first pair of gill arches directly supply the systemic circulation in the head region (anteriorly) and also connect posteriorly to efferent branchial or epibranchial arteries from other gill arches (an epibranchial artery is formed by the anastomosis of two efferent branchial arteries from adjacent gill arches). Systemic arteries do not arise directly from efferent branchial or epibranchial arteries of the second through fourth pair of other gill arches, or from the lateral dorsal aortas. Some fish may have a single anterior dorsal aorta in lieu of the paired lateral aortas.

The internal (IC) and external (EC) carotid arteries, and the hyoidean artery (HY) originate from the first gill arch efferent branchial arteries, usually as the latter makes its posterior turn. The internal carotid travels anterio-dorsally to the brain. The cephalic circle in the brain (BR) is formed by the union of posterior branches of the internal carotids from both sides of the head. The orbito-nasal artery (ON) arises from the internal carotid about midway along the carotid and travels anteriorly. One branch, the nasal artery (N), continues to supply the olfactory organ, the other becomes the optic artery (OT) and continues on to the retina and lentiform body of the eye. The external carotid, or orbital artery, courses dorsally from the efferent branchial artery before branching anteriorly and posteriorly to perfuse the more superficial structures of the head. The hyoidean artery travels anteriorly to the anterior head region.

Arteries to the trunk

The dorsal aorta traverses the longitudinal axis of the body ending in the caudal peduncle anterior to the caudal (tail) fin. While this entire vessel is often referred to as the dorsal aorta, posterior to the peritoneal cavity, it is technically the caudal artery (CA). The dorsal aorta travels just beneath the vertebra and above the dorsal surface of the kidney. As the dorsal aorta diminishes in diameter (there is progressive loss of blood to segmental arteries) and leaves the peritoneal region to become the caudal artery, ventral processes of the spine become fused forming the hemal arch and the caudal artery travels within this arch (the hemal canal), along with the caudal vein and several secondary veins. Longitudinally, both the dorsal aorta and caudal artery take on a characteristic undulating form due to their close association with the vertebrae. In some fish, including rainbow trout, the dorsal wall of the dorsal aorta and caudal artery tightly adhere to the vertebrae and the vessel cannot be dissected free. In other fish, such as eels (*Anguilla*), the vessel is more readily isolated from surrounding tissue. In addition, in some fish such as the rainbow trout, there is a longitudinal elastic ligament in the lumen of the dorsal aorta. This ligament is attached to the dorsal wall of the vessel via the basioccipital bone and forms a dorso-ventral vertical septum that nearly completely divides the long axis of the vessel for up to a quarter of its length. Although its exact function is unclear, it most likely helps propel blood down the dorsal aorta when the trunk undulates during active swimming.

A pair of segmental arteries arise from the dorsal aorta shortly after the union of the lateral aortas and anterior to the anastomosis of the epibranchial arteries with the dorsal aorta (not shown in **Figure 1**). Paired subclavian arteries (SC) arise from the lateral wall of the dorsal aorta just posterior to the anastomoses of the epibranchial arteries. The subclavian arteries run ventrally and supply the pectoral girdle, pectoral fins, and adjacent musculature. Branches of the subclavian arteries may connect directly to the epigastric arteries (EG) or may communicate with them via the coracoid artery (CC). The subclavian arteries often anastomose anteriorly, with the hypobranchial circulation (see below). As the dorsal aorta travels posteriorly from the origin of the subclavian arteries, it gives rise to numerous segmental arteries. The celiac and mesenteric arteries supply the anterior (stomach) and posterior (intestines) viscera, respectively. In some fish, such as rainbow trout, these vessels may arise as a single vessel, the celiacomesenteric artery (CM), but even in the same strain of trout, some individuals may have separate vessels. Small renal arteries (RA) perfuse the kidney and one or two small posterior intestinal arteries (PI) supply the posterior gut (see also **Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation).

Segmental arteries

Segmental arteries rise in association with each vertebra, often in alternating myosepta. These vessels supply blood to the red and white muscle and possibly skin, although the secondary circulation may perfuse the superficial epithelium in the skin. There are three groups of segmental arteries: dorsal, lateral, and ventral. Dorsal and lateral segmental arteries (DSeg and LSeg) and their corresponding veins (DSeg v. and LSeg v.) travel in the septa between myotomes and small branches leave periodically to enter the myotomes and perfuse the muscle fibers and skin. Ventral segmental arteries and veins, more commonly known as the intercostal arteries (ICos) and veins (ICos v.), follow a slightly different pathway but serve a similar purpose.

Dorsal segmental arteries leave the dorso-lateral wall of the dorsal aorta and travel dorsally, briefly skirting laterally around the vertebra (Vb) *en route*. They then accompany the dorsal spines and continue to the dorsal surface. Dorsal segmental arteries of rainbow trout are paired, whereas in many other fish, one of the pair is reduced. Midway along their route, they give rise to perpendicular secondary muscular arteries (SM) that branch profusely to perfuse the dorsal musculature. Further dorsally, tertiary muscular arteries (TM) branch to perfuse the muscle beneath the dorsal fins.

Lateral segmental arteries arise from the dorsal segmental arteries at the level of the ventral border of the vertebrae and run transversely through the horizontal myosepta of the axial muscles to a point just beneath the red muscle. Here they divide into three branches, forming the dorsal, lateral, and ventral intersegmental arteries (DISeg, LISeg, and VISeg). The dorsal intersegmental branch travels dorsally between the muscle and skin, roughly following the path of the myotomes and its fine terminal branches anastomose with the tertiary arteries of the dorsal segmental vessels. The ventral intrasegmental branch follows a similar ventral-ward course and its terminal branches ultimately anastomose with terminal vessels of the epigastric artery (EG). Numerous branches along the length of both dorsal and ventral intersegmental arteries supply the underlying myotomes and superficial skin. The lateral intersegmental artery is considerably shorter and smaller in diameter than the other two and it continues laterally to perfuse the red muscle. The intercostal (ventral segmental) arteries leave more ventrally from the dorsal aorta and briefly travel perpendicularly toward the ribs and then follow the ribs ventrally. Small branches along their length supply the ventro-lateral muscles of the trunk, peritoneum, and ribs. The distal fine branches also anastomose with the epigastric vessels. Intercostal arteries are smaller than the lateral segmental arteries.

Paired epigastric arteries travel longitudinally along the ventral aspect of the fish between the peritoneum and abdominal muscles and link the pectoral and pelvic girdles. They are not true segmental vessels but have numerous capillary anastomoses with the subclavian, intercostal, and ventral intersegmental arteries.

Visceral arteries

The celiacomesenteric artery travels posterioventrally, usually passing to the right of the esophagus, and soon divides into the celiac (anterior) and mesenteric (posterior) branches, although these are also commonly called the intestinal (IN) and gastrointestinal (GI) arteries, respectively. In some fish, the gastrointestinal artery may originate some distance posterior to the intestinal. Other than a few segmental arteries that supply the very posterior gut, the celiacomesenteric artery is the only major visceral artery in rainbow trout.

The intestinal artery travels ventrally and gives rise first to the hepatic artery to perfuse the liver (H) and then turns posteriorly and branches to form the duodenosplenic artery (DS) that perfuses the pyloric cecae and spleen, and ventral intestinal artery (VI) that perfuses the ventral intestine. The branches from the gastrointestinal artery include paired gonadal arteries to the gonads (GO), gastric arteries to the dorsal stomach (GA), gastrosplenic arteries to the stomach and spleen (GS), and dorsal intestinal arteries (DI) to the dorsal aspect of the intestine. In a number of instances, small terminal arteries from different sources anastomose to form a collateral arterial circulation. This is seen in the union of the gastrosplenic and duodenosplenic arteries in the spleen, the gastric and duodenosplenic in the stomach, the dorsal and ventral intestinal arteries along the intestine, and one or two segmental arteries, the posterior intestinal arteries, that travel from the distal dorsal aorta to the rectum and very posterior intestine and anastomose with both dorsal and ventral intestinal arteries. These segmental arteries are unlike the intercostal arteries in that they pass ventrally through the midline of the kidney and travel directly through the peritoneal cavity *en route* to the intestine.

The swimbladder (**see also Design and Physiology of Arteries and Veins**: The Retia and **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes) in physostomes, such as the rainbow trout and eel, is perfused by the swimbladder artery (SA), which branches off the celiacomesenteric artery. This vessel branches into a capillary network in the resorbent portion and then onward, via retia forms a capillary bed in the secretory end. In physoclist fish, the celiac and intercostal arteries supply the secretory and resorbant tissues, respectively. The kidney is supplied by numerous small renal arteries that arise either directly from the dorsal aorta, from segmental arteries, or from the venous renal portal system, as described next.

Anterio-ventral post-gill arteries

Extensions from the efferent branchial arteries of the first, second, and sometimes third and fourth gill arches exit the anterio-ventral portion of the arch and supply the lower jaw, pseudobranch (in rainbow trout), and the heart if the fish has a coronary circulation (**see also Design and Physiology of the Heart**: The Coronary Circulation). Efferent branchial arteries are paired in the anterio-ventral arch because the artery forks around the afferent branchial where the latter enters the gill arch. The lateral, outside fork of the efferent branchial from the first gill arch becomes the mandibular artery (MA). Side branches of this artery supply the lower jaw, whereas a main branch, now the afferent pseudobranch artery (AP), makes an abrupt hair-pin turn and proceeds ventro-posteriorly and then dorsally, following the arc of the operculum, to supply the pseudobranch (P). *En route*, a number of smaller arteries branch from the afferent pseudobranch artery and supply the operculum. In non-salmonids, the afferent pseudobranch artery may originate from the posterio-dorsal arteries via the cephalic circle. The pseudobranch is drained by the efferent pseudobranch artery (EP), which becomes a singular ophthalmic artery (OP) ultimately forming the choroid artery (CH) that perfuses the choroid gland of the eye (**see also Design and Physiology of Arteries and Veins**: The Retia). A short transverse commissural vessel (C), about mid-way between the pseudobranch and choroid, connects the two ophthalmic arteries from the opposite sides of the head.

Ventral extensions from the medial (inside fork) of the efferent branchial arteries from the second pair of gill arches unite with each other to form the median hypobranchial artery (HB). This artery travels posteriorly along the ventral surface of the ventral aorta and it bifurcates as it passes under the bulbus to form the left and right coronary arteries (CO). One or more small branches of the coronary artery may continue posteriorly to anastomose with branches from the subclavian artery.

Venous System

It is common in vertebrate circulatory systems for veins to be closely associated with the respective artery whose blood they return. By convention, these veins are given the same name as their corresponding artery and, unless noteworthy, are not repeated. There are, however, a few exceptions such as the jugular, cardinal, and abdominal veins. These vessels are described below. In addition, there are several aspects of the fish venous system not common to all vertebrates. These include the renal portal system and the venous caudal heart (**see also Design and Physiology of the Heart**: Accessory Hearts in Fishes). These are also described in greater detail later.

Valves are absent from fish arteries (other than those associated with the bulbo-ventricular opening and the conus arteriosus), whereas venous ostial valves are common. These valves, located at the opening of tributary veins into longitudinal veins, are plentiful and prevent retrograde (reverse) flow. They are especially useful during contraction of skeletal muscles and, by virtue of their one-way conduction, act as skeletal-muscle pumps and assist venous return during swimming. Parietal valves are found within longitudinal veins of higher vertebrates and act as anti-gravity valves to prevent blood from being pulled away from the heart by gravity into dependent veins (those lower than the heart). Parietal valves are not found in fish, presumably because they live in a nearly neutrally buoyant environment.

Central Venous System

The central venous system is comprised of the ductus Cuvier (DC) and the large longitudinal (axial) veins that return blood from the head and trunk into the sinus venosus (SV) which lies inside the pericardial cavity. The ductus Cuvier is a U-shaped vessel embedded in the transverse septum, the tissue that separates the pericardial and peritoneal cavities. The opening between the ductus and the sinus venosus is located ventrally near the bottom of the U. From one to several hepatic veins (H v.) also enter the sinus venosus in this region from the peritoneal side. The anterior and posterior cardinal veins (AC v. and PC v.) drain into the dorsal horns of the ductus. In some literature, the ductus may be called the common cardinal vein. Prominent ostial valves are located at the junction of the anterior cardinal veins with the ductus Cuvier, but not between the ductus Cuvier and the sinus venosus.

Venous Drainage of the Head

Three principal veins drain the head, a singular jugular (J v.) and paired anterior cardinals. The jugular vein lies in the midline of the lower jaw, dorsal to the ventral aorta and ventral to the esophagus. The jugular drains the thyroid gland, pharyngeal muscles, and tissues of the lower jaw, the venous drainage from the anterio-ventral ends of the gill arches, and a portion of the pericardium. The jugular vein drains directly into the sinus venosus slightly to the right of the midline. The anterior cardinal veins are the central extensions of the orbito-nasal veins (not shown in **Figure 3**) and drain the snout, olfactory rosettes, eyes, braincase (via anterior, medial, and posterior cerebral veins), and other tissues in this region. The cardinal veins drain into the sinus via the ductus Cuvier.

Venous Drainage of the Trunk

By definition, a portal system contains two vascular networks (typically capillary beds) in series. Two portal systems are unique to fish: the renal portal system and the pseudobranch (see earlier). Another major portal system, which is common to all vertebrates, is the hepatic portal system.

Renal portal system

Much of the venous return from the trunk musculature and skin, and nearly all of it from the post-abdominal region and tail fin, passes through the renal portal system before returning to the heart. Segmental veins in the post-abdominal trunk and primary veins from the caudal fin drain into the central caudal vein (CA v.) located in the hemal arch. This vein also receives flow from secondary vessels in the caudal fin and lateral cutaneous veins (LC v.) via the caudal heart (described below), as well as from the urophysis. As the caudal vein approaches the kidney, it anastomoses with the bladder vein (BL v.) and, within the kidney, a branch from the caudal vein connects with the posterior intestinal vein (PI v.). The bladder may also be connected directly to the posterior cardinal vein via a second vein that passes through the gonads. The direction of flow in these two branches is unclear – toward or away from the caudal vein. If it is away from the caudal vein, it may be an additional portal system in parallel with the renal portal vessels. Nevertheless, the majority of caudal venous blood enters the kidney and in the kidney, the vein ends and blood percolates through the renal parenchyma and around the renal tubules.

Some segmental veins also directly contribute to the renal portal system. In the abdominal region of the trunk, the dorsal and lateral segmental veins anastomose and a short common vessel delivers blood to the renal parenchyma, as do the intercostal veins.

Renal portal blood and post-glomerular renal arterial blood are then collected into the posterior cardinal vein as it passes through the kidney and the blood is delivered to the ductus Cuvier. Commonly, the left posterior cardinal vein is either considerably reduced, or nonexistent, and the right posterior cardinal vein assumes a central position. It then serves as the main conduit for venous return.

Hepatic portal system

Essentially all drainage from the intestines, spleen, and liver (known as the splanchnic or visceral region) ultimately flows into the short hepatic portal vein (HP v.), which then enters the liver and supplies blood to the liver sinusoids. There are numerous interconnections among splanchnic vessels that form a diffuse system with a number of pathways for venous return. For instance, the gastrosplenic vein connects the splenic vein with the lateral gastric vein, the duodenosplenic vein connects the spleen with the pyloric cecae and the duodenum, and venous anastomoses within the spleen form a gastro-dudeno-splenic loop that has several connections with the hepatic portal vein. The dorsal and ventral intestinal veins (DI v. and VI v.) are similarly connected with each other through circumferential veins that encircle the intestine. The dorsal and ventral intestinal veins travel along the respective borders of the intestine and directly anastomose with the hepatic portal vein. Although the hepatic-arterial and hepatic-portal blood mix in the liver sinusoids, the diameter of the celiacomesenteric artery is considerably larger than the hepatic artery and the majority of blood flow through the liver is venous. Blood from the sinuses is collected into one or several very short hepatic veins that drain into the ductus Cuvier.

Cutaneous veins

There are four noticeable longitudinal vessels that run beneath the skin, one each on the dorsal and ventral margins of the fish and one on each side of the fish, essentially beneath the lateral line. Although these vessels appear to be simple return vessels, there are a number of unresolved issues regarding their connections with other vessels: the direction of flow, and why the blood in them often has very few red blood cells. It has been proposed that these may be lymph vessels or veins of the secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy), for a detailed discussion on these possibilities). The more obvious connections to the central venous system are described next.

The abdominal vein extends ventrally from the anus along the ventral midline to the region of the pectoral fins, although there are also reports that a branch continues on to the tail. Near the pectoral fins, the abdominal vein turns laterally and anastomoses with either the left epigastric (EG v.) or subclavian (SC v.) vein or it may drain directly into the ductus Cuvier. The abdominal vein anastomoses repeatedly with the epigastric veins and in the pelvic region with the iliac vein. The dorsal vein follows a similar course beneath the dorsal skin. In the region of the dorsal fin, it often divides into three branches, two of which travel lateral, and one medial, to the fin rays.

Lateral cutaneous veins connect at the caudal end with the caudal heart (CHT) and anteriorly are likely to drain into the ductus Cuvier, although the actual connections remain unclear. The caudal heart is basically a sac-like structure with a vertical baffle that divides the heart into two lateral chambers (**see also Design and Physiology of the Heart**: Accessory Hearts in Fishes). Presumably, it acts as a booster pump to move blood into the caudal vein from the low-pressure secondary veins draining the caudal fin. Spinal nerves alternately contract a sheet of skeletal muscle on either side of the caudal heart, which alternately compresses the baffle into one chamber then the other and forces fluid into the caudal vein. This helps compensate for incompressibility of the caudal vein as the walls of this vessel are imbedded in the bony hemal arch and insulated from muscular contraction. Swimming movements may also contribute to the activity of the caudal heart. Caudal hearts are generally quite small (~1–2 mm in adult rainbow trout) and because their capacity must be limited, their overall importance remains unclear. Furthermore, in non-swimming fish, much of the flow through the lateral cutaneous veins appears to be forward and fluid is delivered to the heart via an anastomosis between the cutaneous vein and the ductus Cuvier.

Hagfish

The vascular anatomy of the most primitive extant fish, the hagfish (class Myxine), is basically similar to that described earlier for teleosts with only a few notable exceptions. Hagfish have more pairs of gills than teleosts, anywhere from five to as many as fifteen pairs, and thus they have more afferent and efferent arteries as well as a distinct gill vascular organization. Other modifications include a single medial anterior dorsal aorta in addition to the paired lateral aortas, a portal heart on the hepatic portal vein that is comprised of true cardiac muscle, complete with its own electrocardiogram. They also have both caudal and anterior cardinal hearts that are powered by skeletal muscle. These are analogous to, but structurally different from, the caudal heart of teleosts. Hagfish do not have a bulbus or conus arteriosus, and they lack a spleen and a renal portal system. Perhaps the most striking feature, however, is the sinus system especially the capacious subcutaneous sinus (**see also Hagfishes and Lamprey**: Hagfishes; **Design and Physiology of the Heart**: Accessory Hearts in Fishes, and **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy). There are numerous small vascular sinuses associated with a variety of tissues, and these ultimately drain into the subdermal blood space that almost completely surrounds the fish from head to tail. This sinus system has been likened to the primitive lymphatic system or the secondary circulation of teleosts.

Elasmobranchs

The elasmobranchs, sharks and skates and rays, typically have five, but may have up to seven, pairs of gill arches and, like hagfish, there are additional branchial arteries to accommodate these gill arches. However, unlike the hagfish, vascular supply to the gills is very similar to that of teleosts. The elasmobranchs lack an operculum and swimbladder, and their conus arteriosus is extensively valved. Most other vascular modifications are relatively minor.

See also: **Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. **Design and Physiology of Arteries and Veins**:

Branchial Anatomy; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of the Heart**: Accessory Hearts in Fishes; Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping. **Hagfishes and Lamprey**: Hagfishes. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 89–120. San Diego, CA: Academic Press.

Forster ME (1998) Cardiovascular function in hagfishes. In: Jørgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 235–258. London: Chapman and Hall.

Gorkiewicz G (1947) Les vaisseauz sanguins des muscles du tronc de la truite (*Salmo irideus* Gibb.). *Bulletin International De L'Academie Polonaise des Sciences et des Lettres Classe des Sciences Mathematiques et Naturelles Serie B: Sciences Naturelles* III: 241–261.

Laurent P and Dunel-Erb S (1984) The pseudobranch: Morphology and function. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, Volume X, Gills, Part B: Ion and Water Transfer*, pp. 285–323. New York: Academic Press.

Lomholt JP and Franko-Dossar F (1998) The sinus system of hagfishes – lymphatic or secondary circulatory system? In: Jørgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 259–272. London: Chapman and Hall.

Muñoz-Chápuli R (1999) Circulatory system: Anatomy of the peripheral circulatory system. In: Hamlett WC (ed.) *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes*, pp. 198–217. Baltimore, MD: The Johns Hopkins University Press.

Priede IG (1975) The blood circulatory function of the dorsal aorta ligament in rainbow trout (*Salmo gairdneri*). *Journal of Zoology, London* 175: 39–52.

Priede IG (1976) Functional morphology of the bulbus arteriosus of rainbow trout (*Salmo gairdneri* Richardson). *Journal of Fish Biology* 9: 209–216.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

Satchell GH (1999) Distinctive attributes of the circulation of elasmobranch fishes. In: Hamlett WC (ed.) *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes*, pp. 218–237. Baltimore, MD: The Johns Hopkins University Press.

Smith LS and Bell GR (1975) *A Practical Guide to the Anatomy and Physiology of the Pacific Salmon,* Canadian Fisheries and Marine Service Miscellaneous Publication 27. Ottawa, ON: Department of the Environment, Fisheries and Marine Service

Thorarensen H, McLean E, Donaldson EM, and Farrell AP (1991) The blood vasculature of the gastrointestinal tract in chinook, *Oncorhynchus tshawytscha* (Walbaum), and coho, *O. kisutch* (Walbaum), salmon. *Journal of Fish Biology* 38: 525–531.

Vogel WOP (1985) Systemic vascular anastomoses, primary and secondary vessels in fish, and the phylogeny of lymphatics. In: Johansen K and Burggren W (eds.) *Cardiovascular Shunts: Phylogenetic, Ontogenetic, and Clinical Aspects*, pp. 143–159. Copenhagen: Munksgaard.

Vogel WOP (1985) The caudal heart of fishes: Not a lymph heart. *Acta Anatomica* 121: 41–45.

Branchial Anatomy

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Gill Arch
Gill Filament
Respiratory Lamellae
Epithelial Cells
Gill Vessels
Innervation of the Gill Arch
Further Reading

Glossary

Abductor muscle A muscle that pulls two objects apart.

Adductor muscle A muscle that pulls one object toward another.

Anastomose To join, plural anastomoses. An anastomosis is a connection joining two blood vessels. This can result from two or more vessels coalescing to form a single vessel, or it can be a shunt created when an artery connects directly to a vein (arteriovenous anastomosis).

Bifurcate To branch evenly. Commonly used to describe the branching of an artery or arteriole into two smaller arteries or arterioles of equal diameter.

Buccal cavity The mouth (oral) cavity at the anterior end of the fish in front of the gills.

Chemoreceptors Type of cell that is specialized to detect chemical stimuli and convert them into signals within the nervous system.

Countercurrent Flowing in opposite directions. Used for an arrangement of vessels (e.g., blood vessels or kidney tubules) such that fluid flows in one direction in one vessel that lies close to another vessel in which the flow is in the opposite direction. This arrangement allows the transfer of molecules or heat from one vessel to another in such a ways as to maintain a gradient along the countercurrent vessels.

Filament The functional unit of the gill containing the respiratory lamellae, ionocytes, mucus cells, and pavement cells. It was formerly called the primary lamella.

Gill raker Projections from the gill arch that prevent particulate material from damaging the gills, and also direct food into the esophagus.

Hemibranch A 'half' branch (gill arch) with only one row of filaments.

Holobranch Two hemibranchs on the same gill arch form one holobranch.

Intrabranchial septum Tissue that runs between, and is attached to, the filaments of both hemibranchs on a gill, thereby restricting their movement.

Lamellae Also known as secondary lamellae, these are attached in rows to the gill filaments. They are the primary sites for gas exchange in fish gills. Each lamella is made up of two epithelial layers separated by pillar cells. Oxygen is taken up by erythrocytes flowing inside the lamellae from water flowing between the lamellae.

Pseudobranch A reduced mandibular gill arch, in elasmobranchs the spiracular gill, in teleosts located on the inside of the operculum. In teleosts with ocular oxygen secretion, blood is supplied to the eye only via the pseudobranch.

Introduction

The fish gill is the primary site for respiration, osmoregulation, acid–base balance, nitrogen excretion, and metabolism of circulating hormones. These diverse and specialized activities are reflected in the anatomical diversity and specialization of the gills. Compared to air, water is 800 times more dense and 60 times more viscous, has 1/30th the capacity to hold oxygen, and allows oxygen to diffuse at 1/8000th the rate. Gill architecture has achieved a delicate balance between a tissue that is thin enough to promote transepithelial transfer of a variety of molecules, yet strong enough to withstand the viscous and often abrasive aquatic environment.

The Gill Arch

The gill develops in the embryo from a series of paired pouches in the lateral walls of the mouth cavity (more commonly called the buccal cavity; **see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms. These pouches form a water pathway from the mouth to the exterior, and the tissue between them becomes the gill arch and supports the gill filaments. In bony fish (teleosts), the gill arch is a relatively simple bow-shaped structure whose ends are attached to the dorsal and ventral surfaces of the buccal cavity and with the curved portion projecting posterio-laterally (**Figures 1**(**a**) and **1**(**b**)). Teleosts have eight gills arranged in pairs with four pairs on either side of the buccal cavity. The pseudobranch, an additional, vestigial gill hemiarch, is also present in most teleosts (notable exceptions being the catfish). The pseudobranch is covered by a thick epithelium; its physiological function is not known.

The gill arch is supported by three bones, which, starting from the ventral aspect, are the hypobranchial (Hb), ceratobranchial (Cb), and epibranchial (Eb) bones (**Figure 1**(**a**)). These bones are hinged to provide flexibility necessary for jaw movement during feeding and respiration. They also serve as the mechanical anchor for the filaments. Gill rakers typically project from the arch on the opposite side of the filaments (**Figures 1**(**a**) and **1**(**b**)). They serve as filters to protect the delicate filaments from large particulate matter, which can also be food that they help funnel into the esophagus. The arch also provides a matrix for gill blood vessels, most notably the afferent and efferent branchial arteries (**Figures 1**(**c**), **2**(**a**), **2**(**b**), *2*(**c**), and **3**(**a**)) and brachial veins.

Each arch supports paired rows of long blade-like filaments. Each row of filaments is called a hemibranch (**Figures 1**(**d**) and **2**(**a**)), and two hemibranchs that share the same arch form a holobranch (**Figure 2**(**a**)). Filaments of one hemibranch alternate with the filaments of the adjacent hemibranch along the arch. Filaments from the two hemibranchs on a single arch face different water channels (gill slits), whereas hemibranchs from adjacent arches (the posterior hemibranch of one arch and the anterior hemibranch of the next arch) share a common water channel. The tips of filaments from adjacent hemibranchs lining the same water channel nearly touch each other and this maximizes water flow across the filament surface (**Figures 2**(**a**) and **2**(**b**)). A cartilaginous rod runs the length of the filament to provide mechanical support (**Figure 3**(**a**)).

In some fish, the filaments are only attached to the gill arch at the filament base. Adductor and abductor muscles mechanically connect the cartilaginous rod in the filament to the arch. The contraction of the abductor muscles spreads the hemibranchs farther apart, whereas the contraction of the adductor muscles moves the tips of the filaments of the hemibranchs on the arch closer to each

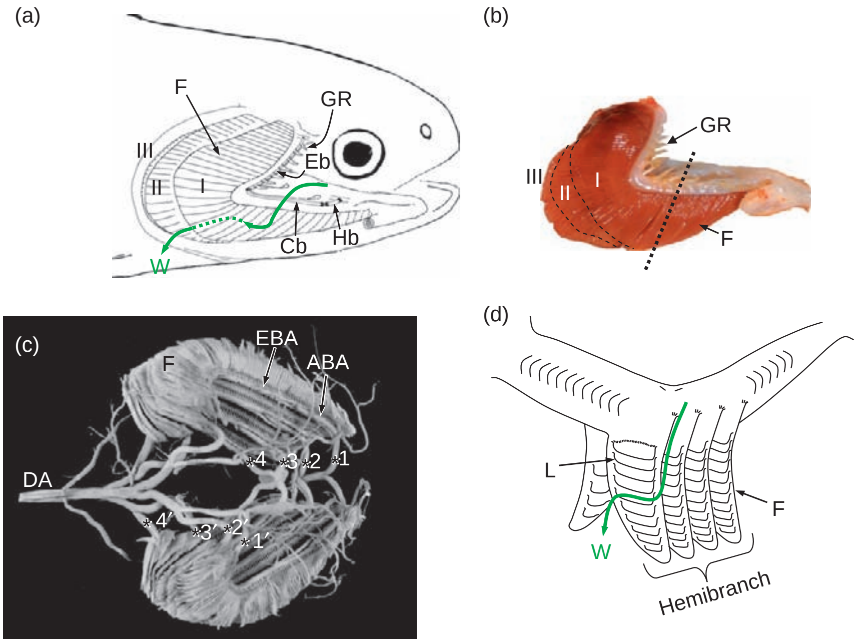


Figure 1 General orientation of the gills. (a) Eight gills line the buccal (mouth) cavity of the fish, four on each side. Three gills, I, II, and III, on the right side of the fish are shown in the figure. The gill arch supports the filaments (Fs) and gill rakers (GRs). Three bones in the arch, hypobrachial (Hb), ceratobrachial (Cb), and epibrachial (Eb), provide strength and are hinged, permitting the gills to move with the jaw during ventilation. Water flows past the gill rakers, over the arch, and between the filaments (green arrows, W). (b) Gills removed from a fish showing three of the four gills (I, II, and III, separated by dashed lines). Dotted line indicates plane of cross section through the arch viewed in **Figure 2**. (c) Dorsal view of a vascular corrosion replica of catfish gills, anterior to right. 1–4, afferent branchial arteries (ABAs) deliver blood to corresponding gill arches (asterisk directly on vessel to left of number); 1′–4′ efferent brachial arteries (EBAs) drain blood from the corresponding gill arches and deliver it to the dorsal aorta (DA). (d) Schematic of a single gill arch showing the relationship of the filaments (Fs) of one hemibranch and the direction of water flow (W; green arrow) between the filaments of one hemibranch and across the respiratory lamellae (L). GRs on the ventral portion of the arch are not shown.

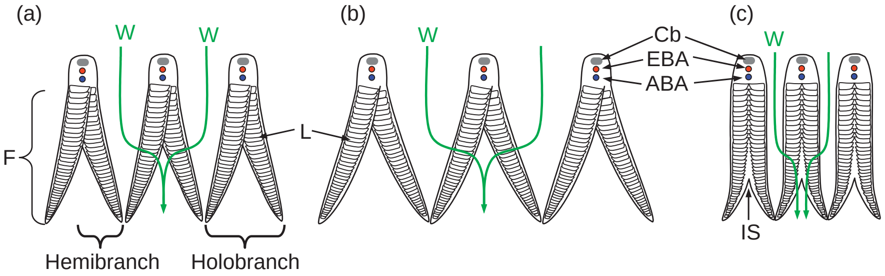


Figure 2 Cross section through three adjacent gill arches showing the freedom of movement of filaments in gills lacking an intrabranchial septum (IS; a, b) compared to the restriction imposed by the IS (c). Filaments on each side of the gill form a hemibranch and two hemibranchs on a single gill arch form a holobranch. Water flows between hemibranchs of adjacent gill arches, passes across the respiratory lamellae (L), and exits between the hemibranchs on the same gill arch. Other abbreviations: Cb, ceratobranchial bone of arch skeleton; ABA, afferent branchial artery; EBA, efferent branchial artery.

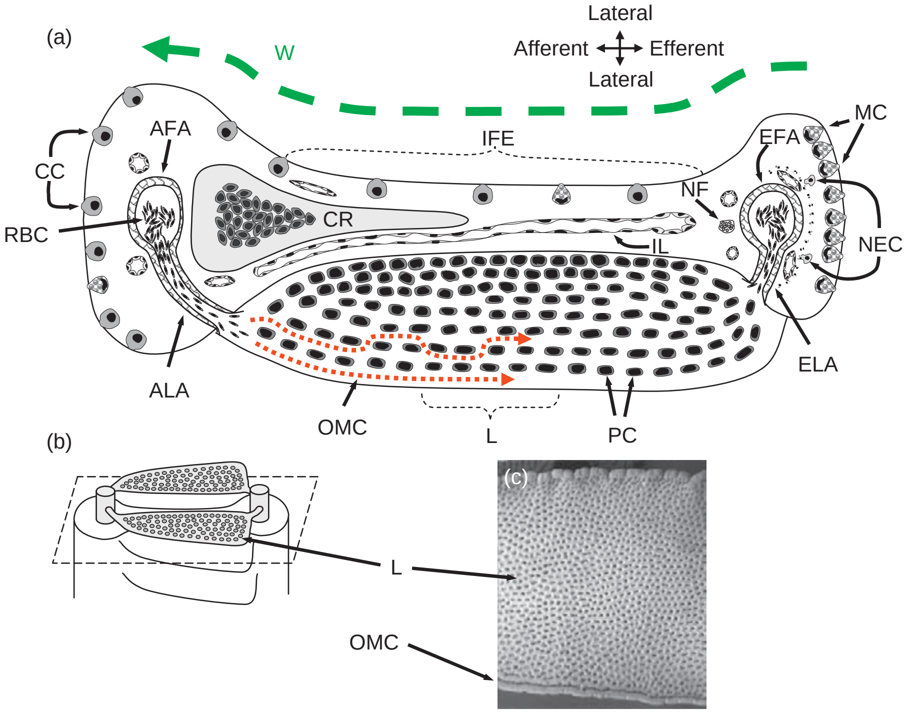


Figure 3 (a) Cross section through a gill filament including a planar section through one respiratory lamella (L); inset (b) shows plane of section. Blood (red blood cells; RBCs) from the afferent filamental artery (AFA) passes through the afferent lamellar arteriole (ALA) and enters the lamellar sinus where it percolates around the pillar cells (PCs) or travels around the periphery of the lamella through the outer marginal channel (OMC). Blood leaves the lamella via the efferent lamellar arteriole (ELA) and is drained from the filament via the efferent filamental artery (EFA). Water flow (W; dashed green arrow) is countercurrent to lamellar blood flow (dotted red arrows). A cartilaginous rod (CR) runs the length of the filament and provides structural support. Ionocytes (chloride cells, CCs) are most prevalent in the epithelium along the afferent edge of the filament and in the interlamellar filamental epithelium (IFE), that is, the filamental epithelium between the lamellae. Mucus cells (MCs) and an occasional sensory neuroepithelial cell (NEC) are more common along the efferent edge of the filament, although the MC can be found throughout the filament. One or several nerve fibers (NFs) are often found near the EFA. Only a portion of the extensive interlamellar vasculature (IL) is shown. (c) Scanning electron micrograph of a lamellar vascular corrosion replica shows the dilated OMC; the numerous dark holes were once occupied by pillar cells.

other (**Figures 2**(**a**) and **2**(**b**)). This motion allows for expansion and contraction of the buccal cavity during respiration and it keeps the filament tips of hemibranchs lining a common water channel in close contact with each other. This creates an effective sieve and forces water to flow across the respiratory surfaces of the gill. Without this apposition, water would too easily flow past the gills without contacting the respiratory exchange surface, creating an anatomical dead space for respiratory water flow.

Other fish have an intrabranchial septum (IS) between, and attached to, the filaments of adjacent hemibranchs on the arch (**Figure 2**(**c**)). The extent of the IS limits the flexibility of the filaments. In fish such as tuna, elasmobranchs, and a few others, the intrabrachial septum extends all the way to the body wall (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics).

Gill Filament

The filament is the functional anatomical unit of the gill and on it are all of the cells and tissues necessary for the various homeostatic activities. The number of filaments on a gill arch increases as fish grow. For example, a 0.35-g rainbow trout (*Onchorhynchus mykiss*) has around 770 total filaments, which increases to approximately 1600 in a 400-g trout. The filament number also varies somewhat from arch to arch; usually, there are more filaments on the first or second arch and often more on the anterior than on the posterior hemibranch.

The filament is a long, flattened knife-blade-like structure that supports numerous respiratory (secondary) lamellae while offering minimal resistance in the respiratory water current. A cross section through the filament is shown schematically in **Figure 3**. By convention, the orientation of the filament is defined by the major blood vessels; the afferent end is nearest the afferent filamental artery (AFA), and the efferent end nearest the efferent filamental artery (EFA). Filaments appear somewhat dumb-bell in shape when viewed in cross section, expanded at the afferent and efferent ends, while the thinner tissue in between supports the respiratory lamellae.

Mechanical support to the filament is provided by a cartilaginous rod near the AFA that extends from the gill arch to the tip of the filament. In some fish, thin spines project perpendicularly from the rod toward the EFA. Presumably, they help stabilize the efferent edge of filament against the incoming water current. The movement of the cartilage rod is controlled by striated and smooth muscle in the arch and base of the filament (see earlier).

The main nerve bundle in the filament nerve usually runs parallel to and near the EFA (**Figure 3**). Additional smaller nerves are found along the AFA. These nerves carry sensory information from cells monitoring the position of the filament (proprioceptors), chemical composition of the water or blood (chemoreceptors), or noxious stimuli (nocioceptors) (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmosensing and **Control of Respiration**: Oxygen Sensing in Fish). Other nerves go to the adductor and abductor muscles and to blood vessels (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes).

Respiratory Lamellae

Respiratory lamellae are thin plate-like structures that spread the blood out into a thin sheet with a minimal diffusion distance between the blood and water. Lamellae project at right angles from both sides of the filament. The outer (free) margins of lamellae from adjacent filaments on the hemibranch nearly interdigitate. This forces respiratory water to flow between the lamellae and over the lamellar surface. In nearly all fish, lamellar blood and respiratory water flow in opposite directions, that is, countercurrent (**Figure 3**(**a**)). This close apposition of adjacent lamellae and countercurrent blood–water flow optimizes gas-exchange efficiency.

Lamellar shape may vary between species and even along a single filament. Most lamellae, however, are in the form of a right triangle with the edge of the lamella facing the incoming water (efferent side) as the right arm. Presumably, this maximizes oxygen loading of the blood just prior to exiting the lamella.

Lamellar pillar cells define the vascular space. They are spool-shaped cells with a large central nuclei and broad cytoplasmic flanges that radiate out from the top and bottom of the cell. The flanges connect pillar cell to pillar cell and in so doing create a flat vascular sinusoid between them (**Figures 3–5**). True endothelial cells are only found at the very outer margin of the lamellar sinus, and they help form the so-called outer marginal channel for blood flow (**Figures 4** and **5**).

The two layers of basement membrane that overlie the pillar cells are connected to each other by numerous collagenous columns (**Figure 5**). This prevents the lamella from ballooning out under the high intravascular pressure. The pillar-cell plasma membrane wraps around these columns, much like a tree growing around a wire fence (**Figure 5**(**b**)). Thus, even though the column remains outside the cell, it is effectively separated from the plasma and cannot initiate a blood clot. Typically, there are 4–10 columns per pillar cell. Myosin filaments within the pillar cell may provide additional mechanical support or they may help regulate blood flow.

Epithelial Cells

At least seven types of cells populate the gill epithelium: pavement, ionocyte, mucus (chloride cells), neuroepithelial, taste, rodlet, and undifferentiated. Pavement cells (**Figure 6**(**a**)) are simple squamous cells. They are relatively thin, often hexagonal, and their surface contains microridges or microvilli believed to help trap a protective coat of mucus. Pavement cells are the most numerous epithelial cells (over 95%) and their primary function is to form a relatively impermeable barrier between the water and tissue. The cell nucleus of lamellar pavement

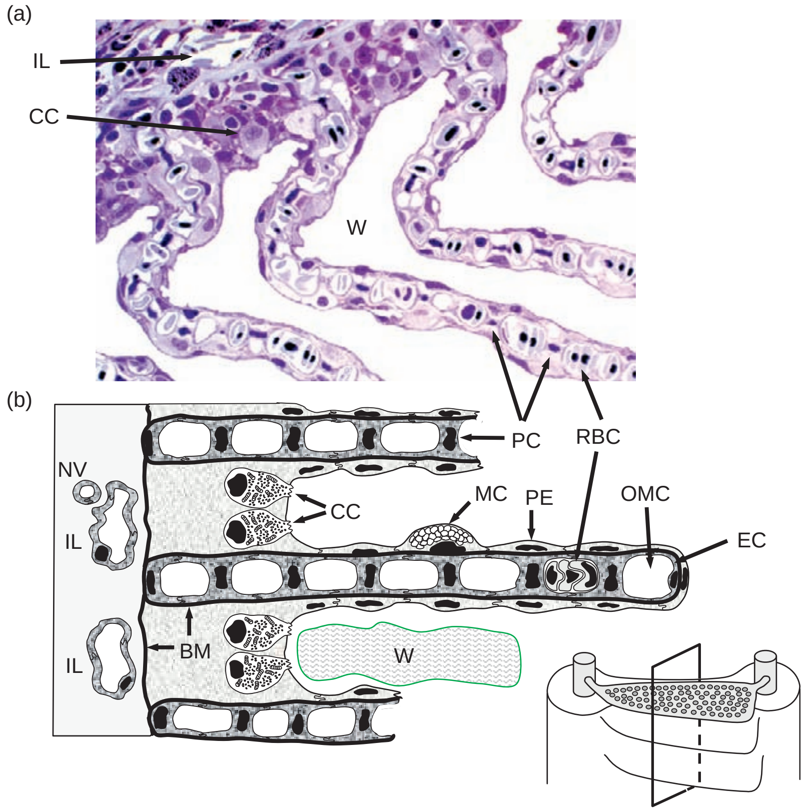


Figure 4 Cross section through several respiratory lamellae ((a), light micrograph; (b), schematic); inset shows plane of section. The lamella consists of two surface layers of pavement cell epithelium (PE) that cover a planar sheet of pillar cells (PCs). Cytoplasmic flanges from adjacent PC form the vascular space (VS), which is occupied by red blood cells (RBCs). The thick nuclear region of the PE is aligned with the pillar-cell body to minimize the blood–water diffusion distance. True endothelial cells (ECs) are only found around the periphery of the outer marginal channel (OMC). Chloride cells (CCs) are common in the interlamellar filamental epithelium but may also be found on the lamella (not shown) along with occasional mucus cells (MCs). The basement membrane (BM) forms a continuous sheet over the lamella between the PE and PC and along the filament interior to the inner margins of the lamella. Filamental nutrient vessels (NVs) and interlamellar (IL) vessels lie beneath the BM of the filament. W, water. Modified from Olson KR (1991) Vasculature of the fish gill: Anatomical correlates of physiological function. *Journal of Electron Microscopy Technique* 19: 389–405.

cells often lies directly above the body of the pillar cell and the thinner, anuclear, region above the pillar-cell flanges and vascular space. This minimizes the diffusion distance between water and blood.

Mucus cells (also called goblet cells) are scattered over the gill arch, filament (they are especially prevalent on the edge of the filament facing the water current), and basal regions of the lamella (**Figures 3** and **6**(**b**)). Mucus secreted by these cells flows over the surface of the gill, and, in addition to its lubricating function, has been reported to have some antibacterial properties. Mucus may even act as a semipermeable barrier to electrolytes. The number and/or size of mucus cells and the rate of mucus secretion may be increased by adaptation to high salinity (seawater), ion-poor water, mechanical abrasion, or a variety of waterborne contaminants. Other cells, called granular cells, embedded in the epithelium may secrete mucus intermittently.

Ion-transporting cells (ionocytes or chloride cells) are most prevalent on the body, or alamellar, portion of the filament, especially along the afferent margin of the filament and in the interlamellar filamental epithelium, that is, the filament epithelium between adjacent lamellae (**Figures 3** and **4**). If ionocytes are present on the lamellar epithelium, they are most commonly located near the filament. Ionocytes are rarely observed on the more lateral areas of the lamella, although they may appear here as well under certain conditions, such as excessively low environmental sodium and chloride. Ionocytes in freshwater fish typically project a broad microvillous surface (**Figure 6**(**c**)) whereas those from saltwater fish are recessed within the crypt just beneath the epithelium and are also associated with accessory cells (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts, and Mechanisms of Ion Transport in Freshwater Fishes). Ionocytes are rarely found along the efferent margin of the filament. All ionocytes have two striking features important for ion regulation: abundant mitochondria and a basolateral membrane that is so extensively invaginated that all but the apical region of the cell appears to be filled with a tubular reticulum.

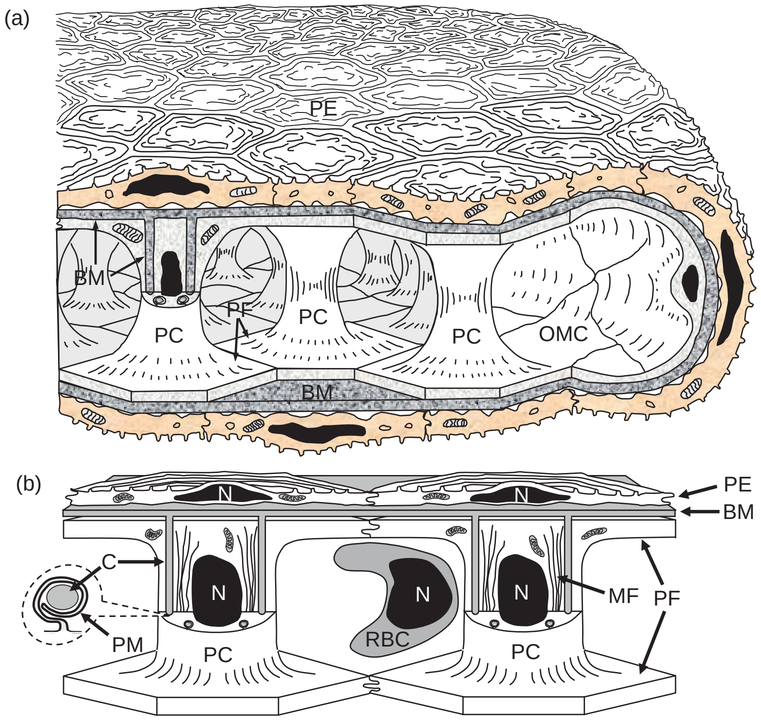


Figure 5 Schematic of a respiratory lamella and pillar cells. Cytoplasmic flanges (PF, arrows) extend from pillar cells (PCs) and surround the vascular space. The basement membrane (BM) separates the PC from the pavement epithelium (PE). The outer marginal channel (OMC) is shown on the right in upper panel. The two layers of BM are connected by numerous columns (C; N) and the PC plasma membrane (PM) wraps around the columns. Myosin filaments (MFs) in the pillar cell provide additional structural support and may be contractile. RBC, red blood cell.

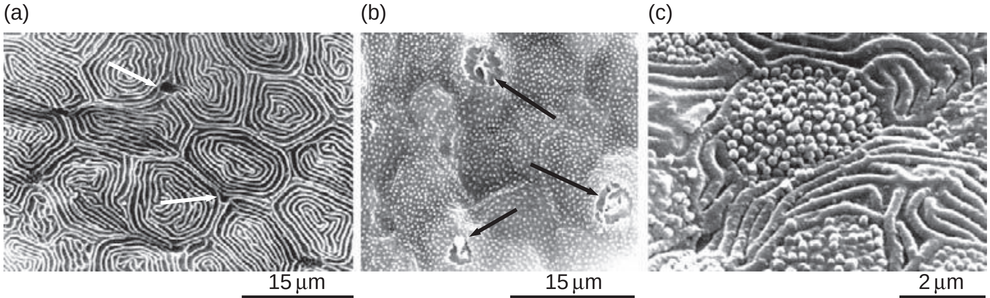


Figure 6 Scanning electron micrographs of gill epithelial cells. Whorled pavement cells (a) are common on arch support tissue and filament body and often interspersed with recessed mucus cells (arrows). (b) Surface mucus cells. A mucus droplet is being secreted from the cell near bottom of micrograph. (c) Microvillous chloride cells of freshwater fish are distinct from adjacent ridged pavement epithelial cells. Modified from Olson KR (1996) Scanning electron microscopy of the fish gill. In: Dutta HM and Munshi JSD (eds.) *Fish Morphology: Horizons of New Research*, pp. 31–46. Lebanon, NH: Science Publishers.

Neuroepithelial cells are also thinly scattered along the efferent margin 50–100 µm beneath the epithelium (**Figure 3**). They serve as chemoreceptor cells and monitor the oxygen tension in both water and blood (**see also Control of Respiration**: Oxygen Sensing in Fish). Interstitial and undifferentiated cells are found throughout the body of the filament. Undifferentiated cells in the interlamellar area are precursor cells for new chloride cells. Undifferentiated cells embedded in the area of the inner margin of the lamella differentiate into lamellar pillar cells, and the lamellae essentially grow outward from the filament as the fish grows.

Taste buds are generally restricted to the arch support tissue and gill rakers; they are rarely found on filaments and are absent from lamellae. Taste buds have two types of sensory cells, light and dark, and a single taste bud may have 10–25 of each (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in

Fishes). Rodlet cells are thinly dispersed on the gill arch, body of the filament, interlamellar filamental epithelium, and basal areas of the lamellae. Rodlet cells appear to be secretory; however, their specific function is unknown.

Gill Vessels

Gill Arch

The arrangement of gill vessels is shown schematically in **Figure 7**. The afferent branchial artery (ABA) enters the anterioventral end of the arch and travels approximately one-quarter to one-third of the length into the arch before branching, one branch turning anteriorly and the other, main branch, continuing ventrally. AFAs arise repeatedly from the ABA and travel to the tips of the filaments. The filament is drained by EFAs and filamental veins. EFAs join the efferent branchial artery (EBA) and exit the arch to become the systemic arterial circulation. This is known as the arterio-arterial pathway. Filamental veins drain into one of the several branchial veins and blood is returned directly to the heart, thus completing the arteriovenous pathway.

The EBA lies anteriodorsal to the ABA except in the anterior end of the arch where it branches in order to pass around the ABA. Here, each branch only drains filaments from its respective hemibranch. The lateral (outside) branch from the first gill arch EBA exits the arch and supplies the jaw, and, in some fish, the pseudobranch. The medial (inside) branch from the second and sometimes third and fourth gill arches also exit the arch and form the hypobranchial (systemic) arterial system that delivers blood to the lower jaw and the heart. These small anterior branches carry only a fraction of the cardiac output. Most of the arterio-arterial flow exits the arch via a single posterio-dorsal EBA; these become the epibranchial arteries of the systemic circulation.

The largest veins in the gill arch (branchial veins) usually travel close to the ABA. They exit the arch from both anterio-ventral and posterio-dorsal ends and drain into the jugular or anterior cardinal veins, respectively. Nutrient supply to the arch tissues, including gill rakers, comes from numerous small arterioles that arise from either the EBA or the EFAs near their junction with the EBA. These arterioles anastomose repeatedly with each other to form the nutrient arteries. Nutrient vessels ultimately drain into the branchial veins. The vascular anatomy of the systemic arterial and venous circulations is described in the article **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns and the origin of the gill nutrient vessels is described in the article **Design and Physiology of Capillaries and**

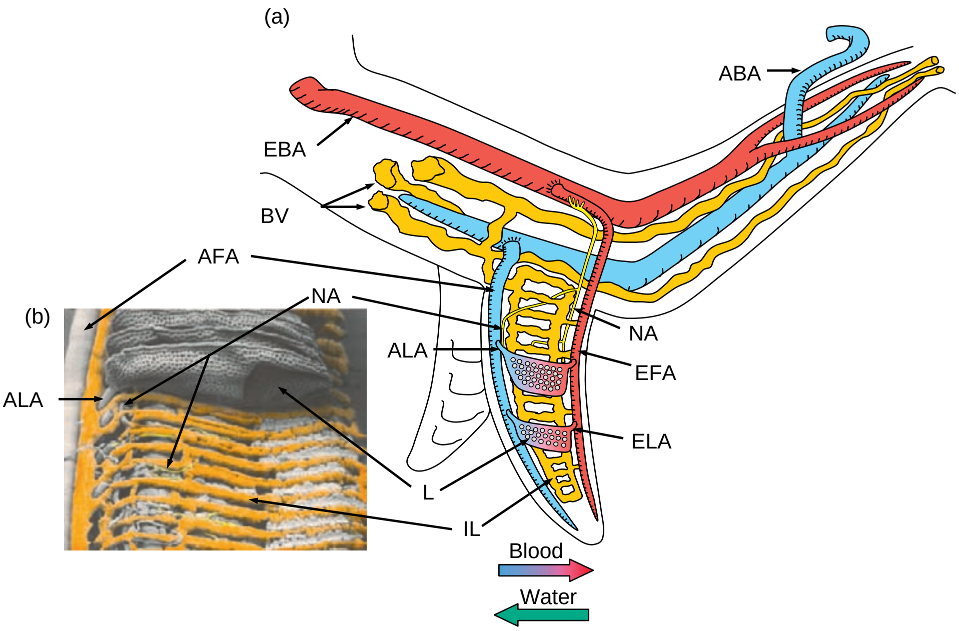


Figure 7 Blood vessels in the gill. (a) The afferent brachial artery (ABA) delivers blood to, and distributes it along, the gill arch. Afferent filamental arteries (AFAs) distribute blood to the respiratory lamellae (L) via afferent lamellar arterioles (ALAs). Oxygenated blood leaves the lamellae through short efferent lamellar arterioles (ELAs), and from there passes into the efferent filamental arteries (EFAs) and then into the efferent branchial artery (EBA). Small nutrient arteries (NAs) originate from the EFA near the base of the filament and reenter the filament. The intra-lamellar vasculature (IL) forms an extensive sinus with the filament that ultimately drains into the branchial veins (BV) in the gill arch. (b) Scanning electron micrograph of a vascular corrosion replica of the filamental circulation. (a) Modified from Olson KR (1991) Vasculature of the fish gill: Anatomical correlates of physiological function. *Journal of Electron Microscopy Technique* 19: 389–405

Secondary Circulation: Secondary Circulation and Lymphatic Anatomy.

Gill Filament

There are three main vascular networks in the gill filament: respiratory, nutrient, and interlamellar. The respiratory pathway consists of the AFA, afferent lamellar arteriole, lamella, efferent lamellar arteriole, and EFA (**Figures 3–5** and **7**). Afferent lamellar arterioles are typically much longer than the efferent lamellar arterioles. The vascular space around the outer margin of the lamella is called the outer marginal channel (**Figures 3**(**a**), **3**(**b**), **3**(**c**), and **5**(**a**)). This pathway is larger in diameter than elsewhere in the lamella. There is some debate over whether the outer marginal channel increases the efficiency of gas exchange because the red cells are exposed to the exchange surface on three sides instead of two, or whether it acts as a shunt to minimize untoward water and salt exchange. Around 15–20% of the lamellar vasculature is embedded in the body of the filament and is not available for gas exchange. The innermost vascular channel of the lamella is called the inner marginal channel, although the pillar cells are packed so close together in this region that neither it is dilated nor it is a direct route across the lamella. This vascular space may, however, be important in metabolizing blood-borne molecules (**see also Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation).

After percolating through the lamella, blood is collected into a short efferent lamellar arteriole that subsequently anastomoses with the lateral wall of the EFA. From here, most of the blood (>90%) drains from the filament into the EBA and continues on to the systemic circulation (**Figure 7**). A highly innervated smooth-muscle sphincter in the EFA, just prior to its anastomosis with the EBA, may be important in regulating blood flow through the filament.

The alamellar body of the filament is perfused by two circulations, a nutrient system and an extensive interlamellar vasculature. Both of these vascular networks lie beneath the basal membrane that envelops the filament (**Figure 4**(**b**)). Nutrient arteries originate from the wall of the EFA near its base or from the EBA (**Figure 7**). These arteries start as small, tortuous arterioles and they repeatedly re-anastomose with each other to form progressively larger nutrient arteries to supply both the arch and filaments. The filamental nutrient artery usually travels singularly (or rarely branched) along and medial to the EFA. Upon entering the filament, one or several branches from the main nutrient artery travels across the filament and these branches then proceed toward the filament tip either through the middle of the filament or along the AFA. In some instances, narrow arterioles arise directly from the medial wall of the peripheral EFA and directly supply nutrient capillaries.

The second vascular system in the filament is the interlamellar system. These vessels have a thinner wall but larger diameter than the nutrient capillaries (**Figure 4**(**b**)). Interlamellar vessels traverse the body of the filament in register with, but between and medial to, the inner margins of the respiratory lamella (**Figures 7**(**a**) and **7**(**b**)). An interlamellar system is found on both sides of the filament along the AFA and around the cartilage support rod. Toward the efferent border of the filament, interlamellar vessels from the two sides come together and form a common system. Often, extensions of the interlamellar vessels wrap around both afferent and EFAs. Interlamellar vessels are supplied by narrow-bore arterioles from the medial wall of the EFA, or by nutrient arterioles. In a few fish, such as eel, *Anguilla anguilla*, and catfish, *Ictalurus punctatus*, a portion of the interlamellar system is supplied by narrow-bore arterioles from the AFA or afferent lamellar arteriole; these vessels are called prelamellar arteriovenous anastomoses (AVAs). However, prelamellar AVAs are rare in most fish. The interlamellar system has also been considered to be part of the secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy). The function of the interlamellar system is unknown.

Innervation of the Gill Arch

Gill nerves (branchial nerves) enter the gill arch via the posterio-dorsal end. These nerves may travel together as a single branch, or in two to three bundles in the arch, usually between the arch skeleton and either the EBA or the basal extension of the EFA. These fibers supply the arch tissues and penetrate into the filaments. Teleost gills are innervated by cranial nerves IX (glossopharyngeal) and X (vagus). These nerves are classified as somatic sensory, visceral sensory, and visceral motor. All are of dorsal root origin and majority of them are sensory. The first gill arch is innervated by the glossopharyngeal and vagus nerves; the remaining arches are innervated by the vagus nerve. Spinal autonomic (sympathetic) nerves travel with the cranial nerves to the gills. Additional details on gill innervation can be found in the article **Brain and Nervous System**: Autonomic Nervous System of Fishes.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Control of Respiration**: Oxygen Sensing in Fish. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns. **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy. **Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine

Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmosensing. **Smell, Taste, and Chemical Sensing**: Morphology of the Gustatory (Taste) System in Fishes. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Bartels H (1998) The gills of hagfishes. In: Jørgensen JJ, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 205–222. London: Chapman and Hall.

Burleson ML (2009) Sensory innervation of the gills: O_2-sensitive chemoreceptors and mechanoreceptors. *Acta Histochemica* 111: 196–206.

Butler PJ (1999) Respiratory system. In: Hamlett WC (ed.) *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*, pp. 174–197. Baltimore, MD: The Johns Hopkins University Press.

Dunel-Erb S, Bailly Y, and Laurent P (1989) Neurons controlling the gill vasculature in five species of teleosts. *Cell and Tissue Research* 255: 567–573.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Hughes GM (1984) General anatomy of the gills. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, Volume X, Gills, Part A: Anatomy, Gas Transfer, and Acid–Base Regulation*, pp. 1–72. New York: Academic Press.

Hughes GM and Morgan M (1973) The structure of fish gills in relation to their respiratory function. *Biological Reviews* 48: 419–475.

Jonz MG and Zaccone G (2009) Nervous control of the gills. *Acta Histochemica* 111: 207–216.

Laurent P (1984) Gill internal morphology. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, Volume X, Part A: Anatomy, Gas Transfer, and Acid–Base Regulation*, pp. 73–183. New York: Academic Press.

Laurent P and Dunel S (1980) Morphology of gill epithelia in fish. *American Journal of Physiology* 238: R147–R159.

Laurent P and Dunel-Erb S (1984) The pseudobranch: Morphology and function. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, Volume X, Gills, Part B: Ion and Water Transfer*, pp. 285–323. New York: Academic Press.

Laurent P and Hebibi N (1989) Gill morphometry and fish osmoregulation. *Canadian Journal of Zoology* 67: 3055–3063.

Nilsson S (1985) Filament position in fish gills is influenced by a smooth muscle innervated by adrenergic nerves. *Journal of Experimental Biology* 118: 433–437.

Olson KR (1991) Vasculature of the fish gill: Anatomical correlates of physiological function. *Journal of Electron Microscopy Technique* 19: 389–405.

Olson KR (1996) Scanning electron microscopy of the fish gill. In: Dutta HM and Munshi JSD (eds.) *Fish Morphology: Horizons of New Research*, pp. 31–46. Lebanon, NH: Science Publishers.

Olson KR (2002) Vascular anatomy of the fish gill. *Journal of Experimental Zoology* 293: 214–241.

Physiology of Resistance Vessels

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction to the Arterial System
Anatomy of Resistance Vessels
Hemodynamics: The Importance of Vascular Resistance on Blood Pressure and Blood Flow
Remote Control of Vascular Resistance
Local Control of Vascular Resistance
Factors That Affect Vascular Resistance
Methods for Measuring Arterial Resistance in Fish
Further Reading

Glossary

Adrenergic A general term pertaining to processes or structures related to catecholamines. For example, neurons that release noradrenaline as transmitter; catecholamines binding to adrenergic receptors (adrenoceptors), and adrenergically activated (catecholamine-activated) physiological processes.

Adrenoceptor A receptor located in the cell membrane that binds the hormones adrenaline and noradrenaline (see adrenergic receptor).

Arterioles Muscular blood vessels that form the terminal end of the arterial circulation. They offer the greatest impediment to blood flow by virtue of their relatively narrow diameter. Their diameter is controlled by neural and endocrine signals and by local factors released from the endothelium or nearby tissue.

Cannula A tube placed into a blood vessel to measure blood pressure or provide a site for injection of drugs. It is sometimes referred to as a catheter as well.

Catecholamine Any of a class of amines derived from a catechol that acts as chemical messangers (a hormone or neurotransmitter). The main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Cholinergic A general term pertaining to processes or structures related to acetylcholine. For example, neurons that release acetylcholine as transmitter. Nicotinic and muscarinic receptors are cholinergic receptors because they bind acetylcholine. Cholinergic synapses are found in both the central and peripheral nervous systems.

Chromaffin cells Cells from the sympathetic-adrenal lineage that synthesize and store catecholamines as well as release them into the blood (via the extracellular fluid).

Heme A large molecule that consists of an iron atom contained in the center of a large heterocyclic organic ring called a porphyrin. It is found in hemoglobin, myoglobin, and cytochromes.

Metarterioles The smallest arterioles and the location of much of the local regulation of blood flow through capillaries.

Perfuse To pump blood or fluid through a tube or blood vessel.

Precapillary sphincters A single smooth muscle wrapped around a metarteriole at the terminal end of the arterial tree and at the entrance to a capillary network.

Introduction to the Arterial System

The resistance vessels derive their name from the fact that they offer the greatest impediment to blood flow. These vessels are usually small arteries and arterioles. Control of their diameter regulates blood flow through tissues and can profoundly impact arterial blood pressure. Resistance vessels are found in both the gills and the systemic circulations; this article emphasizes the latter. Resistance vessels are under the influence of two control systems. The remote control system operates more or less uniformly throughout the body to regulate arterial blood pressure. The local system adjusts blood flow and its pattern of tissue flow to metabolic distribution through tissue capillaries to match tissue metabolic demand. Usually, the larger resistance vessels operate predominately under the influence of the remote system and the smaller arterioles, the local system.

Anatomy of Resistance Vessels

Much of the resistance to blood flow in mammalian tissues is located in small ($<300\,\mu\text{m}$ diameter) vessels. This is assumed to be the case in fish as well. Collectively, these vessels include small arteries, arterioles, and metarterioles.

Resistance arteries and arterioles (~300–30 μm) are characterized by an abundance of smooth muscle wrapped circumferentially around the vessel that allows exquisite control of their diameter. The terminal end of the arteriole (~30–5 μm) has just a single smooth muscle cell encircling it at the very entrance to a capillary bed and is called the precapillary sphincter. Like all fish blood vessels, they are lined by a single layer of endothelial cells and they have less elastic tissues than the larger arteries. Larger arteries may be heavily innervated, but the degree of innervation becomes progressively less in smaller vessels and nerves are usually absent from the smallest arterioles.

Hemodynamics: The Importance of Vascular Resistance on Blood Pressure and Blood Flow

As described in the article **Circulation:** Circulatory System Design: Roles and Principles, central arterial blood pressure is obtained by rearrangement of Darcy's law: $\Delta P = F/R$, where ΔP is the difference between arterial and venous blood pressure, F equals blood flow, which in this case is cardiac output, and R equals vascular resistance. Vascular resistance $R = 8l\eta/\pi r^4$, where 8 and π are geometric constants, and l, η, and r are the vessel length, blood viscosity, and vessel radius, respectively. Thus, arterial blood pressure is directly proportional to cardiac output and inversely proportional to the fourth power of vessel radius. The arterial blood pressure is dispersed equally throughout the body and it is kept relatively constant by a control system that is integrated at the level of the central nervous system. Consequently, the remote control is achieved predominantly through neural reflexes and hormones.

Tissue blood flow is also obtained by a rearrangement of Darcy's law: $F = \Delta P/R$, where flow now is the blood flow through a tissue and ΔP is the difference between arterial and venous blood pressure. Under normal circumstances, arterial pressure is regulated within relatively narrow limits and thus tissue blood flow becomes inversely proportional to the fourth power of vessel radius. As tissue blood flow must be regulated by the needs of individual tissues, it is regulated predominately through local factors, usually metabolic signals, that provide an index of the tissue's metabolic need. As a result, factors that increase arterial or arteriolar resistance decrease blood flow downstream, and increase arterial pressure upstream.

Remote Control of Vascular Resistance

Neural Control

The anatomy and physiology of the fish autonomic nervous system is described in the article **Brain and Nervous System:** Autonomic Nervous System of Fishes. Most systemic blood vessels are innervated by adrenergic (sympathetic) nerves, but they may be lacking in gill vasculature of cyclostomes and elasmobranchs. Only gill blood vessels are innervated by cholinergic nerves. In general, sympathetic nerve stimulation increases systemic vascular resistance and thereby decreases blood flow to those vascular beds where the resistance was increased. This is achieved predominately through activation of α-adrenoceptors located on the vascular smooth muscle cells.

Fish vessels are also innervated by nonadrenergic, noncholinergic (NANC) nerves. Their neurotransmitters include nitric oxide (NO), serotonin (5-hydroxy-tryptamine), purines, and peptides such as vasoactive intestinal peptide (VIP), substance P, tachykinins, and others. The effects of neuronal release of these messengers *in vivo* is an area of active investigation.

Hormonal Control

Catecholamines

Vascular smooth muscle in blood vessels may also receive adrenergic stimulation via the circulating catecholamines, namely epinephrine and norepinephrine (**see also Hormonal Responses to Stress:** Catecholamines). These hormones are released in varying amounts directly into venous blood from chromaffin cells commonly located near the great veins in the trunk, especially the posterior cardinal vein. Chromaffin cells are also found in the hearts of some lower fish. Vascular chromaffin cells are homologous to those that release catecholamines from the mammalian adrenal medulla. The concentration of plasma catecholamines is generally quite low (1–10 nM) and this may not be sufficient to affect vascular resistance, or at best it may provide only a low level of stimulation. Whether or not vascular smooth muscle is stimulated depends on the affinity and type of its adrenergic receptors, as well as the catecholamine concentration in the blood. Severe stress, however, can increase catecholamine release and blood concentrations by 10–100 times, which can significantly increase vascular resistance.

Renin–angiotensin system

The potent vasoconstrictor molecule angiotensin II (ANG II) is formed from the step-wise metabolism of a circulating protein, angiotensinogen, by the enzyme renin, to the inactive 10-amino-acid peptide, angiotensin I, and subsequent metabolism by angiotensin converting enzyme to ANG II. The effects of ANG II appear to be somewhat limited to resistance vessels as large arteries and veins are often insensitive to the hormone. ANG II has two effects on blood vessels. It directly stimulates vascular smooth muscle cells to contract; it also contracts vessels by stimulating norepinephrine release from the adrenergic nerves. In some fish, the indirect effects of adrenergic nerve stimulation are even greater than direct effects. The renin–angiotensin system has been demonstrated in teleosts, elasmobranchs, and lampreys. In teleosts, the renin–angiotensin system is often tonically active in regulating arterial blood pressure. Thus, if the renin–angiotensin system is chemically blocked with the inhibitor that prevents formation of ANG II, such as captopril, blood pressure would decrease without any other change. The renin–angiotensin system does not appear to be tonically active in elasmobranchs.

Natriuretic peptides

Peptides that belong to the family of natriuretic peptides (NPs), including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C natriuretic peptide (CNP; up to four subtypes), and ventricular natriuretic peptide (VNP), have been identified in bony fishes. Hagfish have a single, unique NP and only CNP has been found in elasmobranchs. All NPs are potent vasodilators, and they effectively relax vascular smooth muscle and decrease vascular resistance.

Arginine vasotocin

Arginine vasotocin (AVT) is similar to the mammalian arginine vasopressin (also known as the antidiuretic hormone, ADH). AVT is a potent vasoconstrictor, although it has been shown to relax the hepatic vein of the gar.

Bradykinin

Bradykinins (BKs) are somewhat similar to angiotensins in their structure and biosynthesis. Although bradykinins typically produce vasodilation in mammals, in fish they have both vasodilatory and vasoconstrictory effects. In addition to directly stimulating smooth muscle, bradykinins also stimulate the release of vasodilatory prostaglandins from the endothelium.

Urotensins

The urotensins are members of an ancient family of neuropeptides. Urotensin-I (U-I) produces an indirect α-adrenoceptor-mediated increase in systemic resistance and blood pressure when injected into fish. However, U-I has the opposite effect of relaxing blood vessels *in vivo* or lowering blood pressure *in vivo* when the α-adrenoceptors are blocked. Urotensin II (U-II) is a potent vasoconstrictor of arterial vessels in all fish and its actions are independent of α-adrenoceptor activation. U-II does not appear to affect veins in isolated preparations.

Adrenomedullin

Adrenomedulins (AMs) are a family of vasodilator peptides in mammals. Five AMs have been identified in fish, and some of these are proposed as circulating hormones of head-kidney origin. When injected into fish, they produce a profound drop in blood pressure and appear to be more effective on systemic arteriolar resistance than on gill resistance. Regulation of endogenous AM secretion and its effect on cardiovascular function in fish has not yet been investigated.

Local Control of Vascular Resistance

Metabolites

Local consumption or production of metabolic substrates has direct effects on vascular smooth muscle, thereby coupling blood flow to metabolic needs. A drop in oxygen partial pressure (P_{o_2}), which accompanies an increase in metabolism if blood flow is not increased accordingly, produces vasodilation in many vascular beds and thereby provides a local mechanism to match local capillary blood flow to local metabolism. The notable exception is the gill where hypoxia produces vasoconstriction. An increase in metabolism also increases carbon dioxide (CO_2) production and decreases pH (an increase in H^+ concentration). Both CO_2 and H^+ produce vasodilation. Other vasodilators that are associated with an increase in metabolism, such as adenine nucleotides and other intermediates in oxidative phosphorylation (ATP, ADP, and AMP), appear to be vasodilators in fish, as they are in mammals, but they have been poorly studied in fish.

Paracrine-Mediated Responses

Paracrine signals, liberated by the endothelium, vascular smooth muscle, or even surrounding tissue, diffuse through the interstitial space and stimulate adjacent vascular smooth muscle. Many of these signaling molecules may be more potent than neural or endocrine messengers.

Arachnidonic acid metabolites

Metabolites of arachnidonic acid include the prostaglandins, leukotrienes, and thromboxane. These paracrine signals are released by the vascular endothelium and affect adjacent vascular smooth muscle. Molecules that cause vasodilation are known as endothelium-derived relaxing factors (EDRFs) and those that constrict are known as endothelium-derived contracting factors (EDCFs). Prostaglandin E_2 (PGE_2) and I_2 (PGI_2, prostacyclin) are well-known EDRFs, although PGI_2 has been shown to constrict some experimental preparations. Thromboxane A_2 and its analogs are consistent EDCFs.

Endothelin

Endothelin-1, also released from the endothelium, constricts most, but not all, systemic vessels in experimental preparations. It is one of the most potent constrictors of gill resistance vessels known.

Gasotransmitters

Three gases, nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S), were all originally thought to be toxic molecules but have since been shown in mammals to be important signaling molecules in the cardiovascular system as well as in other tissues. They have been given the name gasotransmitters to reflect their actions parallel with the neurotransmitters. NO is produced by the vascular endothelium and may be secreted by nerves, CO can be produced by both endothelial and vascular smooth muscle cells, whereas all tissues (vascular and extravascular) can produce H_2S. All of these gasotransmitters are rapidly inactivated upon their formation, and, therefore, function primarily as paracrine/autocrine signals. Evidence is emerging that, in many respects, the metabolism and effects of these gasotransmitters are similar in fish.

NO is a potent dilator of fish blood vessels and it inhibits constrictions produced by most stimuli including catecholamines, angiotensin II, AVT, and endothelin-I. In mammals, NO is synthesized by both the endothelium and perivascular nerves, and synthesis from the amino acid L-arginine is under the control of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS), respectively. The presence of nNOS and neuronally mediated release of NO in fish vessels has been well documented. Although there are conflicting reports, most evidence to date suggests that eNOS is not present in fish endothelial cells and that NO is not an EDRF.

CO is produced from the heme of proteins such as hemoglobin, myoglobin, and cytochromes by the action of the enzyme heme oxygenase (HO). Three heme oxygenases have been identified in mammals but only heme oxygenase-2 (HO-2) appears to be an important enzyme in regulating CO production in the vasculature. CO is a vasodilator in mammals and, like NO, activates soluble guanylyl cyclase, which enzymatically produces cyclic guanosine monophosphate (cGMP) and brings about a reduction in intracellular calcium and relaxation. CO also vasodilates fish vessels by increasing cGMP and there is some evidence that there is tonic production of CO in fish. Little else is known of this potentially important vasoactive molecule.

Considerable evidence is accumulating in support of H_2S as an important signaling molecule in fish vessels. H_2S is synthesized from L-cysteine by the enzyme cystathionine γ-lyase (CGL also known as GSE) or cystathionine β-synthase, or from 3-mercaptopyruvate by the enzyme 3-mercaptopyruvate sulfurtransferase (3-MST). All three enzymes have been identified in fish vessels. The effects of H_2S on fish vessels are quite variable; H_2S contracts some vessels, relaxes others, and may even produce biphasic or multiphasic contractions and relaxations. These effects are, for the most part, independent of the endothelium. One consistent observation regarding the effects of H_2S is that they are identical to those produced by hypoxia. It has been proposed that H_2S is the initial oxygen-sensing step, that is, the oxygen sensor in the vasculature. Isolated fish vessels and fish tissues have been shown to produce H_2S when oxygen levels are low but not when oxygen is increased. Recent studies have also shown that H_2S acts solely as a paracrine/autocrine signaling molecule and that it does not exist in the circulation.

Factors That Affect Vascular Resistance

The collective response of the cardiovascular system to stimuli such as exercise, hypoxia, and temperature involves the integration of numerous local and remote factors that regulate vascular resistance. It is often difficult to separate these factors experimentally.

Exercise

Swimming in fish is generally classified as either burst (sprint) or sustained. Burst swimming is brief, anaerobic activity of white muscle and is quite often accomplished without substantial changes in peripheral vascular resistance. Sustained swimming is aerobic and necessitates adjustments in vascular resistance to maintain both adequate perfusion of metabolically active tissue and decreased perfusion of less critical tissues in order to maintain arterial blood pressure (**see also Integrated Response of the Circulatory System:** Integrated Cardiovascular Responses of Fish to Swimming). During sustained swimming, blood flow to skeletal muscle can increase more than fourfold as vascular resistance decreases through local regulatory mechanisms. At higher swimming speeds, blood flow to the gastrointestinal tract is often decreased through remote mechanisms (**see also Design and Physiology of Arteries and Veins:** The Gastrointestinal Circulation). This increase in vascular resistance in the gastrointestinal tract ensures that the heart can pump sufficient blood to maintain, or even slightly elevate, arterial blood pressure.

Temperature

In most fish, as temperature increases so does metabolic rate and this decreases vascular resistance through local mechanisms (**see also Integrated Response of the Circulatory System:** Integrated Responses of the Circulatory System to Temperature). Conversely, a drop in water temperature cools the fish and decreases metabolism, thereby increasing resistance. At very low ambient temperatures, such as those encountered by Antarctic fish, blood viscosity increases and this increase in viscosity will effectively increase vascular resistance. The red-blooded Antarctic icefish solved this problem by remodeling blood vessels to reduce their vascular resistance (they have a larger diameter). Other Antarctic icefish decrease blood viscosity by eliminating red blood cells. While this substantially reduces blood viscosity, these hemoglobin-less icefish must have a considerably higher cardiac output in order to maintain adequate oxygen delivery with blood lacking red blood cells.

Hypoxia

Acute hypoxia in fish, produced experimentally by lowering water P_{O_2} from 100 to 30–50 mmHg, produces reflex fall in heart rate and cardiac output in most fish (**see also Integrated Response of the Circulatory System:** Integrated Responses of the Circulatory System to Hypoxia). In teleosts, arterial blood pressure increases due to activation of the sympathetic nervous system and an increase in peripheral vascular resistance. In elasmobranchs, arterial blood pressure falls indicating that the remote system responses are not sufficient to maintain blood pressure. The crucian carp (*Carassius carassius*) is unique in its ability to maintain normal autonomic control after 5 days of complete anoxia.

Hypovolemia

A decrease in blood volume (hypovolemia) brought about either through hemorrhage or through a dehydrating environment (often accompanying moving from freshwater and saltwater) decreases blood pressure (hypotension), which in bony fish brings about a reflex increase in peripheral vascular resistance. This is usually mediated through activation blood pressure receptors (baroreceptors) located in the gill, which then reflexively activate the sympathetic nervous system. This response may be supplemented by increased production of ANG II or secretion of AVT. Baroreceptor reflexes are greatly reduced or absent in elasmobranchs.

Methods for Measuring Arterial Resistance in Fish

A number of methods have been developed to measure vascular resistance or vasoactivity in fish vessels. These include *in vitro* techniques using isolated blood vessels or perfused organs, or *in vitro* methods to measure vascular resistance in unanesthetized fish.

Isolated Blood Vessels

Myography is the most commonly employed technique to measure vasoactivity in isolated blood vessels. With this procedure, a small (0.5–2 mm diameter) blood vessel is

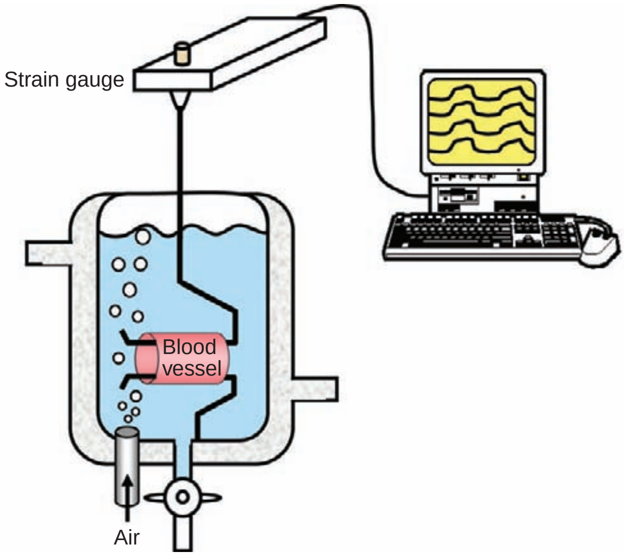


Figure 1 Method for measuring the response of isolated blood vessels using myography. The myograph chamber consists of a water-jacketed vessel that can be filled with physiological buffer and aerated with appropriate gas mixtures. A small piece of a blood vessel is mounted between two wire hooks (dark black lines); the bottom (immobile) hook is attached to the chamber and the upper hook is connected to a sensitive strain gauge. Contraction or relaxation of the vessel is sensed by the strain gauge and the signal is sent to a physiological recorder for data archiving and video screen for observation. Hormones or compounds of interest can be added to the chamber to determine their vasoactivity.

placed between two thin wires, one of which is immobile and the second is connected to a sensitive strain gauge to record slight displacements of the vessel wall (**Figure 1**). The vessel is placed in a temperature-controlled, water-jacketed myograph chamber and aerated with appropriate gas mixtures (typically, room air for fish). Hormones or drugs of interest are added to the chamber and the response (contraction or relaxation) is archived to a computer and displayed on a monitor. The limitation of this system is that it is useful for conductance arteries and can be modified for smaller vessels but does not work well for resistance-size arterioles.

Perfused Organs

The perfused organ technique is useful for measuring vascular resistance of organs or tissues at the level of resistance arterioles. With the constant-flow technique (**Figure 2**), a blood vessel supplying the tissue of interest is cannulated and physiological saline is infused through the tissue with a peristaltic roller pump (as shown in **Figure 2**) or syringe. A pressure transducer connected to the infusion cannula provides information on the input pressure, and vascular resistance is obtained from the equation $R = \Delta P/F$, where ΔP is the difference between input pressure measured with the pressure transducer and venous pressure, which is generally assumed to be zero. F is the flow delivered by the pump. In an alternate method, the constant pressure technique, the physiological saline drains into the tissue through a reservoir elevated above the fish. The height of the reservoir is adjusted to give a constant hydrostatic pressure. Flow is determined by either measuring the change of volume in the reservoir above the fish or collecting the venous effluent draining the fish.

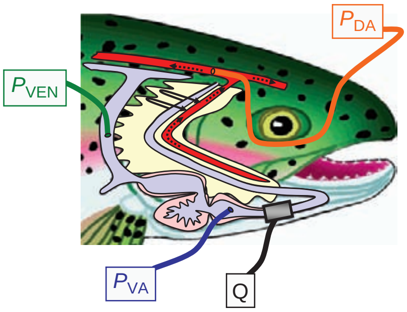


Figure 3 Placement of the pressure cannulas and flow probe for measuring gill and systemic vascular resistance in unanesthetized fish. Ventral aortic pressure (P_{VA}) is recorded from a cannula (blue line) placed in either the bulbus arteriosus (as shown here) or ventral aorta anterior to the flow probe. Dorsal aortic pressure (P_{DA}) is recorded from a cannula (red line) placed through the roof of the mouth and into the dorsal aorta and venous pressure (P_{VEN}) is obtained from a cannula (green line) inserted into the ductus Cuvier. Cardiac output (Q) is measured with a flow probe placed around the ventral aorta (black line).

Intact Fish

Measuring vascular resistance in unanesthetized fish is the most technically challenging procedure. Under anesthesia, blood pressure cannulas are placed in the ventral aorta to record central arterial blood pressure (P_{VA}), the dorsal

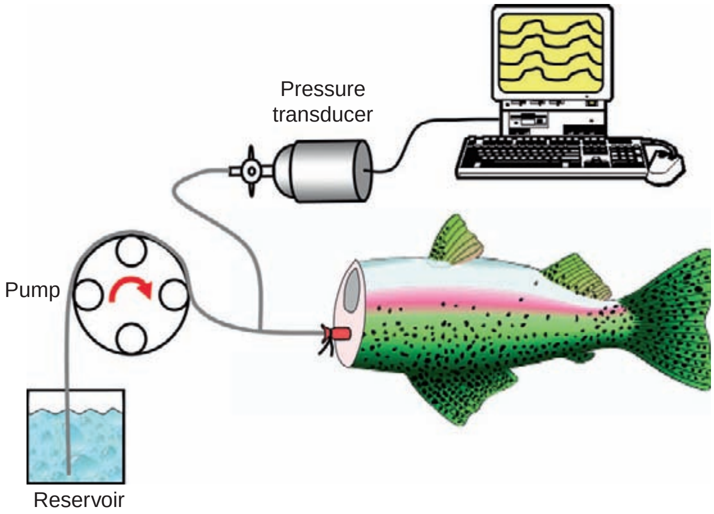


Figure 2 Method for measuring vascular resistance using a perfused organ or tissue. The organ of interest (in this example the trunk of a fish) is cannulated, and a roller pump pumps a physiological saline, containing the compounds of interest from a reservoir into the fish; a pressure transducer connected to the inlet cannula monitors perfusion pressure and the signal is sent to a computer for archiving or a video monitor for observation. Vascular resistance (R) is determined from the equation $R = \Delta P/F$, where ΔP is the difference between input pressure measured with the pressure transducer and venous pressure, which is generally assumed to be zero. F is the flow delivered by the pump.

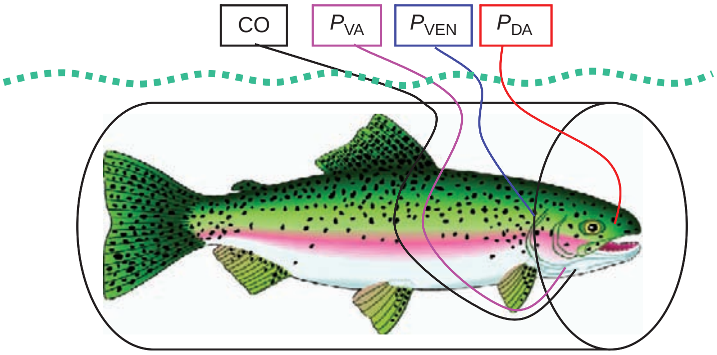


Figure 4 Method for measuring gill and systemic vascular resistance in unanesthetized fish. The fish is instrumented as shown in **Figure 3**. Gill resistance (R_G) is calculated from the equation $R_G = (P_{VA} - P_{DA})/Q$, and systemic resistance (R_S) is calculated from the equation $R_S = (P_{DA} - P_{VEN})/Q$.

aorta to record systemic arterial blood pressure (P_{DA}), and the ductus Cuvier to record central venous pressure (P_{VEN}). A flow probe is placed around the ventral aorta to measure cardiac output (Q; **Figure 3**). The fish is then revived and returned to the water, usually with minimal restraint, while pressure and flow parameters are measured at rest, following either injection of drugs or during other experimental manipulations such as swimming. Gill resistance (R_G) is calculated from the equation $R_G = (P_{VA} - P_{DA})/Q$, and systemic resistance (R_S) is calculated from the equation $R_S = (P_{DA} - P_{VEN})/Q$. Typically, all of these parameters are recorded simultaneously (**Figure 4**).

See also: **Brain and Nervous System:** Autonomic Nervous System of Fishes. **Circulation:** Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins:** The Gastrointestinal Circulation. **Hormonal Responses to Stress:** Catecholamines. **Integrated Response of the Circulatory System:** Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Hypoxia; Integrated Responses of the Circulatory System to Temperature.

Further Reading

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 89–120. San Diego, CA: Academic Press.

Evans DH, Toop T, Donald J, and Forrest JN , Jr. (1993) C-type natriuretic peptides are potent dilators of shark vascular smooth muscle. *Journal of Experimental Zoology* 265: 84–87.

Foster JM, Forster ME, and Olson KR (2008) Different sensitivities of arteries and veins to vasoactive drugs in a hagfish, *Eptatretus cirrhatus*. *Comparative Biochemistry and Physiology – Part C: Toxicology and Pharmacology* 148: 107–111.

Hoagland TM, Weaver L , Jr. Conlon JM, Wang Y, and Olson KR (2000) Effects of endothelin-1 and homologous trout endothelin on cardiovascular function in rainbow trout. *American Journal of Physiology* 278: R460–R468.

Le Mevel JC, Lancien F, Mimassi N, and Conlon JM (2009) Ventilatory and cardiovascular actions of centrally and peripherally administered trout pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) in the unanaesthetized trout. *Journal of Experimental Biology* 212: 3919–3927.

Le Mevel JC, Lancien F, Mimassi N, *et al.* (2008) Central and peripheral cardiovascular, ventilatory, and motor effects of trout urotensin-II in the trout. *Peptides* 29: 830–837.

Nobata S, Ogoshi M, and Takei Y (2008) Potent cardiovascular actions of homologous adrenomedullins in eels. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 294: R1544–R1553.

Olson KR (1992) Blood and extracellular fluid volume regulation: Role of the renin–angiotensin system, kallikrein–kinin system, and atrial natriuretic peptides. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part B: The Cardiovascular System*, pp. 136–232. San Diego, CA: Academic Press.

Olson KR and Donald JA (2009) Nervous control of circulation – the role of gasotransmitters, NO, CO, and H_2S. *Acta Histochemica* 111: 244–256.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

Physiology of Capacitance Vessels

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction to the Venous System
Functions of Veins
Anatomy
The Fahraeus Effect and the Physiology of Venules and Small Veins
Physiological Significance of Regulation of Venous Tone and Compliance
Physiological Factors That Affect the Capacitance Curve and Therefore Cardiac Output
Techniques for Measurement of Venous Compliance and Venous Tone
Further Reading

Glossary

Capacitance The relationship between the volume of fluid in a closed container and its pressure.

Compliance The ability of a blood vessel to stretch and relax. It is defined mathematically as the slope of the change in volume of the vessel divided by the change in pressure, i.e., $\Delta V/\Delta P$.

Edema The accumulation of excess fluid in the space between cells (interstitial space) but outside of the blood vessels.

Euryhaline A fish that can live in both freshwater and saltwater.

Fahraeus effect The observation that hematocrit in small vessels is significantly lower than that in large vessels.

Fahraeus–Lindqvist effect The condition in small blood vessels where viscosity becomes less dependent on hematocrit.

Hematocrit The proportion of blood volume that is occupied by red blood cells.

Hydraulic pressure Pressure imparted on a solution by a piston or pump. This is equivalent to the blood pressure generated by the heart or by vascular smooth muscle.

Hydrostatic pressure Pressure due to the height of a column of fluid directly above the reference point.

Mean circulatory filling pressure The pressure that will exist throughout the cardiovascular system when blood flow has stopped and blood pressure has equilibrated throughout. This pressure is due to the volume of blood in the cardiovascular system and the tension exerted on it by the walls of the blood vessels. This tension is generated by stored energy as well as active tension developed by vascular smooth muscle

Osmotic pressure The hydrostatic pressure produced by the different concentrations of two solutions on opposite sides of a semi-permeable membrane.

Ostial valve A one-way valve in a small vein near the location where it empties into a larger vein. These valves prevent the backflow of blood into the periphery.

Parietal valve A one-way valve in long veins of mammals that prevents the backflow of blood due to gravity.

Resistance The sum of the impediments to fluid flow. Resistance is increased by increased vessel length and fluid viscosity and decreased by increased vessel radius.

Stressed blood volume The volume of blood in the cardiovascular system that distends the vessel walls and creates a positive pressure. Stress blood volume is equal to the total blood volume minus the unstressed blood volume.

Tone The active compression of a blood vessel due to contraction of smooth muscle in the blood vessel wall.

Transmural pressure The pressure difference between the inside and the outside of a vessel.

Unstressed blood volume The volume of blood that fills the dead space of the cardiovascular system without distending the vessels or creating internal blood pressure. This volume is possible because blood vessels do not collapse down to zero volume and therefore can contain blood at zero pressure. This volume is often two-thirds of the total blood volume.

Introduction to the Venous System

The venous system has historically been the least appreciated, if not least understood component of the cardiovascular system. The most obvious function of the veins is to provide a conduit for the return of blood from the capillaries back to the heart. However, studies initially performed in mammals, and more recently in fish, have clearly shown that the venous system is more than a passive participant in blood flow. It is now clear that the veins play an important role in the regulation of cardiac output due to their ability to increase or decrease blood flow into the heart. This is achieved in part through active regulation of the diameter of veins as well as passive factors such as surrounding muscular contractions and elastic recoil of the vessels. As the name implies, most of the blood in the circulatory system is contained in the venous capacitance vessels. This volume is not static, however, and can be mobilized through active and indirect processes according to the fish's needs. The following sections describe these features in detail.

Functions of Veins

The venous system serves three important functions. Veins provide a conduit for blood to flow from the capillaries back to the heart, they act as a storage site for a substantial portion of the blood volume, and by regulating blood flow returning to the heart they profoundly affect cardiac output.

Anatomy

Tiny capillary-size venules drain blood from the capillaries. Through repetitive anastomoses they form progressively larger veins that ultimately drain into the large horseshoe-shaped ductus Cuvier. The ductus Cuvier connects directly to the first chamber, the sinus venosus, of the heart (**see also Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns and **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). Compared to arteries and arterioles, veins and venules are typically very thin–walled because they contain less smooth muscle and elastic tissue. Veins and venules are also larger in diameter than their corresponding arteries and arterioles. Therefore, they offer less resistance to blood flow (**see also Circulation**: Circulatory System Design: Roles and Principles). This means that the loss of blood pressure across the venous circuit is relatively small, generally no more than several mmHg. As a result, blood can leave the capillaries under low blood pressure and still have sufficient potential energy to return to the heart (**see also Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish and **Design and Physiology of the Heart**: Accessory Hearts in Fishes). Any elevation in venous pressure naturally increases capillary pressure, which would cause unwanted leakage of blood plasma across the capillary wall and into the interstitial space. Excessive leakage can produce edema (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange).

In mammals, 70–80% of the blood volume in the systemic circulation resides in the veins. The percent blood volume in the fish venous circulation is unknown, but it is also likely to be considerably larger than the volume of blood in the arteries and capillaries combined given the much larger diameter of the venous compared with arterial vessels. This is undoubtedly the case for certain sharks and rays, which have large central veins. The large volume of blood in the veins represents a pool of blood that the heart can draw on immediately whenever cardiac output increases. In fact, the passive elastic recoil and active contraction of veins can change the blood pressure filling the heart and thereby directly affect cardiac output (**see also Circulation**: Circulatory System Design: Roles and Principles and **Design and Physiology of the Heart**: Physiology of Cardiac Pumping).

Unlike arteries, where valves are absent, veins have one-way valves that ensure movement of blood toward the heart. Mammalian veins have two types of valves, parietal and ostial (**Figure 1**(**a**)). Parietal valves are found in the lumen of longitudinal veins. They are especially important in countering the effects of gravity. The hydrostatic pressure generated by gravity results in a larger venous transmural blood pressure which tends to pool blood at the extremities and limits the flow of blood returning to the heart. The one-way parietal valves prevent backflow of blood downward in the legs when standing, and also limit the expansion of veins because of their connective tissue. Parietal valves are not found in fish veins, presumably because gravitational effects are essentially nil in an aqueous environment (**Figure 1**(**b**)).

This important difference between the venous anatomy of fish and mammals is shown in **Figure 1**(**b**), which illustrates the effects of gravity on the pressure across the wall (transmural pressure) of a vein in a terrestrial animal (air) and one that lives in water. In air, the hydrostatic pressure in a vertical column of fluid increases as a function of the depth of the fluid column (**see also Circulation**: Circulatory System Design: Roles and Principles). Thus, the hydrostatic pressure in the lowest (most dependent) segment of the vessel is the greatest. With minimal external pressure to oppose

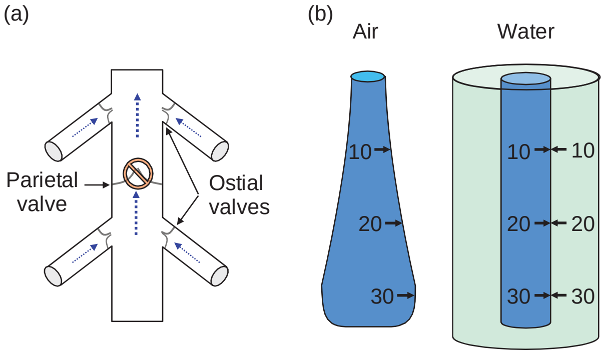


Figure 1 Anatomical and physical properties of veins. (a) Valves in veins ensure one-way blood flow. Ostial valves are found where small veins drain into larger ones and they prevent backflow of blood into the side branches. Parietal valves in the lumen of mammalian veins prevent backflow of blood due to gravity. Parietal valves are not found in fish. (b) Effects of hydrostatic pressure on veins of terrestrial and aquatic animals. In terrestrial animals the hydrostatic blood pressure increases with the length of the veins, and because there is no hydrostatic pressure outside these vessels there is increased venous distention in the lower regions. In fish the intravascular hydrostatic pressure is offset by hydrostatic pressure of the surrounding water and there is no net venous distention (b) Adapted from Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

the internal pressure, the wall of the vessel becomes progressively distended proportional to the transmural pressure, which in this case is the hydrostatic pressure. Any venous blood pressure adds to this hydrostatic pressure to increase the total transmural pressure. To offset this distending pressure, terrestrial animals constrict the smooth muscle layer surrounding the veins to create an external pressure. In a submerged vessel, on the other hand, the increase in hydrostatic pressure in the dependent segment of the vessel is essentially offset by an increase in external hydrostatic pressure from the environment. Thus, the transmural pressure is zero and the vessel cannot distend. As this is the case for fish, it was once thought that fish veins did not need to constrict and they were essentially passive conduits for the return of blood to the heart. However, recent studies have clearly shown that fish veins do indeed constrict and this plays an important role in regulating venous pressure, venous return to the heart, and cardiac output. In fishes, ostial valves are typically found in the lumen of smaller veins near their anastomosis with a larger vein (**Figure 1**(**b**)).

The Fahraeus Effect and the Physiology of Venules and Small Veins

Small veins and venules exhibit a number of characteristics that distinguish them from larger veins. It is well known that as blood passes through progressively smaller vessels into the microcirculation, the hematocrit of blood contained in arterioles, capillaries, and venules that are 200–300 μm diameter or less also decreases. This is known as the Fahraeus effect. This effect can also be produced simply by passing blood through small glass tubing. In mammalian vessels, hematocrit can be decreased from 45% in arteries and veins to 10% or less in the microcirculation. (**see also Circulation**: Circulatory System Design: Roles and Principles).

It has also been shown from studies on mammals that as much as 30–40% of the blood volume is found in the small venules and that these vessels are quite compliant. The importance of the capacitance characteristics of these small vessels is evident when blood is rapidly lost from the vasculature during hemorrhage. Following hemorrhage, the reduced blood volume means that mean circulatory filling pressure is lower and the drop in the mean circulatory filling pressure collapses these vessels. This mobilizes plasma-rich blood from the microcirculation back into heart and provides a near-instantaneous transfusion of blood into the central circulation. This simple biophysical effect can sustain cardiac output until other reflexes set about restoring fluid volume. These responses have also been demonstrated in fish following hemorrhage and show that the fish microcirculation, especially the venules, is functionally similar to that of mammals.

Physiological Significance of Regulation of Venous Tone and Compliance

As described in **Circulation**: Circulatory System Design: Roles and Principles, factors that increase venous pressure increase venous return and cardiac output and, conversely, factors that decrease venous pressure decrease both venous return and cardiac output. With respect to the veins, the major factors that influence the venous blood pressure are blood volume, venous compliance, and venous tone.

An increase in blood volume will increase venous pressure simply because blood vessels cannot expand indefinitely. However, under most circumstances fish do not rapidly alter their blood volume despite the fact that they are continuously faced with water loss (in seawater) or water gain (in freshwater) as a result of large osmotic differences between their blood and the environment (see below and **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). In fact, fishes have excellent regulation of blood volume, with the only exception being hemorrhage. Instead, the mechanical properties of veins, namely venous tone, provide the most important mechanism for rapidly adjusting cardiac output. In fact, as described above, the venous system is important in responding to rapid changes in blood volume that may occur through hemorrhage. Thus, control of venous capacitance and fishes is extremely important, although studies thus far are limited.

Venous capacitance curves, which are frequently derived from unanesthetized animals, provide the most accurate assessment to date of venous responses (see the section titled 'Techniques for measurement of venous compliance and tone'). A typical capacitance curve is shown in **Figure 2**. Experimental points generated from the relationship between mean circulatory filling pressure (x-axis) and blood volume as a percent of total (left y-axis) or actual blood volume (right y-axis) define the capacitance characteristics of the venous system. Venous compliance ($\Delta V/\Delta P$) is the slope of the capacitance line, which is typically measured relative to the normal blood volume (black line, A). Rotation of this line counterclockwise reflects an increase in compliance (blue line, B); compliance is decreased when the line is rotated clockwise. Intersection of the compliance line with the y-axis at 0 mmHg mean circulatory filling pressure provides the unstressed blood volume, which in **Figure 2** is ~67% of the total blood volume or 24 ml kg^{-1} body weight. The difference between total blood volume and unstressed blood volume is the stressed volume, 33% of total, or 11 ml kg^{-1} body weight. In this example, we assume that the total blood volume was 3.5%. Although a number of studies have indicated that some treatments may affect venous compliance (i.e., rotate the compliance curve), there is no experimental evidence on isolated veins that indicate that vascular compliance can be actively increased or decreased. Apparent changes in compliance most likely are the result of a change in blood volume (see below).

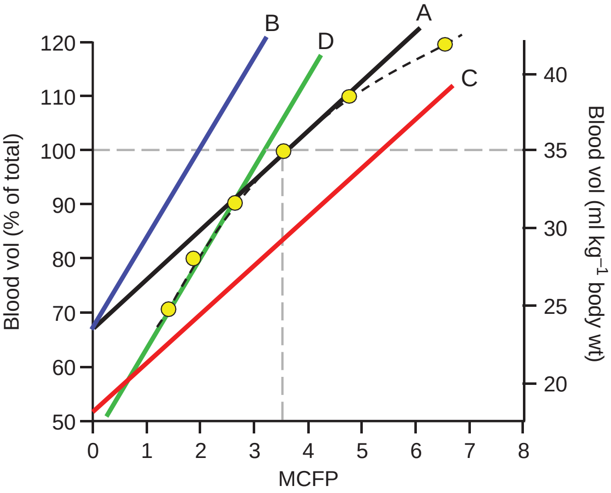


Figure 2 Typical capacitance curve for a rainbow trout. The data points (yellow circles) show the experimentally obtained relationship between mean circulatory filling pressure (MCFP) and blood volume. The slope of the line passing through the capacitance curve at normal (100%) blood volume (line A) is the *in vivo* compliance and the intersection of this line with blood volume at 0 MCFP is the unstressed blood volume, in this case 67% of total blood volume or 24 ml kg^{-1} body weight. The stressed volume is 100–67% or 33% of the total blood volume (11 ml kg^{-1} body weight). An increase in compliance (blue line, B) rotates the compliance curve counterclockwise but does not change the unstressed volume. An increase in vascular tone (red line, C) shifts the compliance curve downward increasing the stressed volume without a change in slope. It can be seen from the figure that if blood volume remains constant, an increase in vascular compliance will lower the mean circulatory filling pressure and decrease cardiac output, whereas an increase in tone will increase mean circulatory filling pressure and increase cardiac output. A decrease in compliance or a decrease in tone will have the opposite effect. If blood volume is decreased experimentally or by hemorrhage, the compliance curve will be shifted to lower blood volumes (green line, D) indicating that compliance has increased, because the veins are now more collapsed, and the unstressed volume has decreased, which reflects the fact that some of the blood has been removed.

The unstressed blood volume, by definition, fills the dead space in the vasculature (as blood vessels cannot passively collapse down to zero volume) and creates no internal pressure. Therefore, it does not contribute to venous pressure. Only the stressed volume is of physiological importance in establishing venous pressure and thereby regulating venous return to the heart and cardiac output. A decrease in blood volume following hemorrhage decreases the stressed volume. For this reason, and in the absence of any regulation of venous smooth muscle, blood loss can have catastrophic consequences on cardiac output. Active constriction of smooth muscle in the veins or external compression of venous vessels that often accompanies contraction of the surrounding skeletal muscle produces a parallel downward displacement of the compliance curve and in doing so shifts blood from the unstressed into the stressed compartment. This increases venous pressure and ultimately cardiac output. Dilation of the veins has the opposite effect and will decrease cardiac output. Some fish hearts create suction during cardiac contraction (via-a-fronte filling; **see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping) and this creates a larger pressure gradient to fill the heart independent of the venous blood pressure.

Physiological Factors That Affect the Capacitance Curve and Therefore Cardiac Output

Hormones and Drugs

Several hormones or drugs that are known to raise arterial blood pressure or increase cardiac output also decrease venous capacitance through a downward displacement of the compliance curve (**Figure 2**), that is, the veins become stiffer. This change in venous compliance has the effect of shifting blood volume from the unstressed into the stressed compartment, thereby increasing mean circulatory filling pressure. Hormones in this list include epinephrine (adrenaline) in rainbow trout (*Oncorhynchus mykiss*), the air breathing fish (*Synbranchus marmoratus*), and the dogfish shark (*Squalus acanthias*), as well as arginine vasotocin (AVT) in rainbow trout. Epinephrine appears to act through α-adrenoceptors (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes and **Hormonal Responses to Stress**: Catecholamines) as synthetic drugs that are specific for α-receptors have similar effects. Furthermore, blockade of α-adrenoreceptors decreases the mean circulatory filling pressure in resting fish, which clearly suggests that there is a continuous stimulation of veins by the sympathetic nervous system (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). This continuous contraction of the veins is sometimes called venous tone, which refers to a constant level of tension developed in the vessel wall.

Drugs that relax blood vessels by stimulating β-adrenoceptors have the opposite effect, that is, they produce an upward displacement of the capacitance and decrease blood in the stressed compartment.

Endothelin-1, a peptide hormone, also increases blood pressure in rainbow trout but it increases venous pressure by decreasing venous compliance (which may be due to fluid retention and volume expansion). Other hormones that are known to increase arterial blood pressure, such as norepinephrine (noradrenaline) and angiotensin II, do not affect vascular capacitance. This indicates that the action of these hormones is achieved through an increase in arterial vascular resistance (**see also Circulation**: Circulatory System Design: Roles and Principles).

Atrial natriuretic peptide (ANP) decreases arterial blood pressure and lowers mean circulatory filling pressure by decreasing venous compliance. Because ANP also promotes rapid loss of water by the kidneys, it is likely that this hormone affects venous compliance indirectly through a reduction in blood volume. The nitric oxide (NO) donor sodium nitroprusside (SNP) also decreases arterial blood pressure, but has no effect on vascular capacitance. Thus, SNP appears to act only on the arterioles.

Exercise

The increase in cardiac output commonly associated with exercise in fish is typically accompanied by an increase in central venous pressure. Furthermore, in both the rainbow trout and European sea bass (*Dicentrarchus labrax*), exercise increases mean circulatory filling pressure. This suggests that the contracting skeletal muscles compress the veins and decreased vascular capacitance. However, this has never been measured directly because of the technical difficulty measuring vascular capacitance in exercising fish.

Temperature

In many fish cardiac output increases as temperature increases. Studies have shown that when temperature is increased from 10 to 16° C, mean circulatory filling pressure also increases in rainbow trout, presumably to enhance venous return, but only up to 13° C. These responses, however, may be species specific as there only appears to be a slight increase in venous tone associated with warming in the Antarctic fish (*Pagothenia borchgrevinki*).

Hypoxia

It has been shown in rainbow trout that moderate hypoxia (lowering water P_{O_2} to approximately 65 mmHg) decreases venous capacitance as a result of an α-adrenergic venous constriction. This is brought about predominantly through an increase in circulating catecholamines (**see also Hormonal Responses to Stress**: Catecholamines). However, profound hypoxia/anoxia relaxes veins through a direct effect on vascular smooth muscle, and this ultimately overrides the effect of any adrenergic stimulation.

Adaptation to Freshwater, Saltwater or High-Salt Diet

Unlike terrestrial animals where loss of body water and salt is a pervasive occurrence, water and salt balance in fish can vary with the environment (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). In freshwater, where the ambient osmolarity is 1 mosm l^{-1} or less and blood osmolarity is nearly 300 mosm l^{-1}, there is strong osmotic gradient for water uptake and these animals must continually regulate against chronic volume expansion. Volume expansion is even more of a problem in freshwater fish fed a high-salt diet as the excess salt increases the tendency to accumulate water. In a saltwater environment where osmolarities are around 1000 mosm l^{-1}, yet plasma osmolarity remains close to 300 mosm l^{-1}, the water and salt gradients are reversed and there is a tendency for volume depletion. The relationship between blood volume and venous capacitance has been studied in euryhaline rainbow trout because of their ability to thrive in all three environments. When freshwater trout are adapted to saltwater, their blood volume decreases as predicted.

This decreases their vascular capacitance and as shown in **Figure 3**(**a**), chronic reduction in blood volume also decreases mean circulatory filling pressure. When freshwater rainbow trout are fed, a diet with excess salt their blood volume increases which in turn increases mean circulatory filling pressure. These alterations in vascular capacitance remain for extended periods, but the long-term effect on cardiac output is unclear.

Passive Responses: The Importance of Venous Compliance

The elastic properties of the veins may also passively contribute to physiological responses. This is perhaps most notable if blood is rapidly removed from the circulation and there is insufficient time for reflexes to restore the lost volume. If blood is removed from the arterial circulation, arterial blood pressure will fall which in turn decreases capillary blood pressure and venous blood pressure. Because of the elastic (compliance) properties of the veins, the drop in venous blood pressure will cause a passive collapse of the veins which in turn shifts the capacitance curve to a new setpoint. This results in the mobilization of blood from the venous compartment toward the heart as illustrated in **Figure 3**(**b**). In this example a 1-mmHg drop in venous pressure mobilizes approximately 12% of the vascular volume (4.2 ml kg^{-1} body weight). A 2-mmHg drop in mean circulatory filling pressure will mobilize almost 25% of the blood volume.

The Importance of the Pericardium

The pericardium is important in venous return in that it helps establish the vis-a-fronte pressure which is a negative pressure created by the contracting heart inside the rigid pericardial vault (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). When the pericardium is opened due to surgery, or as part of an experimental protocol, vascular capacitance decreases due to active venoconstriction to offset the loss of the cardiac suction. While these events do not occur under physiological circumstances, the changes associated with opening the pericardium during experimental procedures need to be understood and accounted for.

The Venous System in Hagfish

Hagfish have one of, if not the largest blood volume per body weight of any vertebrate, some 2–4 times that of mammals or bony fish. As much as 30% of this may reside in the subcutaneous sinus, a large capacious vascular space that lies beneath the skin. It is not known if blood can be mobilized from or stored in this space and thereby affect cardiac output.

Techniques for Measurement of Venous Compliance and Venous Tone

Venous Compliance *In Vitro*

Compliance of a system such as a blood vessel is defined as the change in volume for a given change in pressure

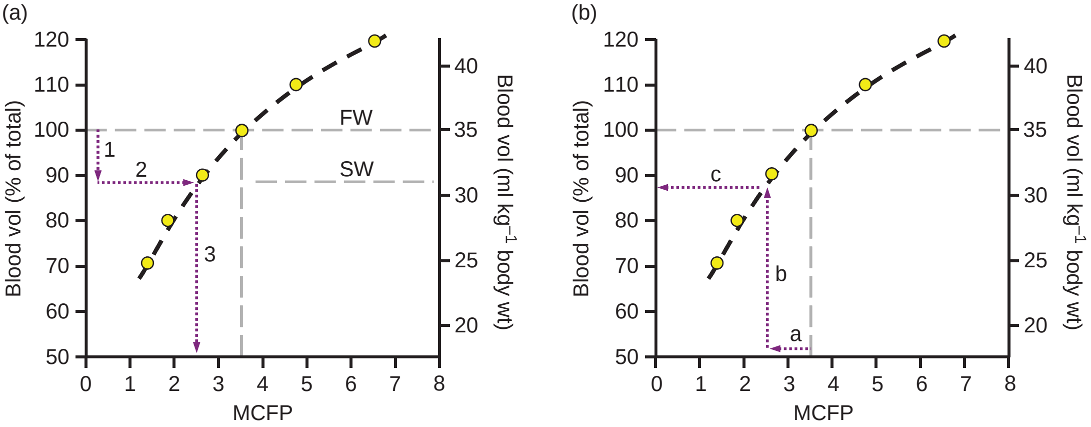


Figure 3 Capacitance curves for a trout showing the effects of freshwater saltwater adaptation (a) and hemorrhage (b). (a) The water–blood osmotic gradients of a fish in freshwater (1–300 mosm l^{-1}) produce chronic volume overload, whereas in saltwater these osmotic ingredients (1000–300 mosm l^{-1}) produce chronic hypovolemia. Thus, the blood volume of a saltwater trout is chronically reduced and this reduces mean circulatory filling pressure (MCFP; lines 1–3). (b) Mobilization of blood volume following acute hemorrhage. In this example, hemorrhage from an artery reduces blood flow into the venous system which in turn lowers venous pressure by 1 mmHg (line a). The vascular capacitance now shifts to a new equilibrium point (line b) that can only be achieved if the venous blood volume is reduced by approximately 12% (4.2 ml kg^{-1} body weight). This 4.2 ml kg^{-1} body weight of blood that is mobilized by the passive recoil of the venous compartment helps sustain cardiac output until the lost volume can be restored. Typically, this blood is mobilized from the microcirculation.

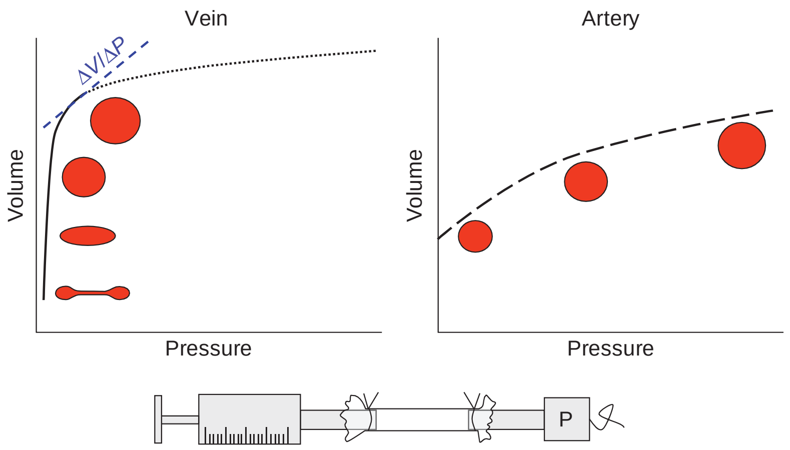


Figure 4 Compliance curves for an isolated vein and artery. Compliance is calculated as the slope of the volume–pressure relationship (Δ*V*/Δ*P*; blue line). Because veins collapse at low pressures, they are initially quite compliant; once distended, their compliance dramatically decreases. Dotted portion of line is obtained at superphysiological pressures. Arteries are considerably (1/25) less compliant than veins but they do not collapse at low pressures. Diagram beneath compliance curves illustrates the method for measuring compliance of isolated vessels. Pressure (*P*) in the vessel is measured as known volumes of fluid are injected.

(i.e., $\Delta V/\Delta P$). The compliance of an isolated blood vessel can be determined by measuring the pressure inside the vessel while known volumes of fluid are injected; compliance is derived from the slope of the volume–pressure curve, as shown in **Figure 4**. Using this approach, it has been determined that fish veins are approximately 25 times more compliant than the corresponding arteries. This difference is similar to that observed between mammalian veins and arteries. It is also evident from the venous compliance curve that veins are very compliant at very low pressures because they are partially collapsed and little pressure is needed to substantially increase blood volume. Once the vessels become distended, compliance decreases dramatically. This degree of distention is not physiological.

Venous Compliance *In Vivo*: Vascular Capacitance Curves

It is far more appropriate, but technically more difficult, to measure venous compliance (and function) *in vivo*. Instead, we measure vascular capacitance. Vascular capacitance curves are constructed from the relationship between blood volume and mean circulatory filling pressure. Mean circulatory filling pressure (also known as zero-flow pressure) is the pressure that will exist throughout the cardiovascular system when there is no blood flow and, therefore, blood pressure in the arterial, capillary, and venous vessels is equal. Experimentally, mean circulatory filling pressure is obtained by either stopping the heart electrically, or with drugs, or by transiently clamping the ventral aorta. During this procedure arterial pressure falls and venous pressure increases as blood flows through the capillaries into the veins. Because arterial and venous pressures do not quite achieve equilibrium during the brief interruption of cardiac output, the venous pressure only approximates mean circulatory filling pressure. (Blood flow must be restored within 10 s to prevent cardiovascular reflexes from responding to the drop in arterial pressure.) Even so, the error is generally minimal. After 15–20 min recovery, a known volume of blood is either removed or added to the fish (using blood from a donor fish), another mean circulatory filling pressure measurement is made, and blood volume is once again quickly returned to normal. Repeating these experiments over a range of blood volumes produces the vascular capacitance curve (yellow data points in **Figures 2** and **3**). Vascular compliance is the slope of the capacitance curve. Usually, compliance is determined from the data points near the normal blood volume (100% blood volume, or 35 ml kg^{-1} body weight in **Figure 2**). The intersection of the compliance line with the *y*-axis when mean circulatory filling pressure equals 0 mmHg provides an estimation of the unstressed blood volume. The stressed blood volume is the difference between total blood volume and unstressed volume.

If an experimental protocol increases vascular compliance, the compliance line will be rotated counterclockwise about the unstressed volume (line B, **Figure 2**). The net effect of this is that at normal blood volume mean circulatory filling pressure will be lower, which will reduce venous return, and cardiac output will decrease. If an experimental protocol increases venous tone, the compliance line will be displaced downward indicating that blood has been shifted from the unstressed to the stressed compartment (line C, **Figure 2**). The net effect of this is that at normal blood volume mean circulatory filling pressure will be greater and this will increase venous return and cardiac output. A decrease in vascular compliance or venous tone will have the correspondingly opposite effects. If blood volume changes during the experimental protocol, this will artificially affect the capacitance curve. As an example if blood volume is decreased experimentally or by hemorrhage, the compliance curve is now shifted to lower blood volumes (line D, **Figure 2**), which has the effect of increasing compliance (because the veins are now more collapsed) and decreasing the unstressed volume (which reflects some of the blood that has been removed).

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange. **Design and Physiology of the Heart**: Accessory Hearts in Fishes; Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping. **Hormonal Responses to Stress**: Catecholamines. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction.

Further Reading

Forster ME, Davison W, Satchell GH, and Taylor HH (1989) The subcutaneous sinus of the hagfish, *Eptatretus cirrhatus*, and its relation to the central circulating blood volume. *Comparative Biochemistry and Physiology – Part A* 93: 607–612.

Olson KR (1992) Blood and extracellular fluid volume regulation: Role of the renin–angiotensin system, kallikrein–kinin system, and atrial natriuretic peptides. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part B: The Cardiovascular System*, pp. 136–232. San Diego, CA: Academic Press.

Olson KR, Conklin DJ, Farrell AP, *et al.* (1997) Effects of natriuretic peptides and nitroprusside on venous function in trout. *American Journal of Physiology* 273: R527–R539.

Olson KR and Hoagland TM (2008) Effects of freshwater/saltwater adaptation and dietary salt on fluid compartments, blood pressure and venous capacitance in trout. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 294: R1061–R1067.

Rothe CF (1986) Physiology of venous return. An unappreciated boost to the heart. *Archives of Internal Medicine* 146: 977–982.

Rothe CF (1991) Vascular capacitance. In: Dulbecco R (ed.) *Encyclopedia of Human Biology*, 2nd edn., vol. 8, pp. 757–765. San Diego, CA: Academic Press.

Rothe CF (1993) Mean circulatory filling pressure: Its meaning and measurement. *Journal of Applied Physiology* 74: 499–509.

Rowell LB (1993) *Human Cardiovascular Control*. New York: Oxford University Press.

Sandblom E and Axelsson M (2005) Baroreflex mediated control of heart rate and vascular capacitance in trout. *Journal of Experimental Biology* 208: 821–829.

Sandblom E and Axelsson M (2006) Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Oncorhynchus mykiss*): Role of neural and circulating catecholamines. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 291: R711–R718.

Sandblom E and Axelsson M (2007) The venous circulation: A piscine perspective. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* 148: 785–801.

Sandblom E, Axelsson M, and Davison W (2009) Circulatory function at sub-zero temperature: Venous responses to catecholamines and angiotensin II in the Antarctic fish *Pagothenia borchgrevinki*. *Journal of Comparative Physiology B* 179: 165–173.

Sandblom E, Axelsson M, and Farrell AP (2006) Central venous pressure and mean circulatory filling pressure in the dogfish *Squalus acanthias*: Adrenergic control and role of the pericardium. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 291: R1465–R1473.

Sandblom E, Farrell AP, Altimiras J, Axelsson M, and Claireaux G (2005) Cardiac preload and venous return in swimming sea bass (*Dicentrarchus labrax* L.). *Journal of Experimental Biology* 208: 1927–1935.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

Satchell GH (1992) The venous system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 141–179. San Diego, CA: Academic Press.

Zhang Y, Weaver L, Jr., Ibeawuchi A, and Olson KR (1998) Catecholaminergic regulation of venous function in the rainbow trout. *American Journal of Physiology* 274: R1195–R1202.

The Retia

ED Stevens, University of Prince Edward Island, Charlottetown, PE, Canada

© 2011 Elsevier Inc. All rights reserved.

What Is a Rete and How Does It Work?
Factors That Affect the Amount of Exchange
Countercurrent Heat Exchangers in Fishes
Countercurrent Multipliers That Serve the Swimbladder and the Retina in Fishes
Evolution of Retia in Fishes
Further Reading

Glossary

Arterial Adjective pertaining to blood that has been oxygenated.

Avascular Adjective pertaining to the lack of blood vessels or capillaries.

Choroid Pertaining to a vascular structure behind the retina, so-called because it resembles the chorion, the membranous structure that surrounds an embryonic mammal and contributes to the development of the placenta.

Countercurrent Flowing in opposite directions. Used for an arrangement of vessels (e.g., blood vessels or kidney tubules) such that fluid flows in one direction in one vessel that lies close to another vessel in which the flow is in the opposite direction. This arrangement allows the transfer of molecules or heat from one vessel to another in such a ways as to maintain a gradient along the countercurrent vessels.

Dorsal aorta The main artery in fishes that delivers oxygenated arterial blood to the body.

Endotherm An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Equilibrium Pertaining to the situation when all forces acting are balanced by others resulting in a stable unchanging system.

Myotome Blocks of white muscle in fish used for burst swimming. When cooked, they separate as flakes.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

pK_a and K_a K_a is the equilibrium constant for the dissociation of a weak acid that is related to the strength of the acid. pK_a is –log10(K_a).

Post-cardinal vein The main vein that returns venous blood to the heart.

Pyloric ceca Blind, finger-like projections stemming from the proximal intestine that contain pancreatic tissue and secrete digestive enzymes, thus facilitating digestion in some species.

Red muscle Striated skeletal muscle that generates ATP aerobically and is used to perform sustained low-intensity swimming. Red muscle has many mitochondria and a good blood supply to provide oxygen and nutrients.

Rete Structure consisting of densely packed blood vessels, mostly with a countercurrent blood flow scheme, arranged to facilitate the exchange of, e.g., heat or oxygen.

Root effect A property of fish hemoglobin in which protons decrease the maximal oxygen saturation of hemoglobin. For practical purposes, it is defined as a reduction of oxygen saturation at atmospheric oxygen tension.

Venous Adjective pertaining to blood that has passed through tissues and has had some of its oxygen removed for metabolism.

Visceral Pertaining to internal organ structures in the abdominal cavity.

What Is a Rete and How Does It Work?

Rete (plural retia) refers to a structure found in fish and other vertebrates that consists of a collection of tubules arranged in parallel. An example of a nonvascular rete is the rete testis, which is a network of tiny tubules of the testicle that carries sperm. In comparative physiology, a rete usually refers to a vascular rete consisting of both arterial and venous vessels. The diameter of the vessels is small (about the size of an arteriole or capillary), and the blood vessels are arranged so that the arterial blood flows in the direction opposite to that of the venous blood (i.e., countercurrent). The juxtaposition of an arterial vessel(s) to a venous vessel(s) allows for the exchange of heat or

Table 1 Number and diameter of blood vessels in the heat exchange and gas exchange retia of fishes

		Body weight	*Length (cm)*	*Arterial vessels number*	*Venous vessels number*	*Arteria diameter (μm)*	*Venous diameter (μm)*
Heat exchange retia							
Central muscle	Skipjack tuna	1.9 kg	1	134 000	121 000	36	84
Central muscle	Black skipjack	1.7 kg		66 800	64 100	54	110
Central muscle	Yellowfin tuna	8 kg				44	116
On liver	Porbeagle shark	88 kg	9	2000	1 sinus	130	
Gas exchange retia							
Choroid rete	Tuna		0.18	7 940 000	8 140 000	10.5	23.2
Swimbladder	Eel		0.77	670 000	360 000	21.6	33.7
Swimbladder	Eel			116 000	88 000		
Swimbladder	Eel		0.4	34 000	22 000	9–10	12

substances between them; hence, they are often called exchangers. In addition, because the blood flows in opposite directions in the two types of vessels, the structures also are called countercurrent exchangers.

In fish, there are two types of countercurrent exchangers: heat exchangers and gas exchangers. Heat exchangers are found in only a few species of fish and are found at various locations in the body. Gas exchangers are found in a wide variety of fishes and are located near the region where gases are secreted into the swimbladder and on the vessels that serve the eyes. The vessels that constitute a heat exchange rete are larger (typically 40–100 μm in diameter) than those in a gas exchange rete (typically 10–20 μm in diameter) (**Table 1**). This article focuses on the structure and the function of vascular retia.

Rete mirabile (plural retia mirabilia) is the correct term for a vascular rete, and because the retia in fish contain both arterial and venous vessels, the more apt term is rete mirabile geminum (rete is Latin for 'net or network', mirabile for 'wonderful' or 'miracle', geminum for 'paired'; plural retia mirabilia geminia). In most of the fish literature, the term rete mirabile geminum is shortened to just rete.

Factors That Affect the Amount of Exchange

Heat and gas exchange between rete vessels take place through conduction and diffusion, respectively. Therefore, the three factors that affect the amount of exchange between the rete vessels are the distance between the vessels, the flow rate in the vessels, and the area available for exchange. Another factor would be the packing of the vessels, but in essentially all vascular retia each arterial vessel is surrounded by venous vessels and vice versa.

Figure 1 illustrates how an exchanger works and the effect of proximity. **Figure 1**(**a**) shows two blood vessels: one containing warm blood flowing from left to right and the other containing cool blood flowing in the opposite direction. Because the blood vessels are distant from each other, there is so little exchange between the two that the temperature remains essentially the same throughout the length of each blood vessel. In **Figure 1**(**b**), the two blood vessels are in proximity; thus, there is an opportunity for heat (or gas) exchange between the two. Different colors represent different temperatures (but also could represent different oxygen partial pressures). The warm blood is

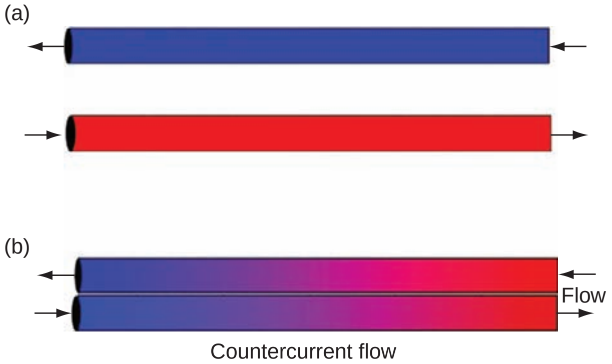


Figure 1 Schematic of a simple exchanger and the effect of proximity of blood vessels. (a) Two blood vessels distant from each other: one containing warm blood (red) flowing from left to right and the other containing cool blood (blue) flowing in the opposite direction. (b) The two blood vessels in proximity.

cooled and the cool blood is warmed as it passes through the exchanger, thus approaching thermal equilibrium. If the exchanger is very efficient, then exchange will be almost complete, and the temperature (or gas partial pressure) at the input side of one vessel will be almost the same as that at the output of the other.

The second factor that affects exchange is the flow rate in the vessels. If the flow in both blood vessels were stopped so that flow rate was zero, then there would be more time for exchange between the vessels and eventually the temperature would reach equilibrium and would be the same in both the vessels throughout their length. At the other extreme, if the flow rate were extremely high, there would be insufficient time for exchange and very little transfer between the vessels would take place. In a living fish, the flow rate lies somewhere between these two extremes. The blood flows in both blood vessels, and heat in the warm vessel warms the blood in the cool vessel and vice versa.

The third factor that affects exchange is the surface area of contact between the vessels. **Figure 1** shows only one artery and one vein. **Figure 2** shows a situation where the total cross-sectional area is the same, but in one case there are two large warm vessels and two large cool vessels (**Figure 2**(**a**)), whereas in the other case there are many small warm vessels and many small cool vessels (**Figure 2**(**b**)).

Although the cross-sectional area is the same in both the cases, there is a larger area available for exchange with the small vessels when compared with the large vessels and thus much more exchange will take place. In actual retia, the blood vessels divide many times so that there are large numbers of arterial vessels, each surrounded by venous vessels, and large numbers of venous vessels, each surrounded by arterial vessels. For example, the large arteries that feed the lateral muscle retia in giant Atlantic bluefin tuna are about 5 mm in diameter, and these divide and subdivide many times so that the vessels in the retia that serve the muscles are only about 0.04 mm in diameter. Even if the subdivision into smaller and smaller blood vessels were such that the total cross-sectional area remained the same, the perimeter of the vessels (i.e., the area available for exchange) would have increased 100 times. In fact, the cross-sectional area of the vessels in the rete is always several times greater than that of the artery that supplies it.

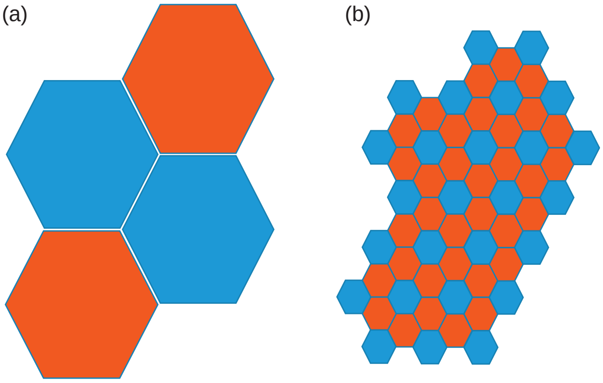


Figure 2 (a) An exchanger consisting of two large warm vessels and two large cool vessels. (b) An exchanger consisting of many small warm and cool vessels, but with the same cross-sectional area as in (a). The contact area for exchange is much greater with many smaller diameter vessels.

The length of the rete vessels also contributes to exchange area: if the vessels are very short, then there is little opportunity for exchange. However, there are some important trade-offs associated with an exchanger with longer vessels of larger diameter as opposed to an exchanger with shorter vessels of smaller diameter. Resistance to blood flow is very sensitive to changes in diameter; it increases with fourth power. Thus, smaller diameter vessels require higher input pressure. On the contrary, long rete vessels require more space for the rete. Moreover, there is no advantage to having vessels longer than that required for exchange to occur. For example, the choroid rete behind the eye in most fishes consists of small-diameter short vessels (there is very little space between the eye and the brain for a rete). In contrast, the visceral rete consists of larger-diameter vessels that are much longer. Thus, all three factors are important for the exchanger to work effectively: proximity of the arterial and venous vessels, flow rate, and subdivision into smaller-diameter vessels to increase the area for exchange. In the example discussed earlier, heat was exchanged between the blood vessels, but in some retia carbon dioxide and oxygen are exchanged – both types of exchangers in fishes are discussed.

Countercurrent Heat Exchangers in Fishes

Although there are about 30 000 species of fish, only about 30 species have well-developed countercurrent heat exchangers; they include tunas, some billfishes, and certain shark species. These fishes have heat exchangers that serve their muscles, their brains and eyes, and their viscera. The exchangers conserve the metabolic heat produced by a particular tissue in that tissue itself; that is, these fishes are warm-bodied, whereas all other fishes have tissue temperatures that are almost identical to that of their environment. The functional significance of endothermy or being warm-bodied is discussed in **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. All animals produce metabolic heat, largely because biochemical reactions are not 100% efficient and much of the energy is lost as heat. Most fishes are cold-blooded owing to the pattern of circulation and the fact that all of the blood passes through the gills for gas exchange. As the venous blood passes through a tissue, its temperature equilibrates with that of the tissue (i.e., it picks

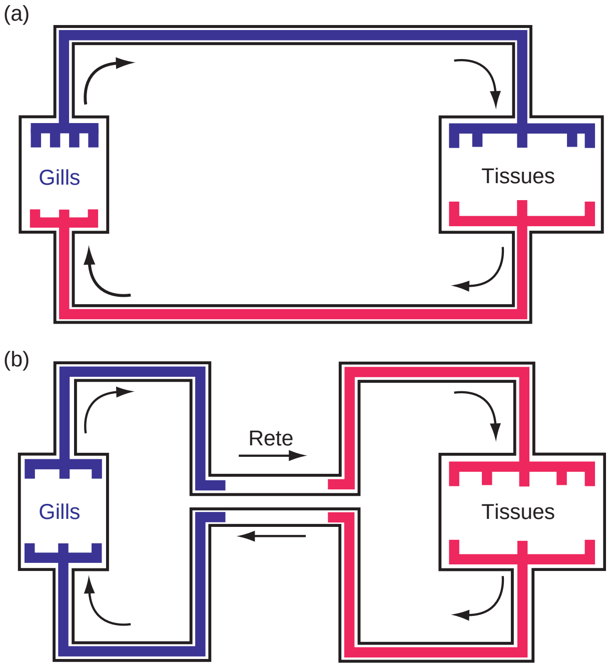


Figure 3 (a) The pattern of circulation in most fishes in which all the metabolic heat produced by the tissues is lost at the gills during gas exchange. (b) The pattern of circulation in fishes with countercurrent heat exchangers – the metabolic heat produced by the tissues is conserved in the tissues that produce it.

up the metabolic heat produced by that tissue and carries it to the gills). The gills have a very large surface area for gas exchange, and the water passing over the gills has a very high heat capacity. Thus, as the blood passes through the gills, the blood temperature equilibrates with ambient water temperature. All the metabolic heat is lost to the ambient water and the arterial blood that delivers the oxygen to the tissues is at the same temperature as the ambient water (**Figure 3**).

Figure 3(**a**) shows the pattern of circulation in most fishes in which all the metabolic heat produced by the tissues is lost at the gills during gas exchange. **Figure 3**(**b**) shows the pattern in tunas in which the metabolic heat produced by the tissues is conserved in the tissues that produce it. Thus, retia on the blood vessels that serve the stomach will conserve heat produced near the stomach and will keep it warm. It is important to appreciate that an individual rete on a tissue conserves the metabolic heat produced by that tissue only.

Heat Exchangers That Serve the Viscera

The first, and still the best, anatomical description of retia in fish is that of Eschricht and Müller published in 1835. They provided an extremely detailed description with exquisite illustrations of the retia that serve the viscera of the giant Atlantic bluefin tuna. These retia are huge, some as large as an orange, and consist of many thousands of arterial and venous blood vessels arranged in parallel. A drawing of a visceral rete from their study is shown in **Figure 4**. Of the 15 species of tunas, only five have visceral retia: *Thunnus thynnus* (Atlantic bluefin), *Thunnus maccoyii* (southern bluefin), *Thunnus alalunga* (albacore), *Thunnus orientalis* (Pacific bluefin), and *Thunnus obesus* (bigeye).

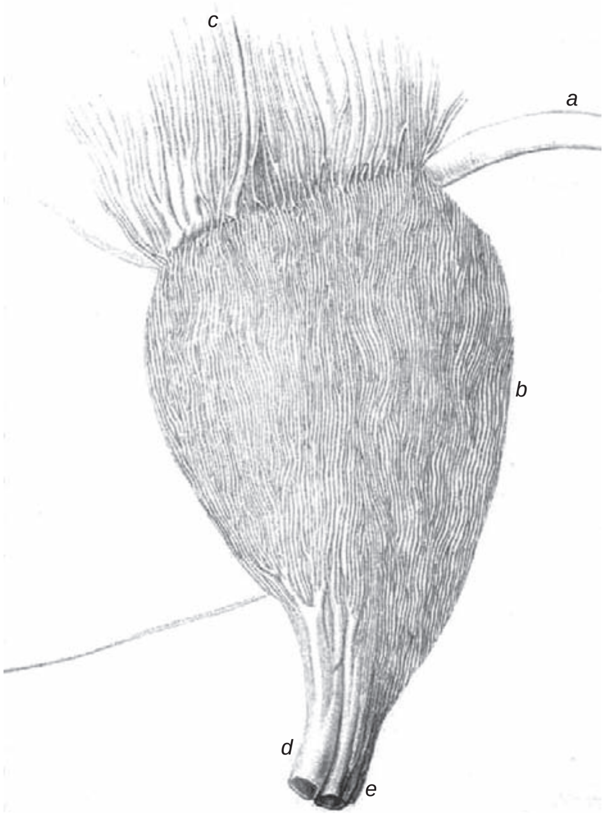


Figure 4 One of the drawings by Eschricht and Müller of a single visceral rete from a giant Atlantic bluefin tuna; the rete vessels are about 7 cm long. (a) Branch of the visceral artery that forms the arterial vessels within the rete. (b) Rete. (c) Branches of the hepatic portal vein that carry the venous blood after passing through the rete. (d) Branch of the visceral portal vein that forms the venous vessels within the rete. (e) Artery formed from arterial rete vessels carrying the arterial blood after passing through the rete.

Figure 5 shows an example of a visceral rete from a 450-kg giant Atlantic bluefin tuna. The rete on the left is still intact and connected to the liver and the pyloric ceca. The rete on the right dissected out of the gut was perfused with saline to remove the blood. The blanched color emphasizes the fact that the vascular tissue making up the rete is translucent white, and it is the blood that gives the intact rete its purplish-red color (in the living animal it would be bright red because the blood in the arterial vessels would be oxygenated).

Although there is only one shark (common thresher) with large retia on their visceral organs similar to those found in bluefin tuna, some other sharks do have retia on

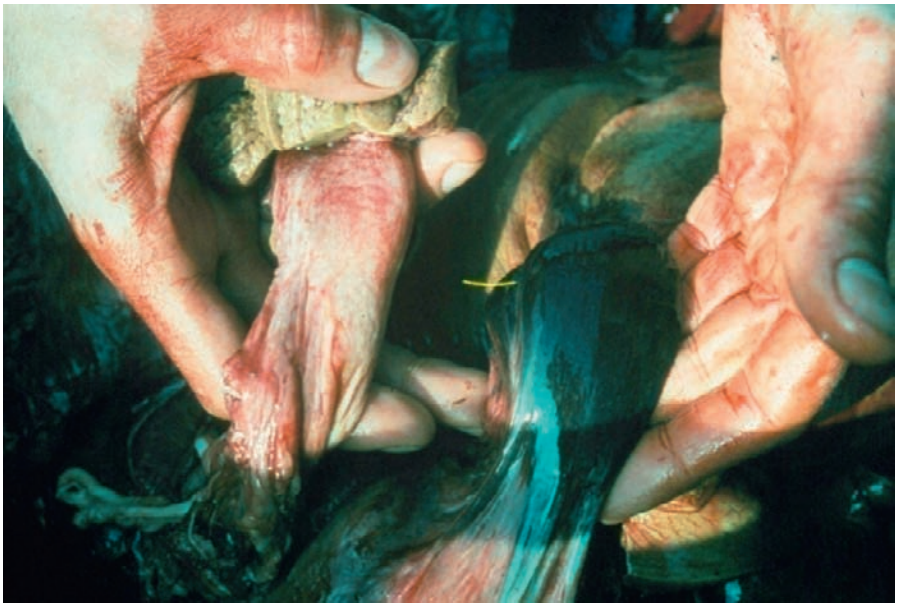


Figure 5 Photograph of visceral retia from a 450-kg giant Atlantic bluefin tuna. (Left) A rete that has been dissected out of the fish and perfused with saline to remove the blood in both the arterial and venous vessels of the rete. (Right) A rete *in situ* and still attached to the viscera.

their livers called suprahepatic retia. The structure of these retia in the lamnid sharks is quite different from that described for the tunas. In these sharks, rather than a grid of arterioles and venules, an array of arterioles pass through a large venous sinus where they are warmed (**Figures 6** and **7**). After being warmed in the venous sinus, the small arterial retial vessels unite to form arteries that supply the stomach and intestines. The main difference between the visceral heat exchangers of the tunas and the lamnid sharks is that in the tunas the liver is not warmed (except by conduction from the viscera), whereas in the lamnid sharks both the liver and the viscera are on the warm side of the visceral rete. The salmon shark also has a separate rete that lies under the kidney and serves to keep it warm.

Heat Exchangers That Serve the Swimming Muscles

The fishes that have retia on their muscles – the tunas, common thresher shark, and lamnid sharks – have their red muscle located centrally rather than in a superficial

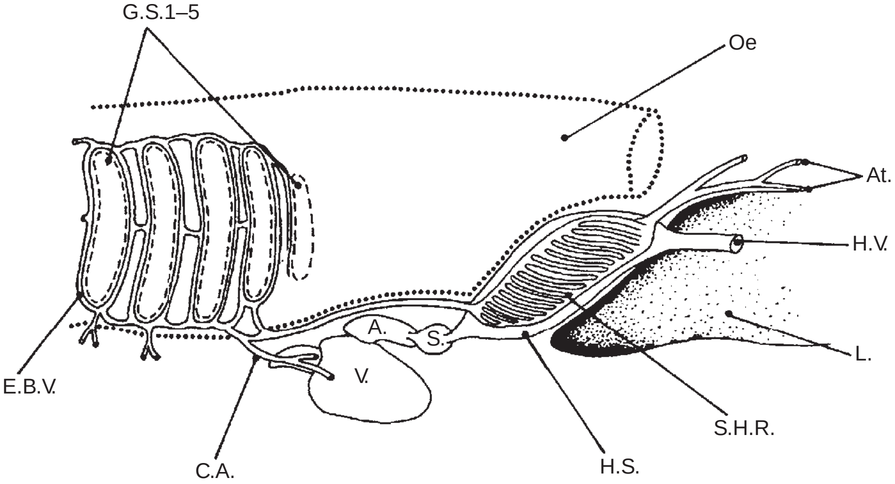


Figure 6 Drawing of the suprahepatic rete in the porbeagle shark to show the pattern of circulation. Cool arterial blood forms many thousands of rete vessels that are bathed in warm venous blood returning from the viscera in a large sinus. At., arteries leaving rete and going to viscera; H.S., hepatic sinus; H.V., hepatic vein; L., liver; Oe, esophagus; S.H.R., suprahepatic rete. Reproduced from Satchell GH (1991) *Physiology and Form of Fish Circulation*. Cambridge: Cambridge University Press.

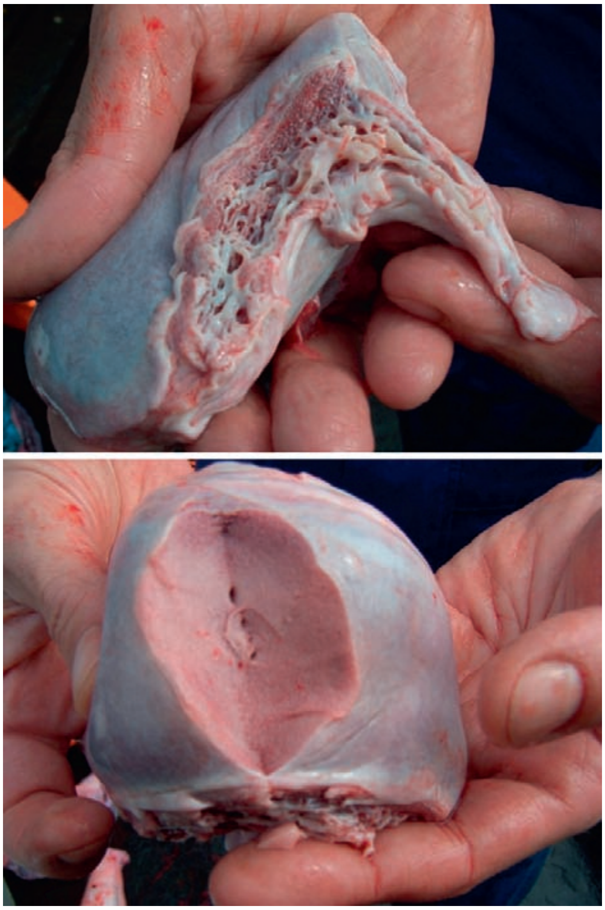


Figure 7 Photographs of the suprahepatic rete from a salmon shark. (Top) Arterial vessels branching as they enter the venous sinus. (Bottom) The same rete from the posterior side showing thousands of arterial vessels within a large venous sinus. Courtesy of Ken Goldman.

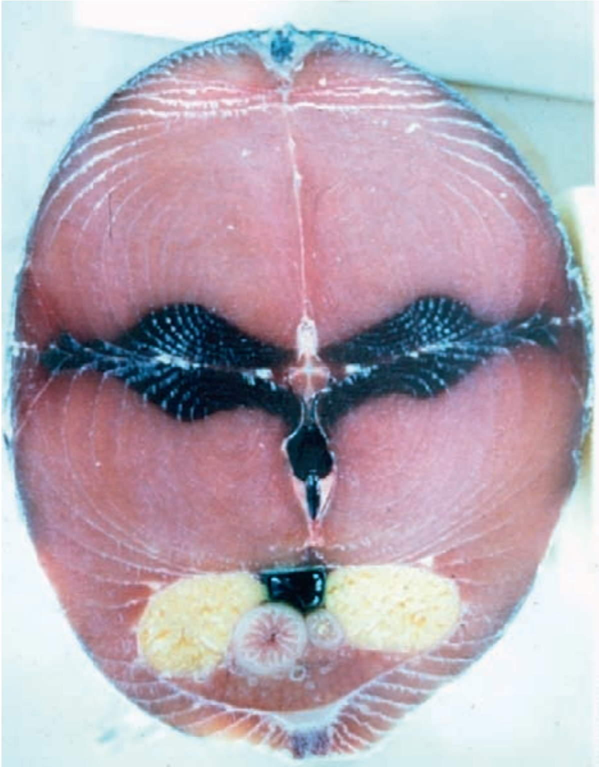


Figure 8 Cross section of a 2-kg skipjack tuna showing the internalized red muscle and the central rete underneath the spinal cord that serves the red muscle. The dorsal aorta and the postcardinal vein are immediately below the rete and are surrounded by kidney tissue.

layer. It is the centrally located red muscle that the retia serve to conserve metabolic heat. Some of these fishes have the retia located centrally, under the spinal cord, and others have the retia located laterally.

Skipjack tuna (*Katsuwonus pelamis*) have the best developed central muscle rete. The location of the rete is shown in **Figure 8**, with a histological slide of the rete region in **Figure 9**(**a**) and a schematic of the slide in **Figure 9**(**b**). After having passed through the gills, the blood in the dorsal aorta is cool and about the same temperature of ambient water. Segmental pre-retial vessels carry blood from the dorsal aorta to the bottom of the rete where they branch many times to form the arterial vessels of the rete. After passing through the rete and picking up the metabolic heat from the venous retial vessels, they unite at the top of the rete at each myotomal segment to form the arteries that supply red muscle. Venous segmental vessels, now warmed in the muscle by its metabolism, return the blood to the top of the rete. There they branch many times to form the venous vessels of the rete. As the blood flows through these venous retial vessels, the heat is transferred to the surrounding arterial vessels. The venous retial vessels, now cooled, unite at the bottom of the rete to form segmental venous vessels that carry the blood to the postcardinal vein. There are about 125 000 arterioles and a similar number of venules in the central rete of a 2-kg skipjack tuna. **Figure 10**(**a**) shows a cross section of the rete vessels to show the large number of parallel rete vessels and **Figure 10**(**b**) shows a higher magnification of the vessels to show how each (small, thick-walled) arteriole tends to be surrounded by (large, thin-walled) venules and vice versa. **Figure 10**(**c**) shows a longitudinal section of five rete vessels to show the diameter relative to the size of the red blood cells. The large number of blood vessels in the rete results in a considerable increase in the total cross-sectional area of the vascular bed; hence, the blood velocity is slower than in the dorsal aorta that supplies it. In fact, the velocity is slowed to about 1/80th of that in the dorsal aorta, and it takes the blood about 1 s to pass through the rete and allow for heat exchange to occur.

Lateral muscle retia are more common and are found in the five species of lamnid sharks, the common thresher shark, and tunas other than *Katsuwonus* (**Table 2**). There is less quantitative information on the number and the size of

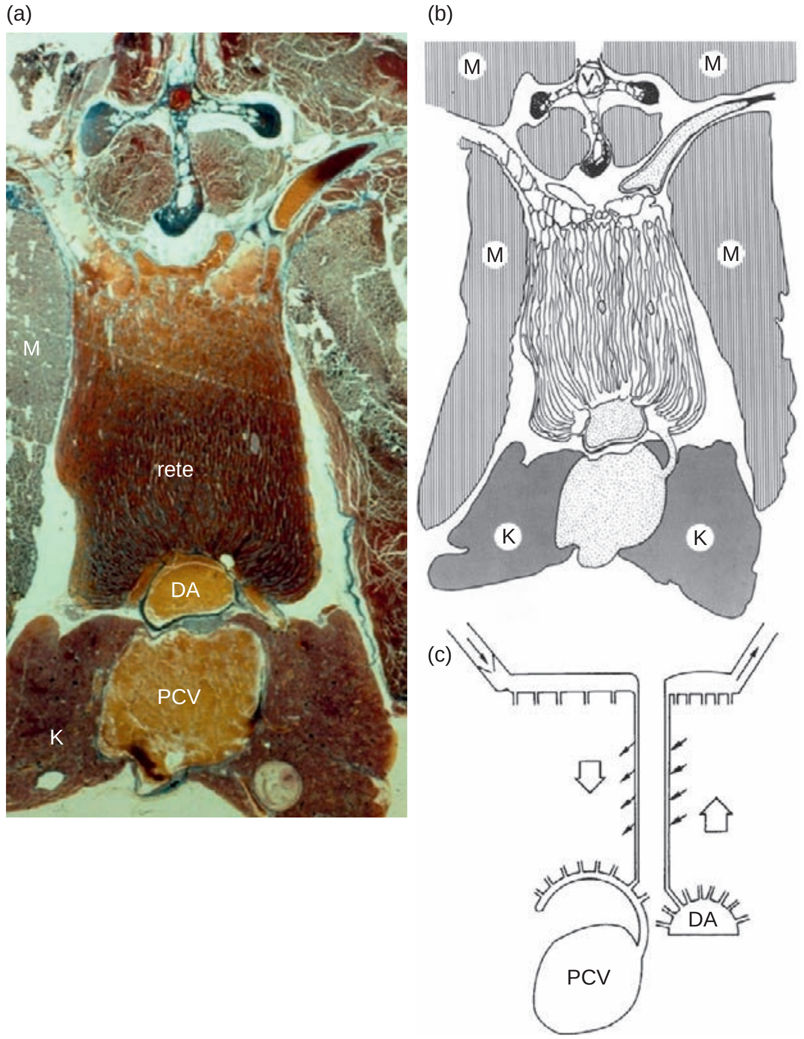


Figure 9 (a) Slide of the central rete of a skipjack tuna. (b) A sketch of the same rete. Compare (a) with diagrammatic sketch in (b) to identify parts of the rete. (c) The pattern of circulation on the right from the DA, through the rete, uniting in segmental arteries at the top of the rete, and on the left from segmental veins, through the rete, uniting in collecting vessel at the bottom of the rete that returns the blood to the postcardinal vein. As the blood passes through the rete, heat is transferred from the blood in warm venous vessels to the blood in the arterial vessels. Thus, heat produced by the muscle is conserved in the muscle. DA, dorsal aorta (containing cool oxygenated arterial blood; K, kidney; M, muscle; PCV, postcardinal vein (containing venous blood cooled in the rete). Adapted with permission of the Company of Biologists.

vessels in these retia than for the central retia. In the tunas these retia are supplied by two lateral arteries on each side of the body, whereas in the sharks they are supplied by only one lateral artery on each side of the body.

Heat Exchangers That Serve the Brain and Eyes

The true tunas (*Katsuwonus*, *Euthynnus*, and *Thunnus*) have a rete on each internal carotid artery to conserve heat in the brain and eyes. Lamnid sharks have a separate blood vessel that carries warm blood from the warm red muscle to a venous sinus near the brain and eyes. Cool arterial blood enters arterial rete vessels within the venous sinus where it is warmed before exiting and going to serve the brain and eyes.

Countercurrent Multipliers That Serve the Swimbladder and the Retina in Fishes

The retia that serve the swimbladder and the retina are talked about together because they consist of vessels of similar size and they use the same mechanism to conserve and secrete oxygen (**Figure 11**). In the swimbladder, the oxygen is released in gas form and contributes to buoyancy. In the eyes, the oxygen is used for metabolism by the avascular retina. Although in both cases the result is

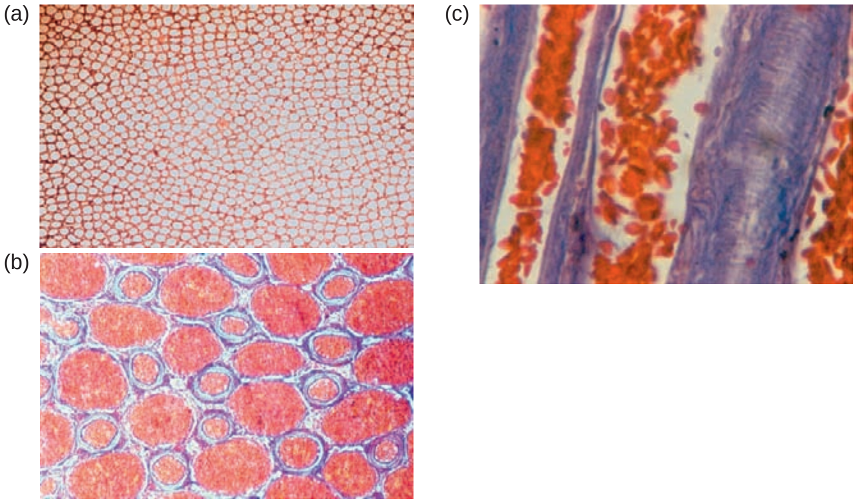


Figure 10 (a) Cross section of the central rete (low-power magnification) of a 2-kg skipjack tuna. The rete has been perfused with saline to remove the blood to show how the vessels are packed together to facilitate exchange. (b) Cross section of the central rete (high-power magnification) of a skipjack tuna. The rete has not been perfused with saline so the red blood cells are still visible. The larger thin-walled vessels are venous and are surrounded by smaller thick-walled arterial vessels. Note that the venous vessels are surrounded by arterial vessels and vice versa. (c) Section of the rete parallel to the vessels in the rete. Individual red blood cells are visible; the diameter of the vessels is almost the same as those of arterioles and venules. Adapted with permission of the Company of Biologists.

Table 2 Similarities and differences in location and size of heat exchange retia in sharks, tunas, and billfishes the salmon shark is the only species that has a separate rete serving the kidney

		EYE	*LAT MUS*	*CENT MUS*	*GUT*
Lamnidae	*Mackerel sharks*				
Carcharodon carcharias	Great white	From RM	Large	No	Large
Isurus oxyrinchus	Shortfin mako	From RM	Large	No	Large
I. paucus	Longfin mako	From RM	Medium	No	Large
Lamna ditropis	Salmon	From RM	Very large	No	Large
L. nasus	Porbeagle	From RM	Large	No	Large
					Kidney
Alopiidae	*Thresher sharks*				
Alopias pelagicus	Pelagic thresher	Large	No	No	No
A. superciliosus	Bigeye thresher	Large	No	No	No
A. vulpinus	Common thresher	Absent	Large	No	Large
Tribe Thunnini	*Tunas*				
Allothunnus fallai	Slender	?	No	Medium	No
Auxis thazard	Frigate	?	1 Small	Medium	No
A. rochei	Bullet	?	1 Small	Large	No
Euthynnus lineatus	Black skipjack	Large	1 Small	Large	No
E. alletteratus	Little tunny	Large	1 Small	Large	No
E. affinis	Kawakawa	Large	1 Small	Large	No
Katsuwonus pelamis	Skipjack	Large	2 Small	Very large	No
Thunnus tonggol	Blackfin	Large	2 Very small	?	No
T. atlanticus	Longtail	Large	2 Very small	?	No
T. albacares	Yellowfin	Large	2 Small	2 Small	Very small
T. obesus	Bigeye	Large	2 Medium	No	Small
T. allanunga	Albacore	Large	2 Large	No	Large
T. orientalis	Pacific bluefin	Large	2 Very large	No	Large
T. maccoyii	Southern bluefin	Large	2 Very large	No	Large
T. thynnus	Atlantic bluefin	Large	2 Very large	No	Large
Xiphiidae	*Billfish*				
Xiphias gladius	Swordfish	Large	No	No	No
Istiophoridae					
Istiophorus platypterus	Sailfish	Large	No	No	No
Makaira nigricans	Blue marlin	Large	No	No	No
M. indica	Black marlin	Large	No	No	No

EYE refers to the heat exchange retia that warm the brain and eyes (these are not the same as the choroid retia that are involved in gas exchange; RM, red muscle); LAT MUS refers to the retia located on the outside of the body wall that warm the red muscle; CENT MUS refers to the rete located centrally below the vertebral column of some tunas; GUT refers to the visceral heat exchange retia.

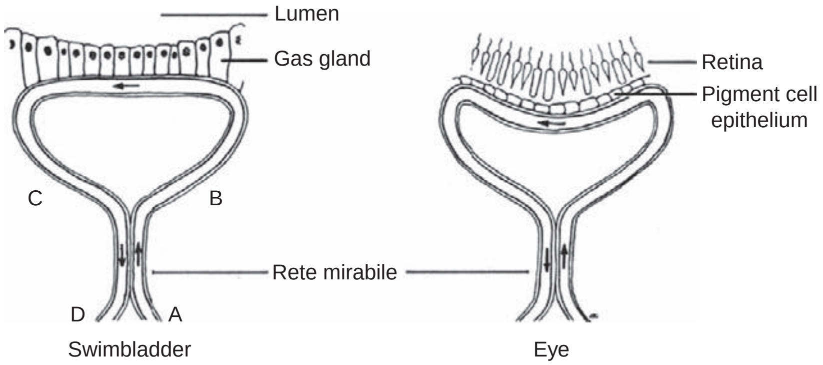


Figure 11 Schematic showing the similarity in the pattern of circulation in the choroid rete that serves the retina and in the rete that serves the swimbladder. Reproduced from Wittenberg JB and Wittenberg BA (1974) The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: Comparison with the swimbladder rete mirabile. *The Biological Bulletin* 146: 116–136.

an increase in oxygen pressure, the mechanism involves the transport of both oxygen and carbon dioxide across the rete.

The motive for conserving oxygen is best illustrated in the swimbladder. For a fish living at a depth of 1000 m, the hydrostatic pressure is 100 atm and, thus, the pressure inside its swimbladder must also be 100 atm. However, the total gas pressure in ambient water is only 1 atm and of oxygen about 0.2 atm; thus, the maximum in arterial blood is 0.2 atm. If this arterial blood went directly to blood vessels in the wall of the swimbladder, it would equilibrate and essentially all of the oxygen in the swimbladder would be lost to the blood and then lost to the water as the venous blood passed through the gills. Therefore, it is essential to conserve the gas that is at high pressure in the swimbladder; the only way to achieve this is with a rete. The mechanism involved in conserving gas is essentially the same as that described earlier for conserving heat. The blood that leaves the swimbladder contains oxygen dissolved at very high pressure (essentially the same as the pressure in the swimbladder). As the venous blood flows through the venous capillaries in the rete, the oxygen diffuses (sometimes called back-diffusion) into the arterial capillaries. After the oxygen passes through the rete, its partial pressure will be almost the same as that in the incoming arterial vessels. Thus, the rete conserves the high oxygen pressure in the swimbladder.

Because there is a large P_{O_2} gradient between the swimbladder and the ambient water, some oxygen will diffuse out and eventually be lost. This gas must be constantly replaced. In addition, gas must be secreted into the swimbladder when a fish descends in the water column and the increase in hydrostatic pressure squeezes the swimbladder to a smaller volume. (Boyle's law states that volume of a gas in an elastic container is inversely proportional to pressure.) To maintain neutral buoyancy, gas must be secreted into the swimbladder to restore its volume. The mechanisms involved in gas secretion are complex and are discussed in detail in **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. Gas secretion involves more than just exchange, and these retia are called countercurrent multipliers because the P_{O_2} achieved by the structure is greater than the input value. An important aspect of the countercurrent multiplier is that the end of the rete forms a loop (**Figure 11**). The repeated recirculation or back-diffusion of oxygen across the rete leads to further increases in P_{O_2}. There are three other traits that must be present for gas secretion to occur. Fishes that employ this strategy have hemoglobin molecules with special properties, the so-called Root effect (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems and **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). These molecules have a reduced capacity to bind oxygen in the presence of acid. Also important is the feature that blood must have a low buffering capacity so that the addition of acid results in a decrease in pH. The third feature is specialized cells that produce lactic acid even though oxygen levels are high. Acidification also occurs because the rete conserves CO_2.

The rete multiplies the P_{CO_2} so that it is high at the gas gland end. The addition of lactic acid (pK_a 3.8) to the blood by the gas gland favors the shift of carbonate and bicarbonate ions to form dissolved CO_2 (pK_a 6.1); both the lactic acid and CO_2 result in a decrease in pH. This acidification results in oxygen release from hemoglobin into solution. Because of the low solubility of oxygen in plasma, there is a large increase in P_{O_2}. This oxygen diffuses out of the venous capillary in the rete and into the arterial capillary where the P_{O_2} is lower. The multiplier effect is achieved because oxygen is repeatedly added, with higher and higher P_{O_2} values on the gas gland side of the rete so that the partial pressure on the gas gland side of the rete is 500-fold higher than that on the incoming side. In the same way that oxygen is multiplied by the rete at the gas gland side, carbon dioxide also is multiplied.

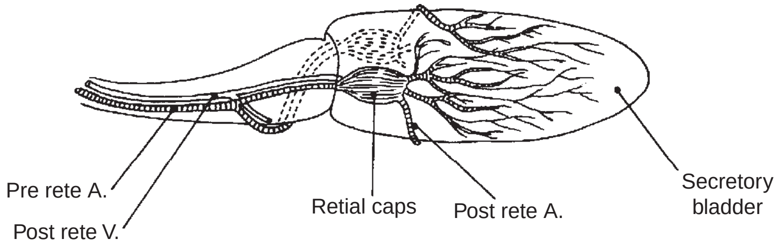
Countercurrent Exchangers That Serve the Swimbladder

In most fishes, the swimbladder is a buoyancy organ; however, in many fishes, the swimbladder is involved in hearing. This is because there is a connection between the hearing organ and the swimbladder, and the bladder amplifies sounds, much like a drum. In some fishes (e.g., the eel), the swimbladder rete is a distinct structure easily visible to the naked eye (**Figure 12**), whereas in most fishes there are many retia that are diffusely located and cover much of the surface of the swimbladder.

Countercurrent Exchangers That Serve the Retina

The rete that serves the retina is called the choroid rete mirabile. The retia serving the eyes and the swimbladder have vessels of similar diameter, but the vessels of the choroid rete are shorter because the P_{O_2} required for diffusion into the retina is not as high as that required for diffusion into the swimbladder when the fish is at depth. The length of the vessels in the choroid rete is almost the same in all fishes, about 0.14 cm; it is independent of the size of the fish (from 2 g to 400 kg). The rete creates a high oxygen partial pressure for diffusion to meet the high metabolic demand of the avascular retina. In most other vertebrates, the retina is well vascularized with an abundant supply of capillaries to deliver oxygen to the metabolically active tissue. In fish, the retina is not vascularized and oxygen demand must be met by diffusion over large distances – hence the need for a high oxygen partial pressure. The distance over which oxygen must diffuse is about 6 times greater than in mammals. Thus, most, but not all, fishes have a rete behind the retina. For example, skate (*Raja ocellata*), sting ray (*Dasyatis centroura*), dogfish (*Mustelus canis*), gar (*Lepisosteus* sp.), and eel (*Anguilla rostrata*) do not have choroid retia, whereas cod (*Gadus morhua*), bluefish (*Pomatomus saltatrix*), menhaden (*Brevoortia tyrannus*), trout (*Oncorhynchus mykiss*), and bowfin (*Amia calva*) have well-developed and large choroid retia. Generally speaking, the fishes that do not have a choroid rete are not visual predators, rather they rely on other senses (chemical and electrosense) to locate their prey.

The choroid rete lies within the eyeball in the choroidal layer retinal cells at the back of the retina. Thus, the schematic diagram in **Figure 13** is slightly misleading because the actual rete lies close to the retina. The ophthalmic artery divides into two branches, one on



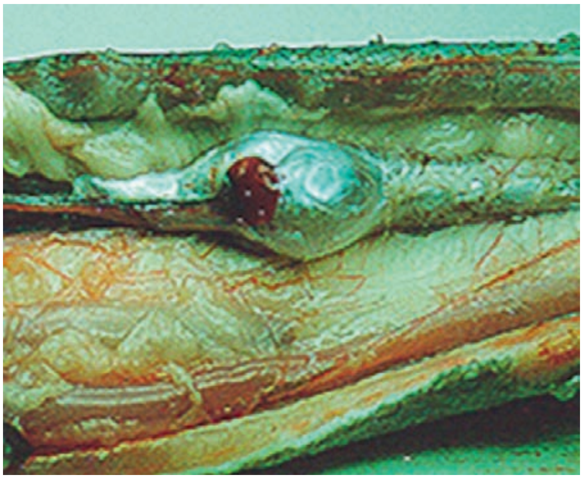


Figure 12 (Top) Schematic showing the large size of the swimbladder retia in the eel (ventral view). Reproduced from Satchell GH (1991) *Physiology and Form of Fish Circulation*. Cambridge: Cambridge University Press. (Bottom) Photograph of the swimbladder rete in the eel. Courtesy of Ben Clemens.

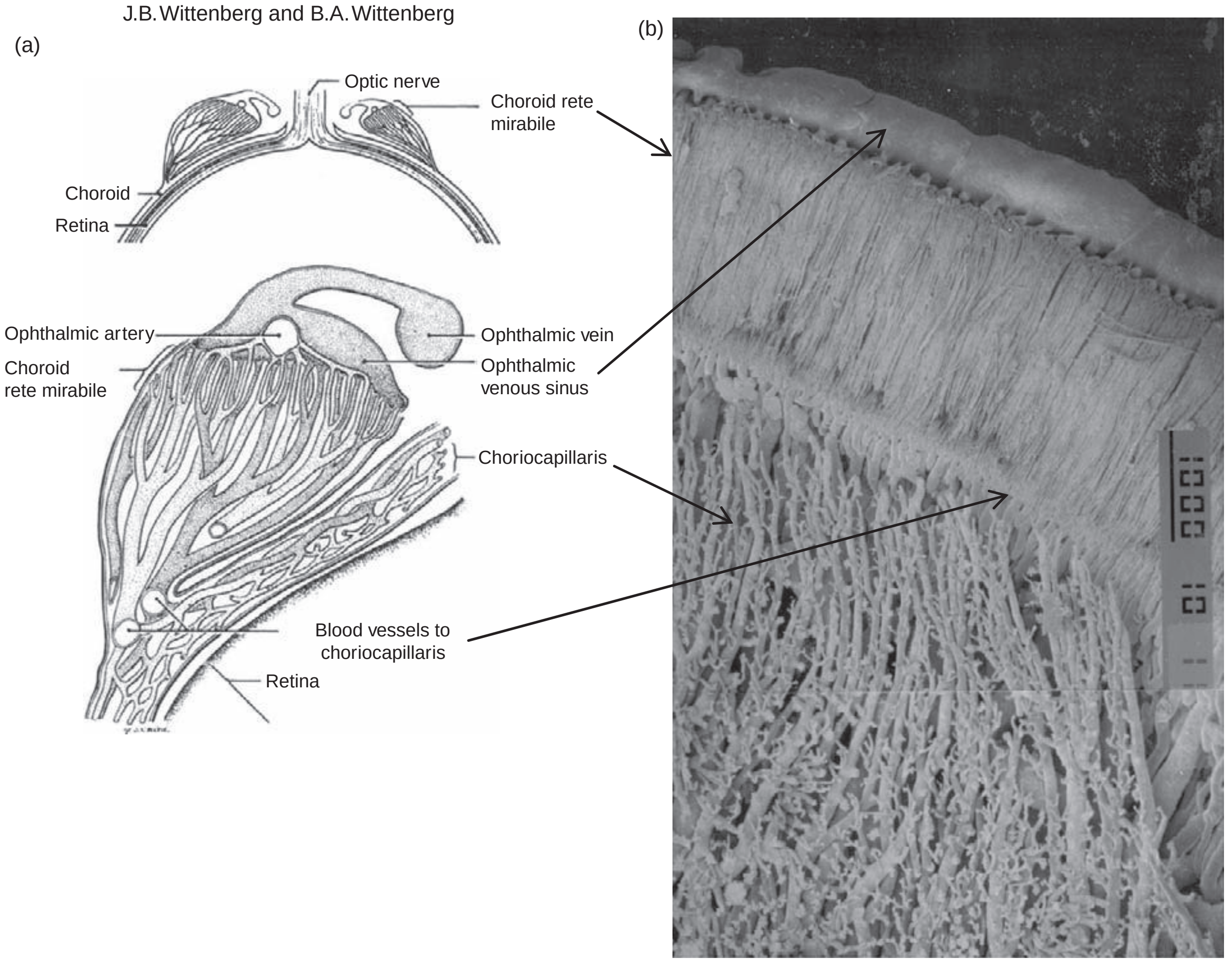


Figure 13 (a) Schematic of the choroid rete. (b) A scanning electron microscopic image of a vascular cast of the choroid rete in a similar orientation showing the same structures as in the drawing. The ophthalmic artery (containing cool arterial blood) divides into thousands of small rete vessels, which then reunite at the bottom of the rete to form the vessels that supply the capillaries of the retina (choriocapillaris). The blood leaving the retina enters the bottom of the rete, passes through it, and then unites at the top to pass blood to the ophthalmic vein. Both oxygen and carbon dioxide are maintained at high partial pressures on the retina side of the rete. Specialized cells on the retinal side of the rete add acid to the blood, resulting in the release of oxygen from hemoglobin and corresponding increase in P_{O_2} (a) Reproduced from Wittenberg JB Wittenberg BA (1974) The charoid rete mirable of the fish eye. 1. Oxygen secretion and structure: Comparison with the swimbladder rete mirable. *The Biological Bulletin* 146: 116–136. (b) Courtesy of Ken Olsen.

each side of the retina. Many smaller arteries branch off from the main ophthalmic artery, and then subdivide many times to form the small vessels of the choroid rete. They then merge to form the larger choriocapillaris vessels that supply oxygen and nutrients to the retina. The location of the choroid rete, the path of blood flow, and a

Table 3 Fishes with well-developed choroid retia tend to have high P_{O_2} values in the eyes

Common name	*Species*	*Size of choroid rete*	P_{O_2} *(mm Hg)*
Dogfish	*Mustelus canis*	Absent	11
Eel	*Anguilla rostrata*	Absent	18
Gar	*Lepisosteus spatula*	Absent	80
Toadfish	*Opsanus tau*	Minute	18
Scup	*Stenotomus versicolor*	Large	484
Menhaden	*Brevoortia tyrannus*	Large	255
Cod	*Gadus morhua*	Large	820
Trout	*Oncorhynchus mykiss*	Large	445

Data from Wittenberg JB and Wittenberg BA (1974) The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: Comparison with the swimbladder rete mirabile. *The Biological Bulletin* 146: 116–136.

scanning electron microscopic image of a vascular cast are shown in **Figure 13**.

The presence of the choroid rete in a particular species of fish is associated with a high P_{O_2} in the eyes. Fishes with well-developed choroid retia have high P_{O_2} values in the eyes (**Table 3**). Without a choroid rete, it would be impossible to achieve a P_{O_2} value higher than that in the arterial blood, which is higher than about 120 mm Hg. The aggregate length of the vessels in the choroid rete of the tuna eye (**Table 1**) is 2.9 km, which gives some idea of the extremely large surface area available for exchange.

Evolution of Retia in Fishes

There has been some recent work by Berenbrink that sheds light on the evolution of retia in fishes (**see also Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems). This work has shown that the structures involved in oxygen secretion evolved very early in the fishes, that the choroid rete evolved first, and that it evolved only once. It first appeared in the ray-finned fishes when the bowfin or a relative (order Amiiformes) diverged from the gars (order Lepisosteiformes) about 250 million years ago. Thus, most teleosts have a choroid rete. However, it has been secondarily lost in a few different groups: for example, elephantnose fishes (Mormyrids), which live in the water with very poor visibility and generate an electric field to 'see' in their murky environment. The evolution of the retia involved in oxygen secretion required a number of associated traits. Most important was a hemoglobin that would release oxygen in the presence of acid. Additionally, specialized cells that produce acid and plasma with low buffering capacity also were essential.

Unlike the choroid rete, the swimbladder rete and other features required for oxygen secretion evolved at least four separate times in the teleost lineage. In every case, the evolution of the swimbladder and its rete was associated with an expansion into a new ecological niche and the accompanying speciation. The evolution of oxygen secretion into the swimbladder is complex because it has two very distinct functions: (1) provides buoyancy and (2) amplifies sound to facilitate hearing. In some groups of fishes, the swimbladder and its rete are important for buoyancy, in some for hearing, and in some for both.

The evolution of the heat exchanger retia occurred much later, about 60 million years ago. It occurred at about the same time in the two very different groups: the lamnid sharks and the tunas. This is an excellent example of convergent evolution. The evolution of these retia is associated with the evolution of a number of other traits that include modification of the structure of the myotomes so that aerobic red muscle used for sustained swimming is internalized; continuous swimming to maintain a vertical position in the water column and to irrigate the gills; high activity of metabolic enzymes, gills with a large exchange area; and regional endothermy. The billfish with retia on the brain and eyes evolved even more recently, about 15 million years ago.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Hearing and Lateral Line**: Physiology of the Ear and Brain: How Fish Hear. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin.

Further Reading

Berenbrink M (2007) Historical reconstructions of evolving physiological complexity: O_2 secretion in the eye and swim bladder of fishes. *Journal of Experimental Biology* 209: 1641–1652.

Bernal D, Dickson KA, Shadwick RE, and Graham JB (2001) Review: Analysis of the evolutionary convergence for high performance swimming in lamnid sharks and tunas. *Comparative Biochemistry and Physiology* 129A: 695–726.

Block BA (1986) Structure of the brain and eye heater tissue in marlins, sailfish, and spearfishes. *Journal of Morphology* 190(2): 169–190.

Block BA (1994) Thermogenesis in muscles. *Annual Review of Physiology* 56: 535–577.

Block BA and Carey FG (1985) Warm brain and muscle temperature in sharks. *Journal of Comparative Physiology* 156: 229–236.

Bone Q and Chubb AD (1983) The retial system of the locomotor muscles in the thresher shark. *Journal of the Marine Biological Association of the United Kingdom* 63: 239–241.

Burne RH (1923) Some peculiarities of the blood vascular system of the porbeagle shark (*Lamna cornubica*). *Philosophical Transactions of the Royal Society London B* 212: 209–257.

Carey FG (1982) A brain heater in the swordfish (*Xiphias gladius*). *Science* 216: 1327–1329.

Carey FG, Kanwisher JW, and Stevens ED (1984) Bluefin tuna (*Thunnus thynnus*) warm their viscera during digestion. *Journal of Experimental Biology* 109: 1–20.

Carey FG and Teal JM (1966) Heat conservation in tuna fish muscle. *Proceedings of the National Academy of Sciences of the United States of America* 56: 1464–1469.

Carey FG, Teal JM, Kanwisher JW, Lawson KD, and Beckett JS (1971) Warm-bodied fish. *American Zoologist* 11: 13–145.

Fudge DS and Stevens ED (1996) The visceral retia mirabilia of tuna and sharks: An annotated translation and discussion of the Eschricht & Müller 1835 paper and related papers. *Guelph Ichthyology Reviews* 4: 1–92.

Graham JB and Dickson KA (2001) Anatomical and physiological specializations for endothermy. In: Block BA and Stevens ED (eds.) *Tuna: Physiology, Ecology, Evolution*, vol. 19, pp. 121–165. New York: Academic Press.

Kishinouye K (1923) Contributions to the comparative study of so-called scombroid fishes. *Journal of the College of Agriculture, Imperial University of Tokyo* 8: 293–475.

Pelster B (2001) The generation of hyperbaric oxygen tensions in fish. *News Physiological Science* 16: 287–291.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. Cambridge: Cambridge University Press.

Stevens ED, Lam HM, and Kendall J (1974) Vascular anatomy of the counter-current heat exchanger of skipjack tuna. *Journal of Experimental Biology* 61: 145–153.

Stevens ED and Neil WH (1978) Body temperature relations of tunas, especially skipjack. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 6. pp. 315–359. New York.

Wittenberg JB and Wittenberg BA (1974) The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: Comparison with the swimbladder rete mirabile. *The Biological Bulletin* 146: 116–136.

The Gastrointestinal Circulation

M Axelsson and H Seth, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
Definition of the GI Tract
Vascularization of the GI Tract
The Liver
The Pancreas
Control of the GBF under Routine Conditions
Factors Influencing the GBF in the Unfed Animal
Concluding Remarks
Further Reading

Glossary

Cardiac output (*Q*) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Catecholamines Any of the group of amines derived from a catechol that has important physiological effects as neurotransmitters and hormones. The main catecholamines are dopamine, adrenaline, and noradrenaline.

Gastrointestinal tract In this article defined as the esophagus, stomach (some fish species lack a stomach) intestine, rectum, and anus. Accessory organs is the liver gallbladder and pancreas.

Hyperemia Increase in blood flow through vasodilation.

In vivo From Latin meaning 'within the living' refers to biological processes or experiments occurring or carried out in living organism as opposed to a partial or dead organism.

Sedentary Contrast to active.

Vasoconstriction A decrease in the diameter of a vessel.

Vasodilator A substance that vasodilates a blood vessel, meaning increases its diameter.

Introduction

The Fish Cardiovascular System

The fish cardiovascular system has a serial arrangement (see **Figure 1**). With the exception of the lungfish, the fish heart is built up of four undivided chambers – the sinus venosus, one single atrium, one single ventricle, and a bulbus arteriosus/cordis (b. arteriosus in teleosts and b. cordis in elasmobranchs) (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes, Accessory Hearts in Fishes, and The Outflow Tract from the Heart). The cardiac chambers are serially connected, and the heart pumps venous blood first into the gills where the blood is oxygenated. Thus, all of the cardiac output (*Q*) goes to the gills. After the oxygenation in the gills, most of the blood leaving the gills enters the dorsal aorta (arterio-arterial route), the main arterial distribution vessel in fishes, which distributes the *Q* to the various parts of the body (the systemic circulation) according to the needs of each tissue. One of the first major arterial distribution paths is the gastrointestinal (GI) tract, which is the focus in this article.

The arrangements of the main vascular beds in fish are interesting from a hemodynamic point of view. When calculating the total vascular resistance, the gills and the systemic circulation (R_{GILL} and R_{SYS} in **Figure 2**) are connected serially and therefore can be added. This means that a change in vascular resistance in the branchial circulation directly affects the dorsal aortic blood pressure, which is the driving pressure for all of the vascular beds that collectively make up the systemic circulation, including that of the GI tract. The systemic circulation can then be subdivided into the GI circulation (visceral) and the somatic circulation, which are connected in parallel (represented by R_{GUT} and R_{SOM} in **Figure 2**), in contrast to the serial connection of branchial and systemic circulation. Thus, the total vascular resistance of the systemic circulation is not the addition of the R_{GUT} and R_{SOM} but instead it is calculated as $1/\text{total} = 1/R_{\mathrm{GUT}} + 1/R_{\mathrm{SOM}}$. This point has to be kept in mind for later in the article when we consider changes in R_{GUT} and R_{SOM}, and how they affect each other.

Definition of the GI Tract

In this article, the GI tract will be loosely defined as a tube that runs from the mouth to the anus in which muscle movements and released enzymes process the food. The

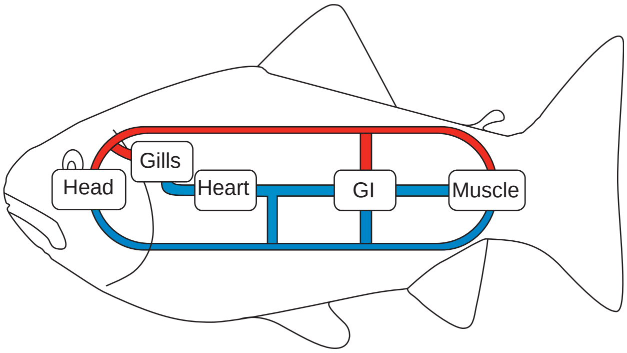


Figure 1 Schematic drawing of the circulatory system in a fish. Note that the heart and gills are placed in series with each other while the GI tract and muscle is connected in parallel. This means that if the blood flow to the GI tract increases, the muscle blood flow will decrease and vice versa unless there is a compensatory increase in overall flow from the heart. Ohm's law states that $V = I \times R$ (V, potential difference in units of volts; R, resistance; I, current). Ohm's law can be applied to the circulatory system as blood pressure = blood flow × vascular resistance, where blood pressure equals V, blood flow equals I, and vascular resistance equals R. This might facilitate the understanding of what happens when blood pressure, blood flow, or vascular resistance changes.

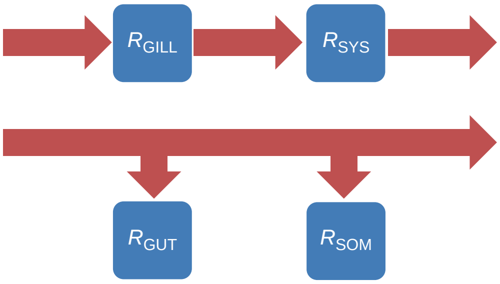


Figure 2 Serially connected resistances. (Top model) The total resistance is additative ($R = R_1 + R_2 + \ldots + R_n$). If the resistances are instead arranged in parallel (bottom model), the total resistance is calculated as $1/TPR = 1/R_1 + 1/R_2 + \ldots + 1/R_n$. In fish the branchial (R_{GILL}) and systemic (R_{SYS}) vascular resistance are serially connected, while the gastrointestinal (R_{GUT}) and somatic vasculature resistance (R_{SOM}) are connected in parallel.

GI tract starts with the mouth followed by the esophagus, stomach, different parts of the intestine, and, finally, the anus. Some fish species (e.g., Cyprinids) lack stomach and the anatomy of the GI tract varies a lot between species and groups (**see also Gut: Anatomy and Morphology**: Development of Fish Gut). The accessory organs associated with the GI tract include the liver, gallbladder, and pancreas, and they are also supplied by the same vascular system that has its origin off the dorsal aorta.

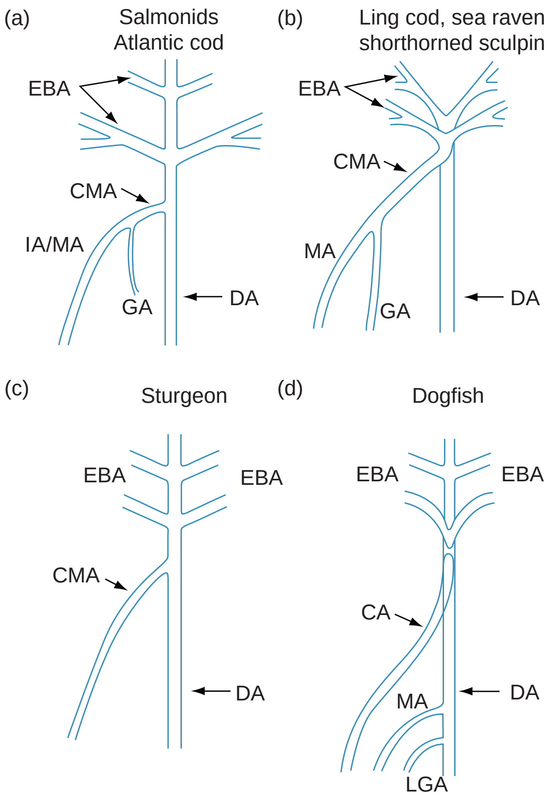


Figure 3 Schematic drawings of the postbranchial vascular tree in different fish species showing where the gastrointestinal blood supply originates. EBA, efferent branchial arteries; CMA, coeliacomesenteric artery; DA, dorsal aorta; GA, gastric artery; IA, intestinal artery; MA, mesenteric artery; LGA, lienogastric artery. Redrawn from Farrell AP, Thorarensen H, Axelsson M, *et al.* (2001) Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry and Physiology Part A* 128: 551–563.

Vascularization of the GI Tract

In elasmobranchs (sharks, rays, and skates), the blood supply of the GI tract resembles the distribution pattern found in mammals with at least three distinct blood vessels, arteria coeliaca, arteria mesenterica superior, and arteria mesenterica inferior (see **Figure 3(d)**). In contrast, in most studied teleosts (bony fishes), the GI tract is supplied by one major vessel, arteria coeliacomesenterica or the coeliacomesenteric artery (CMA), which branches off the dorsal aorta. This vessel then divides into several smaller vessels supplying the gonads, stomach, and

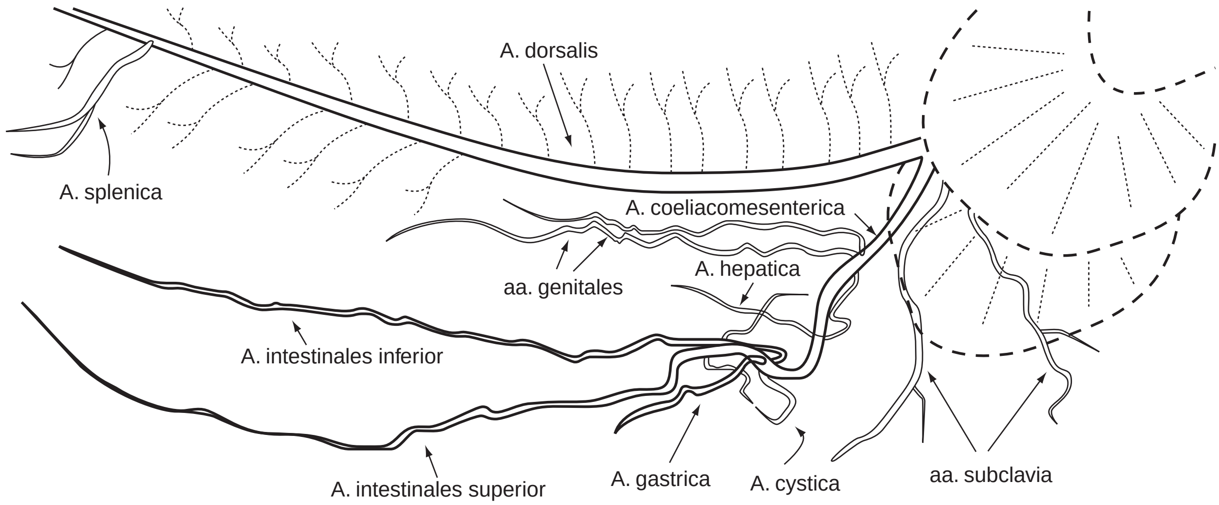


Figure 4 Illustrations of the arterial vascular system in the rainbow trout (*Oncorhynchus mykiss*) with focus on the gastrointestinal blood supply. The figure is based on several corrosion casts. Smaller vessels have been omitted for clarity. A., arteria, aa., arteriae. Redrawn from Seth H, Sandblom E, and Axelsson M (2009) Nutrient-induced gastrointestinal hyperemia and specific dynamic action (SDA) in rainbow trout (*Oncorhynchus mykiss*) – importance of proteins and lipids. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 296(2): R345–R352.

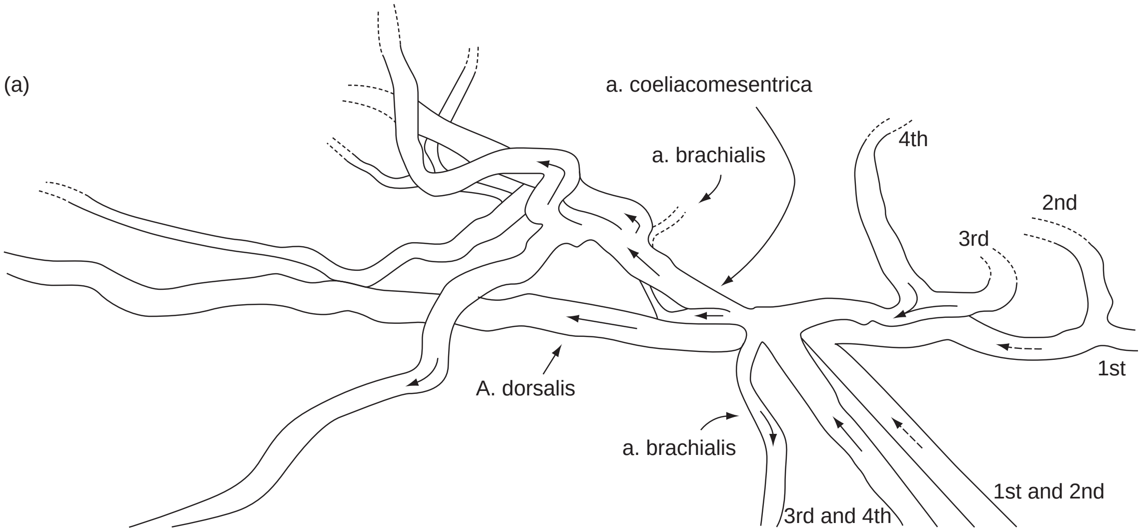
intestine, as well as the liver with blood (**Figures 3**(**a**)–(**c**), **4**, and **5**). Both hagfishes and lampreys have a number of unusual features. Both lack a renal portal system and the kidney is supplied with blood via an arterial connection only. Furthermore, in the lamprey, the intestinal vascular couples have a unique vascular arrangement. Each of these vascular couples consists of an intestinal artery vessel from the dorsal aorta and intestinal vein returning to the posterior cardinal vein. The functional significance of this type of arrangement is unknown. Furthermore, the venous return from the intestine in this group does not enter the hepatic portal system, but returns via a suprarenal venous system to the cardinal vein.

The anatomy of the vasculature of teleost fish, as well as some elasmobranch species, has been investigated using standard *in situ* ocular dissection, with and without color injections of the vascular beds of interest, or the corrosion cast technique (e.g., **Figure 5**). The latter technique normally produces a more detailed picture of the vascular bed and can also be used for electron microscopy imaging looking at the fine details of the vascular casts. In this technique, the vasculature of the euthanized animal is first perfused with saline with an added vasodilator such as sodium nitroprusside. Once the vasculature has been fully rinsed, a two-component epoxi-plastic can be carefully injected and allowed to cure. The organic tissues of the animal are then removed with potassium hydroxide to expose the cast of the vasculature. In some cases, hydrochloric acid is used to remove bony structures that have not been dissolved by the potassium hydroxide treatment (see links in **Figure 5**(**b**)).

This technique has revealed differences between various teleost species, possibly indicating adaptations of the circulation depending on the habitat and feeding regime. In some species the common CMA splits off the dorsal aorta (DA) well behind the junction of the four afferent branchial arteries (see **Figures 3**(**a**), **3**(**c**), and **3**(**d**)), while the CMA vessel branches off very close to this junction in other species (**Figure 3**(**b**)). For example, in the rather sedentary bottom-living shorthorn sculpin (*Myoxocephalus scorpius*), which has a relatively large and very well-vascularized GI tract that receives a large portion of $\dot{Q}$, there seems to be a peculiar anastomose where the branchial arteries unite to form the DA, possibly enabling the animal to direct oxygenated blood from the gills directly to the GI tract (**Figures 3**(**b**) and **5**(**a**)–**5**(**b**)). Whereas in the more active rainbow trout (*Oncorhynchus mykiss*), the CMA branches off the DA well downstream of the branchial arteries (**Figures 3**(**a**) and **4**).

The Liver

All vertebrates have a liver (from the Greek word hepar), which is usually one of the larger organs in the abdominal cavity, accounting for 4–10% of body mass. It is derived from the developing GI tract early in embryonic development. It has a dual blood supply. The arterial supply is derived from branches of the CMA (or one of the mesenteric arteries in elasmobranchs). Some estimates of the arterial blood supply indicate that the liver receives around 2–3% of $\dot{Q}$ via this pathway. The much larger venous supply via the hepatic portal system is constructed of a series of hepatic portal veins (HPVs) draining blood from the GI tract. The arterial blood from the CMA that has first passed through the GI tract drains back to the liver via the HPV and subsequently the



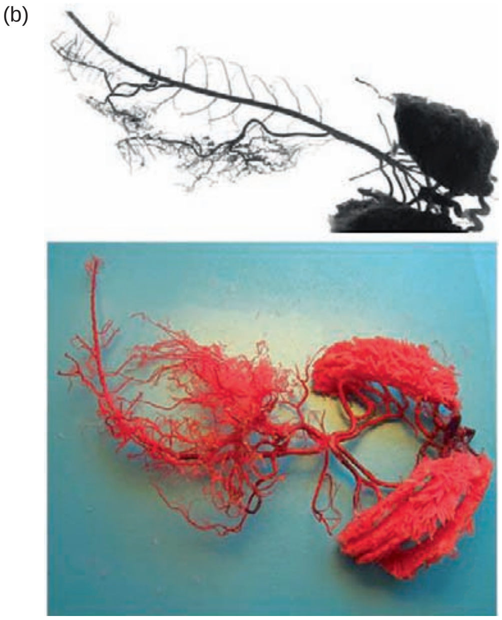


Figure 5 (a) Ventrolateral view of a schematic diagram of the vascular system in the shorthorn sculpin (*Myoxocephalus scorpius*). The figure is based on several corrosion casts. The drawing illustrates the intricate structure of the connection between the efferent branchial arteries (1st, 2nd, 3rd, and 4th) coming from the gills, and the dorsal aorta as well as CMA (supplying the GI tract with oxygenated blood). A., arteria; aa., arteriae. (b) A photo of two corrosion cast made of the vascular bed of the dogfish (*Scualus acanthias*, top) and shorthorn sculpin (lower). The schematic drawing of the major vasculature in **Figures 3**(**d**) and **5**(a) is based on this type of vascular casts. (a) Redrawn from Seth H and Axelsson M (2009) Effects of gastric distension and feeding on cardiovascular variables in the shorthorn sculpin (*Myoxocephalus scorpius*). *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 296(1): R171–R177.

heart via the hepatic veins. The HPV supply has been estimated in a few species to be around 20–30% of $\dot{Q}$. The similarity of this measurement with those for entire GI blood flow (GBF) confirms that the venous output from the GI tract predominantly forms the hepatic portal blood flow into the liver.

The definition of a portal circulation is that the vessels provide a serial connection between two different vascular capillary beds. In the case of the hepatic portal system, the first capillary bed is situated off the GI tract (in the villi), while the second is composed of the wide, fenestrated capillaries of the liver.

In some elasmobranchs, a muscular sphincter is situated on the hepatic veins that return blood from the liver to the heart via the sinus venosus or common cardinal sinuses. These sphincters can constrict with acetylcholine and increase their rate of relaxation with adrenaline. The physiological role of these sphincters is unknown, but clearly they have the potential to affect both the flow of blood leaving the liver and the rate of the venous return to the heart. They could easily work as an external cardiac valve for minimizing the back flow of blood into the liver during the contraction of the sinus venosus. These hepatic sphincters are not unique to elasmobranchs, since inlet and/or outlet hepatic sphincters have been described in mammals, reptiles, amphibians, and other fish species.

The Pancreas

The fish pancreas can be divided into its exocrine and endocrine parts. The exocrine pancreas secretes digestive enzymes into the intestine, while the endocrine pancreas, the Islet of Langerhans, secretes hormones (insulin and glucagons) (**see also Gut Anatomy and Morphology**: Pancreas for more information). The exocrine pancreas is a diffuse organ in teleosts, spread out over the intestinal surface, along the portal vessels and in the overlaying adipose and connective tissue. This means that the pancreas does not have a single distinct blood vessel supplying it. In cyclostomes and most teleosts, the endocrine pancreas is located adjacent to the exocrine pancreas and therefore has a similar diffuse blood supply.

Control of the GBF under Routine Conditions

Under routine conditions, that is, before the ingestion of a meal in a stress-free environment, GBF is regulated to a level sufficient for the housekeeping needs of the GI tract. This routine requirement appears to represent a GBF that is typically 20–40% of routine cardiac output. This percentage is similar to that reported for mammals.

Among fishes, the lowest percentages are reported for sea raven (*Hemitripterus americanus*) and sea bass (*Dicentrarchus labrax*), and the highest for the Atlantic cod (*Gadus morhua*) (see **Table 1** for information on a few selected species and **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion for more detailed information about effects of feeding).

Since the heart cannot simultaneously perfuse all vascular beds at their full capacity, there must be a tight control of each vascular bed to optimize its blood flow and hence oxygen delivery both to its needs and also relative to the needs of other tissues. An example of this balancing act is discussed below for a fish that is swimming while digesting. Regardless of the balancing act that must take place at the level of the whole animal, each vascular bed is not routinely fully perfused, and instead its vessels are constricted to increase their resistance and reduce blood flow to the minimum requirement of the tissue. If tissue activity and oxygen needs increase, so does its blood flow.

In unfed sea raven and red Irish lord (*Hemilepidotus hemilepidotus*), the routine GBF is maintained by an active vasoconstriction of the vasculature in the entire GI tract that is mediated via an α-adrenergic tonus. This was demonstrated by an injection of a drug that blocks α-adrenergic receptors on vascular smooth muscle (the antagonist phentolamine) lowering the vascular resistance of both the celiac and the mesenteric vascular beds in both species. However, in the Atlantic cod only the mesenteric vascular resistance is affected by phentolamine. Therefore, species differences exist among fish species in terms of the details of the control of routine GBF.

This α-adrenergic tonus on the GI vascular beds is most likely chiefly of nervous origin. This has been difficult to definitively verify, primarily because of the difficulty in separating adrenergic effects due to circulating catecholamines from those due to neural tone. However, one study in rainbow trout confirmed that there is in fact a substantial vasoconstrictor tonus on the GI vasculature via the sympathetic innervation. These results were obained by sectioning the sympathetis nerves (via the splanchic nerve) innervating the GI tract. The plasma levels of circulating catecholamines in the species studied to date are likely also too low to significantly stimulate the systemic vascular smooth muscle or the heart. Even so, these rather low concentrations can alter R_{GILL}. In the rainbow trout, especially at cold temperatures, circulating catecholamines may exert an effect, indicating both inter- and intraspecies differences in sensitivity to circulating plasma catecholamines. More studies are needed to resolve these questions.

Factors Influencing the GBF in the Unfed Animal

As noted above, most animals studied so far cannot fully perfuse the entire cardiovascular system at the same time, and integration of the demands of different tissues must occur, with some tissues being prioritized. Exercise, hypoxia, and general stress are good examples of situations where GBF has a lower priority and so GBF is reduced to allow more blood to be directed for fight or flight responses. Below is a short description of the various external stimuli and the effect on the GBF. For more information on the effects of feeding alone, the reader is referred to **Integrated Response of the Circulatory**

Table 1 Postprandial cardiovascular responses, measured as cardiac output and gut blood flow plus the percentage of cardiac output that passes through the coeliacomesenteric artery, in a selection of several different species. The meal sizes used are also given as percentage of body weight.

			Cardiac output (ml min^{-1} kg^{-1})		*Gut blood flow (ml min^{-1} kg^{-1})*		*Gut blood flow/Cardiac output*		
Species	°***C***	***Meal size (%)***	***Routine***	***Feeding (%)***	***Routine***	***Feeding (%)***	***Routine***	***Feeding (%)***	***Source***
Red Irish Lord (*Hemilepidotus hemilepidotus*)	1–9	10–15	24	90	4.1/4.9	112/94	34	40	Axelsson *et al*. (2000)
Sea raven (*Hemitripterus americanus*)	10–12	10–20	18.8	15.4	2.9	100	15	27–30	Axelsson *et al*. (1989)
Short-horn sculpin (*Myoxocephalus scorpius*)	10	8–10	–	50	–	93	–	–	Seth *et al*. (2008)
Sea bass (*Dicentrarchus labrax*)	16	2.9	40	13.5	9.6	71	24	34	Axelsson *et al*. (2002)
Sea bass (only 6h) (*Dicentrarchus labrax*)	22–23	2.7	51.4	22	13.8	82	27	40	Altimiras *et al*. (2008)
Sea bass (*Dicentrarchus labrax*)	19	3	43.4	27%	4.3	160	10	20	Dupont-Prinet *et al*. (1991)
Atlantic cod (*Gadus morhus*)	10–11	2.2–3.5	19*	23	4.1/3.5	72.42	40	52	Axelsson and Fritsche (1991)
Rainbow trout (*Onchorynchus mykiss*)	11–16	2	–	–	4–6	136	-	–	Eliasson *et al*. (2008)
Rainbow trout (*Onchorynchus mykiss*)	9–10	2	–	23–42%	–	156	–	–	Grans *et al*. (2009)
Chinnok salmoon (*Onchorynchus tshawytscha*)	9–10	2	–	–	–	81	–	–	Thorarenson and Farrell (2006)

System: Integrated Responses of the Circulatory System to Digestion.

Stress

The definition of stress is complicated (**see also Hormonal Responses to Stress**: Hormone Response to Stress, Stress Effect on Growth and Metabolism, and Impact of Stress in Health and Reproduction). It can be claimed that stress is any type of external stimuli that affects an organism (a stressor). This wider definition of a stressor could be applied to exercise, hypoxia, hypercapnia, and temperature, for example. However, this is too simplified a view since fish clearly react differently to each of these external stimuli, and there are even different grades of responses. For example, during voluntary aerobic exercise, the classical stress hormones, adrenaline, noradrenaline, and cortisol, typically are not released. Only with more vigorous and exhaustive exercise is a species-specific threshold reached when these stress hormones are released in order to help the system to cope with the higher demand (**see also Hormonal Responses to Stress**: Hormone Response to Stress, Stress Effect on Growth and Metabolism, and Impact of Stress in Health and Reproduction). Thus, it might be more appropriate to apply one of the first definitions of stress given by the Canadian endocrinologist Hans HB Selye in the late 1930s, who described the stress as “the non-specific response of the body to any demand for change.” He argued that stress could be divided into negative stress (distress) and positive stress (eustress). He also argued that stress always involves the hypothalamic–pituitary–adrenal (HPA) axis system.

The fight or flight response also describes the two options that an organism has, either fight the stressor or avoid it. In both cases the body has to prepare for skeletal muscle (locomotory) activity, and the role of the gut can be temporarily put on hold. A number of studies with fish have either noted that a general stress reduces GBF or studied the specific effect of general stress on the GBF. **Figure 6** shows the effects of general stress on GBF in a green sturgeon (*Acipenser medirostris*) equipped with a dual channel telemetric blood flow system. This is a rather typical response that involves a rapid reduction in GBF. The rapid response is likely too fast to be mediated by changes in plasma levels of catecholamines, and probably involves nervous control. Regardless, the mechanism serves to redistribute blood in support of a fight or flight response.

Exercise

In 1982, Whipp defined exercise as “. . . a process which depends upon the transformation of the biochemical energy of ingested food into mechanical energy of muscle contraction and work.” Other definitions have been proposed, but all acknowledge that exercise is an active process that increases the demand for oxygen and nutrients. This demand is met by an increase in blood flow to the activated locomotory muscles. This increase in blood flow is the result of an increase in *Q* and/or a redirection of blood to these muscles away from nonpriority vascular beds.

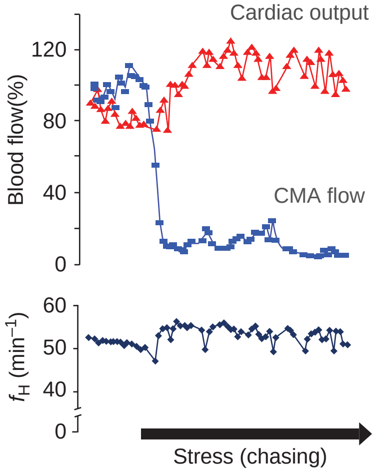


Figure 6 The graph shows the effects of chasing (stress) on *Q* and CMA blood flow (top graph) and f_H (bottom graph) in a green sturgeon (*Acipenser medirostris*) equipped with a dual channel telemetric blood flow system. Note the rapid decrease in CMA blood flow and the increase in *Q* and f_H. Blood flow is directed away from the gastrointestinal tract in order to prepare the animals for fight or flight.

The GI tract is one of the nonpriority vascular beds. Reducing GBF leads to a redistribution of blood toward active locomotory muscles, which could amount to the 30–40% of *Q* it receives in resting, undisturbed fish. Even during moderate exercise, there is a clear reduction in routine GBF (see **Figure 7**). At maximum prolonged swimming speed in a salmonid, the decrease in GBF can be as high as 70–90%, which means that as little as 5% of the routine *Q* would be reaching the GI tract. However, because this reduction in GBF is combined with an increase in *Q*, there is an even larger increase in the total blood flow reaching active muscles. Consequently, GBF is an even smaller fraction of the actual *Q* during exercise.

The reduction in GBF during exercise is via an increase in the vascular resistance of GI tract and mediated by an α-adrenergic mechanism. Obviously, reduced GBF during exercise will reduce the ability for digestion and absorption of nutrients from the gut, dealing meal processing. In seawater fish that drink water to regain water loss across the gills to the hypertonic environment (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction, Mechanisms of Gill Salt Secretion in Marine Teleosts, Mechanisms of Ion Transport in

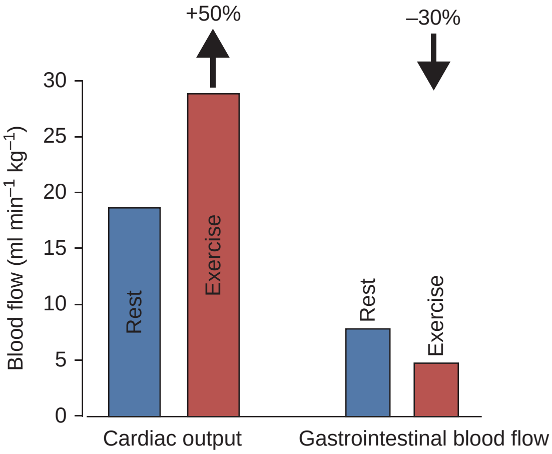


Figure 7 The bar graph shows the redistribution of blood from the GI tract during exercise. The graph is a synthesis of the results from a number of tests in various fish species. During exercise the active muscles have a higher oxygen demand and therefore need more oxygenated blood. This can be achieved by either increasing *Q* or reducing GBF (redistribution), or through a combination of the two as shown in the graph.

Freshwater Fishes, and Water Balance and Aquaporin), there is also a possibility that the osmoregulatory capacity could be compromised during intense exercise.

The effects of exercise after feeding have been tested in a few studies. After feeding, GBF is increased, and this increase is, in most cases, supported by an increase in *Q* (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion). If the fish are then forced to swim, GBF is maintained at a higher level compared to the unfed fish at low swimming speeds but becomes compromised when the fish reaches its maximal swimming capacity. This transition reflects the differences between nonstressful submaximal exercise and anaerobic maximal exercise that mimic the general stress response.

Hypoxia

Hypoxia, which is defined as a lowering of the environmental oxygen availability, may or may not be accompanied by increased carbon dioxide (hypercapnia). In water the amount of physically dissolved oxygen is relatively small, for example, in freshwater at 0 °C and 1 atm, only 10.2 ml oxygen per liter water is available. With increasing temperature and salinity, the available oxygen decreases and at 35 °C and 35 ppt salinity the value is 4.0 ml per liter of water. The levels of both oxygen and carbon dioxide are also highly variable in water compared to air due to the production and consumption with both diurnal and seasonal changes (**see also Hypoxia**: The Expanding Hypoxic Environment). Adding to this low availability of oxygen is the viscosity and density differences between air and water that increase the cost of ventilation dramatically in fish. Responses to hypercapnia *per se* are considered below and here the discussion is restricted to the effects of hypoxia *per se*.

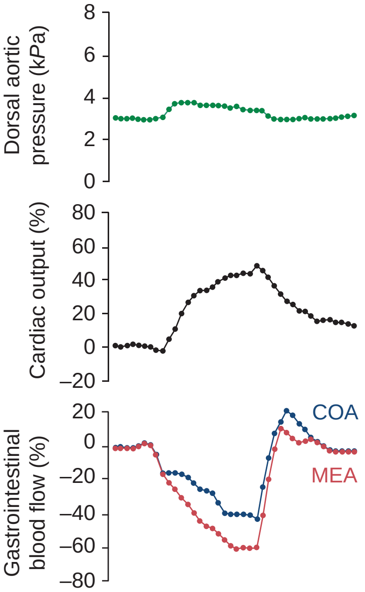


Figure 8 Effects of hypoxia on DA blood pressure (top trace), *Q* (middle trace), and GBF (lower trace) in the Atlantic cod (*Gadus morhua*). Redrawn from Axelsson and Fritsche (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod, *Gadus morhua*. *Journal of Experimental Biology* 158: 181–198.

When fish are exposed to an hypoxic environment, several adjustments to the cardiorespiratory system occur (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia). Fish characteristically undergo a hypoxic bradycardia that may or may not be compensated by an increase in cardiac stroke volume to leave *Q* unchanged (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia). Even so, GBF decreases (see **Figure 8**), as it is not a priority circulation, again due to the increased vascular resistance in the GI tract that is mediated by adrenergic nerves. This vasoconstriction is especially important because dorsal aortic pressure normally increases, which increases the perfusion pressure for both the GI and the systemic circulations. Splenic contraction in some species leads to an increase in circulating red blood cells (**see also Blood**: Cellular Composition of the Blood). The rerouting of blood from the GI tract toward vascular beds that are more dependent on oxygen, together with the increase in total oxygen-carrying capacity due to the spleen contraction and the increase in ventilation, are all aimed at maintaining homeostasis in critical tissues.

Hypercapnia

Carbon dioxide is the end product of the aerobic respiration in both animals and plants. Atmospheric carbon

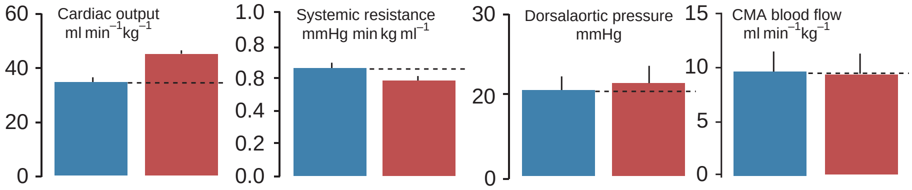


Figure 9 Cardiovascular variables in the white sturgeon (*Acipenser transmontanus*) under control normocapnic conditions (blue bars) and during a 2-h exposure to severe hypercapnia (red bars) P_{CO2} 20 torr at 18 °C. *Q* is increased during the hypercapnic exposure but there is no change in CMA blood flow. Redrawn from Crocker CE, Farrell AP, Gamperl AK, and Cech JJ Jr. (2000) Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *American Journal of Physiology* 279: R617–628.

dioxide levels are increasing from preindustrial levels of around 280 to 370 ppm today. This increase may not in itself be sufficient to affect adult fish, but in combination with other factors such as increased temperature, it could be critical additive stressor. Perhaps more immediately relevant in the fish world is the advent of closed, intense fish aquaculture, which injects oxygen (not air) into the water. Under these situations, carbon dioxide levels in water can easily exceed 10 times normal. Information about the effects of hypercapnia on GBF is scarce. In white sturgeon (*Acipenser transmontanus*) exposed to severe hypercapnia, representative of that seen in an aquaculture setting, CMA blood flow was maintained while overall systemic vascular resistance was lowered and *Q* increased (see **Figure 9**). However, the fish were easily agitated and the decreases in GBF associated with erratic active were deeper and much longer lasting.

Temperature

Little is known about the effects of temperature on the GBF in fish (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature). Studies have indicated that some fish species change their temperature preference after feeding, possibly as a means to increase the efficacy of the GI tract in digesting and absorbing the meal and perhaps also to directly affect GBF. For example, under certain conditions the Bear lake sculpin (*Cottus extensus*), a daytime benthic feeder, migrated into shallower, 10 °C warmer water at night. The consquence of this was a tripled growth rate. In contrast to this, sockeye salmon (*Oncorhynchus nerka*) surface at dusk and dawn to feed, and in between migrate to colder deeper water to minimize routine metabolic rate while digesting their meal and thereby maximizing growth rate.

An interesting observation using free swimming sturgeon equipped with a dual channel blood flow telemetric system showed that GBF did not vary with temperature in fasted animals, while in fed animals there was a strong positive correlation between GBF and temperature. If the temperature increased too much, a typical stress response was triggered, with GBF decreasing presumably to maintain oxygen delivery to other more oxygen-sensitive, priority organs (see **Figure 10**).

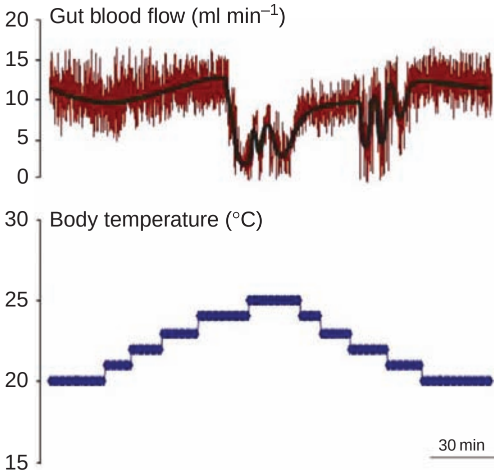


Figure 10 GBF (upper trace, phasic signal in red and average in black) and body temperature (lower panel) in a green sturgeon (*Acipenser medirostris*). The animal was equipped with a dual channel blood flow telemetric system that also records temperature. Temperature was stepwise increased from 20 °C. GBF slowly increases as the temperature increases but is then abruptly decreased, presumably when the temperature passes a critical level and prioritizations have to be made in order to supply the body with enough oxygen.

How preferred temperature changes during infections (exogenous fever) and environmental hypoxia might affect GBF is unknown. A change in preferred temperature during an infection could affect GBF indirectly through a change in temperature, as well as directly through the immune system and the inflammatory processes that follow. The changes in preferred temperature during hypoxia could also affect GBF directly via a stress response or indirectly via a temperature effect, but again this is unknown.

Concluding Remarks

While the above discussion of external stimuli is very interesting, few studies have addressed the question of how the combination of one or more external stimuli will affect GBF. Without GBF, or with a reduced GBF, digestion will cease or slow down, leading to a suboptimal growth and potentially a reduced fitness. In reality, many effects occur simultaneously in the environment, although they were considered separately above. For example, ocean acidification, resulting from elevated atmospheric carbon dioxide levels, is tightly linked to a simultaneous increase in atmospheric and water temperature. Separately, a change in one may not be of sufficient magnitude to be detrimental to fish, but together they can reduce a fish's fitness. Another example is feeding where many species actively search for food and might explore both hypoxic water and different temperatures in combination with the increased demands on the system due to the foraging (exercise) as part of this natural search for food. How these combinations affect the GBF and the capacity of processing food remain largely unknown.

See also: **Design and Physiology of the Heart**: Accessory Hearts in Fishes; Cardiac Anatomy in Fishes; The Outflow Tract from the Heart. **Gut Anatomy and Morphology**: Development of Fish Gut; Pancreas. **Hormonal Responses to Stress**: Hormone Response to Stress; Impact of Stress in Health and Reproduction; Stress Effect on Growth and Metabolism. **Hypoxia**: The Expanding Hypoxic Environment. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion; Integrated Responses of the Circulatory System to Hypoxia; Integrated Responses of the Circulatory System to Temperature. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction; Water Balance and Aquaporin.

Further Reading

Axelsson M, Driedzic WR, Farrell AP, and Nilsson S (1989) Regulation of cardiac output and gut blood flow in the sea raven, *Hemitripterus americanus. Fish Physiology and Biochemistry* 6: 315–326.

Axelsson M and Fritsche R (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod, *Gadus morhua. Journal of Experimental Biology* 158: 181–198.

Crocker CE, Farrell AP, Gamperl AK, and Cech JJ , Jr.. (2000) Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *American Journal of Physiology* 279: R617–R628.

Farrell AP, Thorarensen H, Axelsson M, *et al.* (2001) Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry and Physiology Part A* 128: 551–563.

Gräns A, Albertsson F, Axelsson M, and Olsson C (2009) Postprandial changes in enteric electrical activity and gut blood flow in rainbow trout (*Oncorhynchus mykiss)* acclimated to different temperatures. *Journal of Experimental Biology* 212: 2550–2557.

Seth H and Axelsson M (2010) Sympathetic and parasympathetic regulation of the postprandial gastrointestinal hyperemia in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 213(15): 3118–3126.

Thorarensen H, Gallaugher PE, Kiessling AK, and Farrell AP (1993) Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematrocrit on blood flow distribution. *Journal of Experimental Biology* 179: 115–129.

Thorarensen H, McLean E, Donaldson EM, and Farrell AP (1991) The blood vasculature of the gastrointestinal tract in Chinook, *Oncorhynchus tshawytscha* Walbaum, and coho, *Oncorhynchus kisutch* Walbaum, salmon. *Journal of Fish Biology* 38: 525–532.

Relevant Websites

http://esi.stanford.edu – Environmental Science Investigation.

CIRCULATION

Design and Physiology of Capillaries and Secondary Circulation

Contents

Capillaries, Capillarity, and Angiogenesis
Circulatory Fluid Balance and Transcapillary Exchange
Secondary Circulation and Lymphatic Anatomy

Capillaries, Capillarity, and Angiogenesis

S Egginton and F Syeda, University of Birmingham, Birmingham, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Matching Angiogenesis and Microvascular Function
Concluding Remarks
Further Reading

Glossary

Angiogenesis The process by which an existing microvascular bed expands by growth of new capillaries (the smallest blood vessels) from preexisting vessels.

Anisotropic A capillary bed that is orientated along the axis of the muscle fibers it supplies, without significant meandering.

Arteriogenesis The growth of resistance vessels, vessels that are capable of dynamic adjustments in diameter by way of their contractile smooth muscle layer and innervation. Arteriogenesis usually involves remodeling of the smaller arterioles.

Capillary-to-fiber ratio (*C*:*F*) A commonly used index of the extent of capillarization. It is calculated by dividing the number of capillaries in a field by the number of fibers in the same field. It is not to be confused with either CAF or SF.

Capillaries around a fiber (CAF) The mean number of capillaries surrounding individual fibers.

Capillary density (CD) An index of the functional capacity of the exchange vessels, usually expressed as the number of capillaries per unit area of muscle fibers. It is also often expressed as capillaries per unit area of tissue section, which would also include interstitial tissue, in this case making the meaning of CD less clear.

Capillary length density, J_V(c,f) The length of capillaries per unit volume of muscle. This is calculated by multiplying CD by an estimate of capillary tortuosity.

Capillary surface density, S_V(c,f) An index of the surface area available for respiratory gas, substrate, and metabolite exchange within muscle. It is calculated by multiplying the perimeter of the capillary, *b*(c), by J_V(c,f).

Capillary volume density, V_V(c,f) The instantaneous blood volume within the microcirculation calculated by multiplying capillary cross-sectional area, *a*(c), by $J_V(c,f)$.

Isotropic A capillary bed that runs over the muscle fibers it supplies at varying angles, showing a meandering pattern when viewed in longitudinal sections.

Krogh's radius (*R*) The maximum distance from a capillary that may be adequately supplied by oxygen diffusion, based on hypothetical tissue cylinders. *R* is calculated from the capillary length density as $1/[\pi \cdot J_V(\mathrm{c,f})]^{1/2}$.

Maximum hypothetical diffusion distance (*D*) The maximum distance from a capillary that can be adequately supplied by oxygen diffusion, but without the restrictive and unrepresentative uniform geometry of Krogh's model. Calculated as $\sqrt{[1/\{N(f)a(f)\}/\{\pi N(c)\}]}$, where *N*(f) and *N*(c) are number of fibers or capillaries in the sample area, and *a*(f) is the mean fiber cross-sectional area.

Microvascular unit (MVU) The anatomically connected supply arteriole, exchange capillaries, and drainage venule. These are often longitudinally staggered and/or adjacent MVUs interdigitate in order to even out oxygen tension across a tissue.

Sharing factor (SF) The number of fibers each individual capillary is in contact with.

Stereology An application of geometrical probability theory that permits extrapolation of structural information from *n* to *n*+1 dimensions (e.g., length to surface and area to volume). For example, a systematic random sampling regime is used to quantify muscle fine structure from electron micrographs as volume density (V_V, $\mu m^3 \cdot \mu m^{-3}$), surface density (S_V, $\mu m^2 \cdot \mu m^{-3}$), or numerical density (N_A, μm^{-2}).

Symmorphosis The hypothesis that, in biological organisms, structural design is matched to functional demand.

Vasculogenesis The *de novo* generation of blood vessels from mesenchymal stem cells during embryogenesis.

Introduction

This article is about the anatomical design and plasticity of the microvascular (capillary) supply of tissues, whose primary task is to deliver sufficient oxygen and substrates, and remove waste products. The cardiovascular system has been described in detail elsewhere (**see also Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; **Circulation**: Circulatory System Design: Roles and Principles). Briefly, in fish, it consists of a single-pass circulation that is powered by the heart, a muscular pump of varying complexity. In some cases, the heart requires its own microvascular supply, the coronary microvasculature, to generate adequate power to sequentially perfuse the branchial gas-exchange system followed by the systemic circulation (**see also Design and Physiology of the Heart**: The Coronary Circulation and Cardiac Anatomy in Fishes). The branchial microvasculature is explained in detail in other articles (**see also Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns and **Design and Physiology of Arteries and Veins**: Branchial Anatomy). Therefore, the focus here is on the microvasculature of the skeletal muscle, which comprises up to 80% of the body mass of a fish.

When compared with other vertebrates, oxygenated blood is delivered into the systemic circulation of fishes at relatively low blood pressure, thereby limiting the potential for convective oxygen delivery to peripheral organs. Thus, metabolic error signals, brought about by insufficient substrate delivery or excessive cellular demand, require both anatomical adaptations and physiological responses that improve substrate delivery. The usual vertebrate methods of controlling peripheral arterial resistance and hence tissue-perfusion patterns exist in fishes (sympathetic innervation to constrict pre-capillary vessels, and nitric oxide (NO) and prostaglandin (PG) release to dilate resistance vessels; (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System and **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). Some peculiar vascular arrangements exist, such as a primitive hypobranchial system that provides a dual arterial supply to the locomotory pectoral muscles in some Antarctic teleosts, and is particularly evident in the hemoglobinless icefish (*Chaenichthyidae*). A double supply presumably maximizes delivery of oxygen by perfusion. Overall, vascular adaptations are consistent with improving delivery of blood-borne substances (**Figure 1**).

Capillary Structure

Capillaries are thin tubes that are 4–10 μm in diameter and 500–100 μm in length. They consist of a monolayer of endothelial cells and represent the blood/tissue interface of the circulatory system. Capillary fine structure is closely related to its primary function, diffusive exchange of gases and metabolites, which demands a minimal barrier thickness and a large surface area to facilitate the process of passive diffusion. At the same time, adequate integrity is needed to accommodate the transmural stress of blood perfusion pressure.

At the start of the microvascular unit (MVU), where supply arteries divide into many pre-capillary arterioles, there is a massive increase in total cross-sectional area of blood vessels. As cross-sectional area increases, the velocity of blood flow within the MVU is reduced. This increases the time taken for an erythrocyte to pass through a capillary. The resultant increase in erythrocyte contact time with the capillary endothelial barrier therefore aids oxygen diffusion.

In general, fish have similar capillary dimensions as those of mammals but they have larger blood cells (**see also Blood**: Cellular Composition of the Blood) and a lower body temperature. Even so, the lower deformability of the colder fish erythrocytes does not appear to limit capillary perfusion. Probably more is known about fish capillaries from retia (choroidal, swimbladder, and internalized red muscle), but these are unusual: 4 mm long with endothelial cells up to 4 μm thick, compared with those in skeletal muscle of

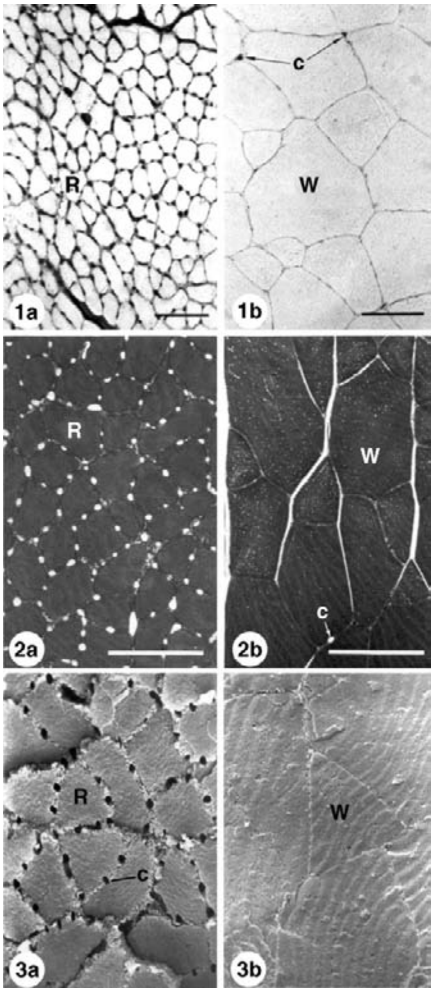


Figure 1 Differences in density of red (R) and white (W) muscle capillary supply in flathead, *Platycepgalus bassensis.* (1) Indian ink-gelatin perfusion followed by cryostat sectioning of frozen tissue (10–12 μm sections, scale bar 100 μm). (2) Fixed muscle, resin embedded (1μm sections, scale bar 100 μm). (3) Scanning electron micrographs of freeze-fractured myotomal muscle (×1400–1500). Note the higher capillary density (c) in red compared to white muscle, and reasonable agreement between estimates of capillary supply derived from the different preparation methods in terms of number per fiber, but not number per fiber area due to tissue shrinkage. Composite of images from Mosse PRL (1978) The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. *Cell and Tissue Research* 187: 281–303.

0.5–1 mm and 0.2–1 μm, respectively (**see also Design and Physiology of Arteries and Veins**: The Retia).

There are only minor differences apparent between fishes and mammals with regard to their basic capillary structure in the skeletal muscle, with the exception of icefish (**Figure 2**). An obvious difference given the higher blood pressure is a thicker endothelium in mammals. However, owing to the low systemic blood pressure, inter-endothelial junctions are likely to be less tight than in the skeletal muscle of other vertebrates, perhaps contributing to the reported higher capillary permeability in fish (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange).

Unlike mammalian muscle, where intermittent perfusion as a result of periodic vasomotion leads to the appearance of collapsed vessels on fixing by immersion, fish muscle capillaries always appear open. This suggests two potential anatomical differences: fish blood vessels may not recoil significantly in the absence of perfusion pressure, which would mean that adventitial filaments join with connective tissue to prevent collapse (as seen with mammalian lymphatic vessels and in the elasmobranch intestine, kidney, and pancreas, as radial strands from the basal lamina surrounding capillaries). Alternatively, there may be continuous blood perfusion of fish capillaries. Observations by intravital microscopy of superficial red muscle in anesthetized fish suggest that the latter is more likely.

Skeletal muscle capillaries are composed of continuous endothelium, but other organ systems have fenestrated capillaries, with or without diaphragms, as befit their function, be it cellular or macromolecular traffic. A tight endothelial blood–brain barrier (BBB) in teleosts appears to be analogous to that in other vertebrates, although elasmobranchs have a glial BBB.

Capillary Function

In mammals, the capillary hydrostatic pressure (developed by the heart and regulated by resistance arterioles) that drives filtration of small molecular weight solutes is opposed by the oncotic pressure of the blood, favoring reabsorption (Starling's forces). It is this balance that regulates fluid exchange. In fishes, the functional consequences of a low blood pressure across the capillary wall would be to reduce any such regulation. However, the capillary wall in fish is unusually permeable to macromolecules and proteins, and 5 times more than mammalian capillaries (filtration coefficient of 5 ml mmHg^{-1}), which therefore allows solute exchange, but at the cost of a reduced oncotic pressure difference (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange). For example, plasma hydrostatic pressure decreasing from ~13 to 3 mmHg along a capillary, with a capillary oncotic pressure of ~8 mmHg, would result in no net fluid movement without a degree of selective permeability and/or effective lymph-like drainage. The consequence is that interstitial fluid has a high protein concentration, but the ease of fluid exchange allows large changes in plasma volume to be tolerated, for example, the half-time of transcapillary fluid exchange is

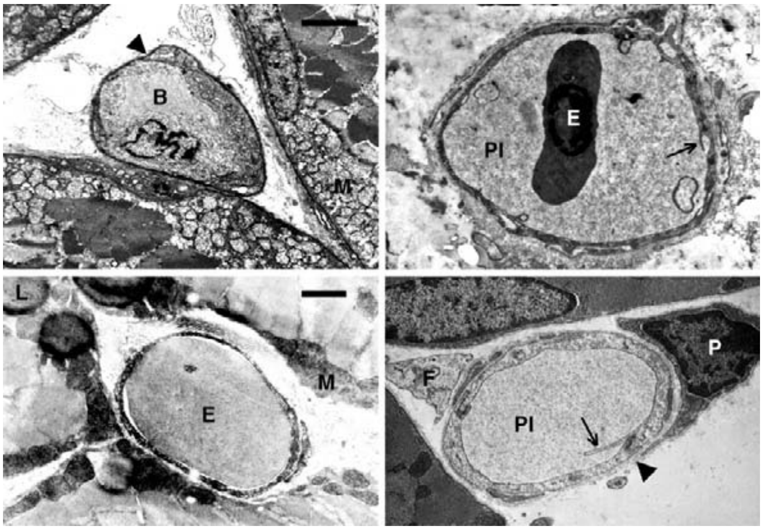


Figure 2 Electronmicrographs of skeletal muscle capillaries from blackfin icefish *Chaenocephalus aceratus* (top left, scale bar = 10 μm), rainbow trout *Oncorhynchus mykiss* (bottom left, scale bar = 5 μm), striped bass *Morone saxatilis* (top right), and rat hindlimb (bottom right) illustrating the similar fine structure, but contrasting endothelial cell thickness and cross-sectional area. B, blood of icefish is essentially plasma; M, mitochondria; L, lipid, E, erythrocyte; PI, plasma proteins; F, fibroblast; P, pericyte; arrowhead, basement membrane; arrow, endothelial cell filopodium. From Egginton S (1990) Morphometric analysis of tissue capillary supply. In: Boutilier RG (ed.) *Vertebrate Gas Exchange from Environment to Cell*, Advances in Comparative and Environmental Physiology, vol. 6, pp. 73–141. Berlin: Springer; Egginton S and Rankin JC (1998) Vascular adaptations for a low pressure/high flow blood supply to locomotory muscles of icefish. In: Di Prisco G, Clarke A, and Pisano E (eds.) *Fishes of Antarctica: A Biological Overview*, pp. 185–195. Berlin: Springer; and Egginton S, Cardirer S, Skilbeck C (2000) Thermal compensation of peripheral oxygen transport in skeletal muscle of seasonally acclimatized front. *American Journal of Physiology* 279: 375–388.

approximately 15 min, allowing rapid replacement of plasma volume following hemorrhage.

In contrast, branchial-exchange capillaries, which are exposed to relatively greater pressure, and the BBB, which is within a rigid container, correspondingly have reduced permeability (reflection coefficient for proteins is close to 1), thus avoiding the deleterious consequences of edema in these tissues.

Oxygen flux is influenced by different capacities of the compartments that regulate the oxygen cascade between the environment and mitochondria – where oxygen is utilized. Tissue capillary density (CD) will largely determine the rate at which oxygen will diffuse from erythrocytes to mitochondria, and the clearance rate of metabolites, by setting the anatomical diffusion distance in red muscle. This is because the rate of diffusion is inversely proportional to the square of the distance traveled, and CD is a primary determinant of diffusion distance.

In red muscle, the maximal hypothetical diffusion distance (*D*) varies between 7.1 μm in Crucian carp (*Carassius carassius*), 15.5 μm in yellow eels (*Anguilla rostrata*), and 25.7 μm, 47.5 μm, and 52.0 μm for sharks (*Galeus*, *Etmopterus*, and *Chimaera*, respectively; **Table 1**). Krogh's radius (*R*) is 13.3 μm for rainbow trout and 12.9 μm for striped bass under cold conditions. The large variability in values of *D* and *R* among species may in part reflect differences in tissue preparation prior to measurements, true interspecific differences in fiber radii, the lifestyle of the fish, and/or oxidative capacity of the muscle. Note that the *in vivo* (physiological) diffusion distances may differ from the measured anatomical diffusion distance with variation in perfusion pressure or intramuscular convection, but *D* or *R* permit reasonable and practicable estimations of diffusive capacity (**Figure 3**).

The efficacy of diffusive exchange may be improved in the more tortuous red muscle capillaries (**Figure 4**) compared to the largely straight vessels in white muscle. Increased capillary tortuosity (reduced anisotropy) within the same muscle length allows for a greater capillary length per muscle. This increases the capillary surface area for diffusion and the capillary transit time for an erythrocyte. Sluggish fish have the most anisotropic capillary bed recorded in aerobic muscle (**Table 2**). The extent of tortuosity may be further developed where there is increased potential for a metabolic error signal – either with reduced blood-oxygen-carrying capacity (e.g., icefish, **Figure 5**) or with increased oxygen consumption, MO_2 (e.g., tuna; **see Pelagic Fishes**: Physiology of Tuna). Other principles are exemplified by studies of skeletal muscle, cardiac muscle, and skin.

Cardiac hypertrophy in male rainbow trout is accompanied by angiogenesis: from a $\mathcal{J}_v$(c,f) of 1620 mm^{-2} in immature fish, an increase in myocyte girth initially reduces this to 1103 mm^{-2}, but then eventually recovers to 1457 mm^{-2}, thus ensuring that the intramuscular

Table 1 Symbols along with their definitions used in the text

Symbol	*Definition*
a	Mean area of object
a(c)	Capillary cross-sectional area
a(f)	Mean fiber cross-sectional area
A	Reference or total area
b(c)	Perimeter of the capillary
c	Capillary
C:F	Numerical capillary to fiber ratio: in stereological notation, N_N(c,f)
CAF	Numerical of capillaries around a fiber: in stereological notation, *N*(c,f)
CD	Numerical capillary density: in stereological notation, N_A(c,f)
D	Maximum hypothetical diffusion distance
f	Muscle fiber
J_v(c,f)	Capillary length density
MO_2	Oxygen consumption
N	Number of object
N_A	Numerical density – number of objects per unit area of reference
N(c)	Number of capillaries in the sample area
N(f)	Number of fibres in the sample area
PO_2	Partial pressure of oxygen (oxygen tension)
R	Radius of krogh's tissue cylinder
S_v	Surface density – surface area of object per unit volume reference
S_v(c,f)	Capillary surface density

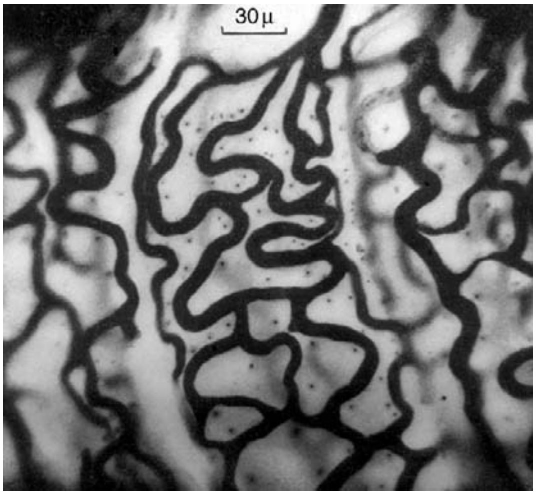


Figure 3 Indian-ink-filled capillary bed in fish red muscle. Note the highly tortuous path for erythrocytes during perfusion, which would increase the transit time compared to straight vessels, and hence maximize diffusive exchange. Scale bar = 30 µm. From Barets A (1961) Contribution a l'étude des systèmes moteurs "lent" et "rapide" du muscle lateral des Teléosténs. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale* 50: 91–187.

diffusion distance remains low enough to maintain adequate oxygenation. Within the compact myocardium of trout sampled at different seasonal temperatures, CD = 1371, 1194, and 2178 mm^{-2} at 4, 11, and 18 °C, respectively. As there is no seasonal variation in myocyte diameter, ventricular CD parallels heart rate and hence is highest at 18 °C, suggesting that angiogenesis in fish cardiac muscles is probably stimulated by different mechanical factors when compared with skeletal muscle.

Fish skin microcirculation is extensive, similar to that of amphibians (e.g., 6 m of capillaries per gram per body mass in common carp *Cyprinus carpio*). The skin may function as an additional respiration site, as with amphibians, which is certainly the case in larval fishes before the gill secondary lamellae are fully developed (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms). Capillaries are found at varying depths within the dermis of different species, but those between the scales and the epidermis may account for up to 30% of oxygen uptake in adults of some fishes. This may have been important in the evolution of air breathing – indeed, aerial exposure of eels leads to hyperaemia (accumulation of blood) of the dorsal fin, although a large proportion of the uptake capacity may be required to support skin metabolism itself.

Vascular Origins

Vasculogenesis occurs in the developing embryo independent of the heart and aorta. The macro- and microvasculature join to form a functional circuit around the time when cutaneous oxygen exchange becomes limiting. The timing of capillary appearance follows the rostral–caudal developmental axis, and, in myotomes, occurs after segmental artery perfusion is evident. It is likely that the intersegmental vessels arise as sprouts from the dorsal aorta, and the arteriogenesis and angiogenesis that originates from these larger vessels is initiated to

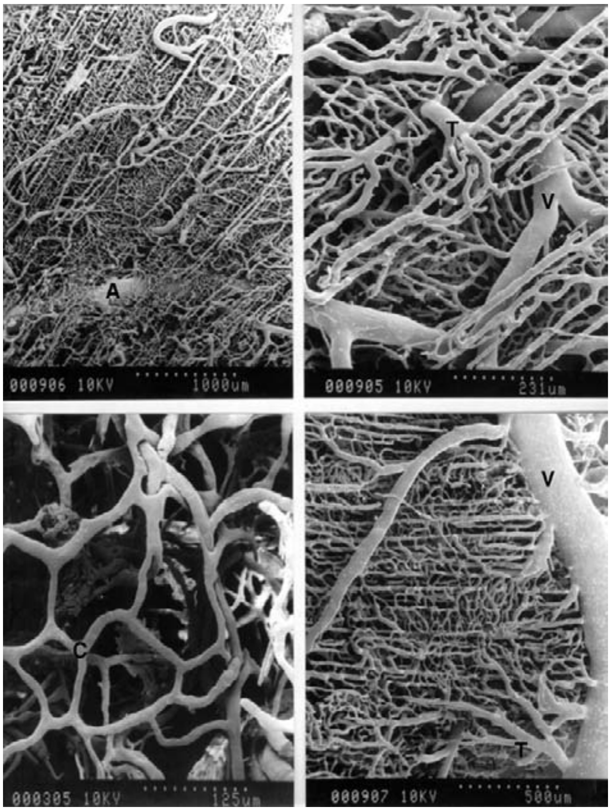


Figure 4 Scanning electronmicrographs of vascular casts from icefish (*Chionodraco hamatus*) pectoral muscle. (Top-left) Feed arteriole (A) lying under a mat of capillaries, some of which branch direct from the parent vessel. (Top-right) The capillary bed covers muscle fibers in an evenly spaced network, orientated with the long axis. (Bottom-left) Loops formed by convergence and divergence of capillaries (C) provide maximal coverage of the fiber surface for diffusive exchange. (Bottom-right) Large drainage venules (V) have joining capillaries as well as terminal venules (T). Muscle fiber axis lies in the horizontal direction. T, terminal arteriole; C, capillary; V, collecting venule. From Egginton S and Rankin JC (1998) Vascular adaptations for a low pressure/high flow blood supply to locomotory muscles of icefish. In: di Prisco G, Clarke A, and Pisano E (eds.) *Fishes of Antarctica: A Biological Overview*, pp. 185–195. Berlin: Springer.

supply muscle with increasing oxygen demand and distance from large vessels or the skin during development. The molecular basis of arteriogenesis becomes clearer from the study of zebrafish embryos, but most of the signals controlling other vascular growth processes tend to be inferred from the dominant mammalian literature. There are few reliable cytochemical markers of capillary endothelium in fishes; so, many studies have utilized resin sections of glutaraldehyde-fixed material (which introduces an unavoidable ~30% shrinkage of tissue). This is a time-consuming method, in part explaining the sparse literature, but it does allow unambiguous identification compared with expression of markers that may vary with developmental age or physiological condition. Morphometric data quantify the structural capillary supply, which correlates with functional capacities, such as potential maximum rates of oxygen delivery and metabolite removal, but says little about the efficiency of exchange at sub-maximal rates. These issues are discussed in detail elsewhere.

Angiogenesis

The signals leading to capillary growth may vary in time and space, may be systemic or local, and may be species specific. Seasonal changes in hormonal status, for example thyroid or growth hormones, likely help match changes in activity with organ function, the responses being moderated or limited by local receptor density. An obvious feedback loop is to induce angiogenesis in response to either increased metabolic demand or reduced oxygen delivery. Both of these triggers likely signal via either local metabolites, for example, adenosine, or transcriptional responses, for example, mediated by hypoxia inducible factor (HIF). Given the generally low $\dot{M}_{O_2}$ for all but tropical species and higher solubility of oxygen in cold water and body fluids, angiogenesis in response to reduced oxygen delivery may be less important in fishes than in endotherms.

The angiogenic response to hypoxia (inadequate oxygen supply to tissues) varies among species, inducing a dramatic angiogenesis in the tench *Tinca tinca* (C:F = 0.9 vs.1.5), a modest response in channel catfish *Ictalurus punctatus* (1.4 vs.1.9), but a slight reduction in microvascular supply in Crucian carp *Carassius carassius* (1.9 vs.1.5).

The angiogenic response may also be initiated either directly or indirectly by local mechanical forces on the endothelium. This is most clearly seen in the skeletal muscle, in response to seasonal cold acclimation. The first prize goes to the Crucian carp, which doubles C:F from 2.2 to an exceptional 4.8 on acclimation from 28 to 2 °C. This growth in capillaries may be a result of the increased frictional forces on the endothelium due to the higher blood viscosity at low temperatures (resulting in elevated shear stress on the luminal face of the endothelium, a known trigger for angiogenesis), and increased rigidity of the nucleated (and therefore large) erythrocytes. In addition, the growth and therefore stretch of muscle fibers (hypertrophy) seen in cold-acclimated fishes imposes a mechanical strain on the abluminal side of the endothelium. Similarly, stretch-induced angiogenesis may govern expansion of the capillary bed during ontogenetic growth (**Figure 5**).

In mammalian skeletal muscle, ontogenetic growth primarily involves fiber hypertrophy, where the increased girth of muscle cells pushes existing capillaries apart, disturbing the normally quiescent endothelium. This stimulates angiogenesis, possibly via mechanotransduction of the lateral strain at vessel

Table 2 Stereological analysis of capillary tortuosity in fish red muscle[a]

Conger eel (*Conger conger*)	–	1.02 (10 °C)	
Rainbow trout (*Oncorhynchus mykiss*)	1.06@4 °C	1.04@11 °C	1.07@18 °C
Skipjack tuna (*Katsuwonus pelamis*)		1.44 (25 °C)	
Nototheniids (*N. coriiceps, G. gibberifrons*)	1.11 (<0 °C)		–
Channichthyids (*C. aceratus, C. rastropinosus*)	1.44 (<0 °C)		–

[a] Random (isotropic distribution) capillary orientation would have a value of 2, orientation along the muscle fiber axis (anisotropic distribution) would have a value of 1

Note that tortuosity is independent of temperature, but increases with the potential metabolic error signal due to either high demand (tuna > trout > eel) or low supply (Channichthyids < Nototheniids).

From Egginton S and Johnson IS (1983) An estimate of capillary anisotropy and determination of surface and volume densities of capillaries in skeletal muscles of the conger eel (*Conger conger* L.). *Quarterly Journal of Experimental Physiology* 68: 603–617; Egginton S, Cardiner S, and Skilbeck C (2000) Thermal compensation of peripheral oxygen transport in skeletal muscle of seasonally acclimatized front. *American Journal of Physiology* 279: 375–388; Mathieu-Costello O (1993) Comparative aspects of muscle capillary supply. *Annual Review of Physiology* 55: 503–525; and O'Brien KM, Skilbeck C, Sidell BD, and Egginton S (2003) Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antartic fishes. *Journal of Experimental Biology* 206: 411–421.

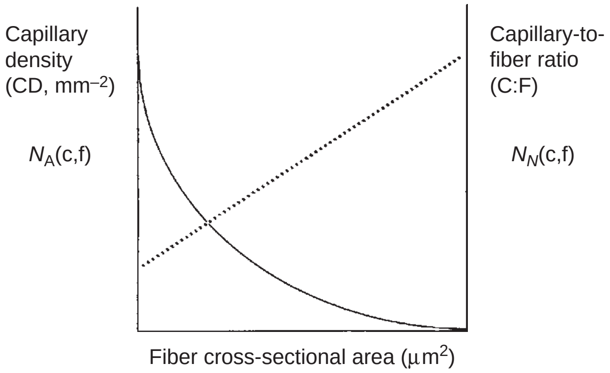


Figure 5 Scaling of capillary supply to skeletal or cardiac muscle during ontogenetic growth, or adaptive remodeling. An angiogenic signal is generated as muscle fibers undergo hypertrophy, leading to an increase in C:F. However, the rate of fiber growth exceeds that of capillary growth, hence CD falls. This may eventually limit muscle activity if other adaptive mechanisms (e.g., altered blood flow or remodeling of the intracellular compartment) become ineffective, leading either to enhanced angiogenesis or impaired physiological performance. From Egginton S (2002) Temperature and angiogenesis: The possible role of mechanical factors in capillary growth. *Comparative Biochemistry and Physiology – Part A* 132: 773–787.

bifurcations. CD decreases exponentially during normal development as capillary growth occurs more slowly than fiber growth, while C:F increases in an approximately linear manner. Consequently, the major determinant of capillary oxygen supply during mammalian development appears to be the mean fiber cross-sectional area, although this relationship may be modified by other angiogenic stimuli.

In contrast, postnatal muscle growth in fishes involves both hyperplasia (growth of new fibers) and hypertrophy (increase in size of existing fibers). Fast-growing, small species rely more on hyperplasia and hypertrophy than slow-growing larger species, which largely rely on hypertrophy. Among fish species, a decrease in CD and an increase in C:F with increasing fiber size is only seen in red muscle, and then only over a relatively small size range (**Figure 6**). Above a mean fiber area of around 1000 μm^2, capillarization appears to be independent of the fiber size. This indicates that mechanisms other than angiogenesis become important at this point, possibly involving reorganization of pathways for intracellular diffusion (e.g., intracellular lipid increases and mitochondrial ribbons are seen). As fiber size varies with body mass, increasing C:F and decreasing CD are seen during ontogenetic growth, irrespective of activity levels.

Matching Angiogenesis and Microvascular Function

It is normally assumed that the role of the microcirculation is primarily one of oxygen delivery, as formalized in the concept of symmorphosis. While this underestimates other roles such as metabolite redistribution, including heat in the case of tunas (**see also Pelagic Fishes**: Physiology of Tuna), it has led to the widely held belief that oxygen supply corresponds with demand, for example, by matching muscle capillarity and mitochondrial content. In mammals, there is indeed a positive correlation between CD (supply) and mitochondrial density (demand). While selective reading of the literature on fishes would support this view, there are sufficient exceptions to demand a reappraisal (**Figure 7**). In addition to scaling of intramuscular diffusion distances, intracellular diffusion distance also exhibits plasticity, revealing a sensitive feedback control that may adjust different parts of the oxygen cascade according to specific demands (**Figure 8**).

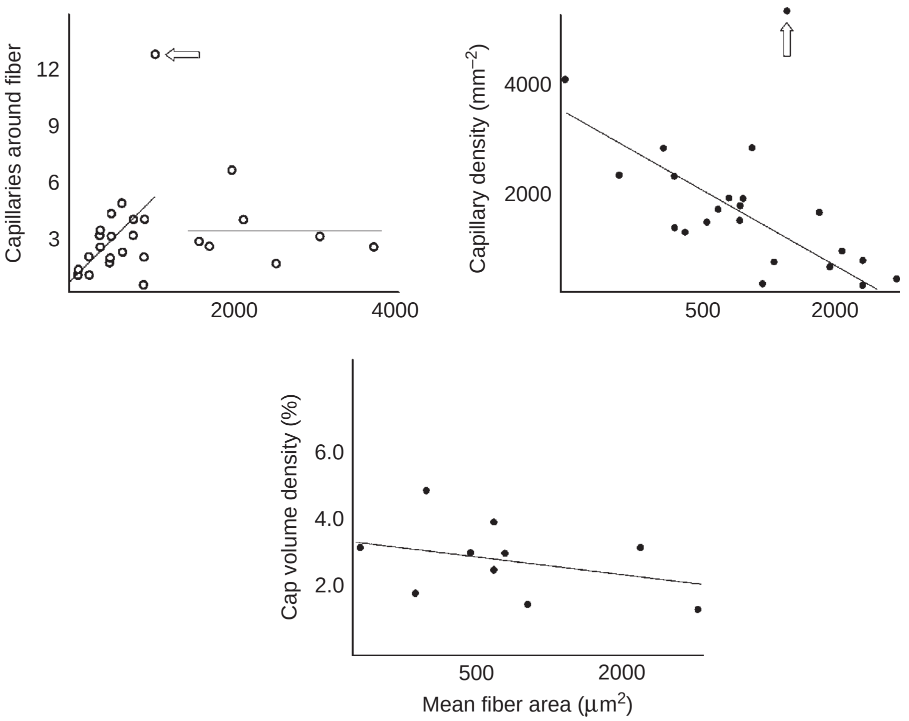


Figure 6 Apparent scaling of capillary supply in red muscle among different fish species (data from various studies using a variety of methods). The number of capillaries adjacent to a fiber increases until a maximum around 1000 μm^2, after which it plateaus and may even decline in species with extremely large fibers (regressions shown with exclusion of data from anchovy, arrow, which is clearly an outlier due to unusual fiber anatomy). By contrast, capillary density decreases with fiber size (top right). Scaling of vessel size, however, results in a relatively invariant capillary volume density (bottom panel). This suggests a tight feedback control of the microcirculation to regulated perfusion capacity while other changes, for example, changes in capillary tortuosity, may regulate diffusion capacity.

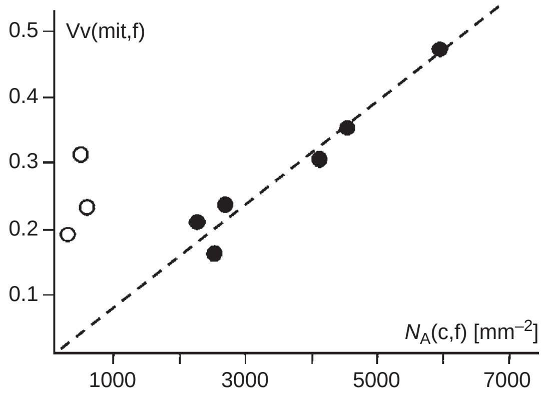


Figure 7 The assumption that oxygen supply and demand must covary, a relationship influential in developing the concept of symmorphosis appears to hold across a range of temperate teleost species (closed circles). However, this may be affected by other considerations, for example, large fiber size and low temperatures associated with sluggish and Antarctic species (open circles).

Skeletal Muscle Capillarity

By far the greatest information exists about the capillarity of skeletal muscle, with a few examples of myocardial capillary supply. These data help to identify the adaptive capacity of muscle and the potential signals involved. Skeletal muscle in the fish trunk is divided into anatomically discrete (red and white), functionally separate (slow and fast), and metabolically distinct (oxidative and glycolytic) regions. The superficial aerobic muscle is highly vascular to sustain low-speed swimming, while the deep anaerobic muscle is reversed for burst activity that requires only a sparse capillary bed. The composition of the fiber types reflects their functions, where red muscle can have a 30-fold greater mitochondrial content and a much higher microvascular supply than white muscle. For example, the capillaries around a fiber (CAF) in the red, pink, and white muscle of the starry sturgeon (*Acipenser stellatus*) are 2.3, 0.9, and 0.2, respectively. The European anchovy *Engraulis encrasicolus* is an outlier with very high values of CAF and CD; the unusually shaped (laterally compressed) muscle fibers allow greater packing of vessels.

An MVU in skeletal muscle spans the myotome of fishes, although there is some doubt whether the arterioles/venules access via the myosepta, as originally suggested from ink-filled preparations. This may vary among species, but if it were the case, then it would prevent staggering of MVUs and the resultant O_2 exchange between vessels. To avoid the decrease in O_2 gradient along a capillary bed impairing aerobic activity

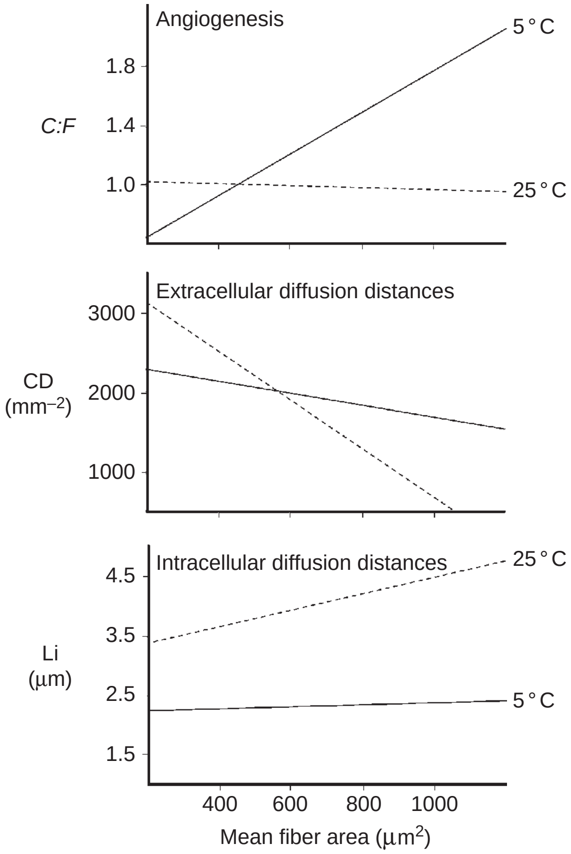


Figure 8 Scaling of intramuscular response (C:F, CD) and intracellular (mitochondrial separation, Li) diffusion distances in red muscle of striped bass acclimated to seasonal extremes of 5 °C and 25 °C. Note that the increase in C:F compensates for decreased O_2 diffusion rate and increased diffusion distance (due to fiber hypertrophy) in the cold to maintain CD, while reduced O_2 flux due to low temperature is compensated by the decrease in Li. The processes identified by the different parameters are indicated for each panel. From Egginton S (1998) Anatomical adaptations for peripheral oxygen transport at high and low temperatures. *South African Journal of Zoology* 33: 119–128.

(contractile activity fueled by oxidative phosphorylation), either fiber mitochondrial content must decrease or muscle CD must increase. The former would seem unlikely along a cell of homogeneous phenotype and, indeed, seems to increase at the myoseptal ends, but there is no quantitative evidence for the latter.

The literature on plasticity of vascular supply to fish muscle is less comprehensive than that for mammals, in part due to the greater diversity of fish species. Skeletal-muscle capillarity changes during ontogenetic growth (as described earlier), during exercise training, and in response to environmental temperature. The similarity with some of these changes described for mammals has generated current interest in identifying whether analogous or homologous angiogenic stimuli are responsible. Although the absolute capillarity is considerably lower in white muscle than in red muscle, the angiogenic potential (i.e., the possibility of increasing capillary supply before reaching the maximum number of capillaries a muscle can accommodate) is relatively greater. For example, striped bass acclimated to 5 °C show an increase of 41% in RM C:F when compared with that at 25 °C, whereas WM increase is 62%; similarly, exercise training of trout produced a 27% increase in CAF for RM but a 95% increase in WM.

However, cross-specific comparisons may have limited value in determining adaptability of the microcirculation because of widely different aerobic capacities and modes of locomotion. For example, the range of RM capillary supply varies from a low in the primitive, deep-water holocephalan ratfish *Hydrolagus colliei* (C:F = 0.3, CD = 118 mm^{-2}) to a high in the fast-swimming European anchovy (CAF = 12.9, CD = 600 mm^{-2}). This means that, in the latter, no myofibril is >8 µm from a capillary, thus permitting a much higher $\dot{M}_{O_2}$ compared with 27.4 µm in dogfish and 47.5 µm in ratfish. Similar caution must be exercised when comparing data using different indices as these may give quite a different interpretation, for example, goldfish RM $N(c,f) = 3.3$ but $N_N(c,f) = 1.1$ at 5 °C.

Due to their myotomal arrangement, the fast muscle (WM) contractions in fish are more isometric than isotonic, which in mammals is not generally seen as a pro-angiogenic stimulus. This may explain the apparently poor training response in this tissue, although the proportional increase may be greater due to much lower initial values. Capillary growth in RM, therefore, may be stimulated by a combination of functional hyperaemia (exercise-induced increase in muscle blood flow), with the more isotonic duty cycle, and the metabolic character of the host tissue. In larval fish, training effects only become evident after the motor program has become fully activated, showing a differential effect between cardiac and skeletal muscle, though whether the usual vertebrate pattern of decreased responsiveness with age also applies to fish has not been investigated. However, the most studied plasticity has been the response to altered temperature.

Thermal Environment

Despite profound effects of temperature on biochemical reaction rates (acute Q_{10} *c.* 2–3) different fish species have successfully colonized niches ranging from <−1.8 °C in Polar seas to >40 °C in East African hot springs. Thus, over evolutionary time, speciation has resulted in fishes with widely different thermal tolerances, within the biological temperature range (freezing point of water to the temperature for protein denaturation), that do not overlap. Cardiac output increases upon maximal sustainable exercise at all temperatures for most fish, with the greatest increase in cardiac performance occurring at the temperature that coincides with the maximum sustainable swimming speed. These, and other data involving, for example, postprandial redistribution of blood flow (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation), suggest that central cardiovascular scope may be a major limiting factor in performance (**see also Circulation**: Circulatory System Design: Roles and Principles). However, diffusion of metabolites and respiratory gases within tissue and cells, which has received little attention since the studies of August Krogh, is also a potentially limiting factor for muscle performance. This would involve adaptive remodeling of muscle fine structure, or the microvascular supply.

Cold Acclimation

In general, fiber size varies inversely with environmental temperature in fishes. This is true both within individuals in response to short-term or seasonal cold-temperature acclimatization (**Figure 9**) and among species inhabiting different latitudes. However, the species ecotype and/or oxidative capacity may modulate this basic relationship, for example, oxidative fibers of the hemoglobinless icefishes are larger than those of congeneric red-blooded nototheniids. Fiber diameter affects the distance oxygen must diffuse to the fiber center and, as mitochondria are found in that location, we must assume that oxygen diffusion is adequate. There are various competing influences on the oxygen-transport cascade, including diffusion distance (influenced by fiber size), diffusivity of oxygen (influenced by cellular composition and temperature), oxygen demand (set by mitochondrial content), and PO_2 gradient (determined by the capillarity). The integrative response may be explored by means of mathematical modeling, which has so far only been done for low temperature, suggesting that angiogenesis is the most effective way of avoiding peripheral limitations in oxygen transport to tissue. The influence of different transit times and capillary tortuosity is unexplored.

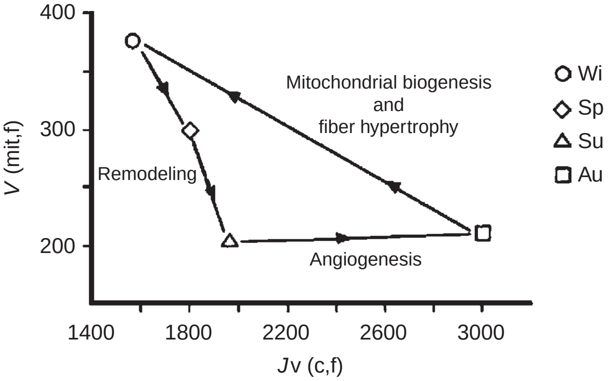


Figure 9 The changing relationship between muscle oxygen delivery (capillary length density) and fiber oxygen consumption (mitochondrial volume) in red muscle of rainbow trout during acclimatization to seasonal differences in environmental temperature. The interspecific relationship obtained from other published values is shown as the linear regression line, suggesting that in winter, trout may be under-supplied and in summer/autumn over-supplied with capillaries. *V*(mit,f), fiber mitochondrial volume; J_V(c,f), capillary length density; Wi, winter; Sp, spring; Su, summer; Au, autumn. Modified from Egginton S, Cardiner S, and Skilbeck C (2000) Thermal compensation of peripheral oxygen transport in skeletal muscle to seasonally acclimatized front. *American Journal of Physiology* 279: 375–388.

Cold Adaptation

Capillary length density, J_V(c,f), is probably the best numerical descriptor of the exchange capacity for oxygen and metabolites between capillaries and muscle. There is an apparent mismatch in supply and demand among Antarctic fishes, lower J_V(c,f) but higher V_V(mit,f) are found in the oxidative pectoral (locomotory) muscles of hemoglobinless icefishes compared to congeneric red-blooded nototheniids, although $\dot{M}_{O_2}$ may be similar. The microcirculation plays a major role in maintaining aerobic-based activity. In addition to changes in surface area due to higher tortuosity, capillary diameter is also adaptive. In red-blooded Antarctic species the total peripheral resistance (TPR) is similar to those of temperate species, but in the icefishes it is only $\sim$20% of the norm. Although much control over tissue perfusion exists at the arteriolar level, and altered blood viscosity influences TPR, given that vessel resistance is proportional to $1/(\text{radius})^4$, capillary diameter also plays a role. It can be calculated that in order to maintain intracellular PO_2, species lacking facilitated diffusion require a 50% greater capillary diameter, or proportionally higher CD.

Changes in muscle fine structure may help compensate for low capillarity and help maintain adequate oxygen diffusion to mitochondria. Striped bass acclimated to low temperatures (5 °C) have a lower capillary:cristae surface area ratio compared to species at 25 °C, while oxygen flux may be maintained in the cold by an increase in intracellular lipid droplets where oxygen is more soluble than it is in aqueous cytoplasm. Although of limited value in most Antarctic species, intracellular lipid may be an important storage site for oxygen and/or act as a low-resistance pathway for oxygen diffusion, for example, the diffusion coefficient for oxygen increases in cold-acclimated striped bass in line with increased lipid content. Given the various possibilities, it is of no surprise that different species adopt differing strategies to overcome the potential limitations of life in the cold (**Figure 10**). The challenge remains to undertake a cost–benefit analysis to identify the relative efficiency of these strategies.

Concluding Remarks

Although specific information and mechanistic explanations in fish lag behind that in other groups, it is clear that there are many similarities but also some distinctive differences between the anatomy and physiology of the microcirculation in fishes and mammals. In part due to

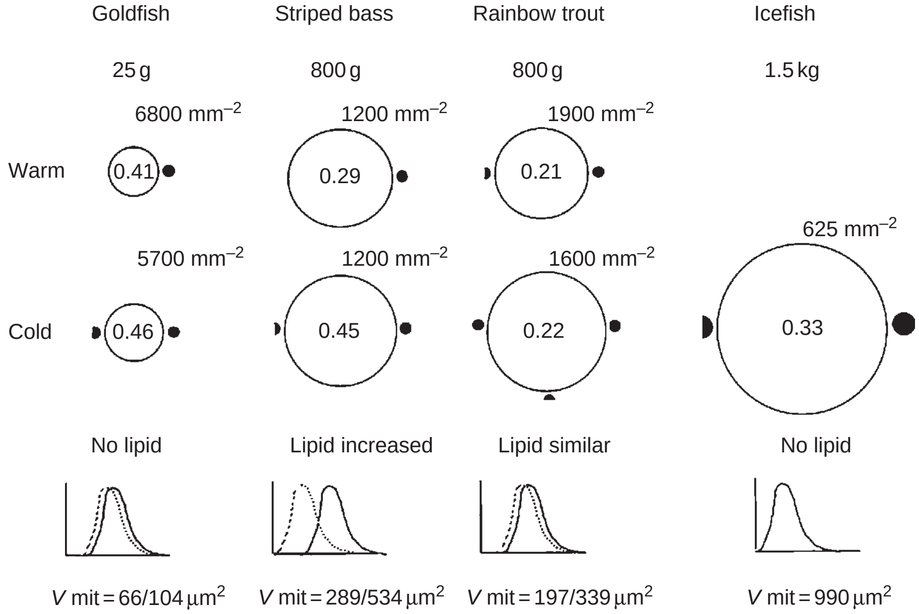


Figure 10 Schematic representation of the varied anatomical adaptations among species (body mass, given under species name) to maintain adequate peripheral oxygen transport at high and low environmental temperatures. Differences in tissue geometry that may be exploited include CD (mm^{-2}), mitochondrial volume density (inside fibers), fiber size (open circles), as well as C:F and capillary size (closed circles). Complementary changes in the intracellular compartment are shown by the involvement of lipid, and the distribution of mitochondrial separation (histograms), with the total mitochondrial content per fiber (μm^2). From Egginton S (1998) Anatomical adaptations for peripheral oxygen transport at high and low temperatures. *South African Journal of Zoology* 33: 119–128.

their aquatic existence, aerobic capacity is largely limited by the structure and functional capacity of the cardiovascular system, which has two major components: systemic cardiac output and local-tissue capillarity. The latter has been quite neglected, and our understanding of fish physiology would be greatly enhanced by more research effort in this area.

See also: **Blood**: Cellular Composition of the Blood. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; Branchial Anatomy; Physiology of Resistance Vessels; The Gastrointestinal Circulation; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes; The Coronary Circulation. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Pelagic Fishes**: Physiology of Tuna. **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms.

Further Reading

Barets A (1961) Contribution a l'étude des systèmes moteurs "lent" et "rapide" du muscle lateral des Teléosténs. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale* 50: 91–187.

Egginton S (1990) Morphometric analysis of tissue capillary supply. In: Boutilier RG (ed.) *Vertebrate Gas Exchange from Environment to Cell*, Advances in Comparative and Environmental Physiology vol. 6, pp. 73–141. Berlin: Springer.

Egginton S (1992) Adaptability of the anatomical capillary supply to skeletal muscle of fishes. *Journal of Zoology, London* 226: 691–698.

Egginton S (1998) Anatomical adaptations for peripheral oxygen transport at high and low temperatures. *South African Journal of Zoology* 33: 119–128.

Egginton S (1999) The influence of environmental temperature on microvascular development of fish. *Zoology – Analysis of Complex Systems* 102: 164–172.

Egginton S (2002) Temperature and angiogenesis: The possible role of mechanical factors in capillary growth. *Comparative Biochemistry and Physiology – Part A* 132: 773–787.

Egginton S and Johnston IA (1983) An estimate of capillary anisotropy and determination of surface and volume densities of capillaries in skeletal muscles of the conger eel (*Conger conger* L.). *Quarterly Journal of Experimental Physiology* 68: 603–617.

Hargens AR, Millard RW, and Johansen K (1974) High capillary permeability in fishes. *Comparative Biochemistry and Physiology – Part A* 48: 675–680.

Hoofd L and Egginton S (1997) The possible role of intracellular lipid in determining oxygen delivery to fish skeletal muscle. *Respiratory Physiology* 107: 191–202.

Hudlická O, Brown MD, and Egginton S (1992) Angiogenesis in skeletal and cardiac muscle. *Physiological Reviews* 72: 369–417.

Jakubowski M (1960) The structure and vascularisation of the skin of the eel and the viparious blenny. *Acta Biologica Cracoviensia* 3: 1–22.

Johnston IA (1982) Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. *Cell and Tissue Research* 222: 325–337.

Johnston IA and Bernard LM (1982) Ultrastructure and metabolism of skeletal muscle fibres in the tench: Effects of long-term acclimation to hypoxia. *Cell and Tissue Research* 227: 179–199.

Krogh A (1959) *The Anatomy and Physiology of the Capillaries*, 422pp. New York: Hafner.

Mathieu-Costello O (1993) Comparative aspects of muscle capillary supply. *Annual Review of Physiology* 55: 503–525.

Mosse PRL (1978) The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. *Cell and Tissue Research* 187: 281–303.

O'Brien KM, Skilbeck C, Sidell BD, and Egginton S (2003) Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antarctic fishes. *Journal of Experimental Biology* 206: 411–421.

Rasio EA, Bendayan M, and Goresky CA (1992) Effect of temperature change on the permeability of eel rete capillaries. *Circulation Research* 70: 272–284.

Rhodin JAG and Silversmith C (1972) Fine structure of elasmobranch arteries, capillaries and veins in the spiny dogfish, *Squalus acanthius*. *Comparative Biochemistry and Physiology – Part A* 42: 59–64.

Satchell GH (1991) *Physiology and Form of Fish Circulation,* 235pp. Cambridge: Cambridge University Press.

Circulatory Fluid Balance and Transcapillary Exchange

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Basic Fluid Compartments
The Important Relationship between Interstitial and Intravascular Compartments
Starling's Forces and Transcapillary Fluid Balance in Fish
Fluid Movement between Intravascular and Interstitial Compartments
Physiological Significance of Transcapillary Fluid Movement
Comparisons of Transcapillary Fluid Flux, Vascular Permeability, and Interstitial and Intravascular Compliance in Fish and Mammals
Where Are the Leaky and Tight Capillaries?
Do Factors other than Starling's Forces Play a Role in Transcapillary Fluid Exchange?
Further Reading

Glossary

Colloid osmotic pressure Unique form of osmotic pressure created by unequal protein concentrations across a membrane or capillary, also called 'oncotic pressure'.

Extracellular fluid The fluid outside of cells. Typically, this includes the plasma and interstitial fluid.

Hematocrit The percentage of packed red blood cells contained in a volume of blood after centrifugation.

Hydraulic pressure Pressure imparted on a solution by a piston or pump. Blood pressure is a form of hydraulic pressure.

Interstitial fluid The fluid that bathes the outside of cells but is not a part of the blood. Interstitial fluid volume is the sum of the volumes of the extracellular fluid and plasma.

Intravascular fluid The noncellular component of blood, that is, the plasma.

Lean-body mass The mass of the body without fat.

Oncotic pressure Unique form of osmotic pressure created by unequal protein concentrations across a membrane or capillary, also called 'colloid osmotic pressure'.

Osmotic pressure The hydrostatic pressure produced by the different concentrations of two solutions on opposite sides of a semi-permeable membrane.

Reflection coefficient A measure of the capillary permeability to protein. Protein-impermeable capillaries have a reflection coefficient of 1, whereas the reflection coefficient of protein-permeable capillaries is 0. Also known as the Staverman coefficient and usually represented by the Greek letter σ.

Starling's forces The vascular and interstitial hydraulic and oncotic pressures that affect fluid movement across the capillary.

Transcellular fluid Fluid in the body that is surrounded by epithelium. This includes the fluid in the lumen of the gastrointestinal tract and secretory glands such as the pancreas and liver, renal tubules, urinary bladder, gallbladder, cerebral spinal fluid, and the fluid in the vitreous and aqueous humor of the eye.

Introduction

The convective delivery of materials to and from the cells, and cardiovascular function in general, relies on sufficient venous return and this, in turn, is dependent on adequate blood volume and vascular tone (**see also Circulation**: Circulatory System Design: Roles and Principles and **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System). Control of fluid volume through drinking, gill and skin exchange of water and salt, and kidney function are discussed in other articles of this enyclopedia (**see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels, **Role of the Kidneys**: The Kidney, **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation, **Role of the Gills**: The Osmorespiratory Compromise, and **Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin). This article discusses the unique relationship between the intravascular and interstitial compartments and the forces that affect movement of fluid between them.

The Basic Fluid Compartments

As described in **Circulation**: Circulatory System Design: Roles and Principles, movement of biologically important molecules occurs by diffusion, convection or, in the case of biomembranes, via selective carriers. The body fluids serve as the highway for molecular trafficking between and within cells, and with the environment. These fluids are divided into four compartments based on their location – intracellular, interstitial, intravascular, and transcellular. Intracellular fluid is the water contained within cell membranes, interstitial fluid bathes the cells, and intravascular fluid is the blood plasma. Transcellular fluid is fluid bounded by an epithelium such as the fluid in the lumen of the intestine, kidney, urinary bladder, gallbladder, salivary glands, pancreatic ducts, cerebral spinal fluid, and intraocular fluid. Collectively, all four compartments are referred to as total body water and interstitial and intravascular water are collectively referred to as extracellular water.

In adult mammals, intracellular water is approximately 60% (by weight) of the lean-body mass (LBM), interstitial water is 16% of LBM, plasma 4% of LBM, and transcellular water varies from 1% to 4% of LBM. Fat only contains 10% water by weight. Therefore, the total body water varies with the amount of body fat and is less than 60%. In newborn mammals, total body water may be as high as 75% of LBM, and much of this is due to an increase in the extracellular fluid volume.

There is more variability (and less certainty) regarding many of these parameters in fish. Most studies have shown that intracellular body water is larger in fish than in mammals. Total body water is approximately 70–75% of a fish's body weight, a value surprisingly similar to that of newborn mammals. In addition, the extracellular fluid volume is larger, approximately 25–30% of body weight, which is also similar to the mammalian neonate. There is considerable species variation in plasma volumes; they tend to be high in cyclostomes (15% of body weight), lower in elasmobranchs (4–5% of body weight), and still lower in teleosts (2–3% of body weight). Some of this variability is due to the uncertainty in the ratio of red blood cells to plasma (hematocrit), which is lower in the small vessels and secondary circulation than in the large vessels, and methodological difficulties associated with escape of markers from the vasculature (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy and **Blood**: Cellular Composition of the Blood). It should also be noted that fluid volume measurements in fish are seldom corrected for body fat and it is likely that lean-body water is perhaps slightly greater than most reported values.

The Important Relationship between Interstitial and Intravascular Compartments

Vascular endothelial cells form a physical barrier between the intravascular and interstitial compartments. This barrier prevents red blood cells from leaving the vascular compartment, but allows free diffusion of most molecules. Mammalian capillaries also restrict diffusion of large proteins such as albumin, fish capillaries may be less restrictive (see below). The volume of fluid in each compartment is dependent upon the net driving pressure across the endothelium (between the plasma and the interstitium) and the relative compliance of each compartment. For instance, the volume in the vascular compartment (V_{vas}) is dependent upon the blood pressure and vascular compliance (**see also Circulation**: Circulatory System Design: Roles and Principles). This is described by the equation: $V_{\mathrm{vas}} = P_{\mathrm{vas}} C_{\mathrm{vas}}$, where P_{vas} is the intravascular pressure and C_{vas} the vascular compliance. Similarly, the volume in the interstitial compartment (V_{ist}) is: $V_{\mathrm{ist}} = P_{\mathrm{ist}} C_{\mathrm{ist}}$, where P_{ist} is interstitial pressure and C_{ist} is interstitial compliance. It is also obvious that compliance is defined as the change in volume for a given change in pressure, that is, $C = \Delta V / \Delta P$. If compliance in both compartments remains constant, then the movement of fluid between the two compartments will depend upon the net pressure difference between them. A change in this driving pressure will affect the movement of fluid across the endothelial cells and the amount of fluid transfer between the intravascular and interstitial spaces will depend upon their respective compliances. Shifting fluid from one compartment to the other can impact the blood volume and ultimately blood pressure on the vascular side and produce edema on the interstitial side.

The net driving pressure across the capillary is the sum of the hydraulic and oncotic pressures acting on each side of the endothelium. Collectively, these pressures are known as Starling's forces, named after their discoverer Ernst Starling. The hydraulic pressure in the plasma is generated by the heart and sustained by the mechanical recoil of the vessels (i.e., compliance; **see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels and **Design and Physiology of the Heart**: Physiology of Cardiac Pumping). The interstitial hydraulic pressure is derived from the relationship between interstitial fluid volume and interstitial compliance. Fluid moves away from a high hydraulic pressure toward a low hydraulic pressure. Oncotic pressure, also called colloid osmotic pressure, is a form of osmotic pressure that is due to plasma proteins. Fluid moves toward the highest oncotic pressure. The influence of gravitational pressure acting on a column of blood, that is, hydrostatic pressure, which is so important in terrestrial animals, especially tall ones, is negligible in fish that are surrounded by water, which has an opposing force (**see also Circulation**: Circulatory System Design: Roles and Principles).

The net driving pressure across the capillary (the sum of the so-called Starling's forces) is described by the general equation: $P_{\mathrm{net}} = (P_{\mathrm{c}} - P_{\mathrm{t}}) - \sigma(\pi_{\mathrm{c}} - \pi_{\mathrm{t}})$, where P_{net} is the net pressure favoring filtration out of a capillary (if positive) or reabsorption of fluid back into it (if negative), P_{c} is the capillary hydraulic pressure (capillary blood pressure), P_{t} is the hydraulic pressure in the interstitial (tissue) space, π_{c} and π_{t} are oncotic pressures in the capillary and interstitial (tissue) fluids, respectively. σ stands for the reflection coefficient (also known as Staverman coefficient), which is an index of the capillary permeability (leakiness) to proteins. In mammals, σ is obtained from the equation: $\sigma = [1 - (L_{\mathrm{prot}}/C_{\mathrm{prot}})]$, where $L_{\mathrm{prot}}/C_{\mathrm{prot}}$ is the ratio of protein concentration in the lymph and plasma. The assumption is made that L_{prot} is equivalent to the protein concentration in interstitial fluid. If $\sigma = 1$, the capillary is impermeable to protein and, therefore, π_{t} will be 0. If $\sigma = 0$, the capillary is freely permeable to protein and there will be no difference in oncotic pressure between plasma and interstitial fluid. When $\sigma = 0$, P_{net} is due only to the difference in hydraulic pressures across the capillary. Values for σ have been determined for many mammalian tissues and range from ~1 in cerebral and renal capillaries to ~0 in the liver where the endothelial cells do not form a continuous barrier.

Figure 1 shows two potential scenarios for fluid movement between vascular and interstitial compartments. In **Figure 1**(**a**) the capillaries are freely permeable to protein ($\sigma = 0$) and the net driving pressure for transcapillary fluid balance is reduced to the difference between hydraulic pressures in the intravascular and interstitial compartments, that is, $P_{\mathrm{net}} = (P_{\mathrm{c}} - P_{\mathrm{t}})$. In **Figure 1**(**b**) the capillaries are relatively protein impermeable ($\sigma \sim 1$) and the net pressure for capillary fluid filtration must take into account the difference in hydraulic and oncotic pressures in the two compartments, that is, $P_{\mathrm{net}} = (P_{\mathrm{c}} - P_{\mathrm{t}}) - \sigma(\pi_{\mathrm{c}} - \pi_{\mathrm{t}})$. **Figure 1**(**b**) reflects the situation in many mammalian vascular beds. In most mammalian systemic tissues P_{c} at the arterial end of the capillary is 35 mmHg and at the venous end of the capillary it is 15 mmHg. P_{t} is close to zero or even slightly subambient. π_{c} is 25 mmHg, π_{t} 1–2 mmHg and σ is close but not equal to 1. Thus, P_{net} favors filtration at the arterial end of the capillary (~+10 mmHg) and reabsorption at the venous end (~–10 mmHg). In mammals, the lymphatic vessels continuously remove what little protein has leaked into the interstitial fluid and keep π_{t} low (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy). Without a functioning lymphatic system the accumulation of protein in the interstitium will upset this balance and P_{net} will now favor fluid moving from the intravascular into the interstitial compartment. In fact, this is the most important function of the lymphatic circulation in mammals.

Starling's Forces and Transcapillary Fluid Balance in Fish

Most research to date suggests that fish do not have a lymphatic system (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary

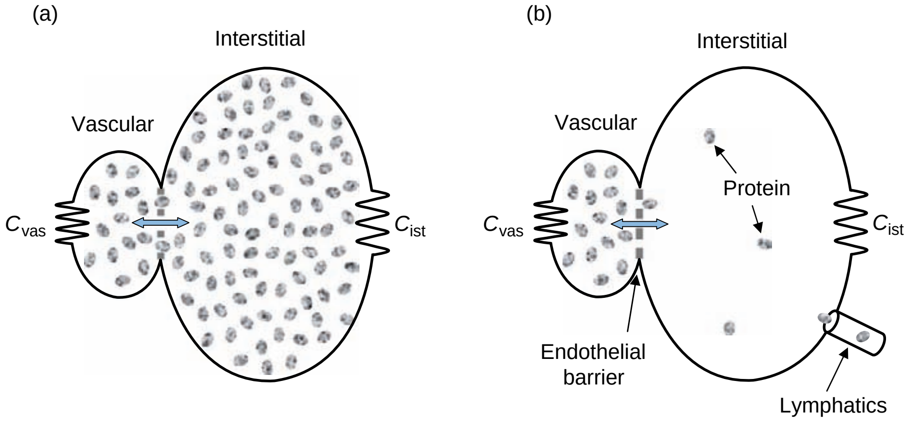


Figure 1 Examples of the forces that affect transcapillary fluid movement between the vascular and interstitial compartments. (a) In vascular beds with a protein permeable endothelium (reflection coefficient ~0), proteins equilibrate throughout both compartments and movement of fluid across the endothelium is governed solely by hydraulic pressures. (b) In vascular beds with a protein impermeable endothelium (reflection coefficient ~1) the net driving pressure is the balance between hydraulic and oncotic pressures in both compartments, that is, Starling's forces. A lymphatic system in (b) is necessary to continuously remove the small amount of protein that leaks into the interstitial fluid and thereby maintain the oncotic pressure gradient. In both examples, a decrease in compliance in one compartment will increase hydraulic pressure in that compartment and favor fluid movement into the other.

Circulation and Lymphatic Anatomy). There is also no compelling evidence that the secondary circulation, which may be the progenitor of the lymphatic system, is integral in removing proteins from the interstitial compartment. Furthermore, the number of studies that have traced the distribution of radioactive protein injected into the vascular system have suggested that protein relatively freely traverses the capillaries in many tissues, that is, they are leaky. Therefore, although there are a few tissues with relatively impermeable endothelial cells, gill and brain, in particular, the whole body σ in fish appears to be fairly close to zero, unlike in mammals. Thus in most fish the factors that determine transcapillary fluid balance are reduced to the difference between intravascular and interstitial hydraulic pressures. Several possible exceptions to this are discussed below.

Fluid Movement between Intravascular and Interstitial Compartments

It is evident from **Figure 1** that a number of factors can affect fluid movement between the vascular and interstitial compartments. In vascular systems with protein-permeable (leaky) capillaries (**Figure 1**(**a**)) an increase in hydraulic pressure in one compartment relative to the other will move fluid out of that compartment, as will a decrease in compliance. In systems where the capillaries are relatively protein impermeable (**Figure 1**(**b**)), transvascular oncotic pressure becomes a factor along with hydraulic pressure and compliance. In these systems, when fluid moves out of one compartment and into the other, the proteins are concentrated in the first compartment and diluted in the second. This decreases the net filtration pressure and retards further fluid movement. There are a number of physiological situations in which the net filtration pressure becomes disturbed and fluid moves from one compartment to the other.

Fluid Reabsorption from the Interstitial Space into the Vasculature

Figure 2 illustrates the mechanism whereby fluid can be reabsorbed from the interstitium into the vasculature. It has been demonstrated in fish and mammals that the

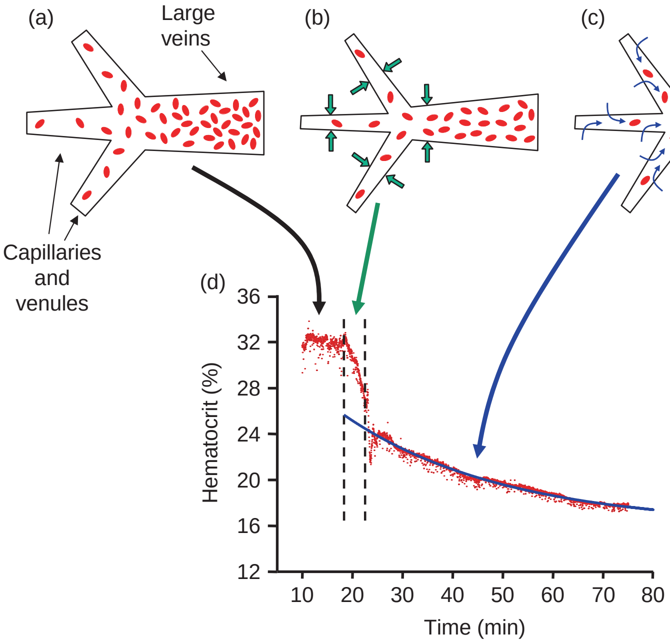


Figure 2 The mechanism of fluid mobilization from the intravascular and interstitial compartments. (a) In the normal situation, the hematocrit in capillaries and small venules is less than that in the larger veins due to the Fahraeus effect. (b) When central venous pressure falls, such as in hemorrhage, the small venules immediately collapse (green arrows) sending plasma-rich blood into the general circulation. (c) A sustained drop in venous pressure shifts the transcapillary fluid equilibrium toward fluid reabsorption and now interstitial fluid is reabsorbed into the capillaries (blue arrows). (d) Actual recording of continuous measurement of hematocrit (red dots) from the aorta of an unanesthetized rainbow trout showing the nearly instantaneous shift of plasma-rich blood from small venules into the larger vessels during a 4 min hemorrage of 35% of the blood volume (dashed vertical lines) and the subsequent reabsorption of interstitial fluid into the vasculature. The blue line illustrates an exponential curve fit to match the decline in hematocrit during interstitial fluid reabsorption. This curve can be used to calculate the rate of fluid flux from the interstitial compartment into the vasculature. (d) Modified from Olson KR, Kinney DW, Dombkowski RA, and Duff DW (2003) Transvascular and intravascular fluid transport in the rainbow trout: Revisiting Starlings forces, the secondary circulation and interstitial compliance. *Journal of Experimental Biology* 206: 457–467.

hematocrit (**see also Blood**: Cellular Composition of the Blood) in capillaries and other small vessels such as venules is considerably lower than that in large vessels, a phenomenon known as the Fahraeus effect (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis). The small venules are also quite compliant and may contain 20–30% of the total blood volume. When blood pressure decreases (**Figure 2**(**b**)), these small vessels immediately collapse, thereby returning the plasma-rich blood into the general circulation. A sustained decrease in blood pressure (**Figure 2**(**c**)) will reduce and subsequently favor fluid reabsorption from the interstitium into the vasculature. This will continue to return fluid to the general circulation. This latter process is more efficient in vascular beds that are protein permeable because it allows the protein to move out of the interstitium with the fluid. In protein-impermeable vascular beds, as fluid is pulled out of the interstitial compartment, the concentration of protein in interstitial fluid increases and in the plasma it decreases. The resulting net change in transvascular oncotic pressure somewhat offsets the change in hydraulic pressure and decreases the amount of fluid that can be absorbed.

Instantaneous hematocrit measurements from an actual experiment on an unanesthetized rainbow trout (*Oncorhynchus mykiss*) subjected to hemorrhage of 35% of its blood volume are shown in **Figure 2**(**d**). The rapid drop in hematocrit (between the two dashed vertical lines) is produced by mobilization of plasma-rich blood that results from the collapse of small venules, as shown in **Figure 2**(**b**). Following this, the continued decrease in hematocrit is produced by reabsorption of interstitial fluid into the capillaries, as shown in **Figure 2**(**c**). This fish was able to recover nearly all of the lost volume within an hour.

Fluid Loss from the Vasculature into the Interstitium

Fluid can be lost from the vasculature into the interstitial compartment through mechanisms, essentially the reverse of those described above. This process is also more efficient in vascular beds with leaky endothelial cells because plasma protein moves with the fluid lost from the vascular compartment and is not concentrated in the plasma. **Figure 3** shows the recovery in plasma, respectively. volume following volume expansion with either isotonic saline or trout plasma. Plasma volume was calculated from the change in hematocrit following volume expansion by addition of either saline or plasma equal to 40% of the initial plasma volume. Fluid movement out of the vasculature was approximately twice as fast as following volume expansion with saline than following volume expansion with plasma. However, at equilibrium the total amount of fluid leaving the vasculature (70% of the injected volume) was identical in both experiments. These results show that proteins slow down the movement of fluid across the vasculature, but they do not affect the total volume exchanged. This would not occur if proteins did not cross the endothelial cells because plasma protein concentration would steadily increase as fluid left the vasculature and the resulting increase in plasma onconic pressure would retain fluid in the plasma.

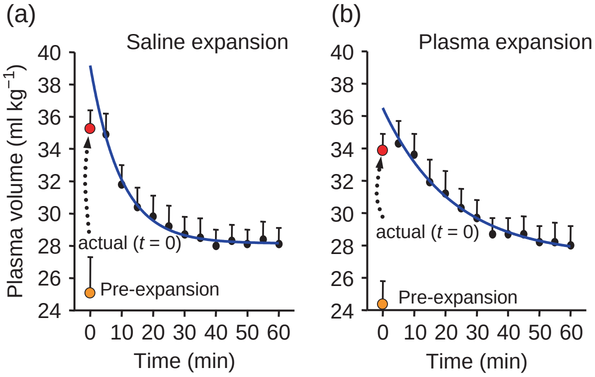


Figure 3 Recovery of plasma volume in unanesthetized trout after volume expansion. (a, b) Restoration of plasma volume following 40% volume expansion with either isotonic saline or trout plasma, respectively. The pre-expansion value (orange circles) indicates the plasma volume prior to volume expansion, actual volume (red circles) is the calculated increase in plasma volume (pre-expansion volume + 40% of pre-expansion volume). The change in plasma volume is calculated based on the change of hematocrit (see also **Figure 2**(**d**)). Fluid moves out of the plasma faster following saline infusion than plasma infusion, however, the total volume of fluid that moves out of the vasculature into the interstitium is identical. This shows that plasma proteins move across the endothelium slower than saline but nevertheless equilibrate between the two compartments. Modified from Olson KR, Kinney DW, Dombkowski RA, and Duff DW (2003) Transvascular and intravascular fluid transport in the rainbow trout: Revisiting Starlings forces, the secondary circulation and interstitial compliance. *Journal of Experimental Biology* 206: 457–467.

Physiological Significance of Transcapillary Fluid Movement

The ability to reabsorb fluid from the interstitial compartment into the vascular compartment provides a significant and rapid safety factor to protect against hemorrhage and it may well be the single most important function of the relatively permeable fish capillaries. This probably explains why fish can replace nearly 100% of their blood volume over the course of 1 h, whereas a blood loss greater than 25% of the total blood volume is fatal to most mammals. Conversely, fish rarely experience a rapid volume overload.

However, the ability to rapidly move plasma out of the vascular space may be advantageous during exercise. Here an increase in blood pressure associated with exercise would shift the plasma out of the vascular compartment and increase hematocrit. This, along with the addition of red blood cells from the spleen (**see also Blood**: Cellular Composition of the Blood), can significantly increase the oxygen-carrying capacity of blood and enhance exercise performance (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

Comparisons of Transcapillary Fluid Flux, Vascular Permeability, and Interstitial and Intravascular Compliance in Fish and Mammals

Although detailed studies on transcapillary fluid balance in fish are limited to rainbow trout, a number of interesting comparisons can be made to the mammalian circulation. Fish capillaries appear quite permeable to plasma protein, which allows for substantial shifts in fluid between intravascular and interstitial compartments. This can explain the high filtration rates across fish capillaries (5 ml mmHg^{-1} kg^{-1} min^{-1}), which are more than 10 times greater than those reported for mammals (0.3 ml mmHg^{-1} kg^{-1} min^{-1}). However, interstitial compliance in fish ($\sim$10 ml mmHg^{-1} kg^{-1}) is similar to that of mammals, as is vascular compliance (3 ml mmHg^{-1} kg^{-1}).

Where Are the Leaky and Tight Capillaries?

A number of studies have examined capillary protein permeability by measuring the distribution of radioactive albumin and red blood cells in tissues following injection of these tracers into the blood vessels. These studies have shown that, relative to red blood cells, high amounts of albumin accumulate in many fish tissues, especially skeletal muscle, gastrointestinal tract, liver, swimbladder, spleen, and skin. The most probable location for the albumin is the interstitial fluid, which is consistent with a leaky endothelium, that is, σ close to zero. Conversely, there appears to be little albumin loss from blood vessels in the gills and brain and this is important physiologically. As described in **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns, blood pumped from the heart must first pass through the gill capillaries before it is distributed to the rest of the body. With this in-series circulation, gill capillary hydraulic pressures are typically 10–20 mmHg higher than capillary hydraulic pressure of any other tissue. If gill capillaries were leaky, this would cause rapid loss of plasma into the environment. The brain is also unique because it is enclosed in a rigid vault. Leaky capillaries would allow plasma to escape into the braincase and the resultant high pressure could directly damage neurons or push against blood vessels and close them off.

There are also some species where the capillaries throughout the body might be relatively impermeable. Perhaps the most notable are tunas and lungfish. Arterial blood pressure in tunas is 2–3 times greater than blood pressures of other bony fish (**see also Pelagic Fishes**: Physiology of Tuna) and protein-permeable capillaries would be disadvantageous. Lungfish appear to possess a functional lymphatic system (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy) and may be able to efficiently remove proteins when they are filtered into the interstitium.

Do Factors other than Starling's Forces Play a Role in Transcapillary Fluid Exchange?

If fish skeletal muscle capillaries are permeable to protein, why does not plasma continue to leak into the interstitial compartment? The answer to this is unclear, but may reside in the skin. As every angler knows, fish skin tightly adheres to the body and cannot be easily pulled away, unlike skin of many mammals. In fact, fish skin is designed to be flexible along the longitudinal axis of the body to permit swimming, but it resists outward stretching. Thus, when blood pressure increases, fluid will initially move into the interstitium but, because the skin does not stretch, interstitial hydraulic pressure will increase and prevent further fluid from entering.

See also: **Blood**: Cellular Composition of the Blood. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; Physiology of Capacitance Vessels. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis; Secondary Circulation and Lymphatic Anatomy. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Integrated Response of the Circulatory System:** Integrated Cardiovascular Responses of Fish to Swimming. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin. **Pelagic Fishes**: Physiology of Tuna. **Role of the Gills**: The Osmorespiratory Compromise. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **Role of the Kidneys**: The Kidney.

Further Reading

Antunes-Rodrigues J, de CM, Elias LL, Valenca MM, and McCann SM (2004) Neuroendocrine control of body fluid metabolism. *Physiological Reviews* 84: 169–208.

Aukland K and Reed RK (1993) Interstitial–lymphatic mechanisms in the control of extracellular fluid volume. *Physiological Reviews* 73: 1–78.

Bushnell PG, Conklin DJ, Duff DW, and Olson KR (1998) Tissue and whole-body extracellular, red blood cell, and albumin spaces in the rainbow trout as a function of time: A reappraisal of the volume of the secondary circulation. *Journal of Experimental Biology* 201: 1381–1391.

Chumlea WC, Schubert CM, Sun SS, *et al.* (2007) A review of body water status and the effects of age and body fatness in children and adults. *Journal of Nutrition, Health and Aging* 11: 111–118.

Duff DW, Fitzgerald D, Kullman D, *et al.* (1987) Blood volume and red cell space in tissues of the rainbow trout, *Salmo gairdneri. Comparative Biochemistry and Physiology* 87A: 393–398.

Duff DW and Olson KR (1989) Response of rainbow trout to constant-pressure and constant-volume hemorrhage. *American Journal of Physiology* 257: R1307–R1314.

Forster ME, Russell MJ, Hambleton DC, and Olson KR (2001) Blood and extracellular fluid volume in whole-body and tissues of the Pacific hagfish, *Eptatretus stouti. Physiological and Biochemical Zoology* 74: 750–756.

Gingerich WH and Pityer RA (1989) Comparison of whole body and tissue blood volumes in rainbow trout (*Salmo gairdneri)* with ^{125}I bovine serum albumin and ^{51}Cr-erythrocyte tracers. *Fish Physiology and Biochemistry* 6: 39–47.

Guyton AC (1995) Interrelationships among interstitial fluid volume, interstitial fluid pressure, interstitial fluid protein concentration and lymph flow. In: Reed RK, McHale NG, Bert JL, Winlove CP, and Laine GA (eds.) *Interstitium, Connective Tissue and Lymphatics*, pp. 167–180. London: Portland Press.

Hargens AR, Millard RW, and Johansen K (1974) High capillary permeability in fishes. *Comparative Biochemistry and Physiology Part A* 48: 675–680.

Hebrank MR and Hebrank JH (1986) The mechanics of fish skin: Lack of an ‘‘external tendon’’ role in two teleosts. *Biological Bulletin* 171: 236–247.

Hillman SS, Degrauw EA, Hoagland T, Hancock T, and Withers P (2010) The role of vascular and interstitial compliance and vascular volume in the regulation of blood volume in two species of anuran. *Physiological and Biochemical Zoology* 83: 55–67.

Lee J-S (2000) 1998 Distinguished lecture: Biomechanics of the microcirculation, an integrative and therapeutic perspective. *Annals of Biomedical Engineering* 28: 1–13.

Olson KR (1992) Blood and extracellular fluid volume regulation: Role of the rennin–angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part B: The Cardiovascular System*, pp. 136–232. San Diego, CA: Academic Press.

Olson KR, Kinney DW, Dombkowski RA, and Duff DW (2003) Transvascular and intravascular fluid transport in the rainbow trout: Revisiting Starlings forces, the secondary circulation and interstitial compliance. *Journal of Experimental Biology* 206: 457–467.

Reed RK (1995) Interstitial fluid pressure. In: Reed RK, McHale NG, Bert JL, Winlove CP, and Laine GA (eds.) *Interstitium, Connective Tissue and Lymphatics*, pp. 85–100. London: Portland Press.

Renkin EM and Tucker VL (1995) Integration of capillary, interstitial and lymphatic function. In: Reed RK, McHale NG, Bert JL, Winlove CP, and Laine GA (eds.) *Interstitium, Connective Tissue, and Lymphatics*, pp. 250–270. London: Portland Press.

Tanaka Y (1979) Whole body transvascular filtration coefficient and interstitial space capacitance. *Japanese Journal of Physiology* 29: 181–193.

Thorson TB (1961) The partitioning of body water in osteichthyes: Phylogenetic and ecological implications in aquatic vertebrates. *Biological Bulletin* 20: 238–254.

Wiig H, Reed RK, and Aukland K (1987) Measurement of interstitial fluid pressure in dogs: Evaluation of methods. *American Journal of Physiology – Heart and Circulatory Physiology* 253: H283–H290.

Secondary Circulation and Lymphatic Anatomy

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Lymph Vessels and the Lymphatic System
History of the Secondary Circulation
Anatomy of the Secondary Circulation
Other Features of the Secondary Circulation
Further Reading

Glossary

Arterioarterial anastomosis An anastomosis is the joining of one blood vessel to another. In an arterio-arterial anastomosis, two arteries or arterioles are connected without an intervening capillary.

Edema An accumulation of fluid in the interstitial space. This results when filtration of fluid across the capillary wall exceeds the capacity of lymph drainage.

Hydraulic pressure Pressure imparted on a solution by a piston or pump. Blood pressure is a form of hydraulic pressure.

Immunohistochemistry Cellular or subcellular localization of specific molecules by staining them with antibodies labeled with a fluorescent or pigmented material.

Indicator dilution method A method to determine the volume of a compartment or space by injecting a known amount of a molecule (indicator) and measuring its final concentration after equilibrium throughout the compartment.

Interstitial fluid The fluid that bathes the outside of cells and is separated from the blood or lymph by an endothelium. Interstitial fluid volume is the sum of the volumes of the extracellular fluid and plasma.

Interstitial space The area surrounding cells and containing interstitial fluid.

Lymph Interstitial fluid that has entered the lymphatic circulation.

Lymphatic circulation A system of blind-end capillaries that collects excess interstitial fluid and proteins filtered from the capillaries and, through a series of progressively larger vessels, returns it to the venous circulation.

Oncotic pressure Unique form of osmotic pressure created by unequal protein concentrations across a membrane or capillary, also called colloid osmotic pressure.

Osmotic pressure The hydrostatic pressure produced by the different concentrations of two solutions on opposite sides of a semipermeable membrane.

Lymph Vessels and the Lymphatic System

The structure and function of the mammalian lymphatic system is well known. The primary function of the lymphatic system is to return protein back to the general circulation after it has leaked from the vascular capillaries into the interstitial spaces. If this protein was not continually removed from the interstitial fluid, its concentration would progressively increase and the osmotic pressure exerted by this protein (colloid osmotic pressure, also known as oncotic pressure) would continue to draw more fluid from the vascular capillaries into the interstitial space and ultimately produce edema. Thus, the presence of lymphatic capillaries maintains a normal balance between hydraulic and oncotic pressures across the vascular capillary wall (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange).

Lymph vessels provide a one-way conduit for return of fluid from the peripheral interstitial spaces back to the circulatory system. Lymph capillaries are blind-end vessels made up of endothelial cells that collect the interstitial fluid. The endothelium has specialized cell junctions that allow the cell margins to act as one-way valves, permitting the entry of interstitial fluid into the lymphatic vessel while blocking its escape (**Figure 1**). Lymph vessels also possess one-way valves in their lumen and periodic compression of smaller lymph vessels or contraction of the larger lymph vessels ensures continuous pumping of lymph from the periphery into progressively larger lymphatic vessels and ultimately into the central systemic veins.

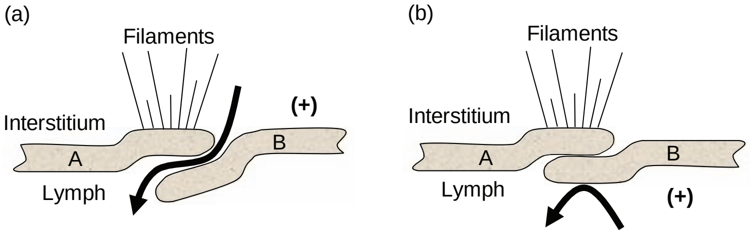


Figure 1 Diagram showing the one-way valve action of endothelial cell junctions in mammalian lymphatic capillaries depending on which direction the net transmural pressure exists (+). (a) An increase in interstitial fluid pressure pushes the endothelial flap B open (down in figure) and permits interstitial fluid to flow into the lymph capillary. Endothelial flap A is connected to the interstitial matrix by thin filaments and cannot move. (b) Compression of the lymph vessel or a decrease in interstitial fluid pressure pushes the endothelial flap B against flap A and prevents back flow of lymph into the interstitium.

This role of the lymphatic system is well known in mammals, birds, reptiles, and amphibians. It was only natural to assume that a lymphatic system was also present in fish, but this question still remains unresolved.

History of the Secondary Circulation

The organization and structure of the fish circulatory system has been the subject of interest and meticulous investigation by anatomists for nearly 200 years. Initially, as often the case for a newly developing field, anatomists viewed the fish circulation from the perspective of the more extensively studied vascular networks in amphibians and mammals, as described above. It is not surprising that these investigators would assume that vessels with a relatively thin wall and few, if any, red blood cells would be part of the lymphatic system and such a set of vessels was frequently observed. Accordingly, the lymphatic system of fish was mapped in great detail (cf. **Figure 2**) culminating in the seminal paper by Kampmeier in 1969 'Evolution and comparative morphology of the lymphatic system'. In the early 1980s, studies by Vogel's group, following up on a brief report from 50 years prior, provided evidence of a direct vascular connection between the arterial system and the so-called lymphatic system. These vascular connections appeared not to anastomose with the lymphatic system but they appeared to be arterio-arterial anastomoses, creating an additional circulatory system. As this second circulation also consisted of arteries, capillaries, and veins, it was called the 'secondary circulation', or in the case of the gills, the 'veno-lymphatic circulation'. These studies and the ones that followed began to question whether or not a true lymphatic system existed in fish, and a hypothesis was developed that the secondary circulation was unique to fish and it was likely the progenitor of a true lymphatic system. Not much evidence has accumulated since then to refute this hypothesis.

Anatomy of the Secondary Circulation

By definition, the secondary circulation is a vascular network consisting of arteries, capillaries, and veins that originate from a major artery in the form of small-bore vessels (arterio-arterial anastomoses) and then re-anastomose to form progressively larger arteries. Like the primary circulation, these larger vessels then feed a second system of arterioles, capillaries, and veins that may even run parallel to the primary circulation. Larger secondary arteries often travel some distance before dividing into a capillary network.

Location of Secondary Vessels

In bony fish, the secondary circulation has been observed in or near external tissues or those that line the body cavity. These include the skin, scales, fins, gills, buccal (mouth) cavity, and peritoneum. The secondary vessels can form complex as well as dense systems, and this is especially true of the gills. Secondary vessels have also been observed in the heat exchangers in tuna (**see also Design and Physiology of Arteries and Veins**: The Retia), but they have not been identified in skeletal muscle, the gastrointestinal tract, liver, kidney, swimbladder, brain, or eye. Secondary circulation has also been observed in gills of non-teleostean species, such as cyclostomes and elasmobranchs, where it is similar to that of bony fish. However, less is known of the secondary system in nongill tissues of these fish.

Arterio-arterial Anastomoses

Secondary vessels usually originate from the dorsal aorta and segmental arteries. Tunas are notable exceptions as the secondary vessels originate from rete arterioles in the

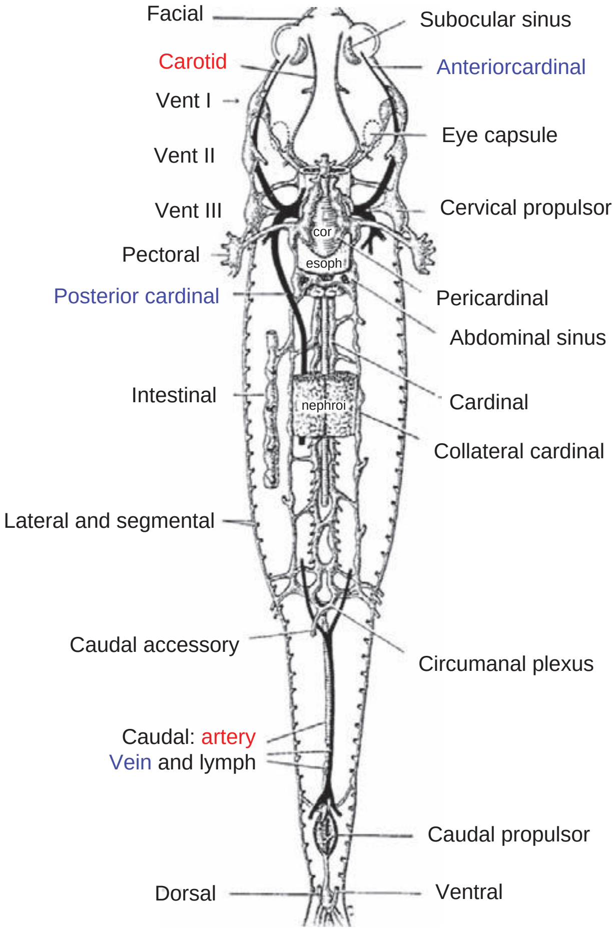


Figure 2 Early interpretation of the so-called lymph circulation in bony fish. The diagram shows the position of lymph vessels (stippled, black lettering) relative to several veins (black, blue lettering) and arteries (cross-lined, red lettering). Vents I, II, and III indicate three levels of communication between the lymphatics and veins in different fish. cor, heart; espoh, esophagus; nephroi, kidney. Modified from Kampmeier OF (1969) *Evolution and Comparative Morphology of the Lymphatic System*. Springfield, IL: Charles C. Thomas.

heat exchanger. Secondary arterio-arterial anastomoses typically emerge perpendicularly from a relatively large primary artery and they follow a long (200–300 μm) and surprisingly tortuous, twisting path before joining with numerous other secondary vessels to form progressively larger arterioles and arteries. Another unique feature of secondary arterioles is the dilated lumen at their origin, forming a funnel-like entrance (**Figure 3(a)**). Microvilli have been reported on the surface of endothelial cells on the parent artery near the origin of the arterio-arterial anastomoses in some fish. These form long microvillous ribbons parallel to the axis of the parent vessel and appear to be guarding the entrance to the secondary arteriole (**Figure 3(c)**). This may physically restrict access of red blood cells into the secondary vessels and contribute to the low hematocrit in the secondary circulation. Leukocytes may also adhere to the microvilli. It has also been suggested that the microvilli provide chemo- or mechanosensory information, but this is yet to be demonstrated experimentally. The tortuous part of the secondary circulation can be embedded within the muscle wall of the primary artery. Thus, it is possible that constriction or dilation of the primary vessel may exert a direct mechanical effect on the arterio-arterial anastomoses as well, perhaps controlling the flow of blood entering the secondary circulation from the primary circulation.

Secondary Vessels in the Systemic Circulation

The translucent body of the glass catfish, *Kryptopterus bicirrhis*, is ideally suited for examining the relationship between primary and secondary circulations in a whole fish. As shown in **Figure 4**, secondary vessels arise from the primary segmental arteries as narrow-bore, tortuous arterioles. These arterioles subsequently re-anastomose to form secondary arteries that enter the ventral fin where they form an extensive secondary capillary network that is restricted to the fins. The capillaries are then drained by secondary veins that ultimately join either with a lateral subcutaneous secondary vein, or less frequently, with the

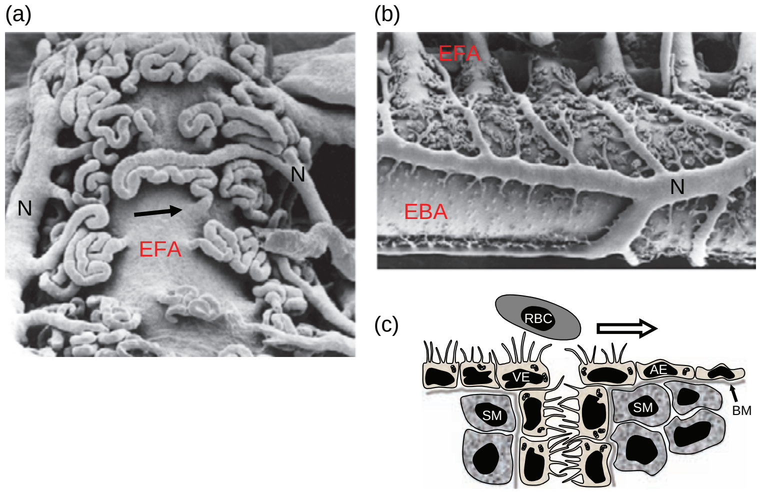


Figure 3 Vascular corrosion replicas from the gill of the climbing perch, *Anabas testudineus*, showing the origin of the secondary arterio-arterial anastomoses. These micrographs of anastomoses are from the gill, but the general anatomy is also typical of systemic secondary arterio-arterial anastomoses. (a) Numerous tortuous vessels arise from the wall of the primary (efferent filamental) artery (arrow) and rejoin to form progressively larger nutrient arteries (N). (b) A large nutrient artery (N) in the gill arch is formed from the anastomosis of numerous tortuous arterioles that arise from both the efferent branchial artery (EBA) and efferent filamental arteries (EFA). (c) Drawing showing the microvillous endothelial cells (VE) lining the efferent filamental artery and preventing a red blood cell (RBC) from entering the narrow-bore vessels that supply the interlamellar system. SM, smooth muscle cells; AE, normal arterial endothelium of the efferent filamental artery; BM, basement membrane. (a, b) Reproduced from Olson KR, Munshi JSD, and Ojha J (1986) Gill microcirculation of the air-breathing climbing perch, *Anabas testudineus* (Bloch): Relationships with the accessory respiratory organs and systemic circulation. *American Journal of Anatomy* 176: 305–320.

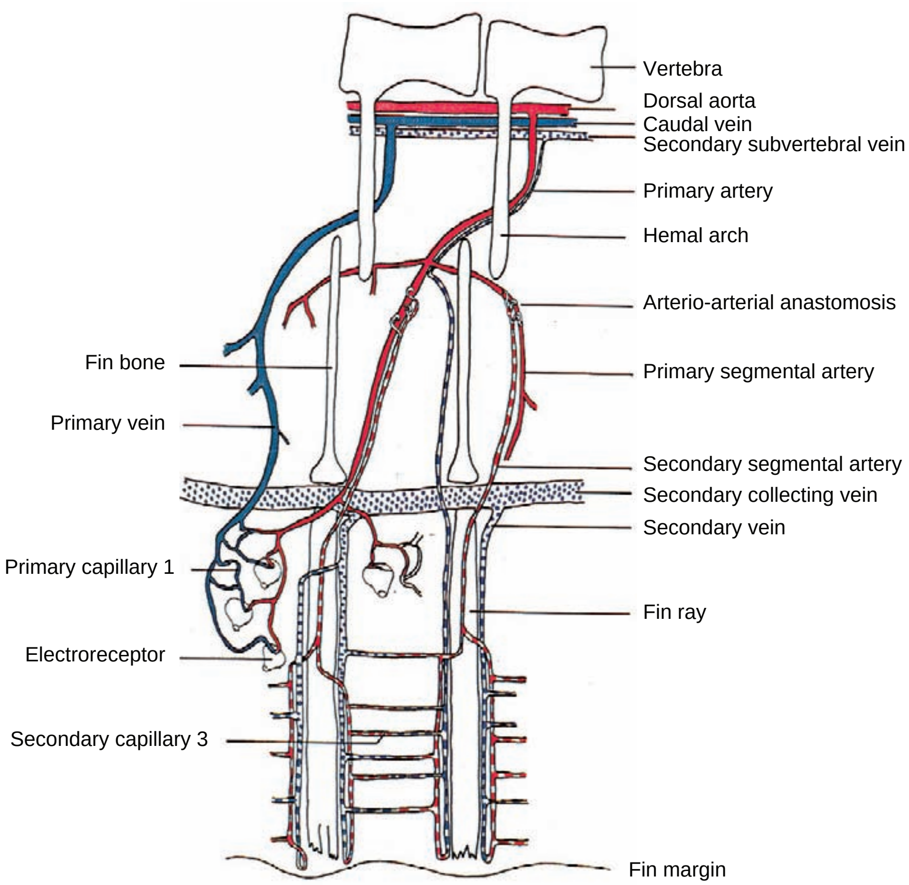


Figure 4 Secondary circulation to the anal fin of the glass catfish. Arterio-arterial anastomoses originate from segmental arteries and form the secondary circulation supplying capillaries in the fin. Secondary capillaries typically drain into large secondary collecting vein or, less commonly, into the secondary subvertebral vein. Reproduced from Steffensen JF, Lomholt JP, and Vogel WOP (1986) *In vivo* observations on a specialized microvasculature, the primary and secondary vessels in fishes. *Acta Zoologica (Stockholm)* 67: 193–200, with permission.

secondary subvertebral vein in the hemal arch. A similar capillary network has been identified in the epithelium that overlies the scales. In the rainbow trout (*Oncorhynchus mykiss*), fluid drains from the secondary circulation of the tail into the caudal heart from where it is then pumped into the caudal vein (**see also Design and Physiology of the Heart**: Accessory Hearts in Fishes). With so much blood reaching the surface of the fish fins and scales, the fish would likely take on a red hue were it not for the fact that there are relatively few red blood cells (1/10th) compared with the primary circulation.

Other investigators have provided slightly different descriptions of the secondary system. In *Blennius pavo* and *Zosterisessor ophiocephalus*, the tortuous arterioles originate from the dorsal, lateral, and ventral segmental arteries as well as from the caudal dorsal aorta. However, these vessels appear to connect directly to secondary veins without an intervening capillary. Thus, this system appears to be an arteriovenous, rather than arterio-arterial circulation.

Lungfish

Lungfish do not have typical secondary vessels and in their place is a well-developed lymphatic system similar to that found in tetrapods. Therefore, lungfishes including the African lungfish (*Protopterus annectens* and *Protopterus dolloi*) and South American lungfish (*Lepidosiren paradoxa*) may represent the transition from a secondary circulation to a true lymphatic circulation. Numerous thin-walled endothelial-lined vessels similar to mammalian blind-end lymphatics are found throughout systemic tissues (except brain) and in the gills of lungfish. These vessels drain into a unique pumping apparatus, the lymphatic micropump. Micropumps are spherical, contractile organs 35–55 μm in diameter. They consist of a single layer of endothelial cells, surrounded by a single muscle cell. The endothelial cells at both the inlet and outlet ends of the pump serve as one-way inlet and outlet valves. The micropumps appear to be suspended within a cell-free interstitium and they contain microfibers that radiate outward from the endothelial cells through perforations in the muscle and extend into the surrounding connective tissue. This gives the appearance of the micropumps being suspended in the interstitial space. Presumably, these microfibers allow the pump to expand by recoil following muscular contraction. Unlike the mammalian lymphatic system, large lymph vessels are absent from lungfish and the red-blood-cell-free lymph is pumped directly from the micropumps into small veins. As many as 105 micropumps per cubic millimeter have been observed in the fin of the South American lungfish.

Zebrafish: A secondary circulation or lymphatic system?

Recent techniques, such as confocal time-lapse imaging of developing vessels and identification of a variety of growth factors important in the development of the mammalian lymphatic system, have been applied to examine vascular development in zebrafish embryos. The time-lapse studies suggest that a true lymphatic system develops in zebrafish and this is supported by immunohistochemical localization of a variety of antibodies to markers of mammalian lymphatic endothelial cells. This raises an interesting question of whether earlier high-resolution anatomical studies mistakenly identified lymphatic vessels as secondary vessels, or whether secondary vessels exist and are indeed progenitors of lymphatic vessels complete with their primordial growth factors.

Non-teleostean fish

The presence of large vascular sinuses filled with low hematocrit blood in the systemic circulation, caudal lymph hearts, and a secondary-like circulation in the gills (see below) all suggest that a systemic secondary circulation is also present in cyclostomes and elasmobranchs. However, arterio-arterial anastomoses similar to those described in teleosts have not yet been observed in these species.

Secondary Vessels in the Gill

There are two vascular networks in the gill, the nutrient and interlamellar pathways, which exhibit some attributes of the secondary circulation. The relationship between these vascular pathways and other the gill vessels is described in detail in **Design and Physiology of Arteries and Veins**: Branchial Anatomy.

The nutrient system arises either from the efferent branchial artery or from the efferent filamental artery near its junction with the efferent branchial artery. Here, numerous narrow-bore, tortuous arterioles arise from the wall of the larger primary vessels and re-anastomose to form progressively larger nutrient arteries that subsequently divide into arterioles and capillaries. These vessels then supply the nonrespiratory tissues of the gill arch and filament (**Figures 3(a)** and **3(b)**), that is, tissues other than the secondary lamellae. While the origin of these nutrient vessels exhibits features of secondary circulation, that is, they originate from small vessels that re-anastomose to form a larger arterial network, they originate directly from the post-gill respiratory circulation, not major systemic arteries and they do not supply a capillary network in parallel with another primary network. Thus, the gill nutrient circulation most closely resembles a primary circulation.

The interlamellar system in the gill filament is considerably different from the nutrient system and has a number of attributes that suggest that it is lymphatic like or at least a progenitor of the lymphatic system, but it too may not be a true secondary system. The interlamellar system in the gill filament was previously called the central sinus, central

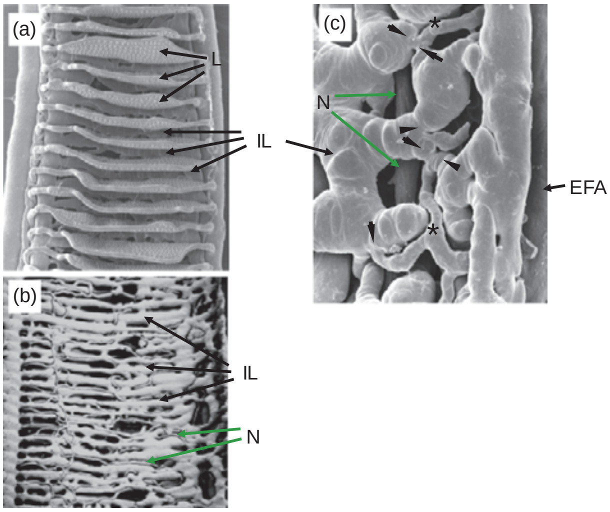


Figure 5 Vascular corrosion replicas showing the relationship between the interlamellar vessels (IL), lamellae (L), and nutrient arterioles (N) in the gill filament. (a) With the lamellae intact, it is clear that the interlamellar system lies between and runs parallel to the inner lamellar margins. (b) With the lamellae removed, the relationship between the larger interlamellar vessels (black arrows) and smaller nutrient arterioles and capillaries (green arrows) is evident. (c) Higher magnification showing the narrow-bore vessels (*) that originate from the efferent filamental artery (EFA) and supply the interlamellar system (arrowheads). The interlamellar system also appears distended in this micrograph. (a, c) Walking catfish, *Clarias batrachus* and (b) rainbow trout. (a, c) Reproduced from Olson KR, Ghosh TK, Roy PK, *et al*. (1995) Microcirculation of the gills and accessory respiratory organs of the walking catfish, *Clarias batrachus*. *Anatomical Record* 242: 383–399, with permission from John Wiley and Sons. (b) From K.R. Olson (unpublished).

venous sinus, or the veno-lymphatic system. The latter is perhaps the most appropriate. The interlamellar system originates primarily from narrow-bore vessels on the medial wall of the efferent filamental artery (**Figure 5**) or efferent lamellar arterioles. These vessels travel partway across the filament and anastomose with the dilated interlamellar system that traverses the filament body (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy). Unlike nutrient capillaries, interlamellar vessels consist of very thin walled endothelial cells and these vessels appear to be quite distensible. Interlamellar vessels contain very few red blood cells and in electron micrographs even the plasma appears to contain less protein (**Figure 6(a)**). The junctions between two endothelial cells are also lymphatic like in that one cell appears to overlap the other (**Figures 6(b)** and **6(c)**). The interlamellar system is drained from the filament into systemic veins that also drain the nutrient capillaries.

Non-teleosts

The detailed accounts of the nonrespiratory gill circulation in cyclostomes are incomplete. In the lamprey, a nutrient system has been described similar to that found in bony fish. In sharks and skates, both interlamellar and nutrient vessels are well developed.

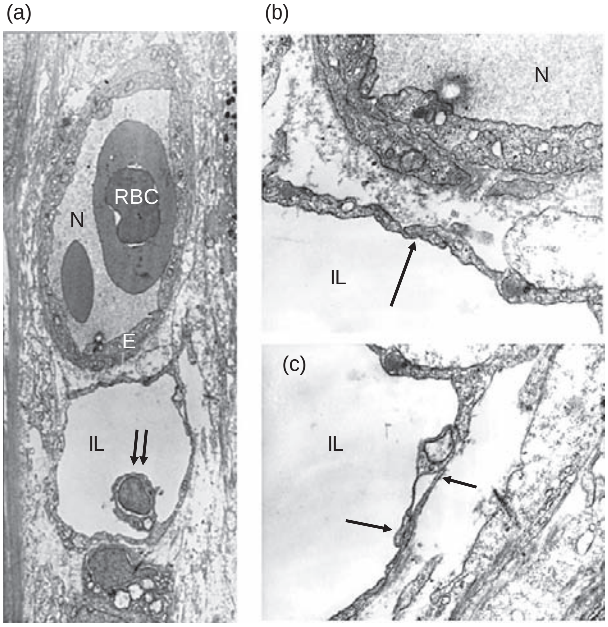


Figure 6 Transmission electron micrographs of the nutrient (N) and interlamellar (IL) vessels in the gill filament of the rainbow trout. (a) Nutrient capillaries contain red blood cells (RBCs), a thick-walled endothelium (E), and the plasma is more electron dense suggesting a high concentration of plasma protein. RBCs are absent from interlamellar vessels, the endothelium is thin-walled, and the nucleus of the endothelial cell (double arrows) bulges into the lumen of the vessel when the vessel is relatively collapsed under low pressure. (b) Higher magnification showing the thick endothelial cells in nutrient capillaries and thin endothelial cells in interlamellar capillaries. The overlapping valve-like arrangement of the interlamellar endothelial cells can be seen. (c) Valve-like interlamellar endothelial cell flaps (arrows) are evident. Reproduced from Olson KR (1996) Secondary circulation in fish: Anatomical organization and physiological significance. *Journal of Experimental Zoology* 275: 172–185, with permission from John Wiley and Sons.

Other Features of the Secondary Circulation

Volume

The volume of the secondary circulation has most often been derived from studies in which dilution of an injected indicator substance is used to estimate volume (indicator dilution method). Typically, these employ radiolabeled red blood cells (assumed to be restricted to the primary circulation) and radiolabeled albumin, or plasma protein tagged with a dye (Evans blue dye) that is assumed to be evenly distributed through both the primary and secondary circulations. In a typical experiment, the indicators are injected into the primary circulation and the volume of the secondary circulation is inferred from the difference between the dilutions of the two indicators. These studies have indicated that the volume of the secondary circulation ranges from 50% to 150% that of the primary circulation.

These values seem excessive given that a secondary circulation has not been identified in skeletal muscle, gastrointestinal tract (including liver and spleen), or brain and these tissues account for the bulk of the fish's mass. Furthermore, other studies have shown that the commonly used indicators of the secondary-circulation volume readily accumulate in skeletal muscle and the gastrointestinal tract. It seems more likely that these indicators have leaked from primary capillaries into the interstitial space. Other studies have confirmed that the primary capillaries of fish are very permeable to protein (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange) and they have suggested that the volume of the secondary circulation may be no more than 20% that of the primary circulation. Clearly, additional work needs to be done in this area.

Pressure

Blood pressure has only been measured in the larger veins of the secondary circulation and, as would be expected from a lymphatic-like system, it is quite low (–3 to +3 mmHg). It is likely that this low pressure is a result of the high resistance in the narrow-bore arterio-arterial anastomoses. Flow into the secondary circulation may be regulated by smooth muscle surrounding these arterio-arterial anastomoses or by direct constriction of the primary artery from which the secondary circulation is derived.

Flow rates have not been measured in the secondary circulation. It is assumed to be lower than that of the primary since flow in the primary circulation is largely determined by the need for oxygen transport (**see also Circulation**: Circulatory System Design: Roles and Principles) and the secondary circulation lacks this role since red blood cells are largely absent. It takes about 20 min for an indicator substance to reach equilibrium between the primary and secondary systems, which suggests that flow in the secondary system may be at least 1/10th of that in the primary circulation given that the primary circulation time is 0.4–2 min (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System).

Physiological Function

The apparent absence of secondary vessels in skeletal muscle and the gastrointestinal tract argues against the importance of this system in returning protein and interstitial fluid back to the primary circulation as done by classical lymphatics. However, not all mammalian tissues have lymphatics (i.e., brain). It is possible that the secondary system is a prototype lymphatic system that has developed to maintain interstitial environment in the most susceptible tissues, that is, those exposed to an osmotically hostile environment. In other tissues, where intravascular pressures are low, a specialized reabsorptive vasculature may not be necessary (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation). The secondary system does not appear to be involved in gas exchange as it is nearly devoid of red blood cells. Despite the inaccessibility to red blood cells, the ability of the endothelial microvilli in the region of the arterio-arterial anastomoses to trap leukocytes may provide immunological protection to the epithelium. Interestingly, fish that have been anesthetized for a long period adopt red coloration to their skin. This may be due to red blood cells entering the secondary circulation that services the skin, scales, and fins.

In summary, fishes are unique among vertebrates in that they possess primary and secondary circulations, with flow reaching the secondary circulation via the primary circulation. While the roles of the primary circulation are well established, those of the secondary system are not. Nutritive and reabsorptive functions are likely, but remain to be confirmed.

See also: **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Branchial Anatomy; The Gastrointestinal Circulation; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis; Circulatory Fluid Balance and Transcapillary Exchange. **Design and Physiology of the Heart**: Accessory Hearts in Fishes. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish; Integrated Control of the Circulatory System. **Pelagic Fishes**: Physiology of Tuna .

Further Reading

Burne RH (1929) A system of fine vessels associated with the lymphatics in the cod (*Gadus morhua*). *Philosophical Transactions of the Royal Society London B* 217: 235–366.

Dewar H, Brill RW, and Olson KR (1994) The secondary circulation of the vascular heat exchangers in skipjack tuna, *Katsuwonus pelamis*. *Journal of Experimental Zoology* 269: 566–570.

Ishimatsu A, Iwama GK, Bentley TB, and Heisler N (1992) Contribution of the secondary circulatory system to acid-base regulation during hypercapnia in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 170: 43–56.

Kampmeier OF (1969) *Evolution and Comparative Morphology of the Lymphatic System*. Springfield, IL: Charles C. Thomas.

Kuchler AM, Gjini E, Peterson-Maduro J, *et al.* (2006) Development of the zebra fish lymphatic system requires VEGFC signaling. *Current Biology* 16: 1244–1248.

Lahnsteiner A, Lametschwandtner A, and Patzner RA (1990) The secondary blood vessel system of segmental arteries and dorsal aorta in *Blennius pavo* and *Zosterisessor ophicephalus.* Histology, fine structure and SEM of vascular corrosion casts. *Scanning Electron Microscopy Inc.* 4: 111–124.

Olson KR (1996) Secondary circulation in fish: Anatomical organization and physiological significance. *Journal of Experimental Zoology* 275: 172–185.

Olson KR, Kinney DW, Dombkowski RA, and Duff DW (2003) Transvascular and intravascular fluid transport in the rainbow trout: Revisiting Starlings forces, the secondary circulation and interstitial compliance. *Journal of Experimental Biology* 206: 457–467.

Satchell GH (1991) *The Physiology and Form of the Circulation*. Cambridge: Cambridge University Press.

Skov PV and Bennett MB (2004) Structural basis for control of secondary vessels in the long-finned eel, *Anguilla reinhardtii*. *Journal of Experimental Biology* 207: 3339–3348.

Skov PV and Steffensen JF (2003) The blood volumes of the primary and secondary circulatory system in the Atlantic cod, *Gadus morhua*, L., using plasma bound Evans Blue and compartmental analysis. *Journal of Experimental Biology* 206: 591–599.

Steffensen JF and Lomholt JP (1992) The secondary vascular system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A*; *The Cardiovascular System*, pp.185–213. San Diego, CA: Academic Press.

Steffensen JF, Lomholt JP, and Vogel WOP (1986) *In vivo* observations on a specialized microvasculature, the primary and secondary vessels in fishes. *Acta Zoologica (Stockholm)* 67: 193–200.

Vogel WOP (1978) Arteriovenous anastomoses on the afferent region of trout gill filaments (*Salmo gairdneri* Richardson, Teleostei). *Zoomorphologie* 90: 205–212.

Vogel WOP (1985) Systemic vascular anastomoses, primary and secondary vessels in fish, and the phylogeny of lymphatics. In: Johansen K and Burggren W (eds.) *Cardiovascular Shunts. Phylogenetic, Ontogenetic, and Clinical Aspects*, pp.143–159. Copenhagen: Munksgaard.

Vogel WOP and Claviez M (1981) Vascular specialization in fish, but no evidence for lymphatics. *Zeitschrift fuer Naturforschung* 36c: 490–492.

Vogel WOP and Mattheus U (1998) Lymphatic vessels in lungfishes (Dipnoi) 1. The lymphatic vessels system in Lepidosireniformes. *Zoomorphology* 117: 199–212.

Yaniv K, Isogai S, Castranova D, *et al.* (2006) Live imaging of lymphatic development in the zebrafish. *Nature Medicine* 12: 711–716.

CIRCULATION

Integrated Control and Response of the Circulatory System

Contents

Integrated Control of the Circulatory System
Central Control of Cardiorespiratory Interactions in Fish
Hormone Metabolism in the Circulation
Integrated Responses of the Circulatory System to Temperature
Integrated Responses of the Circulatory System to Digestion
Integrated Cardiovascular Responses of Fish to Swimming
Integrated Responses of the Circulatory System to Hypoxia

Integrated Control of the Circulatory System

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Control Systems
Integrative Analysis of the Fish Cardiovascular System
Examples of Complex and Simple Cardiovascular Regulation Using Negative Feedback Loops
Examples of Cardiovascular Regulation Using Feedforward Mechanisms
Examples of Cardiovascular Regulation Using Rheostasis
Further Reading

Glossary

Dampening The degree to which a controlled variable is allowed to oscillate. Systems that are tightly controlled are highly damped; a poorly damped system allows the controlled variable to deviate considerably.

Effector A variety of mechanisms that can act upon a controlled system. The heart, blood vessels, and kidney are all effectors of arterial blood pressure.

Feedback transducer A reporting mechanism that monitors a physiological variable and converts this into an output signal, usually an action potential or hormone that can be transmitted back to the controller. Stretch receptors in blood vessel walls detect blood pressure and convert mechanical stretch into action potentials to be sent to the central nervous system.

Feedforward Feedforward systems detect an external disturbance and bring about a change in a controlled variable without monitoring their success.

Feedforward systems Prevent rapid deviations away from the setpoint and typically work in conjunction with negative feedback systems.

Gain The amount of effector response exerted on a system. A system with high gain rapidly restores conditions. Gain is calculated as compensation/error.

Homeostasis The equilibrium or stability of the internal environment of organisms in a changing external environment, maintained by many physiological adjustment mechanisms.

Negative feedback A controlled system response that restores critical values back to their starting or optimal condition.

Neutral zone The range around the setpoint in which the controlled variable can oscillate without initiating an effector response.

Programmed rheostasis A change in the setpoint at some predetermined time or life stage. Parr–smolt transformation accompanying the migration of salmon from freshwater to seawater is an example of programmed rheostasis.

Reactive rheostasis A change in the setpoint that achieves a compromise between opposing factions. For example, during hypoxia blood pressure is regulated downward. While this decreases tissue blood flow, it also decreases the work of the heart and therefore conserves the limited energy.

Rheostasis A change in the setpoint, typically one of two types, reactive and programmed.

Setpoint The ideal value for a controlled variable.

Introduction

Physiology is the study of how an organism regulates its internal environment. A variety of simple to complex control systems are employed to achieve this regulation, and physiological experiments are designed with the ultimate goal of understanding these control systems. The following sections provide an overview of the basic principles of control systems and a few illustrations of how these systems act and interact in the control of the fish cardiovascular system.

Control Systems

The importance of regulating the immediate cellular environment (the *milieu interieur*) for the well-being of all cells in an organism was first realized by Claude Bernard, and the significance of being able to maintain this internal environment within the acceptable limits was delineated by Walter Cannon as the concept of homeostasis. The study of physiological control systems is based on general principles of 'system analysis', commonly employed in engineering. As shown in **Figure 1**, there are three general components to any system: input, laws, and output. The factors acting upon the system are the input, the laws are the properties of the system that will produce a particular output (when unknown these laws are often called the 'black box'), and the output is the ultimate result. If any two of these are known, the third is readily obtainable. For instance, in physiological experiments the input is carefully controlled and the output is analyzed to characterize the laws that govern the response. The key to a well-operating control system is to monitor the output in such a way that it can readily be restored back to some optimal value when external factors cause it to deviate. Control systems operate through feedback loops.

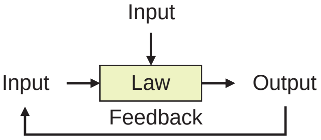


Figure 1 The three components of a controlled system: input, the factors acting upon a system (often external); laws, the intrinsic properties of the system that will produce a particular output (called the 'black box' when unknown); output, the ultimate result. If the result is monitored and can again be readjusted, this becomes a feedback loop. One of the primary goals of science is to identify the laws governing control systems.

Negative Feedback

The basic components of a control system are shown in **Figure 2**. Central to this is the negative feedback loop (yellow in **Figure 2**), which consists of five components. The controlled process is the physiological variable that must be regulated, that is, blood pressure. The feedback transducer is a reporting mechanism that monitors the controlled process (blood pressure) and converts (transduces) the message into another form, usually action potentials or hormones, that can be transmitted to the controller. In the example of blood pressure regulation, this would be stretch receptors in vessel walls that detect the mechanical stretch imposed by blood pressure and convert this into another type of signal, usually nerve action potentials or hormones. The controller receives information from the feedback transducers and compares this to some ideal value, the reference input, also known as the setpoint, and then decides if the controlled process is within an acceptable range. If not, the controller then sends signals to the effectors, which have the ability to make the necessary changes. In the case of blood pressure regulation this may be the heart, blood vessels, kidney, or any other organ that can ultimately affect blood pressure. This feedback loop is a negative feedback system because when the controlled process deviates away from the setpoint the system returns it. Positive feedback loops tend to further drive the system away from the starting point; these are rare in physiology and usually only transient (i.e., blood clotting, action potentials, urination, and defecation reflexes).

Most control systems operate around the desired setpoint, but how tightly the system is controlled may vary. The neutral zone, also known as the dead zone, is the area around the setpoint at which the controlled parameter may change but not initiate a feedback response. The

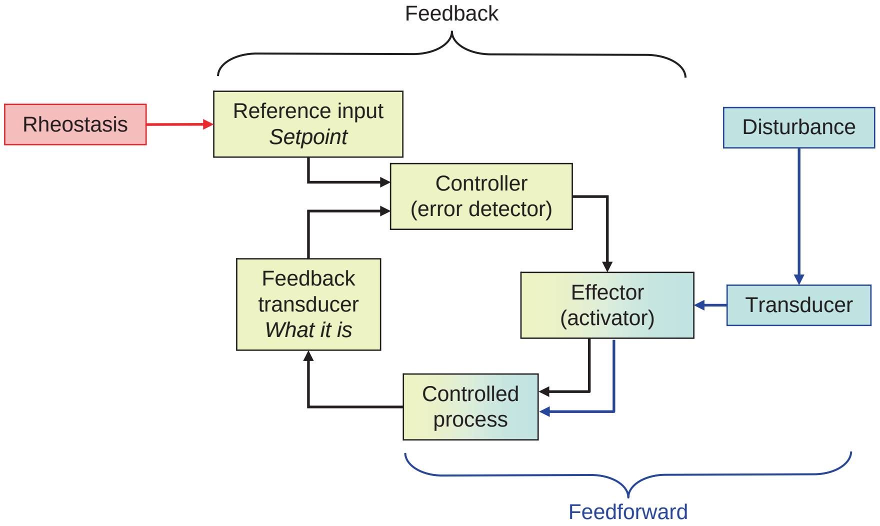


Figure 2 Components of physiological control systems. The basic system relies on a negative feedback loop (in yellow) where the physiological parameter controlled process is monitored by feedback transducers and the signal is sent to the controller and compared to the ideal value, the reference input. If the controlled process has deviated sufficiently away from the ideal, the controller activates mechanisms, effectors, to restore the system. A feedforward system (in blue) detects an outside disturbance and immediately activates the effector to bring about changes in the controlled process. Feedforward systems are anticipatory in that they predict the need for response and initiate the response without directly monitoring their success; thus, they are open-loop systems. Rheostasis (in red) provides a mechanism to change the reference input and thereby adjust the controlled process.

width of the neutral zone determines the dampening, which is defined as the degree of oscillation permitted around the setpoint. The gain of the system refers to how tightly the system is controlled or dampened. Gain can be calculated as the ratio between the amount of correction of an abnormality relative to the amount of abnormality remaining, that is, compensation divided by error. As an example, if after 30% of the blood volume is removed from the fish and its blood pressure falls from 25 to 24 mmHg (net = 1 mmHg) with all control systems intact, but falls from 25 to 15 mmHg (net = 10 mmHg) with all control systems blocked, the compensation is $10 - 1 = 9$ and the error is 1. The gain of this control system is $9/1 = 9$, which is fairly typical. The gain for many physiological systems is between 2 and 10.

Feedforward

Feedforward systems (blue in **Figure 2**) detect an outside disturbance that is a potential threat to the controlled process. However, the transducer sends its signal to the effector, usually without any input from the controller (although these signals often pass through the central nervous system). Activation of the effector changes the controlled process. Feedforward systems are anticipatory in that they predict the need for response and then initiate the response without directly monitoring their success. Thus, feedforward systems are not closed loop because they do not restore the controlled process to the preset value. Rather, they prevent rapid deviations away from optimal. Feedforward systems typically work in conjunction with negative feedback loops.

Rheostasis

Rheostasis (red in **Figure 2**) provides a mechanism to change the setpoint and thereby adjust the value of the controlled process. There are two types of rheostasis: reactive and programmed. Reactive rheostasis is a change in setpoint that is based upon a judgment decision where conditions are such that maintaining the status quo is not in the best interest of the organism. Typically, the new setpoint is some compromise between opposing factions, each of which tends to pull the regulated parameter in the opposite direction. In programmed rheostasis the setpoint changes at some predetermined time or life stage of the animal.

Integration of Control Systems

Most control systems do not operate independently, but are integrated with other control systems. This allows sophisticated regulation of the control processes (while at times makes studying these processes extremely difficult). Even a single controlled process can vary in complexity. Regulation of arterial blood pressure relies upon a highly integrated and extremely complex interaction of numerous effectors that are controlled by multiple areas within the central nervous system (**see also Integrated Control and Response of the Circulatory**

System: Central Control of Cardiorespiratory Interactions in Fish). Conversely, local regulation of blood flow in response to metabolic need is a relatively simple process where an increase in oxygen consumption by tissues reduces the oxygen tension in nearby blood vessels and leads to a direct vasodilation (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels).

Integrative Analysis of the Fish Cardiovascular System

There is considerable detail regarding mammalian cardiovascular control systems and these have been subjected to detailed engineering analysis for over 40 years. Similar analyses have not been performed on fish and this remains as one of the greatest challenges for future research. The following sections provide some examples of control system analysis of the fish cardiovascular system. It is evident that each of these components can be broken down into subcomponents and these can often be further subdivided numerous times.

Regulation of Arterial Blood Pressure

As described in **Circulation**: Circulatory System Design: Roles and Principles, perhaps the most fundamentally regulated parameter by remote control in the cardiovascular system is arterial blood pressure. The primary determinants of arterial blood pressure are cardiac output and total peripheral resistance. Each of these can be further divided into their respective components, resulting in the flow diagram shown in **Figure 3**. The following sections provide a brief overview of these components and references to further details that can be found elsewhere in this encyclopedia.

Cardiac output

The primary determinants of heart rate are parasympathetic inhibition, sympathetic stimulation, mechanical

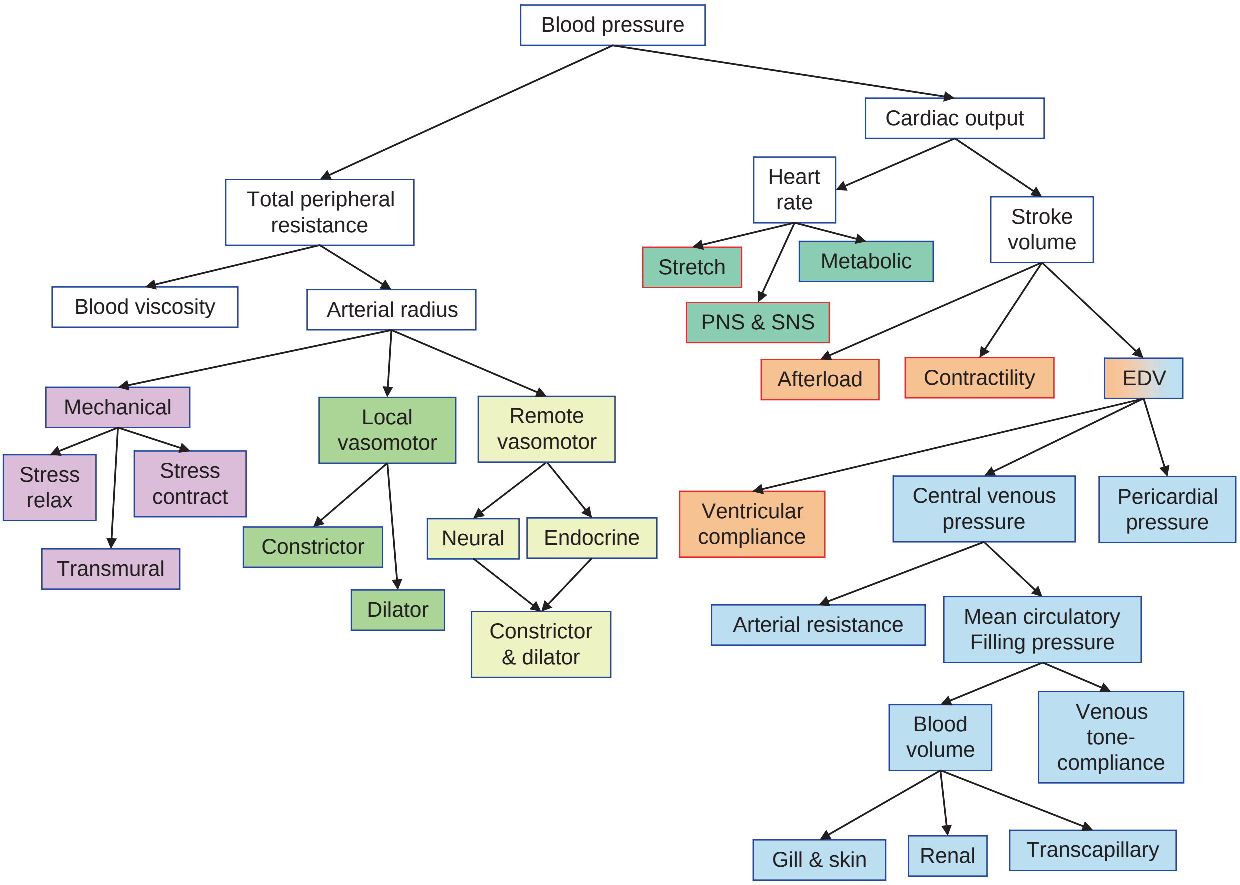


Figure 3 Flow diagram of the factors that affect arterial blood pressure. The cardiac factors that affect stroke volume are shaded in red, extracardiac factors that affect stroke volume are shaded in blue, and factors that affect heart rate are shaded in green. Mechanical factors that affect arterial radius are shaded in lavender, local vasomotor factors in lime green, and remote vasomotor factors in yellow. EDV, end-diastolic volume; PNS, parasympathetic nervous system; SNS, sympathetic nervous system. See text for details.

stretch, and the local chemical environment in the area of the pacemaker cells (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes, Physiology of Cardiac Pumping, **Brain and Nervous System**: Autonomic Nervous System of Fishes, and **Hormonal Responses to Stress**: Catecholamines). Heart rate is slowed by parasympathetic stimulation, increased by sympathetic stimulation, increased by local stretch, and may be decreased by hypoxia, acidosis, or elevated carbon dioxide. Heart rate of many fish is under tonic parasympathetic inhibition and sectioning these nerves alone will produce a modest increase in heart rate due to the faster intrinsic rate of the cardiac pacemaker.

Stroke volume depends upon factors that affect cardiac emptying (e.g., contractility, end-diastolic volume (EDV), and afterload (arterial blood pressure)), cardiac factors that affect filling, including cardiac suction and ventricular compliance, and extracardiac factors that affect filling, namely the mean circulatory filling pressure. Contractility is the strength of contraction independent of the length of the cardiocyte; it is increased by factors such as sympathetic nerve stimulation and elevated intracellular calcium and decreased by factors such as fatigue hypoxia and acidosis (and sometimes parasympathetic nerve stimulation). Afterload (ventral aortic blood pressure) does not affect stroke volume until it becomes approximately 2½ times that of normal ventral aortic pressure, at which point stroke volume will progressively decrease as blood pressure increases. An increase in EDV, within physiological limits, will increase stroke volume due to the increase in strength of contraction when the cardiocytes are stretched. This length–tension relationship is also known as the Frank Starling law of the heart. EDV is directly related to ventricular compliance (an increase in compliance increases EDV, and vice versa). In fish with a rigid pericardium, ventricular contraction decreases pericardial pressure which helps suction blood from the veins into the atrium, a phenomenon known as 'vis a fronte' filling. However, the most important determinant of EDV is central venous pressure and the most important determinant of central venous pressure is the mean circulatory filling pressure. The mean circulatory filling pressure is the 'fullness of the circulation' and is regulated over the short term by changing venous tone (an increase in venous constriction will increase mean circulatory filling pressure), or through rapid adjustments in blood volume by transferring fluid from the interstitial into the intravascular compartment, or vice versa. The mean circulatory filling pressure is also regulated over the long term by changing blood volume through regulation of water and salt balance across the gill, skin, and kidney. In mammals, the ultimate long-term regulator of blood pressure is blood volume, and this appears to be the case in fish as well. Other factors can also transiently increase central venous pressure. For instance, the decrease in arterial resistance accompanying exercise will cause more blood to flow from the arteries into the veins, thereby increasing venous pressure and ultimately cardiac output. For further details on the factors that affect cardiac output, **see Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange, **Circulation**: Circulatory System Design: Roles and Principles, **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels, Physiology of Resistance Vessels, **Design and Physiology of the Heart**: Physiology of Cardiac Pumping, **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts, Mechanisms of Ion Transport in Freshwater Fishes, **Role of the Kidneys**: The Kidney, **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation, and **Role of the Gills**: The Osmorespiratory Compromise.

Total peripheral resistance

The main determinants of total peripheral resistance are blood viscosity and the diameter of small arteries and arterioles. Hematocrit (the relative volume occupied by red blood cells) has the greatest effect on blood viscosity. As hematocrit increases the oxygen-carrying capacity of blood increases but so does blood viscosity. Hematocrit in most fish is around 35, which is a trade-off between the oxygen-carrying capacity and viscosity. High-energy-demand fish such as tuna increase hematocrit to as high as 50 in order to increase oxygen-carrying capacity, but in doing so they also have to increase arterial blood pressure to overcome the elevated viscosity. On the other end of the spectrum, some Antarctic icefish eliminate red blood cells from the circulation altogether in order to reduce viscosity at these very cold temperatures. The loss in oxygen-carrying capacity is compensated for by an increase in oxygen solubility in the plasma due to the cold, and an increase in cardiac output.

The radius of small arteries and arterioles is the primary determinant of total peripheral resistance. Arterial resistance is largely under the influence of both local and remote controlling mechanisms.

Local control

Local control matches blood flow (perfusion) with metabolic demand of the tissue. This is the ultimate purpose of the cardiovascular system. Local control is achieved primarily through vasodilator substances that are consumed by or released from cells as a consequence of tissue metabolism. These typically act upon the smallest resistance vessels and include low oxygen (low P_{O_2}; although low P_{O_2} constricts gill vessels), elevated carbon dioxide, acidosis, elevated potassium, and other metabolites (e.g., ATP, adenosine).

Remote control

The primary function of remote control is to maintain a constant or nearly constant arterial blood pressure to perfuse all tissues, but in crisis situations, such as blood loss due to hemorrhage, the remote system decreases blood flow to less critical tissues (e.g., gastrointestinal tract) in order to maintain sufficient oxygen delivery to oxygen-sensitive critical tissues (e.g., brain and heart). Remote control has both a neural and endocrine component. Neural control is predominantly through a vasoconstriction regulated by the sympathetic nervous system and mediated by catecholamines. However, a variety of other neurotransmitters have also been described, such as nitric oxide (NO), serotonin (5-hydroxytryptamine), purines, and peptides, such as vasoactive intestinal peptide, substance P, tachykinins, and others. Numerous hormonal signals also affect peripheral vascular resistance. The primary vasoconstrictor hormones are angiotensin II, arginine vasotocin, catecholamines, and urotensin II. Vasodilator hormones include the natriuretic peptides and adrenomedullin.

Mechanical factors can also affect arterial radius both passively and actively. Passive compression of blood vessels, which commonly occurs when the skeletal or gastrointestinal muscles surrounding them contract, will increase vascular resistance. Compensatory mechanisms such as an increase arterial blood pressure may be invoked partially to compensate for the increased resistance under these circumstances. Vascular smooth muscle can also respond actively to mechanical stress. An increase in blood pressure will passively dilate blood vessels and decrease their resistance. To overcome this, some vessels such as those of the mammalian kidney constrict in response to mechanical distention in order to prevent excessive decreases in resistance. This constriction may result from an intrinsic response on the smooth muscle cell itself, or may be mediated by stretch of the surrounding underlying endothelial cells and subsequent release of the vasoconstrictor endothelin-1. Whether or not this occurs in fish renal arteries has not been confirmed. Many vessels respond to stretch by stretch relaxation, which is a gradual loss of tone after the vessel has been stretched. This, however, does not appear to have a beneficial effect on regulation of arterial blood pressure. For further information, **see Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels, **Brain and Nervous System**: Autonomic Nervous System of Fishes, and **Hormonal Responses to Stress**: Catecholamines.

Regulation of Tissue Perfusion, a Different Perspective

The example above describes the factors that affect blood pressure which is under the purview of the remote control system. However, one could place any one of these parameters at the top of the flow diagram. This also illustrates both the integrative nature and complexity of these control mechanisms. **Figure 4** prioritizes cardiovascular regulation from the standpoint of local control of nutrient delivery to the tissues. Thus, the main determinants of tissue perfusion are arterial blood pressure and vascular resistance. One can again continue to subdivide these determinants into their respective components and essentially re-create **Figure 3**, albeit from a different perspective. While these diagrams are extremely useful in illustrating the variety of factors involved in controlling particular processes, they do not show the interactive feedback control of these processes. As described above, this has not yet been done in detail in the fish cardiovascular system, but by borrowing principles developed in mammalian physiology we can begin to construct some of these pathways.

Examples of Complex and Simple Cardiovascular Regulation Using Negative Feedback Loops

Baroreceptor-Mediated Restoration of Arterial Blood Pressure Following Hemorrhage

Figure 5 shows the cardiovascular reflexes that accompany hemorrhage. This is a relatively sophisticated feedback system involving both central nervous system, endocrine and local responses. The initial loss in blood volume decreases central venous blood pressure by decreasing mean circulatory filling pressure. This is detected by low-pressure baroreceptors, presumably in or near the heart. In addition, the decrease in venous return sequentially decreases EDV, stroke volume, cardiac output, and arterial blood pressure. The fall in arterial blood pressure is detected by high-pressure baroreceptors in the gill and probably systemic arteries. A variety of reflexes are then initiated to restore both venous and arterial blood pressure. Fluid is rapidly transferred from the interstitial space into the vasculature both passively and actively; the drop in arterial pressure decreases capillary hydraulic pressure which promotes capillary reabsorption of interstitial fluid, and an active arterial vasoconstriction further reduces capillary pressure and augments fluid reabsorption. Long-term restoration of fluid volume is accomplished through regulation of water and salt balance by the gills, skin, and kidney. This, combined with venous constriction, increases central venous pressure and increases filling of the heart (EDV), which increases stroke volume. Stroke volume is also enhanced by an increase in ventricular contractility. An increase in heart rate plus the increase in stroke volume increases cardiac output and this combined with a baroreceptor-mediated increase in peripheral vascular resistance restores arterial blood pressure. This basic

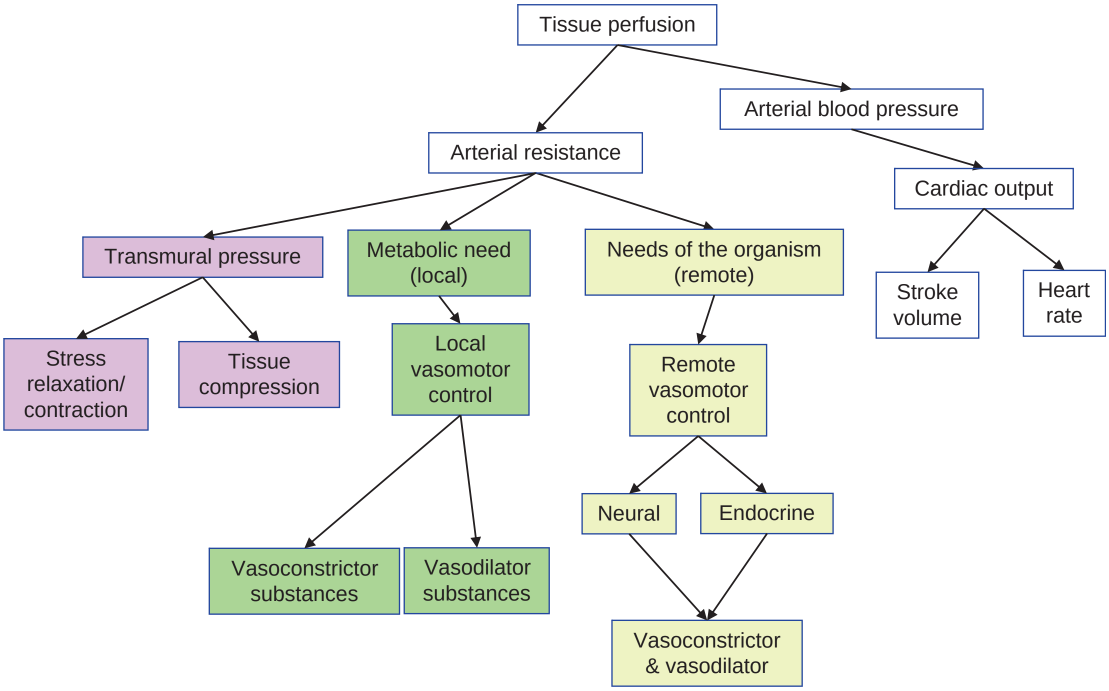


Figure 4 Flow diagram of the factors that affect tissue perfusion. These factors are essentially the same as those that affect arterial blood pressure in **Figure 3**, but rearranged from the perspective of blood flow to tissues. The cardiac factors affecting arterial blood pressure have been abbreviated for simplicity. See text for details.

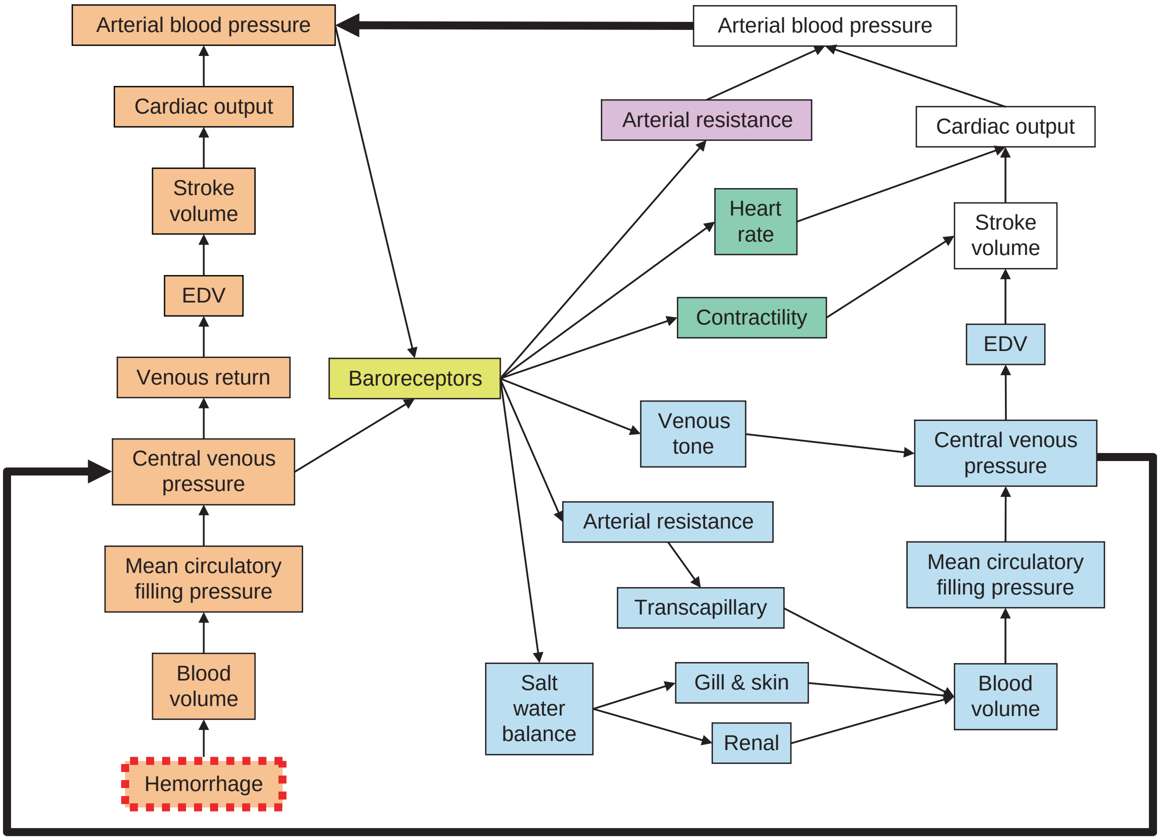


Figure 5 An integrated baroreceptor-mediated negative feedback mechanism for restoring arterial blood pressure following hemorrhage. The immediate effects of hemorrhage are shaded in red. See text for details.

mechanism, with a few minor modifications, can be utilized to respond to hypovolemia accompanying freshwater to saltwater adaptation, or utilized in the opposite direction during saltwater to freshwater adaptation.

Local Regulation of Blood Flow in Response to Oxygen Demand

Figure 6 shows a relatively simple feedback loop in the peripheral vasculature. Here, an increase in tissue metabolism increases oxygen consumption which lowers the partial pressure for oxygen (P_{o_2}) in and around the tissue and nearby blood vessels. The decreased P_{o_2} in the vascular smooth muscle cell is detected by an as-yet unknown O_2 sensing mechanism and results in vasodilation. This increases tissue blood flow and the increased oxygen delivery restores P_{o_2}.

Other Feedback Mechanisms

There are many examples of negative feedback regulation in the cardiovascular system. Two of the most dramatic are the cardiovascular changes accompanying exercise and digestion. Additional information on these can be found in **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming and Integrated Responses of the Circulatory System to Digestion.

Examples of Cardiovascular Regulation Using Feedforward Mechanisms

Feedforward mechanisms anticipate the need for a change in a regulated parameter without directly monitoring that parameter and they are typically transient in nature. There are numerous feedforward responses. One example is the gastric phase of digestion whereupon eating a meal, the distention of the stomach increases arterial blood pressure in anticipation of the need to increase blood flow through the gastrointestinal tract (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion). Another example is the startle reflex which is often initiated by an unexpected environmental cue such as noise. The startle reflex in mammals is characterized by an increase in heart rate and blood pressure which is the opposite of that in fish where heart rate and blood pressure decrease.

Examples of Cardiovascular Regulation Using Rheostasis

Reactive Rheostasis

In reactive rheostasis, the setpoint is changed to accommodate two disparate parameters either of which by itself would have untoward consequences. The cardiovascular response to hypoxia is a good example of reactive rheostasis. When environmental oxygen (P_{o_2}) falls to a critical level, most fish exhibit a decrease in heart rate and arterial blood pressure. This in turn decreases cardiac work and lowers the energy requirements of the heart. Thus, while the blood pressure setpoint is now lowered below the optimal value, the reduction in cardiac work permits conservation of limited resources (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia). Another good example of reactive rheostasis can be found in euryhaline fish moving from freshwater to saltwater (or vice versa). When a fish moves from freshwater to saltwater, it is now faced with hypovolemia due to osmotic loss of water and hypernatremia due to a net inward sodium gradient. Under these circumstances the fish drink seawater to replenish the lost volume but this further increases the sodium load. Typically when this occurs the fish tolerates an elevated plasma sodium load and at the same time maintains blood volume at a slightly lower level. Thus, both the plasma sodium and fluid volume are regulated at slightly different values. Exercise and digestion are also examples of reactive rheostasis in that the blood pressure is elevated in order to provide better perfusion of working muscle in the former, and the gastrointestinal tract in the latter.

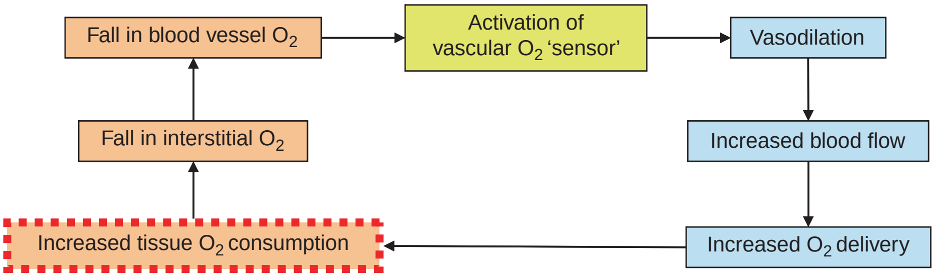


Figure 6 Local negative feedback mechanism for increasing oxygen delivery to tissues in response to increased tissue oxygen demand. The immediate effects of increased tissue metabolism are shaded in red.

Programmed Rheostasis

Programmed rheostasis accompanies a change in life-cycle. A good example of programmed rheostasis is the parr-smolt transformation that accompanies anadromous migration of salmonids. Programmed rheostasis also accompanies transformation of larval to adult phenotypes and the many physiological changes accompany the reproductive cycle, including spawning migrations.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels; Physiology of Resistance Vessels. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes; Physiology of Cardiac Pumping. **Hormonal Responses to Stress**: Catecholamines. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish; Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Digestion; Integrated Responses of the Circulatory System to Hypoxia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Role of the Gills**: The Osmorespiratory Compromise. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **Role of the Kidneys**: The Kidney.

Further Reading

Altimiras J and Axelsson M (2004) Intrinsic autoregulation of cardiac output in rainbow trout (*Oncorhynchus mykiss)* at different heart rates. *Journal of Experimental Biology* 207: 195–201.

Åström KJ and Murry RM (2008) *Feedback Systems: An Introduction for Scientists and Engineers*. Princeton, NJ: Princeton University Press.

Axelsson M and Fritsche R (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua. Journal of Experimental Biology* 158: 181–198.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac function in fish. *Physiological Zoology* 64: 1137–1164.

Farrell AP, Thorarensen H, Axelsson M, *et al.* (2001) Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology* 128: 551–563.

Gamperl AK and Farrell AP (2004) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Gilmour KM and Perry SF (2007) Branchial chemoreceptor regulation of cardiorespiratory function. In: Hara TJ and Zielinski B (eds.) *Fish Physiology, Vol. 25: Sensory Systems Neuroscience*, pp. 97–151. San Diego, CA: Academic Press.

Guyton AC (1995) Interrelationships among interstitial fluid volume, interstitial fluid pressure, interstitial fluid protein concentration and lymph flow. In: Reed RK, McHale NG, Bert JL, Winlove CP, and Laine GA (eds.) *Interstitium, Connective Tissue and Lymphatics*, pp. 167–180. London: Portland Press.

Guyton AC, Carrier O, Jr., and Walker JR (1964) Evidence for tissue oxygen demand as the major factor causing autoregulation. *Circulation Research* 15(supplement 9): 6–69.

Guyton AC, Hall JE, Coleman TG, and Manning RD , Jr. (1990) The dominant role of the kidneys in the long-term regulation of arterial pressure in normal and hypertensive states. In: Laragh JH and Brenner BM (eds.) *Hypertension: Pathophysiology, Diagnosis, and Management*, pp. 1029–1052. New York, NY: Raven Press.

Guyton AC, Jones CE, and Coleman TG (1973) *Circulatory Physiology: Cardiac Output and Its Regulation*. Philadelphia, PA: W.B. Saunders.

Mrosovsky N (1990) *Rheostasis: The Physiology of Change*. New York, NY: Oxford University Press.

Olson KR and Hoagland TM (2008) Effects of freshwater/saltwater adaptation and dietary salt on fluid compartments, blood pressure and venous capacitance in trout. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 294: R1061–R1067.

Rowell LB (1993) *Human Cardiovascular Control*. New York, NY: Oxford University Press.

Sandblom E and Axelsson M (2007) The venous circulation: A piscine perspective. *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology* 148: 785–801.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. New York, NY: Cambridge University Press.

Central Control of Cardiorespiratory Interactions in Fish

EW Taylor, University of Birmingham, Birmingham, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Elasmobranchs
Teleosts
Air-Breathing Fish
The Neural Basis of CRIs
Summary
Further Reading

Glossary

Antidromic stimulation Electrical stimulation of efferent axons in a nerve toward the CNS (i.e., a reversal of their normal direction of conduction of impulses) in order to identify their cell bodies for recordings with microelectrodes inserted into the brainstem.

Capsaicin The chemical irritant in red hot chilli peppers, recognized as stimulating J-receptors in vertebrates that cause changes in ventilation and heart rate to protect the gills or lungs from chemical damage.

Cardiac vagal preganglionic neurons (CVPNs) VPNs that supply the cardiac ganglion.

Cardiorespiratory coupling (CRC) Modulation of cardiac intervals by the respiratory rhythm resulting in specific temporal relationships.

Cardiorespiratory interactions (CRIs) Modulation of cardiac intervals by the respiratory rhythm resulting in an identifiable respiratory component in the pattern of heart rate variability.

Cardiorespiratory synchrony (CRS) Modulation of cardiac intervals by the respiratory rhythm resulting in 1:1 coupling between heart beats and ventilation.

Dorsal motor nucleus of the vagus (DVN) The concentration of cell bodies in a group close to the 4th ventricle in the medulla oblongata of preganglionic neurons supplying efferent axons to the vagus nerve.

Power spectral analysis A statistical analysis of the variability in timing of activity in a rhythmically active system such as heart beat that identifies the separate components contributing to this variability (such as the respiratory cycle).

Respiratory sinus arrhythmia (RSA) Central control mechanism integrating heart rate with the respiration cycle. The increase in heart rate and gating of the baroreceptor response during inspiration described for mammals but lately suggested to be shown by reptiles such as the rattlesnake.

Vagal preganglionic neurons (VPNs) Neurons with their cell bodies in the brainstem that supply efferent axons to ganglia situated on target organs in the viscera such as the heart or intestine.

Vagal tone The chronic inhibitory control of heart rate exerted by the parasympathetic nervous system via the vagus nerve.

Vagus nerve The X cranial nerve (from Greek; vagari, the wanderer) that travels from the brain stem to innervate the heart, respiratory muscles, and chemo- and mechanoreceptors on the gills, as well as much of the intestine and other visceral organs.

Introduction

The jawless fishes (Cyclostomata) ventilate gill pouches with tidal water flow while the jawed fishes ventilate their gills unidirectionally, with water streaming in at the mouth and out over the gills (**see also Hagfishes and Lamprey**: Hagfishes and **Hagfishes and Lamprey**: Lampreys: Energetics and Development). In jawed fishes, there is a close matching of respiratory water flow and cardiac output, according to their relative capacities for oxygen (the ventilation/perfusion ratio), with water flow regulated at 10–20× blood flow. These different water and blood flows are needed to match their relative capacities to carry oxygen, which in water is limited by its solubility but in blood is increased by its reversible combination with hemoglobin in red cells (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). This flow matching has been shown to optimize respiratory gas exchange over a functional countercurrent at the exchange surfaces of the gills (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). As the heart delivers blood directly to the gills along the ventral aorta and water is pumped over the gills by a double-action buccal and opercular or septal pump (**see also Gas Exchange**: Respiration: An Introduction), both these flows are

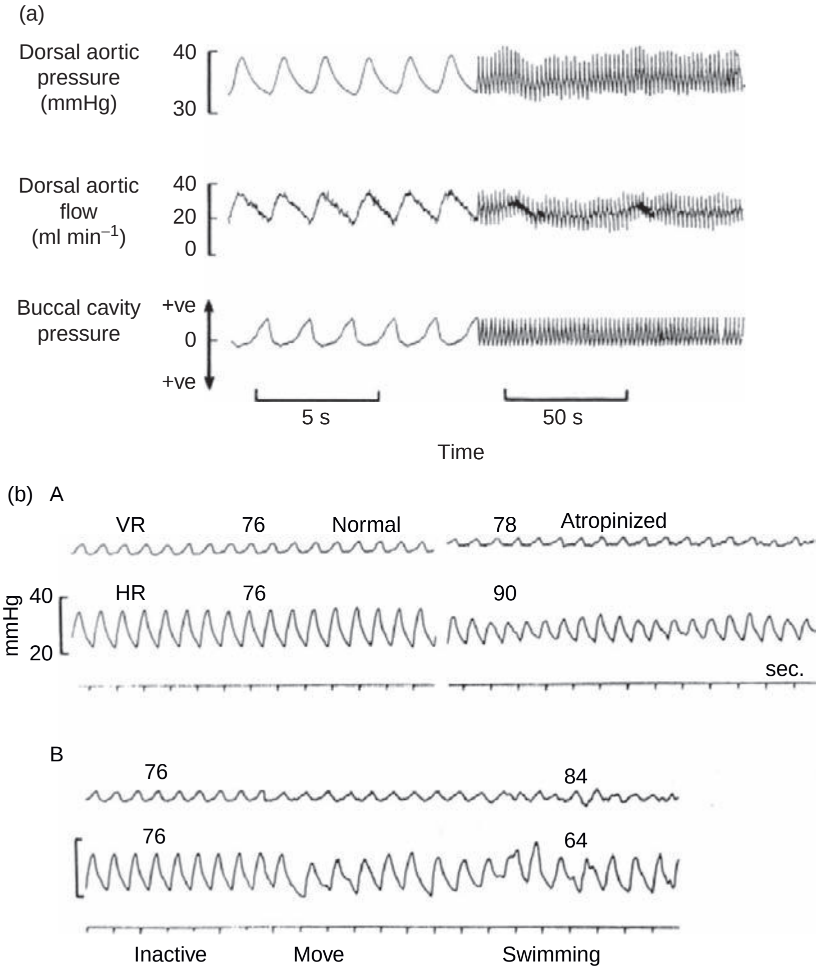


Figure 1 (a) Blood pressure and flow in the dorsal aorta measured from unrestrained cod, *G.* morhua held in aerated seawater are highly pulsatile and often in phase with ventilation, measured as water pressures in the buccal cavity. (b) Ventilation (VR) and heart (HR) rates recorded as pressures from unrestrained and inactive dogfish, *S. canicula* at 23°C show long periods of apparent CRS that is abolished following injection of atropine (A) and lost during a spontaneous period of activity (B). (a) From Jones DR, Langille BL, Randall DJ, and Shelton G (1974) Blood flow in dorsal and ventral aortas of the cod, *Gadus morhua. American Journal of Physiology* 226: 90-95. (b) From Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume 12, Part B*, pp. 343-387. New York: Academic Press.

markedly pulsatile (**Figure 1**). Consequently, close beat-to-beat temporal relationships between them would seem necessary to achieve close matching of flows.

A 1:1 ratio of the cardiac frequency and the ventilatory frequency, termed cardiorespiratory synchrony (CRS), has long been hypothesized as being important for the optimization of respiratory gas exchange in fish. However, recordings of CRS have proved elusive and many fish biologists have denied that it is anything other than a transient effect. Failure to observe CRS may have been due to over-invasive techniques of measurement, and reluctance to accept its existence has now been overcome by a series of long-term observations on Atlantic cod (*Gadus morhua*). Theoretically, CRS should be particularly advantageous during periods of respiratory distress or when conserving energy, which appears to be the case. Fish in normoxia showed a variety of phase relationships between pulsatile blood flow and ventilation, but during progressive hypoxia the heartbeat became synchronized with a specific part of the ventilatory cycle (**Figure 2**). Thus, there is direct evidence for fine control of heart rate, including its beat-to-beat modulation by the respiratory cycle that generates cardiorespiratory interactions (CRIs), culminating in CRS. In addition, more subtle modulation of heart rate by respiratory activity, termed cardiorespiratory coupling (CRC), has been demonstrated by power spectral analysis of cardiac intervals. The relevance of CRC to gas exchange was demonstrated by its abolition following cardiac vagotomy that affected oxygen uptake (**Figure 3**).

The autonomic nervous system is the main short-term modulator of heart rate in fish (**see also Brain and**

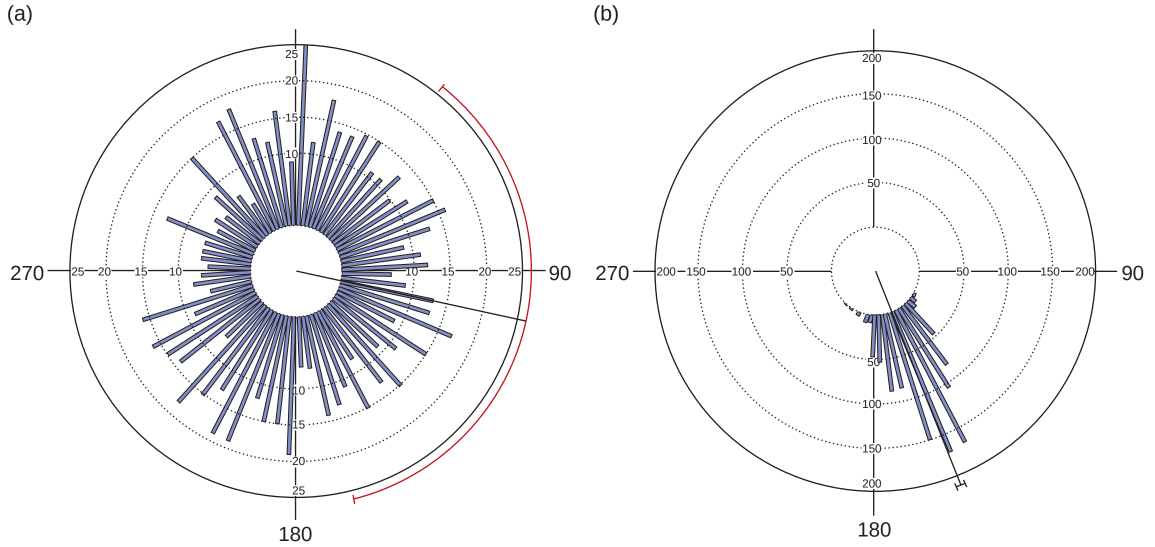


Figure 2 Polar diagrams of the phase relationship between heart beats and the ventilatory cycle in cod. The ventilatory cycle is depicted as a 360-degree circle with the occurrence of heart beats plotted as histograms around this circle. In normoxic fish (a) the heart beats occur at a range of phases but during hypoxia (b) they cluster in a specific phase. Redrawn from unpublished data of L. Jensen, R. Varne, I. Findorf, P. Domenici, DJ. MacKenzie and JF. Steffensen.

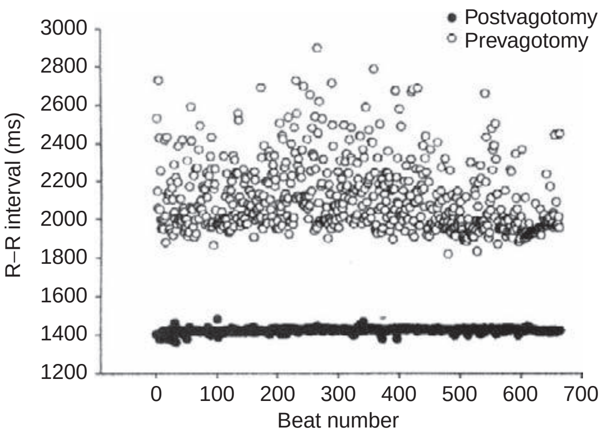


Figure 3 Sculpin, *M. scorpius*: the effect of vagotomy on heart rate variability: in the intact fish heart rate is highly variable. Vagotomy (sectioning of the vagus nerve on both sides of the fish) caused heart rate to rise (cardiac interval R–R was reduced) and to become unvarying, illustrating that the variability was generated via the vagus. From Campbell HA, Taylor EW, and Egginton S (2004) The use of power spectral analysis to determine cardio-respiratory control in the short-horned sculpin *Myoxocephalus scorpius*. *Journal of Experimental Biology* 207: 1969–1976.

Nervous System: Autonomic Nervous System of Fishes). In all fish except cyclostomes, the heart receives inhibitory innervation from the parasympathetic division via the vagus nerve. Removal of cardiac vagal tone in the sculpin (*Myoxocephalus scorpius*), by cutting the cardiac nerves, increases heart rate and decreases cardiac interval. In addition, heart rate is much less variable (see **Figure 3**). An important source of this heart rate variability is modulation by the respiratory rhythm. This respiratory modulation of heart beat is exerted predominantly by variations in inhibitory vagal tone imposed by activity in cardiac vagal preganglionic neurons (CVPNs) within the medulla oblongata. So efferent control via the vagus nerve both slows the heartbeat and couples it to the respiratory cycle. The neural basis of respiratory modulation of heartbeat seems to vary between the major groups of fishes so they are considered separately.

Elasmobranchs

The elasmobranch fishes (class Chondrichthyes) are likely the earliest vertebrates with clearly differentiated parasympathetic and sympathetic components of the autonomic nervous system. They are also the earliest group known to have an inhibitory vagal innervation of the heart (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). However, nervous cardioregulation in elasmobranchs solely depends on variations in the degree of cholinergic vagal tonus on the heart because the sympathetic nervous system does not extend forward into the head so that they have no sympathetic nerves supplying the heart and branchial circulation. Continuous stimulation of the vagus nerve, as well as application of its neurotransmitter acetylcholine, inhibits heart rate. As in other vertebrates, this effect is antagonized by atropine, implying that it is mediated by muscarinic cholinoceptors. A cardiac vagal tonus increases during hypoxia and slows heart rate, is abolished by hyperoxia resulting in an increased heart rate, and is augmented when temperature increases. Furthermore, the long periods of 1:1 synchrony between heartbeats and ventilation that are present in unrestrained dogfish (*Scyliorhinus canicula*) allowed to settle in normoxic seawater are abolished by injection of atropine (**Figure 1**(**b**)).

In the dogfish, the vagus nerve divides to form, at its proximal end, branchial branches 1, 2, 3, and 4, which contain skeletomotor fibers innervating the intrinsic respiratory muscles of gill arches 2, 3, 4, and 5, respectively (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish). The vagus also sends two branches to the heart, on each side of the fish, the branchial cardiac (BC) that arises from the post-trematic, fourth branchial branch of the vagus, and the visceral cardiac (VC) that arises close to the origin of the visceral branch of the vagus (see **Figure 4**). High levels of spontaneous efferent activity can be recorded from the central cut end of a BC branch of the vagus in decerebrate, paralysed dogfish (**Figure 5**). This activity can be attributed to two types of unit. Some units fired sporadically and increased their firing rate during hypoxia. Exposure to hypoxia caused a bradycardia that was abolished by atropine. Injection of capsaicin into the ventilatory stream of the dogfish was accompanied by a marked bradycardia. Both of these stimuli powerfully stimulated activity in the non-bursting units recorded from the central cut end of the BC. Consequently, it is activity in these units that likely causes reflex changes in heart rate, as well as playing a role in the determination of the overall level of vagal tone on the heart. Other, typically larger, units recorded from the BC fired in rhythmical bursts that were synchronous with ventilatory movements or with bursts of activity in respiratory branches innervating the gills, but were unaffected by hypoxia (**Figure 5**). Thus, it is hypothesised that these units showing respiration-related activity may serve to synchronise heartbeat with ventilation in dogfish. Injection of neural tracers located 90% of vagal preganglionic neurons (VPN) in the dorsal motor nucleus of the vagus (DVN) of the dogfish. About 8% of the total population of VPN were in a clearly distinguishable group of cells with scattered distributions outside of the DVN, identified as cardiac VPN (CVPN), innervating the heart via the BC nerve (see **Figure 4** and **Figure 6**(**a**)). They constitute about 45% of CVPN, with the rest located in the DVN. The ray, *Raja clavata*, has only one cardiac vagal branch on each side. This nerve is supplied by CVPN in the DVN and others in lateral locations outside the DVN that again constitute about 45% of total CVPN. This dual location of CVPN

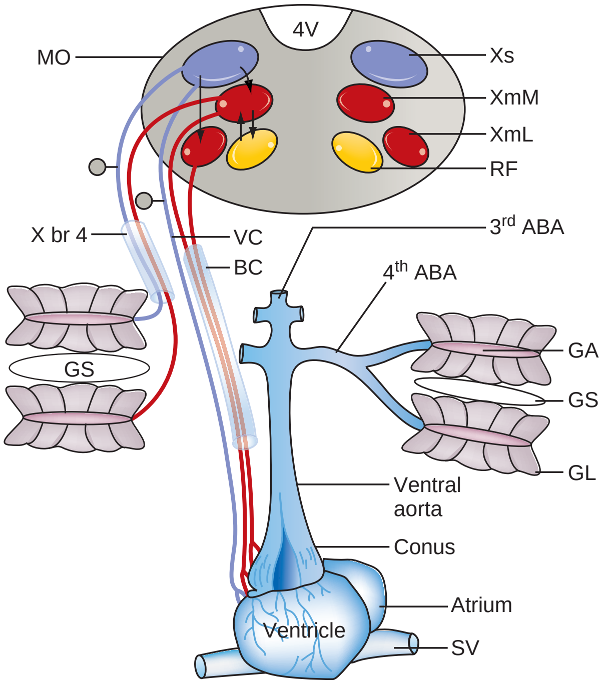


Figure 4 Schematic diagram of the central projections of cardiac and respiratory branches of the X cranial nerve, the vagus to illustrate the nervous control of CRIs in the dogfish. The branches of the vagus are: BC, branchial cardiac branch; VC, visceral cardiac branch; Xbr4, 4th branchial branch. The nuclei in the medulla oblongata (MO) integrating central control are: RF, reticular formation; XmL, lateral vagal motor nucleus; XmM, medial vagal motor nucleus; Xs, vagal sensory nucleus. ABA, afferent branchial artery; GA, gill arch; GL, gill lamellae; GS, gill slit; SV sinus venosus; 4V, 4th ventricle. See text for details of mechanisms.

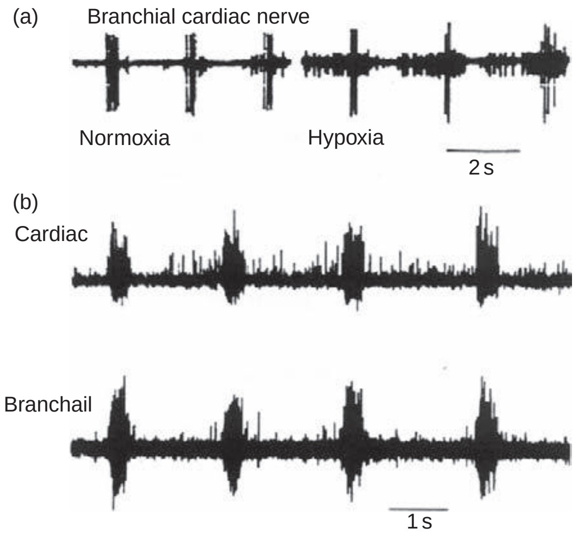


Figure 5 Dogfish: Efferent activity in cardiac and branchial vagi of decerebrate and paralyzed fish. (a) Recordings from individual nerve fibres in a cardiac branch before and after hypoxia (nonbursting activity seems to be responsible for reflex control of heart rate and generation of vagal tone, which increases during hypoxia). (b) Efferent activity recorded simultaneously from the cardiac branch and a branchial (respiratory) branch of the vagus to show sychronous bursts. From Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume 12, Part B*, pp. 343–387. New York: Academic Press.

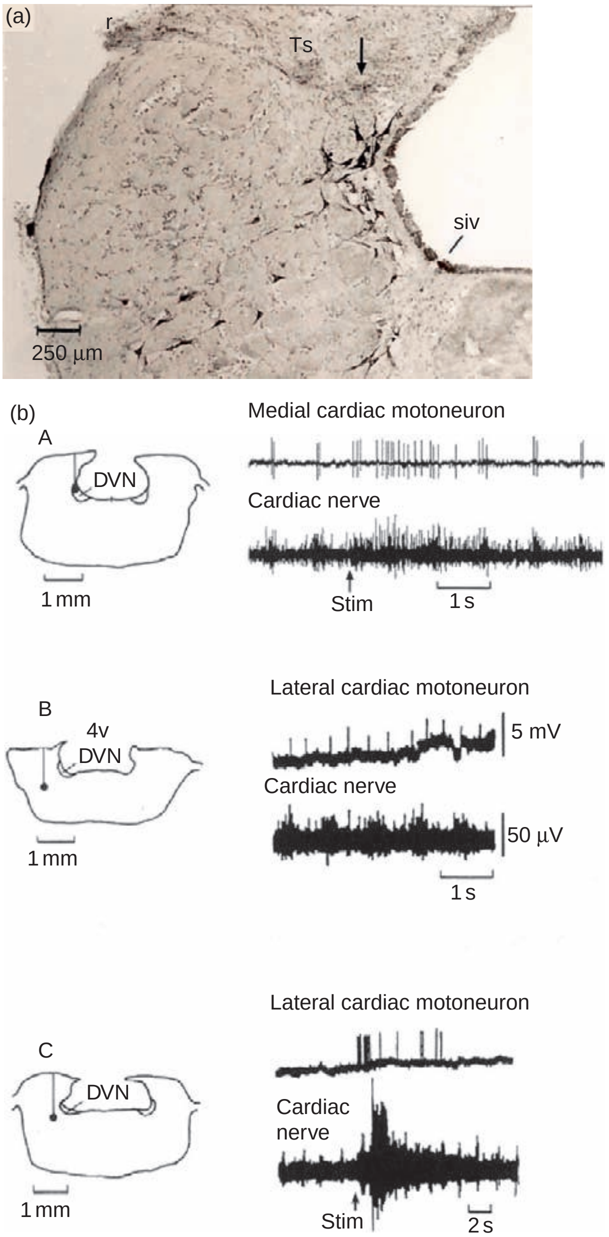


Figure 6 A neuranatomical study revealed two locations for CVPN in the brainstem: a medial group in the dorsal vagal motor nucleus and a scattered ventro-lateral group outside the DVN. (a) A transverse section taken through the brainstem of the dogfish, *S. canicula*, through the midpoint of XmM and XmL (see **Figure 4**) to show CVPN labeled black after application of horseradish peroxidase to the branchial cardiac nerve. They are located in the DVN close to the 4th ventricle (grouped directly below the vertical arrow) and in scattered locations ventro-lateral to the DVN. (r, rootlet of vagus nerve; siv, sulcus intermedius ventralis in the wall of the 4th ventricle; Ts, sensory projection from the cardiac vagus). (b) Dogfish: central recordings of activity in CVPN: extracellular recordings from CVPN in the brainstem of the dogfish, identified by antidromic stimulation of the cardiac nerve followed by dye injection (the course of the electrode and the recording position are shown by the line and filled circle); together with simultaneous recordings of activity in the ipselateral cardiac nerve. A unit located in the DVN (A) fired in bursts that contributed to the respiration-related activity recorded from the cardiac nerve and responded to mechanical stimulation of a contralateral gill septum (stim). Units recorded from lateral locations, outside the DVN, fired regularly (B), sporadically, or were silent (C), except when responding to mechanical stimulation of a gill septum (stim). Fish were decerebrated and paralyzed, with the gills irrigated by a continuous flow of aerated water, removing any phasic influences from peripheral receptors or centers higher in the CNS. From Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume 12*, *Part B*, pp. 343–387. New York: Academic Press.

has been shown to have important functional implications.

In the dogfish, the visceral cardiac branch (VC) has all of its CVPN in the DVN. Efferent activity recorded from the VC was less closely coupled with respiration and this nerve was less effective than the BC in slowing the heart when stimulated peripherally (i.e., toward the heart) with trains of electrical pulses. However, central stimulation of a VC (i.e., toward the brain) with a BC intact caused a major bradycardia, suggesting that the VC carries afferent nerve fibers that innervate the heart and that its primary role may be sensory (see **Figures 4** and **13**).

The separation of efferent cardiac vagal activity recorded from the BC into respiration-related and non-respiration-related units is based on the distribution of their neuron cell bodies in the brainstem, as described above. Extracellular recordings from CVPN identified in the hindbrain of decerebrate, paralyzed dogfish by antidromic electrical stimulation of a branchial cardiac branch, revealed that neurons located in the DVN were spontaneously active, firing in rhythmical bursts that contributed to the respiration-related bursts recorded from the intact nerve (see **Figure 6**(**b**)A). Direct connections between bursting CVPN and RVM are possible in the dogfish hindbrain, as both are located in the DVN with an overlapping rostro-caudal distribution (**Figure 4**; **see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish). As the bursts are synchronous, the innervation of CVPN is likely to be excitatory rather than inhibitory, as described for the mammal, and it is equally possible that a direct drive from a central pattern generator operates both on the RVM and the CVPN (see **Figures 4** and **13**, and figure 2 in **Control of Respiration**: Generation of the Respiratory Rhythm in Fish). Neurons located ventro-laterally outside of the DVN were either spontaneously active, firing regularly or sporadically but never rhythmically (**Figure 6**(**b**)B), or were silent (**Figure 6**(**b**)C). Thus, the two types of efferent activities recorded from the cardiac nerve arise from the separate groups of CVPN, as identified by neuranatomical studies. As activity was recorded from the central cut end of the cardiac vagus, or centrally from CVPN, in the decerebrated, paralyzed dogfish, provided with a constant, nonpulsatile supply of aerated water, the respiration-related activity recorded from CVPN in the DVN must have been generated by central interactions. However, all of the spontaneously active CVPN from both divisions and some of the silent CVPN fired in response to mechanical stimulation of a gill arch (**Figures 6**(**b**)A and C), indicating that stimulation of mechanoreceptors on the gills may recruit CVPN in normally breathing fish, possibly reinforcing central feed-forward control as described for control of ventilation (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish). Thus, in the intact fish, normal breathing movements that stimulate peripheral mechanoreceptors on the gills may, by a reflex pathway, generate activity in CVPN and consequently in the cardiac vagi, affecting heart rate. This implies that the typical reflex bradycardia in response to hypoxia may arise both directly, following stimulation of peripheral chemoreceptors and indirectly, via increased ventilation, which by stimulating branchial mechanoreceptors may increase vagal outflow to the heart. This is reminiscent of, but opposite in kind to, the hypoxic response in the mammal, where stimulation of lung stretch receptors causes an increase in heart rate.

Besides slowing the heart, respiration-related efferent activity in the cardiac vagi may entrain the heart. This possibility was confirmed by peripheral electrical stimulation of branchial cardiac nerves in the prepared dogfish. Although continuous vagal stimulation normally slowed the heart, it proved possible to drive the denervated heart at a rate either lower or somewhat higher than its intrinsic rate with brief bursts of stimuli, delivered down one BC. Interestingly, similar results were obtained from a mammal. In the anaesthetized dog, electrical stimulation of the vagus nerve toward the heart with brief bursts of electrical stimuli caused heart rate to synchronize with the stimulus over a wide range of frequencies.

Teleosts

In teleosts (class Teleostei), the vagus nerve is cardioinhibitory, again through muscarinic cholinoceptors on the heart. Variations in vagal tone affect heart rate and there is an inhibitory vagal tonus that varies with temperature in a variety of teleost species. In addition, the sympathetic chains extend into the head in teleosts, where they contact cranial nerves, forming a vagosympathetic trunk, and adrenergic fibers innervate the heart of some teleosts such as rainbow trout (*Oncorhynchus mykiss*). Thus, teleosts may be considered the earliest group of vertebrates with both sympathetic and parasympathetic control of the heart (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). Even so, work on a range of vertebrates, including mammals, has demonstrated that fine control of the heart likely needed to generate CRIs is predominantly parasympathetic. Accordingly, studies on the mechanisms of respiratory modulation of heart rate in teleosts have focused on cardiac control from the parasympathetic nervous system, via the X cranial nerve, the vagus, and its interactions with activity in the cranial nerves supplying the respiratory muscles, that are inserted around the orobranchial cavity (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish).

A distribution of VPNs and CVPNs similar to that described above for the dogfish is recognized for Atlantic cod and rainbow trout. About 12% of VPNs were located outside the DVNs and although most of these were CVPNs, some of them innervated the gills, possibly supplying vasomotor input to branchial blood vessels. In pacu, *Piaractus mesopotamicus*, CVPNs are distributed in separate nuclei within the DVNs, a ventral group containing about 60% of cell bodies and a dorsal group containing about 40% of cell bodies. In addition, a small number of cell bodies, scattered laterally outside of the DVN, constitute only about 2% of the total number of CVPNs. Therefore, the neuranatomical basis for control of the heart and CRI is inconsistent within the teleost fishes.

Work on teleosts has stressed the importance of peripheral receptor inputs in the genesis of CRS. In rainbow trout, *O. mykiss*, an exact synchrony can develop between ventilation and heart beat during progressive hypoxia. Normoxic heart rate is faster than ventilation and so the hypoxia-induced increase in ventilation rate and the reflex bradycardia converged to produce a 1:1 synchronization of the two rhythms. Both bradycardia and synchrony are abolished by atropine, showing that both are generated by efferent vagal control. In addition, hypoxic heart rate in the trout synchronized with an imposed phasic flow of water rather than the spontaneous breathing efforts of the intubated fish. Clearly, this synchronization was generated by reflex pathways, presumably arising from stimulation of mechanoreceptors on the gills.

Respiration-related bursting activity is absent from the cardiac nerves in resting normoxic pacu, *P. mesopotamicus*, and is only recorded from the cardiac vagus when fish hyperventilate or cough, implying that the bursts arise reflexly, following stimulation of branchial mechanoreceptors. Each burst is accompanied by an increased cardiac interval (**Figure 7**). Brief cessation of water flow to the gills of a lightly anaesthetized fish stimulated a bout of increased ventilatory effort, accompanied by bursts of activity in the cardiac vagus, with consequent transient bradycardia (**Figure 8**). In fish rendered moderately hypoxic by reduction of the flow of water irrigating the gills, a period of spontaneously increased ventilatory amplitude was accompanied by respiration-related bursts of activity in the cardiac vagus, which appeared to recruit the heart (**Figure 9**). As in the dogfish, phasic peripheral stimulation of the cardiac vagus (**Figure 10**) or central stimulation of respiratory branches of cranial nerves VII, IX, and X (**Figure 11**) can entrain the heart over a wide range of frequencies. Thus, increased ventilatory efforts generate bursting activity in the cardiac vagus, which can recruit the heartbeat.

Thus, we are left with apparently conflicting evidence on the mode of generation of CRS. In elasmobranchs, it may be primarily generated by central interactions in inactive, normoxic, or hyperoxic fish when cardiac vagal tone is low. In teleosts, it appears during hypoxia perhaps as a result of stimulation of branchial mechanoreceptors during forced ventilation and is generated reflexly by increased vagal tone. There is, however, some evidence that central interactions may generate some CRS in

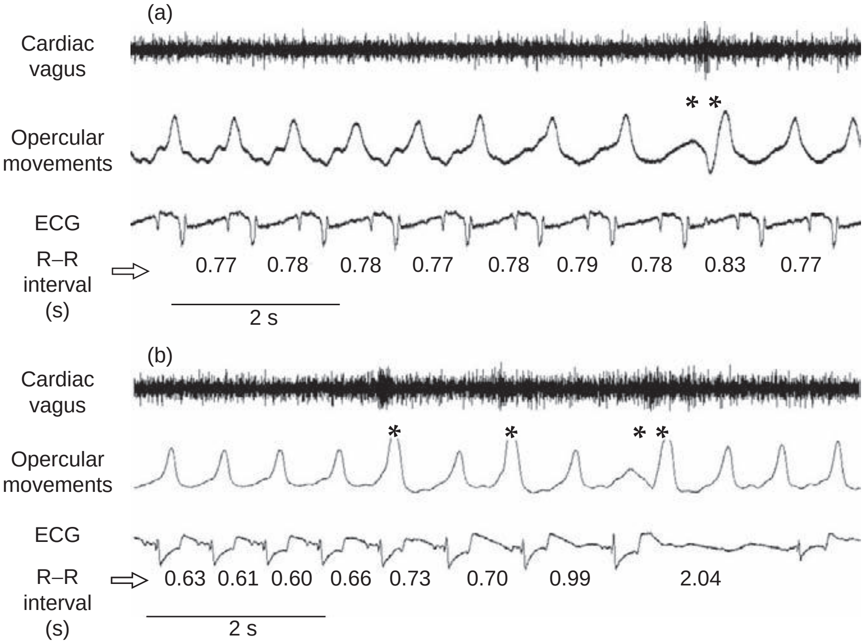


Figure 7 Pacu, *P. mesopotamicus* – recordings from the cardiac vagus, ventilation and ECG. There are no routinely respiration-related bursts in cardiac vagus in normoxic fish. * increased ventilatory effort; ** cough. From Leite CAC, Taylor EW, Guerra CDR, Florindo LH, Belão T, and Rantin FT (2009) The role of the vagus nerve in the generation of cardiorespiratory interactions in a Neotropical fish, the pacu, *Piaractus mesopotamicus Journal of Comparative Physiology A* 195: 721–731.

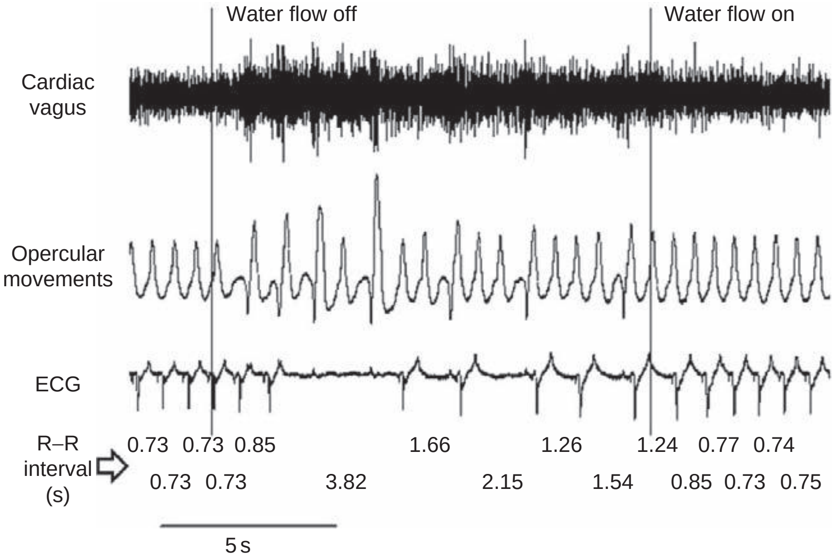


Figure 8 Pacu – effect of stopping the water flow irrigating the gills. Increased activity in cardiac vagus is associated with increased ventilatory effort and generates a bradycardia. From Leite CAC, Taylor EW, Guerra CDR, Florindo LH, Beläo T, and Rantin FT (2009) The role of the vagus nerve in the generation of cardiorespiratory interactions in a Neotropical fish, the pacu, *Piaractus mesopotamicus Journal of Comparative Physiology A* 195: 721–731.

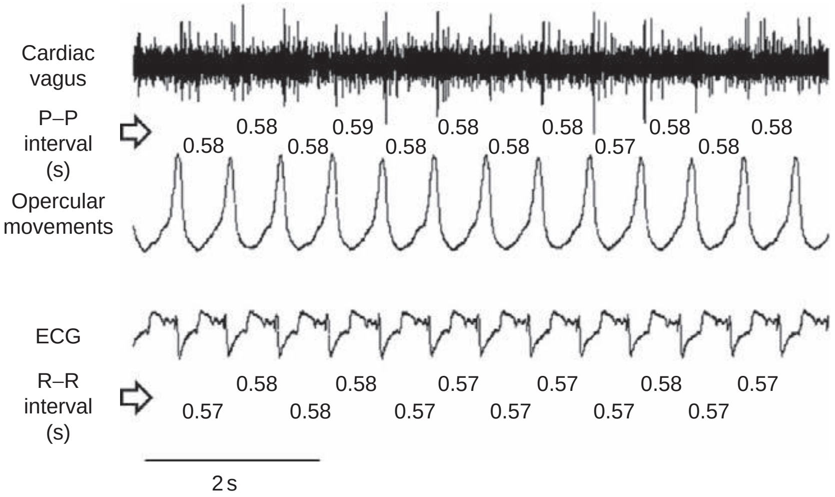


Figure 9 Pacu – restricting water flow increased ventilation amplitude, generated bursting activity in the cardiac vagus that recruited the heart. From Leite CAC, Taylor EW, Guerra CDR, Florindo LH, Beläo T, and Rantin FT (2009) The role of the vagus nerve in the generation of cardiorespiratory interactions in a Neotropical fish, the pacu, *Piaractus mesopotamicus Journal of Comparative Physiology A* 195: 721–731.

teleosts. Recordings of respiration-related bursting activity in the cardiac nerve of pacu were concurrent with bursts in the mandibular branch of the V cranial nerve and both anticipated activity in the respiratory branches of the VII, IX, and X cranial nerves (**Figure 12**(**a**)). This was also the case in recordings from dogfish (see figure 4 in **Control of Respiration**: Generation of the Respiratory Rhythm in Fish). In both elasmobranchs and teleosts, the distribution of motor neurons supplying the V nerve in the brainstem does not overlap with the distribution of CVPN or with neurons supplying the other respiratory nerves (see **Figure 12**(**b**)). In addition, central electrical stimulation of the mandibular branch of the V in pacu is without effect on heart rate, while central stimulation of respiratory branches of the VII, IX, and X cranial nerves either slows or recruits the heart (**Figure 11**). Accordingly, concurrence of bursting activity in the cardiac nerve with activity in the V cranial nerve is very likely to be generated centrally, possibly by inputs to both groups of neurons from the central respiratory pattern

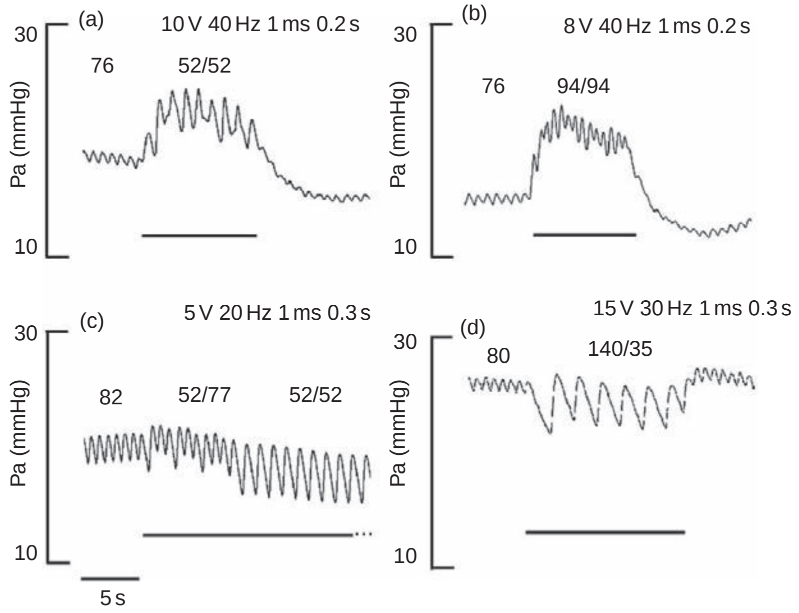


Figure 10 (a–d) Pacu – pulsed efferent stimulation of the cardiac vagus can drive the heart both slower (a, c) or faster (b) than its intrinsic rate. At high stimulation rates the heart was recruited at a simple fraction of the rate (e.g., 1:4) (d). Stimulation protocol is shown above each recording. From Taylor EW, Leite CAC, Florindo LH, Beläo T, and Rantin FT (2009) The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus. Journal of Experimental Biology* 212: 906–913.

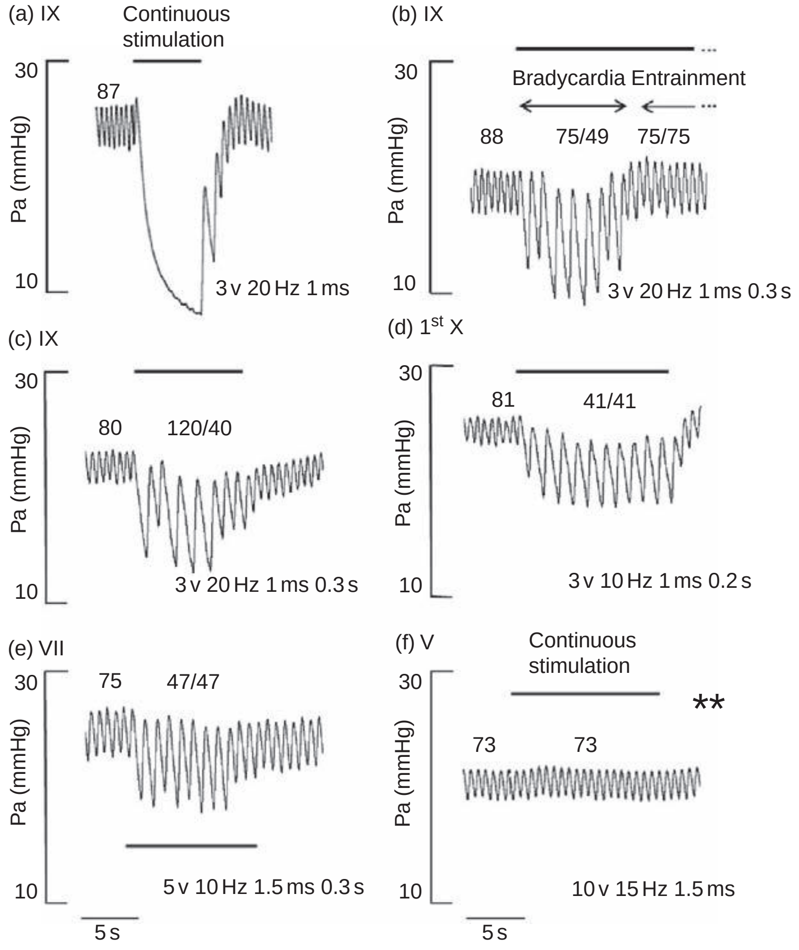


Figure 11 Pacu – effects on heart rate of central stimulation of cranial nerves IX, X, VII, and V supplying respiratory muscles. Continuous stimulation of IX caused cardiac arrest. Pulsed stimulation of VII, IX, and X recruited the heart but stimulation of V was without effect. Stimulation protocol is shown below each recording and the intrinsic and recruited rates are added above each trace. From Taylor EW, Leite CAC, Florindo LH, Beläo T, and Rantin FT (2009) The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus. Journal of Experimental Biology* 212: 906–913.

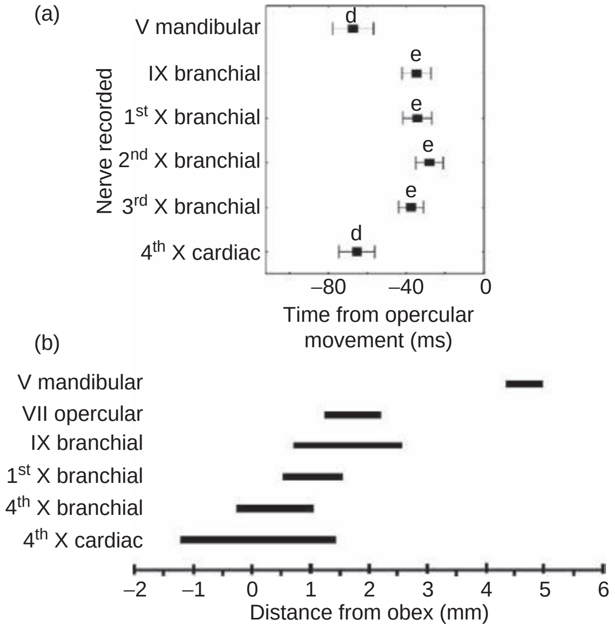


Figure 12 (a) Pacu – recordings of efferent activity in cardiac and respiratory nerves revealed that the bursts of activity in cardiac nerves are synchronous with activity in the V cranial nerve and both anticipate activity in the IX and X cranial nerves (modified from Taylor EW, Leite CAC, Florindo LH, Beläo T, and Rantin FT (2009) The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus. Journal of Experimental Biology* 212: 906–913.). (b) Pacu – rostro-caudal distribution of neurons supplying cranial nerves that innervate respiratory muscles and the CVPN (Xcardiac). All except those supplying the V cranial nerve have an overlapping distribution with CVPN. Modified from Taylor EW, Leite CAC, Florindo LH, Beläo T, and Rantin FT (2009) The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus. Journal of Experimental Biology* 212: 906–913.

generator when respiratory drive is high, as in hypoxia. This possibility is represented in **Figure13** and merits further investigation in dogfish.

Air-Breathing Fish

Many of the primitive ray-finned fishes are air breathers and air breathing also seems to have evolved numerous times within teleosts. The anatomy and physiology of the air-breathing organs vary enormously among species, but its characteristic is that heart rate increases during air breathing.

Changes in heart rate during ventilation are evident in the jeju, *Hoplerythrinus unitaeniatus*, a teleost fish that uses a modified swimbladder as an air-breathing organ. Heart rate decreases during expiration, but increases drastically during the subsequent inhalation that inflates the swimbladder. These heart rate changes are primarily of cholinergic origin, and an in-depth analysis of heart rate variability in this species indicates that the heart rate changes are qualitatively similar to RSA in higher vertebrates. An example of the rapid change in heart rate upon an air breath in *Synbranchus marmoratus* is shown in **Figure 14**. In lungfish (Dipnoi), a vagal innervation of the heart is well established, while the sympathetic chain seems poorly developed. Heart rate does not change much with air breathing but pulmonary ventilation is associated with a marked rise in pulmonary blood flow in all species of lungfish. Control of pulmonary blood flow involves branchial shunts which are neurally regulated by cholinergic vasoconstriction of the pulmonary artery. Lungfish are endowed with pulmonary stretch receptors as well as both central and peripheral chemoreceptors, making it likely that feedback from these receptors is involved. In all of these respects lungfish anticipate the mechanisms described in amphibians and reptiles.

The Neural Basis of CRIs

In mammals heart rate increases during inspiration, a phenomenon known as respiratory sinus arrhythmia (RSA). It is driven by respiration-related fluctuations in the efferent, inhibitory supply to the heart via the cardiac vagus. This is generated centrally by an inhibitory input to CVPNs in the ventro-lateral nucleus ambiguus (NA) from inspiratory neurons in the neighboring ventral respiratory group. The consequent gating of vagal outflow causes heart rate to rise during inspiration. Its functional role in improving pulmonary oxygen uptake, particularly in athletes, has recently been discussed. This subtle relationship between heart beat and ventilation is not restricted to mammals as a similar relationship has been identified by power spectral analysis in rattlesnakes, *Crotalus durrisus*. As detailed above, beat-to-beat control of the heart, necessary to generate CRS, has been reported in both resting dogfish and Atlantic cod as well as in hypoxic trout, species that have CVPNs located both in the DVNs and in a ventro-lateral location outside the DVNs that may constitute a primitive NA. However, in fish the central interactions are between CVPNs and respiratory neurons in the DVNs that drive the CVPNs rather than inhibiting them. The evolution of RSA from CRI in fish, via amphibian and reptilian ancestors, is a subject of immediate interest.

Finally, we are left with an apparent paradox: if the vagus, which is normally inhibitory, delivers pulses of activity to the heart that can override the cardiac pacemaker, how can pulsatile activity mechanistically recruit the heart at rates similar to or even above its intrinsic rate? By its nature, this is likely to be a direct induction of the heart beat rather than an effect on cardiac intervals. When bursts of electrical stimuli were delivered peripherally down the cardiac vagus of the dogfish at 43 bursts min^{-1},

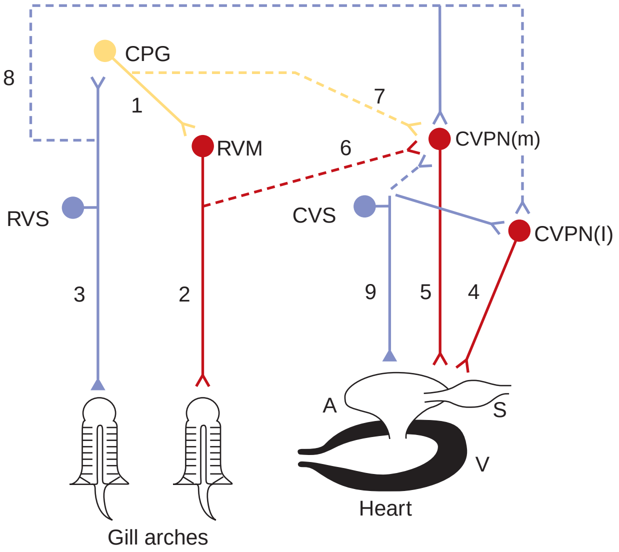


Figure 13 A schematic model of possible connections in the central and peripheral nervous systems generating CRIs in fish. There are several established connections in the nervous control of ventilation: (1) the respiratory central pattern generator neurons (CPG) show endogenous bursting activity that drives respiratory motoneurons (RVM); (2) the RVMs innervate respiratory muscles via the V, VII, IX, and X cranial nerves; (3) the activity of the CPG is modulated by feedback from mechanoreceptors and chemoreceptors located in the orobranchial cavity, innervated by sensory neurons (RVS). Heart rate is controlled by inhibitory input from the vagus nerve that receives axons from CVPNs, which are topographically and functionally separable into: (4) a ventrolateral group (CVPN (l)), some of which fire continuously and may be responsible for reflex changes in heart rate (e.g., hypoxic bradycardia) and for the varying level of vagal tone on the heart, and (5) a medial group (CVPN (m)), which burst rhythmically and may cause the heart to beat in phase with ventilation. Other more speculative connections may determine the activity in CVPN: (6) collateral's from neighboring RVM may have an excitatory effect on bursting CVPN (m) (or release a tonic inhibition); (7) the CPG may connect directly to CVPN (m); (8) stimulation of receptors in the orobranchial cavity, innervated by the, VII, IX, and X cranial nerves but apparently not by the V may directly modify activity in CVPN; (9) stimulation of receptors in the cardiovascular system close to the heart, innervated by vagal sensory neurons (CVS) may affect vagal outflow to the heart. In the dogfish these neurons may project via the visceral cardiac branch of the vagus. This diagram ignores the existence and possible roles of interneurons and inputs from and to higher centres in the CNS. Λ, efferent termination; ▲ afferent termination; S, sinus venosus; A, atrium; V, ventricle. CPG, central pattern generator; CVPN, cardiac vagal motor neuron; CVS, cardiac sensory neuron; RVM, respiratory motor neuron; RVS, respiratory sensory neuron. Modified from Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume 12*, *Part B*, pp. 343–387. New York: Academic Press.

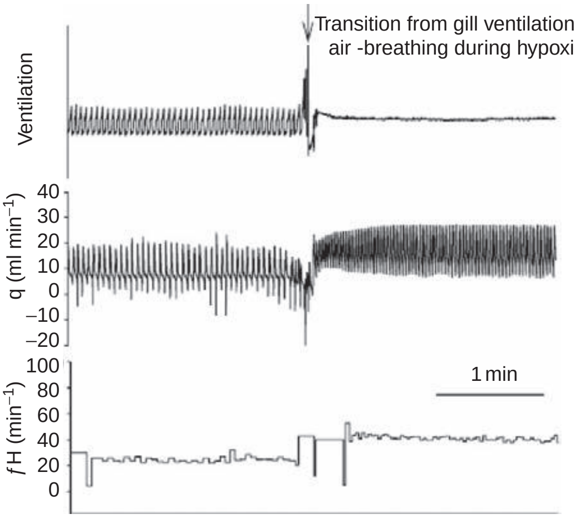


Figure 14 Changes in the blood flow (Q) and heart rate (f_H) during transition from gill ventilation to air breathing in S. *marmoratus* in hypoxic water (P_{O_2} 50mmHg). Gill ventilation in water ceases following the air breath (indicated by the arrow). Heart rate increased 5-fold immediately after the air-breath. Modified from Skals M, Skovgaard N, Taylor EW, *et al.* (2006) Cardiovascular changes under normoxic and hypoxic conditions in the air-breathing teleost *Synbranchus marmoratus:* Importance of the venous system. *Journal of Experimental Biology* 209: 4167-4173.

the heart slowed but to exactly half the bursting rate, implying that it was induced to beat by alternate bursts. Similar data exist for pacu (see **Figure 10**(**d**)). This question has long intrigued mammalian physiologists as well. In mammals, the cardiac vagi show bursting activity of variable frequencies and the heart can be entrained by bursts of electrical activity delivered down the cardiac vagi over a range of stimulation frequencies both lower and higher than the intrinsic heart rate. A relationship between the phase of the cardiac cycle that brief stimulation of the cardiac vagus is delivered and its chronotropic effect has been established in mammals, with the effect of each burst dependent on the phase of the cardiac cycle at which it is applied. Thus, the vagal effect on the heart cannot be measured merely in terms of the amount of acetylcholine delivered per unit time. Detailed analysis of the relationship between pulsatile activity in the cardiac vagus and phases of the cardiac cycle is not yet available for fish.

Summary

Beat-to-beat modulation of heart beat by the respiratory rhythm may be ubiquitous in vertebrates. Studies on fish and mammals and, to a lesser extent, on reptiles and amphibians imply that heart rate and heart rate variability in vertebrates are determined by phasic activity in CVPN. Spontaneous efferent activity in cardiac vagi contains bursts of respiration-related activity. In elasmobranchs this activity seems to originate primarily from central interactions between respiratory neurons and CVPN, and is characterized by relatively low levels of vagal tone on the heart. In teleosts, there may be a fundamental difference in that the bursts seem to be driven reflexly by stimulation of peripheral chemoreceptors and mechanoreceptors when respiratory drive and cardiac vagal tone are high. Ultimately, reflex control from peripheral receptors is important in determining heart rate in all fish and evidence from current investigations suggests that there are elements of central feed-forward control of CRIs in some teleosts. Consequently, it seems probable that variable combinations, relating to conditions and possibly to species differences, of feed-forward control via central interactions plus feedback control from peripheral receptors determine activity in CVPN in fish. This in turn can recruit the heart to the respiratory rhythm, though it may subserve different roles in different groups of fish.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish. **Gas Exchange**: Respiration: An Introduction. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Energetics and Development. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs.

Further Reading

Barrett DJ and Taylor EW (1985) Spontaneous efferent activity in branches of the vagus nerve controlling heart rate and ventilation in the dogfish. *Journal of Experimental Biology* 117: 433–448.

Barrett DJ and Taylor EW (1985) The characteristics of cardiac vagal preganglionic motoneurones in the dogfish. *Journal of Experimental Biology* 117: 459–470.

Barrett DJ and Taylor EW (1985) The location of cardiac vagal preganglionic neurones in the brainstem of the dogfish. *Journal of Experimental Biology* 117: 449–458.

Campbell HA, Taylor EW, and Egginton S (2004) The use of power spectral analysis to determine cardio-respiratory control in the short-horned sculpin Myoxocephalus scorpius. *Journal of Experimental Biology* 207: 1969–1976.

Grossman P and Taylor EW (2007) Toward a proper understanding of respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology* 74: 263–285.

Leite CAC, Taylor EW, Guerra CDR, *et al.* (2009) The role of the vagus nerve in the generation of cardiorespiratory interactions in a Neotropical fish, the pacu, *Piaractus mesopotamicus*. *Journal of Comparative Physiology A* 195: 721–731.

McKenzie DJ, Campbell HA, Taylor EW, *et al.* (2007) The autonomic control and functional significance of the changes in heart rate associated with air breathing in the jeju *Hoplerythrinus unitaeniatus*. *Journal of Experimental Biology* 210: 4224–4232.

Randall DJ (1966) The nervous control of cardiac activity in the tench (*Tinca tinca*) and the goldfish (*Carassius auratus*). *Physiological Zoology* 39: 185–192.

Randall DJ and Smith JC (1967) The regulation of cardiac activity in fish in a hypoxic environment. *Physiological Zoology* 40: 104–113.

Satchell GH (1960) The reflex co-ordination of the heart beat with respiration in dogfish. *Journal of Experimental Biology* 37: 719–731.

Short S, Butler PJ, and Taylor EW (1977) The relative importance of nervous, humoral and intrinsic mechanisms in the regulation of heart rate and stroke volume in the dogfish, *Scyliorhinus canicula. Journal of Experimental Biology* 70: 77–92.

Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume 12, Part B*, pp. 343–387. New York: Academic Press.

Taylor EW (1993) The neuranatomy of central cardiorespiratory control in vertebrates. *Functionsanalyse Biologischer Systeme* 23: 149–158.

Taylor EW and Butler PJ (1982) Nervous control of heart rate: Activity in the cardiac vagus of the dogfish. *Journal of Applied Physiology* 53: 1330–1335.

Taylor EW, Campbell HA, Levings JJ, *et al.* (2006) Coupling of the respiratory rhythm in fish with activity in hypobranchial nerves and with heart beat. *Physiological and Biochemical Zoology* 79: 1000–1009.

Taylor EW, Jordan D, and Coote JH (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiological Reviews* 79: 855–916.

Taylor EW, Leite CAC, Florindo LH, Beläo T, and Rantin FT (2009) The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus*. *Journal of Experimental Biology* 212: 906–913.

Taylor EW, Leite CAC, and Levings JJ (2009) Central control of cardiorespiratory interactions in fish. *Acta Histochemica* 111: 257–267.

Taylor EW, Short S, and Butler PJ (1977) The role of the cardiac vagus in the response of the dogfish, *Scyliorhinus canicula* to hypoxia. *Journal of Experimental Biology* 70: 57–75.

Young JZ (1958) *The Life of Vertebrates*. Oxford: ClarendonPress.

Hormone Metabolism in the Circulation

KR Olson, Indiana University School of Medicine – South Bend, South Bend, IN, USA

© 2011 Elsevier Inc. All rights reserved.

Hormones and the Endocrine System
Types of Hormones
Mechanisms of Hormone Inactivation in Mammals
Mechanisms of Hormone Inactivation in Fish
Methods for Measuring Hormone Inactivation
Further Reading

Glossary

Autocrine A signaling mechanism in which the signaling molecule stimulates the cell from which it was released.

Cardiac output (V_b) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Circulation time The time it takes for blood to make one complete loop around the cardiovascular system.

Endocrine system The signaling system in which the signaling molecule (hormone) is conveyed from the site of release to the target cells by way of the bloodstream.

Endothelial cells Thin cells that line the blood vessels and form a barrier between blood and tissue cells.

Half-time The time it takes for a parameter to decrease by one half ($t_{1/2}$).

Hormones Signaling molecules released by a cell that stimulate cell of origin (autocrine), nearby cells (paracrine), or travel via the bloodstream to distant cells (endocrine).

Hydrophilic hormone Hormones that are more soluble in water than in lipid. These include epinephrine, norepinephrine, serotonin, histamine, and numerous peptides. They typically bind to surface receptors on the cell membrane and activate second messenger systems within the cell.

Lipophilic hormone Hormones that are more soluble in lipid than in water. These include estrogen, progesterone, testosterone, aldosterone, cortisol, thyroid hormones, prostaglandins, and vitamin D. They typically bind to intracellular receptors and act within the cell nucleus.

Neurotransmitter The signaling molecules released by nerves. This is a specialized form of paracrine signaling.

Paracrine A signaling mechanism in which a signaling molecule is conveyed to nearby cells via the interstitial fluid bathing the cells.

Peptides Molecules made by joining from two to as many as several hundred amino acid molecules.

Perfusate A blood substitute that does not contain red blood cells and is often used in isolated organ studies.

Pillar cells Post-like cells in fish gill respiratory lamellae that enclose the blood space in the gas-exchange tissue and prevent the lamellar structure from ballooning out under blood pressure.

Rate constant A unitless number that is equivalent to the fractional decrease of some variable as a function of time. The rate constant (k) is related to the half-time ($t_{1/2}$) by $k=0.693/t_{1/2}$.

Receptor-mediated endocytosis A process in which a molecule binds to a receptor on the cell membrane and then the molecule–receptor complex is internalized.

Hormones and the Endocrine System

The endocrine system and nervous system are the two means of communicating signals between different parts of the body. The endocrine system transmits its signals as chemicals called ‘hormones’ that are carried in the blood. As a result, the endocrine system has the advantage of transmitting its signals to every organ in the body, since all organs receive blood flow. This broad communication between different organs typically takes minutes to hours, possibly days, to reach a maximum response and the system can take equally long to turn off.

The intensity of an endocrine signal is set, in large part, by the concentration of the hormone in the blood, the amount of blood flow directed to an organ, the number of receptors, and the affinity of the receptors for the hormone. Hormone concentrations can be reduced by decreasing their release and through inactivation sites.

In contrast, the nervous system transmits signals within the axon of a nerve cell to very specific areas by way of nerve action potentials, and this action potential is communicated to other cells by means of neurotransmitter chemicals. The nervous system rapidly (<1 s) transmits signals from one area of the body to the next and the signals can be quickly turned on and off. The intensity of the neural signal is encoded in the frequency and pattern of action potentials, and the nature of the neurotransmitter. In some instances, such as the hypothalamus and pituitary, the two systems are combined into a neuroendocrine system. Here, the nerve action potential releases its neurotransmitter hormone directly into the circulation.

Hormones are broadly defined as signal molecules that are secreted by a cell to either communicate directly with that cell (autocrine transmission), communicate with neighboring cells (paracrine transmission), or communicate with distant cells via the bloodstream (endocrine transmission). This article focuses on how endocrine signals are modulated by the removal of hormones from the blood by the cardiovascular system. Therefore, the term hormone is restricted here to the context of endocrine signaling. In this context, hormones are typically classified as circulating or noncirculating. Circulating hormones may make several or many transits around the cardiovascular system because they remain in the circulation longer than the typical circulation time (1–2 min). Noncirculating hormones, however, are rapidly removed from the blood and therefore typically stimulate target tissues immediately downstream from their point of release.

Types of Hormones

The mechanisms of hormonal removal from the blood relate to the chemical characteristics of the hormone, which fall into several categories. Hydrophilic hormones, namely amines and peptides, are water soluble. They are typically synthesized and stored prior to secretion and when secreted they dissolve in the plasma and are transported in the blood without the need for a specific carrier molecule. The amines include: (1) the catecholamines; dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline); (2) the indolamine, serotonin (5-hydroxytryptophan; 5-HT); and (3) histamine. Peptides range from the short, three-amino-acid peptide, thyrotropin-releasing hormone (TRH), to proteins of nearly 200 amino acids, such as growth hormone and prolactin.

The lipophilic hormones include the steroid derivatives of cholesterol (such as sex hormones and adrenal cortical hormones), derivatives of arachidonic acid (such as prostaglandins, leukotrienes, thromboxanes, and prostacyclin), and the thyroid hormones. The steroid derivatives and thyroid hormones are transported in the plasma bound to a carrier molecule because of their very low solubility in plasma. These hormone-specific carrier molecules greatly increase the concentration of hormone that can be transported in blood and also prolong the existence (measured as half-time; $t_{1/2}$) of the hormone in the circulation. Lipophilic hormones, with the exception of thyroid hormones, are generally synthesized immediately prior to secretion.

Plasma hormone concentrations are tightly regulated since their effect is directly related to their concentration. A plasma hormone concentration at any point in time represents a balance between the rates of hormone secretion and inactivation. With a few exceptions, the rate of secretion appears to be the active point of regulation that increases or decreases against a background of constant rate of removal via inactivation. This is analogous to filling a bucket that has a small hole in the bottom; at any point in time, the amount of water in the bucket is controlled by opening and closing the tap filling the bucket. In spite of that, the successful operation of the endocrine system depends upon effectively removing hormones from the circulation because once secretion ceases, $t_{1/2}$ is determined entirely by the rate of inactivation. This is especially true for the noncirculating hormones.

Mechanisms of Hormone Inactivation in Mammals

Most of what is known about hormone inactivation was initially derived from mammalian studies. Several inactivation mechanisms exist and may, or may not be, hormone specific, as summarized in the following paragraphs.

Dilution

If a hormone is released in small, discrete quantities, its concentration will stimulate tissues immediately downstream from the site of release, but the hormone will be progressively diluted due to mixing in the plasma to a point in time when it will no longer be effective distally. Dilution would be greater with secretion into the venous system. Thus, the rate of inactivation is dependent upon tissue blood flow and volume.

Metabolic Inactivation in the Plasma

Enzymes that inactivate hormones can circulate in the plasma or project from the surface of endothelial cells into the plasma. Many peptide hormones are hydrolyzed by a variety of plasma and endothelial proteases that may or may not be peptide specific.

Cellular Uptake from the Plasma and Intracellular Degradation

Uptake may be nonspecific, or may employ selective transcellular transporters or receptors that bind and internalize the hormones. Uptake of lipophilic hormones is generally nonspecific as they are readily dissolved in the plasma membrane, and so there is no need for a special process for cellular uptake (they follow their concentration gradient) prior to intracellular degradation. Amines are typically removed from the circulation by active transport into cells where they are enzymatically degraded. An amine transport mechanism that is relatively nonspecific for different amines, commonly called 'extra-neuronal uptake', or 'uptake-2' accounts for most catecholamine removal from the circulation and may transport histamine and serotonin as well. Receptor-mediated uptake (receptor-mediated endocytosis) occurs through the action of specific hormone receptors, such as the natriuretic peptide receptor-C (NPR-C), which binds a variety of natriuretic peptides and internalizes them for subsequent degradation.

The endothelium, which forms the lining of all vertebrate blood vessels, including those of fish, is a key site of intracellular hormone activation in mammals. The anatomical appearance of endothelial cells as relatively benign, thin barriers lining blood vessels is quite misleading. *In toto*, their mass in mammals is equivalent to that of the liver, but their metabolic potential is enhanced by a much greater surface area.

The location of inactivation sites also critically influences plasma hormone levels. The mammalian pulmonary circulation is in an ideal position to metabolically 'condition' the blood before it is delivered to the systemic circulation as it is the only vascular bed that receives the entire cardiac output in a single pass. Endothelial enzymes projecting into the plasma from the lung endothelial circulation can convert hormones to an active form (angiotensin converting enzyme, ACE, aka kininase I activates angiotensin) and inactivate hormones (kininase II inactivates bradykinin).

Mechanisms of Hormone Inactivation in Fish

Information about hormone inactivation in fishes is in its infancy. The surface area and mass of fish endothelial cells is unknown but probably comparable to that in mammals. The fish gill is the homolog of the mammalian lung and, like its counterpart, has an ideal location for hormone inactivation. The surface area of gill pillar cells, which are functionally homologous to endothelial cells, is also considerable. It is not surprising, then, that we know more about hormone removal and metabolism in the gill than in any other tissue.

Significance of the Gill in Hormone Inactivation

The respiratory surface of the fish gill is greatly elaborated in the form of respiratory (secondary) lamellae to enhance the gas-exchange surface; typically, the lamellar surface area is greater than the total non-gill body surface area. As shown in **Figure 1**, the surface of the pillar cells lining the blood channels even exceeds the respiratory surface area, perhaps by nearly 50%. This substantial surface area provides ample opportunity for contact with blood-borne molecules and subsequent metabolism.

The gill has numerous enzymes and uptake mechanisms to clear hormones from circulation. Many of these are located on the pillar cells (**Figure 1**). ACE is especially concentrated in gill pillar cells and approximately 65% of angiotensin I (ANG I) in the blood is converted to angiotensin II (ANG II) by ACE in a single transit through the gill circulation. Atrial natriuretic peptide clearance receptors (NPR-C) are also prevalent in the lamellae, removing up to 60% of the circulating natriuretic peptide in a single pass through the gill (**Figure 2**). Inhibition of clearance receptors effectively blocks natriuretic peptide uptake from the plasma and produces a drop in blood pressure due to the increased concentration of the vasodilator peptide in the circulation. This suggests that there is a tonic release of natriuretic peptides and because the major site of natriuretic peptide secretion is the heart, their rapid removal by the gill, which is immediately downstream from the heart, is an important filtering process to prevent the peptide from entering the systemic circulation and producing hypotension.

Gill pillar cells also actively accumulate both epinephrine and norepinephrine by way of specific catecholamine transport through mechanisms similar to extra-neuronal uptake. The majority of the catecholamines taken up by the gill are metabolized by intracellular enzymes monoamine oxidase and catechol-*O*-methyl transferase. However, some catecholamines may also be released back into the circulation intact. The gills also show selectivity for the type of amine removed: epinephrine extraction is only 5%, norepinephrine extraction is 16%, and serotonin extraction is over 80%. Thus, while the gills are effective in clearing serotonin from the circulation, they allow epinephrine to circulate almost unimpeded after release from the chromaffin cells into venous blood near the heart. Gills are similarly selective in removing arachidonic acid (essentially 100% extraction) but only remove a small percent of the prostacyclin (15%) and hardly any (<5%) prostaglandin E_2. Thus, in fish with 50–100% gill removal rates on a single pass, the classification of a hormone as circulating or noncirculating depends to a large extent on its ability to traverse the gill microcirculation.

The enzyme cytochrome P450IA1 is also especially prevalent in pillar cells. Enzymes such as this are well known for their detoxification properties. However, members of this family can also metabolize arachidonic

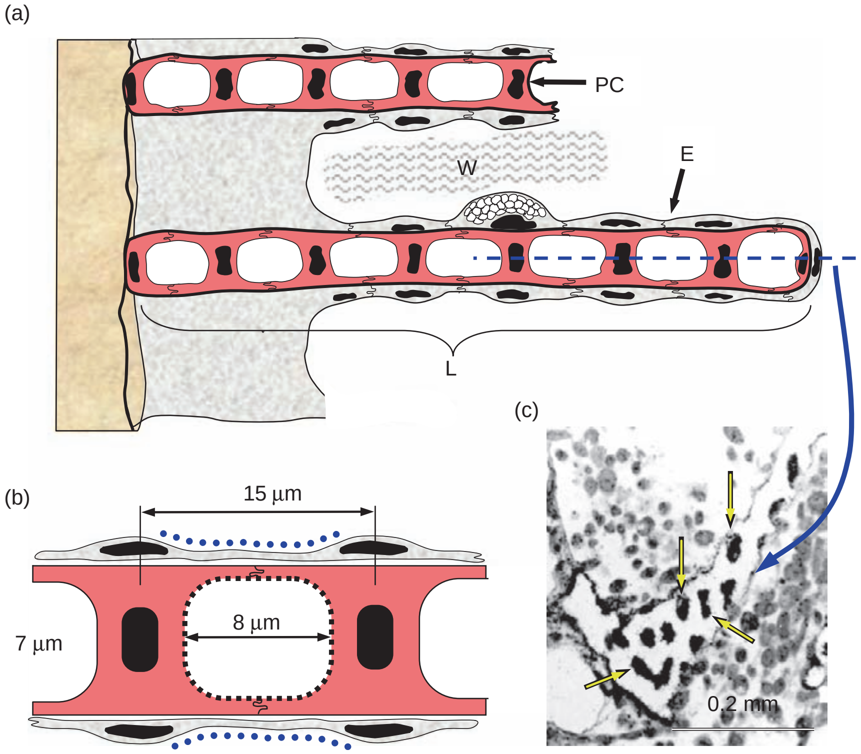


Figure 1 The importance of gill pillar cells in hormone metabolism. (a, b) Cross section through the gill respiratory lamellae (L) showing the arrangement of gill pillar cells (PC, red), epithelium (E), and environmental water (W). The surface area of pillar cells lining the blood space (dashed black line in (b)) is considerably greater than the respiratory surface area of the epithelium (dotted blue lines in (b)). (c) Autoradiograph showing ^{3}H-norepinephrine binding to pillar cells (yellow arrows) in a tangential section through a lamellae, thereby creating a cross section through the pillar cells (blue dashed line in (a) and arrow). (a, b) Modified from Olson KR (1998) Hormone metabolism by the fish gill. *Comparative Biochemistry and Physiology* 119A: 55–65. (c) Modified from Olson (2002) Gill circulation: regulation of perfusion distribution and metabolism of regulatory molecules. *Journal of Experimental Zoology* 293: 320–335.

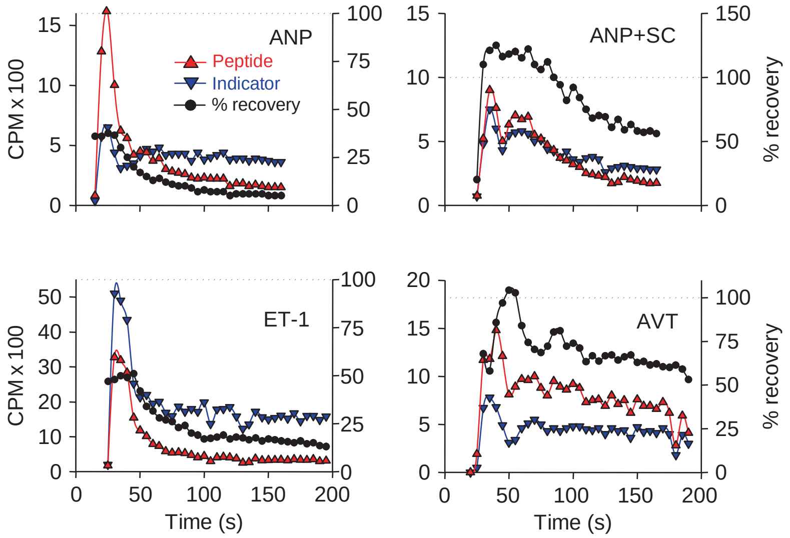


Figure 2 Representative traces showing single-pass recovery (black circles) of radiolabeled peptides (red triangles) and inert volume indicator (blue inverted triangles) from the dorsal aorta of unanesthetized rainbow trout (*Oncorhynchus mykiss*). Indicators were injected into the ventral aorta as a single bolus and 7-μl samples were collected every 5 s from the dorsal aorta. Left ordinate shows radioactivity in counts per minute (CPM); right ordinate is percent recovery of hormone. The spike associated with the initial five to seven samples represents the first pass of radionuclides through the gill. Subsequent radioactivity is due to recirculation and the percent recovery reflects total body extraction. ANP and ET-1 are efficiently removed by the gills in a single pass, whereas inhibiting the clearance receptor greatly inhibited ANP removal and there is little evidence for gill removal of AVT. ANP, atrial natriuretic peptide; ANP+SC, atrial natriuretic peptide plus clearance receptor inhibitor, SC 46542; ET-1, endothelin-1; AVT arginine vasotocin. Modified from Olson KR (1998) Hormone metabolism by the fish gill. *Comparative Biochemistry and Physiology* 119A: 55–65.

acid to form signaling molecules, such as epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). Whether or not these are produced by the gill remains to be determined.

Systemic (Non-Gill) Hormone Inactivation

Comparatively little is known of the metabolic capacity of systemic (non-gill) vasculature, although it undoubtedly contributes to hormone metabolism as well. The relatively low epinephrine extraction by the gill (5%; **Table 2**) would result in an *in vivo* $t_{1/2}$ of over 13 min (**Figure 3**(**b**)) if the gill was the sole site of epinephrine removal. Clearly, this cannot be the case as both the $t_{1/2}$ for physiological inactivation of epinephrine infusion and clearance of radioactive epinephrine (^{3}H-epinephrine) *in vivo* are <5 min (**Table 1**). In fact, this rapid clearance rate indicates that there are multiple systemic inactivation sites for catecholamines. **Figure 2** provides additional evidence for systemic inactivation. In these single-pass experiments, the initial five to seven samples represent the first-pass of radiolabeled peptides across the gill. The appearance of radioactive molecules in later samples results from recirculation through the vasculature and return to the gills. It is evident that even molecules such as arginine vasotocin (AVT), which are not readily extracted by the gill, are still nearly 50% cleared from circulation within 3 min. This could only be accomplished if tissues other than the gills were also actively involved in removing AVT from circulation. ACE also is especially prevalent in systemic tissues. ANG I is completely converted to ANG II in a single transit through many systemic vascular beds.

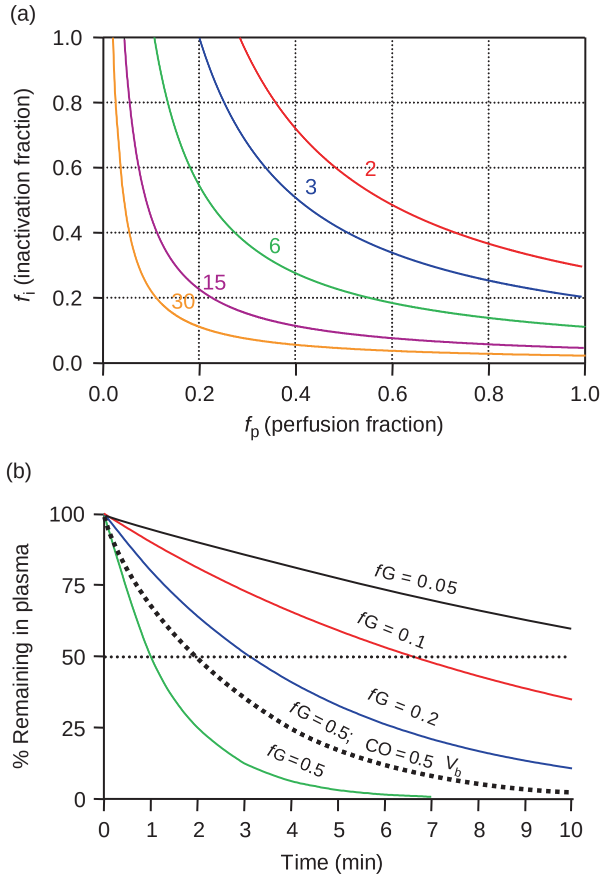


Figure 3 Mathematical expression of hormone inactivation. (a) Relationship between inactivation fraction (f_i) of a tissue and the fraction of cardiac output perfusing that tissue (f_p) for hormones with different $t_{1/2}$ (2, 3, 6, 15, and 30 min). As an example, for a molecule to have a 6-min $t_{1/2}$ in the circulation, gill inactivation fraction would have to be 0.11 (gill f_i is always 1.0), whereas a tissue that receives 20% of the cardiac output ($f_p = 0.2$) would have to have a single-pass inactivation of 45% ($f_i = 0.45$) to produce the same 6-min $t_{1/2}$. The tremendous potential of the gill microcirculation in regulating plasma hormones is evident in these figures. (b) The effect of gill inactivation fraction (f_g) on the theoretical rate of hormone disappearance from plasma. The solid curves assume a ratio of cardiac output (CO; per minute) to blood volume of 1.0. If the gill removes 50% of the hormone in a single pass ($f_g = 0.5$; green line), then $t_{1/2}$ is 1 min, if only 10% is removed in a single pass ($f_g = 0.1$; red line), then $t_{1/2}$ is over 6 min. If the ratio of cardiac output to blood volume (V_b) becomes 0.5 and inactivation fraction remains at 50% ($f_g = 0.5$, CO $= 0.5V_b$; dotted line), then $t_{1/2}$ is 2 min. The black solid line ($f_g = 0.05$) shows the rate of epinephrine disappearance from the circulation based on gill extraction only (see **Table 2**). (a) From Olson KR, Conklin DJ, Conlon JM, *et al*. (1997) Physiological inactivation of vasoactive hormones in rainbow trout. *Journal of Experimental Zoology* 279: 254–264. (b) From Olson KR (1998) Hormone metabolism by the fish gill. *Comparative Biochemistry and Physiology* 119A: 55–65.

Methods for Measuring Hormone Inactivation

Three general methods are used to examine hormone inactivation in fish; physiological inactivation, whole-body uptake, and single-tissue extraction. With the first, the hormone of interest is often infused at a constant rate to change a physiological parameter. When the system reaches steady state, hormone infusion is stopped and the rate of recovery of the physiological parameter is used to provide an index of the overall rate of inactivation of the hormone and recovery of the system. To determine whole-body uptake, the hormone is injected into the fish and blood samples are taken periodically to measure the amount of hormone remaining in the circulation. Single-tissue extraction is measured by injecting a hormone into the arterial circulation of a specific tissue and measuring hormone recovery from the venous effluent or by using isolated perfused organs. Information derived, using these methods, is described in the following sections.

Table 1 Approximate *in vivo* $t_{1/2}$ (min) of common hormones and the volume marker sucrose, determined by physiological inactivation or radionuclide clearance

	Physiological inactivation	Radionuclide clearance
ANG II	3–4	
AVT	18–20	
BK	3–4	
EPI	3–4	1.7–3.3
NEPI	3–4	
Sucrose		1.8–3.3

ANG II, angiotensin II; AVT, arginine vasotocin; EPI, epinephrine; NEPI, norepinephrine.

Physiological Inactivation of Circulating Hormones

The physiological inactivation of circulating hormones entails both biochemical/cellular inactivation and delivery of these hormones to the metabolically active sites. **Figure 4(a)** shows the recovery (decline) of rainbow trout (*Oncorhynchus mykiss*) blood pressure following norepinephrine infusion into the bloodstream. This recovery can be defined as a simple exponential decay:

$$A_t = A_{\mathrm{eq}} + A_i e^{-kt} \qquad [1]$$

where A_t is blood pressure at any time (t), A_{eq} is the blood pressure at equilibrium, that is, resting blood pressure, A_i is the increase in blood pressure produced by the hormone infusion, and k is the rate constant (the fractional change per unit time). Interestingly, the exponential drop in blood pressure when these hormones are administered as a single, bolus injection, displays approximately the same half-time as it does during infusion; thus, a single injection may be a more convenient mechanism to examine $t_{1/2}$. Using these relationships, it was found that the $t_{1/2}$ for the recovery of blood pressure in an intact fish treated with either vasoactive peptides or catecholamines was longer than for the mechanical relaxation of isolated blood vessels *in vitro* when these hormones were removed from the fluid bathing the vessel. Thus, it is evident that the rate-limiting factor for many vasoactive hormones is the circulation time, not the inactivation process *per se*. Some $t_{1/2}$ values for physiological relaxation are shown in **Table 1**.

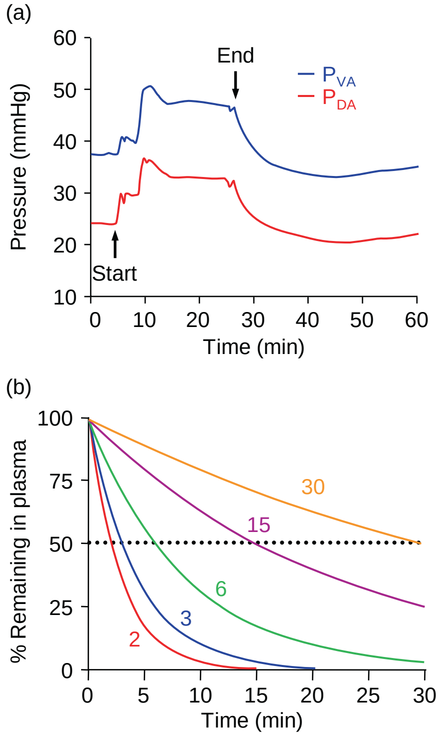


Figure 4 Physiological inactivation of norepinephrine. (a) Norepinephrine is infused into a fish (start) until dorsal aorta (P_{DA}) and ventral aorta (P_{VA}) blood pressures reach a steady state. Infusion is stopped (end) and the exponential decrease in blood pressure is the result of inactivation of circulating norepinephrine and recovery of the heart and blood vessels. (b) Predicted percent hormone remaining in the plasma at different $t_{1/2}$ (2, 3, 6, 15, and 30 min), assuming an exponential decline in hormone concentration and no hormone secretion. From Olson KR, Conklin DJ, Conlon JM, *et al*. (1997) Physiological inactivation of vasoactive hormones in rainbow trout. *Journal of Experimental Zoology* 279: 254–264.

Physiological inactivation of noncirculating hormones can be passively governed by blood flow through the secretory organ, which, in turn, affects the dilution rate. Inactivation of noncirculating hormones can also result from efficient gill metabolism as described later.

Whole-Body Uptake

Removal of hormones from the circulation of an intact fish is most commonly measured by injecting a radiolabeled hormone and monitoring the rate of disappearance of radioactivity through serial blood sampling. Using unlabeled hormone injections is a little more difficult. Hormones with a short $t_{1/2}$, such as catecholamines or some peptides, exhibit a mono-exponential decrease in plasma hormone with a $t_{1/2}$ similar to physiological inactivation measurements (**Table 1**). In fact, the $t_{1/2}$ for clearance of radiolabeled sucrose, an inert marker of mixing and dilution throughout the cardiovascular system and extracellular fluid, is similar to that measured for catecholamines, angiotensin, and bradykinin (**Table 1**). This supports the hypothesis that mixing in the cardiovascular system, not metabolic processes, can be the rate-limiting factor for clearance of certain hormones from the circulation. The impact of $t_{1/2}$ on the percent hormone remaining in the plasma over time is shown in **Figure 2(b)**.

Single-Tissue Extraction

Estimating hormone inactivation by a tissue or organ can be determined in perfused preparations by adding the

Table 2 Fractional extraction of hormones by the rainbow trout (*Oncorhynchus mykiss*) gill *in vivo* (I) or the perfused gill (p)

Class	*Hormone*	*Fractional extraction*
Peptide	ANP (i, p)	0.6, 0.6
	AVT (i)	0.2
	BK (i, p)	0.2, 0.25
	ET-1 (i)	0.55
Amine	EPI (p)	0.05
	NEPI (p)	0.16
	5-HT (p)	0.8
Arachidonic acid	Ar-A (p)	0.99
	PGE_2 (p)	0.4
	PGI_2 (p)	0.13
Purine	ADO (p)	0.4

ANP, atrial natriuretic peptide; AVT, arginine vasotocin; BK, bradykinin: ET-I, endothelin-1; EPI, epinephrine; NEPI, norepinephrine; 5-HT, serotonin; Ar-A, arachidonic acid; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; ADO, adenosine.

Modified from Olson KR (2002) Gill circulation: Regulation of perfusion distribution and metabolism of regulatory molecules. *Journal of Experimental Zoology* 293: 320–335.

hormone to the perfusate and measuring the amount of hormone recovered in venous effluent. The disadvantage of this tissue-specific analysis is that it can potentially fail to reproduce physiological conditions. Half-times of lipophilic hormones may be underestimated due to the absence of carrier molecules in the perfusate, lowering their solubility and stability below that of plasma. In contrast, $t_{1/2}$ of hydrophilic hormones, such as peptides, may be overestimated due to the absence of plasma-born enzymes, such as proteases, that would otherwise inactivate hormones in addition to tissue processes.

Hormone extraction by individual tissues can also be measured *in vivo* using a dual-label technique where the hormone and an inert volume marker are injected simultaneously into the artery supplying the tissue and multiple samples are collected over time from its venous effluent. Values for hormone extraction by the perfused gill are tabulated in **Table 2** and representative experiments are shown in **Figure 2**.

Impact of Single-Tissue Inactivation on Whole-Body Hormone Clearance

The overall rate at which a hormone is removed from the circulation depends on two factors: (1) how efficient a tissue is in inactivating the blood during a single transit through the tissue, and (2) what percent of the total cardiac output passes through this tissue. This is shown graphically in **Figure 3** and can be described mathematically as

$$k_{\mathrm{Tiss}} = -\ln[1 - f_{\mathrm{i}} \cdot f_{\mathrm{p}}] \cdot (\mathrm{CO}/V_{\mathrm{b}}) \qquad [2]$$

where k_{Tiss} is the specific rate constant for each tissue, ln is the natural log, f_{i} is the fractional inactivation of the molecule in a single transit through the tissue (the ratio of the molecule's concentration in venous to arterial blood), f_{p} is the fractional perfusion of the tissue (the ratio of blood flow through the tissue to total cardiac output), CO is cardiac output, and V_{b} is the volume of blood transporting the hormone (usually this is equivalent to plasma volume). The relative importance of tissue blood flow and fractional inactivation is shown in **Figure 3(a)**. It is readily apparent that if a hormone is inactivated by the gill, which is the only tissue in fish to receive the entire cardiac output (i.e., $f_{\mathrm{p}} = 1.0$), the inactivation fraction (f_{i}) will only need to be 0.11 to produce a plasma $t_{1/2}$ of 6 min, whereas inactivation by a systemic tissue, which typically receives no more than 20% of the cardiac output ($f_{\mathrm{p}} = 0.2$), will have to be 55% ($f_{\mathrm{i}} = 0.055$) to achieve the same $t_{1/2}$. The efficacy of the gill in rapidly lowering plasma hormone concentrations at different fractional extractions is shown in **Figure 3(b)**. As an example, if gill fractional extraction (f_{g}) is only 0.2 (20% extraction), the plasma hormone concentration will be halved in less than 4 min.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy.

Further Reading

Gillis CN (1987) Measurement of endothelial metabolic functions *in vivo*. *Annals of Biomedical Engineering* 15: 183–188.

Iversen LL (1975) Uptake of circulating catecholamines into tissues. In: Mountcastle VB, Bloom FE, and Geiger NSR (eds.) *Handbook of Physiology*, pp. 713–722. Washington, DC: American Physiological Society.

Olson KR (1998) Hormone metabolism by the fish gill. *Comparative Biochemistry and Physiology* 119A: 55–65.

Olson KR (2002) Gill circulation: Regulation of perfusion distribution and metabolism of regulatory molecules. *Journal of Experimental Zoology* 293: 320–335.

Olson KR, Conklin DJ, Conlon JM, *et al.* (1997) Physiological inactivation of vasoactive hormones in rainbow trout. *Journal of Experimental Zoology* 279: 254–264.

Olson KR and Duff DW (1993) Single-pass gill extraction and tissue distribution of atrial natriuretic peptide in trout. *American Journal of Physiology* 265: R124–R131.

Olson KR, Lipke DW, Kullman D, Evan AP, and Ryan JW (1989) Localization of angiotensin-converting enzyme in the trout gill. *Journal of Experimental Zoology* 250: 109–115.

Pitt BR (1984) Metabolic functions of the lung and systemic vasoregulation. *Federation Proceedings* 43: 2574–2577.

Pitt BR, Gillis CN, and Hammond GL (1981) Influence of the lung on arterial levels of endogenous prostaglandins E and F. *Journal of Applied Physiology* 50: 1161–1167.

Pitt BR, Hammond GL, and Gillis CN (1982) Comparison of pulmonary and extrapulmonary extraction of biogenic amines. *Journal of Applied Physiology* 52: 1545–1551.

Said SI (1982) Metabolic functions of the pulmonary circulation. *Circulation Research* 50: 325–333.

Stenslokken KO, Sundin L, and Nilsson GE (2006) Endothelin receptors in teleost fishes: Cardiovascular effects and branchial distribution. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 290: R852–R860.

Sultana N, Nag K, Kato A, and Hirose S (2007) Pillar cell and erythrocyte localization of fugu ET(A) receptor and its implication. *Biochemical and Biophysical Research Communications* 355: 149–155.

Turrin M and Gillis CN (1986) Removal of atrial natriuretic peptide by perfused rabbit lungs *in situ*. *Biochemical and Biophysical Research Communications* 140: 868–873.

Integrated Responses of the Circulatory System to Temperature

AK Gamperl, Memorial University of Newfoundland, St. John's, NL, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Acute Temperature Effects on Resting Cardiac Function
Temperature Effects on Maximum Cardiac Function and Scope for Cardiac Output
Responses of the Cardiovascular System to Temperature Acclimation
Adaptations of the Cardiovascular System
Summary
Further Reading

Glossary

Acclimatize (acclimate) Physiological or morphological changes an organism experiences when exposed to chronic (prolonged) changes in environmental conditions.

Bradycardia A slowing of normal heart rate. In fish, this condition is often associated with hypoxia (low environmental oxygen levels).

Critical thermal maximum (CTM) The maximum temperature that can be tolerated by an organism. In fish, this variable is most often measured by acutely increasing the temperature (e.g., by 2 °C h^{-1}) until the fish loses equilibrium.

Diastole A period of the cardiac cycle when cardiac muscle is relaxed or relaxing. It is associated with filling the heart with blood.

Echocardiography A noninvasive procedure that uses sound waves to study the structure and function of the heart. Measurements that can be taken with this technique include chamber/valve sizes, chamber volumes, and blood flows during particular events in the cardiac cycle.

Eurythermal Able to live over a wide range of temperatures.

In situ Being in the original position; used to describe experiments where measurements are taken on an organ (tissue) while they are still located within the animal.

Phenotypic The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.

Phylogenetic Relating to the evolutionary development (history) of an organism.

Stenothermal Describes an organism that cannot live in environments that undergo large changes in temperature.

Temperature quotient (Q_{10}) A measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C. The Q_{10} is calculated as: $Q_{10}= R1/R2^{10\ (T2-T1)}$, where R is the rate at a particular temperature and T the temperature in degrees celsius.

Upper incipient lethal temperature (UILT) Maximum temperature at which 50% of the fish acclimated to a temperature survive after a defined period of exposure to the test temperature.

Introduction

Fish have a truly global distribution, and live in water with temperatures ranging from −1.8 to ~42 °C. For many species, water temperature also varies daily or seasonally, and often by >10 °C. For example, carp species can experience seasonal temperature fluctuations from near 0 °C in winter to 30 °C in summer. The frillfin goby (*Bathygobius soporator*), which lives in tide pools along the east coast of South America, is subjected to diurnal fluctuations from 25 to 40 °C.

Given that the body temperature of fish is normally equivalent to that of the water, and that aerobic metabolic rate (oxygen consumption) is positively correlated with temperature (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism and **Temperature**: Membranes and Temperature: Homeoviscous Adaptation), fish must be able to adjust the delivery of oxygen and energy substrates to meet temperature-dependent changes in tissue demand. In addition, they must try to accommodate these temperature-related demands while providing for other life processes that are critical to the fish's survival and fitness (e.g., growth, digestion, swimming, migration, and reproduction).

In fishes, the oxygen delivered from the gills to the tissues (T_aO_2, arterial oxygen transport) is determined by cardiac output ($\dot{Q}$, the amount of blood pumped by the heart per unit time) and the amount of oxygen carried in the arterial blood (C_aO_2, arterial oxygen content) according to the following equation: $T_aO_2 = \dot{Q} \times C_aO_2$. Since $\dot{Q}$ can vary by up to threefold in fishes, changes in $\dot{Q}$ can have an enormous influence on how much oxygen is delivered to the tissues. In fact, any environmental change that alters the fish's oxygen consumption usually involves a change in $\dot{Q}$. Furthermore, arterial blood is normally nearly fully saturated with oxygen, and it remains this way when temperature changes. Therefore, changes in C_aO_2 are not appreciable compared with $\dot{Q}$ for most changes in temperature.

Over the past four decades, a great deal has been learned about how temperature affects cardiovascular function in fishes. For example, it is well established that resting $\dot{Q}$ changes in concert with temperature until the fish reaches its maximum tolerable temperature. However, maximum cardiac function has an optimum temperature range below the maximum tolerable temperature that is species specific and varies with the fish's thermal history. Moreover, we have begun to appreciate the structural, biochemical, and metabolic changes that allow the fish's cardiovascular system to have a degree of independence from the intrinsic effects of temperature; e.g., those resulting from temperature acclimation, and those that are a consequence of adaptation to a particular thermal environment over evolutionary time (i.e., the result of natural selection).

Acute Temperature Effects on Resting Cardiac Function

Heart rate (f_H) is the easiest and most often measured cardiovascular variable with respect to the effects of temperature on fish cardiac function. As for most rate functions, resting f_H increases with temperature in a nonlinear manner. This is reflected in Q_{10} values, which describe the factorial change in a rate function for a 10 °C temperature change. Many studies indicate that the Q_{10} for f_H is between 1.3 and 3.0 depending on the range of temperatures and species studied. This means that f_H can increase from 30% to threefold with an increase of 10 °C. Regardless, resting f_H increases until just prior to the maximum temperature that the fish can tolerate (this temperature called the upper incipient lethal temperature (UILT) or critical thermal maximum (CTM)).

Temperature increases resting f_H partially through its effects on the membrane permeability of the heart's pacemaker cells and on the ionic currents contributing to the pacemaker's discharge, and thus its intrinsic rate. Further, the pacemaker cells are innervated by cholinergic (inhibitory) and adrenergic (stimulatory) nerves, and temperature influences the neuronal modulation of pacemaker rate. For example, an acute increase in temperature generally decreases cholinergic tone and increases adrenergic tone on the heart, both of which elevate f_H (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

A curious and unexplained observation for fish that are acutely warmed is that cardiac stroke volume (S_V, the amount of blood pumped per heart beat) remains unchanged. Therefore, the increase in $\dot{Q}$ that accompanies warming directly mirrors that in f_H. The reason why this is curious is because fish can often increase S_V by two- to threefold during aerobic exercise. Why resting fish do not increase S_V when acutely warmed is a hotly debated topic, and the major potential explanations are presented below.

1. The heart cannot fill any more during diastole, and this limits S_V. There are three possible reasons for this: (a) as f_H increases, the time available to fill the heart decreases; (b) it is more difficult to fill the heart via venous pressure because the heart muscle cannot relax fully during diastole (i.e., resting tension increases and the muscle becomes stiffer due to elevated intracellular calcium levels); and (c) cardiac filling pressure decreases at high temperatures. The first two explanations remain plausible and supported by experimental work. However, cardiac filling pressure was recently shown to remain unchanged in salmonids at temperatures between 10 and 20 °C, and actually increased substantially when chinook salmon (*Oncorhynchus tshawytscha*) were warmed from 21 to 25 °C.
2. There is residual volume left in the ventricle when the heart finishes contracting (i.e., end-systolic volume increases. This is a very likely occurrence. Studies with isolated cardiac muscle have repeatedly shown that the isometric force developed (the difference between resting tension and peak force) decreases with increasing contraction frequency (termed a negative force–frequency relationship) and with acute warming (e.g., see **Figure 1(a)**). Although catecholamines (adenaline and noradrenaline) can stimulate calcium influx into the myocytes and thus force development, their stimulatory effect is reduced with acute warming in rainbow trout. Finally, increases in $\dot{Q}$ with temperature in most species examined are greater than the decrease in vascular resistance with warming. Consequently, the heart must develop higher blood pressures to eject blood into the circulation at high temperatures, which adds to the challenge of generating tension at higher cardiac frequencies (**see also Design and**

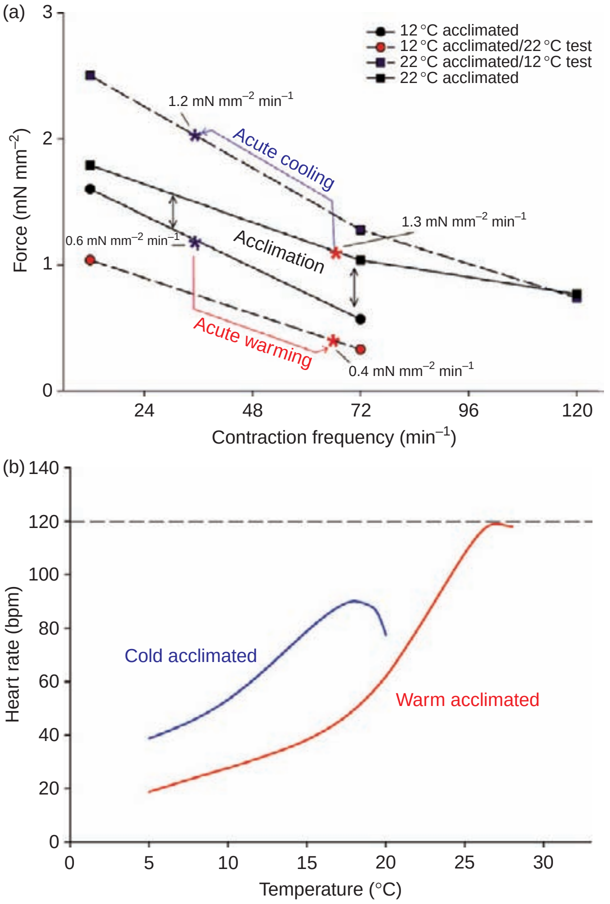


Figure 1 (a) The effect of an acute temperature change and temperature acclimation on isometric force development by isolated ventricular muscle strips from the rainbow trout when paced at various contraction frequencies. The main points are as follows: cold-(12 °C) acclimated ventricular strips (irrespective of test temperature) cannot be paced faster than approximately 72 min^{-1}; acute warming (to 22 °C) severely compromises force development and pumping capacity; warm acclimation (at 22 °C) greatly increases forced developed at a particular temperature, and allows the trout heart to be paced up to approximately 120 min^{-1}; finally, acute cooling increases force development. Asterisk (∗) indicates the approximate heart rate of live rainbow trout at each acclimation temperature, and the numbers associated with each asterisk indicate the heart muscle pumping capacity (product of force development and contraction frequency). (b) Effect of acute changes in temperature on the f_H of cold- and warm-acclimated rainbow trout. 120 beats per minute (bpm) represents the approximately upper limit of f_H in this species. (a) Modified from Shiels HA, Vornanen M, and Farrell AP (2002) The force–frequency relationship in fish hearts – a review. *Comparative Biochemistry and Physiology – Part A* 132: 811–826.

Physiology of the Heart: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

3. Central cardiac control mechanisms favor an increase in f_H. This appears to be a contributing factor because rainbow trout warmed to 24 °C can double S_V and maintained $\dot{Q}$ when f_H is halved by injecting the pharmacological agent zatebradine – a drug that specifically acts to slow down the heart's pacemaker cells without affecting myocardial force development.

Why fish choose to increase resting $\dot{Q}$ exclusively through changes in f_H when acutely warmed, and avoid increases in S_V is unknown. An increase in S_V could improve oxygen delivery to the myocardium and enhance myocardial function at a time when blood oxygen levels returning to the heart become limiting (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia). Further, although a high degree of synchrony between the rate of breathing (ventilation) and f_H has been observed at high temperatures, there is little evidence to support the idea that cardiorespiratory synchrony benefits oxygen transfer across the gills (at least in hypoxia). Thus, elevations in f_H are likely unnecessary for optimal gill oxygen transfer at high temperatures.

Clearly, more research is needed before a definitive answer emerges for this issue. This will require an integrative approach that incorporates techniques such as echocardiography, *in vivo* nerve recordings, and pharmacological intervention.

Temperature Effects on Maximum Cardiac Function and Scope for Cardiac Output

At the whole animal level, temperature tolerance is determined by a fish's metabolic scope (the difference between minimum and maximum metabolic rates). It has been recognized for over 60 years that metabolic scope is greatest within the fish's optimum temperature range, and is reduced as temperature increases or decreases from this range, until it approaches zero at a fish's upper and lower thermal limits.

Of particular relevance to this article is that metabolic scope, aerobic swimming performance, and cardiac scope (the difference between resting and maximum $\dot{Q}$) are very much interdependent when it comes to their responses to temperature. Indeed, *in situ* maximum S_V, cardiac power output ($\dot{Q} \times$ developed pressure), and cardiac scope fall when acclimation temperature is increased beyond a rainbow trout's optimum temperature; this decrease in cardiac function occuring despite increased f_H as temperature increases further.

Based on these findings from work with isolated hearts (i.e., hearts devoid of external nervous or hormonal control), a model was proposed in 1997 to explain the reduction in cardiac scope with temperature. This model proposed that, in a manner similar to metabolic scope, cardiac scope is not maintained across temperatures because resting $\dot{Q}$ continues to increase with temperature to support the elevation in routine metabolic rate until the fish's lethal temperature is reached (as explained in the previous section). In contrast, maximum $\dot{Q}$ (Q_{max}) has an optimum temperature well below the fish's upper temperature limit (see **Figure 2(a)**). Furthermore, the inability to increase or maintain maximum S_V at warm temperatures, unlike f_H, is responsible for the bell-shaped relationship between Q_{max} and temperature (see **Figure 2(b)**).

Interestingly, a recent study showed that sockeye salmon (*Oncorhynchus nerka*), acclimated to 12–14 °C and swum at 75% of their maximum swimming speed (i.e., a swimming speed where aerobic metabolism is near maximal), maintained the S_V recorded at 15 °C (0.7 ml kg^{-1}) while temperature was gradually raised over several hours to 24 °C. Yet, maximum f_H failed to increase after 21 °C, and was equal to that measured in resting fish at 24 °C. These new data (see **Figure 3**) suggest that a different model must be used to explain the temperature dependence of maximum $\dot{Q}$ and cardiac scope (e.g., see **Figure 2(c)**). Based on these data, it appears that maximum f_H is reached at CTM in resting fish and at optimum temperatures in exercising fish, but that maximum S_V can be maintained as temperature approaches the fish's CTM. At present, there is no mechanistic explanation as to how exercising sockeye salmon maintained maximum S_V up to their CTM. The heart's pumping capacity (as determined using isolated ventricular strips) is very limited when acutely exposed to large temperature increases (see **Figure 1(a)**). Further, it has been shown that catecholamines released into the circulation at high temperatures have a reduced ability to support cardiac function.

Responses of the Cardiovascular System to Temperature Acclimation

The studies on maximum cardiac function described above differ in that the *in vivo* work used acute temperature changes, while the perfused heart studies compared temperature-acclimated fish. It is known that, with a reasonable temperature change and allowed adequate time, a fish's cardiac performance can at least partially compensate. This is termed temperature acclimation or acclimatization. Thus, the fish heart has considerable phenotypic plasticity, which enables cardiac function to attain a certain amount of independence from the direct effects of temperature.

There are a number of aspects of cardiac physiology that are known to change appreciably with temperature acclimation. To begin, the heart's intrinsic beating rate (the pacemaker rate) at a given temperature is higher in cold-acclimated individuals (see **Figure 1(b)**). The trade-off is that maximum f_H of isolated hearts from cold-acclimated fish is lower than for warm-acclimated fish. This latter observation has already been related to cellular acclimation, such as a decrease in myocyte action potential duration mediated by an increase in inward Na^+ and outward (rectifying) K^+ currents (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart for ion movements associated with the action potential), a resetting of the cardiac pacemaker rate (again related to increased K^+ currents), and to an enhanced involvement of the sarcoplasmic reticulum (SR) in cellular calcium dynamics. Second, acclimation to warm temperature increases the maximum rate at which the myocardium can be stimulated to contract and the amount of force developed at a particular contraction frequency (see **Figure 1(a)**). This greatly improves myocardial force development as compared to cold-acclimated fish, and especially when compared with the acute effects of warming on myocardial force development. Third, numerous physiological mechanisms (in addition to an increase in intrinsic f_H) have been identified that

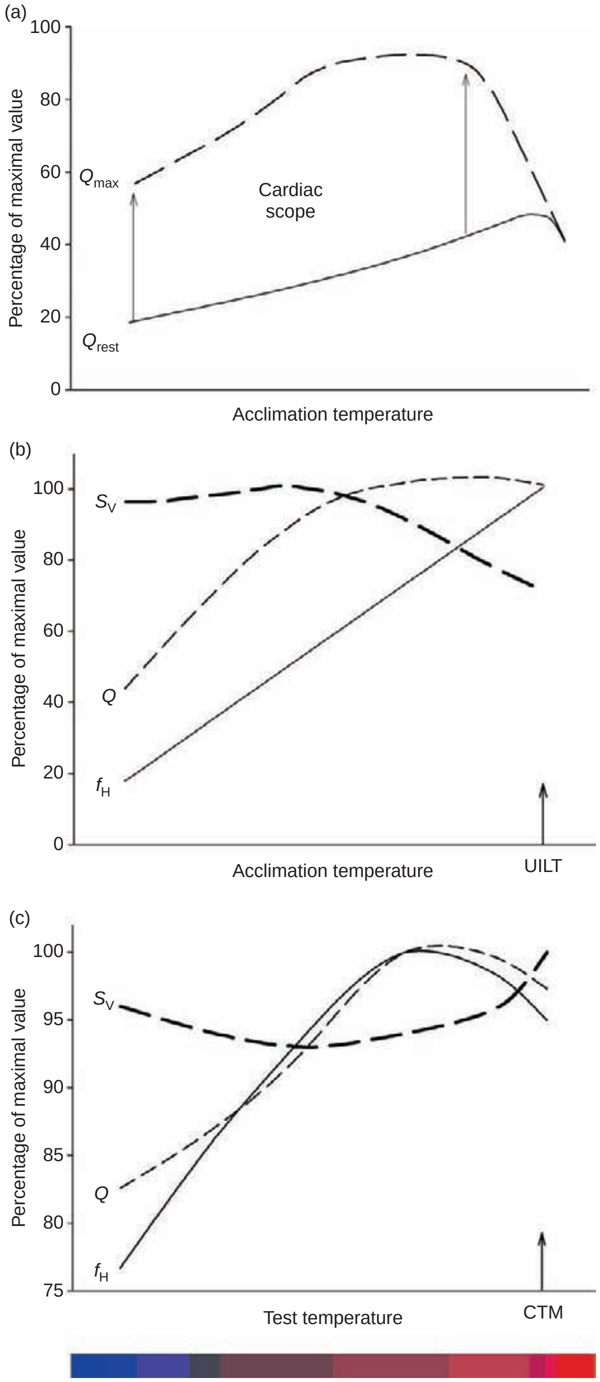


Figure 2 Schematic representations of the influence of acclimation temperature on resting and maximum *Q* (a); and of acclimation temperature (b), and an acute elevation in temperature (by 2 °C h^{-1}) (c), on maximum cardiac variables (heart rate, f_H; cardiac output, *Q*; and stroke volume, S_V). Modified from (a) Farrell (1997) Effects of temperature on cardiovascular performance. In: Wood CM and McDonald DG (eds.) *Society for Experimental Biology Seminar Series, 61: Global Warming: Implications for Freshwater and Marine Fish*, pp. 135–158. Cambridge: Cambridge University Press; (b) Farrell AP, Gamperl AK, Hicks JMT, Shiels HA, and Jain KE (1996) Maximum cardiac performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their upper lethal limit. *Journal of Experimental Biology* 199: 663–672. Gamperl AK, Swafford BL, and Rodnick KJ (2010) Elevated temperature, *per se*, does not limit the ability of rainbow trout to increase ventricular stroke volume. *Journal of Thermal Biology* 36: 7–14. doi:10.1016/j.jtherbio.2010.08.007.

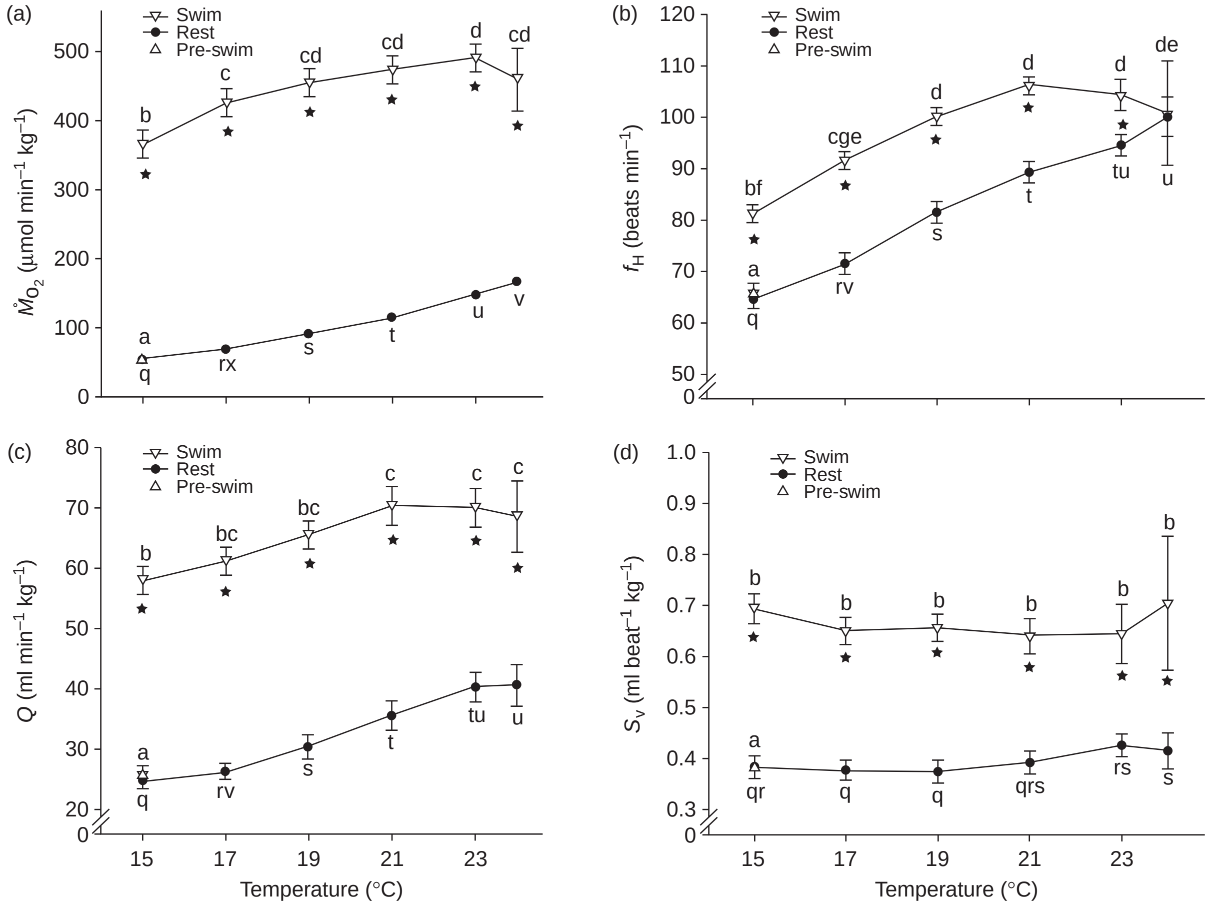


Figure 3 Effect of acute increases in temperature on the resting and swimming oxygen consumption ($\dot{M}_{O_2}$), heart rate (f_H), cardiac output (Q), and stroke volume (S_V) of 12–14 °C acclimated sockeye salmon. The fish were forced to swim constantly at 75% of their maximum aerobic swimming speed. Different letters indicate significant differences between temperatures within the same group (rest or swim). An asterisk (*) indicates a significant difference between resting and swimming fish at a given temperature. Modified from Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, and Farrell AP (2008) The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* 211: 3915–3926.

promote positive thermal compensation of the heart's pumping capacity following cold acclimation. These include an increase in the density of L-type calcium channels in the sarcolemma (the main route for Ca^{2+} entry into the cell), an enhanced ability of catecholamines (adrenaline and noradrenaline) to increase myocardial contractility, and a 20–30% increase in ventricular mass in some species. This cardiac enlargement is mainly due to an increase in the size of the heart's myocytes (i.e., hypertrophy), and is hypothesized to increase S_V (to compensate for the negative rate effects of low temperatures on f_H) and probably force development to overcome the increase in blood viscosity at cold temperatures (see below). Finally, this elevated cardiac function at cold temperatures is supported by an enhanced capacity for aerobic metabolism, especially fatty acid metabolism.

With regards to the effects of acclimation temperature on the nervous control of cardiac function, it appears that the response may depend on the taxonomic group. For example, cholinergic tone decreases and adrenergic tone increases with acclimation temperature in rainbow trout, whereas cholinergic tone increases in the sole (*Solea vulagis*) with acclimation to warm temperature.

Adaptations of the Cardiovascular System

Fishes are a diverse taxonomic group, comprised of over 28 000 species, that employ a variety of life-history strategies, experience a range of thermal regimes/environments, and exhibit quite different thermal preferences and tolerances. Thus, it should be no surprise that the response of cardiac function to changes in temperature varies greatly among species (taxonomic groups). Unfortunately, there are few comprehensive studies of thermal sensitivity/adaptation of circulatory functions and their underlying mechanisms in species other than trout/salmon (with the exception of Antarctic fishes, see below). This makes it difficult to discern whether particular response patterns in cardiac function with temperature are characteristic of particular ecotypes (e.g., stenothermal vs. eurythermal, cold inactive vs. active).

Regardless, a number of strategies have emerged that are utilized by fishes when temperature changes. For example, eurythermal species appear to have a lower f_H at equivalent temperatures, thus preserving their scope to increase f_H with temperature. Although the Atlantic cod (*Gadus morhua*) heart does not exhibit cardiac enlargement at cold temperatures or cold-induced enhancement of intrinsic f_H that is typical of other fishes, and is quite insensitive to adrenergic stimulation, a bell-shaped force–frequency relationship (i.e., positive at low contraction frequencies, negative at high contraction frequencies) appears to allow this species to elevate cardiac function at low temperatures and when acutely warmed (e.g., from 4 to 10 °C).

Some species such as the sea raven (*Hemitripterus americanus*) and the crucian carp (*Carassius carassius*), which are inactive in winter and must conserve available energy, display negative (inverse) thermal compensation of f_H (i.e., f_H is lower at a given temperature). Further, in the cold-acclimated crucian carp, action potential duration is prolonged, there is no contribution of the SR to cellular Ca^{2+} management, and myofibrillar adenosine triphosphatase (ATPase) activity (a major determinant of muscle contraction velocity) is greatly reduced. These adaptations appear to be critical to the survival of this species which experiences anoxia (a complete lack of oxygen) in addition to cold water temperatures during the winter.

With regards to adaptations of the cardiovascular system to temperature, the Antarctic fishes are the only example where the influence of activity and temperature on cardiac function has been put into an appropriate phylogenetic framework. Fish in the Antarctic have been geographically and genetically isolated for approximately 25 million years, and live in frigid, glaciated waters characterized by constant temperatures (-1.8 to -1 °C) and high oxygen content. This exposure to permanent cold conditions has led to interesting phylogenetic specializations.

At these extremely cold temperatures, one of the greatest challenges faced by Antarctic fishes is to compensate for the inverse and exponential relationship between temperature and blood viscosity (a component of a fluid's resistance to flow). This increase in viscosity as temperature decreases is due to a number of factors, including:

1. a temperature-dependent increase in the viscosity of the plasma;
2. the accumulation of antifreeze glycoproteins in the blood to prevent the fish from freezing, the levels of these molecules reaching as high 3% (30 mg ml^{-1}); and
3. an increase in the stiffness of the erythrocytes (red blood cells, RBCs) that makes them less deformable and difficult to push through the vasculature.

To compensate for the increase in viscosity with temperature many Antarctic species have a reduced hematocrit (the proportion of the blood composed of RBCs), approximately one-half that of other teleosts (8–15% vs. ~20–40%). Further, these fishes increase the fluidity of the RBC's cell membrane (by altering fatty acid composition) and decrease the amount of hemoglobin per cell (both of these changes increasing the ease at which the cells can be deformed). Finally, they have arteries and veins that are considerably larger than the norm for vertebrates. Collectively, these changes reduce the blood pressure that the heart must generate, while still providing an adequate T_aO_2 to meet the fish's low metabolic demand at these frigid temperatures.

The challenge of increased blood viscosity also appears to have led to a very unique group of fish, the icefish (family Channichthyidae), which some scientists suggest represent an evolutionary dysadaptation permitted because of the extremely stable Antarctic environment. The icefish have a hematocrit <1%, and thus the blood's oxygen-carrying capacity is only approximately 10% of red-blooded Antarctic fish species. In addition, some of them even lack the oxygen binding/transporting protein myoglobin in their heart and muscles. This presents them with a distinct problem – how to deliver enough oxygen to the tissues?

The Channichthyidae have solved this problem by having a cardiac output 6–15 times greater than other teleosts, a blood volume 2–4 times that seen in red-blooded Antarctic fishes, and a heart and vasculature that are well suited to function as a high-flow, low-pressure system. For example, because f_H is no higher in icefish as compared to other fishes at these temperatures, the relative ventricular mass (i.e., g kg^{-1} of body mass; 0.4%) and S_V of icefishes are 3–4 times greater than those of most teleosts and the red-blood Antarctic fishes (**Figure 4**). Correspondingly, the bulbus arteriosus (see **Figure 4**) is greatly enlarged so that it can accommodate the ejection of such a large S_V. Channichthyids have increased the diameter and density of their vasculature, even more than observed in red-blooded Antarctic species. Finally, to overcome the difficulty of oxygen diffusion in the heart due to the lack of myoglobin, the cellular proliferation of mitochondria is appreciable (a mitochondrial volume density twice that measured in Antarctic red-blooded fishes; ~18% vs. 40%).

Given the specializations in cardiorespiratory function/morphology that have accompanied adaptation to Antarctic waters, one would expect that these fishes have a very limited capacity to tolerate or adapt to

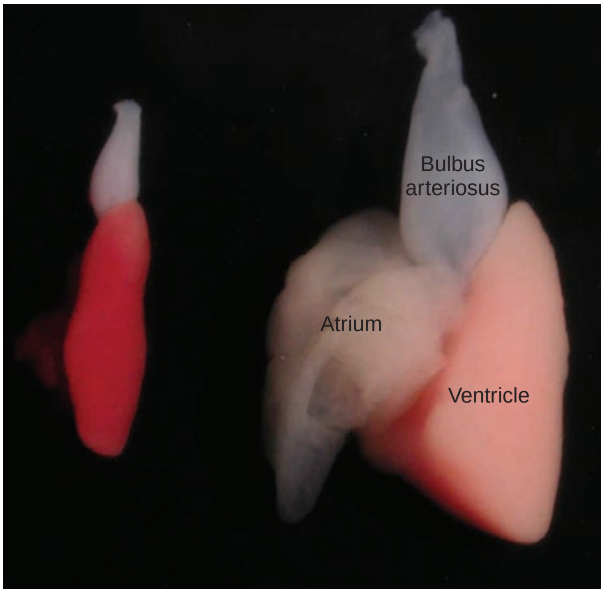


Figure 4 Hearts from approximately 0.25 kg specimens of the red-blooded fish *Trematomus bernacchii* (left) and *Chionodraco hamatus* to illustrate the dramatic increase in heart size displayed by Antarctic icefishes. Photograph courtesy of Dr. Bruno Tota.

temperatures outside their normal thermal range. However, recent studies have shown that the red-blooded Antarctic fish *Pagothenia borchgrevinki* can be acclimated to 4 °C, and that this results in compensatory adjustments in cellular metabolism and cardiovascular function (e.g., see **Figure 5**) that allow these fish to maintain their locomotory performance and cardiac scope until 8 °C. These novel findings for *P. borchgrevinki* suggest that the circulatory system of at least some Antarctic fishes has retained a significant degree of plasticity and that they, and other fish species, may not be as severely impacted by accelerated climate change (global warming) as previously thought.

Summary

Temperature places substantial constraints on the capacity of the fish heart to pump, and thus to support oxygen demand. This is especially true if temperatures are increased or decreased beyond the fish's optimal thermal range over the short term (i.e., acutely), and suggests that temperature-dependent cardiac performance may be the

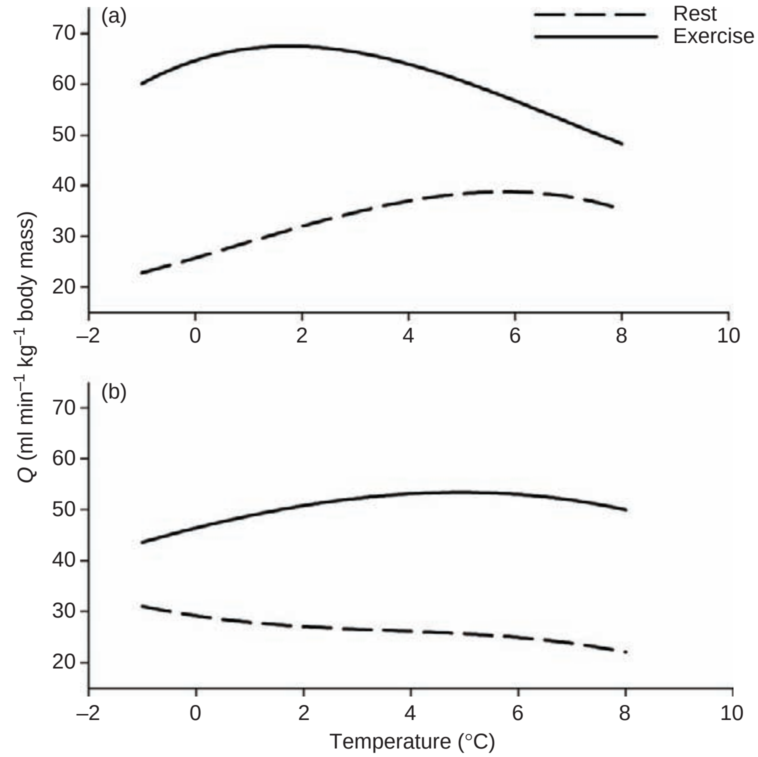


Figure 5 The effect of an acute increase in temperature on resting and maximum (exercise induced) cardiac output (*Q*) in the Antarctic fish *Pagothenia borchgrevinki* acclimated to –1 °C (a) and 4 °C (b). Note the difference in cardiac scope (difference between resting and maximum *Q*) at the higher temperatures in fish acclimated to –1 vs. 4 °C. Modified from Franklin CE, Davison W, and Seebacher F (2007) Antarctic fish can compensate for rising temperatures: Thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *Journal of Experimental Biology* 210: 3068–3074.

basis for the thermal tolerance of certain fishes. However, it is also apparent that fish hearts show great diversity in design, control, and function, and that if temperature changes are prolonged (i.e., over several weeks) fishes can compensate to varying degrees for the negative influences of suboptimal temperatures on cardiovascular function. This adaptation is extreme in Antarctic fishes, but surprisingly, does not prevent their hearts from showing considerable flexibility when exposed to variations in temperature.

See also: **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping. **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Hypoxia. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism. **Temperature**: Membranes and Temperature: Homeoviscous Adaptation.

Further Reading

Aho E and Vornanen M (1998) Ca^{2+}-ATPase activity and Ca^{2+} uptake by sarcoplasmic reticulum in fish heart: Effects of thermal acclimation. *Journal of Experimental Biology* 201: 525–532.

Aho E and Vornanen M (1999) Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout *Oncorhynchus mykiss*: Effects of thermal acclimation. *Journal of Experimental Biology* 202: 2663–2677.

Aho E and Vornanen M (2001) Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). *Journal of Comparative Physiology B* 171: 173–179.

Axelsson M, Agnisola C, Nilsson S, and Tota B (1996) Fish cardio-circulatory function in the cold. In: Portner HO and Playle RC (eds.) *Society for Experimental Biology Seminar Series, 66: Cold Ocean Physiology*, pp. 327–364. Cambridge: Cambridge University Press.

Brett JR (1971) Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of Sockeye salmon (*Oncorhynchus nerka*). *American Zoologist* 11: 99–113.

Clark TD, Sandblom E, Cox GK, Hinch SG, and Farrell AP (2008) Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 295: R1631–R1639.

Driedzic WR, Bailey JR, and Sephton DH (1996) Cardiac adaptations to low temperature in non-polar teleost fish. *Journal of Experimental Zoology* 275: 186–195.

Farrell AP (1997) Effects of temperature on cardiovascular performance. In: Wood CM and McDonald DG (eds.) *Society for Experimental Biology Seminar Series, 61: Global Warming: Implications for Freshwater and Marine Fish*, pp. 135–158. Cambridge: Cambridge University Press.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology, Volume XII, Part A: The Cardiovascular System*, pp. 1–88. London: Academic Press.

Franklin CE, Davison W, and Seebacher F (2007) Antarctic fish can compensate for rising temperatures: Thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *Journal of Experimental Biology* 210: 3068–3074.

Gamperl AK and Farrell AP (2005) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Garofalo F, Pellegrino D, Amelio D, and Tota B (2009) The Antarctic hemoglobinless icefish, fifty five years later: A unique cardiocirculatory interplay of disaptation and phenotypic plasticity. *Comparative Biochemistry and Physiology – Part A* 154: 10–28.

Haverinen J and Vornanen M (2004) Temperature acclimation modifies Na^{+} current in fish cardiac myocytes. *Journal of Experimental Biology* 207: 2823–2833.

Haverinen J and Vornanen M (2007) Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 292: R1023–R1032.

Haverinen J and Vornanen M (2009) Responses of action potential and K^{+} currents to temperature acclimation in fish hearts: Phylogeny or thermal preferences? *Physiological and Biochemical Zoology* 82: 468–482.

Shiels HA, Vornanen M, and Farrell AP (2002) The force–frequency relationship in fish hearts – a review. *Comparative Biochemistry and Physiology – Part A* 132: 811–826.

Shiels HA, Vornanen M, and Farrell AP (2003) Acute temperature change modulates the response of I_{Ca} to adrenergic stimulation in cardiomyocytes. *Physiological and Biochemical Zoology* 76: 816–824.

Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, and Farrell AP (2008) The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming Sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* 211: 3915–3926.

Tiitu V and Vornanen M (2001) Cold adaptation suppresses the contractility of both atrial and ventricular muscle of the crucian carp heart. *Journal of Fish Biology* 59: 141–156.

Vornanen M (1996) Effect of extracellular calcium on the contractility of warm- and cold-acclimated crucian carp heart. *Journal of Comparative Physiology B* 165: 507–517.

Integrated Responses of the Circulatory System to Digestion

H Seth and M Axelsson, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
Blood Supply of the Gastrointestinal Tract
Regulation of the Gastrointestinal Blood Flow during Digestion
Other Factors Influencing the Response of the Circulatory System after Feeding
Further Reading

Glossary

Anabolism Synthesis of materials via metabolic processes; a series of chemical reactions that builds larger molecules from smaller components, usually requiring energy in the process.

Calorie An older unit of energy; today, energy content is normally given in joules (J) and the conversion is: 1 kilocalorie = 4.184 kilojoules.

Cardiac output ($\dot{V}_b$) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Catecholamine Any of a class of amines derived from a catechol that acts as chemical messangers (a hormone or neurotransmitter). The main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Chyme Partly digested food particles mixed with digestive secretions expelled by the stomach into the intestine.

Digestion The process of solubilization and hydrolysis of ingested nutrients in the gastrointestinal canal into constituent molecules and elements suitable for transport across the intestinal wall.

Hyperemia Increase in blood flow through vasodilation.

In vivo From Latin meaning ‘within the living’ refers to biological processes or experiments occurring or carried out in living organism as opposed to a partial or dead organism.

Postprandial Period after ingesting a meal.

Potentiate To promote or strengthen a biochemical or physiological action or effect.

Temporal Relating to time.

Tetrodotoxin A potent neurotoxin that blocks voltage-gated sodium channels.

Vasoconstriction A decrease in the diameter of a vessel.

Vasodilator A substance that vasodilates a blood vessel, meaning increases its diameter.

Introduction

As organisms grew more complex during the evolution, from simple one-celled life forms to increasingly more complex counterparts, there was a demand for a specialization of different tissues and organs. The development of a gastrointestinal tract (**see also Gut Anatomy and Morphology**: Gut Anatomy, **Integrated Function and Control of the Gut**: Intestinal Absorption, Gut Motility, Gut Secretion and Digestion, and Nervous System of the Gut) necessitated a concomitant development of a specialized gastrointestinal (GI) circulatory system to transport oxygen and nutrients to keep the absorptive tissues alive. Moreover, absorbed nutrients must be removed from the gastrointestinal tract and transported to the main nutrient handling organ, the liver, and tissues beyond.

With this organization came a need to coordinate and regulate the gastrointestinal tract and its blood supply in order to optimize the function during a variety of different conditions that might accompany digestion of a meal, such as exercise, coping with hypoxia and other stressful situations.

Because studies with fish show many instances of physiology and control mechanisms that are similar to those in mammals, the basic control systems were thought to be laid down early during vertebrate evolution. Even so, substantial differences still exist between fish and other vertebrate groups.

Blood Supply of the Gastrointestinal Tract

The blood supply to the gastrointestinal canal differs between elasmobranches and teleosts. In teleosts species studied to date, the primary blood supply to the GI tract is via a single major vessel, the coeliacomesenteric artery (CMA), which is the first caudal branch of the dorsal aorta. The CMA then divides progressively to supply the stomach, intestine and liver, as well as the gonads. Consequently, the terms CMA blood flow and GBF are often used interchangeably – in this article, we use GBF.

The blood supply to the GI tract of elasmobranchs (sharks) shows a more typical vertebrate pattern. Three major vessels branch off the dorsal aorta: the celiac artery, which supplies the liver, spleen, and stomach as well as part of the intestine; the anterior mesenteric artery, which supplies most of the small intestine; and the posterior mesenteric artery, which supplies the large intestine.

This article focuses on the regulation of the blood flow to the GI tract in teleosts because there is very little information for other fish groups. As described above the GI tract is typically supplied (with minor exceptions) by one major vessel, the CMA. Its branches supply for example the gonads, stomach and intestine, as well as the liver with blood. There are notable differences in the branching of the CMA among teleost species, possibly indicating special adaptations of the circulation depending on the habitat and feeding regime. This is discussed in more detail in **Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation.

In most fish species studied, the GI tract regularly receives about 30–40% of the total cardiac output. This is a similar proportion to what has been reported for mammals, perhaps suggesting that the GI tract commands about one-third of the metabolic rate of a fish.

After feeding, the GBF increases appreciably. Routine GBF more than doubles (100–160%) depending on the species and the surrounding environment (see **Table 1** for information on a few selected species). It is unclear at this time whether all of this increase in GBF is related to increased oxygen requirements of the GI tract, the transport of nutrients away from the GI tract, or more likely some combination of the two. The regulation of the GBF in unfed and fed fish is now considered.

Regulation of the Gastrointestinal Blood Flow during Digestion

In order to effectively digest, absorb, metabolize, and distribute the absorbed nutrients after a meal, several physiological adjustments must occur. In particular, there is a large need for the cardiovascular system to adjust in response to the increased oxygen demand related to nutrient absorption and anabolic process related to nutrient uptake.

As food enters the GI tract, there is a substantial increase in the total oxygen consumption of the whole organism, which is referred to as the specific dynamic action (SDA) or heat increment (HI). This increase in oxygen consumption is the result of digestion and absorption, but largely protein metabolism. Thus, without any increase in GBF there would be an unsustainable oxygen dept.

While supplying the metabolically active gut with oxygen is clearly one of the more important functions of the cardiovascular system, the increased GBF is also responsible for supplying the GI tract with many secreted compounds (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). The blood along with lymph is also important in transporting away the absorbed nutrients and distributing them to the liver or other tissues such as muscles.

The consequence of this is that there is a need for a firm regulation of the GBF before, during and after feeding in order to optimize all these events.

Different Ways to Increase GBF

While there is a need to substantially increase GBF after feeding, most animals studied so far cannot fully perfused all tissues at one time. Thus, there has to be a mechanism whereby blood flow is locally increased when there is a high demand. At the level of the entire GI tract, there are three ways that this can be achieved. First, the heart can pump more blood and the proportion going to each tissue can remain the same. However, this option may become problematic if there are competing demands, such as exercising skeletal muscle, for increased cardiac output. Second, blood flow can be redistributed toward the GI tract from a less active tissues, by constricting blood vessels in the less active tissue (increased vascular resistance) and dilating those in the GI tract (decreased vascular resistance). Third, there could be a combination of increased cardiac output and blood flow redistribution.

Cardiac output

Cardiac output (**see also Design and Physiology of the Heart**: The Outflow Tract from the Heart, Physiology of Cardiac Pumping, Cardiac Anatomy in Fishes, Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux, Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element, Cardiac Cellular Length-Tension Relationship, Intracardiac Neurons and Neurotransmitters in Fish, and Cellular Ultrastructure of Cardiac Cells in Fishes) can be increased by means of a increase in either stroke volume (SV) or heart rate (fH) or some combination of these two according to the formula: cardiac output = SV × fH. Most fish indeed increase in cardiac output after feeding. Furthermore, in most cases this increase is enough to account for the entire postprandial increase in GBF. This situation contrasts with mammals, where increased cardiac output is minor (see **Figure 1**).

Table 1 Comparison of gut blood flows between different species (routine: post-prandial)

		Meal size	*Cardiac output (ml min^{-1} kg^{-1})*		*Gut blood flow (ml min^{-1}kg^{-1})*		*Gut blood flow/cardiac output*		
Species	C°	(% of *b.w*	*Routine*	*Feeding* (%)	*Routine*	*Feeding* (%)	*Routine*	*Feeding* (%)	*Source*
Red Irish lord (*Hemilepidotus hemilepidotus*)	7–9	10–15	24	90	4.1/4.9	112/94	34	40	Axelsson *et al*. (2000)
Sea raven (*Hemitripterus americanus*)	10–12	10–20	18.8	15.4	2.9	100	15	27–30	Axelsson *et al*. (1989)
Short-horn sculpin (*Myoxocephalus scorpius*)	10	8–10		50		93			Seth *et al*. (2008)
Sea bass (*Dicentrarchus labrax*)	16	2.9	40	13.5	9.6	71	24	34	Axelsson *et al*. (2002)
Sea bass (only 6hr) (*Dicentrarchus labrax*)	22–23	2.7	51.4	22	13.8	82	27	40	Altimiras *et al*. (2008)
Sea bass (*Dicentrarchus labrax*)	19	3	43.4	27	4.3	160	10	20	Dupont-Prinet *et al*. (2009)
Atlantic cod (*Gadus morhus*)	10–11	2.2–3.5	19*	23	4.1/3,5	72/42	40	52	Axelsson and Fritsche (1991)
Rainbow tout (*Oncorhynchus mykiss*)	11–16	2			4–6	136			Eliasson *et al*. (2008)
Rainbow tout (*Oncorhynchus mykiss*)	10–Sep	2		23–42		156			Gräns *et al*. (2009)
Chinook salmoon (*Oncorhynchus tshawytscha*)	9–10	2				81			Thorarensen and Farrell (2006)

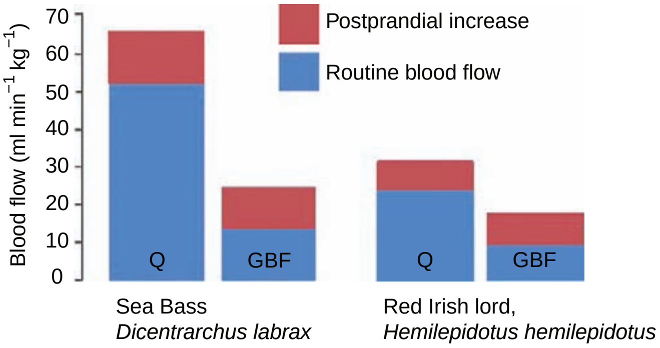


Figure 1 Effect of exercise on cardiac output and gastrointestinal blood flow in two different fish species. Data from Axelsson M, Thorarensen H, Nilsson S, and Farrell AP (2000) Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: Long-term effects of feeding and adrenergic control. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 170: 145–152; and Altimiras J, Claireaux G, Sandblom E, *et al*. (2008) Gastrointestinal blood flow and postprandial metabolism in swimming sea bass *Dicentrarchus labrax*. *Physiological and Biochemical Zoology* 81(5): 663–672.

Redistribution of blood

Redistribution of blood from metabolically less active tissues usually occurs when the vascular resistance of the resistance vessels in the gut decreases, possibly in combination with an increased vasculature resistance in other peripheral tissues. For this to occur, the vessels of the GI tract must vasodilate in response to some stimuli to create a local hyperemia. Since the hyperemia is confined to where food or chyme is present, it is reasonable to speculate that the stimuli are related to the presence of food.

Different Ways of Sensing and Coordinating the Blood Flow with the Presence of Food in the Gastrointestinal Tract

Of substantial importance to the postprandial cardiovascular response is the way in which the GI tract senses the presence of food. Nevertheless, the answer to this question has long eluded scientists and, in large part, still does. We do know that hyperemia is confined to the parts of the GI tract that is filled with food particles or chyme. Thus, there is a selective perfusion of the gut itself. As food enters the stomach, there is a small increase in the blood flow to this region and subsequently when food enters the proximal intestine the blood flow to this region increases (**Figure 2**). Two major hypotheses have been advanced to explain this finding. One hypothesis is that mechanical stimuli are important for the cardiovascular adjustments after feeding; the other is that there is a chemical signal. Working independently or even together, mechanical and chemical stimuli could coordinate the temporal and spatial postprandial patterns seen for GBF. The evidence for each of the sensory modalities is now described.

Mechanical stimuli

When a fish feeds, the stomach or gastric compartment is stretched. The level of the stretching depends on the relative volume of food ingested (often expressed as percentage of body mass) and on the compliance (ability to

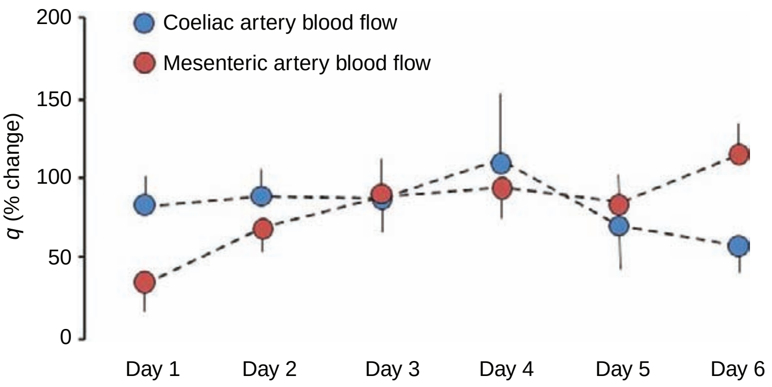


Figure 2 Effect of time on the celiac and mesenteric artery blood flow after feeding. Modified from Axelsson M, Thorarensen H, Nilsson S, and Farrell AP (2000) Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: Long-term effects of feeding and adrenergic control. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 170: 145–152.

stretch) of the stomach. Depending on the feeding habit, meals vary considerably in their relative volume.

A salmonid such as the rainbow trout (*Oncorhynchus mykiss*) that feeds on small prey like insects and larvae will only stretch the stomach to a small extent after a meal. Meals are rarely 1% of body mass and a meal of 2% of body mass typically results in satiation. In contrast, an ambush predator such as the shorthorn sculpin (*Myoxocephalus scorpius*) feeds on larger prey and sometimes consume up to 50–80% of their own body mass. These large meals can lead to a very rapid and profound stretching of the stomach.

Limited knowledge exists concerning the importance of mechanical stimuli in triggering and regulation GBF after feeding. Results show that mechanical stimuli by itself will not induce an increase in GBF. This is true for mechanical stimuli applied specifically to the stomach or the intestine. Even so, when the stomach is distended to mimic feeding, a rather large and rapid increase in dorsal aortic blood pressure occurs, which then persists until the mechanical stimuli is reduced (**Figure 3**). This response occurs when the stomach is artificially distended with a fluid-filled balloon as well as when the animal eats a normal diet, indicating that the stimulus is purely mechanical. Moreover, the pressor response seems to be adapted to produce the same vascular response in species with a very different feeding regime when adjusted to the meal size that is typically ingested.

The pressor response is a result of an adrenergic vasoconstriction that is most likely neural in origin. Thus, mechanical stretch of the GI tract causes a generalized systemic vasoconstriction. Consequently, because dorsal aortic blood pressure is the driving force for GBF, this response is a good starting point to increase GBF. All that is needed now for blood flow to redistribute from a region of high vascular resistance to one with a lower resistance is an accompanying vasodilation locally within the GI tract.

Since a mechanically stimulus alone is insufficient to affect the necessary increase in GBF, another type of stimulus is needed. Chemical stimuli seem to produce this local vasodilator response.

Chemical stimuli

After food enters the intestine, the enzymatic breakdown initiated in the stomach continues and results in the release into the gut lumen of small hydrolyzed products such as amino acids, fatty acids, and carbohydrates. It is these hydrolyzed compounds that are important for the induction of a hyperemia. Placing hydrolyzed food directly into the intestine increases GBF. However, placing unhydrolyzed food in the intestine has no effect at all.

What is far from clear for fish is to what extent different nutrients influence and adjust this response. It is also unclear whether or not the caloric content of the food contributes to the hyperemic response. Nevertheless, given other similarities between teleost species and mammals, we can speculate about the importance of chemical stimuli, especially since the few fish studies conducted so far suggest similarities.

All nutrients are certainly not equally potent in inducing the postprandial hyperemia in mammals and fish. In fact, the relative importance of nutrients is likely to vary with diet. Perhaps, the most important consideration among fish is the dietary composition of what they normally eat. For example, a carnivorous fish such as rainbow trout will most likely show a reduced response to carbohydrates because its diet normally contains fewer

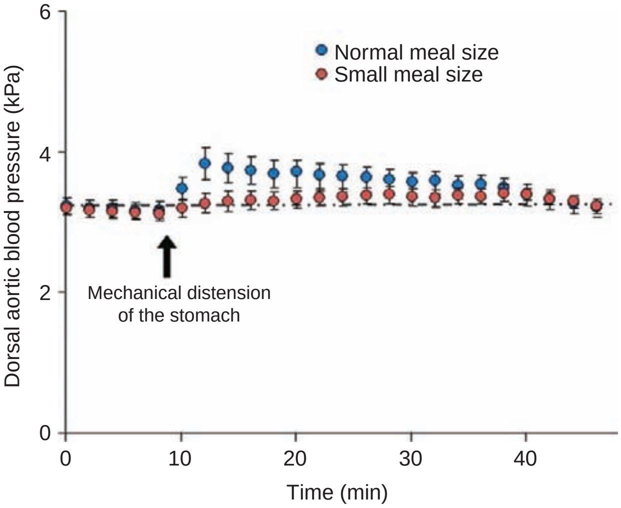


Figure 3 Effect of gastric distension on systemic dorsal aortic blood pressure in rainbow trout. From Seth H, Sandblom E, Holmgren S, and Axelsson M (2008) Effects of gastric distension on the cardiovascular system in rainbow trout (*Oncorhynchus mykiss*). *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 294: R1648–R1656.

carbohydrates relative to proteins and lipids/fats. This contrasts with omnivorous and herbivorous fish like the carps (*Carassius* sp.). Indeed in rainbow trout, a nutrient composition resembling the normal diet is the most effective chemical inducer of the hyperemia, compared to diets rich in either proteins of lipids. There is also a significant temporal difference in how fast the response is induced. This probably reflects the different ways in which nutrients are digested, absorbed, and metabolized.

Another factor influencing the outcome is the caloric or energy content of the different nutrients. Lipids/fats have almost twice the energy density as proteins or carbohydrates. Some results indicate that modest changes in the diet composition while maintaining the same caloric content have no affect on BGF, thus indicating that the caloric content could have an important role. However, it is unclear as to how the caloric content of the diet would be sensed by the gastrointestinal tract. The SDA is larger with a high-protein diet compared to a high-fat diet. Therefore, since the oxygen status of the gastrointestinal tissues is one of the most important regulators of GBF, it is not easy to delineate how the caloric content of the diet would influence the response.

Other factors that in combination to the nutrient composition could affect GBF include the amount of bile secreted, which in mammals potentiates the effects of certain nutrients on GBF.

Integrating the Mechanical and Chemical Stimuli and Relaying the Signal to the Level of the Vessels

Even though we now consider that chemical and mechanical stimuli combine in some way to trigger the postprandial increase in GBF, it is still uncertain in what way these stimuli trigger this response. Several theories have been proposed, some of which are more likely than others. As the knowledge concerning the regulation of GBF increases several of factors, the work in concert to give the overall response will likely emerge as being important.

Extrinsic signals and mechanical stimuli

Mechanical stimuli are most likely sensed by mechano/stretch-receptors in the wall of the stomach. Such receptors are located within the circular muscular layer and relay their signals via the tenth cranial nerve (the vagus) to the central nervous system, where integration and processing can occur. Subsequently, efferent adrenergic pathways to the peripheral systemic vasculature regulate the vascular resistance via α-adrenoceptors (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). A simple reflex pathway such as this would explain the rapidity of the postprandial response, with the vasoconstriction occurring within seconds after the stimulus (**Figure 3**).

Extrinsic signals and chemical stimuli

The signals that trigger the chemically induced GI hyperemia could be either extrinsic or intrinsic to the GI tract. Extrinsic signals would involve pathways outside of the gut, such as the sympathetic or parasympathetic nervous system as well as endocrine substances (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). Extrinsic signals are beneficial in that they enable GBF to be regulated in accordance with the needs of the other tissues of the animal. However, this regulation is not as rapid as and possibly also less sensitive than signals working within the GI tract.

The nervous system. The autonomic nervous system, divided into the sympathetic, parasympathetic, and enteric nervous system based on the anatomical as well as the physiological properties, is of fundamental importance to the regulation of blood flow in an animal. It serves to regulate the supply to different tissues depending on the demand of the different tissues such that tissues with high oxygen consumption receive more blood compared to less active tissues. However, tissues that are crucial to the survival of the animal and need a constant supply of oxygenated blood, such as the heart and brain, rely very little on autonomic control of blood flow, but more on local signals. In fish, the vascular system is for the most part under the control of the sympathetic innervation, as well as circulation catecholamines (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes). Whether vasodilation or vasoconstriction occurs depends on adrenergic receptors that are present in that particular tissue (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). There are, however, deviations from this generalization. The vasculature of the gills is for example heavily dependent on parasympathetic innervation as well as circulating hormones.

During fasting, the tone of the GI resistance vessels is under the control of the sympathetic nervous system via the splanchnic nerve. This is to be expected given that there is a redistribution of blood away from the GI tract during stressful situations such as exercise, hypoxia, or general stress as discussed below and in **Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation. However, the role of the autonomic nervous system during postprandial regulation of GBF is far from clear.

Few studies have been conducted as to reveal an involvement of the autonomic nervous system in the postprandial hyperemia and increase in GBF. Nevertheless, results obtained in mammals, as well as from a few fish studies, indicate that the sympathetic and parasympathetic nervous systems are of little importance to the hyperemia. Sectioning the nerves to the GI tract or blocking the effects of these nerves using antagonistic drugs such as atropine (muscarinic antagonist that blocks the action of the neurotransmitter acetylcholine) has very little influence on the GBF response.

Extrinsic nerves could nevertheless be of importance when several tissues compete for a limited blood supply as is the case when a fish has recently ingested a large meal and is subsequently exposed to some sort of a stressor.

Hormones. Hormones such as cholecystokinin (CCK) and gastrin are probably of importance in regulating and coordinating the GBF given their wide range of functions in the gut, including control of gastric emptying as well as bile production and secretion. The few studies that have been conducted show very differing results and it has not been possible to elude whether the effects are primary or secondary to, for example, an increased pancreatic metabolism.

Although the plasma concentration needed to induce an increase in GBF are well above the plasma levels seen after feeding, it is still possible that these substances work in a paracrine fashion (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). This means that there could be much higher levels of the peptide at certain locations along the gut.

In rainbow trout it has been shown that GBF is indeed increased when CCK is injected intra-arterially within a physiological range, possibly through a decrease in the vascular resistance of the gut. Nevertheless, more work in this field is definitely needed in order to gain insight into the role of hormones and how they function in this perspective.

Intrinsic signals and chemical stimuli

Intrinsic regulation of GBF in response to chemical stimuli would allow a very precise regulation of blood flow according to the present demand. Such regulation would resemble for example the regulation that is seen in and around cerebral arteries in the brain

Metabolite signals and oxygen demand (SDA). The most probable trigger for the chemically induced hyperemia is a change in oxygen tension/content, occurring with increased secretion, absorption, and assimilation. The SDA leads to a lowering of the venous oxygen levels, and the regional hypoxia acts either directly on the vessel or induces the release of other vasoactive metabolites, such as lactate, hydrogen ions, adenosine diphosphate (ADP) and nitric oxide (NO) from the surrounding tissues. This creates a localized hyperemia in the parts of the intestine that are filled with hydrolyzed food. A regional hypoxia could also be sensed by red blood cells, leading to the release of adenosine triphosphate (ATP), thus further contributing to the hyperemia.

Proteins or amino acids account for as much as 70–80% of the SDA, due to the assimilation of proteins through catalytic and analytic processes. Even so, proteins are not necessarily the most potent inducer of the postprandial hyperemia.

Processes such as gut motility and peristalsis (**see also Integrated Function and Control of the Gut**: Gut Motility) produce insignificant increases in oxygen consumption and are also of little importance in inducing a hyperemia. Gut motility can, however, lead to a redistribution of blood within the gut wall, thus perhaps affecting absorption. There are also secondary effects and the gut motility also affects the mixing and propulsion of the food through the GI tract. This influences the absorption and hence the GI blood flow.

A change in the osmolarity of the gut tissue is probably also important. Osmolarity increases as nutrients are hydrolyzed.

Local nerves/paracrine signaling. Local nerves that are intrinsic to the GI tract and as such belong to the enteric nervous system (**see also Integrated Function and Control of the Gut**: Endocrine Systems of the Gut) have the potential to relay signals from a sensor (oxygen levels or osmolarity, etc.) to the vasculature. In a very recent study the postprandial hyperemia was abolished after the inhibition of the enteric nervous system using the voltage-gated sodium channel blocking drug TTX. However, since the intrinsic nerves are involved in virtually every aspect of the gastrointestinal physiology, from gut motility to secretion of digestive enzymes and mucus (**see also Integrated Function and Control of the Gut**: Gut Motility), it is still unclear exactly what function or functions are important. Some can be eliminated, leaving only a handful of possibilities. For example, gut motility and digestive enzymes contribute little to the hyperemia. It is likely, therefore, that some of the more integrative roles of the enteric nervous system are crucial to the ability to increase GBF postprandially (see **Video Clip 1** for a short animation showing the different events described above).

Other Factors Influencing the Response of the Circulatory System after Feeding

In ectotherms, body temperature is directly affected by environmental temperature and these animals can only regulate their body temperature behaviorally. Thus, even minor changes in the environmental temperature can have profound effects of the physiology including digestion and absorption (**Figure 4**). Other factors such as stress, hypoxia, hypercapnia, and exercise also affect the postprandial physiology. There is some information

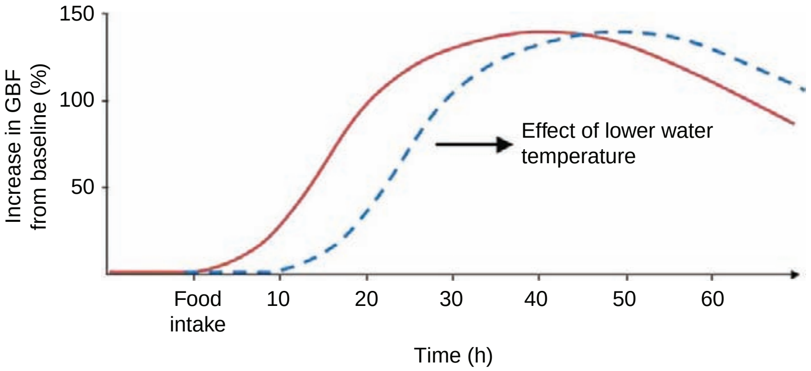


Figure 4 Potential effect of temperature on the gut blood flow postprandially in a fish.

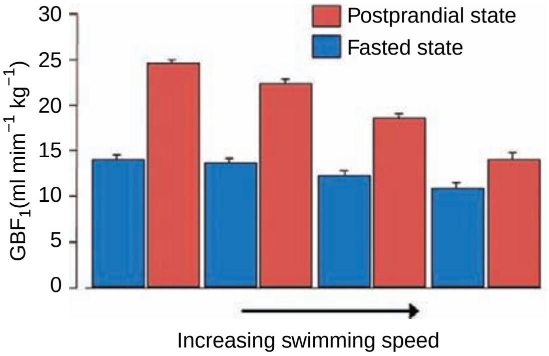


Figure 5 Effect of exercise on gastrointestinal blood flow during routine and postprandial conditions in the sea bass.

available on the effects of this stimulus in fasted animals (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation for more information), but little on the effects in postprandial animals. As stated above, most animals studied so far cannot perfuse the entire cardiovascular system at the same time. Instead, there has to be some integration in response to the demands of the different tissues and the subsequent prioritization. For example, during exercise (**Figure 1**; **see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation for more information about this), GBF is reduced to allow more blood to be directed for fight or flight responses. However, salmonids and several other teleost subgroups seem to protect the gastrointestinal blood flow response after feeding and are thus able to maintain some level of digestion and absorption even during exercise. The consequences of this may lead to a reduced maximal swimming performance. Other species such as the agile sea bass (*Dicentrarchus labrax*) show a clear reduction in postprandial GBF exercise (**Figure 5**), presumably reducing GI metabolism and absorption. The portion of the SDA that is related to processes outside of the GI tract could nevertheless be maintained.

Ultimately, the knowledge of the integrated responses of the circulatory system during digestion in fish is based on relatively few studies and given that there are 30 000+ different fish species, (i.e. about half of all vertebrates), any extrapolation to include more species could be problematic.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels; The Gastrointestinal Circulation. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes; Cardiac Cellular Length-Tension Relationship; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Cellular Ultrastructure of Cardiac Cells in Fishes; Intracardiac Neurons and Neurotransmitters in Fish; Physiology of Cardiac Pumping; The Outflow Tract from the Heart. **Gut Anatomy and Morphology**: Gut Anatomy. **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut; Gut Motility; Gut Secretion and Digestion; Intestinal Absorption; Nervous System of the Gut.

Further Reading

Altimiras J, Claireaux G, Sandblom E, *et al.* (2008) Gastrointestinal blood flow and postprandial metabolism in swimming sea bass *Dicentrarchus labrax*. *Physiological and Biochemical Zoology* 81(5): 663–672.

Axelsson M, Altimiras J, and Claireaux G (2002) Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. *Journal of Experimental Biology* 205: 2891–2896.

Axelsson M, Driedzic WR, Farrell AP, and Nilsson S (1989) Regulation of cardiac output and gut blood flow in the searaven, *Hemitripterus americanus*. *Fish Physiology and Biochemistry* 6: 315–326.

Axelsson M and Fritsche R (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod, *Gadus morhua*. *Journal of Experimental Biology* 158: 181–198.

Axelsson M, Thorarensen H, Nilsson S, and Farrell AP (2000) Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: Long-term effects of feeding and adrenergic control. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 170: 145–152.

Crocker CE, Farrell AP, Gamperl AK, and Cech JJ, Jr. (2000) Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 279: R617–R628.

Farrell AP, Thorarensen H, Axelsson M, *et al.* (2001) Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 128: 551–563.

Secor SM (2008) Specific dynamic action: A review of the postprandial metabolic response. *Journal of Comparative Physiology B* 179(1): 1–56.

Seth H and Axelsson M (2009) Effects of gastric distension and feeding on cardiovascular variables in the shorthorn sculpin (*Myoxocephalus scorpius*). *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 296: R171–R177.

Seth H, Sandblom E, and Axelsson M (2009) Nutrient-induced gastrointestinal hyperemia and specific dynamic action in rainbow trout (*Oncorhynchus mykiss*) – importance of proteins and lipids. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 296: R345–R352.

Seth H, Sandblom E, Holmgren S, and Axelsson M (2008) Effects of gastric distension on the cardiovascular system in rainbow trout (*Oncorhynchus mykiss*). *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 294: R1648–R1656.

Thorarensen H, Gallaugher PE, Kiessling AK, and Farrell AP (1993) Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematrocrit on blood flow distribution. *Journal of Experimental Biology* 179: 115–129.

Relevant Websites

http://esi.stanford.edu – Environmental Science Investigation.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Integrated Cardiovascular Responses of Fish to Swimming

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Aerobic Swimming
Anaerobic Swimming
Summary
Further Reading

Glossary

Cardiac output ($\dot{V}_b$) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Cardiac stroke volume ($\dot{V}_s$) Volume of blood pumped out of the ventricle in a single heartbeat; the difference between ventricular end-diastolic volume and end-systolic volume.

End-diastolic volume Volume of blood in a cardiac chamber at the end of filling (diastole).

End-systolic volume Volume of blood in a cardiac chamber at the end of emptying (systole).

Excess post-exercise oxygen consumption (EPOC) The amount of oxygen that is consumed after exercise has stopped. EPOC represents the oxygen cost of recovering from an exercise bout and includes the replenishment of oxygen stores and metabolites.

Gill secondary lamella(ae) The structures on the gill filaments where the majority of gas exchange occurs between the fish and the surrounding water.

Medulla oblongata Hindmost portion of the brainstem that contains the vagal motor and sensory areas, the respiratory rhythm generator, and cardiovascular control center.

Oxygen consumption ($\dot{M}_{O_2}$) Amount of oxygen consumed by an animal per unit time.

Tissue oxygen extraction The amount of oxygen removed from the blood as it passes through the tissues. It can be calculated from the oxygen content of arterial blood less that of venous blood.

Vis-a-fronte A term for a supplementary cardiac filling mechanism that uses energy directly from ventricular contraction; it is literally the force from in front.

Introduction

Swimming in fishes can be loosely grouped as either pay now (sustained, aerobic swimming activity) or pay later (burst, anaerobic swimming activity) in terms of the cardiovascular system. This simple principle acknowledges that the primary role of the circulatory system is to provide oxygen (O_2) convection to support locomotory muscles either during and/or after activity. Of course, nothing is that simple. The many swimming modes used by fish variably involve anaerobic and aerobic muscle contractions. These contributions are sometimes difficult to separate experimentally. In fact, the prolonged swimming test most commonly used to examine cardiorespiratory responses, the critical swimming speed test, typically involves a gait transition from aerobic to anaerobic swimming as salmonids reach their top speeds. Thus, while pay-as-you-go aerobic swimming is used for most of the prolonged swimming test, the high-speed anaerobic swimming at the end of the test is a pay-later type that can create a substantial, excess post-exercise oxygen consumption (EPOC). Thus, some of the cost of swimming is repaid either after activity has stopped, or at a lower swimming speed when there is capacity to do so. Furthermore, O_2 convection typically lags behind the initial tissue O_2 demand when aerobic swimming starts, creating an initial mismatch between O_2 supply and demand, which also results in an EPOC following aerobic swimming when the debt is repaid.

Nevertheless, the cardiovascular responses following burst activity are qualitatively similar to those during sustained activity. In fact, peak O_2 consumption ($\dot{M}_{O_2}$) for burst and sustained swimming can be similar. An important difference is that the cardiovascular changes that support peak $\dot{M}_{O_2}$ occur during aerobic swimming activity and can be maintained for many hours in some species, whereas peak $\dot{M}_{O_2}$ for burst activity occurs immediately after swimming ceases and decays rapidly, as do the cardiovascular changes. This article first considers the integrated cardiovascular changes that support sustained, aerobic swimming and then briefly turns to those associated with recovery from burst, anaerobic swimming.

Aerobic Swimming

How Is More O_2 Delivered to the Tissues?

The Fick equation for O_2 convection is a useful framework for understanding cardiovascular changes that accompany swimming. It states that $\dot{M}_{O_2}$ is the product of cardiac output ($\dot{V}_b$) and the O_2 extracted from the blood by tissues. Typically, as $\dot{M}_{O_2}$ increases exponentially with swimming speed, there are similar increases in $\dot{V}_b$ and tissue O_2 extraction. Thus, if maximum aerobic swimming results in a fourfold increase in $\dot{M}_{O_2}$, which is easily attainable for many fish species, this increase could come about by doubling both $\dot{V}_b$ and O_2 extraction. The following considers how such increases come about.

How Is $\dot{V}_b$ Increased?

$\dot{V}_b$ is increased by increasing both heart rate (f_H) and cardiac stroke volume ($\dot{V}_s$) in some combination. As described in **Design and Physiology of the Heart**: Physiology of Cardiac Pumping, Cardiac Cellular Length-Tension Relationship, fishes have a remarkable capacity to increase $\dot{V}_s$. For example, during swimming in rainbow trout (*Oncorhynchus mykiss*), $\dot{V}_s$ increases by up to a threefold but f_H increases by only 50%. Tunas, however, mainly increased f_H in response to swimming activity because $\dot{V}_s$ seems to be routinely near its maximum capacity (**see also Pelagic Fishes**: Physiology of Tuna). Other fish groups increase f_H more so than $\dot{V}_s$, while some show similar increases in f_H and $\dot{V}_s$. Thus, fishes use frequency and volume to varying degrees to modulate the heart's output and thereby increase O_2 convection during aerobic swimming. Swimming studies with considerably more fish species are needed before a generalized picture of the interplay of increasing $\dot{V}_s$ and f_H will emerge.

What is already clear, however, is that when athletic fishes swim at maximum $\dot{M}_{O_2}$, their heart is operating at or very near its maximum capacity. In fact, some argue that the circulatory system ultimately limits the maximum level of aerobic swimming.

How Is f_H Increased during Aerobic Swimming?

The cardiorespiratory control centers in the medulla oblongata of the brain (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish) regulate the increase in f_H during swimming by changing autonomic nervous output to the heart (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes), as well as to other organs such as the spleen and head kidney.

Routine f_H is normally under simultaneous but opposing inhibitory (vagal cholinergic) and stimulatory (sympathetic adrenergic) control. Thus, f_H can increase during swimming by reducing or removing vagal cardiac tonus, much like the acceleration of a car going downhill when you remove your foot from the brake. Fish can also press the accelerator to increase f_H during swimming by increasing adrenergic cardiac stimulation. This stimulation may come directly from cardiac sympathetic innervation, if present (as in some teleosts), or from catecholamines released into the circulation from the chromaffin tissue of the head kidney, typically as a result of sympathetic nervous stimulation. However, this humoral mechanism is recruited only in stressful swimming situations (**see also Hormonal Responses to Stress**: Hormone Response to Stress and Catecholamines). Therefore, given that many fish species lack cardiac sympathetic innervation, the extent of routine vagal tone is the primary determinant of how much f_H increases during aerobic swimming. However, handling and surgical stress can reduce vagal cardiac tonus, which means fish should be fully rested and recovered when assessing the contribution of vagal tone to increased f_H.

How Is $\dot{V}_s$ Increased during Aerobic Swimming?

First the conditions for venous return must be such that they fill the heart with more blood (increased end-diastolic volume) between heartbeats. Then, cardiac muscle must contract more forcefully to empty the extra blood in the heart (to keep end-systolic volume small) (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). Cardiac contraction can become more forceful because of either the heart's intrinsic response to increased filling (the Frank–Starling effect; **see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and Cardiac Cellular Length-Tension Relationship) or an adrenergic stimulation of cardiomyocytes (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart). Likely, a combination of several mechanisms increase venous return to the heart during aerobic swimming, including mobilization of blood from venous capacitance vessels, probably as a result of sympathetic vasoconstriction (**see also Design and Physiology of Arteries and Vein**: Physiology of Capacitance Vessels); increased venous blood pressure; a greater suction of blood into the heart created by increased force of cardiac contraction (see *vis-a-fronte* cardiac filling in **Design and Physiology of the Heart**: Physiology of Cardiac Pumping); and skeletal muscle contractions physically compressing veins and massaging blood toward the heart.

How Is Tissue O_2 Extraction Increased during Aerobic Swimming?

Hemoglobin in arterial blood remains at or near full O_2 saturation (for details, **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin and O_2 Uptake and Transport: The Optimal P_{50}) during swimming. Therefore, increased tissue O_2 extraction is produced by reducing the O_2 partial pressure (P_{O_2}) and the O_2 content of venous blood. Normally, as much as two-thirds of the O_2 contained in arterial blood is returned unused in venous blood to the heart. Thus, theoretically, tissue O_2 extraction can increase threefold. In reality, the O_2 content of venous blood never becomes fully depleted by locomotory muscles during aerobic swimming. This is just as well because the last organ to be supplied by the circulatory system in most fishes (**see also Design and Physiology of the Heart**: The Coronary Circulation) is the heart. Many fishes do not have a coronary circulation and the heart therefore depends entirely on the O_2 contained in venous blood.

Where Does Cardiac Output Go to during Aerobic Swimming?

Besides more blood being pumped around the circulatory system, the distribution of blood flow changes during aerobic exercise. Blood flow is redistributed by altering vascular resistance among tissues, which is achieved through vasoactivity, the control of the diameter of arterioles through various neural, humoral, and local mechanisms (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). An increase in $\dot{V}_b$ without any vasoactivity would simply increase blood flow to all tissues by the same percent.

Increasing blood flow to locomotory muscles is achieved in part by decreasing the resistance of its blood vessels (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). How central and local arteriolar control mechanisms are integrated to bring about this vasodilation is unknown. Even so, the potential importance of local control should not be underestimated. For example, increased CO_2 production during aerobic swimming and tissue lactate and H^+ production during anaerobic swimming can lead to vasodilation. Of course, improved muscle blood flow also improves waste removal.

The heart does not have the capacity to fully perfuse all circulations at one time (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). Therefore, blood flow to tissues with a lower O_2 priority must be reduced at some point. In what order fish prioritize circulations during aerobic swimming has yet to be studied. Even so, the integument is clearly a lower-priority circulation that can account for about one-third of $\dot{V}_b$ in fish (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation). Not only is gut blood flow reduced during swimming, this decrease is linearly related with the increase in $\dot{M}_{O_2}$, suggesting tight integration with swimming effort. Integration of blood flow among tissues requires central controls, but their nature is not understood for fish.

The other reason for blood flow redistribution during aerobic swimming is because central arterial blood pressure is tightly regulated (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System). Vasodilation of locomotory muscles would decrease central arterial blood pressure unless there was a corresponding increase in vascular resistance of another tissue or an increase in $\dot{V}_b$ (i.e., one that matched the increase in muscle blood flow). As it turns out, aerobic swimming results in a modest increase in arterial blood pressure. Thus, the decrease in overall systemic vascular resistance associated with blood flow redistribution does not quite offset the increase in $\dot{V}_b$. Therefore, central control of vascular resistance among vascular beds, in concert with increased $\dot{V}_b$, is a hallmark of efficient O_2 convection during activity. Exactly how this is integrated requires further study.

How Is Oxygen Loading at the Gills Improved?

Fish increase gill ventilation rate to bring more water and hence more O_2 to the gill secondary lamellae. However, breathing water with faster and deeper ventilations (**see also Control of Respiration**: The Ventilatory Response to CO_2/H^+ and Generation of the Respiratory Rhythm in Fish) has a substantial O_2 cost. Ram ventilation can help by transferring O_2 costs of ventilation to the locomotory muscles. Some fishes, typically the athletic ones, cease respiratory movements completely when they attain a certain swimming speed and rely solely on ram ventilation. They simply adjust mouth gape to regulate gill ventilation rate.

Circulatory adjustments also benefit gill O_2 uptake during aerobic swimming. These are best appreciated in the context of the Fick equation for diffusion (**see also Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs). When applied to the gills, it states that the rate of O_2 diffusion is proportional to the product of the lamellar surface area available for diffusion (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics and Plasticity in Gill Morphology) and the P_{O_2} gradient, and inversely proportional to the O_2 diffusion distance between the water flowing over the secondary lamella and the blood they contain.

Changes in blood flow to and within secondary lamellae result in a faster rate of O_2 diffusion by increasing the surface area for gas exchange and decreasing the effective diffusion distance. Not all secondary lamellae receive blood flow at rest. Thus, by opening blood flow to all secondary lamellae (termed lamellar recruitment) during swimming can increase the total gill surface area for exchange by as much as 50%. Various vasoactive and physical mechanisms are involved in opening up lamellae (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels). Lamellar recruitment decreases the vascular resistance of the gills because secondary lamellae represent parallel vascular beds (**see also Circulation**: Circulatory System Design: Roles and Principles). While an increase in $\dot{V}_b$ fills these extra lamellae, the increase in $\dot{V}_b$ exceeds the decrease in gill vascular resistance and so ventral aortic blood pressure increases somewhat. This increase in blood pressure and possibly changes in tension of the pillar cells in the secondary lamellae (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy) redistribute blood flow within a secondary lamella, creating a more even blood flow pattern. This redistribution of blood flow within lamellae decreases the overall diffusion distance for O_2 by moving more blood toward regions of the lamella that have a thinner barrier between the blood and the water.

During aerobic swimming, changes in the P_{O_2} of arterial blood leaving the gills are relatively small and have little effect on arterial O_2 concentration (**see also Gas Exchange**: Respiration: An Introduction). Instead, the P_{O_2} gradient across the gills (and hence the rate of O_2 diffusion) increases as a result of a decrease in venous P_{O_2} (see below). Release of stored red blood cells from the spleen (again, under autonomic sympathetic nervous control; **see also Brain and Nervous System**: Autonomic Nervous System of Fishes) can increase the arterial O_2-carrying capacity.

In summary, the increased rate of gill O_2 diffusion required for aerobic swimming comes from a combination of a decrease in venous P_{O_2} to increase the gradient for O_2 diffusion, an increase in the effective surface area for O_2 exchange and a decrease in the overall O_2 diffusion distance. Consequently, fish alter all variables in the Fick equation to improve O_2 diffusion, and then increase the rate at which this O_2 is transported away from the gills to the locomotory muscles by increasing $\dot{V}_b$, releasing more red blood cells into the circulation and redistributing blood flow.

How Is Oxygen Unloading at the Tissues Improved during Aerobic Swimming?

The Fick equation for O_2 diffusion can be applied to the tissues as well. At tissues, the rate of O_2 diffusion is proportional to the product of the tissue capillary surface area and the P_{O_2} gradient, and inversely proportional to the diffusion distance for O_2 between mitochondria and the nearest capillary. The most important circulatory change is increasing blood flow to more capillaries. By doing so, the effective surface area for O_2 diffusion is increased and the O_2 diffusion distance is reduced. Improved diffusion conditions increase tissue O_2 extraction, lowering venous P_{O_2} and O_2 content. However, venous blood rarely becomes fully depleted of O_2 (extreme hypoxia being an important exception). As originally proposed by August Krogh and his wife almost a century ago, this is because any given capillary arrangement for muscle will normally leave a certain amount of O_2 in venous blood because of a diffusion limitation.

Changes to the P_{O_2} at the mitochondia (about 0.1 kPa) and arterial blood are relatively minor by comparison. However, P_{O_2} at the capillary can be drastically altered by increased tissue release of CO_2 and H^+ associated with swimming. Acidification of capillary blood has a profound effect on hemoglobin O_2-binding characteristics (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange), which effectively raises the capillary P_{O_2} and greatly enhances O_2 diffusion into the tissues. Again, a local control mechanism features prominently in O_2 delivery.

The anatomical arrangement of muscle capillaries clearly sets the diffusion conditions for O_2 once all capillaries receive blood flow. Not surprisingly, and in line with their relative O_2 needs, aerobic muscle has a much higher capillary density and lower diffusion distance than anaerobic white muscle (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis). Even so, muscle capillarity in fish is plastic, allowing the anatomical diffusion conditions to be varied. Changes in muscle capillarity are known to occur with temperature acclimation, exercise training, and growth (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis).

Anaerobic Swimming

Swimming modes powered by anaerobic muscle contractions are necessarily brief, but they are typically more powerful than aerobic modes. $\dot{M}_{O_2}$ cannot be measured during such brief swimming events, but is measured afterward. Hence, these swimming modes are loosely grouped as pay later. It is presumed that fish repay the accumulated debt as fast as possible and can recruit the maximum capacity of the cardiovascular system immediately after the swimming bout to do so. Thus, the initial peak $\dot{M}_{O_2}$ during EPOC can approach or equal that reported for maximum aerobic swimming.

Little is known of the cardiovascular changes associated with anaerobic, burst swimming. Those changes that follow burst activity seem to resemble those occurring during aerobic swimming, that is, increased $\dot{V}_b$, f_H, $\dot{V}_s$, arterial blood pressures, and tissue O_2 extraction. The main difference is the decay in these responses following a peak shortly after the activity, unlike during aerobic swimming when steady-state responses prevail. In addition, venous P_{O_2} can decrease below the level seen with maximum aerobic swimming. Visceral blood flow is also reduced when fish struggle, a response that lasts several minutes, even when the burst activity lasts a few seconds (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation). If burst activity is excessive or chronic, loss of gut blood flow could have negative consequences for digestion time, reducing digestion efficiency (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion) and potentially growth.

An obvious cardiovascular problem will accompany particularly strenuous bursts of activity. When a muscle contracts at ~70% of its maximum tension, it generally closes off capillaries as a result of compression. Thus, a vigorous tail beat temporarily starves skeletal muscle of its blood flow, which resumes during muscle relaxation. However, as tail-beat frequency increases, the time available for muscle blood flow decreases at a time when muscles most need more O_2. This mechanical compression event likely contributes to EPOC.

Maintaining $\dot{V}_b$ during extensive vascular compression would increase arterial blood pressure in proportion to the increase in vascular resistance. Thus, fish briefly slow f_H and reduce $\dot{V}_b$ when they initiate a burst swim, even for a couple of tail beats. Anticipating such events requires central control of f_H via the vagus nerve. Some have argued that this response represents an important mechanism to protect the delicate blood vessel gill secondary lamellae that lie between the heart and the locomotory muscle, a position that exposes the gills to any excessive buildup of blood pressure in the dorsal aorta.

Summary

By examining the cardiovascular changes associated with aerobic swimming within the context of Fick equations that govern O_2 convection and O_2 diffusion, it is clear that fish take advantage of a variety of mechanisms to improve O_2 delivery to their locomotory muscles both during and after swimming. The suite of cardiovascular changes that follow anaerobic, burst swimming are quite similar to those that are maintained during aerobic swimming, but is preceded by a protective temporary slowing of f_H. Major studies are still needed to understand how autonomic neural output from the cardiorespiratory centers of the brain and local controls is mechanistically integrated.

See also: **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish; The Ventilatory Response to CO_2/H^+. **Design and Physiology of Arteries and Veins**: Branchial Anatomy; Physiology of Capacitance Vessels; Physiology of Resistance Vessels; The Gastrointestinal Circulation. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis. **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Cardiac Cellular Length-Tension Relationship; Physiology of Cardiac Pumping; The Coronary Circulation. **Gas Exchange**: Respiration: An Introduction. **Hormonal Responses to Stress**: Catecholamines; Hormone Response to Stress. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Integrated Function and Control of the Gut**: Gut Secretion and Digestion. **Pelagic Fishes**: Physiology of Tuna. **Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin; O_2 Uptake and Transport: The Optimal P_{50}. **Ventilation and Animal Respiration**: Efficiency of Gas Exchange Organs; Gill Respiratory Morphometrics; Plasticity in Gill Morphology.

Further Reading

Brill RW and Bushnell PG (2001) The cardiovascular system of tunas. In: Block BA and Stevens ED (eds.) *Tuna Physiology, Ecology and Evolution,* 1st edn., vol. 19, pp. 79–120. San Diego, CA: Academic Press.

Burggren WW, Farrell AP, and Lillywhite H (1997) Vertebrate cardiovascular physiology. In: Dantzler WH (ed.) *The Handbook of Physiology*, pp. 215–308. New York, NY: American Physiological Society.

Bushnell PG, Jones DR, and Farrell AP (1992) The arterial system. In: Hoar WS, Randall DJ, and Farrell AP (eds.) *Fish Physiology Volume XII, Part A, Cardiovascular System*, pp. 89–139. San Diego, CA: Academic Press.

Egginton S (1990) Morphometric analysis of tissue capillary supply. In: Boutilier RG (ed.) *Vertebrate Gas Exchange from Environment to Cell,* Advances in Comparative and Environmental Physiology, vol. 6, pp. 73–141. New York: Springer.

Egginton S (2002) Temperature and angiogenesis: The possible role of mechanical factors in capillary growth. *Comparative Biochemistry and Physiology Part A* 132: 773–787.

Farrell AP (1982) Cardiovascular changes in the unanesthetized ling cod, *Ophiodon elongatus*, during short-term, progressive hypoxia and spontaneous activity. *Canadian Journal of Zoology* 60: 933–941.

Farrell AP (1996) Features heightening cardiovascular performance in fishes with special reference to tunas *Comparative Biochemistry and Physiology* 113A: 61–67.

Farrell AP and Clutterham SM (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *Journal of Experimental Biology* 206: 487–496.

Farrell AP, Eliason EJ, Sandblom E, and Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835–851.

Farrell AP and Jones DR (1992) The heart. In: Hoar WS, Randall DJ, and Farrell AP (eds.), Fish Physiology Volume XII, Part A, The Cardiovascular System, pp. 1–88. San Diego, CA: Academic Press.

Kiceniuk J and Jones DR (1977) The oxygen transport system in trout *(Salmo gairdneri)* during sustained exerices. *Journal of Experimental Biology* 69: 247–260.

Olson KR and Farrell AP (2006) The cardiovascular system. In: Evans DH and Claiborne JB (eds.) *Fish Physiology,* 3rd edn., pp. 119–152. Boca Raton, FL: CRC Press.
Randall DJ, Holeton GF, and Stevens ED (1967) The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *Journal of Experimental Biology* 46: 339–348.
Satchell G H (1991) *Physiology and Form of Fish Circulation*, pp. 1–235. New York: Cambridge University Press.
Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, and Farrell AP (2008) The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* 211: 3915–3926.
Stevens ED and Randall DJ (1967) Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *Journal of Experimental Biology* 46: 307–315.
Stevens ED and Randall DJ (1967) Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *Journal of Experimental Biology* 46: 329–337.

Integrated Responses of the Circulatory System to Hypoxia

AK Gamperl, Memorial University of Newfoundland, St. John's, NL, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Acute Hypoxia
Summary
Further Reading

Glossary

Acclimatize (acclimate) Physiological or morphological changes an organism experiences when exposed to chronic (prolonged) changes in environmental conditions.

Adrenergic receptor A receptor located in the cell membrane that binds the hormones adrenaline and noradrenaline. Binding of these hormones to this receptor initiates a signal cascade that results in a number of changes in cellular physiology.

Anoxia Complete absence of dissolved oxygen in water.

Bradycardia A slowing of normal heart rate. In fish, this condition is often associated with hypoxia (low environmental oxygen levels).

Diastole A period of the cardiac cycle when cardiac muscle is relaxed or relaxing. It is associated with filling the heart with blood.

Hyperplasia Growth by increase in the number of cells of an organ or tissue.

In situ Being in the original position; used to describe experiments where measurements are taken on an organ (tissue) while they are still located within the animal.

Myoglobin An oxygen-binding protein in muscle that resembles hemoglobin in function, and enhances the rate of oxygen diffusion.

Partial pressure of oxygen The pressure of a gas mixture is the sum of the partial pressures of the individual components. P_{O_2} is the partial pressure of oxygen, and is approx. 158 mmHg in fully saturated water at sea level: ~760 mmHg total gas pressure × 20.8% oxygen in the atmosphere.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Systemic vascular resistance An index of the resistance to blood flow in the arterial system. Calculated as arterial blood pressure/cardiac output.

Systole A period of the cardiac cycle when cardiac muscle is contracted or contracting. It is associated with the ejection of blood from the heart.

Teleost A bony fish of the subclass Teleostei, having rayed fins and a swimbladder.

Introduction

Aquatic habitats are subject to variations in many environmental parameters, and one of the most important affecting non-air-breathing vertebrates is dissolved oxygen. Hypoxia, or a subnormal concentration of oxygen, is a phenomenon that occurs in a wide variety of aquatic environments, from the Amazon drainage basin, to ice-covered shallow water bodies in winter and, ever increasingly, to coastal marine areas around the world (including the Black and Baltic Seas, the Gulf of Mexico, and the Gulf of St. Lawrence).

Most fish depend on oxygen contained in the water to supply their metabolic demands. This oxygen is taken up across the fish's gills and then transported to the tissues through the circulatory system. Thus, when water oxygen levels become subnormal (i.e., hypoxic), fish can make a number of adjustments. For example, they will move away from the area of low dissolved oxygen, decrease their activity and thus their requirement for oxygen, or make physiological adjustments that maintain oxygen delivery to the tissues. These changes can include modifications to: (1) ventilation rate or amplitude; (2) gas exchange across the respiratory surface (gills); (3) cardiovascular function; and (4) hemoglobin-oxygen binding and blood-oxygen carrying capacity. Given the critical role that the circulatory system plays in blood oxygen transport, and that fish heart function is solely or partially (for those with coronary arteries, or with lungs or accessory breathing organs) dependent on whatever oxygen is left in the venous blood after it has traversed the fish's other tissues, it is not surprising that the cardiovascular function of fishes during hypoxia has been an active area of research for over 50 years.

This article provides an overview of the cardiovascular responses to hypoxia (anoxia) in fishes. Information is divided into two broad categories for ease of presentation:

1. acute hypoxia, referring to minutes to hours of lowered water O_2 and
2. chronic hypoxia, where decreases in water O_2 levels last days or weeks, that is, long enough for the fish to potentially acclimatize to the limited oxygen conditions through changes in gene and protein expression.

Acute Hypoxia

Heart Rate

There are some taxonomic groups that do not exhibit changes in heart rate (f_H) when exposed to hypoxia (e.g., hagfish and lungfish), or where no clear response pattern to hypoxia has been established (e.g., Antarctic fishes). However, the most common cardiac response to hypoxia is reflex bradycardia (a decrease in f_H). This slowing of the heart is primarily mediated by an increase in inhibitory (cholinergic) nervous tone on the heart's pacemaker cells, and several direct benefits of hypoxic bradycardia to the fish heart have been proposed. These benefits include:

1. improved contractility of the heart muscle (myocardium) due to the negative force–frequency effect (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature);
2. enhanced oxygen delivery to the myocardium due to an increase in the residence time of blood in the heart's chambers (i.e., more time for oxygen diffusion), and stretching of the myocardium (i.e., decreased diffusion distance) in species that also respond to hypoxia with an increase in stroke volume (S_V, see below);
3. more efficient use of available oxygen due to slower rates of pressure development during systole; and
4. an increase in coronary blood flow (and thus oxygen delivery to the heart), due to an increase in the duration of diastole. Diastole is the part of the cardiac cycle where the majority of coronary blood flow occurs (~75–85%) (**see also Design and Physiology of the Heart**: The Coronary Circulation).

In fishes, the partial pressure of oxygen (P_{O_2}, in mmHg) at which bradycardia occurs varies greatly, from 110 mmHg in the dourado (*Salminus maxillosus*) to as low as 25 mmHg for the Amazonian fish (*Hoplias lacerdae*). What environmental factors determine the P_{O_2} at which bradycardia is initiated has not been extensively studied, but data from a number of investigations suggest that it is related to water temperature, and a fish's lifestyle and hypoxia tolerance. For example, the P_{O_2} at which bradycardia was initiated increased from ~20 to 60 mmHg in spangled perch (*Leiopotherapon unicolor*) acclimated to temperatures between 10 and 30 °C. The largemouth bass (*Micropterus salmoides*), which prefers shallow/weedy areas (i.e., a habitat prone to large fluctuations in dissolved oxygen), initiates bradycardia at least 45 mmHg later than the smallmouth bass (*Micropterus dolomieu*) which inhabits deeper and colder waters. Finally, the dourado and rainbow trout (*Oncorhynchus mykiss*), which are very active species that normally inhabit well-oxygenated waters, have thresholds for the induction of bradycardia of >70 mmHg, while those for the hypoxia-tolerant carp (*Cyprinus carpio*) and tench (*Tinca tinca*) are <40 mmHg. These relationships make sense given the increase in metabolism with temperature (two- to threefold change per 10 °C) and the negative influence that temperature has on the solubility of oxygen in water and on the capacity of hemoglobin to carry oxygen (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin).

From a physiological perspective, hypoxia-tolerant species often possess hemoglobins with a lower P_{50} value for hemoglobin-oxygen binding. Without a low P_{50}, the arterial blood would not be fully saturated at low water oxygen tensions, but this important aspect is covered elsewhere in this encyclopedia (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin).

Cardiac Output and Stroke Volume

With the exception of hagfish, all fish examined to date show an increase in S_V when hypoxia-induced bradycardia develops. The difference among species, however, lies in the oxygen level at which they initiate increases in S_V and the extent that the increase in S_V compensates for the effect of bradycardia on cardiac output (Q; the product of f_H and S_V). In general, fish exhibit three patterns as illustrated in **Figure 1**.

In response pattern 1 (e.g., dourado; sea bass, *Dicentrarchus labrax*), S_V increases are either simultaneous with the onset of bradycardia or begin once bradycardia is initiated (often near the limit of hypoxia tolerance). However, these increases in S_V do not compensate for the decrease in f_H and so Q falls almost continuously (albeit slower than f_H) as hypoxia progresses.

In response pattern 2 (e.g., dogfish shark, *Scyliorhinus canicula*; winter flounder, *Pleuronectes americanus*), S_V increases when bradycardia is initiated and this increase maintains Q at, or near, normoxic levels until hypoxia becomes severe.

In response pattern 3 (e.g., rainbow trout; Atlantic cod, *Gadus morhua*), S_V starts to increase well before the onset of hypoxic bradycardia (resulting in a significant increase in Q) and further increases in S_V once bradycardia is initiated maintain Q above normoxic levels until the fish reaches its hypoxic limit.

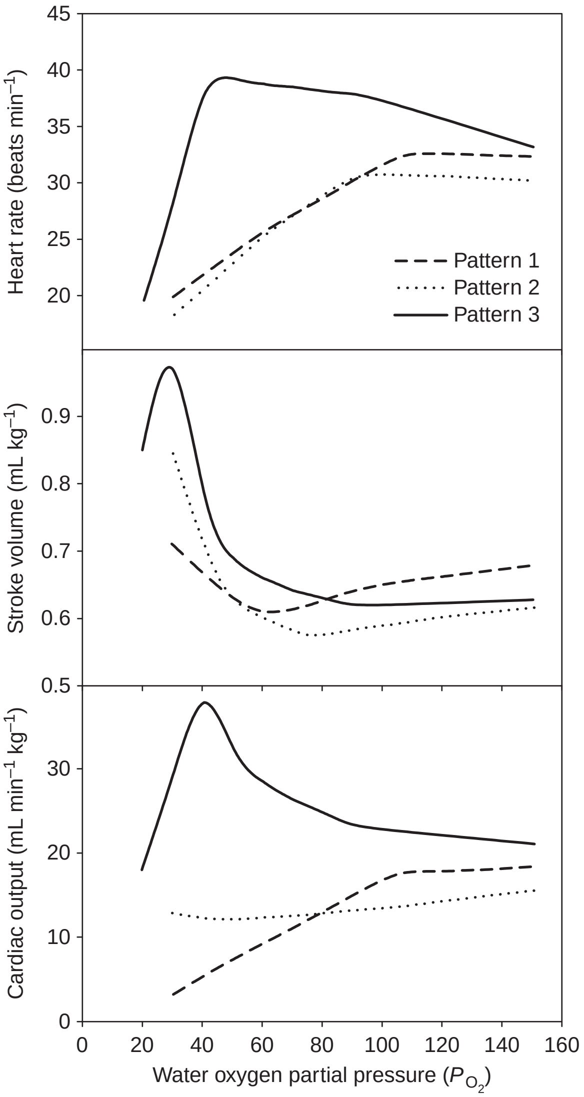


Figure 1 Schematic representation of the three patterns exhibited by fish cardiac parameters when exposed to acute hypoxia.

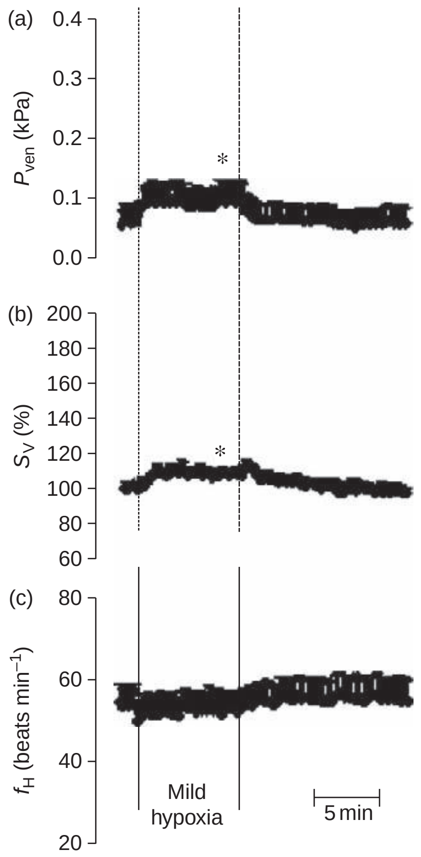


Figure 2 Effect of 8 min of mild hypoxia (P_{O_2} ~ 85 mmHg; section located between dotted lines) on central venous pressure (P_{ven}), stroke volume (S_V), and heart rate (f_H) in rainbow trout (*Oncorhynchus mykiss*). The asterisks indicate a significant difference between the average value for the normoxic period and the average value of the last 2 min of the hypoxic period. Modified from Sandblom E and Axelsson M (2005) Effects of hypoxia on the venous circulation in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology A* 140: 233–239, with permission from Elsevier.

Given the limited number of species on which direct measurements of $\dot{Q}$ and S_V have been performed under well-controlled experimental conditions, the extent that inter-specific variability in the responses of these parameters to environmental hypoxia relates to differences in activity, lifestyle, and/or hypoxia tolerance is unknown. However, we have some understanding of how S_V is controlled during hypoxia in fishes. For example, active regulation of venous tone (primarily through the stimulation of α-adrenergic receptors) is critical for regulating S_V in fishes during hypoxia. Venous pressure and mean circulatory filling pressure increase during hypoxia (e.g., see **Figure 2**), and these parameters are major factors promoting cardiac filling and increases in S_V, and therefore $\dot{Q}$, during hypoxia (**see also Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels).

Vascular Resistance and Arterial Blood Pressure

Generalizations about how these parameters are affected by hypoxia are difficult because of varied protocols and the species-specific nature of the response of arterial blood pressure to hypoxia (there are reports of no change, as well as large increases and decreases in blood pressure). However, considerable knowledge exists on the effects of hypoxia on gastrointestinal (GI) blood flow in teleosts, which then provides important insights into the regulation of systemic vascular resistance (R_{sys}) and blood flow distribution (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels, **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion).

Vascular resistance of vessels supplying the stomach, intestines, and other digestive organs increases by

1.5 times during severe hypoxia, and this decreases blood flow to these organs by 40–60%. The large increases in gut vascular resistance in Atlantic cod do not increase R_{sys} because the rest of the somatic (body) circulation is dilated at these water oxygen levels. This results in a redistribution of blood flow from the GI circulation to the body. Finally, the relative proportion of *Q* reaching the gut of sea bass during hypoxia depends on whether the fish has eaten or not: gut blood flow following exposure to hypoxia decreasing by approx. 50% in unfed sea bass but by only 20% after feeding. This indicates that locally released substances can offset the effects of hypoxia on GI vascular resistance and blood flow (i.e., the response is plastic).

Recent experiments have also revealed that hydrogen sulfide (H_2S) plays a very important role in regulating blood pressure and R_{sys} in fish during hypoxia. This may seem surprising given that H_2S is best known as a noxious and toxic gas. Yet, research has shown that H_2S is synthesized by vascular smooth muscle cells, that concentrations of H_2S in rainbow trout plasma reach levels that produce vasoactive effects in isolated vessels, and that hypoxia and H_2S evoke the same response irrespective of whether the particular vessel's response is contraction or relaxation.

Hypoxia Tolerance of the Heart

Even with the varied cardiac responses to hypoxia among species (see above), and with few exceptions (e.g., see the crucian carp below), there is a lower level of oxygen (i.e., P_{O_2}) at which cardiac function can no longer be sustained. This limit is again species specific, and depends highly on temperature and the cardiac workload being performed when oxygen levels fall. For example, when the *in situ* rainbow trout heart is perfused with saline that has a $P_{O_2} < 5$ mmHg (i.e., a severe level of hypoxia) at 10 °C, resting *Q* slowly falls to just 25% of initial levels within 30 min. By contrast, maximum *Q* cannot be maintained when P_{O_2} is < 70 mmHg, and the heart fails completely when pushed to develop maximum *Q* at P_{O_2} values < 20 mmHg. Further, with intense exercise, the venous blood becomes acidotic (has a lower pH) and hyperkalemic (elevated potassium ion concentration) due to the release of protons and K^+ from the working muscles. These perturbations in blood chemistry raise the P_{O_2} level at which hearts fail when asked to pump maximally to between 35 and 50 mmHg. Because saline does not contain as much oxygen as whole blood, these P_{O_2} thresholds for routine and maximum cardiac performance will be lower *in vivo*, but the results still stress the point that a less severe hypoxia (and associated conditions) impairs maximum cardiac function compared with routine cardiac function.

Temperature and severe hypoxia also have interactive effects on cardiac function. First, although severe hypoxia decreases *in situ* rainbow trout heart performance at all temperatures, the extent of the loss of performance is inversely related to temperature (**Figure 3**). This is not surprising given that water O_2 content decreases with temperature, while metabolic demand (and thus *Q*) generally goes up by a factor of 2–3 with each 10 °C increase. Nonetheless, lactate production increases proportionally with temperature, and this suggests that factors other than insufficient anaerobic energy production contribute to the inverse relationship between cardiac performance and temperature. Indeed, a faster accumulation of waste products likely contributes greatly to the heart's loss of function at high temperatures. These waste products include intracellular inorganic phosphate coming from the breakdown of creatine phosphate to creatine, and reduced intracellular pH associated with lactic acid production.

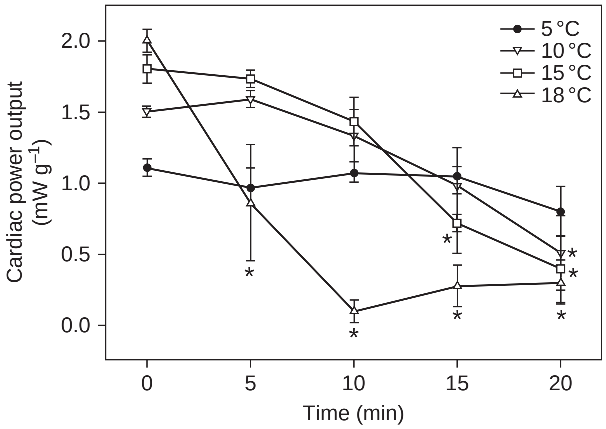


Figure 3 Effect of test temperature on changes in rainbow trout basal cardiac power output during 20 min of anoxic perfusion. An asterisk (*) indicates a value different from pre-anoxic values at a given temperature. Values are means ± SEM, *N* = 6–7. Trout were all acclimated at 10 °C. Note: Cardiac power output = *Q* × output pressure, and is an index of cardiac workload. Modified from Farrell AP and Stecyk JA (2007) The heart as a working model to explore themes and strategies for anoxic survival in ectothemic vertebrates. *Comparative Biochemistry and Physiology A* 147: 300–312, with permission from Elsevier.

Second, cardiac recovery after a hypoxic insult is also inversely related to temperature. This effect is not due to myocardial necrosis (cell damage) or differences in biochemical and energetic parameters, and thus likely caused by myocardial stunning (mechanical dysfunction that persists after reoxygenation due to a decreased responsiveness of myocardial contraction to Ca^{2+}). This decrease in responsiveness is thought to be caused by damage of the contractile proteins by oxygen radicals and/or Ca^{2+} overload, and is dependent on temperature and the duration of anoxia. Thus, the cardiac damage due to severe hypoxia is reduced at a lower temperature.

Finally, circulating catecholamines are very important for supporting cardiac function (i.e., increasing contraction frequency and strength) under conditions that would normally compromise heart function (e.g., hypoxia, acidosis, and hyperkalemia). However, their positive influence on heart function is greatly diminished at high temperatures in rainbow trout. Thus, an inability of adrenergic mechanisms to support cardiac function at high temperatures may limit fish myocardial hypoxia tolerance *in vivo* under conditions that require elevated cardiac performance (e.g., when swimming maximally).

Chronic Hypoxia

Surprisingly, few studies have examined the effects of chronic hypoxia on *in vivo* cardiovascular function. Recently, Atlantic cod were shown to have lower resting and maximum values for S_V and $\dot{Q}$ (by ~28%) after hypoxic acclimation (**Figure 4**). Potential explanations for the decline in pumping capacity of hearts from hypoxic acclimated cod include: (1) myocardial damage by constant exposure to low oxygen conditions;

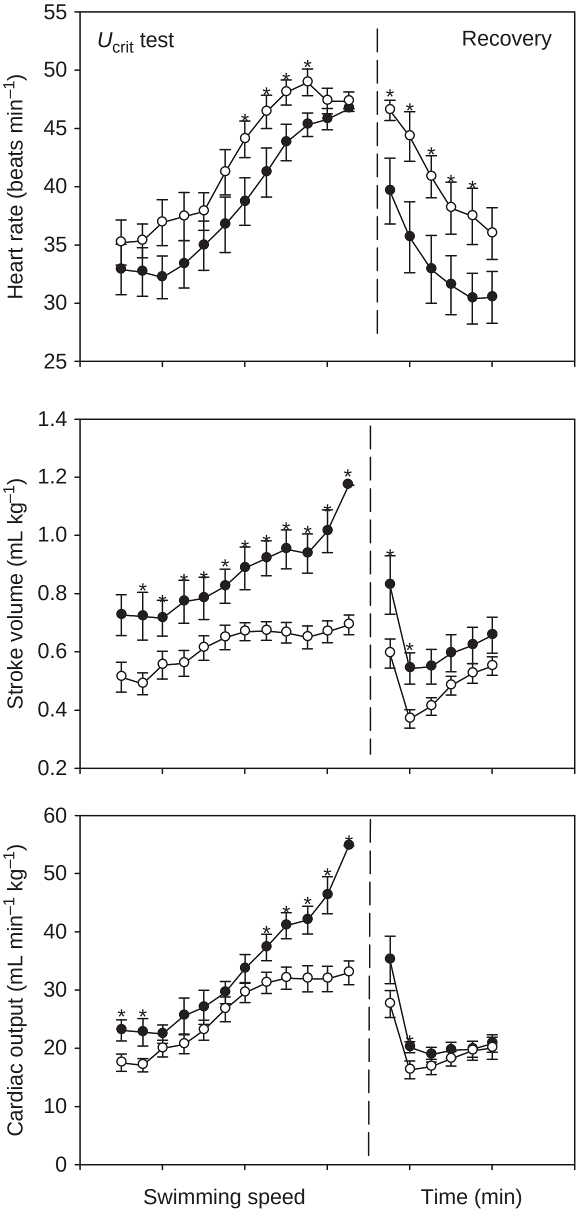


Figure 4 Cardiac parameters and oxygen consumption in normoxic- ($N = 10$, filled symbols) and hypoxic-acclimated (P_{O_2} 60 mmHg; $N = 12$, open symbols) Atlantic cod during a critical swimming speed (U_{crit}) test, and during post-exercise recovery. The asterisk (*) indicates a significant difference ($P < 0.05$) between the normoxic- and hypoxic-acclimated groups at a particular swimming speed. Reproduced from Petersen LH and Gampert AK (2010) Effects of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Attantic cod (*Gadus morhua*). *Journal of Experimental Biology* 213: 808–819, with permission from the Company of Biologists.

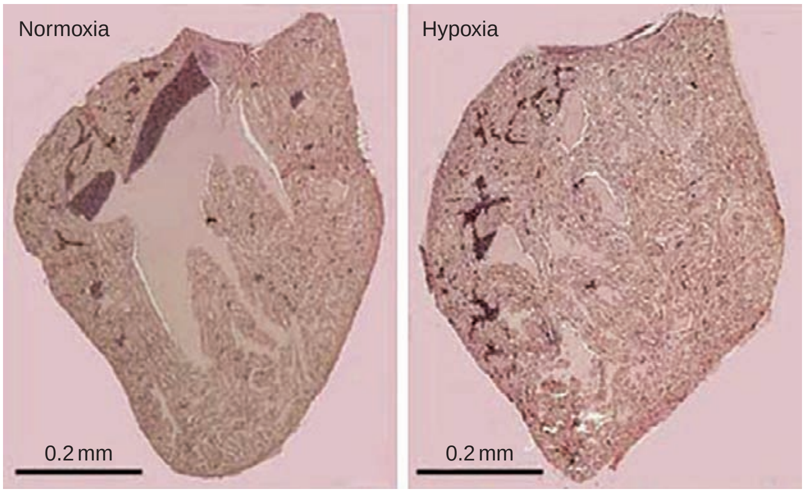


Figure 5 Histological changes in the cichlid (*Haplochromis piceatus*) heart after exposure to chronic constant hypoxia (P_{O_2} ~ 15 mmHg for 3 weeks). Heart of: (a) normoxia-acclimated individual and (b) hypoxia-acclimated individual. Hearts were sectioned and stained with hematoxylin–eosin. Reproduced from Marques IJ, Leito JTD, Spaink HP, *et al.* (2008) Transcriptome analysis of the response to chronic constant hypoxia in zebrafish hearts. *Journal of Comparative Physiology B* 178: 77–92. Reproduced with kind permission from Springer Science and Business Media.

(2) cardiac stunning (see above); and (3) hypoxia-induced myocardial remodeling, without any damage or stunning. In support of this final explanation, acclimation of zebrafish and the cichlid (*Haplochromis piceatus*) to water of 50% O_2 content increased cardiac myocyte density (presumably through hyperplasia) and this resulted in a reduction in the size of the central ventricular cavity (**Figure 5**). Such a decrease in the capacity of the ventricle to fill with blood would contribute to a decrease in maximum S_V, and Q, in hypoxia-acclimated Atlantic cod. Despite the diminished S_V, hypoxic-acclimated Atlantic cod had elevated f_H at rest and during exercise (see **Figure 4**). The mechanism(s) that resulted in the resetting of f_H in hypoxic Atlantic cod is unknown. However, as the f_H of *in situ* (isolated) hearts from hypoxic-acclimated Atlantic cod was similar to that of normoxic-acclimated hearts at resting or at maximal Q, the higher f_H in hypoxic-acclimated cod was clearly a result of altered neural and/or hormonal control. Finally, *in situ* hearts from hypoxia-acclimated Atlantic cod maintained maximum cardiac function longer during severe hypoxia and recover better.

Several strategies can enhance cardiac function during hypoxia, including reducing cardiac ATP demand, increasing cardiac glycolytic ATP production and extending the lower limit at which oxygen can be extracted from the blood. To date, only the first strategy has been identified in most fish species, including the Atlantic cod. Increases in the expression of genes related to glucose transport, uptake, and metabolism are short-lived (<6 days) when Atlantic cod are acclimated to hypoxia, and cardiac lactate dehydrogenase activity, myoglobin, and mitochondrial properties are unchanged. Hearts might reduce ATP demand by decreasing protein synthesis, Na^+K^+ATPase activity, and shortening of the action potential; the latter two possibilities may involve the opening of ATP-sensitive K^+ channels on the muscle cell's surface (i.e., sarcolemmal KATP channels). Finally, it is likely that the improved functional recovery shown by hearts from hypoxic-acclimated cod was at least partially due to an increased ability to protect the myocardium against the negative effects of reactive oxygen species (ROS). Indeed, six genes important for protection against ROS were upregulated in the zebrafish following 21 days of hypoxic acclimation.

Cardiac Function in the Crucian Carp

The crucian carp is the most hypoxia-tolerant fish known, and can survive without oxygen for days to months under the ice of shallow lakes. As such, it seems to be a special case among fishes (**see also Bony Fishes**: Crucian Carp). The exceptional anoxia tolerance of this species is largely attributed to the ability to produce ethanol in skeletal muscle as the major anaerobic end product, and thus avoid the acidosis that is associated with glycolysis. This species also has a unique cardiovascular response to hypoxia (anoxia) as compared to all other fishes studied to date (**Figure 6**).

Crucian carp display a typical reflex bradycardia upon initial exposure to anoxia, with a large compensatory increase in S_V which, in turn, increases Q. However, by 48 h of anoxia, all cardiac parameters return to preanoxic levels and remain stable for at least 5 days. This long-term maintenance of cardiac

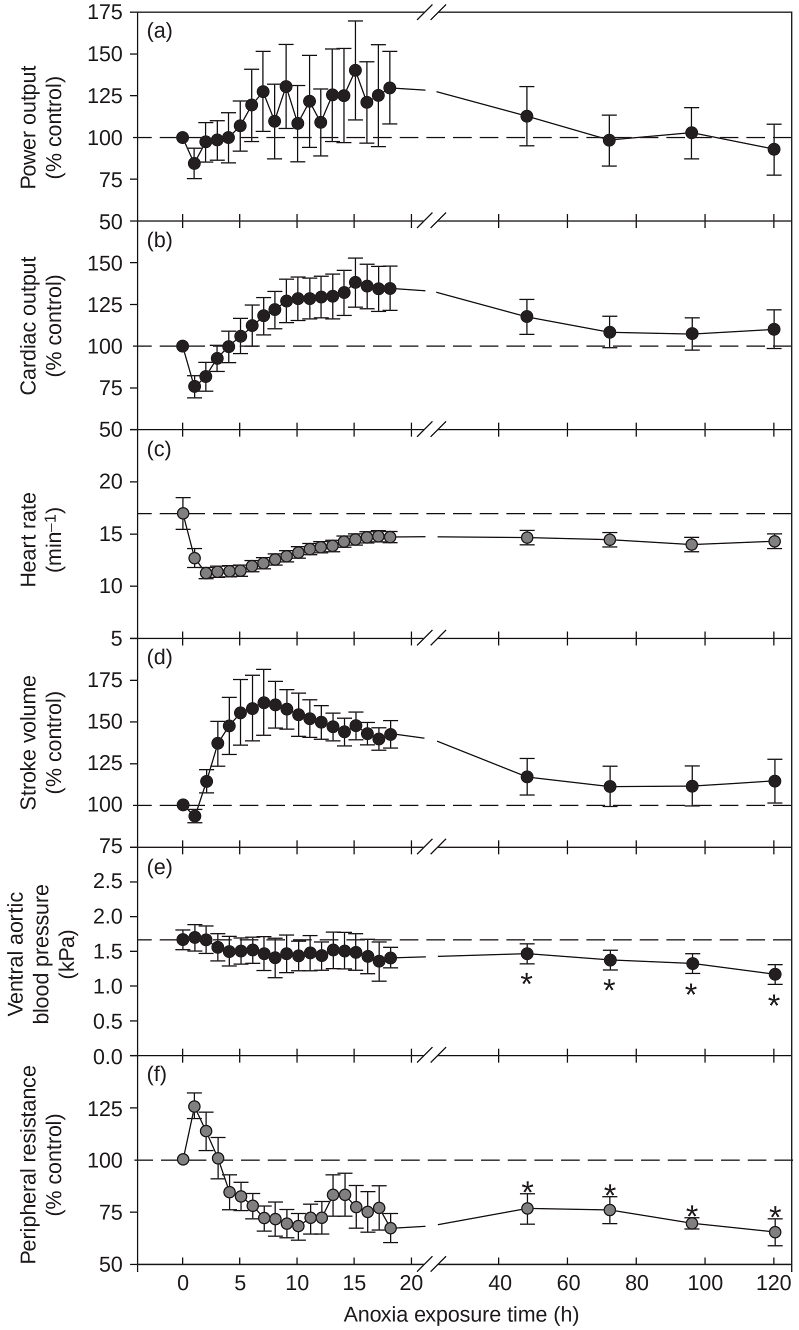


Figure 6 The response of crucian carp cardiovascular function to 5 days of anoxia at 8 °C. Asterisks indicate a significant difference as compared with time 0 (normoxia). Modified from Vornanen M, Steyck JAW, and Nilsson GE (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Richards J (ed.), Brauner CJ and Farrell AP (ser. eds.) *Fish Physiology*, vol. XXVII, pp. 302–360. London: Academic Press, with permission from Elsevier.

function is thought to be important for transporting glucose from the liver to metabolically active tissues, and ethanol from skeletal muscle (where it is produced) to the gills where it can be eliminated from the body. Q is maintained in anoxia because the heart's low ATP demand can be met by its maximal capacity to produce ATP through anaerobic glycolysis. In addition, several factors contribute to the low ATP demand of the crucian carp heart during anoxia. ATP demand is greatly reduced by low winter temperatures; f_H is only approximately one-third of other fishes at these temperatures; this species has low arterial pressures at rest; and both blood pressure and R_{sys} are significantly reduced (by 30–40%) upon exposure to anoxia (see **Figure 6**). Collectively, these characteristics reduce cardiac power output (workload) of the heart, and thus its ATP demand.

Summary

When exposed to hypoxia, fish make a number of adjustments in cardiovascular function (Q, f_{H}, S_{v}, venous pressure, and R_{sys}). These highly depend on the duration (acute vs. chronic) and depth of hypoxia (mild vs. severe), and a species' lifestyle and evolutionary history. However, there are general limits to how long the heart can endure under conditions of oxygen limitation, and this is related to the severity of the hypoxic insult, the workload the heart is subjected to, and temperature.

We have only begun to understand how changes in control mechanisms, gene expression, protein levels/function, signaling cascades, and oxidative and anaerobic metabolism result in intra- and inter-specific differences in myocardial hypoxia tolerance and performance. Such studies will be critical for understanding the plasticity of cardiovascular responses to hypoxia, the evolution of fishes, and which fish will survive as hypoxic zones increase.

See also: **Design and Physiology of the Heart**: The Coronary Circulation. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature. **Temperature**: Effects of Temperature: An Introduction. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin.

Further Reading

Axelsson M, Altimiras J, and Claireaux G (2002) Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. *Journal of Experimental Biology* 205: 2891–2896.

Axelsson M and Fritsche R (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua. Journal of Experimental Biology* 158: 181–198.

Farrell AP (2007) Tribute to Peter Lutz: A message from the heart – why hypoxic bradycardia in fishes? *Journal of Experimental Biology* 210: 1715–1725.

Furimsky M, Cooke SJ, Suski CD, Wang Y, and Tufts BL (2003) Respiratory and circulatory responses to hypoxia in largemouth bass and smallmouth bass: Implications for "live-release" angling tournaments. *Transactions of the American Fisheries Society* 132: 1065–1075.

Gamperl AK and Driedzic WR (2009) Cardiovascular responses to hypoxia. In: Richards J (ed.), Brauner CJ and Farrell AP (ser. eds.) *Fish Physiology*, vol. XXVII, pp. 302–360. London: Academic Press.

Gamperl AK and Farrell AP (2004) Cardiac plasticity in fishes: Environmental influences and intraspecific differences. *Journal of Experimental Biology* 207: 2539–2550.

Gehrke PC and Fielder DR (1988) Effects of temperature and dissolved oxygen on heart rate, ventilation rate and oxygen consumption of spangled perch, *Leiopotherapon unicolor* (Gunther, 1859), (Percoidei, Teraponidae). *Journal of Comparative Physiology B* 157: 771–782.

Hanson LM and Farrell AP (2007) The hypoxic threshold for maximum cardiac performance in rainbow trout *Oncorhynchus mykiss* (Walbaum) during simulated exercise conditions at 18 °C. *Journal of Fish Biology* 71: 926–932.

Hanson LM, Obradovich S, Mouniargi J, and Farrell AP (2006) The role of adrenergic stimulation in maintaining maximum cardiac performance in rainbow trout (*Oncorhynchus mykiss*) during hypoxia, hyperkalemia and acidosis at 10 °C. *Journal of Experimental Biology* 209: 2442–2451.

Keen JE, Vianzon DM, Farrell AP, and Tibbits GF (1993) Thermal acclimation alters both adrenergic sensitivity and adrenoceptor density in cardiac tissue of rainbow trout. *Journal of Experimental Biology* 181: 24–47.

Marques IJ, Leito JTD, Spaink HP, *et al.* (2008) Transcriptome analysis of the response to chronic constant hypoxia in zebrafish hearts. *Journal of Comparative Physiology B* 178: 77–92.

Olson KR, Dombkowski RA, Russell MJ, *et al.* (2006) Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *Journal of Experimental Biology* 209: 4011–4023.

Petersen LH and Gamperl AK (2010) Effects of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology.* 213: 808–819.

Petersen LH and Gamperl AK (2010) *In situ* cardiac function in Atlantic cod (*Gadus morhua*): Effects of acute and chronic hypoxia. *Journal of Experimental Biology.* 213: 820–830.

Sandblom E and Axelsson M (2005) Effects of hypoxia on the venous circulation in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology A* 140: 233–239.

Sandblom E and Axelsson M (2006) Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Onchorhynchus mykiss*): Role of neural and circulating catecholamines. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 291: R711–R718.

Shiels HA, Vornanen M, and Farrell AP (2003) Acute temperature change modulates the response of I_{Ca} to adrenergic stimulation in cardiomyocytes. *Physiological and Biochemical Zoology* 76: 816–824.

Vornanen M, Steyck JAW, and Nilsson GE (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Richards J (ed.), Brauner CJ, and Farrell AP (ser. eds.)Fish Physiology, vol. XXVII, pp. 302–360. London: Academic Press.

THE GUT

The Gastrointestinal Tract: An Introduction

C Olsson and S Holmgren, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Further Reading

Glossary

Apical membrane The side of an epithelial cell facing the outer world; for the gut, this is the lumen.

Autonomic nervous system The nerves controlling the inner organs, operating through reflexes without conscious control.

Basolateral membrane The basal and lateral sides of an epithelial cell facing the internal environment.

Chyme Partly digested food particles mixed with digestive secretions expelled by the stomach into the intestine.

Digestion The process of solubilization and hydrolysis of ingested nutrients in the gastrointestinal canal into constituent molecules and elements suitable for transport across the intestinal wall.

Endocrine Mode of secretion in which a chemical is released by a cell into extracellular fluid and then enters the blood.

Enteric nervous system The part of the autonomic nervous system contained within the gastrointestinal wall.

Enterocyte Columnar adsorptive cell of the intestinal gut epithelium.

Epithelium Relatively flat tissue (composed of one or a few cell layers) that lines cavities and surfaces in multicellular organisms.

Exocrine Mode of secretion in which a chemical is released from a cell into a duct that leads to the outside of the organism (including the lumen of digestive tract).

Extrinsic innervation Innervation that comes from outside the organ. A nerve that is extrinsic to the gut has its cell body outside the gut, but sends its axon to the gut.

Gastroenteropancreatic System of endocrine cells located in the stomach, intestine, and pancreas.

Goblet cell A type of mucus-secreting cell in the epithelium.

Lumen Opening in the center of a tubular organ. Within the digestive system, this space is bordered by a single layer of epithelial cells that create a barrier between outside and inside of the organ.

Microvilli Plasma membrane-bound, cellular extensions that often characterize epithelial cells. They provide for an elaboration of the surface, plasma membrane. Common, for instance, in intestinal cells (enterocytes) that absorb nutrients.

Mucosa Layer in the gut wall facing the lumen.

Myenteric plexus Network of neurons, situated between the longitudinal and circular muscle layers of the gut wall.

Oxyntic cells The acid-secreting cells of the gut.

Paracrine effect When a hormone from an endocrine cell affects neighboring cells.

Peristalsis Propagating movements of gut contents by contraction of gut muscle.

Postprandial Period after ingesting a meal.

Secretion The process of releasing compounds from a cell.

Submucosa Layer between the mucosa and muscle layers in the gut wall.

The gastrointestinal tract plays numerous roles in the maintenance of homeostasis in the fish. This includes food conversion, uptake of nutrients, and osmoregulation. The gut also acts as a barrier against unwanted substances and organisms. This section describes the different aspects of the gut itself, as well as the hormonal and neuronal control systems involved in regulating the gut activities. Although important for providing energy and oxygen to the gut tissues, as well as transporting nutrients from the gut to the rest of the body, gut circulation is discussed in **Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation.

The study of the fish gut has long played an important role in the overall understanding of gut anatomy and control of gut function. The vast number of species, facing a variety of environments and feeding strategies, is reflected in more or less specialized adaptations. Fish as a group also show great similarities with other vertebrates when it comes to the gastrointestinal tract. Significant early anatomical and pharmacological studies on fish gut include, for example, papers by JZ Young, AH Al Hussain, and P Kirtisinghe. The Nobel prize laureate US von Euler together with Östlund isolated one of the first recognized peptidergic transmitters/hormones from the fish intestine. During the period between 1950 and 1970, G Burnstock performed a series of pioneer studies mainly on brown trout (*Salmo trutta*), describing anatomy, innervation, and control of gut motility.

The first article of the section, **Gut Anatomy and Morphology**: Gut Anatomy, gives an anatomical and morphological overview of the gastrointestinal tract in fish, in general, discussing regional characteristics along the gut. It also points out differences among species and groups of fish, such as the presence or absence of a stomach or pyloric appendages (ceca), or different surface enlargement strategies in the intestine. The article concludes by discussing other factors such as food and temperature that may affect gut anatomy and morphology.

Apart from the gastrointestinal tract itself, accessory organs such as the salivary glands, biliary system, and pancreas are often considered a part of the gut. The next article, **Gut Anatomy and Morphology**: Pancreas, describes the anatomy of the latter, including both endocrine and exocrine parts as well as the developmental and evolutionary aspects. It also gives an overview of hormonal and enzymatic pancreatic secretion, and how it is regulated.

Gut activities are tightly regulated to meet the needs of the body, and understanding the control systems is essential to understand the gastrointestinal tract physiology. In **Integrated Function and Control of the Gut**: Nervous System of the Gut, we get an overview of the autonomic innervation of the gut, with special emphasis on the nerves within the gut wall – the enteric nervous system. The structure and distribution of common neurotransmitters are described. General characteristics are outlined, with additional examples from species or groups of fish diverging from the general pattern. Similarly, in **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut, the distribution of endocrine cells releasing local gut hormones to the blood stream is presented. The diverse roles of the hormones are exemplified by selected peptides such as histamine, cholecystokinin (CCK), and ghrelin, and their roles in acid secretion, digestion, and appetite are described.

Gut Anatomy and Morphology: Development of Fish Gut describes early embryological development of the gut, using zebrafish (*Danio rerio*) as a model organism. Much focus is on molecular signals involved in organization of the gut into different regions, as well as in differentiation of the tissues such as smooth muscle and the enteric nerves.

The next three articles describe how food is processed and transported along the gut. While much of our knowledge comes from only a few and mainly teleost species, examples from other fish species, which are included, explain the underlying mechanisms for propagating and nonpropagating contractions involved in breakdown and transport of food. It also describes the control of gut motility (**see also Integrated Function and Control of the Gut**: Gut Motility), with an overview of the current knowledge and an attempt to point to the gaps in our understanding of complex motility pattern. A comprehensive summary of the effects of individual neurotransmitters and hormones, in relation to their distribution in various fish species, is found in **Appendix A** (pp. 1232–1267). **Integrated Function and Control of the Gut**: Gut Secretion and Digestion describes the secretory processes in the stomach, intestine, and pancreas, including water, mucus, acid, and enzyme secretion. The stimuli responsible for increased release are highlighted. Furthermore, digestion of macromolecules (e.g., proteins, carbohydrates, and lipids) is described in detail and related to the type of diet. **Integrated Function and Control of the Gut**: Intestinal Absorption gives a detailed description of the molecular mechanisms behind epithelial transport of carbohydrates, proteins, and lipids. It gives examples of diffusion and active transport as well as discusses the availability and importance of different macronutrients. In addition, regional differences in absorption along the gut are discussed.

Equally important as the uptake of nutrients is the protection provided by the gut against toxic substances and microorganisms. **Integrated Function and Control of the Gut**: Barrier Function of the Gut describes the multiple epithelial barriers, focusing on the intestine. The basis for permeability between and through the epithelial cells is explained, as is the immune system of the gut and its role as a barrier. Examples of bacterial and viral infections via the fish gut are given, and the impact of stress and disease on the barrier functions is discussed.

Supplementary material: Associated with these articles is a spreadsheet that contains extensive lists of the distribution and physiological functions of neurotransmitters and hormones in more than 100 fish species. The spreadsheet is divided into: presence in endocrine cells, presence in nerves in the gut, and effects on gut motility. It also contains the 153 references used to extract this information (**Appendix A** (pp. 1232–1267)).

See also: **Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation. **Gut Anatomy and Morphology**: Development of Fish Gut; Gut Anatomy; Pancreas. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Endocrine Systems of the Gut; Gut Motility; Gut Secretion and Digestion; Intestinal Absorption; Nervous System of the Gut.

Further Reading

Al-Hussaini AH (1949) On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits; anatomy and histology. *Quarterly Journal of Microscopical Science* 90: 109–139.

Al-Hussaini AH (1949) On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits; cytology and physiology. *Quarterly Journal of Microscopical Science* 90: 323–354.

Buddington RK, Krogdahl A, and Bakke-Mckellep AM (1997) The intestines of carnivorous fish: Structure and functions and the relations with diet. *Acta Physiologica Scandinavica Supplement* 638: 67–80.

Burnstock G (1958) Reversible inactivation of nervous activity in a fish gut. *Journal of Physiology* 141: 35–45.

Burnstock G (1958) The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. *British Journal of Pharmacology and Chemotheraphy* 13: 216–226.

Burnstock G (1959) The morphology of the gut of the brown trout (*Salmo trutta*). *Quarterly Journal of Microscopical Science* 100: 183–198.

Burnstock G (1969) Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacological Review* 21: 247–324.

Kirtisinghe P (1940) The myenteric nerve plexus in some lower chordates. *Quarterly Journal of Microscopical Science* 81: 521–539.

von Euler US Östlund E (1956) Occurrence of a substance P-like polypeptide in fish intestine and brain. *British Journal of Pharmacology* 11: 323–325.

von Euler US Östlund E (1957) Effects of certain biologically occurring substances on the isolated intestine of fish. *Acta Physiologica Scandinavica* 38: 364–372.

Young JZ (1933) The autonomic nervous system of selachians. *Quarterly Journal of Microscopical Science* 75: 571–624.

Appendix A

Distribution of endocrine cells

Immunohistochemical localization of hormones in endocrine cells in different fish species

Class/subclass	*Family*	*Species*		*Stomach-less species*
Myxini				
	Myxinidae	*Eptatretus cirrhatus*	New Zealand hagfish	**x**
		Myxine glutinosa	Hagfish	**x**
Cephalaspidomorphi				
	Petromyzontidae	*Lampetra camtschatica*	Arctic lamprey	**x**
		Lampetra fluviatilis	European brook lamprey	**x**
		Lampetra planeri	European river lamprey	**x**
Chondrichthyes				
Elasmobranchii	Rajidae	*Amblyraja radiata*	Thorny skate	
		Leucoraja erinacea	Little skate	
		Leucoraja neavus	Cuckoo ray	
		Raja asterias	Starry ray	
		Raja clavata	Thornback ray	
		Raja microocellata	Small-eyed ray	
		Raja montagui	Spotted ray	
		Raja rhina	Longnose skate	
	Torpedinidae	*Torpedo marmorata*	Spotted torpedo	
	Scyliorhinidae	*Scyliorhinus canicula*	Lesser spotted dogfish	
		Scyliorhinus stellaris	Nursehound	
		Scyliorhinus torazame	Cloudy catshark	
	Hexanchidae	*Heptranchias perlo*	Sharpnose sevengill shark	
	Squalidae	*Squalus acanthias*	Spiny dogfish	
	Squatinidae	*Squatina aculeata*	Sawback angelshark	
Holocephalii	Chimaeridae	*Chimaera monstrosa*	Rabbit fish	
Actinopterygii				
	Polypteridae	*Polypterus senegalus*	Gray bichir	
Chondrostei	Lepisosteidae	*Lepisosteus osseus*	Longnose gar	
Neopterygii: Infraclass Holostei	Amiidae	*Amia calva*	Bowfin	
Neopterygii: Infraclass Teleostei	Pantodontidae	*Pantodon buchholzi*	Freshwater butterflyfish	
	Anguillidae	*Anguilla anguilla*	European eel	
		Anguilla australis	Shortfin eel	
		Anguilla japonica	Japanese eel	
	Cyprinidae	*Carassius auratus*	Goldfish	**x**
		Ctenopharyngodon idella	Grass carp	**x**
		Cyprinus carpio	Common carp	**x**
		Danio rerio	Zebrafish	**x**
		Hypophthalmichthus molitrix	Silver carp	**x**
		Leuciscus idus	Ide	**x**
		Pseudophoxinus antalyae	Sunflower fish	**x**
		Puntius conchonius	Rosy barb	**x**
		Zacco platypus	Freshwater minnow	**x**
	Gyrinocheilidae	*Gyrinocheilus aymonieri*	Chinese algae-eater	
	Characidae	*Hemigrammus ocellifer*	Head-and-taillight tetra	
	Callichthyidae	*Corydoras aeneus*	Bronze corydoras	
	Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	
	Salmonidae	*Oncorhynchus kisutch*	Coho salmon	
		Oncorhynchus mykiss	Rainbow trout	
		Salmo trutta trutta	Brown trout	
	Gadidae	*Gadus morhua*	Atlantic cod	
		Raniceps raninus	Tadpole fish	
	Mugilidae	*Liza saliens*	Leaping mullet	
	Poeciliidae	*Poecilia reticulata*	Guppy	
		Xiphophorus variatus	Variable platyfish	
	Tetraodontidae	*Tetractenos glaber*	Smooth toadfish	**x**
	Paralichthyidae	*Paralichthys dentatus*	Summer flounder	
		Paralichthys olivaceus	Japanese flounder	
	Pleuronectidae	*Hippoglossus hippoglossus*	Atlantic halibut	
		Rhombosolea tapirina	Greenback flounder	**x**
	Scophthalmidae	*Psetta maxima*	Turbot	
	Cottidae	*Myoxocephalus scorpius*	Shorthorn sculpin	
	Scorpaenidae	*Scorpaena porcus*	Black scorpionfish	
	Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	
		Lepomis macrochirus	Bluegill	
	Cichlidae	*Haplochromis* spp.	???	
		Oreochromis mossambicus	Mozambique tilapia	
		Pelvicachromis pulcher	Rainbow krib	
	Gobiidae	*Gillichthys mirabilis*	Longjaw mudsucker	**x**
	Helostomatidae	*Helostoma temminkii*	Kissing gourami	
	Moronidae	*Dicentrarchus labrax*	European seabass	
	Percidae	*Perca fluviatilis*	European perch	
	Sparidae	*Sparus aurata*	Gilthead seabream	
Sarcopterygii				
Dipnoi	Ceratodontidae	*Neoceratodus forsteri*	Australian lungfish	**x***
	Lepidosirenidae	*Lepidosiren paradoxa*	South American lungfish	**x***
	Protopteridae	*Protopterus annectens*	West African lungfish	**x***

* in some studies proximal part is called stomach

Stomach stands for cardiac and pyloric stomach, intestine for the proximal intestine down to the rectum. o, presence of substance.
For references and abbreviations see page 1262–1267.

Tyrosine hydroxylase			*Catecholamines*			*5-HT (serotonin)*			*Bombesin/GRP*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
	o	108									
										o	108
									o	o	20
									o	o	20
									o	o	20
									o	o	20
									o	o	20
									o	o	20
									o	o	20
						o	o	143	o	o	37, 143
						o	o	34			
			o	o?	68	o	o	45, 47, 68	o	o	47, 68
							o	159		o	159
						o	o	29	o		29
									o		126
						o	o	41, 100			
							o	6			
							o	121			
							o	28		o	28
										o	39
							o	94			
						o	o	13, 16	o	o	13, 16, 24, 70, 146
									o	o	23
						o	o	45, 88	o	o	67, 146
							o	28		o	28
							o	6			
							o	6			
						o	o	17, 130			
						o	o	45			
						o		40			
							o	89		o	90
						o		40			
						o		1, 40			
										o	72

Distribution of endocrine cells (*Continued*)

				CGRP		
Class/subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey			
		L. planeri	European river lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. asterias	Starry ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Torpedinidae	*T. marmorata*	Spotted torpedo			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Hexanchidae	*H. perlo*	Sharpnose sevengill shark			
	Squalidae	*S. acanthias*	Spiny dogfish			
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Lepisosteidae	*L. osseus*	Longnose gar			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel			
		A. australis	Shortfin eel			
		A. japonica	Japanese eel			
	Cyprinidae	*C. auratus*	Goldfish			
		C. idella	Grass carp			
		C. carpio	Common carp			
		D. rerio	Zebrafish			
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. antalyae	Sunflower fish		o	39
		P. conchonius	Rosy barb			
		Z. platypus	Freshwater minnow			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Ictaluridae	*A. nebulosus*	Brown bullhead			
	Salmonidae	*O. kisutch*	Coho salmon			
		O. mykiss	Rainbow trout		o	24
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod		o	136
		R. raninus	Tadpole fish			
	Mugilidae	*L. saliens*	Leaping mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Paralichthyidae	*P. dentatus*	Summer flounder			
		P. olivaceus	Japanese flounder			
	Pleuronectidae	*H. hippoglossus*	Atlantic halibut			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot		o	17
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Scorpaenidae	*S. porcus*	Black scorpionfish			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia		o	91
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker			
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Moronidae	*D. labrax*	European seabass	o		40
	Percidae	*P. fluviatilis*	European perch			
	Sparidae	*S. aurata*	Gilthead seabream	o		40
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish		o	72
	Lepidosirenidae	*L. paradoxa*	South American lungfish			
	Protopteridae	*P. annectens*	West African lungfish			

Enkephalin			FMRF			Galanin			Gastrin/CCK		
Stomach	Intestine	Ref.	Stomach	Intestine	Ref.	Stomach	Intestine	Ref.	Stomach	Intestine	Ref.
				o	108						
				o	108					o	119
				o	146						
							o	22		o	150
	o	143							o	o	37, 143
	o	47								o	47, 68
										o	159
o	o	29								o	29
										o	125
										o	96
o	o	41, 100									
	o	91								o	60, 90, 121
	o	120								o	120
										o	110
	o	28, 96								o	28
	o	1, 131, 132		o	127					o	1, 132
										o	94
o	o	96									
										o	96
									o	o	110
o	o	13, 16							o	o	2, 13, 16, 24, 70
									o	o	23
							o	86, 87	o	o	81, 97
	o	44							o	o	44
	o	28, 96								o	28
o	o	96								o	96
									o		95
										o	84
									o	o	17, 130
										o	125
o	o	96								o	96
	o	91								o	90
o	o	96									
	o	149									
									o	o	110
o	o	1, 40, 43							o	o	1, 43

Distribution of endocrine cells (*Continued*)

				Ghrelin		
Class/subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey			
		L. planeri	European river lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. asterias	Starry ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Torpedinidae	*T. marmorata*	Spotted torpedo			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Hexanchidae	*H. perlo*	Sharpnose sevengill shark			
	Squalidae	*S. acanthias*	Spiny dogfish			
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Lepisosteidae	*L. osseus*	Longnose gar			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel			
		A. australis	Shortfin eel			
		A. japonica	Japanese eel	o		83
	Cyprinidae	*C. auratus*	Goldfish			
		C. idella	Grass carp			
		C. carpio	Common carp			
		D. rerio	Zebrafish		?	116
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. antalyae	Sunflower fish			
		P. conchonius	Rosy barb			
		Z. platypus	Freshwater minnow			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Ictaluridae	*A. nebulosus*	Brown bullhead			
	Salmonidae	*O. kisutch*	Coho salmon			
		O. mykiss	Rainbow trout	o		118, 133
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod			
		R. raninus	Tadpole fish			
	Mugilidae	*L. saliens*	Leaping mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Paralichthyidae	*P. dentatus*	Summer flounder	o		26
		P. olivaceus	Japanese flounder			
	Pleuronectidae	*H. hippoglossus*	Atlantic halibut			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Scorpaenidae	*S. porcus*	Black scorpionfish			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia			
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker			
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Moronidae	*D. labrax*	European seabass	o	o	11
	Percidae	*P. fluviatilis*	European perch			
	Sparidae	*S. aurata*	Gilthead seabream			
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish			
	Lepidosirenidae	*L. paradoxa*	South American lungfish			
	Protopteridae	*P. annectens*	West African lungfish			

? nonspecific?

GIP			*Glucagon*			*Insulin*			*NPY*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
	o	42		o	119						
				o	146		o-islet	146		o	146
										o	31, 32
				o	150		o	150			
			o		144						
			o		144						
			o		144				o	o	21, 38
			o	o	143, 50				o	o	38
									o	o	33
			o		144						
o	o	47, 48	o	o	47		o	47			
			o		144						
				o	159						
							o	55		o	55
				o	96						
				o	100				o	o	41
										o	91
	o	120		o	120						
										o	39
				o	1, 131, 132						
				o	94						
				o	96						
				o	111						
			o	o	13, 16, 24, 70, 111				o	o	14, 16, 24
				o	23						
			o		44						
										o	28
			o	o	130						
	o	48							o		46
				o	96						
										o	91
			o		40	o		40			
			o	o	1, 40	o		40			
				o	145		o	145			

Distribution of endocrine cells (*Continued*)

				Neurotensin		
Class/subclass	*Family*	*Species*		*Stomach*	*Intestine*	*Ref.*
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish		(o)	128
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey			
		L. planeri	European river lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. asterias	Starry ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Torpedinidae	*T. marmorata*	Spotted torpedo			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Hexanchidae	*H. perlo*	Sharpnose sevengill shark			
	Squalidae	*S. acanthias*	Spiny dogfish	o	o	47, 128
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Lepisosteidae	*L. osseus*	Longnose gar			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel			
		A. australis	Shortfin eel			
		A. japonica	Japanese eel			
	Cyprinidae	*C. auratus*	Goldfish		o	90
		C. idella	Grass carp			
		C. carpio	Common carp			
		D. rerio	Zebrafish			
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. antalyae	Sunflower fish		o	39
		P. conchonius	Rosy barb		o	131
		Z. platypus	Freshwater minnow			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Ictaluridae	*A. nebulosus*	Brown bullhead			
	Salmonidae	*O. kisutch*	Coho salmon			
		O. mykiss	Rainbow trout			
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod			
		R. raninus	Tadpole fish	o	o	128
	Mugilidae	*L. saliens*	Leaping mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Paralichthyidae	*P. dentatus*	Summer flounder			
		P. olivaceus	Japanese flounder			
	Pleuronectidae	*H. hippoglossus*	Atlantic halibut			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot		o	130
	Cottidae	*M. scorpius*	Shorthorn sculpin	o	o	128
	Scorpaenidae	*S. porcus*	Black scorpionfish			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia		o	90
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker		o	149
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Moronidae	*D. labrax*	European seabass			
	Percidae	*P. fluviatilis*	European perch			
	Sparidae	*S. aurata*	Gilthead seabream	o		1
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish			
	Lepidosirenidae	*L. paradoxa*	South American lungfish			
	Protopteridae	*P. annectens*	West African lungfish			

Opioids			*PACAP*			*Pancreatic polypeptide*			*Somatostatin*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
				o	108						
				o	108					o	108
							o	146		o-islet	146
										o	31, 32
									o		144
									o		144
						o	o	38	o		144
	o	143				o	o	38	o	o	37, 140, 143
									o		144
							o	47	o	o	47, 68
									o		144
							o	159		o	159
									o		29
							o	55	o		55
									o	o	19, 100
							o	120			
							o	150			
				o	61, 117		o	96			
							o	96			
										o	39
							o	1, 132			
							o	94		o	94
									o	o	96
									o		96
							o	110	o		110
				o	113		o	16	o	o	13, 16, 70
				o	113	o	o	46			
						o	o	44	o		44
							o	96			
							o	130	o	o	130
						o	o	46, 139			
									o		40
							o	96			
							o	96	o		96
									o		96
									o		40
							o	110	o		110
							o	1, 43	o		1, 40, 43
										o	72
							o	145		o	145

Distribution of endocrine cells (*Continued*)

Class/subclass	*Family*	*Species*	
Myxini			
	Myxinidae	*E. cirrhatus*	New Zealand hagfish
		M. glutinosa	Hagfish
Cephalaspidomorphi			
	Petromyzontidae	*L. camtschatica*	Arctic lamprey
		L. fluviatilis	European brook lamprey
		L. planeri	European river lamprey
Chondrichthyes			
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate
		L. erinacea	Little skate
		L. neavus	Cuckoo ray
		R. asterias	Starry ray
		R. clavata	Thornback ray
		R. microocellata	Small-eyed ray
		R. montagui	Spotted ray
		R. rhina	Longnose skate
	Torpedinidae	*T. marmorata*	Spotted torpedo
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish
		S. stellaris	Nursehound
		S. torazame	Cloudy catshark
	Hexanchidae	*H. perlo*	Sharpnose sevengill shark
	Squalidae	*S. acanthias*	Spiny dogfish
	Squatinidae	*S. aculeata*	Sawback angelshark
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish
Actinopterygii			
	Polypteridae	*P. senegalus*	Gray bichir
Chondrostei	Lepisosteidae	*L. osseus*	Longnose gar
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish
	Anguillidae	*A. anguilla*	European eel
		A. australis	Shortfin eel
		A. japonica	Japanese eel
	Cyprinidae	*C. auratus*	Goldfish
		C. idella	Grass carp
		C. carpio	Common carp
		D. rerio	Zebrafish
		H. molitrix	Silver carp
		L. idus	Ide
		P. antalyae	Sunflower fish
		P. conchonius	Rosy barb
		Z. platypus	Freshwater minnow
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater
	Characidae	*H. ocellifer*	Head-and-taillight tetra
	Callichthyidae	*C. aeneus*	Bronze corydoras
	Ictaluridae	*A. nebulosus*	Brown bullhead
	Salmonidae	*O. kisutch*	Coho salmon
		O. mykiss	Rainbow trout
		S. trutta trutta	Brown trout
	Gadidae	*G. morhua*	Atlantic cod
		R. raninus	Tadpole fish
	Mugilidae	*L. saliens*	Leaping mullet
	Poeciliidae	*P. reticulata*	Guppy
		X. variatus	Variable platyfish
	Tetraodontidae	*T. glaber*	Smooth toadfish
	Paralichthyidae	*P. dentatus*	Summer flounder
		P. olivaceus	Japanese flounder
	Pleuronectidae	*H. hippoglossus*	Atlantic halibut
		R. tapirina	Greenback flounder
	Scophthalmidae	*P. maxima*	Turbot
	Cottidae	*M. scorpius*	Shorthorn sculpin
	Scorpaenidae	*S. porcus*	Black scorpionfish
	Centrarchidae	*L. gibbosus*	Pumpkinseed
		L. macrochirus	Bluegill
	Cichlidae	*Haplochromis* spp.	???
		O. mossambicus	Mozambique tilapia
		P. pulcher	Rainbow krib
	Gobiidae	*G. mirabilis*	Longjaw mudsucker
	Helostomatidae	*H. temminkii*	Kissing gourami
	Moronidae	*D. labrax*	European seabass
	Percidae	*P. fluviatilis*	European perch
	Sparidae	*S. aurata*	Gilthead seabream
Sarcopterygii			
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish
	Lepidosirenidae	*L. paradoxa*	South American lungfish
	Protopteridae	*P. annectens*	West African lungfish

Tachykinins			VIP		
Stomach	Intestine	Ref.	Stomach	Intestine	Ref.
				o	108
	o	108		o	108, 129
	o	76			
o		76		o	42, 48
				o	48
o	o	37		o	142, 143
o	o	47, 73, 82		o	47, 129
	o	159			
o		29			
			o		127
o	o	41			
				o	9, 121
	o	120			
	o	61, 117		o	117
				o	9
	o	28		o	28
	o	39		o	39
	o	131		o	131, 132
o	o	2, 13, 16, 70			
	o	77, 79, 88	o	o	98
				o	129
o	o	44			
	o	28			
o	o	17, 130		o	17, 130
				o	129
				o	9
	o	90		o	91
	o	96			
	o	149		o	149
	o	96			
o	o	1, 43			
	o	72			
	o	76			

Innervation of the myenteric plexus

Immunohistochemical localization of neutransmitters in nerve cell bodies (nc) and nerve fibers (nf) in different fish species

Class/subclass	***Family***	***Species***		***Stomach-less species***
Myxini				
	Myxinidae	*Eptatretus cirrhatus*	New Zealand hagfish	**x**
		Myxine glutinosa	Hagfish	**x**
Cephalaspidomorphi				
	Petromyzontidae	*Lampetra camtschatica*	Arctic lamprey	**x**
		Lampetra fluviatilis	European brook lamprey	**x**
		Lampetra planeri	European river lamprey	**x**
		Petromyzon marinus	Great sea lamprey	**x**
Chondrichthyes				
Elasmobranchii	Rajidae	*Amblyraja radiata*	Thorny skate	
		Leucoraja erinacea	Little skate	
		Leucoraja neavus	Cuckoo ray	
		Raja clavata	Thornback ray	
		Raja microocellata	Small-eyed ray	
		Raja montagui	Spotted ray	
		Raja rhina	Longnose skate	
	Rhinobatidae	*Rhinobatos typus*	Giant shovelnose ray	
	Scyliorhinidae	*Scyliorhinus canicula*	Lesser spotted dogfish	
		Scyliorhinus stellaris	Nursehound	
		Scyliorhinus torazame	Cloudy catshark	
	Heterodontidae	*Heterodontus portusjacksoni*	Port Jackson shark	
	Hemiscyllidae	*Hemiscyllium ocellatum*	Epaulette shark	
	Squalidae	*Squalus acanthias*	Spiny dogfish	
	Squatinidae	*Squatina aculeata*	Sawback angelshark	
Holocephalii	Chimaeridae	*Chimaera monstrosa*	Rabbit fish	
Actinopterygii				
	Polypteridae	*Polypterus senegalus*	Gray bichir	
Chondrostei	Acipenseridae	*Acipenser transmontanus*	White sturgeon	
	Lepisosteidae	*Lepisosteus platyrhincus*	Gar pike	
Neopterygii: Infraclass Holostei	Amiidae	*Amia calva*	Bowfin	
Neopterygii: Infraclass Teleostei	Pantodontidae	*Pantodon buchholzi*	Freshwater butterflyfish	
	Anguillidae	*Anguilla anguilla*	European eel	
		Anguilla australis	Shortfin eel	
	Engraulidae	*Engraulis japonicus*	Japanese anchovy	
	Cyprinidae	*Carassius auratus*	Goldfish	**x**
		Cyprinus carpio	Common carp	**x**
		Danio rerio	Zebrafish	**x**
		Hypophthalmichthus molitrix	Silver carp	**x**
		Leuciscus idus	Ide	**x**
		Puntius conchonius	Rosy barb	**x**
	Gyrinocheilidae	*Gyrinocheilus aymonieri*	Chinese algae-eater	
	Characidae	*Hemigrammus ocellifer*	Head-and-taillight tetra	
	Callichthyidae	*Corydoras aeneus*	Bronze corydoras	
	Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	
		Salmo trutta trutta	Brown trout	
	Gadidae	*Gadus morhua*	Atlantic cod	
		Pollachius pollachius	Pollack	
		Raniceps raninus	Tadpole fish	
	Lotidae	*Ciliata mustela*	Fivebeard rockling	
	Mugilidae	*Aldrichetta forsteri*	Yellow-eye mullet	
	Poeciliidae	*Poecilia reticulata*	Guppy	**x**
		Xiphophorus variatus	Variable platyfish	**x**
	Tetraodontidae	*Tetractenos glaber*	Smooth toadfish	**x**
	Pleuronectidae	*Platichthys flesus*	Flounder	
		Pleuronectes platessa	European plaice	
		Rhombosolea tapirina	Greenback flounder	**x**
	Scophthalmidae	*Psetta maxima*	Turbot	
	Soleidae	*Solea solea*	Common sole	
	Cottidae	*Myoxocephalus scorpius*	Shorthorn sculpin	
	Platycephalidae	*Platycephalus bassensis*	Sand flathead	
	Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	
		Lepomis macrochirus	Bluegill	
	Cichlidae	*Haplochromis* spp.	???	
		Oreochromis mossambicus	Mozambique tilapia	
		Pelvicachromis pulcher	Rainbow krib	
	Gobiidae	*Gillichthys mirabilis*	Longjaw mudsucker	**x**
	Helostomatidae	*Helostoma temminkii*	Kissing gourami	
	Labridae	*Centrolabrus exoletus*	Rock cock	**x**
		Ctenolabrus rupestris	Goldsinny-wrasse	**x**
		Labrus berggylta	Ballan wrasse	**x**
		Labrus mixtus	Cuckoo wrasse	**x**
	Moronidae	*Dicentrarchus labrax*	European seabass	
	Percidae	*Perca fluviatilis*	European perch	
	Uranoscopidae	*Uranoscopus japonicus*	Japanese stargazer	**?**
Sarcopterygii				
Dipnoi	Ceratodontidae	*Neoceratodus forsteri*	Australian lungfish	**x***
	Lepidosirenidae	*Lepidosiren paradoxa*	South American lungfish	**x***
	Protopteridae	*Protopterus annectens*	West African lungfish	**x***

* in some studies proximal part is called stomach

Stomach stands for cardiac and pyloric stomach, intestine for the proximal intestine down to the rectum.
For references and abbreviations see page 1262–1267.

VAChT/ChAT			Tyrosine hydroxylase			Catecholamines			5-HT (serotonin)		
Stomach	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*
										nf	108
										nf, nc	52, 108
										nf, nc	158
							nf, nc	15		nf, nc	15, 108
										nf, nc	143
										nf, nc	108
										nf	49
										nf	36
						nf	nf	68	nf	nf	68
										nf	159
									nf	nf	29
				nf	41						
						nf	nf	7		nf, nc	6, 7
							nf	7		nf, nc	7, 52
										nf, nc	117
										nf, nc	28
						nf, nc		135	nf, nc	nf, nc*	16, 71
						nf	nf	7		nf, nc	7
	nf nc	88					nf	75		nf, nc	75, 88
						nf	nf	7, 8		nf, nc	7, 8
										nf, nc	28
							nf	6, 7		nf, nc	6, 7
										nf	19, 151
							nf	7			
									nf, nc*	nf, nc*	17, 130
						nf	nf	151		nf	151
						nf		7		nf, nc	6, 7
										nf, nc	89
										nf	107

* nc not specified in what region

Innervation of the myenteric plexus (*Continued*)

				NOS		
Class/subclass	***Family***	***Species***		***Stomach***	**Intestine**	***Ref.***
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey			
		L. planeri	European river lamprey			
		P. marinus	Great sea lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Rhinobatidae	*R. typus*	Giant shovelnose ray			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Heterodontidae	*H. portusjacksoni*	Port Jackson shark			
	Hemiscyllidae	*H. ocellatum*	Epaulette shark			
	Squalidae	*S. acanthias*	Spiny dogfish	nf, nc	nf, nc	114
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Acipenseridae	*A. transmontanus*	White sturgeon			
	Lepisosteidae	*L. platyrhincus*	Gar pike			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel	nf	nf	41
		A. australis	Shortfin eel			
	Engraulidae	*E. japonicus*	Japanese anchovy	nf, nc	nf, nc	35
	Cyprinidae	*C. auratus*	Goldfish		nf, nc	27
		C. carpio	Common carp			
		D. rerio	Zebrafish		nf, nc	62, 117
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. conchonius	Rosy barb			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Salmonidae	*O. mykiss*	Rainbow trout	nf, nc	nf, nc	101
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod	nf, nc	nf, nc	86, 114
		P. pollachius	Pollack			
		R. raninus	Tadpole fish			
	Lotidae	*C. mustela*	Fivebeard rockling			
	Mugilidae	*A. forsteri*	Yellow-eye mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Pleuronectidae	*P. flesus*	Flounder			
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot			
	Soleidae	*S. solea*	Common sole			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Platycephalidae	*P. bassensis*	Sand flathead			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia			
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker			
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Labridae	*C. exoletus*	Rock cock			
		C. rupestris	Goldsinny-wrasse			
		L. berggylta	Ballan wrasse			
		L. mixtus	Cuckoo wrasse			
	Moronidae	*D. labrax*	European seabass	nf, nc	nf, nc	123
	Percidae	*P. fluviatilis*	European perch			
	Uranoscopidae	*U. japonicus*	Japanese stargazer			
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish			
	Lepidosirenidae	*L. paradoxa*	South American lungfish			
	Protopteridae	*P. annectens*	West African lungfish			

GABA			Bombesin/GRP			CGRPz			Enkephalin		
Stomach	**Intestine**	**Ref**	**Stomach**	**Intestine**	**Ref.**	**Stomach**	**Intestine**	**Ref.**	**Stomach**	**Intestine**	**Ref.**
				nf, nc	158		nf, nc	158			
				nf, nc	108		nf, nc	108			
				nf	108		nf	108			
			nf	nf	83						
			nf	nf	20						
			nf	nf	19, 20						
			nf	nf	19, 20						
			nf	nf	19, 20						
			nf	nf	19, 20						
			nf	nf	83						
			nf	nf	49, 141						
			nf	nf	36, 37						
			nf, nc	nf, nc	68						
			nf	nf	141						
				nf, nc	159						
			nf		29				nf	nf	29
			nf		69						
			nf	nf	41	nf	nf	41	nf, nc*	nf, nc*	96, 41
							nf, nc	91		nf	91
				nf	19						
							nf, nc	117			
				nf	28						
										nf	131, 132
										nf	96
			nf	nf	19, 146						
			nf	nf	19, 67, 146		nf, nc	136	nf		19
			nf		19						
			nf		19						
			nf		19						
				nf	28					nf	28
			nf	nf	19				nf		19
			nf, nc		19				nf		19
				nf	96						
							nf, nc	91		nf, nc	91
									nf	nf	96
										nf	149
										nf	96
				nf	19					nf, nc	19
				nf	19					nf, nc	19
				-	19					nf, nc	19
				-	19					nf, nc	19
			nf	nf	19				nf, nc	nf, nc	19
							nf	72			
										nf	107
										nf	107

*nc not specified in what region

Innervation of the myenteric plexus (*Continued*)

				Galanin		
Class/subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey		nf, nc	22
		L. planeri	European river lamprey			
		P. marinus	Great sea lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Rhinobatidae	*R. typus*	Giant shovelnose ray	nf	nf	131
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Heterodontidae	*H. portusjacksoni*	Port Jackson shark	nf	nf	124
	Hemiscyllidae	*H. ocellatum*	Epaulette shark	nf	nf	124
	Squalidae	*S. acanthias*	Spiny dogfish			
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Acipenseridae	*A. transmontanus*	White sturgeon	nf, nc		22
	Lepisosteidae	*L. platyrhincus*	Gar pike			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel			
		A. australis	Shortfin eel			
	Engraulidae	*E. japonicus*	Japanese anchovy			
	Cyprinidae	*C. auratus*	Goldfish		nf, nc	91
		C. carpio	Common carp			
		D. rerio	Zebrafish			
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. conchonius	Rosy barb			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Salmonidae	*O. mykiss*	Rainbow trout			
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod	nf, nc	nf	86, 87
		P. pollachius	Pollack			
		R. raninus	Tadpole fish			
	Lotidae	*C. mustela*	Fivebeard rockling			
	Mugilidae	*A. forsteri*	Yellow-eye mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Pleuronectidae	*P. flesus*	Flounder	nf, nc	nf, nc	25
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot	nf, nc	nf	25
	Soleidae	*S. solea*	Common sole	nf, nc	nf, nc	25
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Platycephalidae	*P. bassensis*	Sand flathead			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia		nf, nc	91
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker			
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Labridae	*C. exoletus*	Rock cock			
		C. rupestris	Goldsinny-wrasse			
		L. berggylta	Ballan wrasse			
		L. mixtus	Cuckoo wrasse			
	Moronidae	*D. labrax*	European seabass			
	Percidae	*P. fluviatilis*	European perch			
	Uranoscopidae	*U. japonicus*	Japanese stargazer			
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish		nf	72
	Lepidosirenidae	*L. paradoxa*	South American lungfish		nf	107
	Protopteridae	*P. annectens*	West African lungfish			

Gastrin/CCK			*NPY*			*Neurotensin*			*Opioids*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
			nf	nf	19						
			nf	nf	19						
			nf	nf	19						
			nf		19						
			nf	nf	21, 38						
nf		33	nf	nf	38						
			nf		33						
nf	nf, nc	65									
	nf	159									
						nf, nc	nf, nc	69			
			nf	nf	41						
	nf	60, 90		nf	91		nf	90			
	nf	19		nf	19						
	nf, nc	28					nf	28			
							nf	131			
			nf	nf	14, 19	nf		19			
nf		19, 81		nf	19						
nf		19	nf		19						
nf		19	nf		19						
nf		19	nf	nf	19						
				nf	28		nf, nc	28			
							nf	96			
			nf		19	nf		19			
						nf	nf	130			
nf	nf	19	nf		19, 46	nf	nf	19			
						nf	nf	96			
							nf	90			
							nf	149			
							nf	96			
							nf	19			
							nf	19			
							nf	19			
							nf	19			
			nf	nf	19	nf	nf	19			
	nf	71					nf	71			
	nf	107		nf	107		nf	107			
	nf	107					nf	107			

Innervation of the myenteric plexus (*Continued*)

Class/Subclass	*Family*	*Species*		*PACAP*		
				Stomach	*Intestine*	*Ref*
Myxini						
	Myxinidae	*E. cirrhatus*	New Zealand hagfish			
		M. glutinosa	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. camtschatica*	Arctic lamprey			
		L. fluviatilis	European brook lamprey			
		L. planeri	European river lamprey			
		P. marinus	Great sea lamprey			
Chondrichthyes						
Elasmobranchii	Rajidae	*A. radiata*	Thorny skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. clavata	Thornback ray			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
		R. rhina	Longnose skate			
	Rhinobatidae	*R. typus*	Giant shovelnose ray			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
		S. stellaris	Nursehound			
		S. torazame	Cloudy catshark			
	Heterodontidae	*H. portusjacksoni*	Port Jackson shark			
	Hemiscyllidae	*H. ocellatum*	Epaulette shark			
	Squalidae	*S. acanthias*	Spiny dogfish			
	Squatinidae	*S. aculeata*	Sawback angelshark			
Holocephalii	Chimaeridae	*C. monstrosa*	Rabbit fish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Chondrostei	Acipenseridae	*A. transmontanus*	White sturgeon			
	Lepisosteidae	*L. platyrhincus*	Gar pike			
Neopterygii: Infraclass Holostei	Amiidae	*A. calva*	Bowfin			
Neopterygii: Infraclass Teleostei	Pantodontidae	*P. buchholzi*	Freshwater butterflyfish			
	Anguillidae	*A. anguilla*	European eel			
		A. australis	Shortfin eel			
	Engraulidae	*E. japonicus*	Japanese anchovy			
	Cyprinidae	*C. auratus*	Goldfish			
		C. carpio	Common carp			
		D. rerio	Zebrafish		nf	61, 117
		H. molitrix	Silver carp			
		L. idus	Ide			
		P. conchonius	Rosy barb			
	Gyrinocheilidae	*G. aymonieri*	Chinese algae-eater			
	Characidae	*H. ocellifer*	Head-and-taillight tetra			
	Callichthyidae	*C. aeneus*	Bronze corydoras			
	Salmonidae	*O. mykiss*	Rainbow trout	nf, nc	nf, nc	113
		S. trutta trutta	Brown trout			
	Gadidae	*G. morhua*	Atlantic cod	nf, nc	nf, nc	113
		P. pollachius	Pollack			
		R. raninus	Tadpole fish			
	Lotidae	*C. mustela*	Fivebeard rockling			
	Mugilidae	*A. forsteri*	Yellow-eye mullet			
	Poeciliidae	*P. reticulata*	Guppy			
		X. variatus	Variable platyfish			
	Tetraodontidae	*T. glaber*	Smooth toadfish			
	Pleuronectidae	*P. flesus*	Flounder			
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Scophthalmidae	*P. maxima*	Turbot			
	Soleidae	*S. solea*	Common sole			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Platycephalidae	*P. bassensis*	Sand flathead			
	Centrarchidae	*L. gibbosus*	Pumpkinseed			
		L. macrochirus	Bluegill			
	Cichlidae	*Haplochromis* spp.	???			
		O. mossambicus	Mozambique tilapia			
		P. pulcher	Rainbow krib			
	Gobiidae	*G. mirabilis*	Longjaw mudsucker			
	Helostomatidae	*H. temminkii*	Kissing gourami			
	Labridae	*C. exoletus*	Rock cock			
		C. rupestris	Goldsinny-wrasse			
		L. berggylta	Ballan wrasse			
		L. mixtus	Cuckoo wrasse			
	Moronidae	*D. labrax*	European seabass			
	Percidae	*P. fluviatilis*	European perch			
	Uranoscopidae	*U. japonicus*	Japanese stargazer		nf, nc	104
Sarcopterygii						
Dipnoi	Ceratodontidae	*N. forsteri*	Australian lungfish			
	Lepidosirenidae	*L. paradoxa*	South American lungfish			
	Protopteridae	*P. annectens*	West African lungfish			

PP			Somatostatin			Tachykinins			VIP		
Stomach	Intestine	Ref	Stomach	Intestine	Ref	Stomach	Intestine	Ref	Stomach	Intestine	Ref
						nf		76		nf	41, 48
										nf	134
				nf	49					nf, nc	49
			nf		36	nf		36	nf, nc	nf, nc	36, 37, 142, 143
			nf	nf	68	nf, nc	nf, nc	47, 73, 82	nf, nc	nf, nc	47, 68, 114, 129
										nf, nc	159
						nf	nf	29	nf	nf	29
									nf, nc	nf, nc	69
									nf	nf	127
									nf	nf	96
			nf	nf	41	nf	nf	19, 41	nf	nf	19, 41
							nf	90		nf, nc	9, 91
							nf	19		nf	19
							nf	61, 117		nf	96, 117
										nf	9
							nf	28		nf, nc	28, 96
				nf	1, 131, 132		nf	131		nf	1, 131, 132
									nf	nf	96
										nf	96
										nf	96
						nf	nf	16, 19, 70	nf, nc	nf, nc	16, 19, 70
										nf	7
						nf, nc	nf, nc	19, 75, 77, 79, 88	nf, nc	nf, nc	19, 75, 86, 96, 98, 113, 114
						nf	nf	19	nf	nf	19
						nf	nf	19	nf	nf	19, 129
						nf		19	nf	nf	19
							nf	28		nf	28
						nf	nf	19	nf	nf	19
									nf		19
						nf	nf	17, 130	nf	nf	17, 130
						nf		19	nf	nf	19, 129
										nf	9
									nf	nf	127
									nf	nf	96
										nf, nc	91
							nf	149		nf	149
									nf	nf	96
							nf	19		nf	19
							nf	19		nf	19
							nf	19		nf	19
							-	19		nf	19
						nf	nf	19	nf	nf	19
				nf	72		nf	72		nf	72
				nf	107		nf?	76		nf	107
				nf	107, 145		nf	107		nf	107

Effect of different signaling substances on motility

Class/subclass	*Family*	*Species*		*Stomach-less species*	*Acetylcholine*	
					Stomach	*Intestine*
Myxini						
	Myxinidae	*Myxine glutinosa*	Hagfish	**x**		+
Cephalaspidomorphi						
	Petromyzontidae	*Lampetra fluviatilis*	European river lamprey	**x**		**(−)**
Chondrichthyes						
Elasmobranchii	Dasyatidae	*Pteroplatytrygon violacea*	Pelagic stingray			
	Rajidae	*Amblyraja radiata*	Thorny skate			+
		Dipturus batis	Blue skate			+
		Leucoraja erinacea	Little skate			
		Leucoraja neavus	Cuckoo ray		+	**SI (+) R+**
		Raja brachyura	Blond ray		+	**SI (+) R+**
		Raja clavata	Thornback ray		+	**SI (+) R+**
		Raja eglanteria	Clearnosed skate			
		Raja microocellata	Small-eyed ray		+	**SI (+) R+**
		Raja montagui	Spotted ray		+	**SI (+) R+**
	Scyliorhinidae	*Scyliorhinus canicula*	Lesser spotted dogfish		+	**(+)**
	Squalidae	*Squalus acanthias*	Spiny dogfish		+	
Actinopterygii						
	Polypteridae	*Polypterus senegalus*	Gray bichir			
Neopterygii: Infraclass Teleostei	Anguillidae	*Anguilla anguilla*	European eel			+
	Cyprinidae	*Cyprinus carpio carpio*	Common carp	**x**		+
		Danio rerio	Zebrafish	**x**		+
		Rutilus rutilus	Roach	**x**		
		Tinca tinca	Tench	**x**		+
	Salmonidae	*Oncorhynchus mykiss*	Rainbow trout		+	+
		Salmo salar	Atlanitc salmon			+
		Salmo trutta trutta	Brown trout		+	+
	Esocidae	*Esox lucius*	Northern pike			
	Lophiidae	*Lophius piscatorius*	Angler		+	+
	Gadidae	*Gadus morhua*	Atlantic cod		+	+
	Pleuronectidae	*Ammotretis rostratus*	Longsnout flounder	**x**		+
		Platichthys flesus	Flounder			
		Pleuronectes platessa	European plaice			+
		Rhombosolea tapirina	Greenback flounder	**x**		+
	Cottidae	*Myoxocephalus scorpius*	Shorthorn sculpin		+	+
	Blenniidae	*Lipophrys pholis*	Shanny		+	+
		Neogobius fluviatilis	Monkey goby			
	Haemulidae	*Haemulon flavolineatum*	French grunt		+	+
		Labrus berggylta	Ballan wrasse			+
	Mullidae	*Mullus barbatus*	Red mullet			
	Percidae	*Perca fluviatilis*	European perch			
Sarcopterygii						
Dipnoi	Lepidosirenidae	*Lepidosiren paradoxa*	South American lungfish	**x***		

* in some studies proximal part is called stomach

Stomach stands for cardiac and pyloric stomach, intestine for the proximal intestine down to the rectum. SI, spiral intestine, R, rectum +, contraction/stimulation; −, relaxation/inhibition; no, no response.

For references and abbreviations see page 1262–1267.

	Adrenaline			*Dopamine*			*5-HT (serotonin)*		
Ref.	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*
66, 76, 152		+/–	66, 152					**no**	152
76								+	80
	+	+	154						
76									
152		+/–	152						
	+	-	103						
156, 157	+/–	**Sl** + **R** –	156, 157	+	**Sl no**	52, 156	+/–	**Sl no R**+	156, 157
156, 157	+/–	**Sl** + **R** –	156, 157		**Sl no**	156	+/–	**Sl no R**+	156, 157
155, 156, 157	+/–	**Sl** + **R** –	154, 155, 156, 157		**Sl no**	156	+/–	**Sl no R**+	155, 156, 157
	+	-	103						
156, 157	+/–	**Sl** + **R** –	156, 157		**Sl no**	156	+/–	**Sl no R**+	156, 157
156, 157	+/–	**Sl** + **R** –	156, 157		**Sl no**	156	+/–	**Sl no R**+	156, 157
154, 155, 156	+/–	+	154, 155, 156		**Sl no**	156	+/–	**Sl no**	155, 156
109, 152	+	+/–	103, 109, 152				+		109
152	+	-	105, 152					+	152
160		-	160					+	160
61									
12		-	12						
65, 76, 92, 146, 161	+		92, 161				+	+	64, 65, 76, 92
137								+	137
30	+	-	30				+	+	30
152, 153	-	-	152, 153					+	152
75, 88, 106, 146, 152	+	–/+*	75, 106, 152	-		56	+	+	75, 88, 152
57		-	57						
152		-	152					+	152
57		-	57						
53		-	53						
51									
18		-	18						
152		-	152					+	152

* inhibition followed by contraction in some prep.

Effect of different signaling substances on motility (*Continued*)

				Nitric oxide		
Class/subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*M. glutinosa*	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. fluviatilis*	European river lamprey			
Chondrichthyes						
Elasmobranchii	Dasyatidae	*P. violacea*	Pelagic stingray			
	Rajidae	*A. radiata*	Thorny skate			
		D. batis	Blue skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. brachyura	Blond ray			
		R. clavata	Thornback ray			
		R. eglanteria	Clearnosed skate			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
	Squalidae	*S. acanthias*	Spiny dogfish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Neopterygii: Infraclass Teleostei	Anguillidae	*A. anguilla*	European eel			
	Cyprinidae	*C. carpio carpio*	Common carp			
		D. rerio	Zebrafish		–	62
		R. rutilus	Roach			
		T. tinca	Tench			
	Salmonidae	*O. mykiss*	Rainbow trout	–	–	54, 118
		S. salar	Atlanitc salmon			
		S. trutta trutta	Brown trout			
	Esocidae	*E. lucius*	Northern pike			
	Lophiidae	*L. piscatorius*	Angler			
	Gadidae	*G. morhua*	Atlantic cod	–	–	85, 112, 118
	Pleuronectidae	*A. rostratus*	Longsnout flounder			
		P. flesus	Flounder			
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Blenniidae	*L. pholis*	Shanny			
		N. fluviatilis	Monkey goby			
	Haemulidae	*H. flavolineatum*	French grunt			
		L. berggylta	Ballan wrasse			
	Mullidae	*M. barbatus*	Red mullet			
	Percidae	*P. fluviatilis*	European perch			
Sarcopterygii						
Dipnoi	Lepidosirenidae	*L. paradoxa*	South American lungfish			

Purines			*Histamine*			*Bombesin/GRP*			*CGRP*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
				(+)	152						
+/–	**SI no R -**	156, 157									
+/–	**SI no R -**	156, 157									
+/–	**SI no R -**	155, 156, 157									
+/–	**SI no R -**	156, 157									
+/–	**SI no R -**	156, 157									
+/–	**SI no**	155, 156									
							+	102			
				no	152						
							no	162			
			no	**no**	148						
			no	**no**	148						
+/–*		64				+		64, 146			
				no	137						
			(+)	(+)	30						
			+	+	148						
+	–	153		**no**	152						
	–	75		+	152	+	–	67, 75, 146		–	136
	+/–	99									
				+	152						
						+		147			
			no	**no**	148						
				no	18						
				+	152						
			+	+	148						
			+	+	148						

*dose-dependent response

Effect of different signaling substances on motility (*Continued*)

				Galanin		
Class/subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*M. glutinosa*	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. fluviatilis*	European river lamprey			
Chondrichthyes						
Elasmobranchii	Dasyatidae	*P. violacea*	Pelagic stingray			
	Rajidae	*A. radiata*	Thorny skate			
		D. batis	Blue skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. brachyura	Blond ray			
		R. clavata	Thornback ray			
		R. eglanteria	Clearnosed skate			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
	Squalidae	*S. acanthias*	Spiny dogfish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Neopterygii: Infraclass Teleostei	Anguillidae	*A. anguilla*	European eel			
	Cyprinidae	*C. carpio carpio*	Common carp			
		D. rerio	Zebrafish			
		R. rutilus	Roach			
		T. tinca	Tench			
	Salmonidae	*O. mykiss*	Rainbow trout			
		S. salar	Atlanitc salmon			
		S. trutta trutta	Brown trout			
	Esocidae	*E. lucius*	Northern pike			
	Lophiidae	*L. piscatorius*	Angler			
	Gadidae	*G. morhua*	Atlantic cod	+		87
	Pleuronectidae	*A. rostratus*	Longsnout flounder			
		P. flesus	Flounder			
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Blenniidae	*L. pholis*	Shanny			
		N. fluviatilis	Monkey goby			
	Haemulidae	*H. flavolineatum*	French grunt			
		L. berggylta	Ballan wrasse			
	Mullidae	*M. barbatus*	Red mullet			
	Percidae	*P. fluviatilis*	European perch			
Sarcopterygii						
Dipnoi	Lepidosirenidae	*L. paradoxa*	South American lungfish			

Gastrin/CCK			*Ghrelin*			*NPY*			*Neurotensin*		
Stomach	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***	***Stomach***	***Intestine***	***Ref.***
+	**SI + Rno**	10							**no**	**no**	10
+	**SI + Rno**	10							**no**	**no**	10
+	**SI + Rno**	10							**no**	**no**	10
	+	3									
				+	116						
+/–		115							**?**		64
+	+	81					+	74, 138		**no**	75

? variable response

Effect of different signaling substances on motility (*Continued*)

				Opioid		
Class/Subclass	***Family***	***Species***		***Stomach***	***Intestine***	***Ref.***
Myxini						
	Myxinidae	*M. glutinosa*	Hagfish			
Cephalaspidomorphi						
	Petromyzontidae	*L. fluviatilis*	European river lamprey			
Chondrichthyes						
Elasmobranchii	Dasyatidae	*P. violacea*	Pelagic stingray			
	Rajidae	*A. radiata*	Thorny skate			
		D. batis	Blue skate			
		L. erinacea	Little skate			
		L. neavus	Cuckoo ray			
		R. brachyura	Blond ray			
		R. clavata	Thornback ray			
		R. eglanteria	Clearnosed skate			
		R. microocellata	Small-eyed ray			
		R. montagui	Spotted ray			
	Scyliorhinidae	*S. canicula*	Lesser spotted dogfish			
	Squalidae	*S. acanthias*	Spiny dogfish			
Actinopterygii						
	Polypteridae	*P. senegalus*	Gray bichir			
Neopterygii: Infraclass Teleostei	Anguillidae	*A. anguilla*	European eel			
	Cyprinidae	*C. carpio carpio*	Common carp		+	93
		D. rerio	Zebrafish			
		R. rutilus	Roach			
		T. tinca	Tench			
	Salmonidae	*O. mykiss*	Rainbow trout	+		64
		S. salar	Atlanitc salmon			
		S. trutta trutta	Brown trout			
	Esocidae	*E. lucius*	Northern pike			
	Lophiidae	*L. piscatorius*	Angler			
	Gadidae	*G. morhua*	Atlantic cod		+/-	75
	Pleuronectidae	*A. rostratus*	Longsnout flounder			
		P. flesus	Flounder			
		P. platessa	European plaice			
		R. tapirina	Greenback flounder			
	Cottidae	*M. scorpius*	Shorthorn sculpin			
	Blenniidae	*L. pholis*	Shanny			
		N. fluviatilis	Monkey goby			
	Haemulidae	*H. flavolineatum*	French grunt			
		L. berggylta	Ballan wrasse			
	Mullidae	*M. barbatus*	Red mullet			
	Percidae	*P. fluviatilis*	European perch			
Sarcopterygii						
Dipnoi	Lepidosirenidae	*L. paradoxa*	South American lungfish			

PACAP			Somatostatin			Tachykinins			VIP		
Stomach	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*	*Stomach*	*Intestine*	*Ref.*
							no	76			
							no	76			
						+		76			
							+	152			
						+	**SI (+)R+**	10	**no**	**no**	10
						+	**SI (+)R+**	10	**no**	**no**	10
						+	**SI (+)R+**	10	**no**	**no**	10
				+/-	102	+		73		**R -**	102
							+	127			
				no	162		+	162, 163, 165, 166		**no**	162
	-	61					+	61			
	-	118	+/-		58, 64	+	+	64, 71, 76, 92	**?**	-	58, 64, 118
							+	137			
-	-	112, 118	-	+/-	59, 75	+	+	77, 79, 88	**- /no***	**no**	59, 75, 78, 112
							+	152			
							+	152			
							+	76			

? variable response
* different studies

Systematics

The systematics and nomenclature is based on Fishbase and Tree of Life

Class/subclass		Order	Family	Species		Stomach-less species
Myxini						
		Myxiniformes	Myxinidae	*Eptatretus cirrhatus*	New Zealand hagfish	x
				Myxine glutinosa	Hagfish	x
Cephalaspidomorphi						
		Petromyzontiformes	Petromyzontidae	*Lampetra camtschatica*	Arctic lamprey	x
				Lampetra fluviatilis	European brook lamprey	x
				Lampetra planeri	European river lamprey	x
				Petromyzon marinus	Great sea lamprey	x
Chondrichthyes						
Elasmobranchii	Batoidea	Rajiformes	Dasyatidae	*Pteroplatytrygon violacea*	Pelagic stingray	
			Rajidae	*Amblyraja radiata*	Thorny skate	
				Dipturus batis	Blue skate	
				Leucoraja erinacea	Little skate	
				Leucoraja neavus	Cuckoo ray	
				Raja asterias	Starry ray	
				Raja brachyura	Blond ray	
				Raja clavata	Thornback ray	
				Raja eglanteria	Clearnosed skate	
				Raja microocellata	Small-eyed ray	
				Raja montagui	Spotted ray	
				Raja rhina	Longnose skate	
			Rhinobatidae	*Rhinobatos typus*	Giant shovelnose ray	
		Torpediniformes	Torpedinidae	*Torpedo marmorata*	Spotted torpedo	
	Selachimorpha	Carcharhiniformes	Scyliorhinidae	*Scyliorhinus canicula*	Lesser spotted dogfish	
				Scyliorhinus stellaris	Nursehound	
				Scyliorhinus torazame	Cloudy catshark	
		Heterodontiformes	Heterodontidae	*Heterodontus portusjacksoni*	Port Jackson shark	
		Hexanchiformes	Hexanchidae	*Heptranchias perlo*	Sharpnose sevengill shark	
		Orectolobiformes	Hemiscyllidae	*Hemiscyllium ocellatum*	Epaulette shark	
		Squaliformes	Squalidae	*Squalus acanthias*	Spiny dogfish	
		Squatiniformes	Squatinidae	*Squatina aculeata*	Sawback angelshark	
Holocephalii		Chimaeriformes	Chimaeridae	*Chimaera monstrosa*	Rabbit fish	
Actinopterygii						
		Polypteriformes	Polypteridae	*Polypterus senegalus*	Gray bichir	
Chondrostei		Acipenseriformes	Acipenseridae	*Acipenser transmontanus*	White sturgeon	
		Lepisosteiformes	Lepisosteidae	*Lepisosteus osseus*	Longnose gar	
				Lepisosteus platyrhincus	Gar pike	
Neopterygii: Infraclass Holostei		Amiiformes	Amiidae	*Amia calva*	Bowfin	
Neopterygii: Infraclass Teleostei	Osteoglossomorpha	Osteoglossiformes	Pantodontidae	*Pantodon buchholzi*	Freshwater butterflyfish	
	Elopomorpha	Anguilliformes	Anguillidae	*Anguilla anguilla*	European eel	
				Anguilla australis	Shortfin eel	
				Anguilla japonica	Japanese eel	
	Clupeomorpha	Clupeiformes	Engraulidae	*Engraulis japonicus*	Japanese anchovy	
	Ostariophysi	Cypriniformes	Cyprinidae	*Carassius auratus*	Goldfish	x
				Ctenopharyngodon idella	Grass carp	x
				Cyprinus carpio	Common carp	x
				Danio rerio	Zebrafish	x
				Hypophthalmichthys molitrix	Silver carp	x
				Leuciscus idus	Ide	x
				Pseudophoxinus antalyae	Sunflower fish	x
				Puntius conchonius	Rosy barb	x
				Rutilus rutilus (Leuciscus rutilus)	Roach	x
				Tinca tinca	Tench	x
				Zacco platypus	Freshwater minnow	x
			Gyrinocheilidae	*Gyrinocheilus aymonieri*	Chinese algae-eater	
		Characiformes	Characidae	*Hemigrammus ocellifer*	Head-and-taillight tetra	

For references and abbreviations see page 1262–1267.

References
108
42 52 66 76 108 119 128 129 152
146 158
15 22 31 32 76 80 108
22 76 143 150
108
154
20 41 42 48 76 83
152
20 103
19 20 52 156 157
144
156 157
10 19 20 48 134 154 155 156 157
103
10 19 20 156 157
10 16 20 156 157
20 83
131
144
21 38 49 141 144 154 155 156
33 36 37 38 50 140 142 143
33 34
124
144
124
3 45 47 48 65 68 73 82 102 103 109 114 128 129 152
141 144
159
29 127
22
55
69
125 126 127
96
19 41 96 100 105 152
67
83
35
7 9 27 52 60 90 91 121
120
19 110 150 160 162 163 165 166
61 62 96 116 117
9
28 96
39
1 127 131 132
148
12 148
94
96
96

(*Continued*)

Systematics (*Continued*)

Class/subclass		*Order*	*Family*	*Species*		*Stomach-less species*
		Siluriformes	Callichthyidae	*C. aeneus*	Bronze corydoras	
			Ictaluridae	*A. nebulosus*	Brown bullhead	
	Protacanthopterygii	Salmoniformes	Salmonidae	*O. kisutch*	Coho salmon	
				O. mykiss	Rainbow trout	
				S. salar	Atlanitc salmon	
				S. trutta trutta	Brown trout	
		Esociformes	Esocidae	*E. lucius*	Northern pike	
		Lophiiformes	Lophiidae	*L. piscatorius*	Angler	
	Paracanthopterygii	Gadiformes	Gadidae	*G. morhua*	Atlantic cod	
				P. pollachius	Pollack	
				R. raninus	Tadpole fish	
			Lotidae	*C. mustela*	Fivebeard rockling	
	Acanthopterygii	Mugiliformes	Mugilidae	*A. forsteri*	Yellow-eye mullet	
				L. saliens	Leaping mullet	
		Cyprinodontiformes	Poeciliidae	*P. reticulata*	Guppy	**x**
				X. variatus	Variable platyfish	**x**
		Tetraodontiformes	Tetraodontidae	*T. glaber*	Smooth toadfish	**x**
		Pleuronectiformes	Paralichthyidae	*P. dentatus*	Summer flounder	
				P. olivaceus	Japanese flounder	
			Pleuronectidae	*A. rostratus*	Longsnout flounder	**x**
				H. hippoglossus	Atlantic halibut	
				P. flesus	Flounder	
				P. platessa	European plaice	
				R. tapirina	Greenback flounder	**x**
			Scophthalmidae	*P. maxima*	Turbot	
			Soleidae	*S. solea*	Common sole	
		Scorpaeniformes	Cottidae	*M. scorpius*	Shorthorn sculpin	
			Platycephalidae	*P. bassensis*	Sand flathead	
			Scorpaenidae	*S. porcus*	Black scorpionfish	
		Perciformes	Blenniidae	*L. pholis*	Shanny	
			Centrarchidae	*L. gibbosus*	Pumpkinseed	
				L. macrochirus	Bluegill	
			Cichlidae	*Haplochromis* spp.	???	
				O. mossambicus	Mozambique tilapia	
				P. pulcher	Rainbow krib	
			Gobiidae	*G. mirabilis*	Longjaw mudsucker	**x**
				N. fluviatilis	Monkey goby	**???**
			Helostomatidae	*H. temminkii*	Kissing gourami	
			Haemulidae	*H. flavolineatum*	French grunt	
			Labridae	*C. exoletus*	Rock cock	**x**
				C. rupestris	Goldsinny-wrasse	**x**
				L. berggylta	Ballan wrasse	**x**
				L. mixtus	Cuckoo wrasse	**x**
			Moronidae	*D. labrax*	European seabass	
			Mullidae	*M. barbatus*	Red mullet	
			Percidae	*P. fluviatilis*	European perch	
			Sparidae	*S. aurata*	Gilthead seabream	
			Uranoscopidae	*U. japonicus*	Japanese stargazer	**?**
Sarcopterygii						
Dipnoi		Ceratodontiformes	Ceratodontidae	*N. forsteri*	Australian lungfish	**x***
		Lepidosireniformes	Lepidosirenidae	*L. paradoxa*	South American lungfish	**x***
			Protopteridae	*P. annectens*	West African lungfish	**x***

* **in some studies proximal part is called stomach**

References
96
110
111
2 13 14 16 19 24 54 58 64 65 70 71 76 92 101 113 115 118 133 135 146 161
137
7 23 30
148
152 153
19 45 46 56 59 67 74 75 77 78 79 81 85 86 87 88 89 97 98 106 112 113 114 118 136 138 146 152
19
19 128 129
19
7 8
44
28 96
96
6 7
26
95
57
84
19 25 99
19 151 152
6 7 57
17 25 130
25
15 19 45 46 48 53 128 129 139 147
6 7
40
51
9
125 127
96
89 90 91
96
149
148
96
18
19
19
19 152
19
11 40 123
148
19 110 148
1 40 43
104
71 72
76 107
107 145

Abbreviations

CCK	cholecystokinin
ChAT	choline acetyltransferase
CGRP	calcitonin gene-related peptide
GABA	gamma-amino butyric acid
GIP	gastric inhibitory peptide
GRP	gastrin-releasing peptide
NOS	nitric oxide synthase
NPY	neuropeptide Y
PACAP	pituitary adenylate cyclase-activating polypeptide
VAChT	vesicular acetylcholine transporter
VIP	vasoactive intestinal polypeptide

References

1 Abad ME, Peeze Binkhorst FM, Elbal MT, and Rombout JHWM (1987) A comparative immunocytochemical study of the gastro-entero-pancreatic (GEP) endocrine system in a stomachless and a stomach-containing teleost. *General and Comparative Endocrinology* 66: 123–136.

2 Aldman G and Holmgren S (1987) Control of gallbladder motility in the rainbow trout, *Salmo gairdneri*. *Fish Physiology and Biochemistry* 4: 143–155.

3 Aldman G, Jönsson AC, Jensen J, and Holmgren S (1989) Gastrin/CCK-like peptides in the spiny dogfish, *Squalus acanthias*; concentrations and actions in the gut. *Comparative Biochemistry and Physiology* 92C: 103–109.

4 Al-Mahrouki AA and Youson JH (1998) Immunohistochemical studies of the endocrine cells within the gastro-entero-pancreatic system of Osteoglossomorpha, an ancient teleostean group. *General and Comparative Endocrinology* 110: 125–139.

5 Al-Mahrouki AA and Youson JH (1999) Ultrastructure and immunocytochemistry of the islet organ of osteoglossomorpha (Teleostei). *General and Comparative Endocrinology* 116: 409–421.

6 Anderson C (1983) Evidence for 5-HT-containing intrinsic neurons in the teleost intestine. *Cell and Tissue Research* 230: 377–386.

7 Anderson C and Campbell G (1988) Immunohistochemical study of 5-HT-containing neurons in the teleost intestine: Relationship to the presence of enterochromaffin cells. *Cell and Tissue Research* 254: 553–559.

8 Anderson CR (1990) The ultrastructure and distribution of 5-HT-containing neurons in the intestine of a teleost fish, *Aldrichetta forsteri*. *Cell and Tissue Research* 259: 379–387.

9 Andreozzi G, de Girolamo P, Affatato C, Antonucci R, Russo P, and Gargiulo G (1997) VIP-like immunoreactivity in the intestinal tract of fish with different feeding habits. *European Journal of Histochemistry* 41: 57–64.

10 Andrews PLR and Young JZ (1988) The effect of peptides on the motility of the stomach, intestine and rectum in the skate (*Raja*). *Comparative Biochemistry and Physiology* 89C: 343–348.

11 Arcamone N, Neglia S, Gargiulo G, Esposito V, Varricchio E, Battaglini P, De Girolamo P, and Russo F (2009) Distribution of ghrelin peptide in the gastrointestinal tract of stomachless and stomach-containing teleosts. *Microscopy Research and Technique* 72: 525–33.

12 Backman L (1917) Untersuchungen über die Automatie des Schleiendarmes und dessen Beeinflüssung durch Adrenalin. *Zeitschrift für Biologie* 67: 307.

13 Barrenechea MA, López J, and Martínez A (1994) Regulatory peptides in gastric endocrine cells of the rainbow trout *Oncorhynchus mykiss*: general distribution and colocalizations. *Tissue and Cell* 26: 309–321.

14 Barton CL, Shaw C, Halton DW, and Thim L (1992) Rainbow trout (*Oncorhynchus mykiss*) neuropeptide Y. *Peptides* 13: 1159–1163.

15 Baumgarten HG, Björklund A, Lachenmayer L, Nobin A, and Rosengren E (1973) Evidence for the existence of serotonin-, dopamine-, and noradrenaline-containing neurons in the gut of *Lampetra fluviatilis*. *Zeitung für Zellforschung* 141: 33–54.

16 Beorlegui C, Martinez A, and Sesma P (1992) Some peptide-like colocalizations in endocrine cells of the pyloric caeca and the intestine of *Oncorhynchus mykiss* (Teleostei). *Cell and Tissue Research* 269: 353–357.

17 Bermúdez R, Vigliano F, Quiroga MI, Nieto JM, Bosi G, and Domeneghini C (2007) Immunohistochemical study on the neuroendocrine system of the digestive tract of turbot, *Scophthalmus maximus* (L.) infected by *Enteromyxum scophthalmi* (Myxozoa). *Fish and Shellfish Immunology* 22: 252–263.

18 Bernheim F (1934) Action of drugs on the isolated intestine of certain teleost fish. *Journal of Pharmacology and Experimental Therapeutics* 50: 216–222.

19 Bjenning C and Holmgren S (1988) Neuropeptides in the fish gut. A study of evolutionary trends. *Histochemistry* 88: 155–163.

20 Bjenning C, Farrell AP, and Holmgren S (1991) Bombesin-like immunoreactivity in the gut and heart region of elasmobranch fish (skates), and the *in vitro* effect of bombesin on coronary vessels from the longnose skate, *Raja rhina*. *Regulatory Peptides* 35: 207–219.

21 Bjenning C, Hazon N, Balasubramaniam A, Holmgren S, and Conlon JM (1993) Distribution and activity of dogfish NPY and peptide YY in the cardiovascular system of the common dogfish. *American Journal of Physiology* 264: R1119–R1124.

22 Bosi G, Di Giancamillo A, Arrighi S, and Domeneghini C (2004) An immunohistochemical study on the neuroendocrine system in the alimentary canal of the brown trout, *Salmo trutta*, L. 1758. *General and Comparative Endocrinology* 138: 166–181.

23 Bosi G, Shinn AP, Giari L, Arrighi S, and Domeneghini C (2004) The presence of a galanin-like peptide in the gut neuroendocrine system of *Lampetra fluviatilis* and *Acipenser transmontanus*: An immunohistochemical study. *Tissue and Cell* 36: 283–292.

24 Bosi G, Shinn AP, Giari L, Simoni E, Pironi F, and Dezfuli BS (2005) Changes in the neuromodulators of the diffuse endocrine system of the alimentary canal of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), naturally infected with *Eubothrium crassum* (Cestoda). *Journal of Fish Diseases* 28: 703–711.

25 Bosi G, Bermudez R, and Domeneghini C (2007) The galaninergic enteric nervous system of pleuronectiformes (*Pisces, Osteichthyes*): An immunohistochemical and confocal laser scanning immunofluorescence study. *General and Comparative Endocrinology* 152: 22–29.

26 Breves JP, Veillette PA, and Specker JL (2009) Ghrelin in the summer flounder: Immunolocalization to the gastric glands and action on plasma cortisol levels. *Comparative Biochemistry and Physiology* 152A: 268–272.

27 Brüning G, Hattwig K, and Mayer B (1996) Nitric oxide synthase in the peripheral nervous system of the goldfish, *Carassius auratus*. *Cell and Tissue Research* 284: 87–98.

28 Burkhardt-Holm P and Holmgren S (1989) A comparative study of neuropeptides in the intestine of two stomachless teleosts (*Poecilia reticulata, Leuciscus idus melanotus*) under conditions of feeding and starvation. *Cell and Tissue Research* 255: 245–254.

29 Burkhardt-Holm P and Holmgren S (1992) A study of the alimentary canal of the brachiopterygian fish *Polypterus senegalensis* with electron microscopy and immunohistochemistry. *Acta Zoologica (Stockholm)* 73: 85–94.

30 Burnstock G (1958). The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. *British Journal of Pharmacology and Chemotherapy* 13: 216–226.

31 Cheung R, Andrews PC, Plisetskaya EM, and Youson, JH (1991) Immunoreactivity to peptides belonging to the pancreatic polypeptide family (NPY, aPY, PP, PYY) and to glucagon-like peptide in the endocrine pancreas and anterior intestine of adult lampreys, *Petromyzon marinus* – an immunohistochemical study. *General and Comparative Endocrinology* 81: 51–63.

32 Cheung R, Ferreira LCG, and Youson JH (1991) Distribution of two forms of somatostatin and peptides belonging to the pancreatic polypeptide family in tissues of larval lampreys, *Petromyzon marinus* – An immunohistochemical study. *General and Comparative Endocrinology* 82: 93–102.

33 Chiba A, Honma Y, and Oka S (1995) Ontogenetic development of neuropeptide Y-like-immunoreactive cells in the gastro-enteropancreatic endocrine system of the dogfish. *Cell and Tissue Research* 282: 33–40.

34 Chiba A (1998) Ontogeny of serotonin-immunoreactive cells in the gut epithelium of the cloudy dogfish, *Scyliorhinus torazame*, with reference to coexistence of serotonin and neuropeptide Y. *General and Comparative Endocrinology* 111: 290–298.

35 Chusovitina SV, and Varaksin, AA (2003) NO-ergic innervation of digestive tract of Japanese anchovy (*Engraulis japonicus*). *Morfologiia* 123: 50–53.

36 Cimini V, Van Noorden S, Giordano-Lanza G, Nardini V, McGregor GP, Bloom SR, and Polak JM (1985) Neuropeptides and 5-HT immunoreactivity in the gastric nerves of the dogfish (*Scyliorhinus stellaris*). *Peptides* 6(supplement 3): 373–377.

37 Cimini V Van Noorden S, and Polak JM (1989) Co-localization of substance P-, bombesin- and peptide histidine isoleucine PHI-like peptides in gut endocrine cells of the dogfish *Scyliorhinus stellaris*. *Anatomy and Embryology* 179: 605–614.

38 Cimini V, Van Noorden S, and Sansone M (1992) Neuropeptide y-like immunoreactivity in the dogfish gastroenteropancreatic tract: light and electron microscopical study. *General and Comparative Endocrinology* 86: 413–423.

39 Cinar K, Senol N, and Ozen MR (2006) Immunohistochemical study on distribution of endocrine cells in gastrointestinal tract of flower fish (*Pseudophoxinus antalyae*). *World journal of gastroenterology* 12: 6874–6878.

40 De Girolamo P, Lucini C, Vega JA, Andreozzi G, Coppola L, and Castaldo L (1999) Co-localization of Trk neurotrophin receptors and regulatory peptides in the endocrine cells of the teleostean stomach. *Anatomical Record* 256: 219–226.

41 Domeneghini C, Radaelli G, Arrighi S, Mascarello F, and Veggetti A (2000) Neurotransmitters and putative neuromodulators in the gut of *Anguilla anguilla* (L.). Localizations in the enteric nervous and endocrine systems. *European Journal of Histochemistry* 44: 295–306.

42 Ekblad E, Alumets J, Ekman R, Falkmer S, Håkanson R, and Sundler F (1985) Two distinct VIP precursors in cartilaginous fish? *Peptides* 6(supplement 3): 383–388.

43 Elbal MT, and Agulleiro B (1986) An immunocytochemical and ultrastructural study of endocrine cells in the gut of a teleost fish, *Sparus auratus* L. *General and Comparative Endocrinology* 64: 339–354.

44 Elbal MT, Lozano MT, and Agulleiro B (1988) The endocrine cells in the gut of *Mugil saliens* Risso, 1810 (Teleostei): An immunocytochemical and ultrastructural study. *General and Comparative Endocrinology* 70: 231–246.

45 El-Salhy M, Wilander E, and Lundqvist M (1985) Comparative studies of serotonin-like immunoreactive cells in the digestive tract of vertebrates. *Biomedical Research* 6: 371–375.

46 El-Salhy M (1984) Occurrence of polypeptide YY (PYY) and pancreatic polypeptide (PP) in the gastrointestinal tract of the bony fish. *Biomedical Research* 5: 441–444.

47 El-Salhy M (1984) Immunocytochemical investigation of the gastro-entero-pancreatic (GEP) neurohormonal peptides in the pancreas and gastrointestinal tract of the dogfish *Squalus acanthias*. *Histochemistry* 80: 193–205.

48 Falkmer S, Ebert R, Arnold R, and Creutzfeldt W (1980) Some phylogenetic aspects on the enteroinsular axis with particular regard to the appearance of the gastric inhibitory polypeptide. *Frontiers in Hormone Research* 7: 1–6.

49 Faraldi G, Canepa M, Borgiani L, and Zanin T (1990) Identification of pancreatic glucagon cells in the cartilaginous fish *Scyliorhinus canicula* by ultrastructural immunocytochemistry. *Basic and Applied Histochemistry* 34: 199–208.

50 Faraldi G, Bonini E, Farina L, and Tagliafierro G (1988) Distribution and ontogeny of glucagon-like cells in the gastrointestinal tract of the cartilaginous fish *Scyliorhinus stellaris* (L.). *Acta Histochemistry* 83(1): 57–64.

51 Goddard JS (1973) The effects of cholinergic drugs on the motility of the alimentary canal of *Blennius pholis* L. *Cellular and Molecular Life Sciences* 29: 974–975.

52 Goodrich JT, Bernd P, Sherman D, and Gershon MD (1980) Phylogeny of enteric serotonergic neurons. *Journal of Neurology* 190: 15–28.

53 Gräns A and Olsson C unpublished.

54 Green K and Campbell G (1994) Nitric oxide formation is involved in vagal inhibition of the stomach of the trout (*Salmo gairdneri*). *Journal of Autonomic Nervous System* 50: 221–229.

55 Groff KE and Youson JH (1997) An immunohistochemical study of the endocrine cells within the pancreas, intestine, and stomach of the gar (*Lepisosteus osseus* L.). *General and Comparative Endocrinology* 106: 1–16.

56 Groisman SD and Shparkovskii IA (1989) The effect of dopamine and dopa on the electrical activity of stomach muscles in the skate *Raja radiata* and cod *Gadus morhua*. *Zhurnal Evolyutsionnoi Biokhimii i Fiziologii* 25: 505–511.

57 Grove DJ and Campell C (1979) The role of extrinsic and intrinsic nerves in the coordination of gut motility in the stomachless flatfish *Rhombosolea tapirina* and *Ammotretis rostrata* Guenther. *Comparative Biochemistry and Physiology* 63C: 143–159.

58 Grove DJ and Holmgren S (1992) Intrinsic mechanisms controlling cardiac stomach volume of the rainbow trout (*Oncorhynchus mykiss*) following gastric distension. *Journal of Experimental Biology* 163: 33–48.

59 Grove DJ and Holmgren S (1992) Mechanisms controlling stomach volume of the Atlantic cod (*Gadus morhua*) following gastric distension. *Journal of Experimental Biology* 163: 49–63.

60 Himick BA and Peter RE (1994) Bombesin acts to suppress feeding behavior and alter serum growth hormone in goldfish. *Physiology and Behavior* 55: 65–72.

61 Holmberg A, Schwerte T, Pelster B, and Holmgren S (2004) Ontogeny of the gut motility control system in zebrafish *Danio rerio* embryos and larvae. *Journal of Experimental Biology* 207: 4085–4094.

62 Holmberg A, Olsson C, and Holmgren S (2006) The effects of endogenous and exogenous nitric oxide on gut motility in zebrafish *Danio rerio* embryos and larvae. *Journal of Experimental Biology* 209: 2472–2479.

64 Holmgren S (1983) The effects of putative non-adrenergic, non-cholinergic autonomic transmitters on isolated strips from the stomach of the rainbow trout. *Salmo gairdneri*. *Comparative Biochemistry and Physiology – Part C* 74: 229–38.

65 Burka JF, Blair RMJ, and Hogan JG (1989) Characterization of the muscarinic and serotoninergic receptors of the intestine of the rainbow trout (*Salmo gairdneri*). *Canadian Journal of Physiology and Pharmacology* 67: 477–482.

66 Holmgren S and Fänge R (1981) Effects of cholinergic drugs on the intestine and gallbladder of the hagfish *Myxine glutinosa* L with a report on inconsistent effects of catecholamines. *Marine Biology Letters* 2: 265–277.

67 Holmgren S and Jönsson AC (1988) Occurrence and effects on motility of bombesin-related peptides in the gastrointestinal tract of the Atlantic cod, *Gadus morhua*. *Comparative Biochemistry and Physiology* 89C: 249–256.

68 Holmgren S and Nilsson S (1983) Bombesin-, gastrin/CCK-, 5-hydoxytryptamine-, neurotensin-, somatostatin-, and VIP-like immunoreactivity and catecholamine fluorescence in the gut of the elasmobranch, *Squalus acanthias*. *Cell and Tissue Research* 234: 595–618.

69 Holmgren S and Nilsson S (1983) VIP-, bombesin-, and neurotensin-like immunoreactivity in neurons of the gut of the holostean fish, *Lepisosteus platyrhincus*. *Acta Zoologica (Stockholm)* 64: 25–32.

70 Holmgren S, Vaillant C, and Dimaline R (1982) VIP-, substance P-, gastrin/CCK-, bombesin-, somatostatin-, and glucagon-like immunoreactivities in the gut of the rainbow trout, *Salmo gairdneri*. *Cell and Tissue Research* 223: 141–153.

71 Holmgren S, Grove DJ, and Nilsson S (1985) Substance P acts by releasing 5-hydroxytryptamine from enteric neurons in the stomach of the rainbow trout, *Salmo gairdneri*. *Neuroscience* 14: 683–693.

72 Holmgren S, Fritsche R, Karila P, Gibbins I, Axelsson M, Franklin C, Grigg G, and Nilsson S (1994) Neuropeptides in the Australian lungfish *Neoceratodus forsteri*: Effects *in vivo* and presence in autonomic nerves. *American Journal of Physiology* 266: R1568–R1577.

73 Holmgren S (1985) Substance P in the gastrointestinal tract of *Squalus acanthias*. *Molecular Physiology* 8: 119–130.

74 Jensen J and Conlon JM (1992) Characterization of peptides related to neuropeptide tyrosine and peptide tyrosine-tyrosine from the brain and gastrointestinal tract of teleost fish. *European Journal of Biochemistry* 210: 405–410.

75 Jensen J and Holmgren S (1985) Neurotransmitters in the intestine of the Atlantic cod, *Gadus morhua*. *Comparative Biochemistry and Physiology* 82C: 81–89.

76 Jensen J and Holmgren S (1991) Tachykinins and intestinal motility in different fish groups. *General and Comparative Endocrinology* 83: 388–396.

77 Jensen J, Holmgren S, and Jönsson AC (1987) Substance P-like immunoreactivity and the effects of tachykinins in the intestine of the Atlantic cod, *Gadus morhua*. *Journal of Autonomic Nervous System* 20: 25–33.

78 Jensen J, Axelsson M, and Holmgren S (1991) Effects of substance P and vasoactive intestinal polypeptide on gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *Journal of Experimental Biology* 156: 361–373.

79 Jensen J, Karila P, Jönsson AC, Aldman G, and Holmgren S (1993) Effects of substance P and distribution of substance P-like immunoreactivity in nerves supplying the stomach of the cod *Gadus morhua*. *Fish Physiology and Biochemistry* 12: 237–247.

80 Johnels AG and Östlund E (1958) Anatomical and physiological studies on the enteron of *Lampetra fluviatilis* (L.). *Acta Zoologica (Stockholm)* 39: 9–12.

81 Jönsson AC, Holmgren S, and Holstein B (1987) Gastrin/CCK-like immunoreactivity in endocrine cells and nerves in the gastrointestinal tract of the cod, *Gadus morhua*, and the effect of peptides of the gastrin/CCK family on cod gastrointestinal smooth muscle. *General and Comparative Endocrinology* 66: 190–202.

82 Kågström J, Axelsson M, Jensen J, Farrell AP, and Holmgren S (1996) Vasoactivity and immunoreactivity of fish tachykinins in the vascular system of the spiny dogfish. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 270: R585–R593.

83 Kaiya H, Tsukada T, Yuge S, Mondo H, Kangawa K, and Takei Y (2006) Identification of eel ghrelin in plasma and stomach by radioimmunoassay and histochemistry. *General and Comparative Endocrinology* 148: 375–382.

84 Kamisaka Y, Totl and GK, Tagawa M, Kurokawa T, Suzuki T, Tanaka M, and Rønnestad I (2001) Ontogeny of cholecystokinin-immunoreactive cells in the digestive tract of Atlantic halibut, *Hippoglossus hippoglossus*, larvae. *General and Comparative Endocrinology* 123: 31–37.

85 Karila P and Holmgren S (1995) Enteric reflexes and nitric oxide in the fish intestine. *Journal of Experimental Biology* 198: 2405–2411.

86 Karila P and Holmgren S (1997) Anally projecting neurons exhibiting immunoreactivity to galanin, nitric oxide synthase and vasoactive intestinal peptide, detected by confocal laser scanning microscopy, in the intestine of the Atlantic cod, *Gadus morhua*. *Cell and Tissue Research* 287: 525–533.

87 Karila P, Jönsson A-C, Jensen J, and Holmgren S (1993) Galanin-like immunoreactivity in extrinsic and intrinsic nerves to the gut of the Atlantic cod, *Gadus morhua*, and the effect of galanin on the smooth muscle of the gut. *Cell and Tissue Research* 271: 537–544.

88 Karila P, Shahbazi F, Jensen J, and Holmgren S (1998) Projections and actions of tachykininergic, cholinergic, and serotonergic neurones in the intestine of the Atlantic cod. *Cell and Tissue Research* 291: 403–413.

89 Kiliaan AJ, Joosten HWJ, Bakker R, Dekker K, and Groot JA (1989) Serotonergic neurons in the intestine of two teleosts, *Carassius auratus* and *Oreochromis mossambicus*, and the effect of serotonin on transepithelial ion-selectivity and muscle tension. *Neuroscience* 31: 817–824.

90 Kiliaan A, Holmgren S, Jönsson AC, Dekker K, and Groot J (1992) Neurotensin, substance P, gastrin/cholecystokinin and bombesin in the intestine of the tilapia (*Oreochromis mossambicus*) and the goldfish (*Carassius auratus*): Immunochemical detection and effects on electrophysiological characteristics of the mucosa. *General and Comparative Endocrinology* 88: 351–363.

91 Kiliaan AJ, Holmgren S, Jönsson AC, Dekker K, and Groot JA (1993) Neuropeptides in the intestine of 2 teleost species (*Oreochromis mossambicus, Carassius auratus*) – localization and electrophysiological effects on the epithelium. *Cell and Tissue Research* 271: 123–134.

92 Kitazawa T (1989) 5-Hydroxytryptamine is a possible neurotransmitter of the non-cholinergic excitatory nerves in the longitudinal muscle of rainbow trout stomach *Salmo gairdneri*. *British Journal of Pharmacology* 98: 781–790.

93 Kitazawa T, Hoshi T, Temma K, and Kondo H (1986) Effects of morphine and methionine-enkephalin on the smooth muscle tonus and the contraction induced by transmural stimulation in the carp (*Cyprinus carpio*) intestinal bulb. *Comparative Biochemistry and Physiology* 84C: 299–305.

94 Ku SK, Lee JH, and Lee HS (2004) Immunohistochemical study on the endocrine cells in gut of the stomachless teleost, *Zacco platypus* (Cyprinidae). *Anatomia, Histologia, Embryologia* 33: 212–219.

95 Kurokawa T, Suzuki T, and Andoh T (2000) Development of cholecystokinin and pancreatic polypeptide endocrine systems during the larval stage of Japanese flounder, *Paralichthys olivaceus*. *General and Comparative Endocrinology* 120: 8–16.

96 Langer M, Van Noorden S, Polak JM, and Pearse AGE (1979) Peptide hormone-like immunoreactivity in the gastrointestinal tract and endocrine pancreas of eleven teleost species. *Cell and Tissue Research* 199: 493–508.

97 Larsson L-I and Rehfeld JF (1977) Evidence for a common evolutionary origin of gastrin and cholecystokinin. *Nature* 269: 335–338.

98 Larsson L-I, Polak JM, Buffa R, Sundler F, and Solcia E (1979) On the immunocytochemical localization of the vasoactive intestinal polypeptide. *Journal of Histochemistry and Cytochemistry* 27: 936–938.

99 Lennard R and Huddart H (1989) Purinergic modulation in the flounder gut. *General Pharmacology* 20: 849–853.

100 L'Hermite A, Ferrand R, Dubois MP, and Andersen AC (1985) Detection of endocrine cells by immunofluorescence method in the gastroenteropancreatic system of the adult eel, glass-eel, and Leptocephalic larva (*Anguilla anguilla* L.). *General and Comparative Endocrinology* 58: 347–359.

101 Li ZS and Furness JB (1993) Nitric oxide synthase in the enteric nervous system of the rainbow trout, *Salmo gairdneri*. *Archives of Histology and Cytology* 56: 185–193.

102 Lundin K, Holmgren S, and Nilsson S (1984) Peptidergic functions in the dogfish rectum. *Acta Physiologica Scandinavica* 121: 46A.

103 Lutz BR (1931) The innervation of the stomach and rectum and the action of adrenaline in elasmobranch fishes. *Biological Bulletin* 61: 93–100.

104 Matsuda K, Kashimoto K, Higuchi T, Yoshida T, Uchiyama M, Shioda S, Arimura A, and Okamura T (2000) Presence of pituitary adenylate cyclase-activating polypeptide (PACAP) and its relaxant activity in the rectum of a teleost, the stargazer, *Uranoscopus japonicus*. *Peptides* 21: 821–827.

105 Nilsson S and Fänge, R (1967) Adrenergic receptors in the swimbladder and gut of a teleost (*Anguilla anguilla*). *Comparative Biochemistry and Physiology – Part C: Comparative Pharmacology* 23: 661–664.

106 Nilsson S and Fänge R (1969) Adrenergic and cholinergic vagal effects on the stomach of a teleost (*Gadus morhua*). *Comparative Biochemistry and Physiology* 30: 691–694.

107 Nilsson S and Holmgren S (1992) Autonomic nerve function and cardiovascular control in lungfish. In: Wood SC, Weber RE, Hargens AR and Millard RW (eds.) *Physiological Adaptations in Vertebrates*, pp. 377–395. New York, Basel, Hong Kong: Marcel Dekker.

108 Nilsson S and Holmgren S (1998) The autonomic nervous system and chromaffin tissue in hagfishes. In: Jörgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) *The Biology of Hagfishes*, pp. 480–495. London, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman and Hall.

109 Nilsson S and Holmgren S (1983) Splanchnic nervous control of the stomach of the spiny dogfish, *Squalus acanthias*. *Comparative Biochemistry and Physiology – Part C* 76: 271–276.

110 Noaillac-Depeyre J and Hollande E (1981) Evidence for somatostatin, gastrin and pancreatic polypeptide-like substances in the mucosa cells of the gut in fishes with and without stomach. *Cell and Tissue Research* 216: 193–203.

111 Nozaki M, Miyata K, Oota Y, Gorbman A, and Plisetskaya EM (1988) Colocalization of glucagon-like peptide and glucagon immunoreactivities in pancreatic islets and intestine of salmonids. *Cell and Tissue Research* 253: 371–375.

112 Olsson C and Holmgren S (2000) PACAP and nitric oxide inhibit contractions in proximal intestine of the Atlantic cod, *Gadus morhua*. *Journal of Experimental Biology* 203: 575–583.

113 Olsson C and Holmgren S (1994) Distribution of PACAP (pituitary adenylate cyclase-activating polypeptide)-like and helospectin-like peptides in the teleost gut. *Cell and Tissue Research* 277: 539–547.

114 Olsson C, and Karila P (1995) Coexistence of NADPH-diaphorase and vasoactive intestinal polypeptide in the enteric nervous system of the Atlantic cod (*Gadus morhua*) and the spiny dogfish (*Squalus acanthias*). *Cell and Tissue Research* 280: 297–305.

115 Olsson C, Aldman G, Larsson A, and Holmgren S (1999) Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* 202: 161–170.

116 Olsson C, Holbrook JD, Bompadre G, Jonsson E, Hoyle CH, Sanger GJ, Holmgren S, and Andrews PL (2008) Identification of genes for the ghrelin and motilin receptors and a novel related gene in fish, and stimulation of intestinal motility in zebrafish (*Danio rerio*) by ghrelin and motilin. *General and Comparative Endocrinology* 155: 217–226.

117 Olsson C, Holmberg A, and Holmgren S (2008) Development of enteric and vagal innervation of the zebrafish (*Danio rerio*) gut. *Journal of Comparative Neurology* 508: 756–770.

118 Olsson C and Holmgren S, unpublished.

119 Östberg Y, Van Noorden S, Everson Pearse AG, and Thomas NW (1976) Investigations on polypeptide hormone containing cells in the intestinal mucosa of a cyclostome *Myxine glutinosa*. *General and Comparative Endocrinology* 28: 213–227.

120 Pan QS and Fang ZP (1993) An immunocytochemical study of endocrine cells in the gut of a stomachless teleost fish, grass carp, Cyprinidae. *Cell Transplantation* 2: 419–427.

121 Pederzoli A, Trevisan P, and Bolognani Fantin AM (1996) Immunocytochemical study of endocrine cells in the gut of goldfish *Carassius carassius* (L.) var. *auratus* submitted to experimental lead intoxication. *European Journal of Histochemistry* 40: 305–314.

122 Pederzoli A, Bertacchi I, Gambarelli A, and Mola L (2004) Immunolocalisation of vasoactive intestinal peptide and substance P in the developing gut of *Dicentrarchus labrax* (L.). *European Journal of Histochemistry* 48: 179–183.

123 Pederzoli A, Conte A, Tagliazucchi D, Gambarelli A, and Mola L (2007) Occurrence of two NOS isoforms in the developing gut of sea bass *Dicentrarchus labrax* (L.). *Histology and Histopathology* 22: 1057–1064.

124 Preston E, Mcmanus CD, Jonsson AC, and Courtice GP (1995) Vasoconstrictor effects of galanin and distribution of galanin containing fibres in three species of elasmobranch fish. *Regulatory Peptides* 58: 123–134.

125 Rajjo IM, Vigna SR, and Crim JW (1988) Cholecystokinin immunoreactivity in the digestive tract of bowfin *Amia calva*, bluegill *Lepomis macrochirus*, and bullfrog *Rana catesbeiana*. *General and Comparative Endocrinology* 70: 133–144.

126 Rajjo IM, Vigna SR, and Crim JW (1989) Immunocytochemical localization of vasoactive intestinal polypeptide in the digestive tracts of a holostean and a teleostean fish. *Comparative Biochemistry and Physiology* 94C: 411–418.

127 Rajjo IM, Vigna SR, and Crim JW (1989) Immunohistochemical localization of bombesin-like peptides in the digestive tract of the bowfin, *Amia calva*. *Comparative Biochemistry and Physiology* 94C: 405–409.

128 Reinecke M, Carraway RF, Falkmer S, Feurle G, and Forsmann WG (1980) Occurrence of neurotensin-immunorective cells in the digestive tract of lower vertebrates and deuterostomian invertebrates. *Cell and Tissue Research* 212: 173–183.

129 Reinecke M, Schluter P, Yanaihara N, and Forssmann WG (1981) VIP immunoreactivity in enteric nerves and endocrine cells of the vertebrate gut. *Peptides* 2: 149–156.

130 Reinecke M, Müller C, and Segner H (1997) An immunohistochemical analysis of the ontogeny, distribution and coexistence of 12 regulatory peptides and serotonin in endocrine cells and nerve fibres of the digestive tract of the turbot, *Scophthalmus maximus* (Teleostei). *Anatomy and Embryology* 195: 87–102.

131 Rombout JHWM, and Reinecke M (1984) Immunohistochemical localization of (neuro)peptide hormones in endocrine cells and nerves of the gut of a stomachless teleost fish, *Barbus conchonius* (Cyprinidae). *Cell and Tissue Research* 237: 57–65.

132 Rombout JHWM, van der Grinten CPM, Peeze Binkhorst FM, Taverne-Thiele JJ, and Schooneveld H (1986) Immunocytochemical identification and localization of peptide hormones in the gastro-entero-pancreatic (GEP) endocrine system of the mouse and a stomachless fish, *Barbus conchonius*. *Histochemistry* 84: 471–483.

133 Sakata I, Mori T, Kaiya H, Yamazaki M, Kangawa K, Inoue K, and Sakai T (2004) Localization of ghrelin-producing cells in the stomach of the rainbow trout (*Oncorhynchus mykiss*). *Zoological Science (Tokyo)* 21: 757–762.

134 Sakharov DA and Salimova NB (1980) Serotonin neurons in the peripheral nervous system of the larval lamprey *Lampetra planeri* a histochemical, microspectrofluorimetric and ultrastructural study. *Zool. Jb. Physiol.* 84: 231–239.

135 Santer RM and Holmgren S (1983) An ultrastructural and fluorescence histochemical study of the myenteric plexus of the stomach of the rainbow trout *Salmo gairdner*i. *Acta Zoologica* 64: 55–66.

136 Shahbazi F, Karila P, Olsson C, Holmgren S, Conlon JM, and Jensen J (1998) Primary structure, distribution, and effects on motility of CGRP in the intestine of the cod *Gadus morhua*. *American Journal of Physiology–Regulatory Integrative and Comparative Physiology* 44: R19–R28.

137 Wartman CA, Mokler JM, Briand HA, Ireland WP, and Burka JF (1999) Effects of acclimation temperatures of 7 and 14 °C on the contractility of Atlantic salmon (*Salmo salar*) intestinal smooth muscle *in vitro*. *Fish Physiology and Biochemistry* 21: 277–284.

138 Shahbazi F, Holmgren S, Larhammar D, and Jensen J (2002) Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod *Gadus morhua*. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 282: R1414–R1421.

139 Stefan Y, Dufour C, and Falkmer S (1978) Mise en évidence par immunofluorescence de cellules à polypeptide pancréatique (PP) dans le pancréas et le tube digestif de Poissons osseux et cartilagineux. *Les Comptes Rendus de l'Académie des sciences (Paris)* 286: 1073–1075.

140 Tagliafierro G, Faraldi G, and Bandelloni R (1985) Distribution histochemistry and ultrastructure of somatostatin-like immunoreactive cells in the gastroenteric tract of the cartilaginous fish *Scyliorhinus stellaris*. *Histochemical Journal* 17: 1033–1042.

141 Tagliafierro G, Zaccone G, Bonini E, Faraldi G, Farina L, and Fasulo S (1987) Bombesin-like immunoreactive cells in the gastro-intestinal tract of some lower vertebrates. *Regulatory Peptides* 19: 141 (abstract).

142 Tagliafierro G, Bonini E, Faraldi G, Farina L, and Rossi GG (1988) Distribution and ontogeny of VIP-like immunoreactivity in the gastro-entero-pancreatic system of a cartilaginous fish *Scyliorhinus stellaris*. *Cell and Tissue Research* 253: 23–28.

143 Tagliafierro G, Rossi GG, Bonini E, Faraldi G, and Farina L (1989) Ontogeny and differentiation of regulatory peptide and serotonin-immunoreactivity in the gastrointestinal tract of an elasmobranch. *Journal of Experimental Zoology* Supplement 2: 165–174.

144 Tagliafierro G, Farina L, Faraldi G, Rossi GG, and Vacchi M (1989) Distribution of somatostatin and glucagon immunoreactive cells in the gastric mucosa of some cartilaginous fishes. *General and Comparative Endocrinology* 75: 1–9.

145 Tagliafierro G, Carlini M, Faraldi G, Morescalchi AM, Putti R, DellaRossa A, Fasulo S, and Mauceri A (1996) Immunocytochemical detection of islet hormones in the digestive system of *Protopterus annectens*. *General and Comparative Endocrinology* 102: 288–298.

146 Thorndyke M and Holmgren S (1990) Bombesin potentiates the effect of acetylcholine on isolated strips of fish stomach. *Regulatory Peptides* 30: 125–135.

147 Thorndyke MC, Holmgren S, Nilsson S, and Falkmer S (1984) Bombesin potentiation of the acetylcholine response in isolated strips of fish stomach. *Regulatory Peptides* 9: 350.

148 Valette G and Augereau P (1958) Réactivité des muscles lisses des poissons a l'histamine et a d'autres agents contracturans (5-hydroxytryptamine, acétylcholine et chlorure de baryum). *Journal of Physiology* 50: 1067–1074.

149 Van Noorden S and Patent GJ (1980) Vasoactive intestinal polypeptide-like immunoreactivity in nerves of the pancreatic islet of teleost fish, *Gillichtys mirabilis*. *Cell and Tissue Research* 212: 139–146.

150 Van Noorden S and Pearse AGE (1974) Immunoreactive polypeptide hormones in the pancreas and gut of the lamprey. *General and Comparative Endocrinology* 23: 311–324.

151 Watson AHD (1979) Fluorescent histochemistry of the teleost gut: Evidence for the presence of serotonergic neurones. *Cell and Tissue Research* 197: 155–164.

152 von Euler US and Östlund E (1957) Effects of certain biologically occuring substances on the isolated intestine of fish. *Acta Physiologica Scandinavica* 38: 364–372.

153 Young JZ (1980) Nervous control of gut movements in *Lophius*. *Journal of the Marine Biological Association of the United Kingdom* 60: 19–30.

154 Young JZ (1933) The autonomic nervous system of selachians. *Quarterly Journal of Microscopical Science* 75: 571–624.

155 Young JZ (1980) Nervous control of stomach movements in dogfishes and rays. *Journal of the Marine Biological Association of the United Kingdom* 60: 1–17.

156 Young JZ (1983) Control of movements of the stomach and spiral intestine of *Raja* and *Scyliorhinus*. *Journal of the Marine Biological Association of the United Kingdom* 63: 557–574.

157 Young JZ (1988) Sympathetic innervation of the rectum and bladder of the skate and parallel effects of ATP and adrenalin. *Comparative Biochemistry and Physiology* 89C: 101–107.

158 Yui R, Nagata Y, and Fujita T (1988) Immunohistochemical studies on the islet and the gut of the Arctic lamprey, *Lampetra japonica*. *Archives of Histology and Cytology* 51: 109–119.

159 Yui R, Shimada M, and Fujita T (1990) Immunohistochemical studies of peptide- and amine-containing endocrine cells and nerves in the gut and the rectal gland of the ratfish *Chimaera monstrosa*. *Cell and Tissue Research* 260: 193–201.

160 Kitazawa T, Temma K, and Kondo H (1986) Presynaptic alpha-adrenoceptor mediated inhibition of the neurogenic cholinergic contraction of the isolated intestinal bulb of the carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology* 83C: 271–277.

161 Kitazawa T, Kondo H, and Temma K (1986) Alpha 2-adrenoceptor-mediated contractile response to catecholamines in smooth muscle strips isolated from rainbow trout stomach (*Salmo gairdneri)*. *British Journal of Pharmacology* 89: 259–266.

162 Kitazawa T, Hoshi T, and Chugun A (1990) Effects of some autonomic drugs and neuropeptides on the mechanical activity of longitudinal and circular muscle strips isolated from the carp intestinal bulb (*Cyprinus carpio*). *Comparative Biochemistry and Physiology* 97C: 13–24.

163 Kitazawa T, Kondo H, and Temma K (1988) Presence of a substance P-like peptide in an acid extract of the intestinal bulb of the carp (*Cyprinus carpio*). *British Journal of Pharmacology* 95: 39–48.

164 Campbell G, and Gannon BJ (1976) The splanchnic nerve supply to the stomach of the trout, *Salmo trutta* and *S. gairdneri*. *Comparative Biochemistry and Physiology* 55C: 51–53.

165 Kitazawa T, Kimura A, Furahashi H, Temma K, and Kondo H (1988) Contractile responses to substance P in isolated smooth muscle strips from the intestinal bulb of the carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology* 89C: 277–285.

166 Kitazawa T, Kudo K, Ishigami M, Furuhashi H, Temma K, and Kondo H (1988) Evidence that a substance P-like peptide mediates the non-cholinergic excitatory response of the carp intestinal bulb (*Cyprinus carpio*). *Naunyn-Schmiedeberg's Archives of Pharmacology* 338: 68–73.

THE GUT

Gut Anatomy and Morphology

Contents
Gut Anatomy
Pancreas
Development of Fish Gut

Gut Anatomy

C Olsson, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Anatomy of the Gastrointestinal Tract
Morphology
Species Differences
Other Factors That May Affect Gut Morphology
Concluding Remarks
Further Reading

Glossary

Epithelium Relatively flat tissue (composed of one or few cells layers) that lines cavities and surfaces in multicellular organisms.

Mucosa The layer in the gut wall facing the lumen.

Myenteric plexus The network of neurons, situated between the longitudinal and circular muscle layers of the gut wall.

Oxyntic cells The acid-secreting cells of the gut.

Submucosa The layer between the mucosa and muscle layers in the gut wall.

Introduction

In general terms, the anatomy of the gastrointestinal tract is similar in most groups of vertebrates, with the gut consisting of a saclike stomach and an elongated tube-like intestine. Likewise, the morphology is similar in most species and regions of the gut. However, at a more detailed level, considerable variations exist both between and within vertebrate classes. These variations largely depend on different feeding strategies and choice of food. In fish as a whole, including cyclostomes, as well as cartilaginous and bony fishes, consistent differences exist between the different groups, as will be explored in more detail below. However, even within the groups, there are variations between families and species.

The gut performs multiple tasks, including osmoregulation and defense against pathogens. The most important role, however, is the breakdown, transportation, and absorption of food and nutrients. The different regions of the gut have specialized tasks, reflected in, for example, the thickness of the muscle layers or the type of epithelial cells present. The morphology may also be affected by external factors, such as the type of food, feeding state, season, or pathogens, leading to up- or downregulation of different signals and, eventually, tissues.

General Anatomy of the Gastrointestinal Tract

A discussion of the gastrointestinal tract or the gut commonly refers to the entire tract, from the mouth to the anus (**Figure 1**). In addition to the gastrointestinal tract itself, the salivary glands, biliary system, and pancreas are often included in the term. These are the so-called accessory organs, which are similar in origin and/or are involved in food processing. The organization of these organs is not covered in this article; for a more detailed

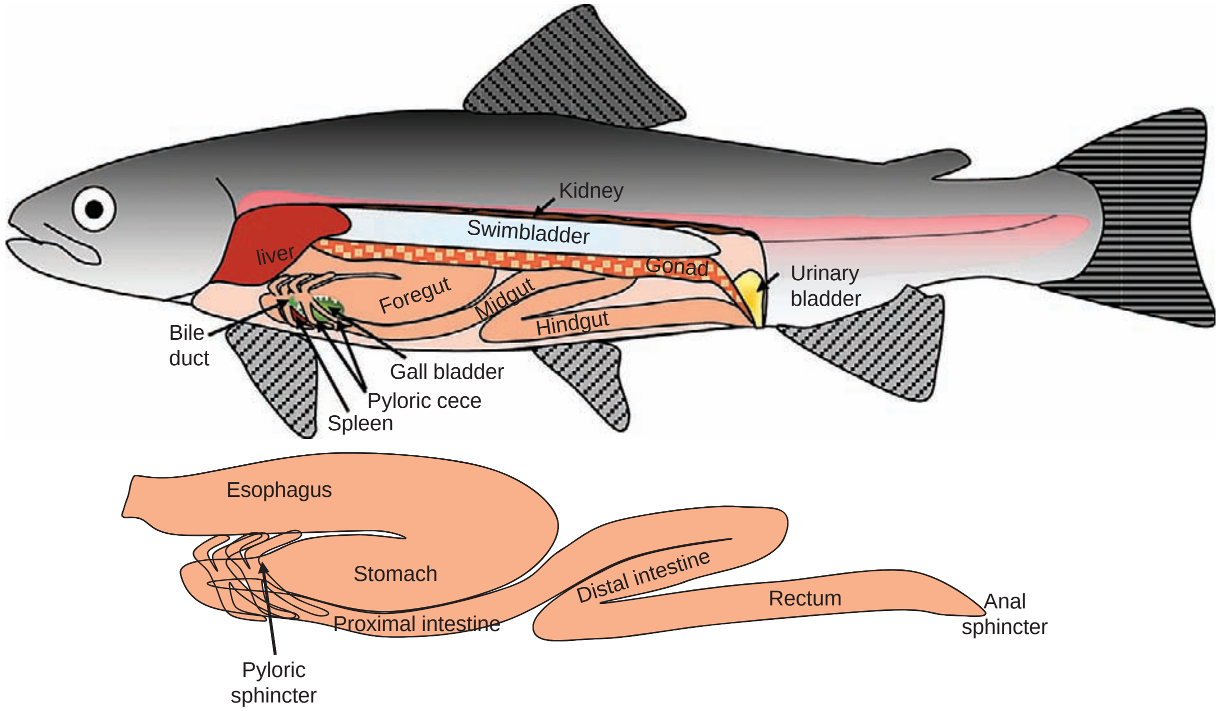


Figure 1 Schematic drawing of the gastrointestinal tract and accessory organs in a teleost (rainbow trout, *Oncorhynchus mykiss*). Courtesy of Albin Gräns.

description of the pancreas **see Gut Anatomy and Morphology**: Pancreas.

The gastrointestinal tract does not develop uniformly but separate regions have slightly different origins (**see also Gut: Anatomy and Morphology**: Development of Fish Gut). The diverse cell types present in the gut wall (see below) also have different origins, including all three germ layers. Of course, the gastrointestinal tract is not an isolated organ but is connected with the rest of the body via blood and lymphatic vessels, and also via autonomic nerves. The nerves as well as circulating and local metabolites and hormones play essential roles in the control and regulation of the gut, in order for it to achieve its many tasks.

Using a gross division of the gut, it is common to refer to the headgut, foregut, midgut, and hindgut. These regions may then be further subdivided. The nomenclature of the different regions of the fish gut varies between authors. Some stick with the mammalian classification, while others use different, usually more relevant, labeling that is more appropriate to fish anatomy and physiology.

Headgut

The headgut includes the mouth and pharynx and is only mentioned briefly here. The fish mouth looks very different among species, partially reflecting the choice of food. Consider, for example, a broad shark mouth, with plenty of sharp teeth, compared with the toothless narrow jaws of a carp fish (*Cyprinidae*). Another extreme is the cyclostomes, with their round, jawless mouth with sharp teeth. The cyclostomes, such as lampreys (*Lampetra*) and hagfish (*Myxine*), use their mouth and teeth to attach to and feed on dead or dying fish. Other fish species may use their teeth for grasping, tearing, or crushing, or to capture and hold prey (**see also Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology).

The position of teeth can also vary. For example, cyprinids have pharyngeal teeth, used to grind mostly the vegetarian food. Most fish also have a tongue that sometimes may aid in the breakdown of the food and hagfish even has teeth on its tongue. Often, however, the tongue is quite immobile.

Foregut

The foregut includes the esophagus and stomach. The esophagus is generally short and wide, and designed to transport food from the mouth to the stomach. However, a substantial number of fish, including cyclostomes, lungfish, and cyprinids, lack a stomach. In such cases, the esophagus empties directly into the intestine (see below).

The stomach serves both as a reservoir and as an important site of degradation of the food, aided by the release of acid and enzymes. It can usually be divided into a proximal and a distal part, often called cardiac and pyloric stomach, respectively. The cardiac stomach corresponds roughly to the fundus and corpus of the human stomach, while the pyloric part is similar to the antrum in both appearance and function, having, for example, thicker muscle layers.

Although a distinct anatomical divider (sphincter) between the stomach and the esophagus often may not exist in fish, the muscle in that region can still be kept contracted, ensuring that stomach contents under normal circumstances will not be transported back to the esophagus. An exception is during vomiting, which has been observed in several fish species. The other end of the stomach has a well-defined sphincter, the pyloric sphincter, which separates the stomach from the proximal intestine (**Figure 1**).

Midgut and Hindgut

In most mammals, there is a clear difference in diameter between the small and large intestine, making the nomenclature very useful. In most fish, however, this is not the case. Furthermore, the mammalian small intestine is divided into three parts (duodenum, jejunum, and ileum) based mostly on histological features. Again, the situation in fish in general is less clear.

The commonly used nomenclature for fish divides the intestine into four regions. The proximal, middle, and distal intestine (collectively termed the midgut) correspond to the small intestine, while what is often called rectum (hindgut) is most likely both the colon and rectum (i.e., the large intestine) (**Figure 1**).

No obvious macroscopic features separate the proximal, middle, and distal intestine. Often the division is arbitrary, corresponding to roughly one-third each. In some species (e.g., elasmobranchs and sturgeons), part of the intestine contains spiral valves making a distinction between regions easier. The bile duct enters the gut in the proximal intestine. In teleost fish, this region often contains one or many appendages called 'pyloric ceca'. However, the hindgut is commonly separated from the rest of the intestine by a valve (ileorectal) and is distinguished by differences in the appearance of the mucosa.

The gut generally ends with an anal sphincter. In elasmobranchs and lungfish, the hindgut ends with a cloaca, which is a common cavity into which the digestive, urinary, and reproductive tracts open.

Morphology

Looking at a cross section of any region of the gastrointestinal tract, it is obvious that the wall consists of several tissue layers (**Figure 2**). Their relative and absolute thicknesses vary between species and regions. Again, the nomenclature used in mammalian literature does not translate well to the fish gut wall, with some layers being less well defined or absent in fish.

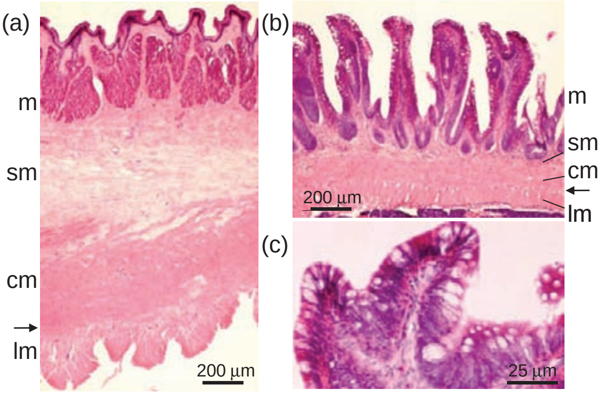


Figure 2 Cross sections of the gastrointestinal tract of shorthorn sculpin (*Myoxocephalus scorpius*) stained with hematoxylin eosin. (a) Cardiac stomach. (b) Proximal intestine. (c) Higher magnification of the epithelium in the proximal intestine. Note the high number of goblet cells. Arrows indicate myenteric plexus. m, mucosa; sm, submucosa; cm, circular muscle layer; lm, longitudinal muscle layer.

Mucosa

The innermost layer (facing the gut lumen) is the mucosa (**Figure 2**), consisting of a single layer of epithelial cells generally in addition to a dense layer of connective tissue (lamina propria) and a thin muscle layer (muscularis mucosa). To increase the surface area of the intestine, the mucosa generally makes folds rather than the finger-like protrusions (villi) seen in mammals. The epithelial cells include, for example, oxynticopeptic cells, mucus cells, endocrine cells, and enterocytes, and there are often clear regional differences in their distribution.

Hydrochloric acid and pepsin are both secreted in the stomach, and interact in the breakdown of proteins. In contrast to mammals, where the parietal cells (oxyntic) release acid while chief cells release pepsin, generally only one cell type in fish is responsible for the release of both acid and pepsinogen. Accordingly, these cells are called oxynticopeptic (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). These gland cells are found in gastric pits mainly in the cardiac part of the stomach.

Mucus is essential to protect the gastric lining from the digestive juice and mucus-secreting cells are found throughout the gastrointestinal tract. The appearance of the cells varies depending on gut region. In the esophagus and intestine, the so-called goblet cells are abundant, a name that corresponds to their cup-like appearance. This is the result of large amount of mucus present in the apical part of the cell causing this part to bulge out, at the expense of other neighboring cell types. In contrast, in the stomach, mucus-secreting cells lie close to each other and hence do not have room to bulge.

Endocrine cells release numerous different hormones that may either act locally or enter the circulation. The cells may respond to chemicals (food components) in the lumen. For more details on endocrine cells, the reader is referred to **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut. Enterocytes are the cells that absorb nutrients. They are most prominent in the intestine. They have microvilli at their luminal border, to further increase the absorbing surface. The mucosal layer also consists of granular cells likely to be involved in immune responses. For more details on this topic in this encyclopedia, **see also Integrated Function and Control of the Gut**: Barrier Function of the Gut and Intestinal Absorption.

Submucosa

The submucosa lies below the mucosa and consists mainly of connective tissue. It also contains blood and lymph vessels and a relatively high number of nerve fibers that innervate the blood vessels as well as the mucosa. A well-defined submucous nerve plexus similar to that seen in most mammals is generally absent. The submucosa is comparably thick in the stomach of most species, while it is thinner, or even regarded to be absent, in the intestine (**Figure 2**).

Smooth Muscle

In general, two layers of smooth muscle are seen in the stomach and intestine – one circular and the other longitudinal (**Figure 2**). However, deviations from this basic plan occur. For example, in many elasmobranchs, there may be a more or less well-defined oblique layer, situated between the circular layer and the submucosa. This may occur in some, but usually not in all, regions, with some species variations.

In teleosts, as well as in elasmobranchs, the inner circular layer is usually thicker than the outer longitudinal layer. This difference is most prominent in the stomach, especially in the pyloric region. A recent study on yellow catfish (*Pelteobagrus fulvidraco*) reported a 5:1 thickness ratio between the circular and longitudinal layers of the stomach. In the intestine, the circular layer is usually still the thickest, but the overall wall thickness is thinner compared with the stomach (**Figure 2**).

In addition, there are usually striated muscles in the esophagus. Whether these are exclusive to this region and whether they are found in the entire esophagus depends on the species. In many species, there is a gradual transition from striated muscle in the esophagus to smooth muscle in the stomach.

Myenteric Plexus

Between the two muscle layers, there is a dense network of nerve cell bodies and fibers – the myenteric plexus (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut). The plexus is well developed in all fishes. The nerve cell bodies send processes (axons) to all the other layers, innervating muscle cells, blood vessels, and glands.

In mammals, the plexus also contains glial cells and interstitial cells of Cajal (ICCs). Enteric glial cells outnumber the neurons by up to 4 times and have mainly a supporting role, although they may also be involved in signaling. ICCs exist as several morphological subtypes, serving as pacemakers (i.e., they set the frequency of contraction in the smooth muscle) and also conveying signals from nerves to muscles.

So far, there have been few and inconsistent reports on the presence and appearance of these cell types in different fishes. Using mammalian glial markers, positive cells are present in the fish gut; however, their appearance seems to differ. There are no reports of functional studies on the role of enteric glia in fish. Although ICC-like cells have been histologically demonstrated in some species, functional studies of these cells are lacking.

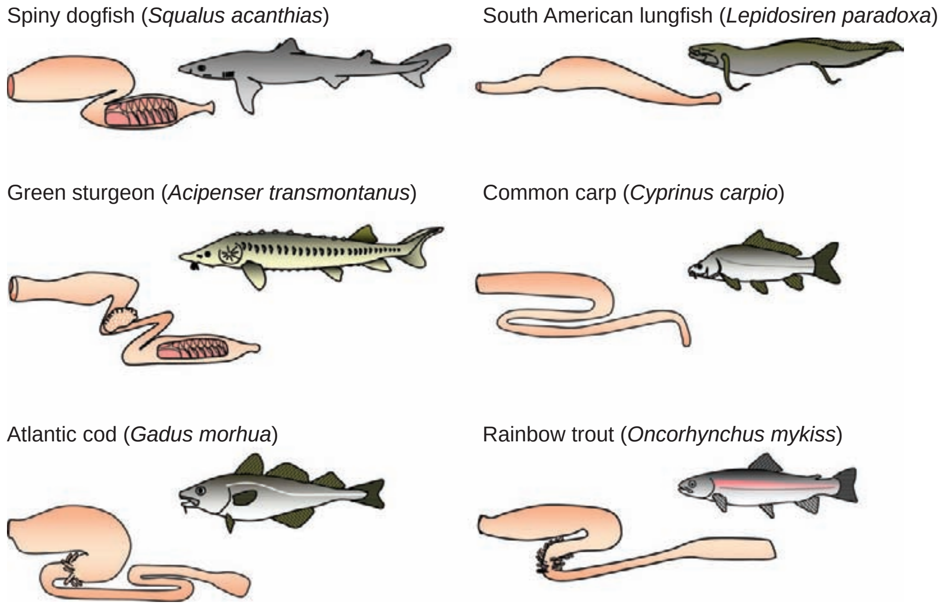
Species Differences

Considering that the total number of fish species exceeds 20 000, it is not surprising that the above description of the basic anatomy of the gut cannot be applied universally (**Figure 3**). Fish live in diverse habitats and rely on very different food sources. While few fish are true herbivores, many rely on a diet consisting of a mixture of plant material and different types of prey. Carnivorous fish may depend on other fish species or different invertebrates, such as insects, mollusks, or crustaceans. Some fish feed mainly on plankton. The composition of the food imposes different demands on digestion, including anything from the types of teeth to the types of enzymes needed.

Fish also differ in feeding frequency. Some fish, such as Northern pike (*Esox lucius*), may capture a large prey at intermittent intervals, while others, such as goldfish (*Carassius auratus*), are nibblers, continuously ingesting small quantities of food. Some main differences between species and groups are described in more detail in the following.

Stomach

Both size and form of the stomach vary widely between different species. The simplest form is a straight tube, seen, for example, in pike. In many other species, there is a bend, resulting in an inner (lesser) and an outer (greater)



Spiny dogfish (*Squalus acanthias*)

South American lungfish (*Lepidosiren paradoxa*)

Green sturgeon (*Acipenser transmontanus*)

Common carp (*Cyprinus carpio*)

Atlantic cod (*Gadus morhua*)

Rainbow trout (*Oncorhynchus mykiss*)

Figure 3 Schematic drawings exemplifying differences in general anatomy of the gastrointestinal tract in different fish species. See **Figure 1** for nomenclature. Courtesy of Albin Gräns.

curvature. The stomach may be further subdivided into U- and Y-shaped stomachs (**Figures 3–5**). The latter, present for example in European eel (*Anguilla anguilla*) and Atlantic cod (*Gadus morhua*), is characterized by a blind sac (or cecum), while U-shaped stomachs like that in salmonids and sharks are more like a siphon. The sac-like stomach usually indicates species that eat large pieces at a time, requiring a stomach that can expand for storage purposes. Nibblers, on the other hand, do not have the same need, and usually have a U-shaped stomach, or may even lack a stomach altogether.

Stomachless Species

A large number of fish species lack a stomach, which is unlike other vertebrate groups. For instance, the cyprinids, a large teleost family consisting of over 2700 species, including carp (*Cyprinus carpio*), goldfish, and zebrafish (*Danio rerio*), are all stomachless. Stomachless species also occur in other teleost families, such as *Labridae* and *Gobidae* and others (one estimation says that up to 50 families may contain some stomachless species). In addition, cyclostomes, like hagfish and lamprey, and lungfish also lack a proper stomach.

To say that a species is stomachless, one must first define a stomach. Most stomachless species have some kind of enlargement of the gut that may look more or less like a stomach that serves for storage. This is often called the ‘intestinal bulb’. However, in contrast to a stomach (in most species), this intestinal bulb does not contain acid- or pepsinogen-secreting epithelial cells. Instead, stomachless species rely mainly on the release of trypsin into the intestine for breakdown of proteins.

A more distinctive feature of stomachless species is the location of the entry of the bile duct. As the duct enters the gut in the proximal intestine, this takes place immediately distal to the esophagus, in contrast to species with a stomach, where it opens after the pyloric sphincter (see **Figure 1**).

Intestine

The length of the intestine also reflects feeding habits. A vegetarian diet is more difficult to digest and, hence, there is a need for a longer intestine to extend processing time. The length of the gut has been measured and compared with the total body length in several species. The ratio varies 100-fold, ranging from 0.4 to almost 40. The species with the longer gut are generally omni- or herbivores, but there are exceptions. A study of three cyprinid species from different feeding habits showed that the carnivore (gudgeon; *Gobio gobio*) had the shortest intestine, the herbivore (common carp) had the longest, and the omnivore (roach; *Rutilus rutilus*) was in between. However, both the omnivore and the herbivore had shorter intestines compared with the average omnivorous or herbivorous species, indicating that other factors, such as species, influence gut length as well.

The differences in gut length between herbivores and carnivores can also be seen during development, when many species change in diet (from carnivorous to more omnivorous). This is generally accompanied by a change in relative gut length. However, there are several other, probably more important, ways to increase the surface area of the gut.

Pyloric Ceca

The pyloric ceca are finger-like appendages found at the proximal intestine in most teleosts. The epithelial layer of the ceca does not differ from the rest of the intestine and it is believed that they increase the absorptive surface. They also may prolong transit time.

The number and size of the ceca vary considerably among teleosts, from absence in stomachless species to up to around a 1000 (**Figures 3** and **5**). Again, a correlation may or may not exist with food type. A general observation is that the ceca are more abundant in carnivores. Even so, the carnivorous wolffish (*Anarhichas*) as well as most elasmobranchs lack pyloric ceca. For example, in cod and sculpin (e.g., *Myoxocephalus* sp.), the ceca sit in a ring at the very proximal end of the intestine. In salmonids, they are more dispersed, covering most of the proximal intestine (**Figures 4** and **5**). In sturgeons (*Acipenser*), the ceca are fused together, forming a multilobed structure. All these species are also carnivorous. Few studies have measured the total cecal surface area or correlated it with intestinal length.

Spiral Valve

Spiral valves, or a spiral intestine, are found in elasmobranchs, lungfish, and chondrosteans (e.g., sturgeon). The spiral valves are not valves as such, but are mucosal (and submucosal) foldings, running along the intestine in a spiral pattern, thereby increasing the epithelial (absorptive) area without affecting the length of the intestine. The valves also delay the passage of chyme.

Spiral valves are mainly seen in species with a short straight intestine. In sharks and ray, the valves occupy the proximal part of the intestine, while in sturgeons they begin halfway down the intestine. The length of the spiral intestine and the number of turns may also differ within a species. The outer diameter of the spiral intestine of white sturgeon is ~1.5 times the diameter of the proximal intestine, and it is even larger in most elasmobranchs.

The spiral intestine in sturgeons is the region with highest nutrient uptake.

Other Factors That May Affect Gut Morphology

As discussed above, the food choice is often reflected in the morphological adaptations of the gut. However, diet also has direct short-term effects on gut morphology. The composition of the diet, like for instance relative protein, fat, and carbohydrate contents, may affect cellular properties such as membrane composition, which, in turn, depending on cell type, may affect uptake and signaling.

Diet composition may also affect different cell populations differently. For example, goblet cells increase in number when crude protein content of the food is increased, whereas the mucosa and muscle layers tend to decrease in thickness.

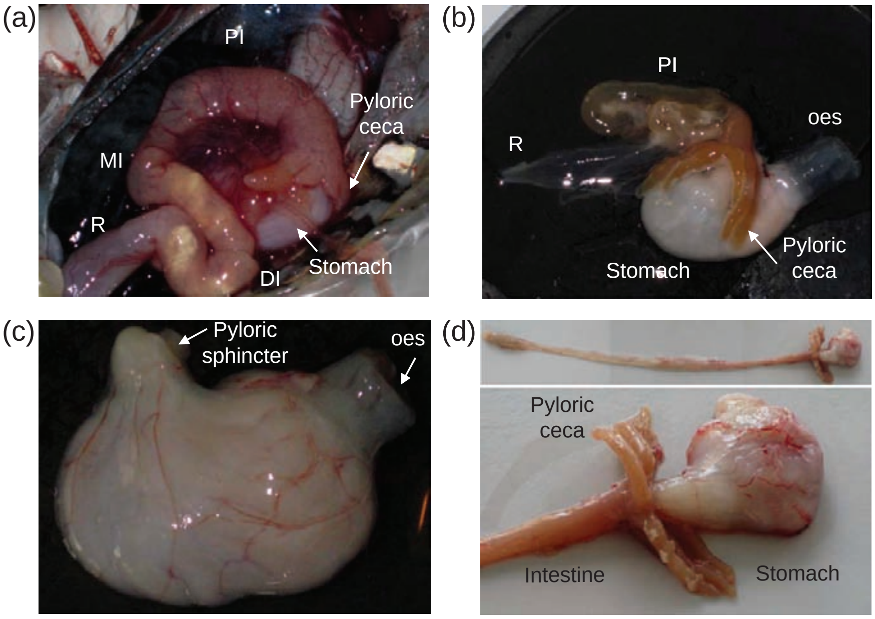


Figure 4 The gastrointestinal tract of Arctic sculpin (*Myoxocephalus scorpioides*) (a), Arctic staghorn sculpin (*Gymnocanthus tricuspis*) (b, c), and shorthorn sculpin (*Myoxocephalus scorpius)* (d). Sculpins have a Y-shaped stomach and a ring of pyloric ceca (note the difference between species in (a) and (b)). DI, distal intestine; MI, mid-intestine; PI, proximal intestine; oes, esophagus; R, rectum.

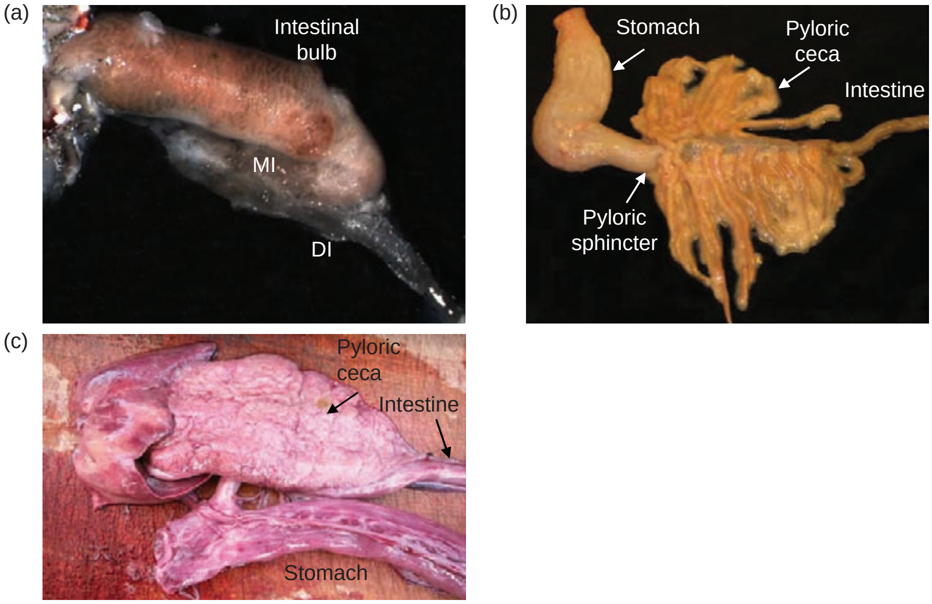


Figure 5 The gastrointestinal tract of: (a) zebrafish (*Danio rerio*) (note the absence of stomach and pyloric ceca); (b) rainbow trout (*Oncorhynchus mykiss*) (note the U-shaped stomach and the widespread pyloric ceca); and (c) wahoo (*Acanthocybium solandri*) (note elongated stomach and numerous pyloric ceca). (c) Courtesy of Fredrik Jutfelt.

In farmed fish, the composition of the diet is essential for optimal growth and there is continuous research to find new and more economic food sources. However, some alternatives have serious drawbacks because they disrupt the mucosa.

Many fishes encounter large seasonal variations in food availability; consequently, they may experience shorter or longer periods of starvation on a regular basis. This also will likely affect gut morphology reversibly, often observed as a decrease in intestinal length and thickness. For example, when salmon return to their natal river to spawn, they stop feeding and the gut atrophies. Some species are more easily affected than others. Similarly, developmental stage and health status play important roles. Other factors that influence the impact of starvation are hormonal levels and environmental parameters, such as temperature and water quality.

Changes in body temperature, due to seasonal or other changes in the water temperature, can also by itself affect gut morphology. In experiments with short-term, cold-acclimated channel catfish (*Ictalurus punctatus*), an increase in intestinal length and weight was observed. In carp, there was no effect on the intestinal length but an increase in mucosal surface area was seen. The increase in intestinal parameters could possibly compensate for a less-efficient uptake at lower temperatures.

Seasonal variations usually depend on a combination of factors, including temperature and food availability; seasonal changes in hormone levels may also play an important role.

Finally, various disease states can also cause morphological disruptions. Several studies have shown serious effects on the gut mucosa, caused by infections of common pathogens (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut).

Concluding Remarks

The anatomy and morphology of the fish gastrointestinal tract follow a general vertebrate pattern. However, with fish being so diverse, there are many special adaptations to different environments and behaviors. Although the type of food and feeding strategies play an important role, genus- and family-specific traits may be more important in determining the appearance of the gut in species-specific manners.

See also: **Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **Gut Anatomy and Morphology**: Pancreas; Development of Fish Gut. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Endocrine Systems of the Gut; Gut Secretion and Digestion; Intestinal Absorption; Nervous System of the Gut. **The Gut:** The Gastrointestinal Tract: An Introduction.

Further Reading

Adam H (1963) Structure and histochemistry of the alimentary canal. In: Brodal A and Fänge R (eds.) *The Biology of Myxine*, pp. 256–288. Oslo: Universitetsförlaget.

Al-Hussaini AH (1949) On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: Anatomy and histology. *Quarterly Journal of Microscopical Science* 90: 109–139.

Al-Hussaini AH (1949) On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: Cytology and physiology. *Quarterly Journal of Microscopical Science* 90: 323–354.

Bishop C and Odense PH (1966) Morphology of the digestive tract of the cod, *Gadus morhua*. *Journal of the Fisheries Research Board of Canada* 23: 1607–1630.

Blake IH (1936) Studies on the comparative histology of the digestive tube of certain teleost fishes. III. A bottom-feeding fish, the sea robin (*Prionotus carolinus)*. *Journal of Morphology* 60: 77–102.

Buddington RK, Krogdahl A, and Bakke-Mckellep AM (1997) The intestines of carnivorous fish: Structure and functions and the relations with diet. *Acta Physiologica Scandinavica Supplement* 638: 67–80.

Burnstock G (1959) The morphology of the gut of the brown trout (*Salmo trutta)*. *Quarterly Journal of Microscopical Science* 100: 183–198.

Cao XJ and Wang WM (2009) Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anatomia Histologia Embryologia* 38: 254–261.

Chatchavalvanich K, Marcos R, Poonpirom J, Thongpan A, and Rocha E (2006) Histology of the digestive tract of the freshwater stingray *Himantura signifer* Compagno and Roberts, 1982 (*Elasmobranchii, Dasyatidae)*. *Anatomy and Embryology* 211: 507–518.

Holmgren S and Nilsson S (1999) Digestive system. In: Hamlett WC (ed.) *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*, pp. 144–173. London: Johns Hopkins University Press.

Stevens CE and Hume D (1995) *Comparative Physiology of the Vertebrate Digestive System*. Cambridge: Cambridge University Press.

Sullivan MX (1905) The physiology of the digestive tract of elasmobranchs. *American Journal of Physiology* 15: 42–45.

Pancreas

MA Caruso and MA Sheridan, North Dakota State University, Fargo, ND, USA

© 2011 Elsevier Inc. All rights reserved.

The Pancreas Has Many Functions
Structure of the Adult Pancreas
Embryological Development of the Pancreas
Evolution of the Pancreas
Hormones of the Pancreas
Enzymes of the Pancreas
Regulation of Pancreatic Secretion
Concluding Remarks
Further Reading

Glossary

Brockmann body Large aggregate of endocrine pancreatic tissue found especially within teleost fish.

Endocrine Mode of secretion in which a chemical is released by a cell into extracellular fluid then enters the blood.

Enteropancreatic System of endocrine cells located in the intestine and pancreas.

Exocrine Mode of secretion in which a chemical is released from a cell into a duct that leads to the outside of the organism (including the lumen of digestive tract).

Gastroenteropancreatic cells A system of endocrine cells located in the stomach, intestine, and pancreas.

Hydrolyze Chemical reaction in which a water molecule is involved in the process of cleaving a macromolecule by donating hydrogen and hydroxide ions.

Ontogeny Changes in the phenotype of an individual during its life span. Ontogeny is the outcome of a developmental program, encoded in an organism's genes, as well as interactions between the genotype of the individual and the environments it encounters, together with any maternal effects mediated through the egg itself.

Phylogeny Modification of the structure and function of a species or group of species throughout their evolutionary history.

Principal islet Large accumulation of endocrine tissue encircled by a fine rim of exocrine tissue.

Secretogog A chemical that stimulates a cell to secrete another chemical.

The Pancreas Has Many Functions

The pancreas plays a central role in digestion, metabolism, growth, and development in vertebrates. The functions of the pancreas are essential for integrating physiological and developmental events within the organism as well as for coordinating such events with the environment. The pancreas has two chief functions: to secrete enzymes into the digestive tract and to secrete hormones into the bloodstream.

Fish have been used as experimental models from the dawn of research on pancreatic function in the late nineteenth century to the present. The study of fish has provided essential information about the structure and evolution of the pancreas, as well as about the structure, biosynthesis, evolution, and function of the hormones and enzymes it produces. For example, fish insulin was one of the first vertebrate insulins to be isolated. Furthermore, the yield of this hormone from fish pancreata (formal plural form of pancreas) has remained better than that from any other group, including mammals, despite sophisticated extraction techniques. This article describes the basic aspects of the structure, development, and evolution of the pancreas of fish. We also discuss the principal hormones and enzymes produced by the pancreas and highlight their major actions.

Structure of the Adult Pancreas

The anatomy of the gastrointestinal tract and associated organs, including the pancreas, greatly varies among different species of fish (**see also Gut Anatomy and Morphology:** Gut Anatomy for more details on gut anatomy). The pancreas of fish, similar to that of all vertebrates, has two major components: an exocrine component and an endocrine component. The cells of the exocrine component form ducts closed at one end, the open ends of which connect to the lumen of the gastrointestinal tract. A common feature of exocrine glands is their ducted nature. The ducts provide avenues through which the secretory products of the exocrine pancreas, primarily digestive enzymes, reach the lumen of the gut. As discussed subsequently, the exocrine pancreas produces a number of enzymes.

The cells of the endocrine component typically form closely packed balls of cells called 'islets', so named because they resemble small islands in a microscopic section. A common feature of endocrine glands is their ductless nature. The secretory products of the endocrine pancreas, hormones, are first released by cells into the extracellular fluid before they enter the capillaries, and are distributed throughout the organism via the blood. In general, four different types of islet cells are present, each defined by the hormones they produce. These include glucagon (produced by α cells), insulin (produced by β cells), somatostatin (produced by δ cells), and peptide YY (PYY) (produced by F cells).

In some groups of fish, especially the elasmobranchs (sharks and rays), the pancreas is well developed and possesses clusters of endocrine cells arranged within a compact organ containing extensive exocrine tissue. Among the bony fish (teleosts), the pancreas typically is not a distinct organ, but aggregates of exocrine and endocrine cells are dispersed between or on internal organs and blood vessels. Pancreatic tissue, especially exocrine tissue, may surround or be found within the submucosa of the intestine and pyloric ceca, and in liver, gall bladder, and/or adipose tissue.

In many groups of teleosts, the endocrine cells are organized into one or two principal islets (often called the Brockmann bodies) plus a number of accessory or satellite islets. The Brockmann bodies (**Figure 1**) can exceed 500 μm in diameter, which is 5 times the size of a typical islet in a mammalian pancreas. The relatively unique structure of the teleost pancreas, in which the endocrine portion is anatomically separated from the endocrine portion coupled with the large size of the islet, has made it an extremely useful model for pancreatic research. Notably, recent work on transplantation of fish pancreata into mice shows promise as a cure for diabetes mellitus.

Pancreatic tissue, especially islet tissue, is well vascularized and innervated. This enables an extensive communication network in which hormones can interact with islet cells to influence the production/secretion of other hormones as well as with nearby exocrine cells to influence the production/secretion of digestive enzymes. Both sympathetic and parasympathetic nerves innervate the pancreatic tissue to release adrenaline and acetylcholine, respectively, as well as neuropeptides (e.g., cholecystokinin (CCK) and galanin), all of which affect hormone and digestive enzyme production/release.

Embryological Development of the Pancreas

The pancreas develops from the gastrointestinal endoderm (which together with mesoderm and ectoderm make up the three primary germ layers), and begins with the budding of the gut endocrine cells from the mucosal epithelium. Unlike the development of the Agnathan or jawless fish (hagfish and lamprey) pancreas that originates from the epithelium of the intestine or bile duct, the Gnathostome (vertebrates with jaws) pancreas expands from the dorsal and ventral outgrowths of the small intestine. For example, the teleost pancreas buds laterally to the foregut and spans the intestinal wall innervated with blood vessels. The nature of the contributions from the ventral and dorsal regions of the gut plays a role in the type of islet organ that is found in each species of teleost, and, in more recent Gnathostomes, this consists of one dorsal and two ventral outgrowths. Exceptions are noted in which zebrafish (*Danio rerio*) pancreata develop from only a single cell mass located on the dorsal gut epithelium (**see also Gut Anatomy and Morphology:** Development of Fish Gut). Both endocrine and exocrine cells arise from the same endodermal rudiment, and, generally, the endocrine cells start to differentiate before the exocrine cells.

All four of the different islet cell types are derived from the same precursor stem cell. The sequence of appearance of the islet cell types during embryonic development follows in order as β cell, α cell, δ cell, and F cell. Co-expression of different hormones by the same cell is often observed at early stages. In rainbow trout (*Oncorhynchus mykiss*), islets are diffusely distributed in the adipose tissue that surrounds the pyloric ceca and the spleen, which may give rise to hormonal secretion from these organs. Development after hatching involves growth in pancreas size and an elevation in enzyme concentration.

Evolution of the Pancreas

Fish provide critical insight into the evolution of pancreas and the hormones as well as the enzymes it secretes. In almost all aquatic and terrestrial Gnathostomes, four different endocrine cells (α, β, δ, and F) are present in the pancreas. The exception here is the Agnathans that contain a primitive gastroenteropancreatic (GEP) system, more accurately referred to as the enteropancreatic (EP) system as indicated by no definable stomach (**see also Gut Anatomy and Morphology:** Gut Anatomy). Agnathans also display an uncommon feature in which endocrine and exocrine cells are segregated. For example, larval lampreys possess a one-hormone islet organ that is then followed by a three-hormone gland in adult lamprey. Hagfish have a separate islet organ at the junction of the

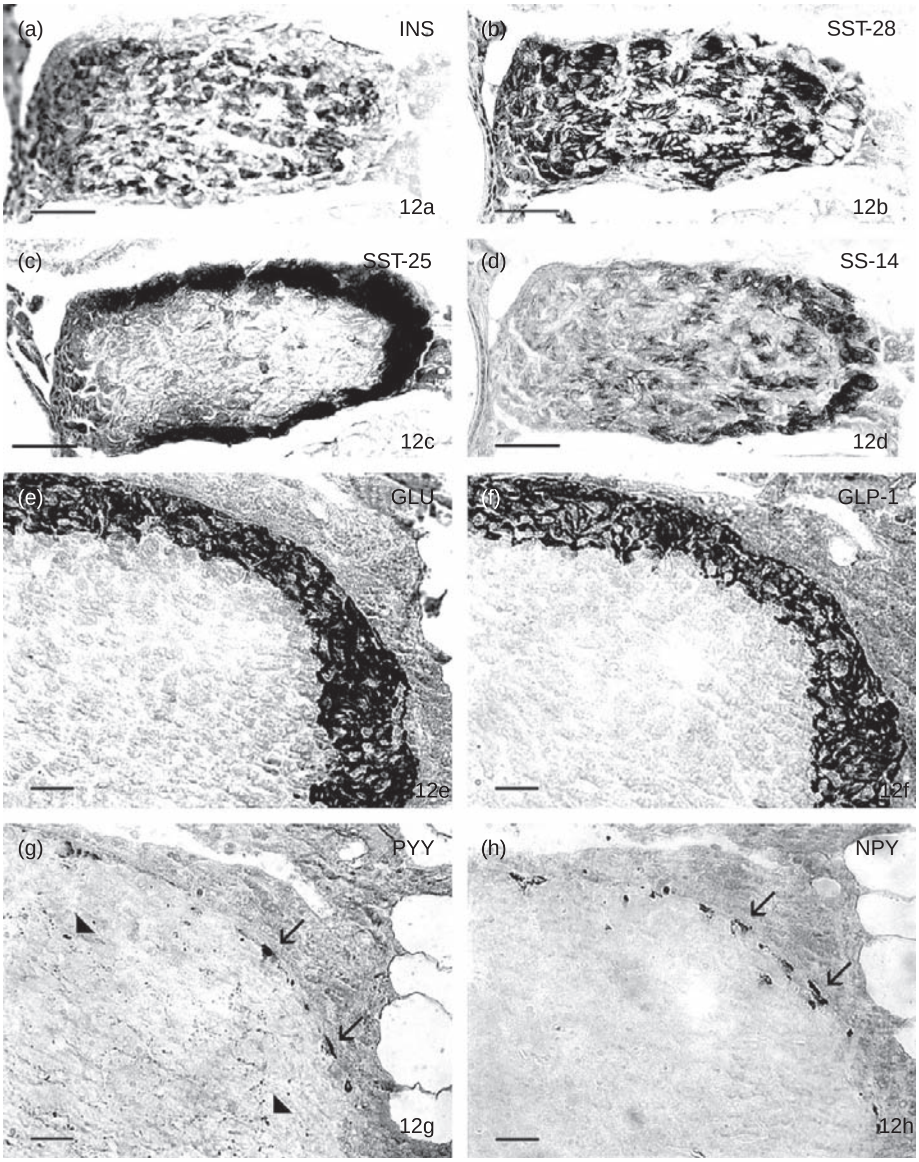


Figure 1 Consecutive longitudinal sections of the principal islet (Brockmann body) of a 51-day-old sea bream (*Sparus aurata*) larva showing (a) insulin-, (b) somatostain (SS)-28 (1–12)-, (c) SS-25-, and (d) SS-14-immunoreactive cells. Detail of the islet periphery shows coexistence of (e) glucagon- and (f) GLP-1-like immunoreactivities or (g) PYY- and (h) NPY-like immunoreactivities. Arrows point out identical cell profiles. Arrowheads in (e) indicate PYY immunoreactive nerve fibers. Scale = 20 μm. Reproduced from Navarro MH, Lozano MT, and Agulleiro B (2006) Ontogeny of the endocrine pancreatic cells of the gilthead sea bream, *Sparus aurata* (teleost). *General and Comparative Endocrinology* 148: 213–226, with permission from Elsevier.

gut and bile duct that consists almost entirely of insulin cells with a relatively low number of somatostatin-secreting cells. Other somatostatin cells are found in the bile duct, whereas glucagon and PYY cells are found in the gut mucosa.

The chondrichthyes are phylogenetically the oldest group of living vertebrates with a combined exocrine–endocrine pancreas. Their islet cells, which have a close relationship with ducts of the exocrine components of the gland, frequently form an outer layer around ductules. The pancreas of chondrichthyes contains only insulin, glucagon, and somatostatin cells; PYY cells are located in the gut.

A well-defined Brockmann body is present in almost all teleosts. The phylogenetic development of islet tissue seems to be toward at least a single, large principal islet or Brockmann body independent or nearly independent of exocrine elements located near the gall bladder or spleen. Phylogenetically, the islet hormones appear long before the emergence of the islet organ. For example, an insulin-like substance occurs in several groups of invertebrates (which lack pancreata), including mollusks and insects. The order of appearance of hormones over the course of animal evolution is insulin, somatostatin, glucagon, and then PYY (**Figure 2**).

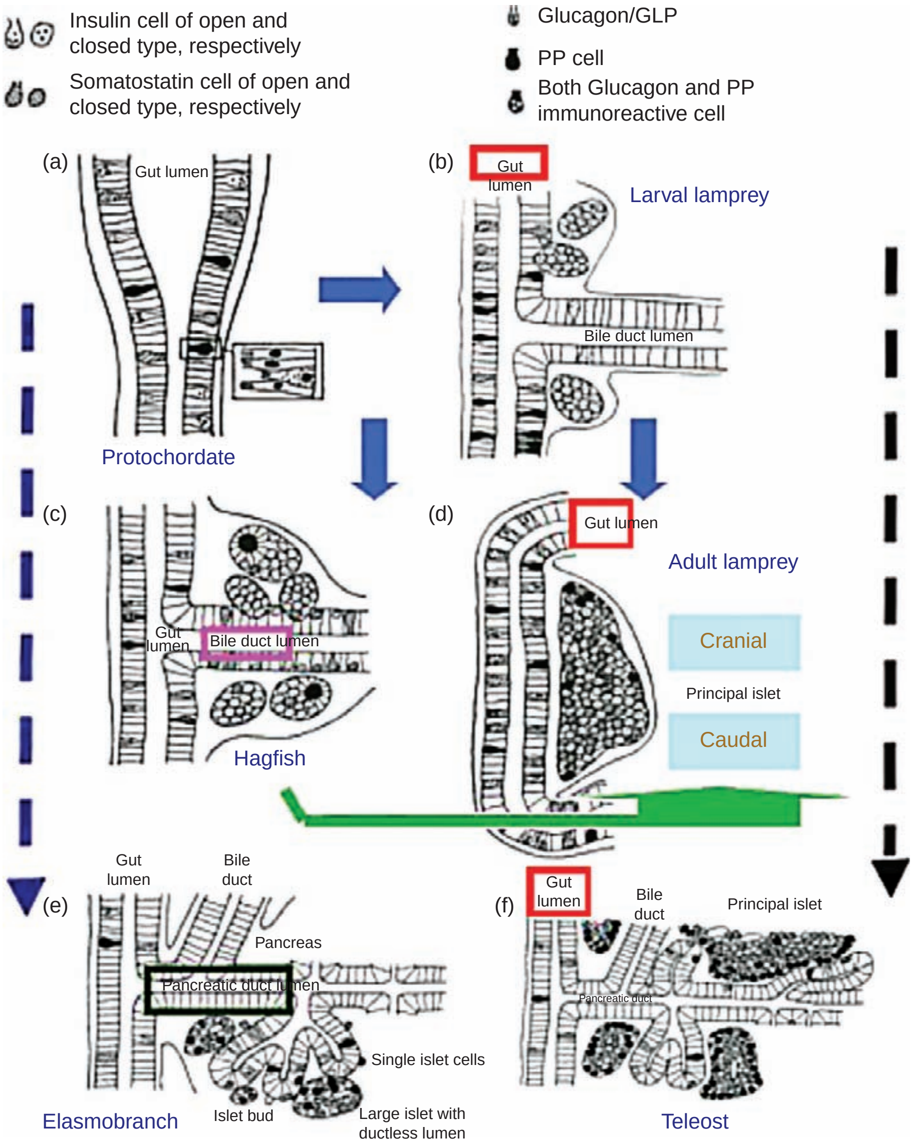


Figure 2 Representation of endocrine cell types in the epithelium of the gut, bile duct, pancreatic ducts, and islet tissue of a protochordate (a) and various groups of fish, including larval (b) and adult (d) lamprey, hagfish (c), elasmobranchs (e), and teleosts (f). Arrows indicate the possible lines of phylogenetic development of islet tissue from protocordates, in which the endocrine cells are confined to the gut, to the larval lamprey or hagfish. The larval lamprey islets give rise to the adult cranial principal islet, whereas the caudal principal islet can arise from the larval bile duct, similar to the situation in hagfish. The islets of elasmobranchs originate from the pancreatic ducts while those of teleosts arise from the gut. It should be noted that glucagon-family peptides are confined to the gut epithelium in hagfish and lamprey, but in elasmobranchs and teleosts, cells producing glucagon-family peptides also are incorporated into islet tissue. Reproduced from Youson JH (2006) Peripheral endocrine glands. I. The gastroenteropancreatic endocrine system and the thyroid gland. In: McKenzie DJ, Farrel AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 26: Primitive Fishes*, pp. 381–455. New York: Academic Press, with permission from Elsevier.

Hormones of the Pancreas

As mentioned above, the four main pancreatic hormones (insulin, somatostatin, glucagon, and PYY) are expressed by separate cells in most adult fish. Additional hormones may co-localize with these in some cell types. The hormones play important roles in metabolism as well as in control of other secretory processes (see **Table 1**).

The relative abundance of each cell type has been estimated, with α cells being 25–40%, β cells 30–50%, δ cells 15–20%, and an undetermined amount of F-cell types. The relative hormone content in coho salmon (*Oncorhynchus kisutch*), from most to least, is insulin, somatostatin (SS)-25, glucagon-like peptide 1 (GLP-1), glucagon, SS-14, and PP. Within the islets of actinopterygians, the β and δ cells usually occupy the central region,

Table 1 Physiological actions of pancreatic hormones produced in fish

Hormone	*Principal action*	*Example*
Insulin	Metabolism	Aids in nutrient uptake (e.g., amino acids) and glycogenesis
	Growth	Promotes anabolism through lipogenesis, myogenesis, and osteogenesis
	Cellular differentiation	Necessary in the formation of the developing pancreas and gut
	Secretotropic	Glucagon-like-peptide (GLP), secretin, cholecystokinin (CCK)
Glucagon	Metabolism	Initiates glycogenolysis in liver and muscle tissue and lipolysis
Somatostatin	Metabolism	Promotes lipolysis and glycogenolysis
	Growth	Inhibits the proliferation of intestinal muscosa and osteogenesis
	Cellular differentiation	Inhibits the differentiation of cartilage and osteocytes
	Secretotropic	Modulation of insulin, glucagon, and exocrine pancreas
	Transport	Modulation of intestinal transport of Na^+ and Cl^-
Peptide YY	Metabolism	Stimulates appetite, gastric emptying
Glucagon-like-peptide 1	Metabolism	Promotes glycogenolysis and gluconeogenesis, promotes satiety

while α cells and F cells occupy the peripheral border (**Figure 1**). Close to the β cells are D2 cells that produce SS-14, whereas the SS-25-producing D1 cells are located in the periphery of the islets near α cells. PYY and neuropeptide Y (NPY) are located in the periphery of the islets. The proximity of the different islet cell types suggests the existence of specific interactions between islet hormones to regulate production/secretion.

Insulin

The first to appear in development is insulin, which is synthesized and secreted from β cells. Insulin, which is mainly involved in nutrient homeostasis and also has cell proliferative (mitogenic) effects. Insulin is synthesized from a large precursor, preproinsulin, that contains a 24-amino-acid signal sequence, followed by a B-chain (30 amino acids), a C-peptide of variable length (~28–42 amino acids), and an A-chain (21 amino acids). Preproinsulin is processed in a stepwise manner by first removing the signal sequence to form proinsulin, and then prohormone convertases cleave the C-peptide to form bioactive insulin. C-peptide and some proinsulin are secreted together with insulin and enter the circulation. Extrapancreatic production of insulin has been suggested for decades, but only recently has evidence been provided that it is synthesized in the brain and adipose tissue of rainbow trout.

Insulin mediates carbohydrate metabolism in fish (e.g., stimulates glucose uptake and glycogen synthesis); however, this role is minimal compared with its action on protein metabolism (e.g., stimulates amino acid uptake and protein synthesis; decreases protein turnover and inhibits gluconeogenesis from amino acids). Amino acids are also more potent secretagogs of insulin than glucose. These observations may reflect the diet of fish, at least that of those species most studied, which is rich in amino acids and poor in carbohydrates.

Insulin also enhances lipogenesis (e.g., stimulates fatty acid uptake, fatty acid synthesis, and triacylglycerol synthesis) and inhibits lipolysis (i.e., hydrolysis of stored triacylglycerol). During periods of fasting, insulin counteracts the reduction of fuel supplies by conserving muscle and adipose tissue. Plasma insulin levels depend on dietary protein level and nutritional state, and display seasonal variation.

Glucagon

Glucagon antagonizes the metabolic actions of insulin. It typically consists of 29 amino acids and is highly conserved among vertebrates. Glucagon is also synthesized from a prohormone, proglucagon, which is expressed in the pancreas and other tissues (e.g., intestine), and, depending on the presence and activity of processing enzymes, glucagon or GLP-1 may be produced. Glucagon and GLP-1 are both produced by α cells in the pancreas and may also co-localize with PYY in this cell type. GLP-1 is produced primarily in the intestine. Glucagon promotes glycogenolysis and gluconeogenesis in liver and muscle, and results in increased plasma glucose. Glucagon also stimulates lipolysis from fat stores and elevated plasma fatty acid levels. Interestingly, GLP-1 and the related hormones such as gastric inhibitory polypeptide (GIP) are secretagogs of insulin, while glucagon inhibits insulin release.

Somatostatin

Somatostatin has many effects on growth, development, and metabolism. Fish possess multiple genes encoding somatostatin, and, depending on the proteolytic processing of the precursors, several hormonal variants may be produced, including SS-14 and SS-25. Somatostatin is produced and secreted from several locations, including the central nervous system, stomach, intestine, and δ cells of the endocrine pancreas. In some cases, it is co-secreted with CCK and NPY. The two subpopulations of δ cells in teleosts, D1 and D2 cells, produce different forms of somatostatins.

Somatostatin is a major player in the regulation of digestion and metabolism because of its inhibitory effects on hormones of the endocrine pancreas (e.g., insulin and glucagon) and intestine, as well as on acid release from the stomach and enzyme secretion from the exocrine pancreas. Somatostatin also has direct effects on metabolism by stimulating lipolysis.

Peptide YY

PYY affects a number of processes associated with feeding and digestion in fish. This family of pancreatic polypeptides also includes NPY and fish pancreatic peptide (PY). All of these peptides display similar characteristics, with a high frequency of conserved amino acids, size, and three-dimensional (3D) structure. PYY has been identified in a number of piscine species, including the lamprey, and is secreted from the pancreas, as well as from the gastrointestinal tract and nervous tissue. It has an orexigenic effect in fish and aids in gastric emptying, whereas NPY is made predominately in nervous tissue, and stimulates food intake when administered to the brain but not when administered peripherally. Unlike NPY, PYY inhibits food intake; however, similar to NPY, PYY inhibits gastric and exocrine pancreatic secretions.

Enzymes of the Pancreas

The exocrine pancreas secretes enzymes, such as proteases, carbohydrases, and lipases, necessary for the digestion of protein, carbohydrate, and fat (**see also Integrated Function and Control of the Gut:** Gut Secretion and Digestion). Pancreatic enzymes in fish are secreted into the intestine through the pancreatic or hepatic ducts and have characteristics similar to those of the corresponding enzymes in other vertebrates (**Table 2**). In particular, trypsin, chymotrypsin, amylase, pancreatic elastase, pancreatic lipase, endothelial lipase, phospholipase, lipoprotein lipase, carboxypeptidase A and B, carboxylesterase, and carboxylic ester hydrolase exist in fish. Considering the evolutionary and life-history patterns of fish, involving highly variable diets and extreme variation in habitat, fish enzymes present certain qualities that complement and distinguish themselves from those of mammals.

Peptidases

Carnivorous fish rely heavily on the intake and assimilation of amino acids; consequently, proteases are a critical component of metabolism and growth. The protein-digesting endopeptidases, trypsin, chymotrypsin, and elastase, all are derived from precursor molecules or proenzymes. This ensures that they are activated only when present in the lumen of the small intestine. Although there exist slight variations in the size of certain peptidases, most share a high percentage of nucleotide identity with enzymes from mammals, However, where variability does exist, it is most likely due to differences specialized for the temperature variances experienced by the many different species of fish. Certain pancreatic proteases (e.g., elastase and trypsin) in some species of fish have higher catalytic efficiencies than the corresponding enzymes in mammals (when measured at the same (cold) temperature).

Lipases

Lipids, a highly diverse group of biomolecules, play important roles in fish. Their metabolism is accomplished

Table 2 Physiological actions of pancreatic enzymes produced in fish

Enzyme	*Principal action*
Trypsin	Hydrolyzes the carboxyl terminal of amino acids lysine and arginine
Chymotrypsin	Hydrolyzes the carboxyl terminal of amino acids tyrosine, tryptophan, and phenylalanine
Pancreatic elastase	Catalyzes the catabolism of elastin, fibrin, hemoglobin, and albumin
Carboxypeptidase	Hydrolyzes the carboxyl terminal of a peptide bond
Carboxylic ester hydrolase	Hydrolyzes carboxylic acid esters, resulting in the formation of an alcohol and a carboxylate
Pancreatic lipase (triacylglycerol lipase)	Hydrolyzes ester linkages of triglycerides
Endothelial lipase	Hydrolyzes SN-1 acyl chain of phospholipids
Phospholipase	Hydrolyzes phospholipids into fatty acids and other lipophilic substances
Lipoprotein lipase	Hydrolyzes very low density lipoproteins (VLDL) into fatty acids and glycerol
Carboxyl ester lipase	Hydrolyzes cholesteryl esters, tri-, di-, and mono-acylglycerols, phospholipids, lysophospholipids, and ceramide
Amylase	Hydrolyzes starch into di- and trisaccharides

by a large number of lipases produced by several organs, including the pancreas, liver, pyloric ceca, and stomach. Pancreatic lipase or colipase-dependent lipase is the primary lipolytic enzyme found in most fish species. Carboxyl ester lipase (CEL), also known as bile-salt-dependent lipase, is a critically important pancreatic lipase because it hydrolyzes several lipid molecules, including triacylglycerols, phospholipids, cholesteryl esters, wax esters, and vitamin esters. This enzyme depends on bile salt for hydrolysis of water-insoluble substrates, whereas soluble substrates may be hydrolyzed in the absence of bile salt.

Homogenates of Atlantic cod (*Gadus morhua*) pancreatic tissue reveal that fish lipases hydrolyze triglycerides containing unsaturated fatty acids at higher rates than those with saturated fatty acids. Certain ester bonds with myristic and palmitic acid are resistant to hydrolysis. Moreover, rapid release of glycerol from the reaction mixture indicates that Atlantic cod lipase is able to hydrolyze bonds in all positions of the triglyceride.

Other enzymes that depend on bile salts and other minerals (e.g., Ca^{2+}) include the phospholipases. Choline and glycerophospolipids are hydrolyzed more efficiently than ethanolamine and inositol phospholipids. Specific activities and substrate specificities are affected differently by calcium and bile-salt concentrations.

Carbohydrases

Amylase activities of pancreatic tissue and intestinal contents show great variation. In several fish species, intestinal amylase activity correlates positively with dietary carbohydrate level and feeding intensity. The ability to adapt amylase secretion to match carbohydrate level in the diet and feed intake is restricted to herbivorous and omnivorous fish.

Regulation of Pancreatic Secretion

The secretion of pancreatic hormones and enzymes is controlled by neural innervation and by hormones and metabolites. Many of the regulating hormones come from neighboring pancreatic or GEP cells. Notably, many of the hormones produced in the pancreas are also produced by the gut (e.g., GIP, glucagon, GLP, NPY, PYY, and somatostatin; **see also Integrated Function and Control of the Gut:** Endocrine Systems of the Gut) and help in coordination of digestion and metabolism. The control of islet hormone release is discussed in **Integrated Function and Control of the Gut**: Gut Secretion and Digestion and Nervous System of the Gut the focus of this section is on the exocrine portion of the pancreas.

Nutrients in the lumen of the gastrointestinal tract stimulate the secretion of pancreatic enzymes. Breakdown products from macronutrient lysis are more potent activators of enzyme secretion than whole feed itself. Most importantly, single amino acid mixtures increase enzyme secretion, whereas intact protein with a similar amino acid composition release little to no enzymes. Studies with rainbow trout indicate that fish regulate their pancreatic enzyme stores according to specific diet composition. Proteolytic activity of pancreatic tissue, measured 24 h after the last meal, increases with increasing levels of dietary protein.

The luminal environment (e.g., nutrients, low pH, and ionic composition) also stimulates the secretion of several GEP hormones that in turn affect the exocrine pancreas and other digestive processes. For example, secretin from the intestine stimulates the release of bicarbonate from the exocrine pancreas that is used to buffer gastric acid emptying from the stomach. In addition, CCK and secretin are released from the gastrointestinal tract to stimulate the release of exocrine pancreatic enzymes (e.g., pancreatic phospholipase) that aid in the digestion of food.

Numerous interactions between and among GEP hormones also exist. Insulin stimulates the release of GLP-1 that acts synergistically on the gastrointestinal tract and other tissues, recruiting the uptake of nutrients (**see also Integrated Function and Control of the Gut**: Intestinal Absorption). Inhibitory regulators from endocrine pancreatic cells, such as somatostatin, PP, and PYY, inhibit the release of secretin and CCK. GLP-1 and PYY also inhibit gastric secretion and motility, while GLP-2 stimulates growth and digestion. Somatostatin also modulates the intestinal active transport of Na^+ and Cl^- ion uptake, aiding in maintaining osmoregulatory homeostasis. Overall, the GEP exhibits a dynamic relationship regulating various physiological processes throughout food intake, digestion, nutrient absorption, and intermediary metabolism (**Table 1**).

Concluding Remarks

The study of fish has provided considerable insight into the structure, function, and evolution of the pancreas. Because of their diversity, fish offer a unique account of the phylogeny of the pancreas. Species differ in the structure of their pancreas, ranging from a discrete organ containing both endocrine and exocrine tissue to a diffuse collection of endocrine and exocrine tissue distributed on/in several internal organs. Enzymes produced by the pancreas help digest food, whereas hormones produced by the pancreas regulate numerous processes associated with growth, development, and metabolism, including control of pancreatic enzyme secretion and other digestive processes.

See also: **Gut Anatomy and Morphology:** Development of Fish Gut; Gut Anatomy. **Integrated Function and Control**

of the Gut: Endocrine Systems of the Gut; Gut Secretion and Digestion; Intestinal Absorption; Nervous System of the Gut. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Brinn JE, Fletcher DJ, and Epple A (1986) Ontogeny and phylogeny of the islet organ. *Anatomical Record* 214: 447–448.

Burglund GI, Smalas AO, Outzen H, and Willassen NP (1998) Purification and characterization of pancreatic elastase from North Atlantic salmon. *Molecular Marine Biology and Biotechnology* 7: 105–114.

Duguay SJ and Mommsen TP (1994) Molecular aspects of pancreatic peptides. In: Sherwood NM and Hew CL (eds.) *Fish Physiology, Vol. XIII: Molecular Endocrinology of Fish*, pp. 225–271. New York: Academic Press.

Epple A and Brine JE (1987) *The Comparative Physiology of the Pancreatic Islets*. Berlin: Springer.

Kurtovic I, Marshall SN, Zhao X, and Simpson BK (2009) Lipases from mammals and fishes. *Reviews in Fisheries Science* 17: 18–40.

Nelson LE and Sheridan MA (2006) Gastroenteropancreatic hormones and metabolism in fish. *General and Comparative Endocrinology* 148: 116–124.

Plisetskaya EM (1989) Physiology of fish endocrine pancreas. *Fish Physiology Biochemistry* 7: 39–48.

Plisetskaya EM (1990) Recent studies of fish pancreatic hormones – selected topics. *Zoological Science* 7: 335–353.

Volkoff H, Unniappan S, and Kelly SP (2009) The endocrine regulation of food intake. In: Bernier N, Van Der Kraak G, Ferrell A, and Brauner C (eds.) *Fish Physiology, Vol. 28: Fish Neuroendocrinology*, pp. 421–465. New York: Academic Press.

Youson JH (2006) Peripheral endocrine glands. I. The gastroenteropancreatic endocrine system and the thyroid gland. In: McKenzie DJ, Farrel AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 26: Primitive Fishes*, pp. 381–455. New York: Academic Press.

Youson JH and Al-Mahrouki AA (1999) Ontogenetic and phylogenetic development of the endocrine pancreas (islet organ) in fishes. *General and Comparative Endocrinology* 116: 303–335.

Youson JH, Al-Mahrouki AA, Amemiya Y, *et al.* (2006) The fish endocrine pancreas: Review, new data, and future research directions in ontogeny and phylogeny. *General and Comparative Endocrinology* 148: 105–115.

Development of Fish Gut

KN Wallace, Clarkson University, Potsdam, NY, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Specification of Endoderm
Development of the Digestive Tract: Formation of the Pharynx, Esophagus, and Intestine
Determination of the Anterior-to-Posterior Position of the Liver and Pancreas
Differentiation of Digestive Organs
Concluding Remarks
Further Reading

Glossary

Basement membrane The extracellular component attached to the epithelial layer, consisting of connective tissue made up of a complex of short filamentous proteins, e.g., collagens. Epithelial cells adhere to the extracellular matrix by expressing cell adhesion molecules on their basal surface.

Blastoderm The layer of cells that develops on the surface of the yolk, giving rise to the cells from which the embryo develops.

Ectoderm The germ layer positioned on the outside of the embryo after gastrulation, giving rise to epidermis, neurons, and neural crest.

Endoderm The germ layer positioned on the inside of the embryo after gastrulation, giving rise to the lining of the digestive tube, respiratory tube, and accessory digestive organs such as the liver and pancreas.

Gastrulation The migration and rearrangement of cells after embryo undergoes proliferation to increase cell number (cleavage stage) in order to create three germ layers.

Germ layers The three cell layers created after gastrulation: ectoderm on the outside, mesoderm in the middle, and endoderm most internal.

Lumen The opening in the center of a tubular organ. Within the digestive system, this space is bordered by a single layer of epithelial cells, which create a barrier between outside and inside of the organ.

Mesoderm The germ layer positioned between the endoderm and ectoderm, which gives rise to notochord, dermis, muscle, blood, bone, and kidney.

Patent In this article, patent refers to a continuous open lumen, which occurs once the digestive organs connect with each other.

Primitive gut tube A simple epithelial tube that is closed at both ends. In zebrafish, this only encompasses the intestine, whereas other organs associated with the gut form from separate groups of cells.

Somites The blocks of cells that form at regular intervals from the paraxial mesoderm (tissue on either side of the embryonic midline) in an anterior-to-posterior direction; these go on to form repeating muscles, cartilage, dermis, tendons, and vascular cells.

Specification Taking on characteristics of a particular cell type as determined by gene expression and morphology.

Introduction

Development of the fish digestive system has gained wide attention over the past few decades. Zebrafish (*Danio rerio*) have become the subject of intense study for everything from early patterning to the details of organ development, and will be the model system used in this article to describe formation of the digestive system. While the genetics and development of the zebrafish digestive system are similar to other vertebrates, there are also differences and each step is not representative of all vertebrates. For example, zebrafish do not develop a stomach or pyloric ceca.

Zebrafish embryogenesis is completed in 5 days, after which the digestive system is functional and contains a full complement of tissues and cell types found in other vertebrates. As with other vertebrates, the fish digestive system is formed from all germ layers. Further changes occur in juvenile fish before the adult form is achieved.

The zebrafish digestive system develops in three general phases. The first phase involves specification of the endoderm and mesoderm, which forms the majority of cells within the digestive system. Few endodermal cells are specified; hence, the second phase involves endodermal proliferation to create enough cells to form the digestive tube and associated digestive organs, such as

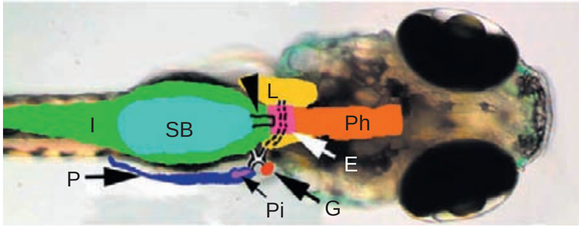


Figure 1 Zebrafish digestive anatomy. The pharynx (Ph) connects to a short muscular esophagus (E) which connects to the intestine (I). The liver (L) straddles the esophagus. The hepato-pancreatic duct enters the anterior intestine connecting the liver, gall bladder (G), and pancreas (P). The pancreas has a single islet (Pi) with the tail containing exocrine tissue. Reproduced from Wallace KN and Pack M (2003) Unique and conserved aspects of gut development in zebra fish. *Developmental Biology* 255: 12.

liver, pancreas, and gall bladder. However, the endoderm does not form a continuous sheet to fold into a primitive gut tube, as with other vertebrates. Instead, the digestive organs form separately and later join to create a patent tract. The third phase involves a dramatic decrease in cell proliferation between the third and fourth day of embryogenesis, and a maturation of cell types within the epithelium and mesenchyme. Differentiation of enteric neurons completes the formation of a functional digestive tract by the end of embryogenesis (**Figure 1**).

Specification of Endoderm

After fertilization, the single cell divides on top of the yolk. As cells become more numerous, they begin to migrate over the yolk cell in a manner that would be akin to pulling a knit cap over one's head. Deep cells form closest to the yolk and a thin enveloping layer develops on the outside. When cells reach the halfway point on the yolk (50% epiboly), gastrulation begins with deep cells separating into the hypoblast layer (endoderm and mesoderm) between the yolk and epiblast cells (ectoderm). Endoderm and mesoderm are specified around the entire margin, followed by migration to the embryonic midline. As migration to the midline proceeds, endoderm and a portion of the mesoderm undergo further specification to form specific digestive organs.

When the embryo reaches 50% epiboly, cells at the blastoderm margin undergo a series of signaling events prior to and during gastrulation to differentiate endodermal from mesendodermal cells. Signaling through the nodal pathway has been shown to be essential for this process. Nodal-related genes, *squint/ndr1* and *cyclops/ndr2*, are expressed in both mesodermal and endodermal progenitors around the blastoderm margin. With the aid of the co-receptor one-eyed pinhead (oep), these nodal-related genes are required for specification of endoderm and mesoderm. The products of *squint/ndr1* and *cyclops/ndr2* are members of the transforming growth factor (TGF)-β superfamily of ligands, which bind to type II TGF-β receptors and allow binding to type I TGF-β receptors, activating the cytoplasmic kinase. Kinase activation leads to phosphorylation of the transcription factors Smad 2 and 3. Smad 2 and 3 then bind Smad 4 and enter the nucleus to repress or activate downstream genes, leading to endoderm specification.

Within the developing endoderm, nodal signaling results in expression of several transcription factors. These transcription factors appear to work in parallel with each other to induce and maintain expression of the transcription factor Sox32/Casanova, which is critical for differentiation of endoderm. Sox32/Casanova in the endoderm is critical for expression of endoderm markers, such as *sox17* and *foxA2/axial*.

Development of the Digestive Tract: Formation of the Pharynx, Esophagus, and Intestine

While zebrafish develop a pharynx, esophagus, and intestine, they (and some other species) do not develop a stomach. However, most species develop a stomach by the hatching period (**see also Gut Anatomy and Morphology**: Gut Anatomy) for more details on anatomy), with gastric glands developing later in the posthatching period.

The Intestine Is First to Begin Organizing

While the pharynx, esophagus, and intestine share a common lumen by the third day of zebrafish embryogenesis, they initially develop as separate organs (**Figure 2**). The intestine first forms a group of radially organized cells in a short anterior region at 21 h postfertilization (hpf) (**Figure 2(a)**). Later, at 26 hpf, a stretch of endoderm at the posterior end of the future intestine becomes radially organized (**Figure 2(b)**. In addition, at 26 hpf, endodermal cells throughout the intestine begin to polarize with basement membranes forming between developing epithelial cells and surrounding mesoderm. At 34 hpf, the entire intestine has formed a continuous tube with an observable lumen (**Figure 2(c)**). This is closed at both ends and has been referred to as a 'primitive gut tube' but, unlike other vertebrates, it only encompasses the intestine rather than the entire digestive tract. At this stage, intestinal epithelial cells have distinct apical and basolateral surfaces, with more pronounced basal laminin as well as apical β-actin and alkaline phosphatase. Cadherin is expressed on the lateral surface, indicating the presence of desmosomal junctions.

In contrast, tight junctions have not yet developed at 34 hpf. As epithelial cells mature into the second day, the tight junction protein ZO-1 develops in irregular patches at

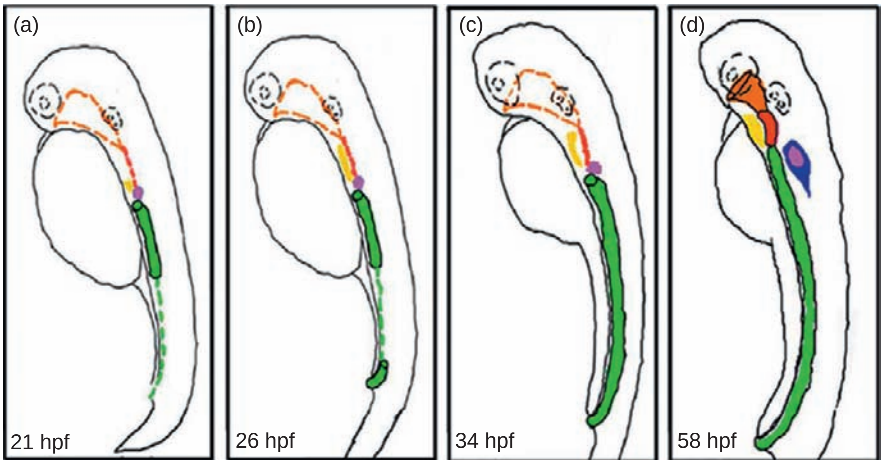


Figure 2 Sequence of digestive organ formation. At 21 hpf, a short anterior region of the intestine develops radial organization (solid green (a)) while both the liver (yellow) and endocrine pancreas (purple) can be identified molecularly. At 26 hpf, a short posterior region of the intestine becomes radially organized (solid green (b)). At 34 hpf, the entire intestine is radially organized, epithelial cells have become polarized and a lumen is observed throughout the intestine (solid green (c)). By 58 hpf, the pharynx (orange) and esophagus (red) have formed and the entire digestive tract is patent (d). Adapted from Wallace KN and Pack M (2003) Unique and conserved aspects of gut development in zebrafish. *Developmental Biology* 255: 12.

the apical–lateral junctions. Expression of ZO-1 matures by 74 hpf when distinct desmosomes and tight junctions are observed by transmission electron microscopy.

Throughout intestinal development, the epithelial layer continues to be comprised of a single layer of cells attached to the basement membrane (**Figures 3(a)** and **3(b)**). The cells, which were cuboidal early on (**Figure 3(a)**), begin developing a more columnar appearance throughout the third day. During the fourth and fifth days, the epithelial layer adopts a folded configuration throughout the length of the intestine (**Figure 3(b)**). At the base of the folds, epithelial cells are cuboidal while being surrounded by columnar neighbors. As shown by electron microscopy, epithelial cells also form a well-developed brush border between 74 and 120 hpf (**Figures 3(c)** and **3(d)**) ((**see also Integrated Function and Control of the Gut:** Barrier Function of the Gut) for more details on the intestinal epithelium).

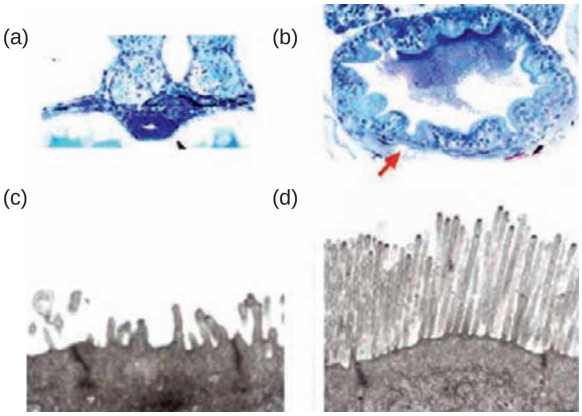


Figure 3 Organization of the intestinal epithelium. At 50 hpf, epithelial cells are cuboidal (a) but by the end of embryogenesis, these cells have a columnar appearance (b). The epithelium becomes folded with cells remaining cubodial at the base of the folds (red arrow in (b)). Between 74 and 120 hpf, the apical surface develops well-differentiated microvilli (c and d). Adapted from Wallace KN, Akhter S, Smith EM, and Pack M (2005) Intestinal growth and differentiation in zebrafish. *Mechanisms of Development* 122: 157–173.

Organization of the Pharynx and Esophagus

The anterior endoderm, giving rise to the pharynx and esophagus, does not organize until after 34 hpf. At this stage, polarization of the epithelium begins with laminin developing in the basement membrane. At 50 hpf, the pharynx develops a bilayer appearance, forming a thin tube spanning the width of the endoderm, with polarized epithelial cells expressing cadherin on the lateral surfaces. Farther posterior, the esophagus and hepatic duct form, with polarized cells expressing laminin at the basement membrane and cadherin on the lateral surfaces.

During the second day, the pharynx becomes rounder and thicker with an obvious lumen and pharyngeal arches develop at the lateral sides to support gills. The esophageal lumen becomes wider during this period, becoming patent with the intestine. At 58 hpf, the posterior end of the pharynx has no apparent lumen before transitioning into the esophagus. A lumen in the posterior pharynx opens by 74 hpf, creating a patent digestive tract up to the posterior end of the intestine, which does not open until 96 hpf.

Intestinal Epithelial Proliferation

Intestinal tube organization involves a period of high cell proliferation. Over 40% of the epithelial cells undergo DNA replication (S-phase of the cell cycle). As junctional complexes mature during the second and third day, proliferation decreases. As epithelial cells differentiate, proliferation decreases to <10% on the fourth day and <5% on the fifth day.

Cell proliferation is evenly distributed throughout the intestinal epithelium from tube formation at 34 hpf to initiation of folding at day 4. During the fifth day, proliferation becomes restricted to the base of the folds, which is a feature of the adult intestine.

Determination of the Anterior-to-Posterior Position of the Liver and Pancreas

After gastrulation, endoderm migrates to the embryonic midline. Endodermal cells are arranged in a flat sheet in two groups on either side of the embryonic midline at 14 hpf. Cells between somites 1 and 3 give rise to liver and pancreas. Duplicate columns of three cells are present on either side of the embryonic midline. Medial cells are opposed to each other at the midline, followed by lateral 1 and lateral 2 cell columns. Medial cells primarily give rise to endocrine pancreas and intestine. Lateral 1 cells give rise to exocrine pancreas, intestine, and some endocrine pancreatic cells; lateral 2 cells give rise to exocrine pancreas, intestine, and liver cells. By 18 hpf, endodermal cells have completed migration to the midline.

As endodermal cells migrate to the midline, regionally expressed signals specify differentiation of individual digestive organs along the anterior-to-posterior axis. One of these is a gradient of retinoic acid (RA) in the mesoderm. The loss of RA prevents pancreas and liver precursors from developing without affecting other endodermal organs. Ectopic RA converts pharyngeal precursors into pancreas. Other factors, such as the transcription factor Cdx4, limit the development of the pancreas. Normally, expression of Cdx4 in an endodermal gradient creates an inhibitory region, providing a posterior limit to the pancreas. Removal of *cdx4* allows pancreas precursors to form at more posterior positions.

Other paracrine factors from the surrounding tissue also initiate signal transduction cascades to promote liver and pancreas differentiation. For example, expression of *wnt 2b*, a ligand for the transmembrane frizzled receptor, in the surrounding lateral plate mesoderm, inhibits degradation of β-catenin. Accumulated β-catenin binds to other transcription factors to alter transcription and promote liver differentiation. Similarly, *bmp2b* promotes pancreas differentiation. Binding of *bmp2b* to type I and II TGF-β receptors results in phosphorylated Smad entering the nucleus, resulting in the absence of *pdx-1* expression in liver progenitors. Cells farther away, still expressing *pdx-1*, become exocrine pancreas and intestine. RA signaling is required between 8 and 13 hpf, *bmp2* at 14 hpf, and *wnt 2b* between 16 and 25 hpf.

Differentiation of Digestive Organs

The teleost gastrointestinal tract forms similar structures to other vertebrates (**Figure 4**). In the zebrafish intestine, there is a single layer of epithelial cells attached to a mucosal layer followed by a layer of circular muscle, enteric neurons, and a longitudinal smooth muscle layer (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut and **Gut Anatomy and Morphology**: Gut Anatomy).

Intestinal Epithelial Differentiation

The intestinal epithelial layer differentiates into absorptive, goblet and enteroendocrine cells. Specialized cells, which pinocytose molecules from the lumen, also form within a posterior region of the intestine.

The epithelium in zebrafish is organized into folds. There are no villi or crypts. Proliferation of stem cells occurs at the base of the folds. Cells migrate from the base to the top of the folds, differentiating into one of the three main cell types. At the top of the fold, epithelial cells undergo apoptosis.

Beginning at 74 hpf, the majority of epithelial cells differentiates into enterocytes, which express Na^+/K^+ adenosine phosphatase (ATPase) on the basolateral surface and the sodium phosphate co-transporter protein (NaPi) on the apical surface. Expression of both transporters develops in an anterior-to-posterior gradient. Goblet cells differentiate by 74 hpf, growing in size and mucin content throughout the second half of embryogenesis. Enteroendocrine cells differentiate by 96 hpf.

Juxtacrine signaling has been shown to be important in the choice between different intestinal epithelial cell fates. Membrane-bound signals, such as Delta or Jagged ligand, signal to neighboring cells upon binding to the transmembrane receptor, Notch. The conformation of ligand-bound Notch is altered and the cytoplasmic portion is cleaved to act as a transcription factor, thus altering the fate of the receiving cell. Notch signaling between developing epithelial cells is involved in the choice between enterocytes and secretory cells. Expression of DeltaD ligand in enteroendocrine cells appears to laterally inhibit neighboring epithelium from adopting the same fate.

Enteric Neuron and Intestinal Smooth Muscle Differentiation

Enteric neural precursor cells enter the zebrafish digestive system at 32 hpf (**Figure 5**(**a**)). They migrate from the neural crest in two streams into the pharynx, posterior to the ear. They migrate along the edge of the pharynx into the esophagus and intestine, reaching the posterior intestine by 66 hpf (**Figure 5**(**b**)). This is followed by circumferential migration.

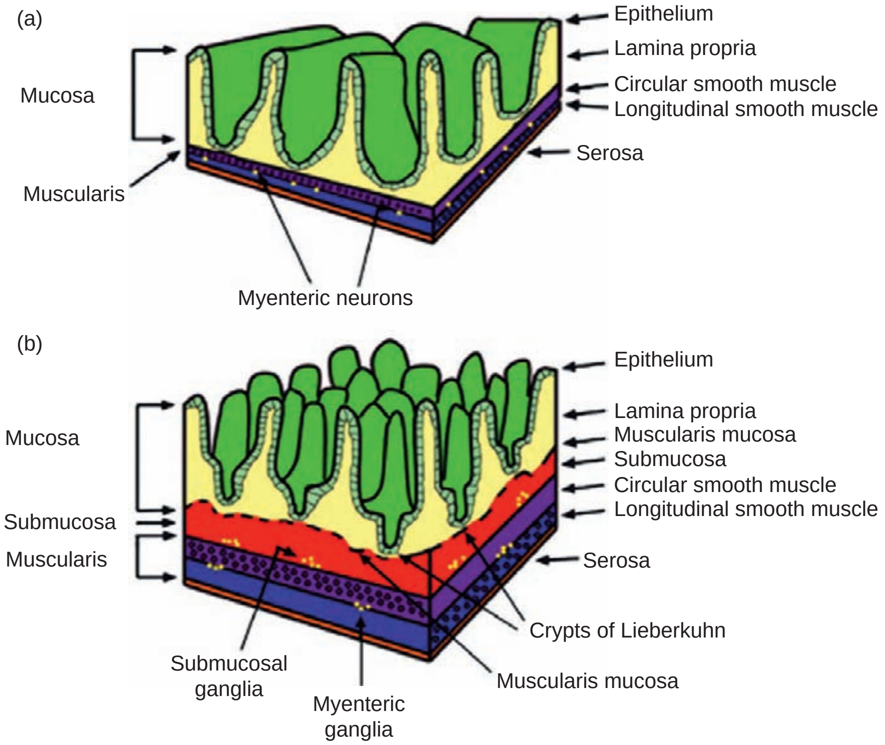


Figure 4 Comparison of teleost intestine to mammalian intestine. The teleost intestine (a) has broad irregular folds rather than individual villi as in mammals (b) and lacks crypts. The teleost has no muscularis mucosa and only a thin layer of connective tissue separates the base of the folds from the circular and smooth muscle layers. Enteric neuronal cell bodies are located between the circular and longitudinal smooth muscle but do not form ganglia as is mammals. Adapted from Wallace KN, Akhter S, Smith EM, and Pack M (2005) Intestinal growth and differentiation in zebrafish. *Mechanisms of Development* 122: 157–173.

During the second day, when migration is a prominent activity, enteric precursors proliferate at high rates (55% of the cells in S-phase at 50 hpf and 44% at 58 hpf). The proliferation rate is lower for anterior enteric precursors, where enteric neurons are differentiating as shown by the general neural marker Hu C/D (**Figure 5(c)**). At 64 hpf, proliferation is equal throughout the intestine (**Figure 5(d)**). During the third day, as more enteric precursors differentiate, the proliferation rate continues to decrease to 18% by 82 hpf.

By 50 hpf, a few enteric neurons are differentiating in the esophagus and anterior intestine. Differentiation of enteric neural precursors continues farther posterior during the second day. During this period, enteric neurons differentiate in two lines along the lateral surface of the intestine. By 74 hpf, enteric neurons begin differentiating around the circumference of the anterior intestine but continue to differentiate in lateral lines in the posterior. Later in the third day, enteric neurons have begun to differentiate around the circumference of both the anterior and the posterior intestine, increasing in numbers until the end of embryogenesis. During enteric neural development, a lower density of neurons develops in the anterior intestine as compared with the posterior.

As circular smooth muscle begins differentiating at 69 hpf, the number of enteric neurons increases dramatically and axons begin to project (**Figure 5(e)**). Later, as longitudinal smooth muscle begins to differentiate (**Figure 5(f)**), there is another dramatic increase in enteric neuron numbers. The largest increase in enteric neurons occurs as the muscle matures between 93 and 98 hpf, resulting in the formation of about one-third of the total neurons.

Neuronal subtypes (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut) form at different times. Neurons expressing neuronal nitric oxide synthase (nNOS) are the first to develop at 60 hpf. As shown by expression of nNOS RNA, these neural precursors have an even distribution throughout the intestine, and the numbers increase relatively evenly throughout development. In contrast, serotonin-expressing neurons are present in low numbers on the third day and only increase during the fourth and fifth day. In addition, serotonin neurons develop in an anterior-to-posterior direction. Each of these neural subtypes develops a lower density in the anterior intestine.

The genetics of enteric development have been conserved. Major known regulators of enteric neuronal precursor migration and proliferation play a role in migration and differentiation of enteric neurons within the zebrafish intestine. Antisense knockdown of transcription,

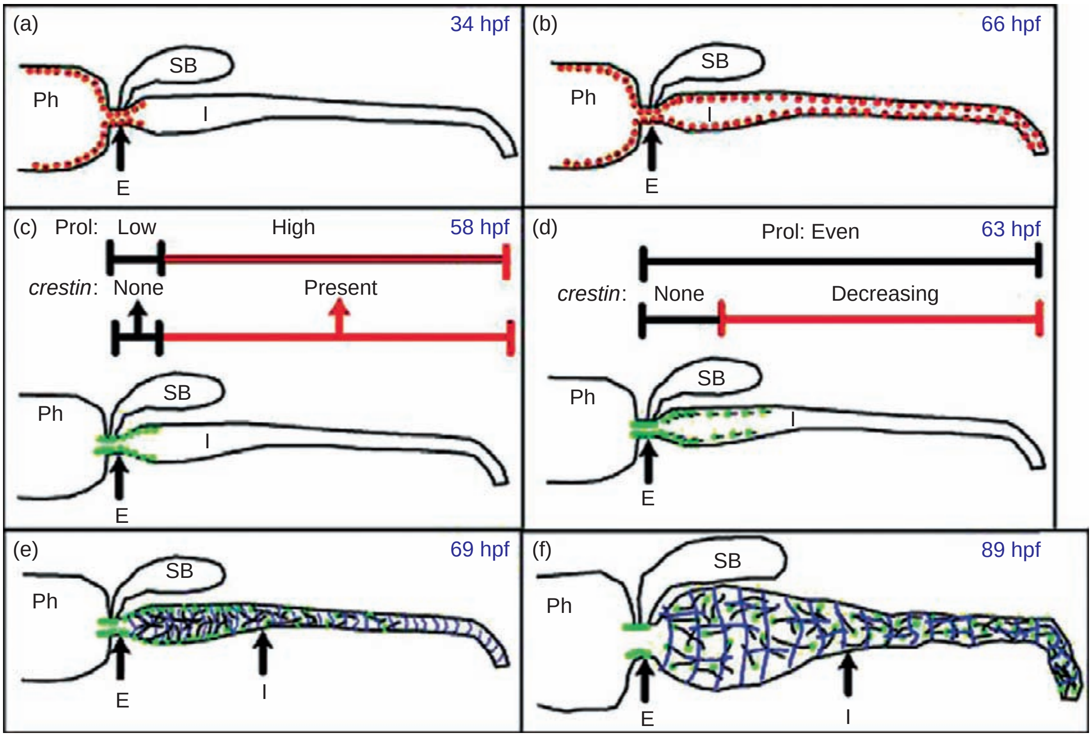


Figure 5 Development of enteric neurons. (a) Enteric neuronal precursors enter the intestine by 34 hpf. (b) Enteric precursors migrate along the lateral sides of the intestine, reaching the posterior by 66 hpf. During the second day of embryogenesis, enteric neurons begin differentiating in the esophagus and anterior intestine (c). This region also shows loss of *crestin* expression (a marker for undifferentiated neural crest) and significantly lower proliferation rates than posterior enteric precursors. At 63 hpf, enteric neurons begin differentiating more posteriorly as *crestin* expression decreases and enteric neuronal precursor proliferation becomes even throughout the intestine. At 69 hpf (e), there is a significant increase in enteric neuron numbers, they begin projecting axons, and there is a coordinated circular smooth muscle development. At 89 hpf (f), longitudinal smooth muscle develops with a second significant increase in enteric neuronal numbers. A third of the enteric neurons differentiate as the longitudinal muscle matures between 93 and 96 hpf. Ph, pharynx; E, esophagus; SB, swimbladder; I, intestine; red dots, enteric neuronal precursors; green dots, enteric neurons; blue lines, differentiating smooth muscle; black lines, axons. Reproduced from Olden T, Akhtar T, Beckman SA, and Wallace KN (2008) Differentiation of the zebrafish enteric nervous system and intestinal smooth muscle. *Genesis* 46: 484–498.

using morpholinos to each of the glial-cell-line-derived neurotrophic factor (GDNF) signaling components, results in lower numbers of enteric neurons, which leads to varying degrees of aganglionosis in the posterior intestine. TGF-β related ligand GDNF activates the RET receptor tyrosine kinase upon binding the growth factor receptor alpha (GFRa)1subunit. The activation of RET is responsible for regulation of enteric nervous system proliferation, differentiation, survival, and migration.

In addition, loss of expression of *sonic hedgehog* (*shh*) in the endoderm or *hand2* in the mesenchyme affects the expression of smooth muscle myosin heavy chain (*myh11*) in the developing smooth muscle, which also results in reduction of enteric neuronal precursor proliferation.

Differentiation of the Pancreas

The pancreas develops both the exocrine and the endocrine tissues. Exocrine cells develop a network of ducts that collect digestive enzymes to drain into the anterior intestine. Among fish, there is diversity in distribution of endocrine tissue. Many teleosts have a diffuse distribution of small islets within exocrine tissue, while other species tend to have principal islets (**see also Gut Anatomy and Morphology:** Pancreas). Like other cyprinids, adult zebrafish have a pancreas comprised of a diffuse network of both endocrine and exocrine tissues surrounding the intestine. The embryonic zebrafish pancreas, however, develops with a single islet at the anterior head surrounded by a body of exocrine tissue, which forms a posterior tail.

At 17 hpf, before the endoderm completes migration to the midline, insulin-expressing cells differentiate in bilateral groups adjacent to the midline. By 18 hpf, they merge to form the majority of the endocrine pancreas. The islet develops in an organization similar to amniotes with central β-cells and α-, δ-, and ε-cells in the periphery.

Later, a second group of endodermal cells differentiate ventral to the developing esophagus, forming the majority of the exocrine pancreas. The two groups join at 48 hpf, forming a single islet surrounded by exocrine cells. At this stage, there is no observable differentiation of ducts or acini and the tissue is considered protodifferentiated. At 60 hpf, ducts and acinar cells can be observed but these isolated cells are not yet organized into distinct glands and ducts. By 72 hpf, exocrine tissue proliferates and grows in the posterior direction, forming a distinct tail from the head of the pancreas, which contains the single islet. Ducts and acini organize from isolated groups of cells rather than the continuous branching that occurs in mammals. By 120 hpf, acinar cells are fully polarized and there is an organized network of ducts that drain individual acini.

Notch signaling is a key regulator of the fate of the pancreatic cells. As with the differentiation of other cell types, activation of Notch signaling plays a role in maintaining cells in an undifferentiated state. During pancreas differentiation, Notch signaling is required at multiple stages to maintain cells in the appropriate precursor state. Several Notch signals (*deltaA*, *jagged2*, and *jagged1b*) are expressed during pancreas development, acting at different times and places within the developing pancreas to permit the differentiation of both endocrine and exocrine cell types.

Differentiation of the Liver

As mentioned above, liver endoderm becomes specified as early as 14 hpf. During the early phase, the liver increases in size, but bile duct formation does not begin until 70 hpf. Biliary cells are first observed between 50 and 60 hpf, and a branching network of biliary ducts is observed by 80 hpf. Formation of the biliary network is not dependent on the presence of vasculature as in mammals.

The zebrafish liver has a tubular organization. Here, small bile ducts reside within hepatocyte tubules, which are surrounded by fenestrated sinusoidal endothelial cells (**Figure 6**(**a**)). In contrast, the mammalian liver is organized around a central vein surrounded by bilayered hepatic plates and radially organized groupings of portal veins, hepatic arteries, and bile ducts (**Figure 6**(**b**)).

Differentiation of hepatoblasts and the biliary tree has been demonstrated to be dependent on Notch signaling, using a combination of ligands and receptors. Genes for the Notch receptors *notch 1a*, *1b*, *2*, *and 5* along with the ligands *jagged 1*, *2*, *and 3* are expressed during bile duct formation at 2 dpf. Morpholino knockdown of *jagged 2 and 3* disrupts intrahepatic biliary development, whereas knockdown of *jagged 1* does not. Compound knockdown of the receptor genes *notch 2* and *5* also affects intrahepatic biliary development. Additional signals (such as *onecut 3* and *hnf*) also play a role in intrahepatic biliary development.

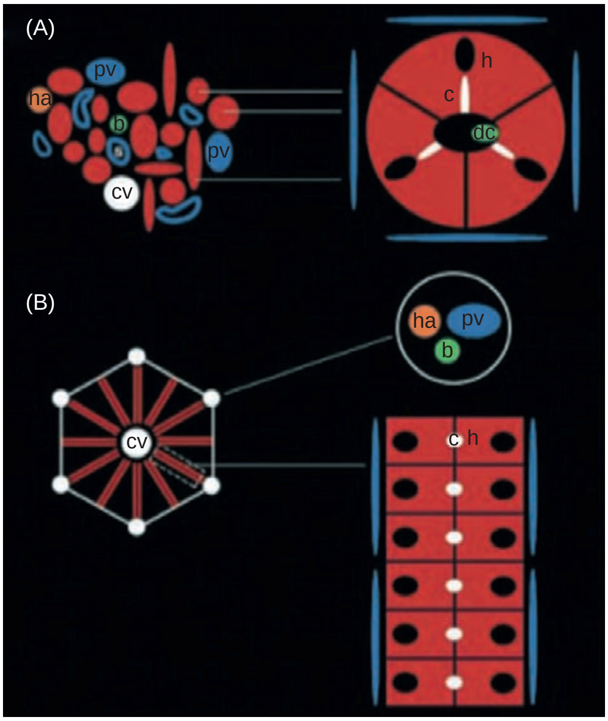


Figure 6 Structure of the teleost liver. The teleost liver (A) has a tubular organization with small bile ducts (dc) residing in the center of hepatocytes and anastomoses with three unicellular hepatocyte canaliculi (c). Hepatocytes are surrounded by fenestrated endothelia (blue). The portal vein (pv), hepatic artery (ha), and bile ducts (b) are not grouped around the bilayered hepatocytes (h) and central vein (cv) as in mammals (B). Organization of the teleost liver is not as apparent in cross section due to longitudinal, transverse, and oblique sections of tubular structures (A). Adapted from Lorent K, Yeo SY, Oda T, *et al*. (2004) Inhibition of Jagged-mediated Notch signaling disrupts zebrafish biliary development and generates multi-organ defects compatible with an Alagille syndrome phenocopy. *Development* 131: 5753–5766.

Concluding Remarks

There are differences between development of zebrafish and other vertebrate digestive systems. However, there are also many striking similarities, in both structure and genetic pathways, between the digestive systems of many vertebrates. This suggests that even though species are separated by wide evolutionary distances, a common pathway for digestive development may emerge for vertebrates.

See also: **Gut Anatomy and Morphology**: Gut Anatomy; Pancreas. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Nervous System of the Gut. **The Gut**: The Gastrointestinal Tract: An Introduction

Further Reading

Burzynski G, Shepherd IT, and Enomoto H (2009) Genetic model system studies of the development of the enteric nervous system, gut motility and Hirschsprung's disease. *Neurogastroenterology and Motility* 21: 113–127.

Chung WS, Shin CH, and Stainier DY (2008) Bmp2 signaling regulates the hepatic versus pancreatic fate decision. *Developmental Cell* 15: 738–748.

Crosnier C, Vargesson N, Gschmeissner S, *et al.* (2005) Delta–Notch signaling controls commitment to a secretory fate in the zebrafish intestine. *Development* 132: 1093–1104.

Fukuda K and Kikuchi Y (2005) Endoderm development in vertebrates: Fate mapping, induction and regional specification. *Development Growth Differentiation* 47: 343–355.

Kinkel MD and Prince VE (2009) On the diabetic menu: Zebrafish as a model for pancreas development and function. *BioEssays* 31: 139–152.

Lorent K, Yeo SY, Oda T, *et al.* (2004) Inhibition of Jagged-mediated Notch signaling disrupts zebrafish biliary development and generates multi-organ defects compatible with an Alagille syndrome phenocopy. *Development* 131: 5753–5766.

Matthews RP, Lorent K, and Pack M (2008) Transcription factor onecut3 regulates intrahepatic biliary development in zebrafish. *Developmental Dynamics* 237: 124–131.

Ober EA, Verkade H, Field HA, and Stainier DY (2006) Mesodermal Wnt2b signaling positively regulates liver specification. *Nature* 442: 688–691.

Olden T, Akhtar T, Beckman SA, and Wallace KN (2008) Differentiation of the zebrafish enteric nervous system and intestinal smooth muscle. *Genesis* 46: 484–498.

Poulain M, Furthauer M, Thisse B, Thisse C, and Lepage T (2006) Zebrafish endoderm formation is regulated by combinatorial Nodal, FGF and BMP signaling. *Development* 133: 2189–2200.

Reichenbach B, Delalande JM, Kolmogorova E, *et al.* (2008) Endoderm-derived Sonic hedgehog and mesoderm Hand2 expression are required for enteric nervous system development in zebrafish. *Developmental Biology* 318: 52–64.

Schier AF and Talbot WS (2005) Molecular genetics of axis formation in zebrafish. *Annual Review of Genetics* 39: 561–613.

Shin D, Shin CH, Tucker J, *et al.* (2007) Bmp and Fgf signaling are essential for liver specification in zebrafish. *Development* 134: 2041–2050.

Tao T and Peng J (2009) Liver development in zebrafish (*Danio rerio*). *Journal of Genetics and Genomics* 36: 325–334.

Wallace KN, Akhter S, Smith EM, and Pack M (2005) Intestinal growth and differentiation in zebrafish. *Mechanisms of Development* 122: 157–173.

Wallace KN and Pack M (2003) Unique and conserved aspects of gut development in zebrafish. *Developmental Biology* 255: 12–29.

Ward AB, Warga RM, and Prince VE (2007) Origin of the zebrafish endocrine and exocrine pancreas. *Developmental Dynamics* 236: 1558–1569.

Warga RM and Stainier DY (2002) The guts of endoderm formation. *Results and Problems in Cell Differentiation* 40: 28–47.

Yee NS, Lorent K, and Pack M (2005) Exocrine pancreas development in zebrafish. *Developmental Biology* 284: 84–101.

Zecchin E, Filippi A, Biemar F, *et al.* (2007) Distinct delta and jagged genes control sequential segregation of pancreatic cell types from precursor pools in zebrafish. *Developmental Biology* 301: 192–204.

THE GUT

Integrated Function and Control of the Gut

Contents
Gut Motility
Gut Secretion and Digestion
Intestinal Absorption
Barrier Function of the Gut
Nervous System of the Gut
Endocrine Systems of the Gut

Gut Motility

A Gräns and C Olsson, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

What Is Gut Motility?
Different Motility Patterns Are Present in the Gut
Cellular Mechanisms behind Motility
Gut Motility Is Tightly Controlled
Development of Gut Motility and Control
Integrated Effects on Gut Motility
Concluding Remarks
Further Reading

Glossary

Chyme Partly digested food particles mixed with digestive secretions expelled by the stomach into the intestine.

Enteric nervous system The part of the autonomic nervous system contained within the gastrointestinal wall.

Peristalsis Propagating movements of gut contents by contraction of gut muscle.

Postprandial Period after ingesting a meal.

Propagating contractions Directional contraction waves in the gut in fasted and feed animals.

Slow waves Periodic depolarizations and repolarizations in gut muscle cell membranes which do not always result in an action potential.

Standing contractions Local nonpropagating contractions involved in mixing of the gut contents.

What Is Gut Motility?

The concept gut motility includes mixing and propulsive activity that is achieved by contraction and relaxation of smooth muscle in the gut wall. Gut motility plays an essential role in the breakdown and transport of food in the gastrointestinal tract, and occurs also in fasted states, when no food is present in the gastrointestinal tract. To facilitate digestion, the ingested food is mixed so that enzymes and acid can gain access to all food particles. Likewise, food needs to be transported from the mouth, where digestion starts, along the stomach and intestine where it is further digested before nutrients are eventually absorbed.

To ensure optimized processing of the food, all these different activities need tight control and coordination. The control systems are complex and we know only a fraction of the pathways involved. In this article, we review the fragmented literature available on gut motility in fish.

Different Motility Patterns Are Present in the Gut

The type of motility depends on the feeding status of the animal as well as the region of the gut. There are few *in vivo* studies of gastrointestinal motility in fish, limiting our knowledge of what patterns exist and when they occur.

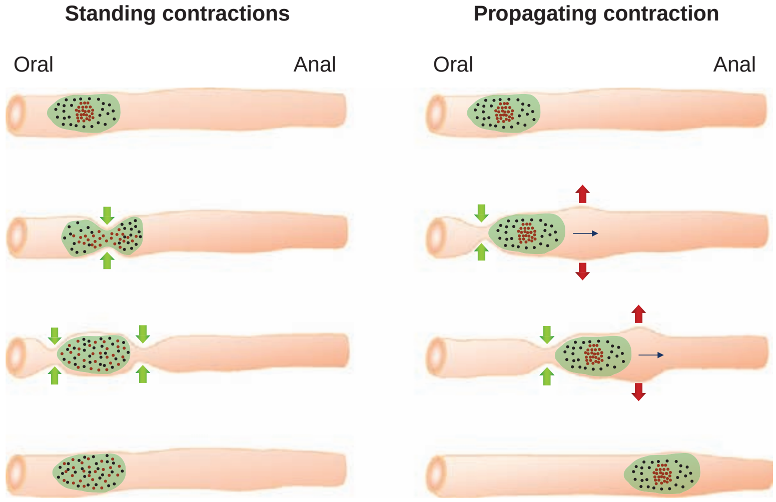


Figure 1 Different motility patterns of the gut. Local standing contractions mix the gut contents while propagating contractions move gut contents in an oral-to-anal direction. Green arrows indicate contractions, red arrows relaxation.

In most vertebrates, food intake induces propagating contractions of varying frequency and amplitude (**Figure 1**). In addition, local standing contractions mix the food. Propagating contractions also are seen in the fasted state but their appearance often differs from the postprandial (after food intake) patterns. Motility patterns also include the relaxation that may occur in response to distension of the gut wall (accommodation) or by anticipation of food (receptive relaxation).

So far, in fish, most studies on gut motility are *in vitro* recordings of muscle contractions, focusing more on specific control mechanisms than trying to reveal normally occurring motility patterns under natural situations. When using isolated muscle strip preparations, it is impossible to tell if a contraction is local or propagating. Some resolution can be achieved by using longer gut segments or intact organs, but still the situation is far from what occurs *in vivo* because the gut is detached from many of its control systems (see below). To accurately distinguish between propagating and nonpropagating contractions and to make comparisons with mammalian motility patterns, *in vivo* studies are needed in fish. These may include several different methods from pressure balloons inserted into the gut lumen or force transducers attached to the serosa to X-ray monitoring or video recordings.

Propagating Contractions

Propagating contractions in fish have been observed *in vivo* mainly in larvae. The transparent larvae are well suited for noninvasive studies of gut physiology and pharmacology in living fish. Using video-microscopy on zebrafish (*Danio rerio*) larvae, contractions are seen traversing the intestine in an oral-to-anal direction at a frequency of approximately one contraction-wave per minute and a speed of 0.1–0.5 cm min^{-1} (**Figure 2**; **Video Clip 1**).

Other studies involve *in vitro* or *in situ* experiments. In the late 1950s, G. Burnstock performed a series of experiments on brown trout (*Salmo trutta*) identifying different contraction patterns in isolated intestine and stomach. Propagating contractions were observed visually, moving at ~2 cm min^{-1}. Judging from his figures, the contraction frequency was approximately one per 5–6 min. The contraction waves were most prominent in fish that were fed prior to the experiments. Similarly, balloon distension and/or transmural electrical stimulation of rainbow trout (*Oncorhynchus mykiss*) and Atlantic cod (*Gadus morhua*) intestine *in vitro* trigger contraction waves traveling at approximately 3.5 cm min^{-1}. The contraction frequency in both stomach and intestine of unfed fish usually is about one every 2 min.

Postprandial propagating contractions (often simply referred to as peristalsis) are characteristically fine-tuned interactions between the muscles immediately anal and oral to the food bolus. Moreover, in fish, stimulation at one point causes oral contractions and a simultaneous anal relaxation, but the exact roles of the circular and longitudinal muscle layers are not known.

In fasted/unfed mammals, propagating contractions are often referred to as migrating motor complexes (MMCs). MMCs consist of three distinct phases, with only one phase containing regular (migrating) contractions. These contractions differ from the postprandial propagating contractions by running for longer distances and at a lower speed. They may play an important housekeeping function, cleaning the gut from unwanted debris and bacteria when normal peristaltic movements are absent. In continuous feeders such as sheep and guinea pig, the distinction between MMCs and postprandial movements is less obvious. Propagating contractions observed in unfed fish, including young zebrafish larvae, are possibly the equivalent to mammalian MMCs. As different feeding strategies exist among fish, it is likely that motility patterns vary accordingly.

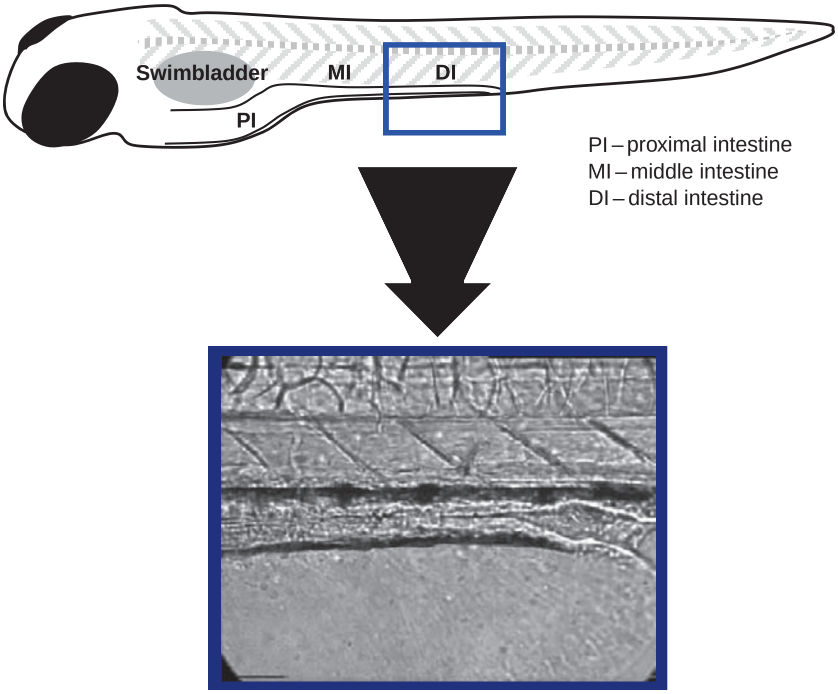


Figure 2 Propagating contractions in the distal intestine from a zebrafish (*Danio rerio*) larva at 7 days postfertilization (dpf). (see **Video Clip 1**; please note the events in the video are sped up, the time velocity is around 20 μms^{-1}. **Video Clip** courtesy of Dr. Anna Holmberg).

Although the propagating contractions normally run in the oral-to-anal direction, it is not uncommon to see retrograde (anal-to-oral) contractions. Zebrafish and Atlantic halibut (*Hippoglossus hippoglossus*) larvae have regular retrograde contractions, mainly in the proximal gut. The purpose of these movements is not fully understood but perhaps they mix gut contents and fill the pyloric ceca. Retroperistalsis is also seen in lesser-spotted dogfish (*Scyliorhinus canicula*) and has been suggested that it may contribute to vomiting.

Local Contractions

Transport of food (chyme) in the gut is a discontinuous process. To improve digestion, the chyme needs to be mixed with enzymes. To facilitate absorption, chyme has to be in close contact with the epithelial cells. Hence, propagating movements are interrupted by local, standing contractions in the postprandial state.

The propulsion rate varies along the gut, with muscular sphincters function acting as dividers between different regions (**see also Gut Anatomy and Morphology**: Gut Anatomy). Both propulsion rate and the state of the sphincters are controlled by an orchestra of subconscious signals. The sphincters are normally tonically contracted, keeping them closed, and open in the presence of an appropriate stimulus. For example, gastric emptying (the process where stomach contents are released into the intestine) normally occurs in smaller portions at a rate adjusted so that the food can be handled by the small intestine. The rate is ultimately determined by the state of the pyloric sphincter situated between the distal part of the stomach and the proximal intestine. If the sphincter is contracted, very little chyme is released while relaxation of the muscle results in opening of the sphincter.

Cellular Mechanisms behind Motility

Gut motility occurs when the smooth muscle in the gut wall contracts and relaxes in a reasonably well-coordinated fashion. The smooth muscle cells are connected to each other via gap junctions, allowing adjacent cells to contract in synchrony. The muscle layer generally has a basal tone of activity.

Muscle cells contract when the depolarization reaches a threshold, resulting in an action potential. Contraction may also occur if intracellular calcium levels increase without a change in membrane potential. Relaxation, on the other hand, occurs when the cell is hyperpolarized or if calcium levels decrease. So far, few, if any, studies on fish have examined the electrophysiological events in gut muscle cells and little is known about the ion channels involved. However, extracellular recordings of enteric electrical activity are a useful way of recording contractile activity *in vivo* in fish as in mammals (**Figure 3**).

In mammals, gut smooth muscle cells depolarize and repolarize in a rhythmic fashion, without any external stimuli. This spontaneous slow-wave activity, however, does not originate in the muscle cells but in the so-called interstitial cells of Cajal (ICCs) from which the electrical activity spreads to muscle cells via gap junctions. Muscular

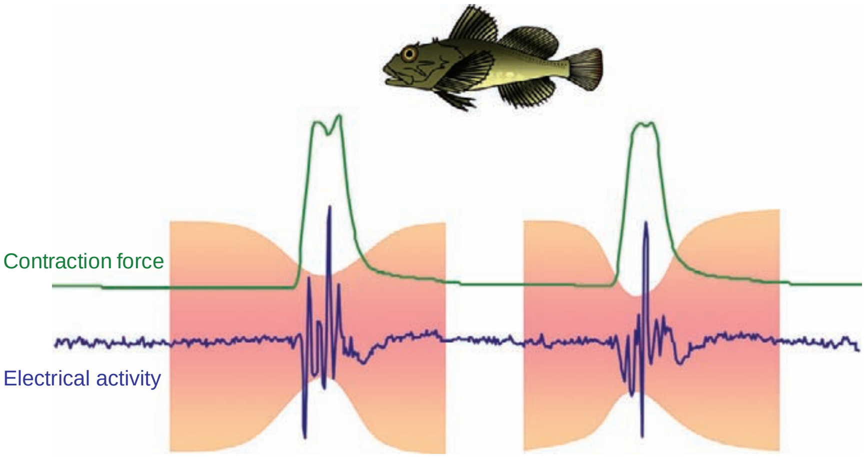


Figure 3 Correlation between electrical activity and mechanical contractions in the gut smooth muscle of shorthorn sculpin (*Myoxocephalus scorpius*).

slow waves often need additional stimuli from nerves or hormones to effect contractions. ICCs are present in fish and we hypothesize that they play a similar role in initiation of gut motility, although this has not been tested.

Gut Motility Is Tightly Controlled

The complex motility patterns of the gut depend on factors including the intrinsic properties of the smooth muscles as well as intrinsic and extrinsic nerves and hormones (**Figure 4**). In addition, environmental factors, such as temperature and pH, play an important role in the control, for example, by affecting the contractility of smooth muscles. Many fish species encounter large variations in the environment and are adapted to handle them. The impact of seasonal changes on gut motility is, however, poorly understood and should be further investigated. Furthermore, the type of food ingested changes motility.

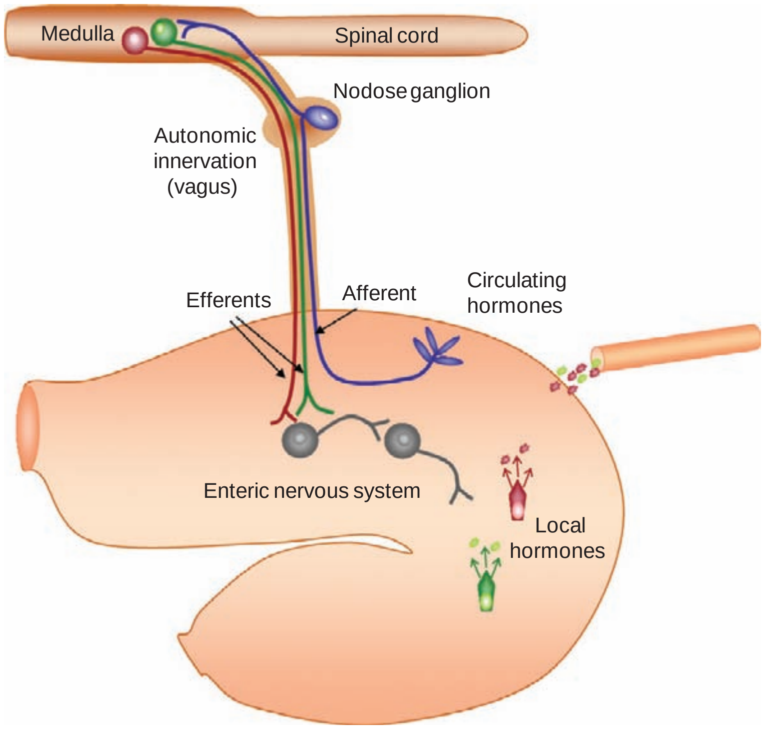


Figure 4 Schematic drawing of nervous and hormonal control of gut motility. Afferent extrinsic neurons (blue) respond to chemical and mechanical stimuli in the stomach, stimulating efferent autonomic pathways (illustrated by the vagal innervation). The efferent nerves may then stimulate (green) or inhibit (red) the enteric nervous system (gray). Inhibitory (red) and excitatory hormones (green) may be released from local endocrine cells or reach the gut via the circulation.

The main player in the control of gut motility is the enteric part of the autonomic nervous system. The enteric nervous system comprises all nerve cells intrinsic to the gut, that is, they have their cell bodies within the gut wall and one of its functions is to contract or relax gut muscle. An important characteristic of the enteric nervous system is its ability to work independently of central input, which means it contains the components to make up entire reflexes (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut). This does not exclude extrinsic input as an important part of the control system, especially for modification and coordination of gut motility between different regions.

In addition to neuronal control, local endocrine cells release hormones that affect muscle activity. The effect can be direct or indirect, either via neighboring cells in a paracrine fashion or by release to the general circulation. Likewise, circulating hormones released elsewhere in the body may affect the gut.

The relative importance of the different control pathways varies among the different motility patterns and situations. Experimental setups seldom allow an integrated estimation of all possible influences. For example, *in vitro* preparations of isolated organs and muscle strips essentially exclude the involvement of circulating hormones and extrinsic innervation. The rhythmic contractions observed under basal conditions in such setups are often called spontaneous because they occur in untreated preparations. However, strictly speaking, this is not correct because they usually depend on some stimulus external to the muscle cell. Application of the sodium-channel blocker tetrodotoxin (TTX) may reduce or completely abolish the activity, indicating that it is nerve dependent.

In mammals, postprandial activity is affected by both intrinsic and extrinsic nerves, as well as by hormones. The extrinsic innervation seems to be less important for basal activity like MMCs. In either case, it is the underlying slow-wave activity that determines the maximal contraction frequency. TTX greatly reduces contraction frequency in intact zebrafish larvae, indicating the involvement of nerves in the initiation and/or maintenance of propagating motility. However, TTX affects both intrinsic and extrinsic neuronal pathways and it cannot be deduced which is its primary effect. On the other hand, TTX also reduces propagating contractions in, for example, isolated Atlantic cod intestine, suggesting that enteric nerves play an important role. Likewise, zebrafish mutants that lack or have reduced numbers of enteric neurons show reduced propagating activity.

Nervous Reflexes within the Gut

Intrinsic and extrinsic reflexes, as well as release of hormones, are initiated by the presence of food in the gut. The actual stimuli may be either chemical or mechanical, activating different types of sensory cells. In fish, few studies have looked at the types of stimuli involved in reflexes. Balloon distension is one way of mimicking mechanical stimulation. Electrical stimulation has also been used in some studies but this is a very artificial stimulus that does not accurately correspond to the normal postprandial distension. To study chemical stimulation, different breakdown products of the food (e.g., fat, proteins, or low pH) may be introduced in the gut *in vitro* or *in vivo*. Using a three-compartment bath, Karila and Holmgren showed that stimulation of the Atlantic cod intestine in one region resulted in contractions anally and relaxation orally, indicating that one stimulus affects both ascending and descending enteric nerves at the same time (**Figure 5**).

The role of the extrinsic innervation of the fish gut is poorly investigated and may vary between species. In mammals, extrinsic reflexes control gastric receptive relaxation and accommodation. In contrast, in teleosts, accommodation of the stomach takes place in the absence of vagal input, indicating that the enteric neurons perform at least some of the tasks that are under extrinsic control in mammals. Electrical stimulation of the vagus revealed mainly inhibitory fibers in salmonids and primarily excitatory fibers in Atlantic cod and plaice (*Pleuronectes platessa*) stomach. In some species, a combination of inhibitory and excitatory vagal fibers has been suggested. Similarly, excitatory effects exist in elasmobranchs but most studies support an inhibitory vagal effect. The splanchnic nerve(s) similarly have excitatory and inhibitory functions in both teleosts and elasmobranchs with species-specific variations.

Pharmacological Studies of Gut Motility

The fish gut has been studied extensively for the presence of signaling substances. Many of these substances occur in both nerves and endocrine cells, but this speaks little to function. An appropriate receptor is also needed for an effect. Although many transmitters are purely inhibitory or excitatory, others depend on the target receptor. For excitatory effects on gut motility, they may be manifested as an initiation of a muscle contraction or as an enhancement of the amplitude and/or frequency of rhythmic contractions. The opposite is true for inhibition.

Pharmacological studies, mostly with isolated gastrointestinal preparations, have discovered much about the effects of different signaling substances. The lack of fish-specific agonists and antagonist, however, has slowed progress. Some signaling molecules, such as acetylcholine, catecholamines, and nitric oxide, are identical across species, while peptides can vary appreciably. Today, the

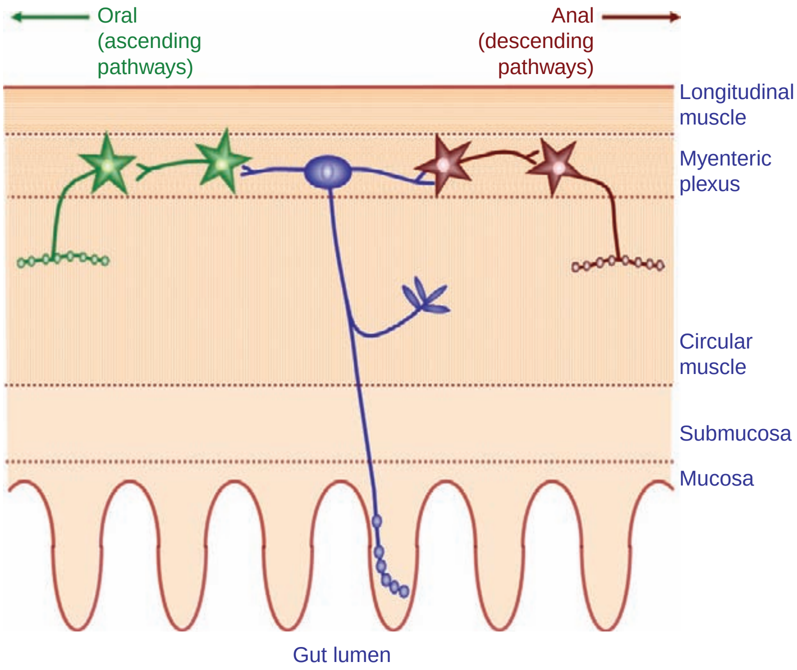


Figure 5 Schematic drawing of ascending (green) and descending pathways (red) in the peristaltic reflex, triggered by a common sensory neuron (blue) that may respond to chemical (luminal content, or hormones released from mucosal endocrine cells) or mechanical (stretching of the muscle layer) stimuli.

sequences of several fish peptides are known but still very few peptide receptor antagonists have worked in fish.

Rainbow trout and Atlantic cod are well studied in this regard. Some control mechanisms are similar throughout vertebrates, but with fish being such a large and diverse group, differences do occur among species and different parts of the gut. For a summary of the effects of different neurotransmitters and hormones in different fish species, see supplementary material (**Appendix A** (pp. 1232–1267)).

Excitatory and Inhibitory Signals in the Gut

Perhaps the most important neurotransmitter controlling gut motility is acetylcholine. In most teleosts and elasmobranchs, acetylcholine has an excitatory effect on the gut. Acetylcholine (or the more stable analog carbachol) may increase contraction amplitude, frequency or basal tone, or cause a combination of these effects in isolated muscle strips. This effect is likely achieved by a direct effect of ascending enteric motoneurons acting via muscarinic receptors because the response is often reduced by atropine but not affected by TTX. Acetylcholine is also involved in distension-induced reflex contractions in Atlantic cod and rainbow trout stomach.

Cholinergic innervation also includes ascending and descending interneurons as well as extrinsic (vagal) fibers. The contribution of these nerves to the overall cholinergic response is less well studied. In addition, little is known about the cholinergic control of fish gut motility *in vivo*.

Another important group of excitatory transmitters present in motoneurons is the tachykinins. Both substance P and neurokinin A (NKA) induce or enhance contractions in muscle strips from thorny skate (*Amblyraja radiata*), South American lungfish (*Lepidosiren paradoxa*), gray bichir (*Polypterus senegalus*), and rainbow trout, while they have no effect in Atlantic hagfish (*Myxine glutinosa*) or lamprey (*Lampetra fluviatilis*). The route of action of the tachykinins varies between species (see **Figure 6**) and may be direct on the smooth muscle or indirect via cholinergic or serotonergic neurons. So far, there are no reports of the effects on gut motility of scyliorhinins, two tachykinins first discovered in elasmobranchs.

The most widely examined inhibitory substances in fish and other vertebrates are members of the vasoactive intestinal polypeptide (VIP) family and nitric oxide. The effect of VIP, its relative pituitary adenylate cyclase activating polypeptide (PACAP), and nitric oxide on isolated gut preparations is mainly a reduction of rhythmic contractions. However, species and regional differences exist. In Atlantic cod, all three transmitters coexist to a large extent in enteric neurons and they may be released in response to slightly different stimuli. While PACAP reduces intestinal contractions in isolated muscle strips, neither mammalian nor cod VIP have any effect. In contrast, VIP reduces gastric contractions. However, VIP does not affect the response to distension in the Atlantic

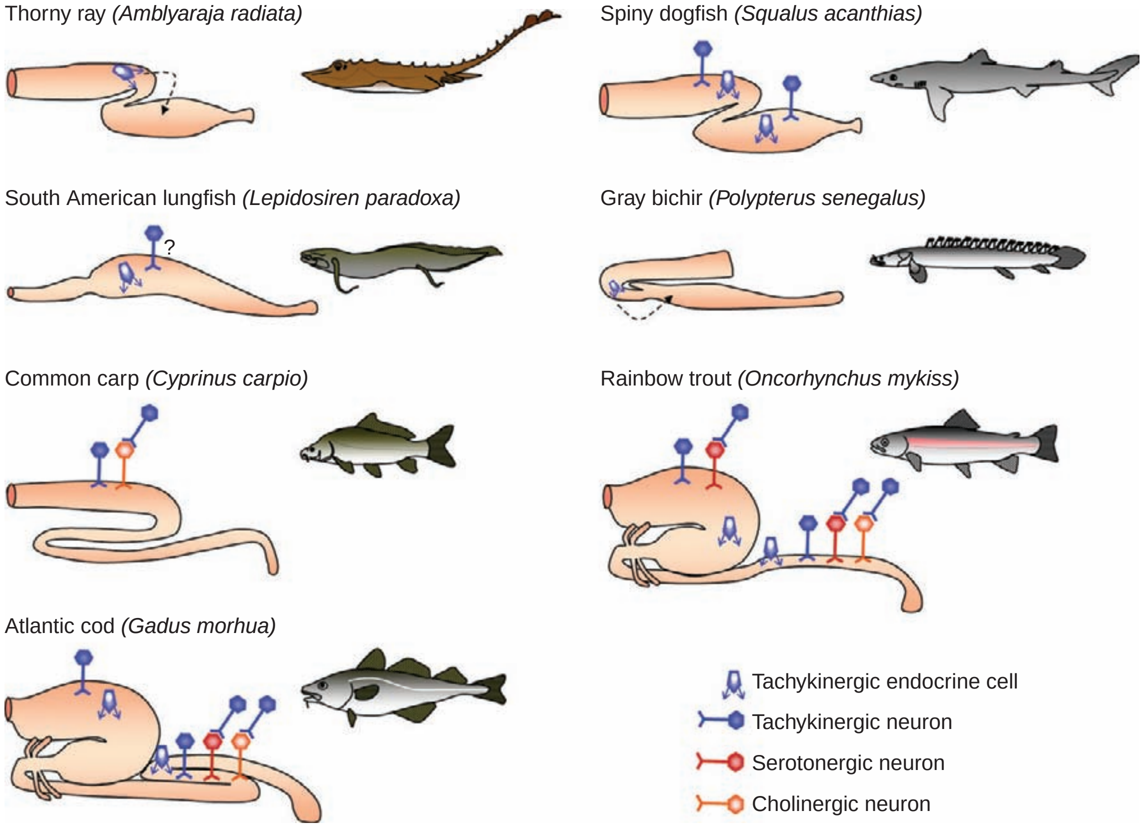

Thorny ray *(Amblyaraja radiata)*
Spiny dogfish *(Squalus acanthias)*
South American lungfish *(Lepidosiren paradoxa)*
?
Gray bichir *(Polypterus senegalus)*
Common carp *(Cyprinus carpio)*
Rainbow trout *(Oncorhynchus mykiss)*
Atlantic cod *(Gadus morhua)*
Tachykinergic endocrine cell
Tachykinergic neuron
Serotonergic neuron
Cholinergic neuron

Figure 6 Effects of tachykinins on gut motility in various species of fish. The tachykinins may be released from enteric neurons or local endocrine cells and may either have direct effect on the smooth muscle, or via release of acetylcholine or serotonin from other enteric neurons. Modified from Jensen J and Holmgren S (1994) The gastrointestinal canal. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 119–167. Chur, Switzerland: Harwood.

cod stomach, but causes relaxation in similar experiments in rainbow trout. Furthermore, VIP does not affect motility in any gut region of skate (*Raja* sp.), while inhibition is seen in the lesser-spotted dogfish rectum.

Exogenous nitric oxide lowers basal tension and abolishes rhythmic contractions in muscle strips from several species and regions. In addition, there is usually a nitrergic tone because blocking the synthesis of nitric oxide with L-arginine analogs increases contraction amplitude and basal tension. Nitrergic innervation mainly involves descending motoneurons (and possibly interneurons).

As the effects of inhibitory substances are most easily observed on rhythmically contracting preparations and those contractions would be decreased or abolished by TTX, few studies have managed to reveal where in a reflex VIP/PACAP and nitric oxide are released. If one would make parallels to the mammalian control systems, the most likely suggestion would be descending motoneurons. In the study on rainbow trout stomach mentioned above, it also was suggested that VIP might act via inhibition of excitatory (serotonergic) motoneurons. Judging from the distribution of VIP and PACAP, it also may be released from extrinsic neurons as well as from local endocrine cells.

Another important gut transmitter is serotonin (5-hydroxytryptamine, 5-HT). It is present in a large proportion of enteric neurons in many fish species, and may also be released from mucosal endocrine cells. Serotonin causes contractions of the gut in teleosts. In elasmobranchs, the response is less clear-cut and large variations occur both within and between different regions of the gut (**Appendix A** (pp. 1232–1267)). The route of action is far from clear with both direct and indirect effects reported depending on species. In rainbow trout, for example, serotonin is not blocked by atropine or TTX, indicating a direct effect on the smooth muscle. In Atlantic cod and several other species, an indirect pathway is suggested because the effect is at least partly reduced by TTX. In mammals, serotonin is released from endocrine cells, in response to chemical stimuli in the lumen, and acts as a stimulus for intrinsic sensory neurons. Whether it plays the same role in fish is not established but the serotonergic neurons may, to some extent, include a sensory population. They may also be excitatory descending interneurons.

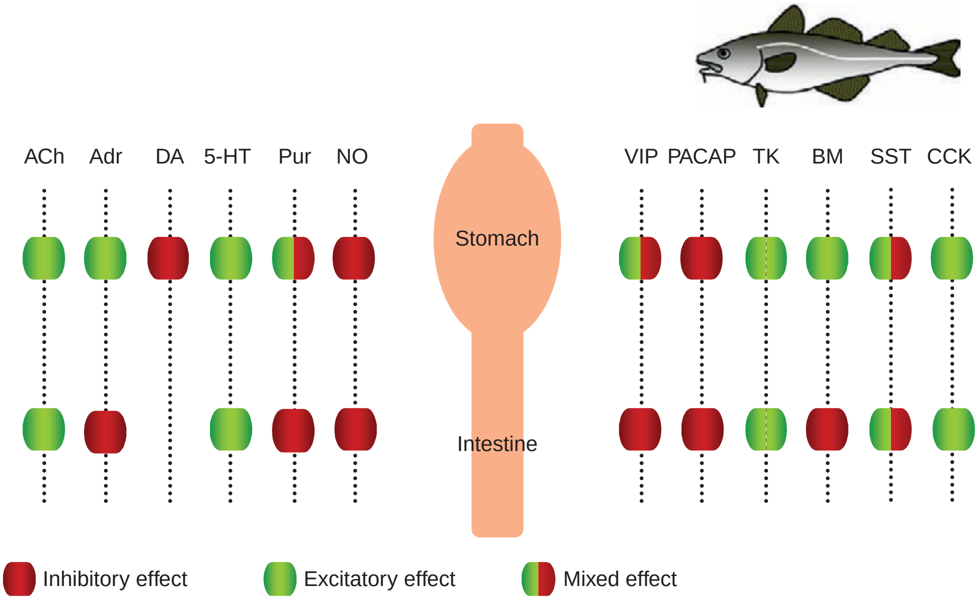


Figure 7 Summary of the effects of individual signaling substances in Atlantic cod (*Gadus morhua*). 5-HT, serotonin (5-hydroxytryptamine); ACh, acetylcholine; Adr, adrenaline; BM, bombesin; CCK, cholecystokinin; DA, dopamine; NO, nitric oxide; PACAP, pituitary adenylate cyclase-activating polypeptide; Pur, purines; SST, somatostatin; TK, tachykinins; VIP, vasoactive intestinal polypeptide.

The effect of catecholamines, like adrenaline and noradrenaline, is mainly extrinsic, via the sympathetic innervation, but electric stimulation of the splanchnic nerves gives variable results depending on species and region. In elasmobranchs, adrenaline usually contracts the stomach and intestine, but relaxes the rectum. In teleosts, splanchnic nerve stimulation and/or application of adrenaline/noradrenaline generally cause contraction of the stomach and relaxation of the intestine in species such as rainbow trout, brown trout, and European eel (*Anguilla anguilla*). However, in Atlantic cod and anglerfish (*Lophius piscatorius*), mixed responses or relaxation occur after adrenergic stimulation. In isolated Atlantic hagfish intestine, adrenaline gives weak and inconsistent effect.

Other signaling substances have been tested including neurotransmitters such as gastrin-releasing peptide (GRP), bombesin, calcitonin gene-related peptide (CGRP) and adenosine, as well as hormones such as cholecystokinin (CCK), somatostatin, histamine, and ghrelin. Results are often less than clear-cut. **Figure 7** summarizes the effects of the signaling substances discussed above on the gut of Atlantic cod, which is probably the best-studied fish species (**Appendix A** (pp. 1232–1267)).

Development of Gut Motility and Control

Young fish larvae mainly depend on internal sources of nutrients (the yolk) and different species start to feed (external feeding) at different stages. Zebrafish larvae have emerged as a model organism for developmental studies on gut motility, not only in fish but also as a more general model for vertebrate development (**see also Gut Anatomy and Morphology**: Development of Fish Gut). Under standard conditions, the larvae usually start to feed at 5–6 days postfertilization (dpf). Gut contractions start at 3 dpf as uncoordinated contractions that soon develop into more rhythmic patterns. At the same time, neuronal control mechanisms apparently begin to be functional with acetyl-choline and nitric oxide being the first to occur and to be involved in the control of contraction frequency. Other transmitters (e.g., various peptides) soon follow.

Integrated Effects on Gut Motility

The coordination of contractions and relaxation in the entire gut depends on the integration of many diverse signals. While the *in vitro* effects of individual neurotransmitters and hormones are relatively well understood, information about the interactions between them and their participation in complex reflex pathways is still limited. Translation to the *in vivo* situation must be done with great caution.

Gastric Emptying Rate

One of the best-studied integrated mechanisms *in vivo* is gastric emptying. In fish this has usually been done by postmortem examinations of the residual gut content, but also more sophisticated methods such as X-ray analysis have been used. The rate of gastric emptying is affected by numerous factors, including extrinsic factors such as meal size, feeding frequency, food composition, and temperature. In Atlantic salmon, larger meal-particle size delays gastric emptying. In rainbow trout, the half-emptying time is approximately 1 day but the lag phase before emptying depends on the dryness of the food. Gut blood flow responses in rainbow trout also depend on the composition of food injected directly into the intestine. As there is a correlation between gut blood flow and intestinal contraction frequency, one can speculate that gut motility is also affected by food composition.

Water temperature also affects gastric emptying rate. When the ambient temperature decreases, appetite, gut motility, digestion rate, and secretion all decrease, thus slowing down the gastric emptying rate. Different species acclimated to different temperatures may, however, show similar gastric emptying rates. Sea bass (*Dicentrarchus labrax*) acclimated to 16 °C, rainbow trout acclimated to 10 °C, and the Antarctic species bald notothen (*Pagothenia borchgrevinki*) living in −1.8 °C all have a half-emptying time of about 24 h. The physiological mechanisms adjusting for this somewhat surprising similarity are unknown.

The gastric emptying rate in fish also greatly varies between individuals and between species. For example, in juvenile lemon shark (*Negaprion brevirostris*), the stomach is emptied after 24 h. Intraspecies variations not only depend largely on food composition but also depend on factors such as position in the hierarchy with dominant individuals showing longer gastric emptying time compared with subordinate individuals.

The intestinal brake is a feedback mechanism that slows down gastric emptying rate even when there is food present in the stomach. This includes contraction of the pyloric sphincter and reduced contraction frequency in the stomach. The trigger of the brake is the amount and composition of food entering the intestine that in turn stimulates endocrine and nervous responses. One of the most important signals in mammals seems to be the release of CCK from endocrine cells in the proximal intestine in response to fat and acid in the proximal intestine. Moreover, in rainbow trout, CCK prolongs gastric emptying time. Other neurotransmitters and hormones have been suggested as potential factors involved in the intestinal brake, with gastrin and serotonin being two strong candidates.

Concluding Remarks

The lack of *in vivo* studies has hampered the knowledge of gut motility patterns in fish, how they are triggered, and propagated. Based on what we know, there are many similarities with mammals. The enteric nervous system is essential for the control of smooth muscle activity but local hormones also play important roles.

See also: **Gut Anatomy and Morphology**: Gut Anatomy. **Integrated Function and Control of the Gut**: Nervous System of the Gut. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Burnstock G (1958) Reversible inactivation of nervous activity in a fish gut. *Journal of Physiology* 141: 35–45.

Burnstock G (1958) The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. *British Journal of Pharmacology and Chemotherapy* 13: 216–226.

Gräns A, Albertsson F, Axelsson M, and Olsson C (2009) Postprandial changes in enteric electrical activity and gut blood flow in rainbow trout (*Oncorhynchus mykiss)* acclimated to different temperatures. *Journal of Experimental Biology* 212: 2550–2557.

Grove DJ and Holmgren S (1992) Intrinsic mechanisms controlling cardiac stomach volume of the rainbow trout (*Oncorhynchus mykiss)* following gastric distension. *Journal of Experimental Biology* 163: 33–48.

Grove DJ and Holmgren S (1992) Mechanisms controlling stomach volume of the Atlantic cod *Gadus morhua* following gastric distension. *Journal of Experimental Biology* 163: 49–63.

Holmberg A, Holmgren S, and Olsson C (2007) Enteric control. In: Finn RN and Kapoor BG (eds.) *Fish Larval Physiology*, pp. 553–572. Enfield, NH: Science Publishers.

Holmgren S and Olsson C (2009) The neuronal and endocrine regulation of gut function. In: Bernier NJ, Van Der Kraak G, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 28: Fish Neuroendocrinology*, pp. 467–512. Burlington: Academic Press.

Holmberg A, Olsson C, and Hennig GW (2007) TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (*Danio rerio)* larvae. *Journal of Experimental Biology* 210: 1084–1091.

Holmberg A, Schwerte T, Fritsche R, Pelster B, and Holmgren S (2003) Ontogeny of intestinal motility in correlation to neuronal development in zebrafish embryos and larvae. *Journal of Fish Biology* 63: 318–331.

Jensen J and Holmgren S (1994) The gastrointestinal canal. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 119–167. Chur, Switzerland: Harwood.

Karila P and Holmgren S (1995) Enteric reflexes and nitric oxide in the fish intestine. *Journal of Experimental Biology* 198: 2405–2411.

Lutz BR (1931) The innervation of the stomach and rectum and the action of adrenaline in elasmobranch fishes. *Biological Bulletin* 61: 93–100.

Olsson C, Aldman G, Larsson A, and Holmgren S (1999) Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* 202: 161–170.

Olsson C and Holmgren S (2001) The control of gut motility. *Comparative Biochemistry and Physiology A* 128(3): 481–503.

Underhay JR and Burka JF (1997) Effects of pH on contractility of rainbow trout (*Oncorhynchus mykiss)* intestinal muscle *in vitro*. *Fish Physiology and Biochemistry* 16: 233–246.

von Euler US and Östlund E (1957) Effects of certain biologically occurring substances on the isolated intestine of fish. *Acta Physiologica Scandinavica* 38: 364–372.

Young JZ (1983) Control of movements of the stomach and spiral intestine of *Raja* and *Scyliorhinus*. *Journal of the Marine Biological Association of the United Kingdom* 63: 557–574.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Gut Secretion and Digestion

Å Krogdahl, A Sundby, and AM Bakke, Norwegian School of Veterinary Science, Oslo, Norway

© 2011 Elsevier Inc. All rights reserved.

Introduction
Secretion
Digestion
Concluding Remarks
Further Reading

Glossary

Chyme Partially digested food particles mixed with digestive secretions.

Digestion The process of solubilization and hydrolysis of ingested nutrients into constituent molecules and elements suitable for transport across the intestinal wall.

Emulsification A process by which lipid and water can be mixed with the aid of emulsifiers, compounds with both lipophilic and hydrophilic areas such as phospholipids and bile acids.

Ligand (*ligare* (Latin): to bind) A molecule that binds to another molecule, such as an enzyme substrate, cofactor or signaling molecule.

Microbiota In this context, the microorganisms that inhabit the gastrointestinal tract, also called microbial flora and microflora.

Secretion The process of releasing compounds from a cell.

Introduction

Digestion is the mechanical and chemical breakdown (enzymatic hydrolysis) of food into smaller components to a form that can be absorbed by the intestinal epithelium. Digestion requires interaction between the dietary nutrients and a range of digestive enzymes and other components, which are secreted from various organs and tissues of the alimentary tract. The components secreted from the stomach, liver (via the gall bladder), and exocrine pancreas are of major importance for enzymatic hydrolysis of complex food polymers, namely proteins, carbohydrates, and fats, into smaller fragments. For proteins and carbohydrates, the final stages of hydrolysis are catalyzed by enzymes located in the brush-border membrane of the enterocytes (**see also Integrated Function and Control of the Gut**: Intestinal Absorption). Vitamins and minerals need to be released from the food matrix and made soluble before they can be absorbed. Any condition impairing interaction between nutrients and digestive components will limit digestion and absorption of the nutrient. Both chemical and physical characteristics of the diet as well as intestinal conditions can influence digestion.

Secretion

Secretion occurs all along the alimentary tract from the mouth to the anus (see **Figure 1**). These secretions are water mixed with mucins and a range of proteins, lipids, and other biologically active compounds. Levels and types of secreted components are specific and optimal for the various regions of the alimentary tract for their various digestive functions. The secreta are also necessary for conveyance and solubilization of nutrients. Mucus aids passage and mixing of chyme, and protects the epithelia against harsh dietary components, including the mucosal surface from its own acid, alkaline, and enzyme secretions. The mucus also protects the entire organism against microbes and chemicals that might be detrimental to its health and well-being. In addition to aiding nutrient digestion and absorption, water and ion secretions are vital for osmoregulation in fish. Mechanical and chemical stimuli derived from the meal induce secretory responses, via humoral and neural signals.

Water and Mucus

Mucus secretions come from mucus-producing cells (**Figure 2**) in the foregut, intestine, liver, and pancreas. Mucus contains water and mucins. Mucins are highly glycated proteins with great water-holding capacity and are produced by specialized epithelial cells located in the mucosal lining all along the alimentary tract (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut). Fish appear to lack salivary glands and instead have salivary cells dispersed among the epithelial cells. The number of mucin-secreting cells varies between the esophageal, gastric, and intestinal sections and so does

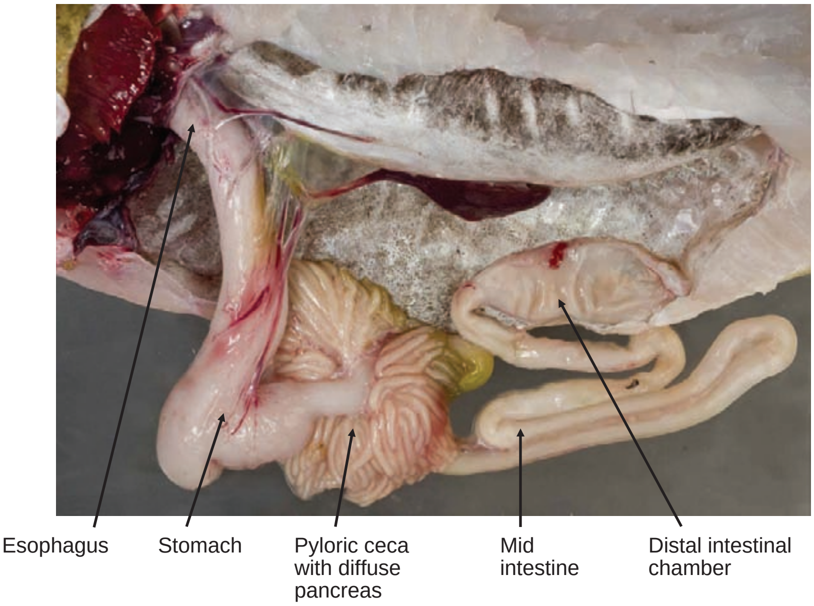


Figure 1 An overview of the gastrointestinal tract of Atlantic cod (*Gadus morhua*) caught in the Oslo fjord. Courtesy of D Griffiths.

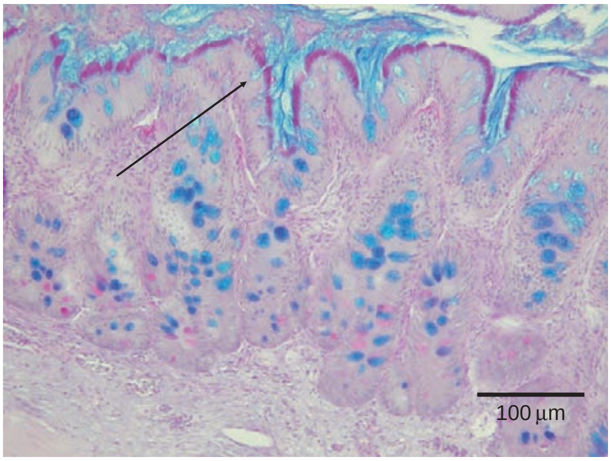


Figure 2 Section from the distal intestine of Atlantic cod stained with alcian blue/periodic acid–Schiff reaction (AB–PAS). The mucin secreted into the lumen and located in the apically situated goblet cells is stained blue, whereas the brush border of the enterocytes and the goblet cells situated deeper in the intestinal folds are stained magenta.

the flow of mucus, from low in the mouth to high in the intestinal regions. The quality of gut mucosubstances can vary with factors such as feeding habits, diet composition, fish species, and developmental stage.

The mucus of the gastrointestinal (GI) tract may also contain immunoglobulins and antimicrobial components such as enzymes and peptides, as well as organic compounds with antioxidative characteristics. Dead cells sloughed off from the surface also add proteins and enzymes to the mucus. The secretion of mucus may be helpful in removing harmful bacterial products by a washout effect. Mucus is kept close to the surface of the intestine by the glycocalyx, which consists of glycoprotein filaments extending from the interior of absorptive cells to the exterior forming a buffering network above the cell surfaces.

Stomach Secretions

In fish species with true stomachs, the central fundic and distal pyloric regions have secretory functions, whereas the most anterior cardiac region does not. Simple and branched gastric glands in the secretory regions are lined with mucus-secreting cells. The so-called oxynticopeptic cells are located at the base of the glands (**Figure 3**) secreting both hydrochloric acid (HCl) and pepsinogen, the precursor of the proteolytic enzyme pepsin. The ultrastructure of the oxynticopeptic cells reflects their dual purpose: apically located tubulovesicular networks secrete HCl, whereas more basally located zymogen granules contain pepsinogen granules. The secreted HCl contributes to the conversion of pepsinogen to pepsin.

Distension of the stomach wall as food enters is the initial stimulus for increased secretion. Following feeding, gastric pH increases rapidly from about 2 to 5 because water and food dilute or buffer the acidic gastric secretions. After feeding, increased gastric acid secretion gradually re-acidifies the stomach contents. The time taken for re-acidification is dependent on meal size. The response of gastric acid secretion to fasting varies among fish species, with most species secreting acid continuously while others periodically cease secretions during fasting. In the latter, stomach pH becomes close to neutral

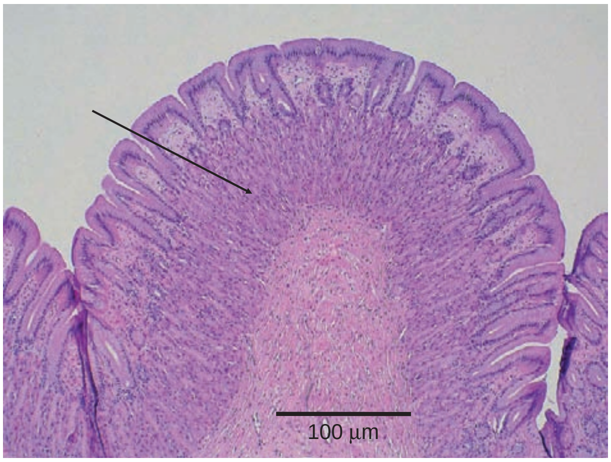


Figure 3 Section (hematoxylin and eosin stained) from the stomach of Atlantic cod. The arrow points to the oxynticopeptic glandular area below the mucosal folds. Courtesy of M Penn.

between meals. Mucus secreted in the stomach has a basic pH creating a thin surface layer protecting the epithelium from acid and proteolysis.

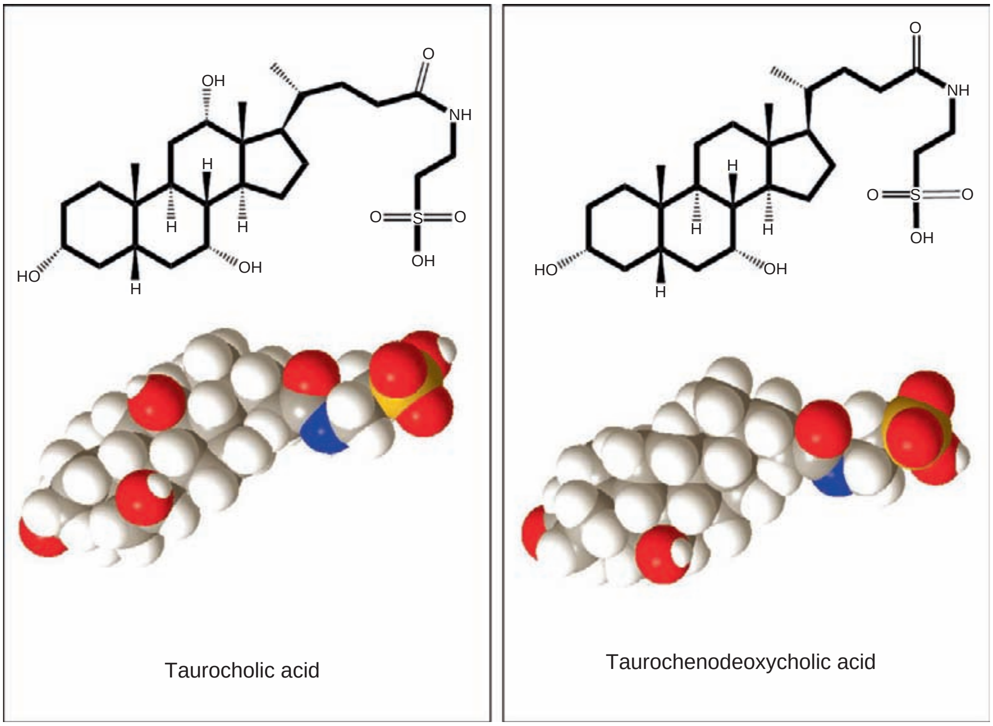
Secretion from the Intestine and Accessory Organs

Chyme enters the intestine and is subsequently mixed with secretions from the intestine and accessory organs, the pancreas and liver/gall bladder. The secretions include electrolytes, especially bicarbonate from the pancreas, liver, and intestine, which are essential to neutralize the acidic pH of the chyme coming from the stomach. This modification of the chemical environment enhances the conditions necessary for the pancreatic and intestinal digestive enzymes to work effectively.

The pH in the intestinal contents of most fishes is in the range from 7.5 to 8.5, which is more alkaline than in mammals. The secreted bicarbonate is also important in osmoregulation in seawater fishes by mediating ion and water absorption in the intestine. The pH may decrease in the distal intestine, most likely due to microbial production of short-chain fatty acids from indigestible carbohydrates.

Bile

Bile is produced by hepatocytes in the liver. It is subsequently released into small liver ducts known as canaliculi that converge and drain into the gall bladder where it is stored. Bile acids are acidic steroids with powerful detergent properties. The primary bile acids, cholic and chenodeoxycholic acids, are formed from cholesterol, and a large proportion of the bile acids are conjugated with taurine to form taurocholic acid and taurochenodeoxycholic acid (**Figure 4**), but some are conjugated with glycine. These primary bile acids may be further modified in the intestinal lumen by bacterial enzymes to form secondary bile acids. The extent and significance of such modifications in fish are largely unknown.



Taurocholic acid

Taurochenodeoxycholic acid

Figure 4 The structure of the bile acid conjugates taurocholic and chenodeoxytaurocholic acid. Courtesy of H Grav.

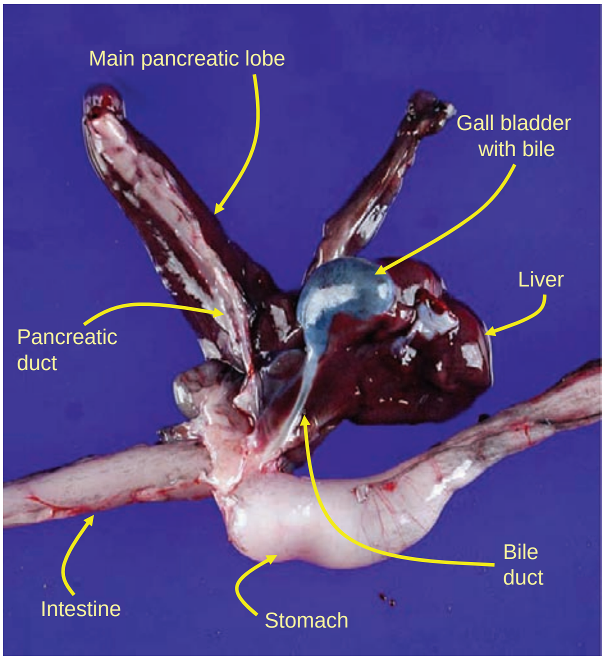


Figure 5 Liver with gall bladder and the main pancreatic lobes in sturgeon. Courtesy of F Dapra.

During fasting, the volume of bile in the gall bladder increases and the bile becomes more concentrated and darker in color (**Figure 5**). After a meal, the gall bladder is typically nearly empty and light in color. The bile acids are presumed mainly to be reabsorbed in the distal regions of the intestine and returned to the liver in the so-called entero-hepatic circulation. Bile acids aid digestion by emulsifying dietary lipids and forming primary micelles, and are necessary for the action of the main lipase in fish. The bile also contains phospholipids and cholesterol, and is a medium for the excretion of many endogenous and toxic waste products that have often been modified and conjugated in the liver to increase their lipid solubility.

Pancreatic juice

Acinar cells of the exocrine pancreas (**see also Gut Anatomy and Morphology**: Pancreas) contain zymogen granules, which store the digestive enzymes produced in the rough endoplasmic reticulum (**Figure 6**). These granules contain proteolytic enzymes: trypsin, chymotrypsin, elastase, and carboxypeptidase A and B; lipases: pancreatic lipase, co-lipase, and phospholipase involved in lipid digestion; carbohydrase: α-amylase for hydrolyzing α1–4 polysaccharides such as glycogen; as well as DNAse and RNAse. Some characteristics of the enzymes are given in **Table 1**. Signals from the gut trigger zymogen granule release into tubules that converge into pancreatic ducts, which are finely structured and numerous in fish such as salmonids with diffusely located pancreatic tissue. These ducts carry the enzymes into the lumen of the pyloric ceca and/or proximal intestine, or into the bile duct(s).

The proteolytic enzymes and co-lipase are stored and secreted as pro-enzymes that need to be activated in the intestinal lumen before they can hydrolyze feed components, whereas the lipases and α-amylase are released in active forms. The cascade of events that leads to activation of the pro-enzymes is initiated by enterokinase secreted from intestinal cells (**Figure 7**). Enterokinase activates trypsinogen to form trypsin, which in turn activates the other pro-proteases.

Pancreatic proteolytic enzymes have been isolated and characterized from several fish species. One enzyme may

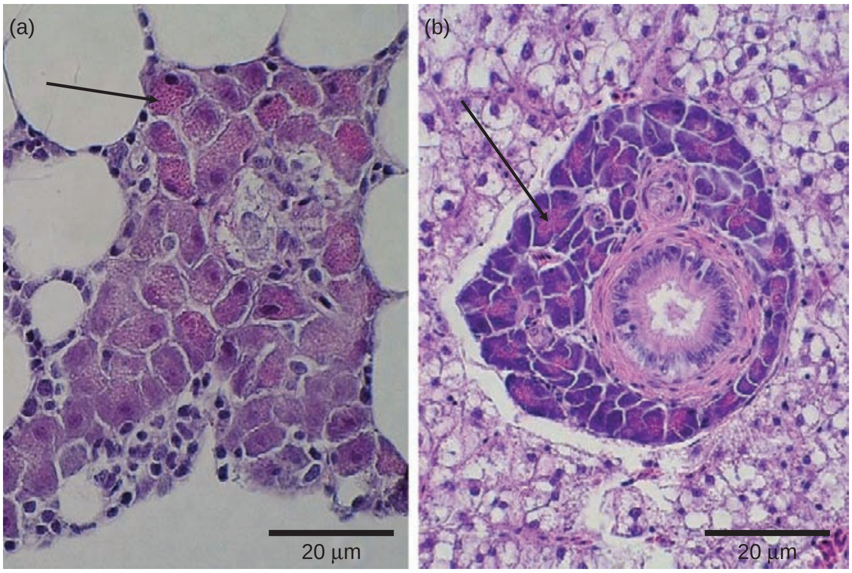


Figure 6 Section (hematoxylin and eosin stained) showing pancreatic tissue embedded in a lipid depot in Atlantic salmon (a) and in liver tissue in the sea bass (b). Courtesy of M Penn.

Table 1 Characteristics of digestive enzymes secreted by the gastrointestinal tract and its accessory organs

Enzyme	*Species investigated*	*Tissue*	*pH optimum*	*Substrate specificity*
Endopeptidases				
Pepsin	Sea bass (*Dicentrarchus labrax*)	Stomach	3	Tyr, Phe, Leu
Trypsin	Atlantic salmon (*Salmo salar*), capelin (*Mallotus villosus*), Atlantic cod (*Gadus morhua*)	Pancreas	7–10	Arg, Lys
Chymotrypsin	Atlantic salmon	Pancreas	7–9	Tyr, Trp, Phe, Met, Leu,
Elastase 1 and 2	Atlantic salmon, spiny dogfish (*Squalus acanthias*), common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*)	Pancreas	7–9	Ala, Gly, Ser
Exopeptidases				
Carboxypeptidase A	Atlantic salmon, Atlantic cod	Pancreas	7–10	Val, Leu, Ile, Ala
Carboxypeptidase B	Atlantic salmon, Atlantic cod	Pancreas	7–10	Arg, Lys
Lipases				
Bile-salt-activated triacylglycerol lipase	Atlantic cod, European turbot (*Psetta maxima*)	Pancreas	7–10	Ester bond in triacylglycerides, esp. in the 1- and 3-positions
Co-lipase-activated triacylglycerol lipase				
Phospholipase A	Sea bream (*Sparus aurata*)	Pancreas	8–9	Phospholipids
Amylase	Atlantic salmon, rainbow trout (*Onchorynchus mykiss*), common carp	Pancreas	4–9	α-1,4-bonds in starch

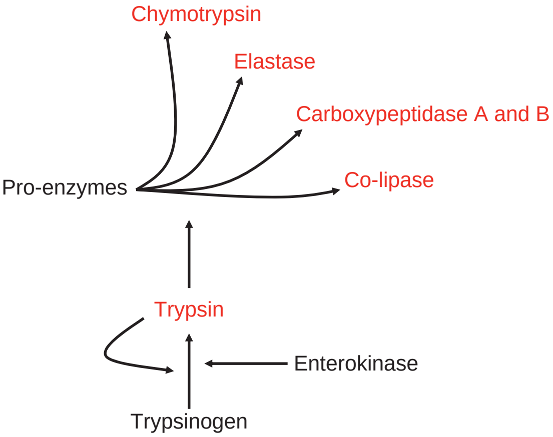


Figure 7 Illustration of activation of pro-enzymes secreted from the pancreas. Enterokinase activates trypsinogen to trypsin, which activates the other pro-enzymes. Trypsin also shows autoactivation.

have several forms in one species, forms that differ in specificity and specific activity. For example, α-amylase has several isoforms in tilapia (*Oreochromis niloticus*). This molecular diversity reflects adaptation to varied but specific feeds and environments. When activities of an enzyme from various species are analyzed at the same temperature, the enzyme from the Antarctic icefish (*Chionodraco hamatus*), for example, is generally more efficient compared to other fishes and endotherms adapted to warmer temperatures. The enzymes act in concert; trypsin, chymotrypsin, and elastase hydrolyze internal peptide bonds in the protein, whereas the carboxypeptidases cut one amino acid at a time from the carboxyterminal end of peptide chains.

The bile-salt-dependent triacylglycerol lipase, with highest specificity for fatty acids in position 1 and 3 of the triacylglyceride molecule, is the dominant lipase in fish. It can also hydrolyze wax esters and phospholipids. Fish also secrete phospholipase A_2, which hydrolyzes cholesterol esters as well as phospholipids, and possibly also the monoglycerides with a fatty acid in the 2 position remaining from the action of the triacylglycerol lipase. Wax-ester hydrolase and cholesterol-ester hydrolase occur in some fish species, but their significance is unknown. The pancreas is the major source of lipases in fish, but the intestinal wall also produces and may release lipases.

The enzyme characteristics differ between species. For example, the pH optimum for α-amylase in six sparid species has been observed to vary between 4 and 9. Atlantic salmon (*Salmo salar* L.) seem to have a defective α-amylase resulting in enzyme activities in the chyme below detection limit when analyzed with starch as substrate. The defect may be related to deletion of a peptide sequence in a substrate-binding site and possibly also an alteration in a sequence important for intracellular protein transport. Chitinase and cellulase activities exist in the GI tract of some fish species, but whether these originate from endogenous sources is debated. However, a chitinase seems to exist in the spiny dogfish (*Squalus acanthias*), suggesting that at least some elasmobranchs can produce chitinase.

Enzymes provided by prey animals may play important roles in nutrient digestion in predatory fish, particularly in larval/juvenile stages. Moreover, microbiota may add to the digestive processes, although microbial activity in fish is lower than in endothermic animals.

Nutrient delivery into the intestine is the most important stimulus of exocrine pancreatic secretion. Diet composition and nutrient digestibility influence secretion of specific enzymes and other factors. For example, the pancreas is stimulated to deliver a juice with higher levels of trypsin by diets containing a high level of protein, protein with low digestibility, and/or components that inhibit proteases, that is, the anti-nutritional factors in some plant feedstuffs known as trypsin inhibitors. A high-fat diet will stimulate higher lipase secretion. The relationship between a high-carbohydrate diet and an α-amylase secretion is more complicated, at least in fish species such as Atlantic salmon.

Digestion

Physical degradation of food can start in the mouth where food is torn apart and crushed by teeth, buccal pads, and/or gill rakers. Ingested food passes quickly to the foregut (**see also Gut Anatomy and Morphology**: Gut Anatomy). In the stomach, muscle contractions secure close contact with enzymes and emulsifiers, which solubilize the main nutrients. Stomach contractions and relaxation of the pyloric sphincter allow chyme to pass into the intestinal tract. Water-soluble components may leave the stomach earlier than fat, and small solid particles leave earlier than larger ones. In stomachless fish, such as common carp (*Cyprinus carpio*), food reaches the intestine before solubilization and emulsification take place to any extent. Some species, such as the gillaro (*Salmo stomachicus*), have a gizzard, a blind muscular sac, which continues the physical breakdown of the food with strong muscular contractions. The proximal intestine and the pyloric ceca, when present, contribute to about two-thirds of total nutrient absorption. However, and in contrast to the situation in mammals and birds, the distal-most and anal regions of fish gut are equipped with enterocytes and brush border, and have absorptive capacity for most nutrients. An overview of nutrient digestion along the digestive tract is given in **Figure 8**.

Newly hatched fish larvae often lack a fully developed GI tract regarding structure and function. They have a simple tube with one loop. All fish may be considered carnivorous and, in the wild, feed on zooplankton until the GI tract is fully differentiated. Maturation progresses at different rates in different species. In some species, such as salmonids and seawolf (*Anarhichas lupus*), the GI tract is well differentiated at hatch, making them capable of eating dry food at first feeding. Most cultivated fish species, however, need

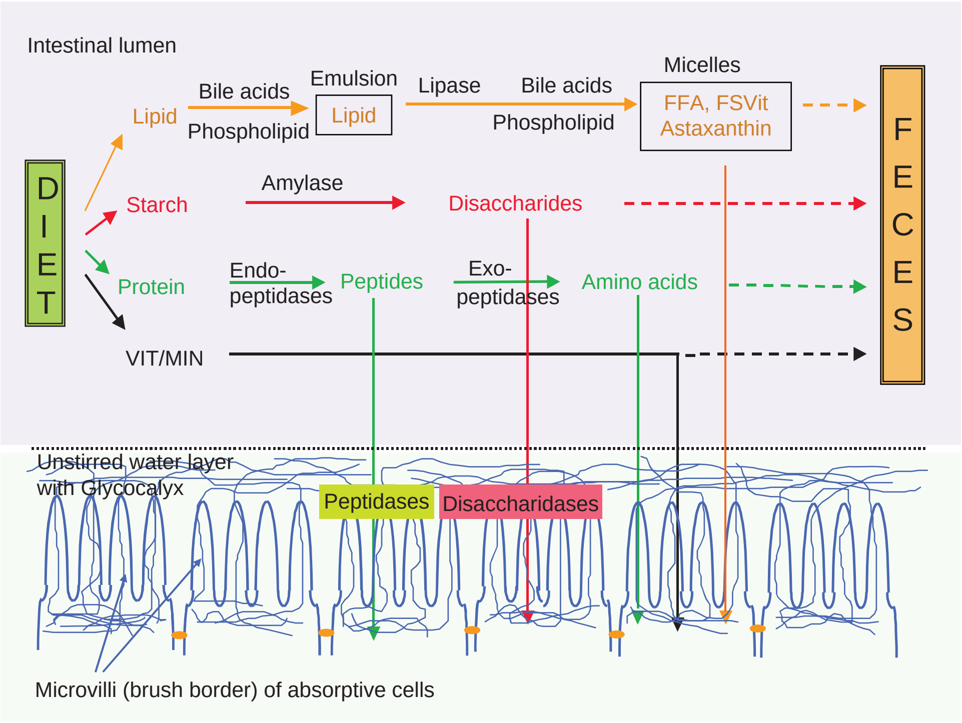


Figure 8 An illustration of digestive processes regarding the main nutrients along the digestive tract of fish. FFA, free fatty acids; FSVit, fat-soluble vitamins (see text for detailed explanation).

live feed the first weeks or months of life. In any case, dietary composition must fulfill the fish's changing nutrient needs and digestive capacity as they develop.

Protein Digestion

Natural prey contain a variety of proteins, as do formulated feeds for fish in cultivation, that vary greatly in aminoacid composition. Their digestibility is influenced by the three-dimensional structure and water solubility. The secretions from the wall of the digestive tract proximal to the intestine and water taken in with the feed moisten the feed to allow initiation of protein digestion. In species with a stomach, proteins are precipitated and hydrolysis is initiated by the action of HCl and pepsin secreted from the stomach wall. Gastric processes yield partially denatured and hydrolyzed protein and peptides in an acid solution, which are emptied into the proximal part of the intestine for further hydrolysis. In stomachless fish, protein digestion is initiated in the proximal intestinal compartment.

The array of proteolytic enzymes present in digestive juices sequentially hydrolyze the proteins and peptides into ever-smaller peptides and free amino acids, until they have molecular sizes suitable for transport across the brush border (see **Figure 8**). The different proteases are characterized according to the position of the peptide bond upon which they act, inside the protein or at the ends, as endo- and exopeptidases, respectively. Enzymes catalyzing the hydrolysis of peptides located at the carboxylic end are called carboxypeptidases, whereas aminopeptidases act on the opposite end. Proteases also show specificity regarding the amino acid at the peptide bond they hydrolyze (see **Table 1**).

Peptides longer than three amino acids are broken down by different classes of peptidases (endopeptidases, aminopeptidases, and carboxypeptidases) localized in the brush-border membrane of enterocytes in the vicinity of the amino acid/peptide transporters of the epithelial cells.

Some proteins entering the intestine either from the food or via the secretions are quite resistant to proteolysis and may be taken up intact by enterocytes by endocytosis in the distal section of the intestine. Intracellularly, they may trigger immune responses that may protect the fish from alien compounds or stimulate hypersensitivity reactions as found in salmonids fed soybean meal. Endogenous proteins are digested later in the digestive processes than food proteins and some, such as enzymes, may be recirculated. The digestive apparatus for protein digestion seems to be regulated according to the protein level of the diet. Protein in most fish food, live prey or formulated, has digestibility in the range of 70–98%. Formulated feeds in general have the lowest values.

Carbohydrate Digestion

Carbohydrates in food for fish in the wild comprise glycogen from the muscle and liver of prey animals, glucose in prey blood, and trehalose and chitin in blood and exoskeleton of crustaceans and insects. Plant sources of carbohydrates, such as phytoplankton, grasses, and algae, ingested by herbivore and omnivore species, may contain some digestible mono- and disaccharides, such as glucose, fructose, and sucrose. However, most carbohydrates in plants eaten by fish are polysaccharides including cellulose, hemicelluloses, alginates, pectins, mucilages, and chitin. Carbohydrates in formulated feeds range from highly digestible mono-, di-, and oligosaccharides to insoluble and indigestible hemicelluloses and cellulose. Starch, in the two forms of amylose and amylopectin, is the main carbohydrate in formulated feed serving both as energy source and as pellet binder. Amylose is a single-stranded molecule with several thousand glucose molecules that form a helix not easily attacked by α-amylase, whereas the amylopectin is highly branched and easily hydrolyzed by the enzyme.

All fish species investigated to date can digest glucose, fructose, sucrose, glycogen, and starch (**Figure 8**). However, most starches are digested in fish only after gelatinization, and amylopectin is more digestible than amylose. Hydrolysis of starch and glycogen is initiated in the proximal intestine by the action of pancreatic α-amylase, producing di- and oligosaccharides. Intestinal α-amylase activity varies greatly among species and is highest in herbivores. Amylase expression has been observed to be regulated according to dietary starch level in some species such as sea bass (*Dicentrarchus labrax*) but not in others (Atlantic salmon and Atlantic cod (*Gadus morhua*)). In many species, the α-amylase activity appears to be the limiting step in starch digestion. Starch digestibilities range from 20% to 99% depending on species, level in the diet, food intake, temperature, etc. Generally, digestibility decreases with increasing starch level, increasing food intake, and decreasing temperature. Herbivorous species digest starch better than omnivores and carnivores, which have lower α-amylase activities and starch digestibilities.

Di- and oligosaccharides are hydrolyzed into their constituent monosaccharides by various brush-border enzymes, such as maltase, sucrase, and trehalase. Brush border maltase activity is often much higher than the other enzymes, 10 times higher in salmonids. Whether expression of these enzymes varies with dietary carbohydrate level of the diet is unclear from the available literature. However, the total capacity can increase by an increase in tissue mass without any change in specific activity or activity per tissue weight. Glucose, galactose, and fructose reach the blood by specific transporters in the brush border and basolateral membrane or by diffusion.

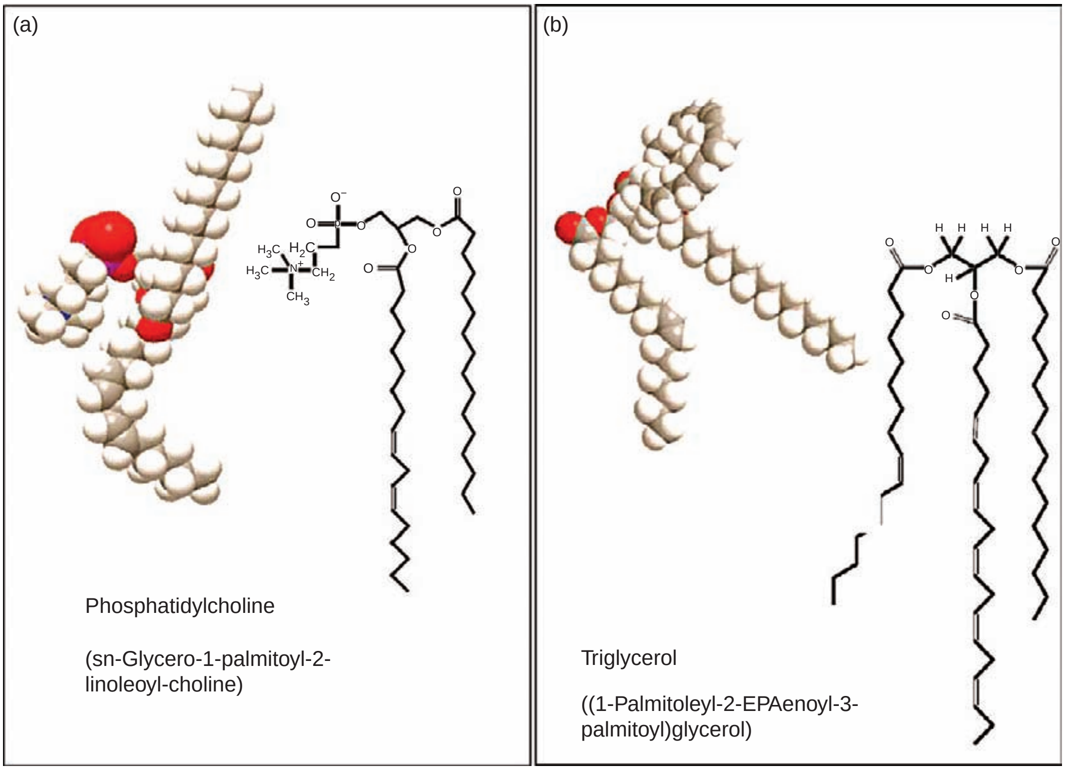
Polysaccharides other than glycogen and starch are not digested by endogenous digestive enzymes. However, some species of the microbiota in the distal intestinal compartments may secrete polysaccharidases that can make some of the energy and the carbon skeleton of undigested plant polysaccharide sources available for the fish. Herbivorous fish species generally show the highest numbers and the greatest species variability of intestinal microbes. However, based on knowledge gained from investigations with grass carp (*Ctenopharyngodon idella*) and other herbivore species, the quantitative importance of plant polysaccharides for growth of fish in the wild is assumed to be low.

Dietary glucose and sucrose are well absorbed by all fish species. However, the capacity for glucose metabolism seems limited in many species. High levels of mono- and disaccharides therefore cause blood glucose and insulin to become elevated and the liver enlarged with glycogen.

Lipid Digestion

The main dietary lipids in fish diets are triglycerides, phospholipids, and wax esters (**Figure 9**). Phospholipids dominate in zooplankton used as larval foods and possibly also in food for herbivorous fish. Wax esters form a heterogeneous group of esters with alcohols other than glycerol and a large range of fatty acids. Waxes may serve as energy storage in prey such as planktonic crustaceans. Triglycerides dominate natural prey of mature carnivorous fish and in most formulated feeds. Fish can hydrolyze and absorb all three categories of lipids. In fish with distinct stomachs, the kneading movements of the stomach muscles, low pH, and solubilization of proteins release lipids from the diet particles. The hydrophobicity of the lipids causes them to aggregate into lipid droplets. Emulsifying compounds, such as phospholipids and certain amphiphilic proteins supplied either from the diet or from fluids secreted from the stomach wall, keep the droplets small and suitable as substrate for lipases present in the intestinal tract. A deficient supply of emulsifiers may cause oil accumulation in the stomach and regurgitation of lipid.

Lingual and gastric lipase are absent in most fish. Hence, in most fish, lipid hydrolysis is initiated when the chyme reaches the proximal intestine and is mixed with bile and pancreatic juice. **Figure 10** illustrates lipid digestion in the intestine. However, in pacu (*Colossoma macropomum*), lipase is present in secretions along the whole length of the GI tract. The bile acids further stabilize the emulsified fat. Free fatty acids, glycerol, and lysophospholipids are the major products of the concerted action of the lipases, although minor amounts of mono- and diglycerides are present in the intestinal



Phosphatidylcholine

(sn-Glycero-1-palmitoyl-2-linoleoyl-choline)

Triglycerol

((1-Palmitoleyl-2-EPAenoyl-3-palmitoyl)glycerol)

Figure 9 Illustration of the main lipid components of fish diets: (a) phospholipid (phosphatidylcholine, lecithin) with palmitic and linoleic acid and (b) triacylglycerols with palmitic (C16:0), eicosapentaenoic (C20:5), and palmitoleic (C16:1) acid. Courtesy of H Grav.

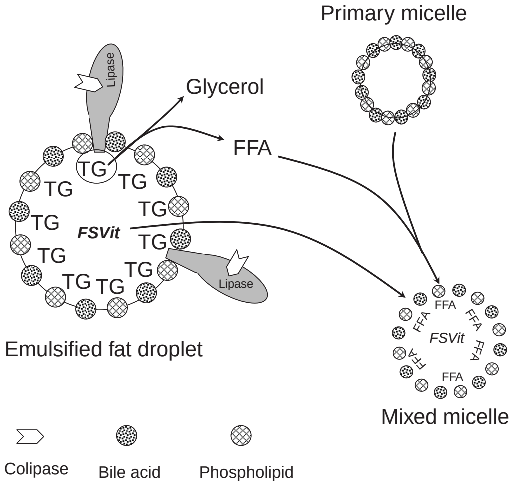


Figure 10 An illustration of the principal steps in lipid digestion in the gastrointestinal tract of fish. BS, bile salts; FFA, free fatty acids; FSVit, fat-soluble vitamins; PL, phospholipid; TG, triglyceride (see text for detailed explanation).

chyme. The long-chain fatty acids that are released are incorporated into primary micelles, which are mixes of bile acids and phospholipids. The micelles absorb the more lipophilic compounds such as fat-soluble vitamins, carotenoids, and cholesterol ester to form secondary micelles. In the vicinity of the brush border, the micelles disintegrate, catalyzed by a descending pH gradient in the glycocalyx causing massive protonation and release of the fatty acids. The short-chain fatty acids, such as C10:0 and C12:0, seem to escape incorporation into micelles and are rapidly absorbed in the proximal part of the intestine. Whether lipase secretion is adjusted to lipid levels of the diet is not clear. Lipid digestibility in fish ranges from 50% to 98% and is highest for marine-based foods.

Vitamins and Carotenoids

Present knowledge on the fate of vitamins along the GI tract in fish is almost nonexistent. However, processes preceding delivery to the intestinal wall for absorption are likely similar to those in mammals. If so, the fat-soluble vitamins A, D, E, and K, as well as carotenoids, follow the emulsified lipid droplets from the foregut to the intestine. As fatty acids are released and incorporated into the micelles to form mixed micelles, these vitamins are picked up by the mixed micelles. As the micelles disintegrate in the unstirred water layer, the vitamins are released close to the brush border. Any condition that interferes with emulsification and micelle formation will interfere with the availability for absorption of fat-soluble vitamins. Chyme conditions such as pH and redox potential affect availability for absorption of water-soluble vitamins.

Minerals

Very little information is available regarding the fate of minerals along the digestive tract in fish, but is likely very similar to that taking place in mammals. Minerals may be present in food in dissociated and solubilized form such as iron in the prey's blood which is bound to a heme molecule. Other minerals are present in the food as solid salts. An example is calcium in the skeleton of prey fish. Formulated feeds are most often supplemented with minerals in solid state. Solubility of mineral salts in digestive juices varies from insoluble to highly soluble depending on the elements involved, crystal structure, and particle size. Solid minerals may become solubilized as a result of the combined action of acid and alkali, digestive enzymes, and intestinal contractions.

Whether an element is absorbed or not also depends on chemical form, valency, and concentration in the chyme. Some elements are inorganic, while others are organically bound and may need to be released from the organic molecule before they can be absorbed. The level in the diet of ligands is very important for mineral availability. Ligands are divalent anions that bind divalent cations such as Ca^{2+}, Fe^{2+}, and Mg^{2+}. Some amino acids bind minerals and make them more available for absorption, whereas other components such as phytate have negative effects on availability for absorption. Antioxidants, for example, vitamin C with the power to reduce the valence state of minerals, affect the availability of minerals for absorption. Vitamin C has positive effects on iron availability but negative for copper. Interactions between minerals in the chyme may also affect availability. For many of the minerals, absorption is regulated according to requirement of the animal by control of carrier-protein production. This is the case for calcium and iron.

Concluding Remarks

Efficient nutrient digestion requires the secretion of a large number of digestive compounds in the right proportions as well as their coordinated conveyance along the digestive tract. Many of the processes and mechanisms are only partially known even for the most-studied fish species. Progress in aquaculture and the need for alternative nutrient sources have created an urgent need for a better understanding of digestive processes in fish.

See also: **Gut Anatomy and Morphology**: Gut Anatomy; Pancreas. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Intestinal Absorption. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Choat JH and Clements KD (1998) Vertebrate herbivores in marine and terrestrial environments: A nutritional ecology perspective. *Annual Review of Ecology and Systematics* 29: 375–403.

Cyrino JEP, Bureau DP, and Kapoor BG (2008) *Feeding and Digestive Functions of Fishes*. Enfield, USA: Science Publishers.

Gelineau A and Boujard T (2001) Oral administration of cholecystokinin receptor antagonists increase feed intake in rainbow trout. *Journal of Fish Biology* 58: 716–724.

Johnson LR (ed.) (2006) *Physiology of the Gastrointestinal Tract*, 4th edn. New York: Academic Press.

Krogdahl Å. and Bakke-McKellep AM (2005) Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology* 141: 450–460.

Krogdahl A, Hemre GI, and Mommsen TP (2005) Carbohydrates in fish nutrition: Digestion and absorption in postlarval stages. *Aquaculture Nutrition* 11: 103–122.

Niot I, Poirier H, Tran TTT, and Besnard P (2009) Intestinal absorption of long-chain fatty acids: Evidence and uncertainties. *Progress in Lipid Research* 48: 101–115.

O'Dell BL and Sunde RA (eds.) (1997) *Handbook of Nutritionally Essential Mineral Elements*. New York: Marcel Dekker Inc.

Olsson C and Holmgren S (2001) The control of gut motility. *Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology* 128: 481–503.

Rucker RB, Suttie JW, McCormick DB, and Machlin LJ (eds.) (2001) *Handbook of Vitamins*, 3rd edn. New York: Marcel Dekker.

Rust MB (2002) Nutritional physiology. In: Halver JE and Hardy RW (eds.) *Fish Nutrition*, 3rd edn., pp. 367–452. San Diego: Academic Press.

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11: 107–184.

Volkoff H, Canosa LF, Unniappan S, *et al.* (2005) Neuropeptides and the control of food intake in fish. *General and Comparative Endocrinology* 142: 3–19.

Wapnir RA and Teichberg S (2002) Regulation mechanisms of intestinal secretion: Implications in nutrient absorption. *Journal of Nutritional Biochemistry* 13: 190–199.

Zambonino Infante JL and Cahu CL (2007) Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: Applications to diet formulation. *Aquaculture* 268: 98–105.

Intestinal Absorption

KS Sundell, University of Gothenburg, Gothenburg, Sweden

I Rønnestad, University of Bergen, Bergen, Norway

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Intestine as a Transporting Epithelium
Transepithelial Transport of Macronutrients
Protein Absorption
Lipid Absorption
Carbohydrate Absorption
Intestinal Ion and Water Transport
Micronutrient Absorption
Regionalization of Intestinal Absorption
Concluding Remarks
Further Reading

Glossary

Agastric An organism without a defined stomach.
Amphipathic A molecule with one hydrophilic and one hydrophobic end.
Apical The tip of something, an apex. An apical membrane of an epithelial cell faces the outer world; in the case of the gut, it faces the lumen.
Basolateral The part of a cell or layer of cells lying closest to the blood supply; faces the internal environment.
Divalent A divalent cation has a net charge of +2.
Electrochemical gradient A difference in concentration and charge between two solutions separated by a semi-permeable membrane, including a plasma epithelial membrane, that produces a net movement of an ion by diffusion.
Endocytosis Cellular ingestion of fluid, substances, or particles through the formation of vesicles of the cell membrane.
Enterocyte Columnar adsorptive cell of the intestinal gut epithelium.
Hyperglycemia High blood sugar concentration.
Lipolytic The enzymatic activity that hydrolyzes lipids into smaller breakdown products.
Macronutrient Nutrients needed in large amounts.
Micronutrient Nutrients needed in very small amounts.
Monovalent A monovalent cation has a net charge of +1.

Introduction

The main function of the fish intestine is to absorb nutrients, ions, and water in order to provide the animal with energy and building blocks for growth and development. For fish in seawater, the important role in fluid absorption results in continuous drinking to ensure a net uptake of water to match water loss across the gills and other epithelia (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). However, in freshwater fishes, this route of fluid absorption is trivial because water is continuously gained across the gills.

The process of absorption means a net transfer of material from the intestinal lumen to the circulation. This is accomplished by the single cell layer of columnar absorptive cells (enterocytes) lining the intestine, the epithelium (**see also Gut Anatomy and Morphology**: Gut Anatomy and **Integrated Function and Control of the Gut**: Barrier Function of the Gut). This article presents the functional morphology and physiology of the intestinal epithelium and its transport mechanisms and further discusses the role of the intestine as a multifunctional transporting epithelium. The article focuses primarily on macronutrient uptake. The specific role of the intestine in ion regulation is further considered in **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation.

The Intestine as a Transporting Epithelium

Substances move across the intestinal epithelium through simple diffusion, facilitated diffusion, or primary and secondary active transport. When a substance follows its electrochemical gradient, the small, uncharged, lipid-soluble molecules diffuse directly across the lipid bilayer (simple diffusion); the larger, charged, water-soluble molecules pass either between the cells or with the help of carrier or channel proteins in the membrane (facilitated diffusion; **Figure 1**).

If the substance is to be moved against its electrochemical gradient, energy is required. This can be

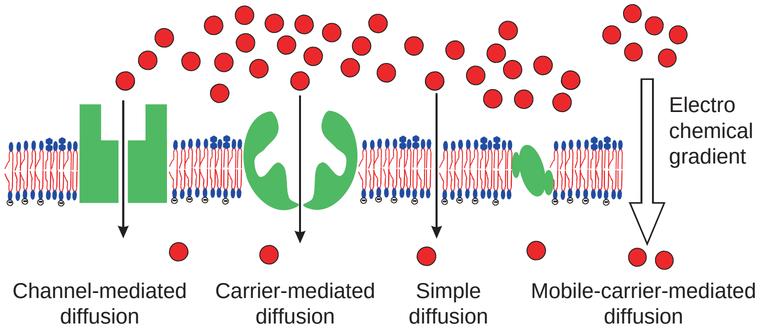

Electro chemical gradient
Channel-mediated diffusion
Carrier-mediated diffusion
Simple diffusion
Mobile-carrier-mediated diffusion

Figure 1 The main pathways for passive diffusion, that is, movement along an electrochemical gradient, of a substance across a lipid bilayer.

obtained through primary active transport, where a membrane-bound transporting protein is linked to an adenosine triphosphatase (ATPase) or through secondary active transport, which is especially important for nutrient uptake. Secondary active transport utilizes energy stored in an existing electrochemical gradient of another substance. The major primary active transporter of the intestinal epithelium is sodium–potassium ATPase (Na^+/K^+-ATPase), which functions as the primary transporter of Na^+ and K^+ and creates a sodium gradient that drives several secondary transports into the cell. It is also electrogenic.

Enterocytes are polarized, which is a prerequisite for the directed transfer of molecules across the epithelium. Polarization means that the plasma membrane, and thereby the whole enterocyte, is divided into two main regions: apical and basolateral. The apical side, which faces the intestinal lumen, has a brush border. Its surface area is greatly enlarged by the presence of many microvilli (**Figures 2** and **3**). The brush-border membrane is separated from the rest of the plasma membrane by tight junctions that create the main barrier for diffusion and prevent lateral diffusion of the transporters along the plasma membrane. The basolateral membrane faces the

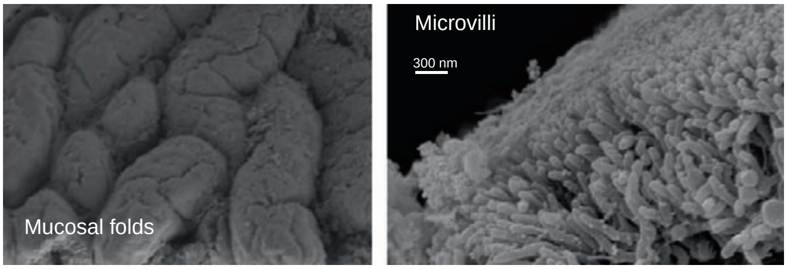


Figure 2 Scanning electron micrographs of intestinal epithelium of Atlantic salmon showing the structure of the mucosa, including mucosal folds and microvilli. Photographs: courtesy of Y Kamisaka and H Kryvi, Department of Biology, University of Bergen, Norway.

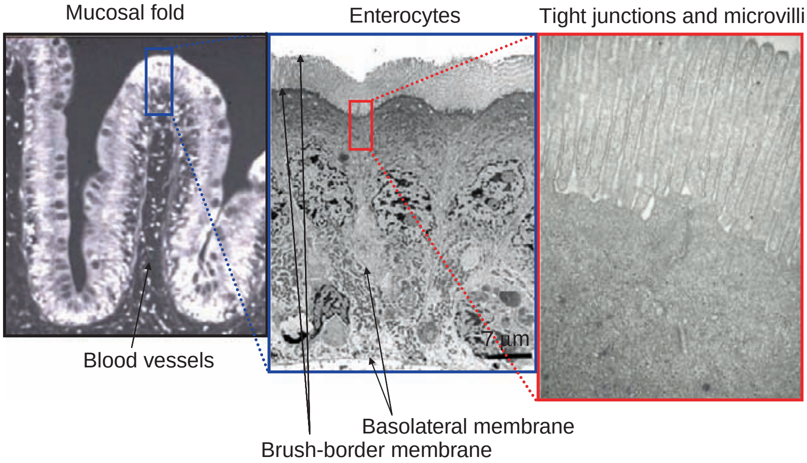


Figure 3 Light and transmission electron micrographs of fish intestinal epithelium showing organization and structure of the intestinal transporting epithelial cell layer.

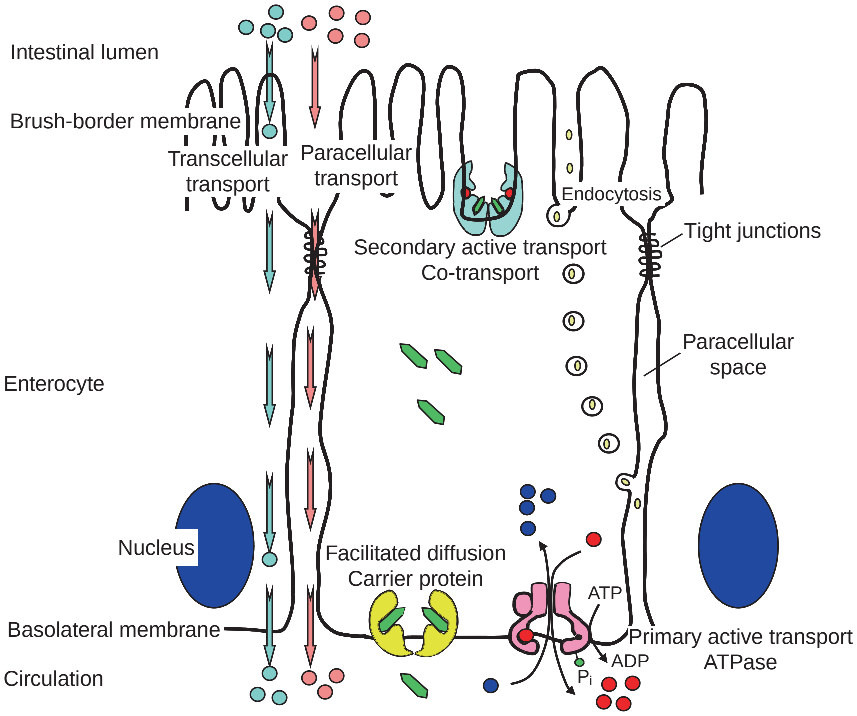


Figure 4 Pathways for absorption across the intestinal epithelium including examples of transporting enzymes inserted in the brush-border and basolateral membranes of the epithelial cells.

neighboring cells and basal membrane, and constitutes the interface to the circulation (**Figures 3** and **4**).

Substances can be taken up via two main pathways: (1) transcellularly through the epithelial cells or, (2) paracellularly, between the epithelial cells passing the tight junctions (**Figure 4**) (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut). Transcellular transport comprises (1) transport across the brush-border membrane, (2) transfer through the cytosol, and (3) transport across the basolateral membrane. All three steps depend on direction and size of the electrochemical gradients as well as on the presence of specific transporters or the diffusion properties of the lipid bilayer. Together, these properties create a highly efficient and selective transport apparatus (**Figure 4**).

Transepithelial Transport of Macronutrients

Macronutrients are the main nutritional components of food: proteins and their digestion products, peptides and amino acids (AAs); lipids and their degradation products such as diacylglycerides, monoacylglycerides, glycerol, and free fatty acids; and carbohydrates.

Depending on species, developmental stage, habitat, and dietary history, the individual macronutrients differ in their importance. Fish are traditionally regarded as glucose intolerant, and in many species AAs are more insulinotropic (inducing insulin release from the pancreas) than glucose. The low and variable ability to digest and utilize carbohydrates as energy source, as well as the apparent hyperglycemia lasting several hours after a glucose load, strengthens the indications that carbohydrates are less important for fish. Thus, in most fish, proteins are usually regarded as the main limiting factor for growth and lipids as the main source of energy.

However, many omnivores and herbivores can utilize dietary carbohydrates, and glucose is indeed an important energy source for certain fish tissues such as the brain. In herbivores, digestion of carbohydrate-rich food often depends on symbiotic microorganisms in the gut, which use anaerobic fermentation to acquire energy for their own use. The fermentation breakdown products are mainly short-chain and volatile fatty (or carboxyl) acids such as butyric, propionic, and acetic acid, which easily diffuse across the epithelium due to their lipid solubility. Of these, only propionic acid can be converted to glucose in the circulation. This and the observations that several herbivorous fish depend on lipid-rich food sources such as diatoms (unicellular phytoplankton) for growth and reproduction suggest that glucose probably does not meet the total energy requirements throughout the life cycle of herbivorous species.

Protein Absorption

Amino Acid Absorption

The smallest breakdown products of the dietary proteins are AAs. After a meal, the concentration of free AAs in the intestinal lumen increases. AA uptake is mainly transcellular, through Na^+-dependent, secondary active, co-transport. Paracellular diffusion may occur if luminal concentrations are very high.

The AA transporters are a diverse group of membrane proteins that share several properties. They typically exhibit broad substrate specificity, so that several AAs may be transported by one type. More than 20 different dietary AAs can be divided into acidic, basic, neutral, and imino acids; in mammals, each group of AAs seems to have its own specific Na^+-dependent co-transporters. Not much is known about the molecular mechanisms of AA transporters in fish, but the four main groups of Na^+-dependent transporters are present in the brush-border membrane of European eel (*Anguilla anguilla*), rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), and the marine bream (*Boops salpa*) (**Figure 3**). Furthermore, sodium-independent AA transporters (i.e., H^+-dependent neutral amino acid brush-border membrane transporters) have been identified in fish and mammals.

Within the absorbing cell, AAs are either metabolized to yield energy for the cell, used in protein synthesis, or transferred unchanged through the enterocyte and into the hepatic portal system for transport to the liver and beyond. Glutamine is a particularly important AA for enterocyte metabolism, serving as a major source of energy for cells in tissues with a high turnover, that is, containing rapidly dividing populations of cells, such as the intestinal epithelium.

AA excretion across the basolateral membrane of the enterocyte occurs through passive diffusion, mainly facilitated by membrane-bound carrier proteins. These require an outwardly directed gradient of AAs from the enterocytes to the circulation. In addition to these Na-independent carrier mechanisms, Na^+-dependent, secondary active transporters (as described above for brush-border membrane) are also found in the basolateral membrane. These transporters carry AA from the circulation into the cell, probably to supply AA to the enterocytes for intracellular energy and protein synthesis between meals and during periods of starvation (**Figure 5**).

Essential AAs generally show a higher uptake rate across fish brush-border membrane than nonessential AAs. Furthermore, the affinities of the Na^+-dependent AA transporters in fish are generally higher than the corresponding mammalian transporter. The higher uptake efficiency of fish is believed to be correlated to their relatively short intestinal length compared with mammals.

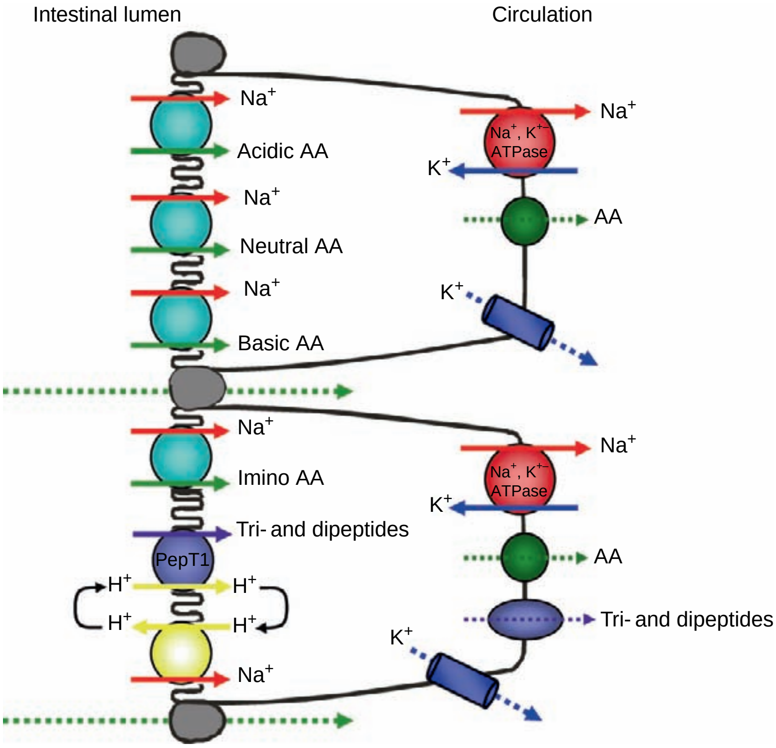


Figure 5 Probable mechanisms for absorption of amino acids and di- and tripeptides across the intestinal epithelium of fish. Pep T1, oligopeptide transporter 1.

Di- and Tripeptide Absorption

Protein digestion into single AAs is not always complete prior to absorption. Recent studies of several vertebrate species suggest that the entry of di- and tripeptides across the apical membrane of the enterocytes is an important route of absorption of digested proteins. Evidence for specific proton-coupled peptide transporters in the brush-border membrane of both mammals and fish is accumulating.

Dietary proteins differ in their AA composition and thus also in the composition and properties of the peptides produced by hydrolysis. The oligopeptide transporter 1 (PepT1) transports di- and tripeptides, and has extremely broad substrate specificity. A detailed analysis of mammalian PepT1 shows that it is a Na^+-independent transport coupled to active proton transport. The mammalian transporter responds to extracellular pH with an optimum of between 4.5 and 6.5, depending on the net charge of the transported substrate. In contrast, the few fish PepT1 characterized to date show increased transport rates with alkaline extracellular pH. After entering the enterocytes, the peptides are normally hydrolyzed to free AAs by cytosol peptidases. However, small peptides that are resistant to hydrolysis may be excreted at the basolateral membrane and enter the systemic circulation (**Figure 5**).

The relative nutritional importance of AA versus di- and tripeptide absorption seems highly variable among fish species with different feeding habits. In salmon, maximal rates of peptide transport, measured *in vitro* (using an everted sleeve method), are low compared with reported transport rates for AAs. At the same time, *in vivo* data suggest that di- and tripeptides are absorbed more rapidly. However, the limiting step for AAs absorption seems to be neither in the AA transporters nor in the di- and tripeptide transporter because the maximal capacity of the transporters is generally higher than the substrate concentrations available in the intestinal lumen.

Absorption of Intact Proteins

Fish enterocytes can also absorb intact proteins by endocytosis (**Figure 4**). This occurs primarily in the posterior intestine, also called the 'hindgut' (**see also Gut Anatomy and Morphology**: Gut Anatomy). The proteins probably bind to a specific receptor at the brush-border membrane that initiates invagination of the membrane, and transport vesicles are formed. Within the enterocytes, the endocytosed vesicles can fuse with lysosomes, whereupon the proteins are degraded by enzymes. The products, peptides and AAs, are released into the cytosol. The vesicles may also be transported through the cytosol, fuse with the basolateral membrane, and secrete the intact proteins into the circulation.

Both larvae and adult fish can absorb intact protein through endocytosis. In larvae of some viviparous fish, the hindgut protrudes and functions as an organ analogous to the mammalian placenta because it can absorb digested nutrients and intact proteins directly from the mother. Absorption of intact proteins in the hindgut may be a general feature in larval fish as it is present in oviparous fish. This mechanism may be related to the lack of a stomach prior to metamorphosis in many fish larvae and therefore absorption and intracellular digestion of intact proteins in the intestine is suggested as an important route that supplies the larvae with AAs. Ultimately, the reported processing rates of proteins in the hindgut are too low to satisfy the high metabolic and anabolic demands for AA of rapidly growing fish larvae, which may in part explain the special nutrient demands of most first-feeding agastric fish larvae. Endocytotic absorption of intact proteins in adult fish has been suggested to be important for recirculation of secreted proteins (e.g., digestive enzymes such as trypsin), absorption of proteins that have not been digested, and, probably most importantly, as a way to present antigens to the mucosal immune system (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut).

Lipid Absorption

Micelle Formation and Importance

Dietary lipids are emulsified by bile salts and form into droplets containing neutral lipids (mainly triacylglycerol) and an outer layer of polar lipids in a ratio of approximately 30:1 (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). This facilitates lipolytic action of digestive enzymes by reducing the tension at the oil–water interface, thereby increasing the concentration of lipids at the interface. Lipases, released from the pancreas, hydrolyze triacylglycerides into monoacylglycerides, diacylglycerides, and free fatty acids. The breakdown products, in turn, form smaller vesicles and interact with bile salts to form mixed micelles. In the mixed micelles, the lipid components are surrounded by taurocholate and other bile salts.

In marine fish, in particular, another important lipolytic pathway exists. These fish eat high amounts of dietary wax esters and triacylglycerides containing high proportions of highly unsaturated fatty acids (HUFAs). The typical marine lipids are mostly hydrolyzed by bile-salt-activated lipases, yielding small breakdown products: glycerol, free fatty acids, and long-chain fatty acids (LCFAs), which are also recruited into bile-salt-containing micelles.

The absorption of the lipid breakdown products can be considered simple as lipid-soluble molecules that can easily dissolve in the lipid bilayer of the brush-border

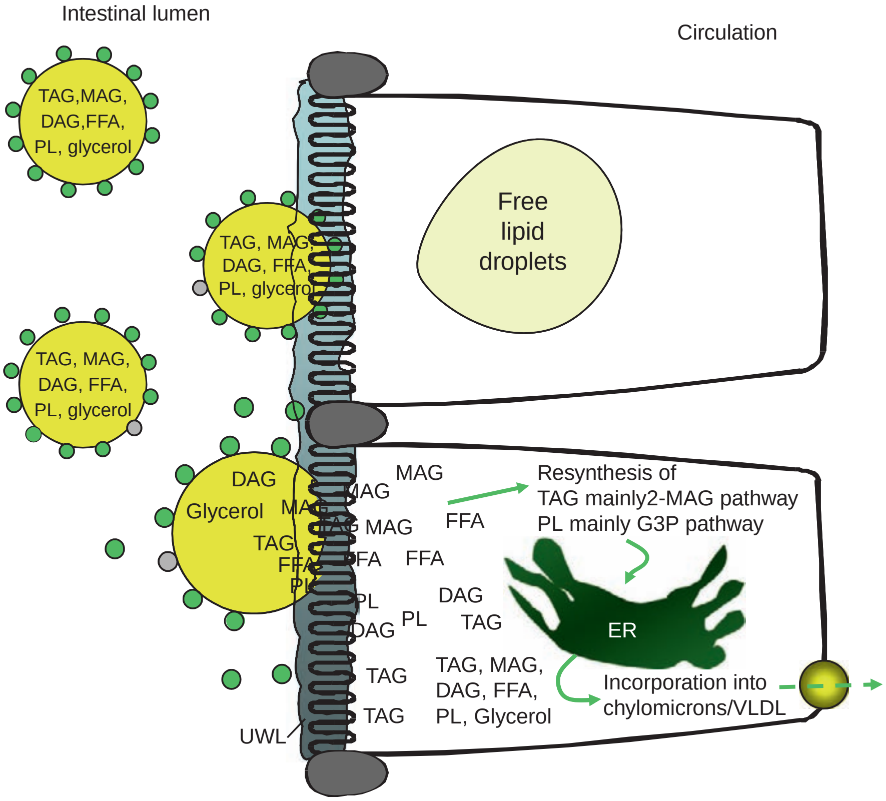

Intestinal lumen
Circulation
TAG,MAG, DAG,FFA, PL, glycerol
TAG, MAG, DAG, FFA, PL, glycerol
TAG, MAG, DAG, FFA, PL, glycerol
Free lipid droplets
DAG
Glycerol
MAG
TAG
FFA
PL
MAG
MAG
MAG
FFA
FFA
FFA
PL
DAG
PL
DAG
TAG
TAG
TAG
Resynthesis of TAG mainly2-MAG pathway PL mainly G3P pathway
ER
TAG, MAG, DAG, FFA, PL, Glycerol
Incorporation into chylomicrons/VLDL
UWL

Figure 6 Intestinal absorption of lipids from micelles in the intestinal lumen. The different lipid components are taken up into the enterocytes and resynthesized to triacylglycerides (TAGs) and phospholipids (PLs) in the endoplasmic reticulum (ER). The newly synthesized lipids are packed together and covered with proteins to form chylomicrons and very low density lipoproteins (VLDLs) that are secreted to the circulation. FFA, free fatty acids; MAG, monoacylglycerides; DAG, diacylglycerides; G3P, glyceraldehyde-3-phosphate; UWL, unstirred water layer.

membrane and enter the cell. However, the cell surface is covered by mucus which binds a lot of water and are creating a so-called unstirred water layer (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut). This layer will constitute a major barrier to lipid absorption. Micelle formation is therefore essential, as it increases the water solubility of the lipids and facilitates the passage through this unstirred water layer (**Figure 6**).

Uptake across the Brush-Border Membrane

Passive diffusion

At the brush-border membrane, the micelles dissociate and the lipid constituents are absorbed into the cell. The dissociation is facilitated by the creation of an acidic environment by Na^+/H^+ exchangers in the brush-border membrane. The subsequent uptake of the lipid components across the brush-border membrane is believed to occur through passive diffusion at high luminal concentrations and via carrier-mediated or active mechanisms at low luminal concentrations. Passive diffusion of fatty acids increases with increasing chain length up to 16 carbon atoms long, as well as with increasing degree of saturation. Thus, LCFAs and HUFAs may depend more on carrier-mediated or active uptake.

Fatty-acid-transporting proteins

In mammals, three main types of facilitating proteins are known for fatty acid absorption: fatty acid translocases, fatty-acid-transporting proteins, and plasma membrane fatty-acid-binding proteins. No fatty-acid-binding or -transporting proteins have been identified to date in the intestinal brush-border membrane of fish, but rainbow trout and Atlantic salmon muscle and liver cells express these proteins. Cytosolic fatty-acid-binding proteins, within the enterocytes, are also of great importance for fatty acid absorption across the intestinal mucosa. The binding proteins will unload the brush-border membrane of fatty acids and thus maintain a high inwardly directed concentration gradient for diffusion of fatty acids into the cell. Similarly, no cytosolic fatty-acid-binding proteins have been demonstrated in fish intestinal cells, although several isoforms exist in muscle, liver, heart, and visceral fat (**Figure 6**).

Intra-enterocytic resynthesis

The mechanism of transfer of lipids and fat products further into the circulation appears to differ in one aspect between mammals and fish. In mammals, the shorter fatty acids (<12 carbon atoms) can be transferred directly across the basolateral membrane into the lymphatic system from which they are eventually emptied into the systemic circulation. However, most studies suggest that fish do not possess a true lymphatic system. Instead, they have a so-called secondary circulation (**see also Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy), but this is not present in the intestine. Thus, there seems to be no pathway for a direct transfer of short-chain lipid products into a secondary circulation in fish.

Considering all LCFAs in mammals and possibly all lipid products in fish, a resynthesis of triacylglycerides and polar lipids in the endoplasmic reticulum of the enterocytes is required for lipid absorption. Two main pathways for lipid resynthesis have been described: the 2-monoacylglycerides pathway mainly leads to triacylglyceride reconstitution, while the glycerol-3-phosphate pathway yields both triacylglycerides and phosphatidylcholine. Both pathways are present in fish enterocytes, but the 2-monoacylglyceride pathway appears to be dominant.

Following absorption and resynthesis, triacylglycerides and phosphatidylcholine are mainly incorporated into large particles called ‘chylomicrons’ and/or very low density lipoproteins that contain a core of triacylglycerides surrounded by phosphatidylcholine, cholesterol, and apolipoproteins. Assembly into large, protein-covered particles increases water solubility, thus facilitating blood lipid transport to other target tissues for energy utilization and storage (**Figure 6**).

Carbohydrate Absorption

Dietary carbohydrates are mainly absorbed as monosaccharides. The fish intestine can absorb a range of these monomers, including glucose, fructose, galactose, and the breakdown product of chitin, *N*-acetyl-glucoseamine. In mammals, the monosaccharides glucose and galactose are taken up across the brush-border membrane through secondary active co-transport by the sodium-dependent glucose transporter, SGLT1. Fructose is transported by a member of the facilitative glucose transporter family, GLUT5 (**Figure 7**). In addition, during high carbohydrate loads another protein from this family, GLUT2, is expressed in the brush-border membrane, facilitating entry of monosaccharides into the enterocytes. After transfer through the enterocyte, the monosaccharides cross the basolateral membrane via facilitated diffusion through GLUT2, which is constitutively expressed in this membrane.

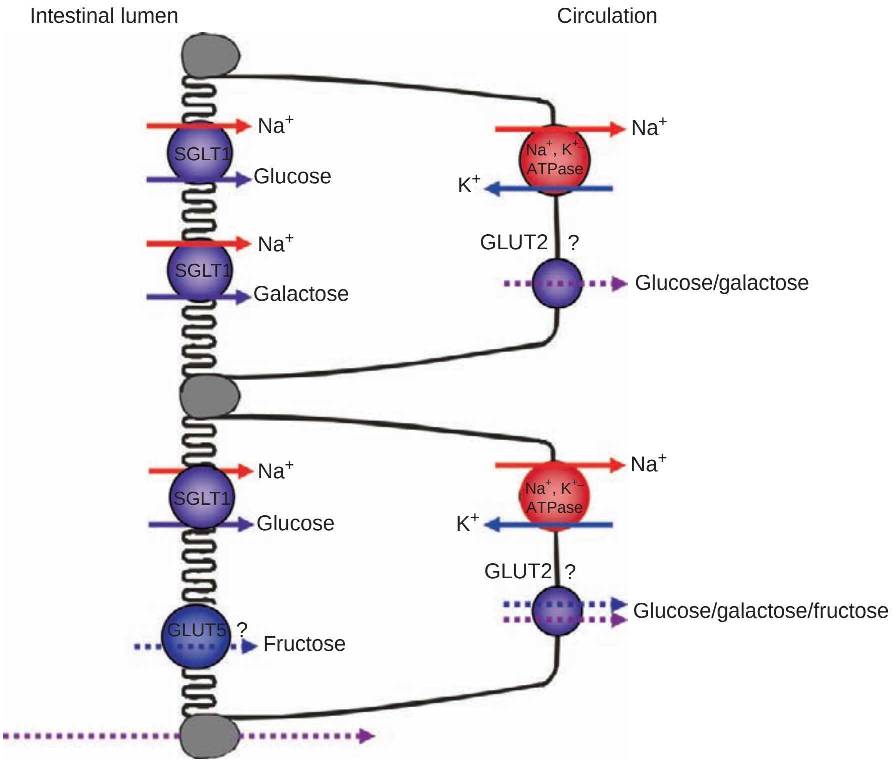


Figure 7 Probable mechanisms for absorption of monosaccharides across the intestinal epithelium of fish. SGLT, sodium-dependent glucose transporting protein; GLUT, glucose transporter.

The entry of glucose and galactose across the brush-border membrane of the fish intestine is SGLT1 driven in all species examined to date. Several members of the GLUT family, including GLUT2 and GLUT5, have been demonstrated in the intestine of several fish species, but their precise location and mechanisms are unknown. However, the mechanisms of carbohydrate absorption are believed to be very similar to those described in mammals (**Figure 7**).

Intestinal Ion and Water Transport

As stated earlier in this article, the gastrointestinal tract is a key organ not only for food digestion and nutrient uptake, but also for regulation of ion and water uptake and maintenance of homeostasis. In seawater, the intestine absorbs ions to create an osmotic gradient that in turn creates an inwardly directed water flux. The main driving force is the same as for the uptake of nutrients, that is, the Na^+ gradient created by Na^+/K^+-ATPases in the basolateral membrane. According to the present model for intestinal ion-coupled water transport, apically located $Na^+/K^+/2Cl^-$ co-transporters drive the uptake of K^+ and Cl^- against their gradients by coupling to the inwardly directed Na^+ gradient. Basolaterally located chloride (cystic fibrosis transmembrane conductance regulator (CFTR)) and potassium channels then permit these ions to flow along their electrochemical gradient into the paracellular spaces between the enterocytes. In combination with the Na^+/K^+-ATPases, which are located in the intestine mainly in the lateral parts of the basolateral membrane, with a higher density toward the tight junction area, creates an osmotic gradient. The highest osmotic concentration in the gradient faces the tight junctions and this osmotic gradient is the driving force for the inwardly directed water flux (**Figure 8**). From this perspective, the presence and possible role of aquaporins, that is, water channels, in the apical and brush-border parts of the enterocyte plasma membrane, are of greatest interest. Indeed, recent research has positively demonstrated the messenger RNA (mRNA) expression of at least four

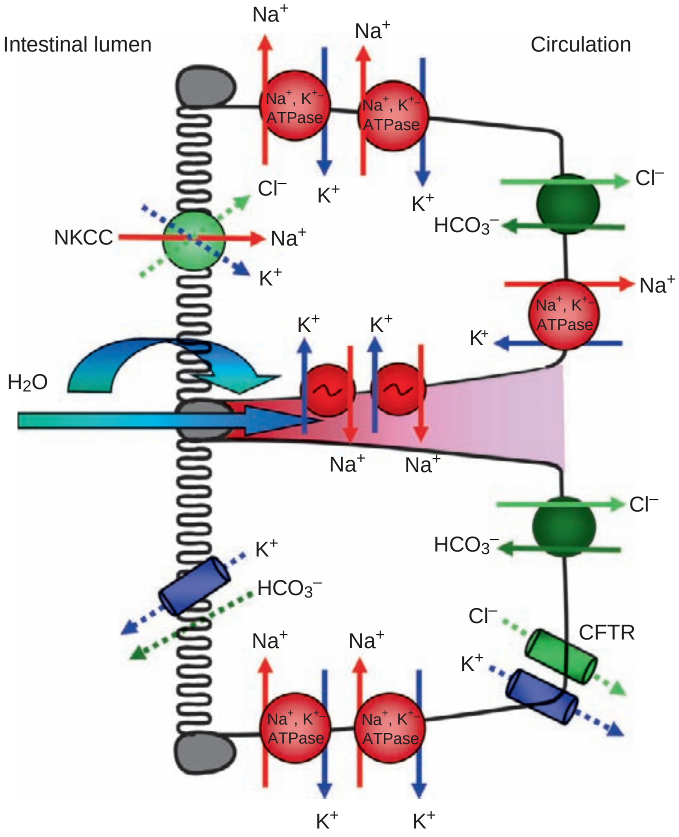


Figure 8 Mechanisms for intestinal fluid uptake. Active transport and passive flow of monovalent ions across the brush-border and basolateral membranes of intestinal cells. The ion transport creates a high salt gradient in the intercellular spaces between the intestinal cells. This gradient passively attracts water that flow across the tight junctions and/or through the cells, creating a flow of water from the intestinal lumen to the circulation. NKCC, sodium, potassium, chloride co-transport; CFTR, cystic fibrosis transmembrane conductance regulator.

different isoforms of the aquaporin family in enterocyte plasma membranes of Atlantic salmon (*Salmo salar*), Japanese eel (*Anguilla japonica*), European eel (*A. anguilla*), sea bass (*D. labrax*), and the gilthead sea bream (*Sparus aurata*), though the exact role of these water channels in the intestinal water transport remains to be elucidated (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin).

Micronutrient Absorption

In addition to monovalent ions, several divalent ions and minerals are of great importance for the physiology and performance of the fish and are thus essential for the organism. These ions are often needed in minuscule amounts, and are called 'micronutrients'. Typical micronutrients such as calcium, phosphorous, zinc, selenium, iron, copper, and iodine, as well as vitamins are necessary for the production of enzymes, hormones, and other substances essential for growth and development. Most micronutrients are absorbed by active mechanisms in the intestine but for Ca^{2+} the gills are also an important tissue with primary active transport through Ca-ATPases present in the chloride cells (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes).

Regionalization of Intestinal Absorption

Intestinal absorption starts immediately after the pyloric sphincter, in the first region of the intestine that also possesses the surface-enlarging pyloric ceca (**see also Gut Anatomy and Morphology**: Gut Anatomy). An absorption gradient is present along the length of the intestine of all fish. Water-soluble nutrients, apart from full-sized proteins, are mostly absorbed in the pyloric ceca and the midgut. Gene expression studies and protein identification methods such as immunohistochemistry have shown that the same types of membrane-bound transporters are present in the pyloric ceca as in the rest of midgut. The pyloric ceca contribute, in a surface-area-dependent way, to the absorption of AAs, di- and tripeptides, as well as monosaccharides. Thus, the more numerous and larger the pyloric ceca are, the larger the surface area and the larger the percentage of nutrient absorption that takes place in these projections. This correlates well in both carnivores and herbivores. In fish with

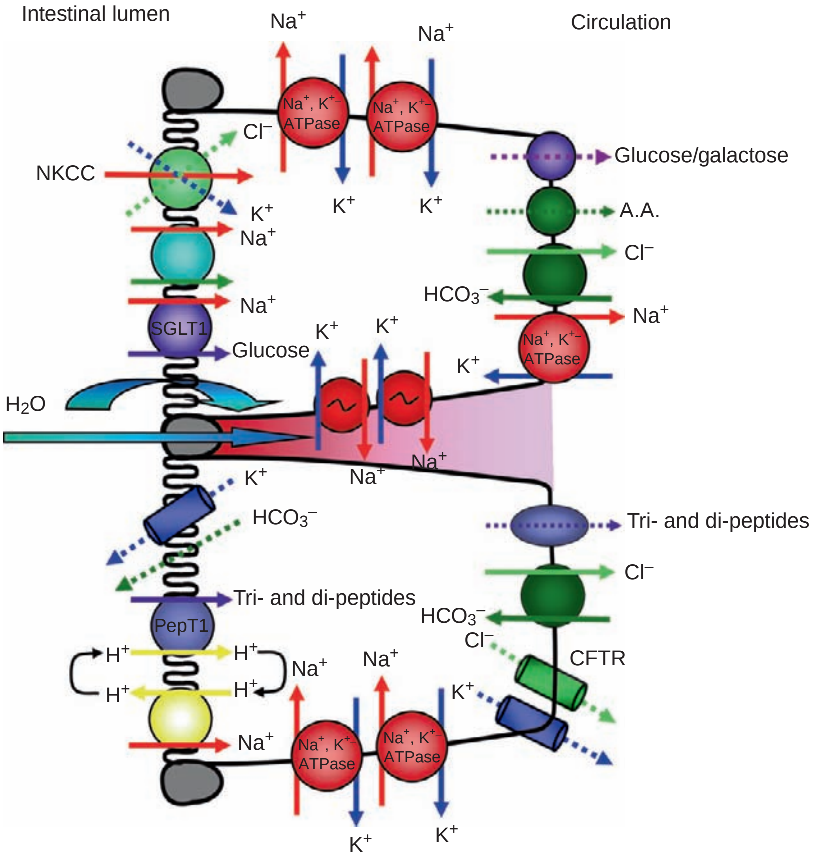


Figure 9 Summary picture describing the ion- and water-soluble nutrient-transporting mechanisms of intestinal cells.

numerous pyloric ceca, such as the salmonids, the absorption of glucose, AAs, and di- and tripeptides in this region can reach 65–80% of total nutrient absorption. Endocytotic transport of intact proteins, on the other hand, mostly occurs in the hindgut.

The absorption of fatty acids partly follows the same regional uptake pattern as water-soluble nutrients. However, there are indications of a regionalization depending on fatty acid chain length; medium-chain fatty acids are mainly absorbed in the pyloric ceca, while LCFAs are absorbed in the midgut. Furthermore, intracellular lipid droplet accumulation, which has been observed in several studies, usually takes place in the pyloric ceca and proximal parts of the intestinal tract.

In many marine species, ion and water absorption along the digestive tract starts in the esophagus. At first, water diffuses from the circulation to the esophageal lumen to dilute the high-salinity seawater, causing a major loss of water from the circulation. The diffusion of water continues to some extent in the stomach and when the ingested water enters the intestine its salinity is reduced to 30–50% of that of the ingested seawater. In other species, such as the European and Japanese eel in particular (but also in the Atlantic cod and several marine flounder species), the esophagus is only slightly permeable to water and instead there is an active uptake of Na^+ and Cl^- in this region of the gut. This reduces the flow of water from the circulation as most desalinization takes place through the active absorption of Na^+ and Cl^-.

In the intestine, Na^+ and Cl^- absorption is active; it creates the osmotic gradient that passively attracts water flux and creates an inwardly directed flow of fluid. This active ion-coupled fluid uptake is higher in the more proximal parts of the intestine. Active and passive uptake of divalent ions also takes place along the length of the intestine, but to a much lower extent than for Na^+ and Cl^-. The concentration of monovalent ions in the lumen thus falls along the intestinal axis, more rapidly at the beginning, while the concentration of several of the divalent ions increases (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation).

Concluding Remarks

The fish intestine is the unique example of the tissue with a constant trade-off between optimal capacity for osmoregulation and nutrient uptake (**Figure 9**). Increased demands for osmoregulation can reduce energy utilization for growth and performance and vice versa. A basic understanding of the physiological mechanisms underlying transport functions and of factors that influence and regulate this balance is of fundamental scientific interest. This is also important in applied aquaculture situations, where it could lead to improved food conversion ratios and energy utilization, resulting in better development, growth, and performance of the fish.

See also: **Design and Physiology of Capillaries and Secondary Circulation**: Secondary Circulation and Lymphatic Anatomy. **Gut Anatomy and Morphology**: Gut Anatomy. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Gut Secretion and Digestion. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Water Balance and Aquaporin. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Buddington RK and Diamond JM (1987) The pyloric cacea of fish: A "new" absorptive organ. *American Journal of Physiology* 252: G65–G76.

Buddington RK, Krogdahl Å., and Bakke-Mckellep AM (1997) The intestine of carnivorous fish: Structure and functions and the relations with diets. *Acta Physiologica Scandinavica* 638: 67–80.

Clements KD and Raubenheimer D (2006) Feeding and nutrition. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn, pp. 46–82. New York: Taylor and Francis.

Collie NL and Ferraris RP (1995) Nutrient fluxes and regulation in fish intestine. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes,* vol. 4, pp. 221–239. Amsterdam: Elsevier.

Ganapathy V, Brandsch M, and Leibach FH (1994) Intestinal transport of amino acids and peptides. In: Johnston LR (ed.) *Physiology of the Gastrointestinal Tract*, pp. 1773–1794. New York: Raven.

Goncalves AF, Filipe L, Castro C, *et al.* (2007) Is there a compromise between nutrient uptake and gas exchange in the gut of *Misgurnus anguillicaudatus*, an intestinal air breathing fish? *Comparative Biochemistry and Physiology D* 2: 345–355.

Jutfelt F, Olsen RE, Björnsson BTh, and Sundell K (2007) Parr–smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture* 273: 298–311.

Larsen EH, Mjöberg N, and Nielsen R (2007) Application of the Na^+recirculation theory to ion coupled water transport in low- and high resistance osmoregulatory epithelia. *Comparative Biochemistry and Physiology A* 148: 101–116.

Leaver M, Bautista JM, Björnsson BT, *et al.* (2009) Towards fish lipid nutrigenomics: Current status and prospect for finfish aquaculture. *Reviews in Fisheries Sciences* 16: 73–94.

Lee VHL (2000) Membrane transporters. *Pharmaceutical Sciences* 11: S41–S50.

Loretz CA (1995) Electrophysiology of ion transport in teleost intestinal cells. In: Wood CM and Shuttleworth TJ (eds.) *Fish Physiology Vol. 14. Cellular and Molecular Approaches to Fish Ionic Regulation*, pp. 25–56. San Diego, CA: Academic Press.

Ringö E, Ström E, and Tabacheck JA (1995) Intestinal microflora of salmonids: A review. *Aquaculture Research* 26: 773–789.

Scott GR, Schulte PM, and Wood CM (2007) Plasticity of osmoregulatory function in the killifish intestine: Drinking rates, salt and water transport, and gene expression after freshwater transfer. *Journal of Experimental Biology* 209: 4040–4050.

Sundell K, Jutfelt F, Agustsson T, *et al.* (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr–smolt transformation of Atlantic salmon, *Salmo salar. Aquaculture* 222: 265–285.

Tipsmark CK, Sorensen KJ, and Madsen SS (2010) Aquaporin expression dynamics in osmoregulatory tissue of Atlantic salmon during smoltification and seawater acclimation. *Journal of Experimental Biology* 213: 368–379.

Tocher DR, Bendiksen EÅ., Campbell PJ, and Bell JG (2008) The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture* 280: 21–34.

Tso P, Nauli A, and Lo CM (2004) Enterocyte fatty acid uptake and intestinal fatty-acid binding protein. *Biochemical Society Transactions* 32: 75–78.

Verri T, Romano A, Barca A, *et al.* (2009) Transport of di- and tripeptides in teleost fish intestine. *Aquaculture Research* X: 1–13.

Barrier Function of the Gut

F Jutfelt, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Epithelium
The Intestinal Barrier
The Barrier Function of Different Intestinal Regions
Translocation of Pathogens across the Barriers
The Barrier in Stress and Disease
Species Differences
Concluding Remarks
Further Reading

Glossary

Apical The tip of something, an apex; an apical membrane of an epithelial cell faces the outer world; in the case of the gut, it faces the lumen.

Basal The side of the epithelium (or epithelial cells) facing the circulation.

Commensal bacteria The normal nonpathogenic bacterial flora.

Enterocyte Columnar adsorptive cell of the intestinal gut epithelium.

Epithelium Relatively flat tissue (composed of one or a few cells layers) that lines cavities and surfaces in multicellular organisms.

Goblet cell A type of mucus-secreting cell in the epithelium.

Lamina propria A layer of connective tissue, vessels, and immune cells under the gut epithelium.

Microvilli Plasma membrane-bound, cellular extensions that often characterize epithelial cells. Provide for an elaboration of the surface, plasma membrane. Common, for instance, in intestinal cells (enterocytes) that absorb nutrients.

Mucosal fold Surface-enlarging structure of the epithelium, sometimes referred to as villi.

Oral tolerance The ability of the gut immune system to avoid reaction to harmless antigens.

Tight junction Protein complexes that close the space between enterocytes.

Introduction

The gastrointestinal lumen contains microorganisms, some that are pathogenic, and toxins that would kill the fish if allowed to enter the circulation. What the fish needs, therefore, is a protective barrier to keep unwanted agents out, yet minimize the interference with other intestinal epithelial functions, such as nutrient, ion, and water uptake. This article is an overview of how the multiple functional layers of barrier protect the fish and it discusses factors that can affect these functions. Most research has focused on the barrier functions of the intestine and less on the esophagus and stomach. Moreover, the research has focused on a very few teleost species, and even fewer nonteleost fish. We present a consensus view for the teleost intestine, with additional examples from other fish groups when available.

The Epithelium

The epithelium is the cell layer that marks the border between the outside (lumen) and inside (tissue) of the animal (**Figure 1**). The epithelium typically consists of a monolayer of epithelial cells linked at their apical ends by attachment proteins. The cytoskeletons of the epithelial cells are attached to adjacent cells by desmosomes and cadherins. Tight junctions keep the cell membranes of neighboring cells in close contact and reduce diffusion through the paracellular space (**Figure 2**). Despite the physical barrier for diffusion across the epithelium, numerous substances and particles can cross the epithelium. Ions and hydrophilic nutrients are generally actively taken up by membrane-bound transport proteins, while lipophilic substances diffuse passively over cell membranes. Small hydrophilic molecules diffuse across the epithelium via the paracellular route, that is, through tight junctions and the paracellular space. Larger hydrophilic molecules, particles, bacteria, and viruses are mainly transported by active transcytosis (endocytosis at the apical membrane and exocytosis at the basolateral) through epithelial cells (**Figure 2**), because they are too large to pass through tight-junction pores.

The Intestinal Barrier

The intestinal epithelial barrier can be divided into the extrinsic, intrinsic, and immunological barriers.

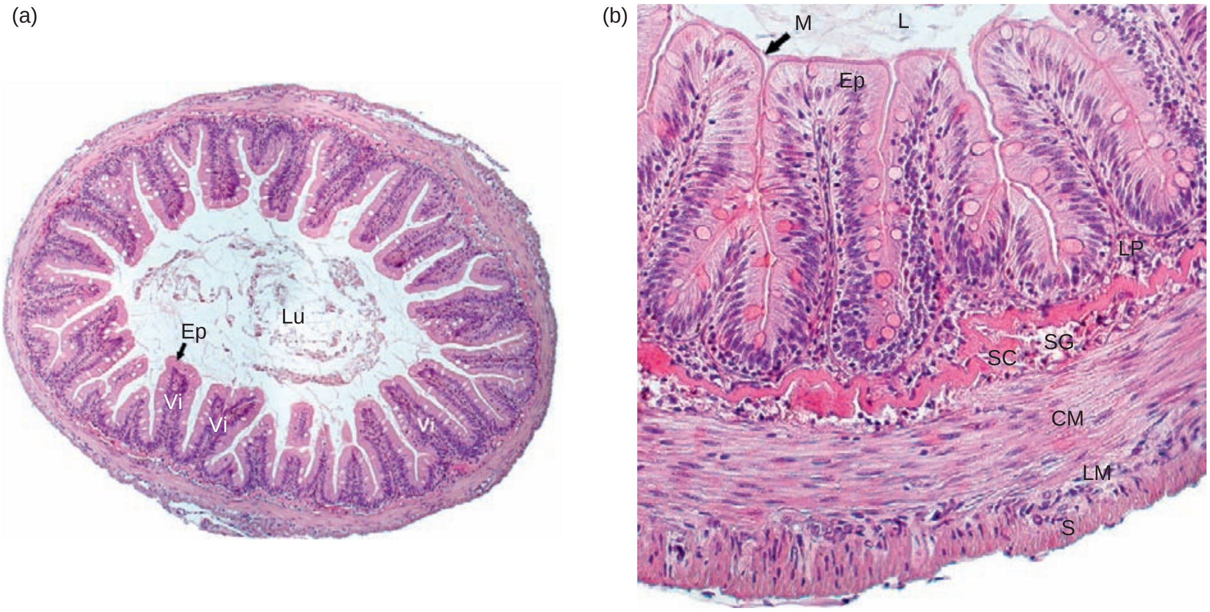


Figure 1 (a, b) Cross section of the proximal intestine in Atlantic salmon (*Salmo salar*) stained with eosin–hematoxylin. Note the numerous mucosal folds (villi) (Vi), lumen with luminal contents (Lu) and the epithelium (Ep) lining the mucosal folds. (b) Mucus (M), stratum compactum (SC), stratum granulosum (SG) with mast cells, circular muscle (CM), longitudinal muscle (LM), and serosa (S). Per Fahlberg and F. Jutfelt.

Extrinsic Barrier

Pathogens entering the gut face a number of obstacles. In the stomach, hydrochloric acid is secreted, killing most microorganisms that enter the gut. In the fish intestinal lumen, commensal bacteria numbering roughly 10^5–10^8 cells ml^{-1} (compared to 10^{12} in mammals) inhabit every ecological niche and reduce the growth of pathogenic bacteria by competing for nutrients and microhabitat resources. Commensals also inhibit the growth of other microorganisms, including pathogens, by secreting bactericidal substances, such as peptide bacteriocins, hydrogen peroxide, and lactic acid. The host tolerates commensal bacteria for their role in protection against pathogens and possible nutritional benefits using regulated interactions with the immune system. However, if the epithelial barriers fail, opportunistic commensal microorganisms infect the host.

The epithelium also has its own secretions to counter pathogens and toxins. Mucus, a viscous fluid containing mucin glycoproteins, is secreted by goblet cells (**Figure 3**). The continuous flow of mucus from the epithelium into the lumen physically removes harmful agents from the epithelium as well as protects the epithelium from mechanical wear. The mucins contain mimics of cell surface receptors to which bacterial adhesion factors attach, after which the bacteria are transported with the flow of mucus away from the epithelium. Viruses, however, are not blocked, but can diffuse through the mucus gel. Antimicrobial factors, such as antimicrobial peptides, reactive oxygen species, and hydrogen peroxide, are secreted into the lumen along with the mucus. Intestinal goblet cells contain antibacterial peptides, at least in some fish species, suggesting that the epithelial surface can be additionally protected by these peptides (**see also The Skin:** Hagfish Slime).

Intrinsic Barrier

The intrinsic barrier is the primary physical barrier between the self and the nonself, keeping the luminal and interstitial fluids separate. The intrinsic barrier consists of epithelial cells and cell junctions (**Figures 4** and **5**). In the esophagus, the epithelium can be mono- or multilayered, depending on the species, while the stomach and intestinal epithelia are monolayered. The epithelium is attached at its basal side to the basement membrane (**Figure 2**), a multilayered collagenous structure that may form a partial barrier for large particles that have crossed the epithelium.

The cells of the intestinal epithelium comprise mainly columnar absorptive cells (enterocytes), as well as goblet cells, endocrine cells and immune cells (e.g., macrophages, neutrophils, lymphocytes, natural killer (NK)-like cells, and rodlet cells) (**Figures 3** and **6**). On the apical membrane, the enterocytes have surface-enlarging microvilli, which are covered in glycocalyx (glycoproteins). In the African lungfish (*Protopterus aethiopicus*), the intestinal epithelium also contains ciliated cells. Intestinal epithelial cilia are absent in most vertebrate groups, and the function of these cells in the lungfish epithelium remains unknown. As an interesting side note, the appearance of intestinal cilia is a pathological condition in humans.

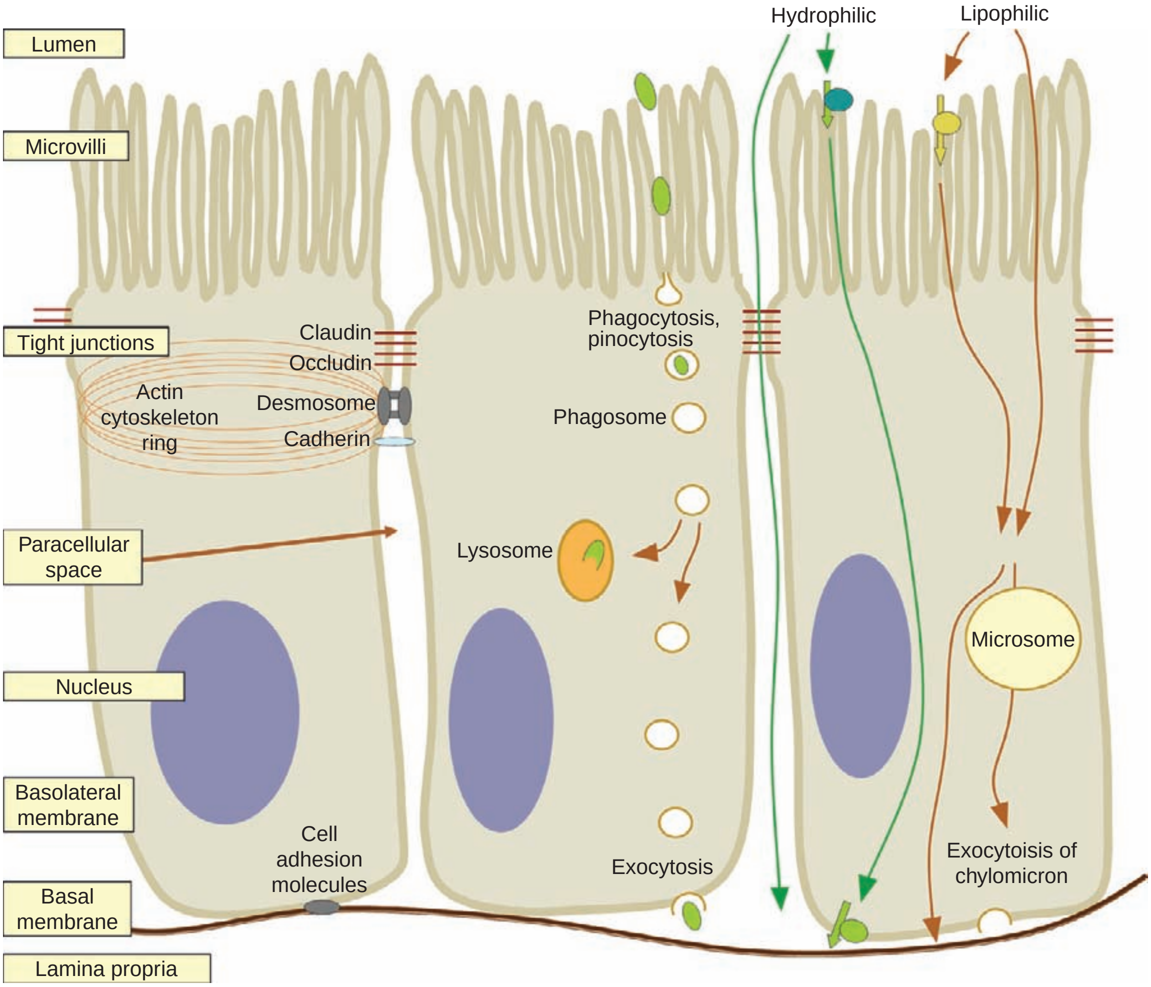


Figure 2 Schematic drawing of three enterocytes. (a) The morphology of an enterocyte, the nomenclature used in the present article, and the attachment protein complexes. (b) Endocytosis and exocytosis of macromolecules/particles and lysosomal digestion in an enterocyte. (c) Routes of transport across the epithelium for lipophilic and hydrophilic substances: the paracellular diffusion, transporter mediated, transcellular transport of both hydro and lipophilic substances, and cross-membrane diffusion of lipophilic molecules. Lipid nutrients can be modified in microsomes before exocytosis. From Jutfelt F (2006) The Intestinal Epithelium of Salmonids: Transepithelial Transport, Barrier Function and Bacterial Interactions. PhD Thesis, University of Gothenburg, Sweden.

The tight-junction protein complexes, where occludin and claudins form the extracellular domains, act as the main diffusion barrier between the epithelial cells (**Figures** 2 and 5). The pore sizes between the protein strands allow only small molecules through and the protein regions exposed to the lumen are negatively charged, leading to cation selectivity. The tight-junction permeability is regulated and is modified according to the current needs of the fish. There are also long-term changes taking place during the development of the fish, such as the tightening of the paracellular barrier when salmonids become smolts in preparation for seawater migration.

Paracellular permeability of the epithelium is often quantified by passive diffusion of small hydrophilic marker molecules through tight junctions, while transcellular permeability is measured by lipophilic molecules diffusing over both the apical and basolateral membranes. Transcellular diffusion of lipophilic substances can be modified by alterations of the membrane lipid composition, such as those seen after a change in diet. Larger hydrophilic molecules, particles, bacteria, and viruses are probably transported through active transcytosis through epithelial cells, as they are too large for tight-junction pores. An epithelium can thus have different permeabilities, depending upon which marker is used. This is often reflected by epithelia with a strong barrier for one molecular size and lower barrier for molecule or particle of another size (**see also Temperature:** Membranes and Temperature: Homeoviscous Adaptation).

Immunological Barrier

Although the extrinsic and intrinsic barriers offer effective protection, the exclusion of harmful agents is never absolute. Therefore, an additional immunological

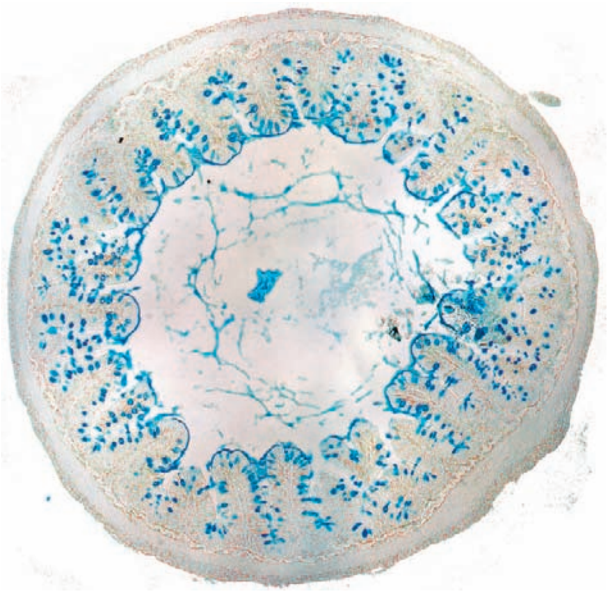


Figure 3 Cross section of the proximal intestine in Atlantic salmon stained for mucus. The mucus (blue) is visible inside goblet cells and as a layer of secreted mucus covering the mucosal folds and filling the spaces between mucosal folds. Per Fahlberg and F. Jutfelt.

barrier is required. Both the innate immune system (e.g., macrophages, neutrophils, and mast-like cells) and the adaptive immune system (the B- and T-cell-like lymphocytes) are present in the intestinal wall. The epithelium and the lamina propria contain phagocytotic cells (e.g., macrophages and neutrophils), which phagocytose and digest harmful agents that have crossed the epithelium (**Figure 6** (**see also Cellular, Molecular, Genomics, and Biomedical Approaches:** Molecular Fish Pathology)).

Enterocytes actively sample luminal contents through endocytosis and release particles and antigens at the basal membrane. Such transcytosis has been demonstrated in several fish classes, including lampreys, sharks and rays, sturgeons, and many teleosts. In teleosts, antigen sampling, processing, and presentation to lymphocytes have speculatively been suggested to occur within the epithelium, lamina propria, and stratum granulosum (**Figure 1**), possibly performed by cells such as macrophages, neutrophils, and/or by the enterocytes themselves. The purpose for this sampling of molecules, particles, and live microorganisms has been suggested to be early detection of pathogens and subsequent production of antibodies (immunoglobulins (Igs)) against the pathogens. The other important role of immune sampling of luminal content is induction of oral tolerance to harmless antigens in order to avoid unnecessary immune stimulation. In other words, this sampling process functions to fine-tune the immune system to the current need.

The intestinal immune system can, after exposure to a luminal antigen, induce a systemic increase in antibodies against the antigen that may confer protection in subsequent exposures. In mammals, antibodies are also secreted into the lumen for protection against microorganisms. Teleost skin mucus contains IgM as a part of the skin barrier function, but the importance of secreted Igs in the intestinal lumen of fish has not been demonstrated conclusively. IgM is present in the intestinal mucosal fold, but has low resistance against proteolytic degradation, which could lead to rapid inactivation if secreted into the intestinal lumen.

Harmful organisms are detected by toll-like receptors (TLRs). When TLRs are stimulated, possibly together with danger signals (other receptors for detecting tissue damage), cytokines mediate an inflammation signal that

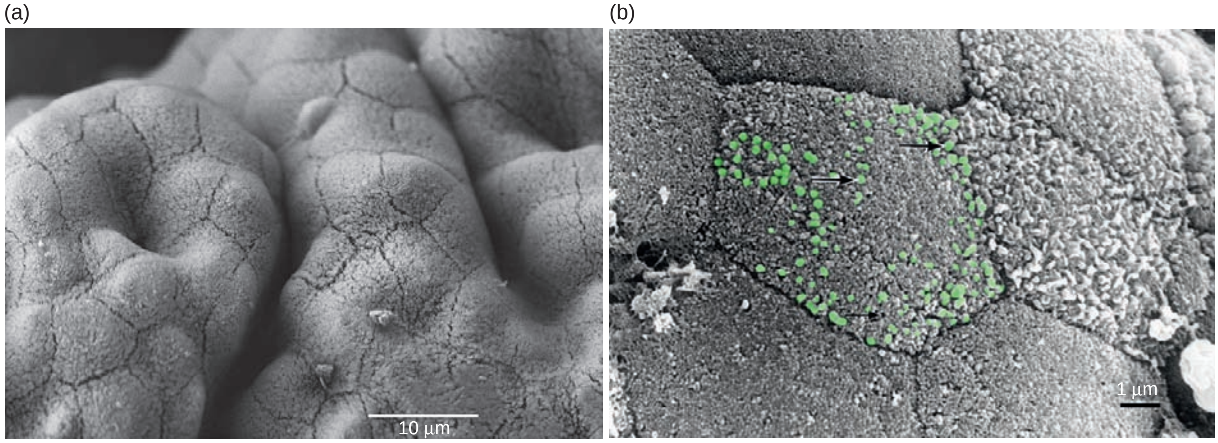


Figure 4 Scanning electron micrographs of the epithelial surface from the luminal viewpoint of proximal intestines from Atlantic cod (*Gadus morhua*) (a) and Arctic charr (*Salvelinus alpinus*) (b). The polygonal patterns are the junctions of bordering enterocytes. The fine granular structures are microvilli. (b) Microvilli-associated bacteria (arrow) are false colored in green on the central enterocyte. (a) Courtesy of Rolf Erik Olsen, Einar Ringø, and Reidar Myklebust. (b) Modified from Ringø E, Olsen RE, Mayhew TM, and Myklebust R (2003) Electron microscopy of the intestinal microflora of fish. *Aquaculture* 227: 395–415.

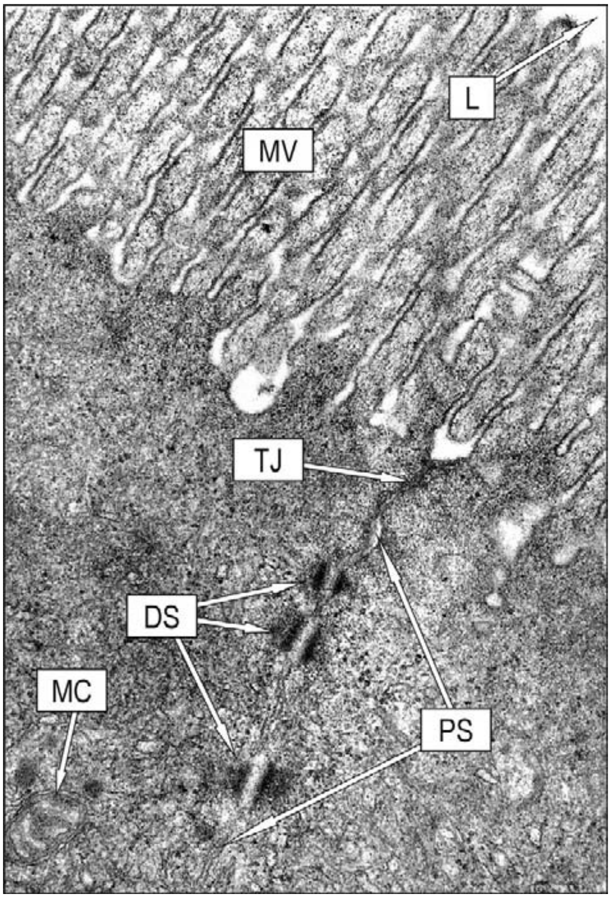


Figure 5 Transmission electron micrograph (TEM) of the anterior intestinal brush border membrane from rainbow trout (*Oncorhynchus mykiss*). L, lumen; MV, microvilli; TJ, tight junctions; DS, desmosomes; PS, paracellular space; MC, mitochondria. Courtesy of Prof. Kristina Sundell.

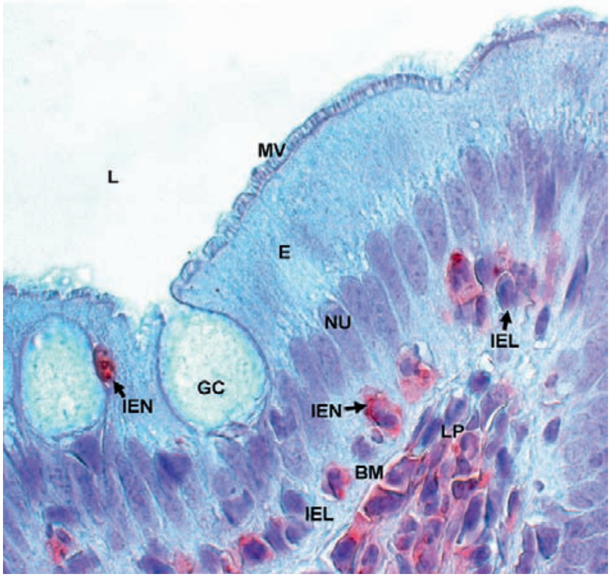


Figure 6 Light microscopy of the intestinal wall in Atlantic salmon (*Salmo salar*). Immunohistochemical staining for neutrophils (red) and general Giemsa staining. L, lumen; MV, microvilli; E, enterocyte; NU, nucleus; GC, goblet cell; LP, lamina propria; IEN, intra epithelial neutrophil (in red); IEL, intra epithelial lymphocyte; BM, basement membrane. Reproduced from Sundh H, Olsen RE, Fricadell F, *et al*. (2009) The effect of hyperoxygenation and reduced flow in fresh water and subsequent infectious pancreatic necrosis virus challenge in sea water, on the intestinal barrier integrity in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 32: 687–698.

activates and attracts cells of the immune system. Such mobilization of neutrophils into the epithelium and mast-like cells into the stratum granulosum has been shown in several teleosts, demonstrating the plasticity of the immunological barrier.

The Barrier Function of Different Intestinal Regions

The gastrointestinal tract is functionally and morphologically divided into distinct regions in most fish groups, and the barrier function varies between the regions. As the uptake function of the esophagus and the stomach is small (European eel, *Anguilla anguilla*, is one exception), the barrier can be strong without interfering with transport. In the absorbing parts of the gut, there are also large differences in barrier function between regions.

In teleosts, the pyloric ceca and anterior intestine are the main nutrient-absorbing regions. They contain similar luminal contents and perform the same nutrient and ion transports, and thus have similar barrier properties (**Figure 7**). The lumen in these regions contains high nutrient concentrations, typically relatively low numbers of bacteria, and high paracellular permeability.

In the posterior intestine, however, bacterial numbers are higher and free nutrient concentrations drop. Here, the diffusion of hydrophilic molecules across the epithelium is low, as measured per cm^2 of the intestinal wall, in the species examined (including salmonids, sea bass, sculpins, and Atlantic cod). This could be caused not only by smaller pore sizes or fewer pores in the tight junctions, but also due to a lower epithelial area because of differences in folding between regions. As the nutrient concentration drops along the intestinal tract and bacteria and bacterial toxins rise in concentration, the need for a tighter barrier increases.

The low tight-junction permeability of the posterior regions could also serve the function of enhancing water extraction by lowering the leakage of Na^+ from the epithelial paracellular space back into the lumen. Particle and macromolecular transport in the posterior region, on the other hand, is high, at least in salmonids. This can be visualized in transmission electron micrographs (TEMs) (**Figure 8**) and in fluorescence micrographs (**Figure 9**).

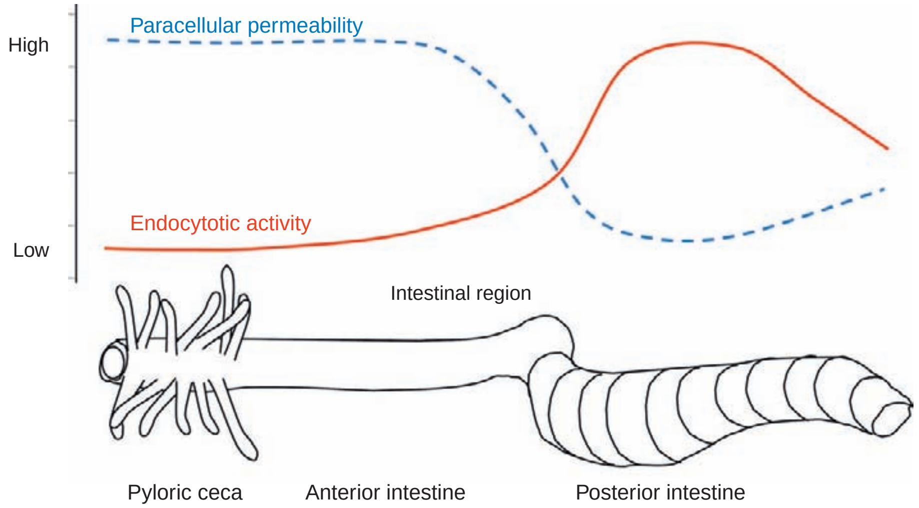


Figure 7 Epithelial barrier function of the regions of the rainbow trout (*Oncorhynchus mykiss*) intestine. The data are from *in vitro* using chamber measurements.

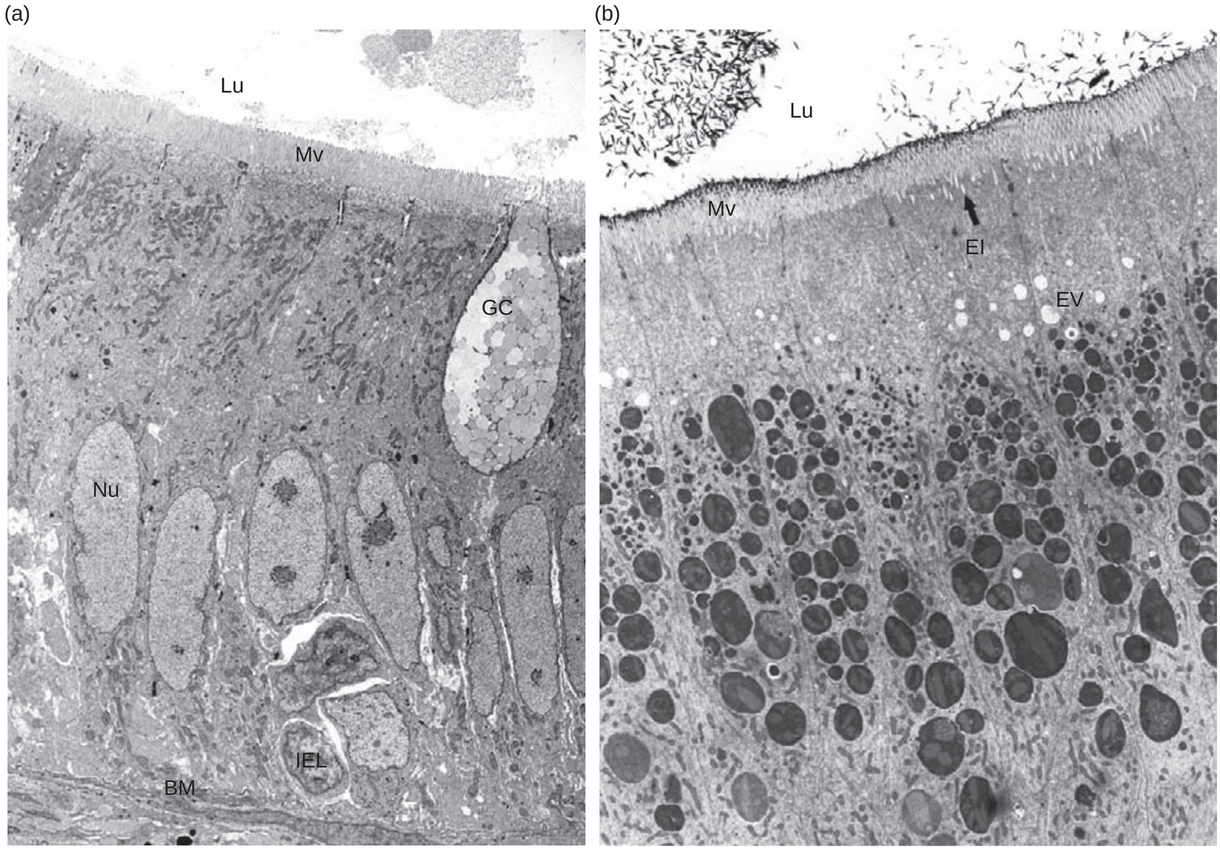


Figure 8 Transmission electron micrograph (TEM) of the epithelium in the anterior (a) and posterior (b) regions of the Atlantic salmon (*Salmo salar*) intestine. (a) Note the intraepithelial lymphocyte (IEL) in close contact with two other intraepithelial cells, possibly neutrophils or macrophages. (b) Endocytotic vesicles (EV) in the apical half of the enterocytes, small budding endocytotic invaginations (EI), and vesicles at the base of the microvilli (arrow). GC, goblet cell; MV, microvilli; Lu, lumen; BM, basement membrane. Courtesy of Olsen R, Ringø E, and Myklebust R.

In many fish larvae, and probably adults of some species, particle transport is of nutritional importance.

Another suggested reason for high endocytotic activity is the more active sampling of antigen by the immune system in the posterior intestine, both for detection of pathogens and for induction of oral tolerance to harmless antigens. The rectum has low permeability and low active transport.

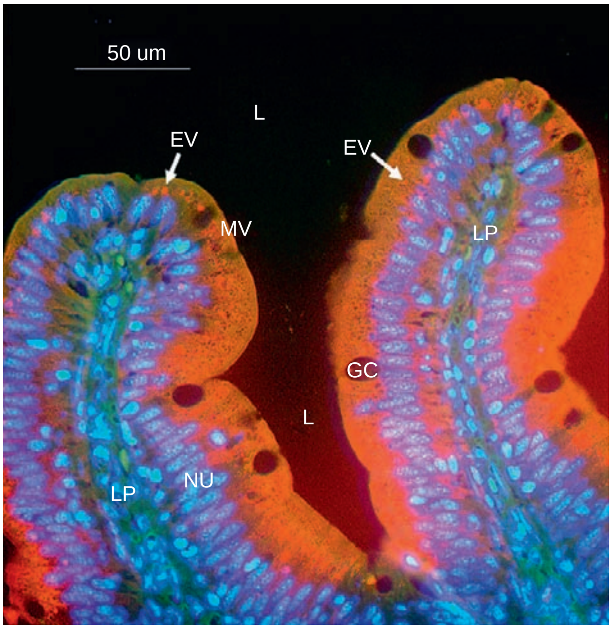


Figure 9 A micrograph showing macromolecular uptake by the intestinal epithelium in brown trout (*Salmo trutta*) parr visualized by the use of Texas-red-labeled albumin using a triple fluorescence filter. The absorbed albumin is seen as red vesicles in the apical half of the enterocytes (arrows), while DAPI-stained cell nuclei are seen in blue and general tissue fluorescence as green. L, lumen; MV, microvilli; NU, nucleus; GC, goblet cell; LP, lamina propria; EV, endocytotic vesicle. Lars Sonesson and F. Jutfelt.

Translocation of Pathogens across the Barriers

An infectious pathogen can enter the fish through the skin, gills, or the gut. The quantitative relevance of these routes has not been measured for any fish pathogen, but both bacteria and viruses can translocate through the intestinal epithelium and infect the host. The pathogen can cross the epithelial barriers in several ways: paracellularly through tight junctions, transcellularly through transcytosis or the cytoplasm (without a vesicle), or through damaged areas of the epithelium. All of these routes are used by different pathogens infecting mammals through the gastrointestinal tract, but less is known for fish pathogens.

Bacteria

Vibrio anguillarium and *Aeromonas salmonicida* are Gram-negative bacteria that can cross the epithelium through transcytosis (**Figures 10** and **11**). Both are pathogenic to several fish species. In addition, both pathogens cause desquamation (shedding of epithelia). This not only could be caused by direct damage from bacterial toxins, but may also be a defense strategy by the fish to expel infected cells. A model for breaching the intestinal barrier by *A. salmonicida* has been proposed (**Figure 11**), where the final result is disruption of the epithelium as well as translocation of the bacteria into the circulation.

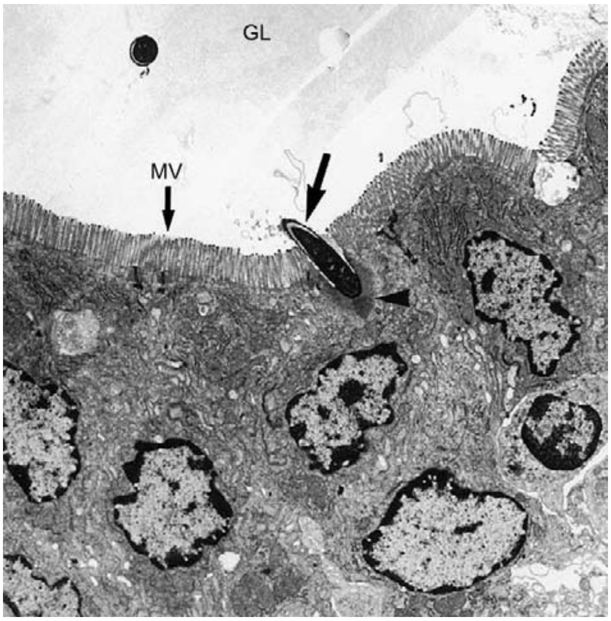


Figure 10 Transmission electron micrograph (TEM) micrograph of the epithelium of a pyloric cecum in Atlantic salmon (*Salmo salar*). The large arrow points at a rod-like cell, partially internalized into an enterocyte. The microorganism was likely a very large bacterial cell. An electron dense area (arrowhead) of the cytoplasm is surrounding the invagination, possibly comprising proteins involved in the internalization process. MV, microvilli. Modified from Ringø E, Olsen RE, Mayhew TM, and Myklebust R (2003) Electron microscopy of the intestinal microflora of fish. *Aquaculture* 227: 395–415.

Virus

Viruses can enter fish through the intestinal route. In Atlantic salmon, the pathogenic infectious pancreatic necrosis (IPN) virus is found in the intestinal epithelium in early stages of infection. IPN virus can cross the intestinal epithelium and enter the circulation *in vitro* with maintained virulence. This demonstrates both the actual crossing of the extrinsic and intrinsic barriers of the epithelium, as well as the failure of the immune system to neutralize translocated virions in the lamina propria. In addition, the epithelial permeability is increased, while the ion-transporting capacity is decreased after 1 h of the virus in the lumen. This implies that the epithelium either is damaged or reacts rapidly to the pathogen.

During IPN-virus infections in Atlantic salmon *in vivo*, the intestinal epithelial barrier function can be severely decreased, leading to increased translocation of bacteria. It is possible that the failure of the intestinal barrier is the main cause of death by IPN virus.

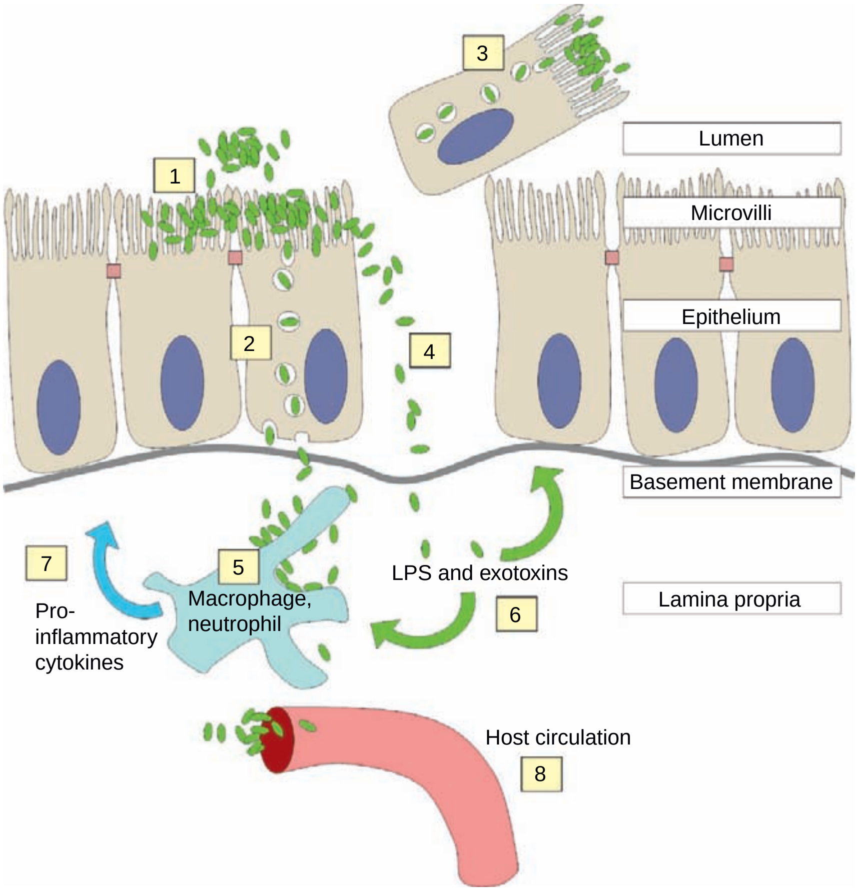


Figure 11 Suggested model for translocation of *A. salmonicida* across the intestinal epithelium in salmonids. (1) Attachment of *A. salmonicida*; secreted exotoxins negatively affect the transepithelial ion fluxes. (2) Transcellular transport. (3) Shedding of enterocytes creates holes in the epithelium. Bacterial extracellular proteases may be involved in the detachment. Apoptotic or necrotic processes may also cause shedding. (4) Entry of bacteria through damaged epithelium. (5) Bacteria have mechanisms for counteracting the innate immune system. (6) Lipopolysaccharides (LPSs) and other bacterial substances are detected by mucosal cells that release cytokines, leading to decreased barrier function. Exotoxins cause tissue damage directly. (7) Cytokines may cause inflammation, increase bacterial translocation, and induce apoptosis. (8) Entry of bacteria into the intestinal vascular system. Extracellular toxins cause host symptoms and death.

The Barrier in Stress and Disease

Disturbances to the general homeostasis of the fish, as with other physiological systems, can affect the intestinal barrier. Many types of stressors weaken the epithelial barrier function. In European eel, the stomach epithelium shows reduced surface area and increased paracellular space in response to social stress. Acute stress also increases the paracellular permeability in salmonids and Atlantic cod. This effect is stronger in fasting fish, demonstrating that the ability of the epithelium to cope during stress is reduced in food-deprived fish. The effect of stress on the epithelium could stem from reduced blood flow to the gut, ultimately resulting in ischemia and tissue damage. However, such serious barrier malfunction would probably cause sepsis and mortality, which usually does not happen. Cortisol, which is released during acute stress, may have a direct effect on the epithelial barrier by increasing tight-junction permeability as well as directly modulating ion transport.

Chronic stress can also lead to a disturbed barrier. Many long-term stressors, including water hyperoxia and hypoxia, suboptimal water temperature, suboptimal diet composition, fasting, starvation, and infection, can impair intestinal barrier functions (**Figure 12**). The reduction in barrier function is significant even in low-intensity long-term stress, when other stress and welfare parameters may fail to detect changes. In fact, the paracellular permeability has been suggested as a new and reliable indicator for chronic stress. Reliable physiological or biochemical indicators for chronic stress have been elusive.

The inclusion of vegetable ingredients in some diets reduces the integrity of the intestinal barrier in carnivorous Atlantic salmon. Vegetable lipids can cause lipid accumulation within the enterocytes to

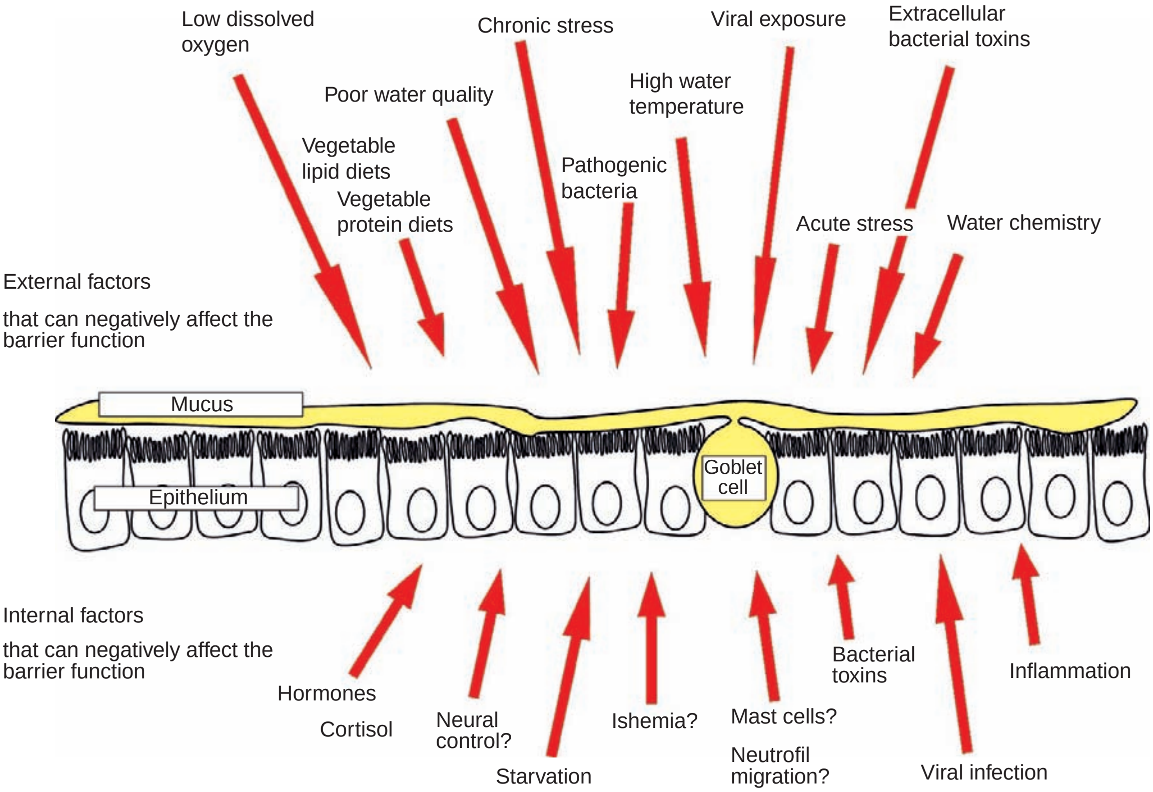


Figure 12 Summary of known and suspected factors that disrupt the integrity of the intestinal epithelium and reduce the barrier function.

such an extent that the cells rupture, causing mild barrier dysfunction. Vegetable proteins in the diet, especially soybean meal, can cause inflammation in the posterior intestine and increase the permeability of the epithelium by a factor of 2–5. The increased permeability is caused by saponins, the amphiphilic-membrane-disturbing molecules in the soybean, increasing the amount of antigens that cross the epithelium, and, in turn, initiate the inflammation. Furthermore, inflammation *per se* can cause even stronger reductions in barrier function, as discussed above.

Does a reduced intestinal epithelial barrier function lead to disease? In mammals, increased epithelial permeability causes many diseases and infections. Similarly, in fish, disease can arise from increased permeability. Soybean-meal-induced barrier dysfunction can cause enteritis, as discussed above, as well as mucosal cancer. There can also be an increased risk of infection by pathogens whenever the barrier function is compromised, regardless of the underlying reason. Fish can be chronic carriers of pathogenic bacteria in the lumen for long periods without showing clinical symptoms, yet, when the fish are subjected to acute stress, the pathogen is able to infect the fish. A plausible explanation is increased translocation through the intestinal epithelium. However, it can also be a change in the immune system that allows the bacteria to survive and proliferate after translocation.

Species Differences

Given the large number of fish species and environments to which they are adapted, the variations in barrier function strategies are probably large. Most research on the intestinal barrier function has been performed on a few species, including salmonids, cod, cyprinids, European eel, sculpins, seabass, and hammerhead shark; hence, the interspecies variation is not well described. As the contents of the intestinal lumen of fish from different environments differ immensely in aspects, such as salinity, pH, temperature, toxins, oxygen concentration, nutrient composition, composition of microorganisms, and exposure to pathogens in the lumen, the barrier strategies used by the different species can potentially provide information on how these factors are handled. They could even be considered as model organisms for research on human medical conditions.

Several aspects of the barrier function in mammals first appeared in fish; allowing us to trace the evolutionary origin of certain mechanisms. For example, mucosal lymphoid nodes, such as Peyer's patches in mammals and bursa of Fabricius in birds, are usually not considered to have appeared before the tetrapods; however, based on histological examinations, the American paddlefish (*Polyodon spathula*) have structures resembling Peyer's patches. However, the physiology of these structures has not been investigated. Sharks can also have lymphoid aggregations in the spiral valve that may have a function in lymphocyte maturation and antigen presentation.

Concluding Remarks

Three functional layers of barriers in the intestine contribute to maintaining homeostasis of the fish by keeping out unwanted substances and organisms. The extrinsic barrier, consisting of luminal secretions and microorganisms, inhibits growth of pathogens as well as their attachment to the epithelium. The intrinsic barrier, consisting of the epithelial cells and the tight junctions, is the main physical barrier that marks the border between the self and the nonself. The immunological barrier is needed to neutralize harmful agents from crossing the aforementioned barriers. In addition, the immune system of the intestine samples the luminal contents for modulation of immune responses.

Most aspects of barrier function are actively controlled. Increase in mucus production and infiltration of immune cells occur when the epithelium senses pathogens or tissue damage. The barrier function is disturbed by factors, such as suboptimal water temperature, hypoxia, low water quality, suboptimal diet, acute and chronic stress, exposure to pathogens, and starvation. This is of great importance for research on health and welfare of fish, disease transmission, vaccination, husbandry conditions in aquaculture, water transport mechanisms, as well as for the general understanding of fish physiology in health and disease.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches:** Fish as Model Organisms for Medical Research; Molecular Fish Pathology. **Gut Anatomy and Morphology:** Gut Anatomy. **Hormonal Control of Metabolism and Ionic Regulation:** Corticosteroids. **Hormonal Responses to Stress:** Impact of Stress in Health and Reproduction. **Integrated Function and Control of the Gut:** Gut Secretion and Digestion; Intestinal Absorption. **Temperature:** Membranes and Temperature: Homeoviscous Adaptation. **The Gut:** The Gastrointestinal Tract: An Introduction. **The Skin:** Hagfish Slime.

Further Reading

Bakke-Mckellep AM, Penn MH, Salas PM, *et al.* (2007) Effects of dietary soybean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress. *British Journal of Nutrition* 97: 699–713.

Fänge R (1984) Lymphomyeloid tissues in fishes. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening* 145: 143–162.

Hart S, Wrathmell AB, Harris JE, and Grayson TH (1988) Gut immunology in fish: A review. *Developmental and Comparative Immunology* 12: 453–480.

Jutfelt F (2006) The Intestinal Epithelium of Salmonids: Transepithelial Transport, Barrier Function and Bacterial Interactions. PhD Thesis, University of Gothenburg, Sweden.

Jutfelt F, Olsen RE, Björnsson BT, and Sundell K (2007) Parr–smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture* 273: 298–311.

Jutfelt F, Olsen RE, Glette J, Ringo E, and Sundell K (2006) Translocation of viable *Aeromonas salmonicida* across the intestine of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 29: 255–262.

Jutfelt F, Sundh H, Glette J, *et al.* (2008) The involvement of *Aeromonas salmonicida* virulence factors in bacterial translocation across the rainbow trout, *Oncorhynchus mykiss* (Walbaum), intestine. *Journal of Fish Diseases* 31: 141–151.

Knudsen D, Jutfelt F, Sundh H, *et al.* (2008) Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition* 100: 120–129.

Olsen RE (1999) Lipid digestibility and ultrastructural changes in the enterocytes of arctic charr (*Salvelinus alpinus* L.) fed linseed oil and soybean lecithin. *Fish Physiology and Biochemistry* 21: 35–44.

Olsen RE, Sundell K, Hansen T, *et al.* (2002) Acute stress alters the intestinal lining of Atlantic salmon, *Salmon salar* L.: An electron microscopical study. *Fish Physiology and Biochemistry* 26: 211–221.

Ringø E, Jutfelt F, Kanapathippillai P, *et al.* (2004) Damaging effect of the fish pathogen *Aeromonas salmonicida* ssp. *salmonicida* on intestinal epithelium enterocytes of Atlantic salmon (*Salmo salar* L.). *Cell and Tissue Research* 318: 305–311.

Ringø E, Olsen RE, Mayhew TM, and Myklebust R (2003) Electron microscopy of the intestinal microflora of fish. *Aquaculture* 227: 395–415.

Rombout JHWM and Joosten PHM (1998) Immunology of fishes. In: Pastoret PP, Griebel P, Bazin H, and Goaverts A (eds.) *Handbook of Vertebrate Immunology*, pp. 3–62. San Diego, CA: Academic Press.

Sundell K, Jutfelt F, Agustsson T, *et al.* (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out of season parr–smolt transformation of Atlantic salmon, *Salmo salar*. *Aquaculture* 222: 265–85.

Sundh H (2009) Chronic Stress and Intestinal Barrier Function. PhD Thesis, University of Gothenburg: Sweden.

Sundh H, Olsen RE, Fricadell F, *et al.* (2009) The effect of hyperoxygenation and reduced flow in fresh water and subsequent infectious pancreatic necrosis virus challenge in sea water, on the intestinal barrier integrity in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 32: 687–698.

Nervous System of the Gut

S Holmgren and C Olsson, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Autonomic Nerves Innervate the Gut
Anatomy of the Gut Nervous System
Neurotransmitters and Their Receptors
Concluding Remarks
Further Reading

Glossary

Autonomic nervous system A collection of motor neurons (ganglia) ordered segmentally from cranial to caudal levels. The system controls the inner organs (viscera) through reflexes without conscious control.

Cranial autonomic nerve Autonomic nerve starting in the cranial (head) region of the CNS.

Enteric nerve The nerve that is intrinsic to the gut; the cell body is contained within the gut wall.

Enteric nervous system The part of the autonomic nervous system contained within the gastrointestinal wall.

Extrinsic innervation Innervation that comes from outside the organ. A nerve that is extrinsic to the gut has its cell body outside the gut, but sends its axon to the gut.

Ganglion A collection of nerve cell bodies of similar function. A ganglion is a type of nucleus but often refers to structures in the peripheral nervous system. An exception is the basal ganglia that are nuclei of the telencephalic subpallium.

Glial cells Cells surrounding nerve cells that provide support and nourishment.

Nerve plexus A network of branching nerve fibers in or between tissue layers.

Spinal autonomic nerve Autonomic nerve initiating from the spinal cord region of the CNS.

Autonomic Nerves Innervate the Gut

Nerves control many processes in the gut, such as secretion of digestive juices and mixing and propulsion of food. Gut nerves are part of the involuntary autonomic nervous system (ANS). The innervation of the fish gut is dense and, as in all vertebrates, uses a large number of different neurotransmitters for signaling. Indeed, the nervous system of the gut functions as a small local brain: the nerves form elaborate, interacting reflex pathways, comprising sensory mechanisms, networks of relaying interneurons, and final motor or secreto-motoneurons.

A large division of the innervation of the gut is intrinsic, that is, the nerve cells are located entirely within the gastrointestinal wall. This enteric nervous system (ENS) is, to a large extent, capable of an adequate control of the gut on its own. The gut is also innervated by extrinsic autonomic nerves. These have their nerve cell bodies outside the gut, either in the central nervous system (CNS) or in ganglia (aggregation of cell bodies) along the nerves to the gut, and send their axons to the gut in the extrinsic nerves. The extrinsic innervation plays important roles in coordinating gut activity and relaying information, for example, about hunger and pain to the CNS.

This article outlines the similarities and differences in the anatomy of the gut innervation and the occurrence of different transmitters between different fish groups and in comparison to other vertebrates.

Anatomy of the Gut Nervous System

The general anatomy of the gut nervous system is very similar in all vertebrates, but fish deviate from the general plan in some features, either as a group or as individual species. This mainly is due to differences in general anatomy, such as the lack of a stomach, which is a common feature in many fish families, or the presence of pyloric ceca (**see also Gut Anatomy and Morphology**: Gut Anatomy).

The Gut Nervous System is Well Developed

The vertebrate ENS on average contains similar numbers of nerve cells as the spinal cord, and also contains enteric glial cells, surrounding the enteric neurons. Judging from the few (teleost) fish studies where the number of neurons in the ENS has been calculated, the overall density of nerve cell bodies in the teleost gut is similar to that in small mammals like mice and guinea pig.

In fish, the majority of the enteric nerve cell bodies is located in the myenteric plexus, between the longitudinal

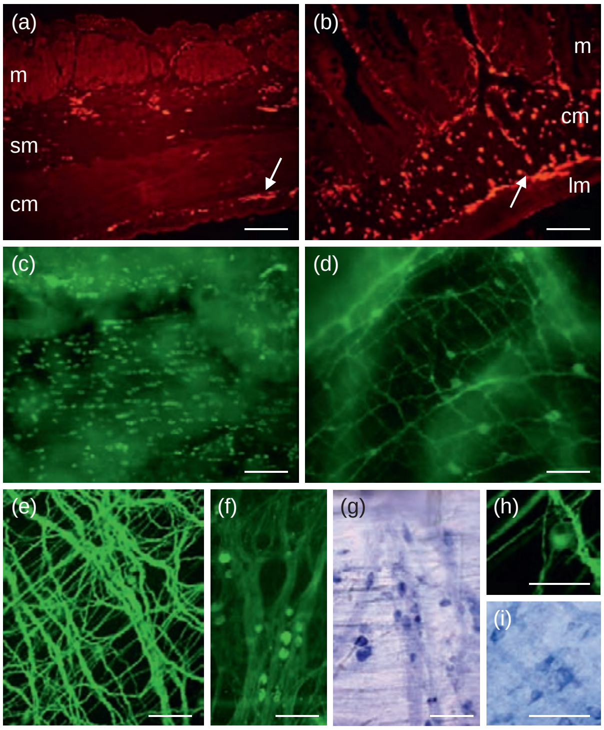


Figure 1 Innervation of the gut wall in shorthorn sculpin (*Myoxocephalus scorpius*) (a,b, e,f, h), zebrafish (*Danio rerio*) (c,d) and Atlantic cod (*Gadus morhua*) (g, i) demonstrated using a mixture of antibodies against human neuronal protein (Hu) and acetylated tubulin (AcT) (a,b, c–f, h), Hu alone (c) or NADH-diaphorase staining (g, b). (a) Nerve fibers in the myenteric plexus (arrow) of the cardiac stomach. (b) Dense innervation of the myenteric plexus (arrow), circular muscle layer and mucosa of the proximal intestine. (c–i) Whole mounts of the intestinal myenteric plexus showing nerve cell bodies and nerve fibers. m, mucosa; sm, submucosa; cm, circular muscle layer; lm, longitudinal muscle layer. Scale = 50 μm (d, h), 100 μm (b, c, e–g, i), 200 μm (a).

and circular muscle layers (**Figures 1(a)** and **1(b)**); (**see also Gut Anatomy and Morphology**: Gut Anatomy). In general, the submucous plexus found in other vertebrates is either missing in fish or limited to a few scattered cell bodies. The enteric nerve cell bodies in fish typically occur singly or as two to three cells together, scattered over the myenteric plexus (**Figures 1(c)–1(g)**), rather than forming ganglia of several cells as in tetrapods. In general, there seem to be more nerve cell bodies per surface area in the intestine than in the stomach.

The size of the enteric nerve cell bodies is also in the same range as for mammals of similar body mass. The diameter of the cell bodies range from 10 to 100 μm in fish such as brown trout (*Salmo trutta*) and Atlantic cod (*Gadus morhua*) with the majority of cells being around 15–20 μm. In smaller species such as zebrafish (*Danio rerio*), the nerve cells are generally smaller (**Figures 1(c)** and **1(d)**). Fish enteric neurons seem to be more uniform than mammalian neurons; the majority of cells have a smooth cell body and most often appear to have only one (long) process (**Figures 1(f)–1(h)**). However, cells similar to the two dominating cell types present in mammals, Dogiel type I (cells with flattened dendrites and one axon) and Dogiel type II (smooth multipolar cells), are found in cyclostomes and some elasmobranch and teleost species. In mammals, the different morphologies correspond to different functions, but this has not been established in fish.

The axons of enteric neurons form a network of smaller and larger nerve bundles, which connect neurons within the myenteric plexus, and innervate muscle, glands, and blood vessels. With the exception of cyclostomes (and in particular, myxinoids), this network is well developed among fish. The axons are predominantly varicose, with the ability to release transmitter substances along their entire length.

Extrinsic Nerves from the CNS Coordinate

Except in cyclostomes, extrinsic nerves reach all parts of the gastrointestinal tract and its associated organs such as the pancreas and gallbladder. The axons form either autonomic nerves transmitting impulses from the CNS to the gut, or sensory nerves transmitting impulses in the other direction, from the gut to CNS. The extrinsic innervation includes both cranial and spinal nerves, but fish generally lack the concentrated outflow of fibers in the sacral/pelvic region that is common in tetrapods (**Figures 2** and **3**).

The cranial autonomic fibers (also called parasympathetic fibers) to the gut run mainly in the visceral branches of the paired vagus nerve. In elasmobranchs, they may also be found in the facial and glossopharyngeal nerves (**Figure 2**). A cranial autonomic pathway has a preganglionic neuron with the cell body in the brainstem. This synapses with a postganglionic neuron either somewhere along the vagus nerve or within the gut wall. In most fish species, the vagal innervation reaches the esophagus, stomach, and proximal intestine. However, in some species, vagal fibers innervate the esophagus only, while in others they may reach far back along the intestine.

The splanchnic nerves to the gut are part of the spinal autonomic system (also called the sympathetic system). Spinal autonomic pathways consist of a preganglionic neuron with its cell body in the spinal cord, connecting to a postganglionic neuron with, in fish, its cell body in the sympathetic chain ganglia just outside the spinal cord. Postganglionic axons run in the splanchnic nerves to the gut.

The number of splanchnic nerves and the distribution of the spinal autonomic innervation vary among fish. In cyclostomes, the innervation mainly reaches the hindgut. In most elasmobranchs, there are several splanchnic nerves to the gut (**Figure 2**). The anterior pair innervates the posterior stomach and anterior intestine. The most anterior outflow of spinal fibers often joins the vagus, forming a vago-sympathetic trunk running to the gut. A varying number of mid- and posterior splanchnic

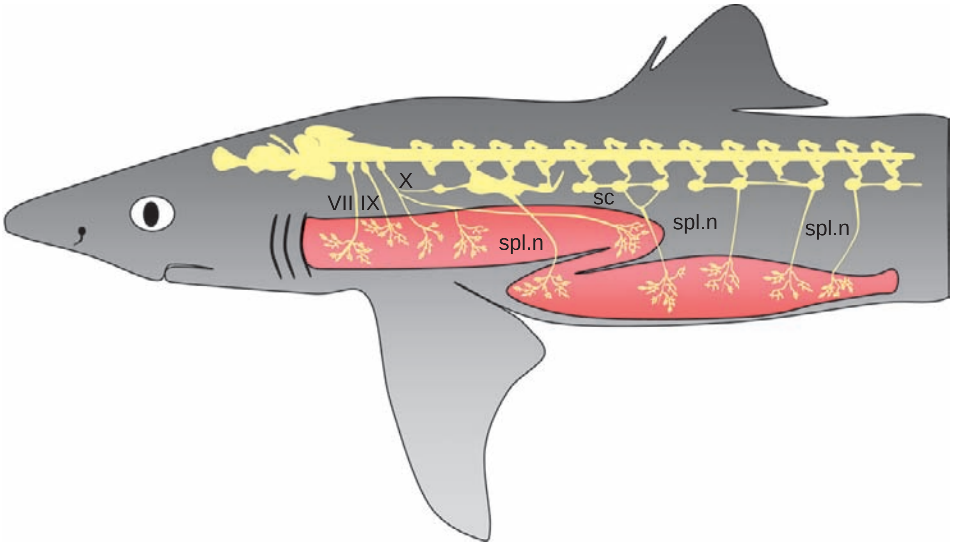


Figure 2 Schematic picture of the extrinsic autonomic innervation of the gut in an elasmobranch. Cranial outflow (parasympathetic nerves) is via cranial nerves X (vagus) and to some extent IX (glossopharyngeal) and VII (facial). Spinal (sympathetic) pathways synapse in ganglia in the sympathetic chain (sc) and then run in a number of splanchnic nerves (spl. n) to the gut. Courtesy of Albin Gräns, redrawn from Nilsson S (1983) *Autonomic Nerve Function in the Vertebrates*. Berlin: Springer.

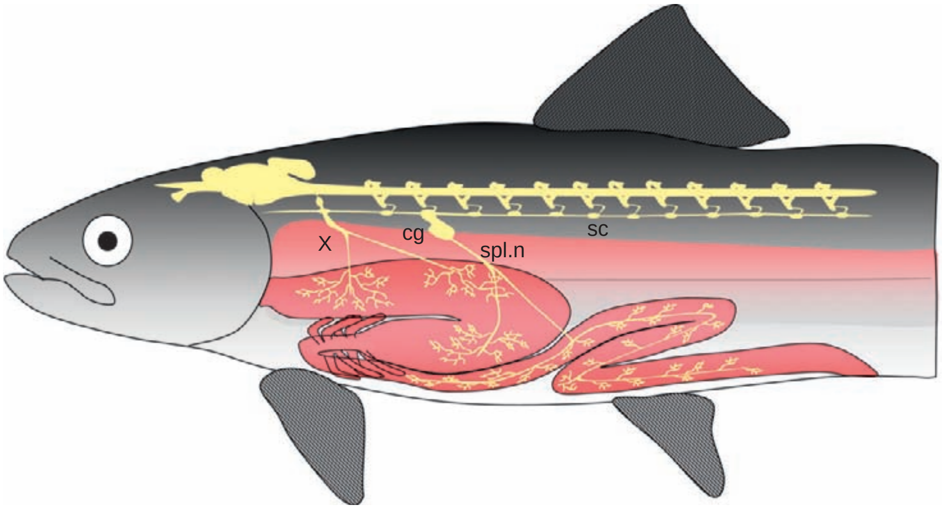


Figure 3 Schematic picture of the extrinsic autonomic innervation of the gut in a teleost. Cranial outflow (parasympathetic nerves) is via cranial nerves X (vagus). Spinal (sympathetic) pathways synapse in the large celiac ganglion (cg) in the sympathetic chain (sc) and then run in the splanchnic nerve (spl. n) to the gut. Courtesy of Albin Gräns, redrawn from Nilsson S (1983) *Autonomic Nerve Function in the Vertebrates*. Berlin: Springer.

nerves innervate the spiral intestine and rectum. In teleosts, the anterior splanchnic nerve divides into several branches, innervating the posterior stomach and most of the intestine (**Figure 3**). In addition, in teleosts, as in elasmobranchs, spinal fibers enter the gut via the vagi.

The vagi and the splanchnic nerves also contain a large number of sensory fibers. Vagal sensory fibers usually have their cell bodies gathered in a nodose ganglion somewhere along the vagus. Splanchnic sensory fibers have their cell bodies in the dorsal root ganglia of the spinal cord.

Neurotransmitters and Their Receptors

The effect of a nerve cell may be either inhibitory or excitatory, depending on the transmitter(s) it releases and the type of receptors and second messenger systems present on/in the target cell. Immunohistochemistry has revealed a large number of neurotransmitters in fish gut nerves. In several cases, the chemical structure of these transmitters is known. It appears that all transmitters present in the mammalian gut are found in a corresponding but not always identical version in fish. In addition, the relative abundance of a transmitter and its distribution along the gut may vary in comparison with mammals, as well as among different groups, families, or species of fish. Distribution and abundance of nerves expressing a certain transmitter, as well as presence of its receptors, are plastic and can change with feeding status, food type, changes in hormone levels, and in the environment. Some studies show that infections and inflammation can alter gut innervation in fish.

The most investigated signal substances that affect the performance of the gut, and their receptors are presented in the following. **Figure 4** shows the distribution of some of the best-studied transmitters in the gastrointestinal tract of shorthorn sculpin (*Myoxocephalus scorpius)* (**Appendix A** (pp. 1232–1267)).

Acetylcholine

Pharmacological and physiological studies suggest that acetylcholine dominates the control of gut functions in fish, like in mammals. However, histochemical evidence of its presence is so far only indirect. For example, the synthesizing enzyme, choline acetyl transferase (ChAT), and the transporter that moves acetylcholine into vesicles in the nerve terminal, vesicular acetylcholine transporter (VAChT), have been demonstrated in the intestine of zebrafish and Atlantic cod. Acetylcholine is proposed to be released from enteric motor- and interneurons. A large population of vagal fibers is cholinergic in most teleosts, but elasmobranchs and some teleosts appear to lack a vagal cholinergic innervation of the gut; in these species, cholinergic nerves are exclusively enteric.

Acetylcholine acts via nicotinic or muscarinic receptors. Several teleost subtypes and subunits have been sequenced, and a muscarinic M3-like subtype appears to be important in the control of gut motility in rainbow trout (*Oncorhynchus mykiss).*

Amines

Amines constitute a group of several signaling substances, including catecholamines (adrenaline, noradrenaline, and dopamine), serotonin (5-hydroxytryptamine; 5-HT), and histamine. Catecholamines and serotonin act as neurotransmitters in the fish gut.

Catecholamine-containing nerves have been demonstrated in the gut by the presence of the synthesizing enzymes, tyrosine hydroxylase (TH) or phenylethanolamine-N-methyl transferase (PNMT), or by making the catecholamines fluoresce. Fibers surround nerve cell bodies in the myenteric plexus and innervate blood vessels in the submucosa as well as major vessels to the gut. In some species, fibers are observed in the muscle layers. As in mammals, adrenergic nerve fibers are believed to be

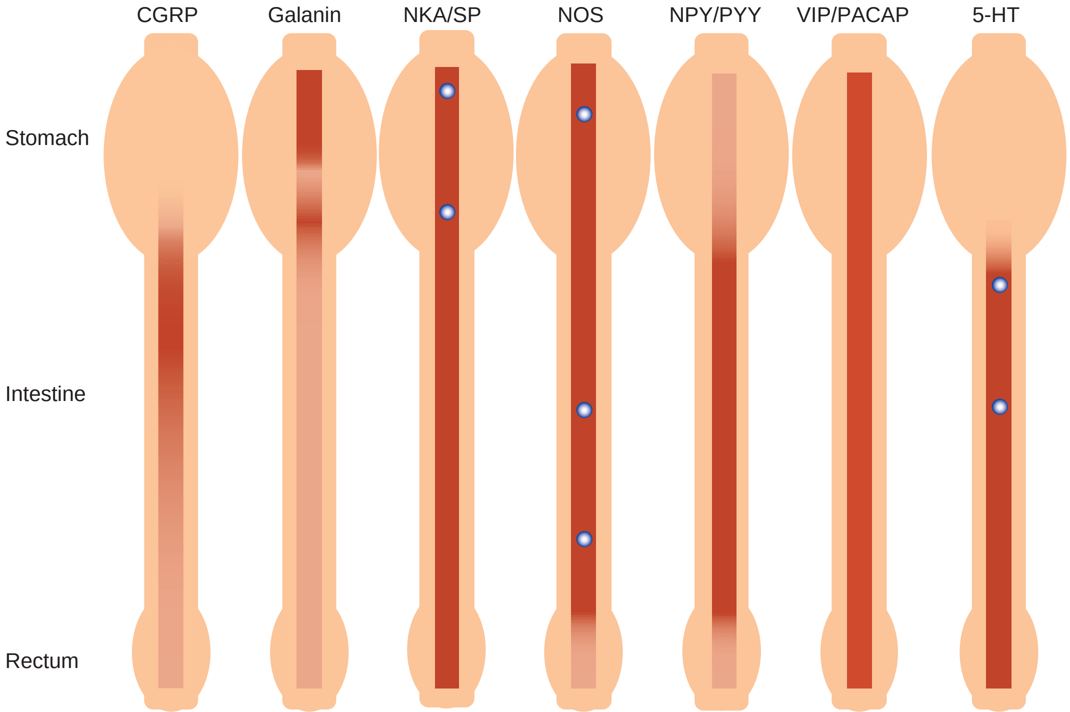


Figure 4 Schematic representation of the presence of immunoreactive material in different nerve fibers (vertical bars) and nerve cell bodies (circles) in gastrointestinal tract of a teleost, the shorthorn sculpin (*Myoxocephalus scorpius*. Based on immunohistochemical data from Holmgren S and Olsson C (2009) The neuronal and endocrine regulation of gut function. In: Bernier NJ, Van Der Kraak G, Farrell AP, and Brauner CJ (eds.) *Fish Physiology*, *Vol. 28: Fish Neuroendocrinology*, pp. 467–512. Burlington: Academic Press.

mainly extrinsic (spinal autonomic), but TH-expressing nerve cell bodies were recently found in the gut of several teleost species, suggesting an enteric subpopulation of the catecholamine nerves.

In most vertebrates, including elasmobranchs, noradrenaline is the dominant catecholamine in adrenergic neurons. However, adrenaline dominates in teleosts and holosteans. Dopamine levels are usually lower, and the release of dopamine from gut nerves in fish, although implicated in both lamprey and some teleosts, is poorly investigated.

Pharmacological experiments suggest the presence of alpha- and beta-adrenoceptor subtypes both in stomach and intestine, and an alpha-2-like receptor is found in the intestine of the Japanese eel (*Anguilla japonica*). The pharmacological properties of these fish receptors appear to differ considerably from those of corresponding mammalian receptors.

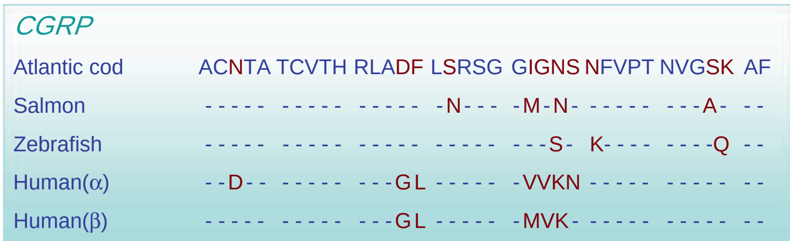
Serotonin is expressed in gut neurons or in endocrine cells of the mucosa, with strikingly different proportions between the two populations in different species. In cyclostomes, serotonin is only neuronal. In elasmobranch species, it is rarely found in nerves but commonly in endocrine cells. In sturgeons (*Chondrostei*), serotonin is found in both endocrine and nerve cells. Teleosts show large interspecies variation, from an entirely neuronal presence in some cyprinid fish, through mixed populations, to a presence in endocrine cells only in the European eel (*Anguilla anguilla*). When present, nerve cell bodies are located in the myenteric plexus, sending fibers within the plexus and to the muscle layers. At least seven classes of serotonin receptors exist and members of most of the classes have been isolated from different fish species although little is known about their distribution in the gut.

Neuropeptides

Neuropeptides are small- to medium-sized peptides, consisting of from a few to up to 40 amino acids. Like most true transmitters, they are stored in vesicles in the nerve terminals. They also act as modulators of the action of other transmitters and typically coexist with other transmitters in the terminals. Many neuropeptides are found in gut endocrine cells as well (**see also Integrated Function and Control of the Gut**: Intestinal Absorption). Neuropeptides of the same evolutionary origin are said to form families. Gene duplications and subsequent mutations leading to amino acid substitutions, have led to these groups of neuropeptides of similar structure and overlapping functions.

Calcitonin gene-related peptide (CGRP). It is a 37-amino acid peptide, with at least two isoforms (CGRP1 and CGRP2) in teleosts (**Figure 5**). CGRP nerves are found in teleosts, lamprey, and lungfish. Fibers in the anterior parts of the gut (stomach and proximal intestine) are often extrinsic, reaching the gut via the vagus. Enteric nerve cell bodies, and hence an intrinsic innervation, are present more posteriorly. In Atlantic cod, there is a dense innervation of the myenteric plexus, the circular muscle layer, and the mucosa. Few fibers only innervate vessels in the gut wall. A CGRP-receptor has been cloned from the bastard halibut (*Paralichthys olivaceus*).

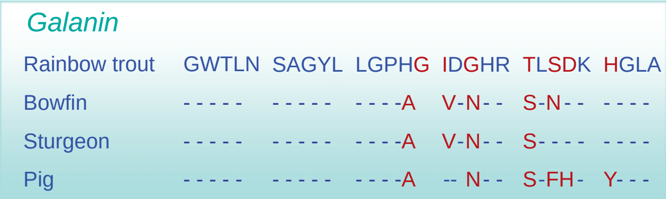
Galanin. It is a highly conserved 29-amino acid peptide that has been isolated from ancient fish groups as well as teleosts and elasmobranchs (**Figure 6**). Galanin usually occurs only in nerve cells and fibers but not in endocrine cells. Most nerve cells are found in the myenteric plexus with some present also in the vagus nerve, projecting their axon either toward the CNS or toward



CGRP

Atlantic cod	ACNTA	TCVTH	RLADF	LSRSG	GIGNS	NFVPT	NVGSK	AF
Salmon	-----	-----	-----	-N---	-M-N-	-----	---A-	--
Zebrafish	-----	-----	-----	-----	---S-	K----	----Q	--
Human(α)	--D--	-----	---GL	-----	-VVKN	-----	-----	--
Human(β)	-----	-----	---GL	-----	-MVK-	-----	-----	--

Figure 5 The amino acid sequence of calcitonin gene-related peptide (CGRP) from some fish species and a mammal (human). Dash denotes the same amino acid in this position as in Atlantic cod. Note the large similarity in all species, in particular in the N-terminal (left) end of the peptide.



Galanin

Rainbow trout	GWTLN	SAGYL	LGPHG	IDGHR	TLSDK	HGLA
Bowfin	-----	-----	----A	V-N--	S-N--	----
Sturgeon	-----	-----	----A	V-N--	S----	----
Pig	-----	-----	----A	--N--	S-FH-	Y---

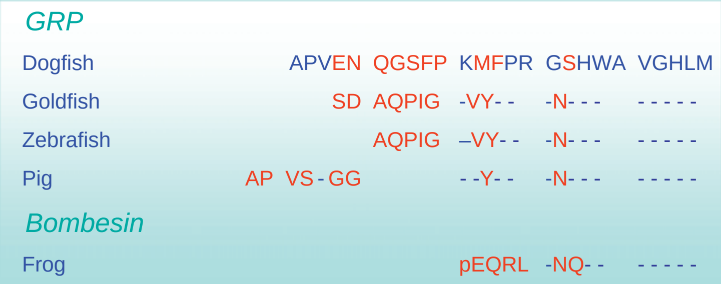
Figure 6 The amino acid sequence of galanin from some fish species and a mammal (pig). Dash denotes the same amino acid in this position as in rainbow trout. Note the large similarity in all species, in particular in the N-terminal (left) end of the peptide.

the gut. Most fibers run in the mucosa and submucosa; however, some also occur in muscle layers, indicating effects on both secretion and motility. The density of innervation often decreases from stomach to rectum (**Figure 4**). Galanin fibers innervating gut blood vessels are reported in elasmobranch species and in the Australian lungfish (*Neoceratodus forsterii*).

Peptides of the *GRP (gastrin releasing peptide)/bombesin* family all share the same seven C-terminal amino acids (**Figure 7**). GRP has been sequenced from elasmobranch and teleost species, for example, lesser-spotted dogfish (*Scyliorhinus canicula*), zebrafish, and goldfish (*Carassius auratus*). GRP/bombesin-immunoreactive gut nerves are found in all major fish groups, including lampreys, elasmobranchs, teleosts, and lungfish. The fibers are present along the whole gut, and cell bodies are found in the myenteric plexus of spiny dogfish (*Squalus acanthias)* and shorthorn sculpin, indicating that the nerves may be enteric in many species. In some species, like lungfish and bowfin (*Amia calva*), only a few fibers are found, while others, like spiny dogfish, have a dense innervation. In several elasmobranchs, gut perivascular nerve fibers express a GRP-like peptide.

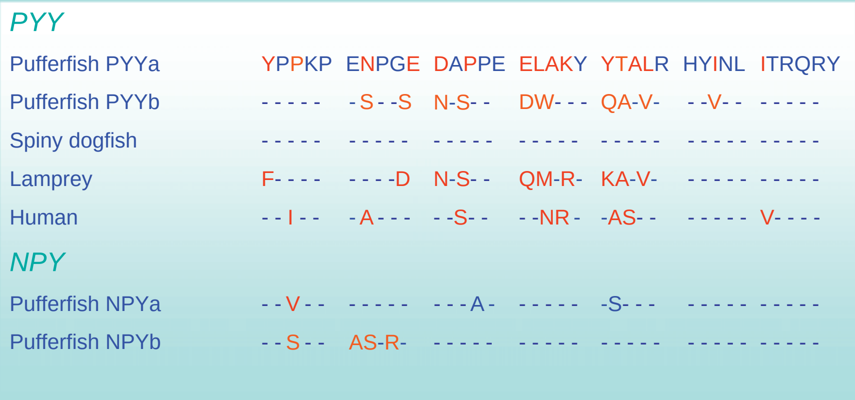
The closely related peptides *NPY (neuropeptide Y) and peptide PYY (peptide tyrosine tyrosine)* evolved early from an ancestral NPY (**Figure 8**). Evolution has led to two forms of each peptide: NPYa and NPYb, and PYYa and PYYb (formerly pancreatic polypeptide, PP). It has recently been established that NPY is primarily present in the CNS, while PYY is expressed in gut nerves. However, histochemical studies rarely conclusively distinguish between these peptides. Gut nerves containing NPY/PYY-like material are commonly found in elasmobranch and teleost species (**Figure 4**), but not in cyclostomes. The absence of enteric nerve cell bodies in various species studied to date suggests the nerves might be exclusively extrinsic. Nerve fibers are present in the plexa and the muscle layer and some surround blood vessels. At least seven types of NPY/PYY-receptors are present in vertebrates, but the expression in fish gut is little investigated.

Tachykinins form a large group of neuropeptides. The best studied are *substance P* and *neurokinin A* (NKA), which come from the same gene, via different splicing. Several unique fish peptides have also been isolated, for example, the *scyliorhinins* that were first found in the elasmobranch *Scyliorhinus* (**Figure 9**).



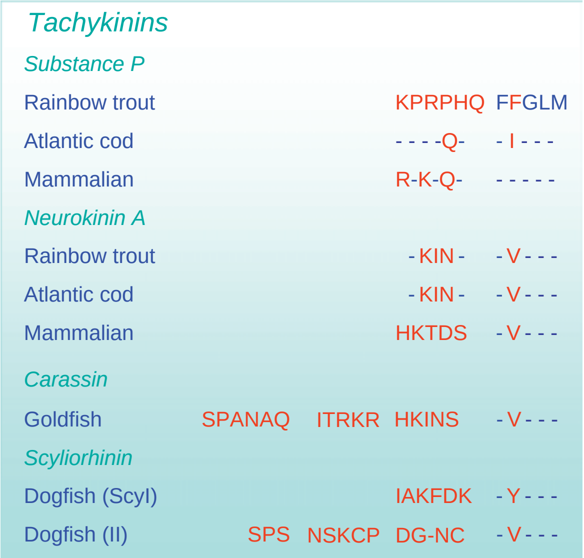
GRP						
Dogfish		APVEN	QGSFP	KMFPR	GSHWA	VGHLM
Goldfish		SD	AQPIG	-VY--	-N---	-----
Zebrafish			AQPIG	–VY--	-N---	-----
Pig	AP	VS-GG		--Y--	-N---	-----
Bombesin						
Frog				pEQRL	-NQ--	-----

Figure 7 The amino acid sequences of gastrin-releasing peptide (GRP) from some fish species and a mammal (pig) and of the amphibian skin peptide bombesin. Dash denotes the same amino acid in this position as in dogfish. Note the similarity between species in the C-terminal end (right) of the peptide.



PYY							
Pufferfish PYYa	YPPKP	ENPGE	DAPPE	ELAKY	YTALR	HYINL	ITRQRY
Pufferfish PYYb	-----	-S--S	N-S--	DW---	QA-V-	--V--	-----
Spiny dogfish	-----	-----	-----	-----	-----	-----	-----
Lamprey	F----	----D	N-S--	QM-R-	KA-V-	-----	-----
Human	--I--	-A---	--S--	--NR-	-AS--	-----	V----
NPY							
Pufferfish NPYa	--V--	-----	---A-	-----	-S---	-----	-----
Pufferfish NPYb	--S--	AS-R-	-----	-----	-----	-----	-----

Figure 8 The amino acid sequences of peptide tyrosine tyrosine (PYY) and neuropeptide Y (NPY) from some fish species and a mammal (human). In pufferfish (*Takifugu rubripes*), PYY is found in gut nerves and NPY in the brain. Dash denotes the same amino acid in this position as in pufferfish. Note the large similarity between species in particular the C-terminal (right) end of the peptide.



Tachykinins				
Substance P				
Rainbow trout			KPRPHQ	FFGLM
Atlantic cod			- - - -Q-	- I - - -
Mammalian			R-K-Q-	- - - - -
Neurokinin A				
Rainbow trout			- KIN -	- V - - -
Atlantic cod			- KIN -	- V - - -
Mammalian			HKTDS	- V - - -
Carassin				
Goldfish	SPANAQ	ITRKR	HKINS	- V - - -
Scyliorhinin				
Dogfish (Scyl)			IAKFDK	- Y - - -
Dogfish (II)	SPS	NSKCP	DG-NC	- V - - -

Figure 9 The amino acid sequences of different tachykinins from some fish species and mammals. Dash denotes the same amino acid in this position as in rainbow trout substance P. Note the C-terminal, where four out of five amino acids are common, which is characteristic for tachykinins.

Numerous substance P/NKA-immunoreactive neurons are present in the gut of representatives of all fish groups except cyclostomes. Enteric nerve cell bodies are usually common, indicating an intrinsic origin, although some tachykinin-immunoreactive fibers may well be extrinsic (**Figure 4**). Fibers are particularly dense in the myenteric plexus in all gut regions, suggesting a presence in local sensory neurons and/or interneurons, but are also seen in other gut layers and around gut vessels.

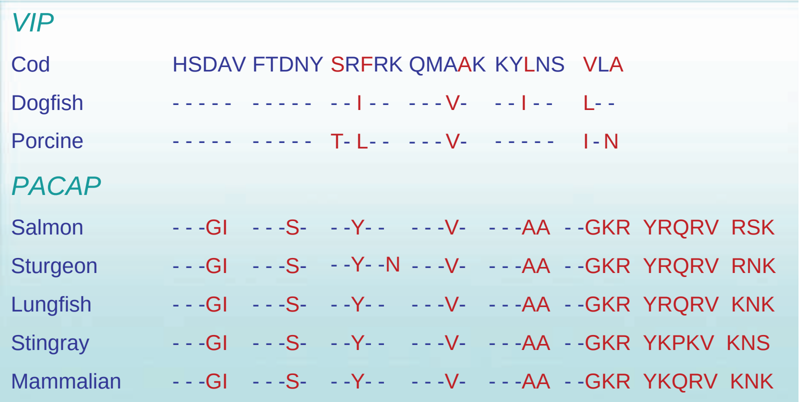
The three known tachykinin receptor types, neurokinin (NK) 1–3, have all been found in fish genomes. The type(s) expressed in the gut have not been conclusively established, but functional data suggest the presence of at least NK1-receptors. In general, NK1-receptors are most sensitive to substance P while NK2-receptors prefer NKA.

VIP (vasoactive intestinal polypeptide) and PACAP (pituitary adenylate cyclase activating peptide) are two peptides of the secretin-glucagon superfamily, which often exist together in gut neurons. VIP contains 28 amino acids, while PACAP can exist in two forms, a full-length peptide containing 38 amino acids and a C-terminally truncated 27-amino-acid version. Both VIP and PACAP have been sequenced from several teleost and elasmobranch species (**Figure 10**).

VIP and PACAP exist in gut nerves of most fish species examined, except cyclostomes where it is only present in endocrine cells. However, VIP was discovered first and is the better studied of the two. VIP fibers innervate all layers of the gut wall and, more sparsely, vessels running on and in the gut tissues. Few VIP-immunoreactive nerve cell bodies are usually observed.

VIP and PACAP show 100% co-localization in enteric neurons in Atlantic cod, rainbow trout, and spiny dogfish and generally interact with the same receptors (VPAC1, VPAC2, and/or PAC1). There are species differences in the distribution of receptor types: while pufferfish species express VPAC2 and PAC1 in the gut, only VPAC1 is found in the goldfish and one PAC1 isoform in zebrafish intestine.

Other neuropeptides. Some peptides are more common in mucosal endocrine cells, but occur also in small populations of nerves in fish. Among these is cholecystokinin (CCK), which is found in nerves of the dogfish rectum and in the lungfish foregut. The single type of receptor (CCK-X) demonstrated in fish may be the common ancestral form of the mammalian CCK-A and CCK-B receptors. Similarly, somatostatin, commonly present in



VIP								
Cod	HSDAV	FTDNY	SRFRK	QMAAK	KYLNS	VLA		
Dogfish	- - - - -	- - - - -	- - I - -	- - - V-	- - I - -	L- -		
Porcine	- - - - -	- - - - -	T- L- -	- - - V-	- - - - -	I - N		
PACAP								
Salmon	- - -GI	- - -S-	- -Y- -	- - -V-	- - -AA	- -GKR	YRQRV	RSK
Sturgeon	- - -GI	- - -S-	- -Y- -N	- - -V-	- - -AA	- -GKR	YRQRV	RNK
Lungfish	- - -GI	- - -S-	- -Y- -	- - -V-	- - -AA	- -GKR	YRQRV	KNK
Stingray	- - -GI	- - -S-	- -Y- -	- - -V-	- - -AA	- -GKR	YKPKV	KNS
Mammalian	- - -GI	- - -S-	- -Y- -	- - -V-	- - -AA	- -GKR	YKQRV	KNK

Figure 10 The amino acid sequences of vasointestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) from some fish species and a mammal (human). Dash denotes the same amino acid in this position as in VIP from Atlantic cod. Note the large similarities, in particular in the N-terminal (left) end of the peptides, between peptides within the VIP and PACAP groups, and between the two groups.

endocrine cells, is found in nerve populations in the spiny dogfish rectum, in the nursehound (*Scyliorhinus stellaris*) stomach and in the intestine of the teleost *Barbus conchonius* (rosy barb).

Nitric Oxide

Nitric oxide is synthesized in nerves by neuronal nitric oxide synthase (nNOS), and may be one of the most common neurotransmitters. The sequence of nNOS has been determined in several fish species, including zebrafish, rainbow trout, and fugu (*Takifugu rubripes*). The presence of NOS is the most commonly used marker for NO-producing (nitrergic) nerves. In several teleosts and in spiny dogfish, NOS is common in the myenteric plexus throughout the gut, often present in over 50% of the nerve cell bodies. Fibers reach all layers, with the highest density in the circular muscle layer. No nitrergic neurons are found in hagfish.

Other Transmitters

Some substances, like amino acids and purines, are transmitters in mammals, but are so far little investigated in fish. Amino acids like glutamate and γ-amino butyric acid (GABA) are the most common transmitters in the brain, but so far there are only a few reports of their findings in the fish gut. The purines adenosine and adenosine triphosphate (ATP), commonly used in the general metabolism of all cells, is concentrated by specific transporters into secretory vesicles in certain nerves, and subsequently released to act as transmitters in the gut. They act through two main types of receptors, P1-receptors stimulated by adenosine, or P2-receptors interacting with ATP and other nucleotides. Both P1- and P2-receptors are found in fish gut.

Coexistence and Chemical Coding

Nerve cells normally express a combination of different transmitters, and the combination of transmitters sometimes gives an indication of the function. However, little is known about the patterns of coexistence in nerves innervating the fish gut, and whether these change with changing circumstances, such as during long periods of fasting. This lack of information may at least in part depend on a lack of specific antisera produced in different hosts, which is needed for simultaneous staining for different markers.

CGRP in combination with substance P often indicates extrinsic sensory neurons in mammals, and this combination also is found in about 20% of the substance P-immunoreactive nerve fibers in the gut of the Australian lungfish. In contrast, few or no fibers co-express the two peptides in investigated teleosts or elasmobranchs. In Arctic lamprey (*Lampetra japonica*), CGRP coexists with serotonin in mucosal nerve cells and fibers.

Cells containing VIP and PACAP often co-express NOS. For example, in Atlantic cod and spiny dogfish, around 50% of the VIP-immunoreactive neurons contain NOS. In other species, some VIP neurons co-express galanin, and most galanin-immunoreactive nerves are also ChAT-immunoreactive, that is, they synthesize acetylcholine.

Concluding Remarks

Large and fundamental similarities exist between fish and other vertebrates in the general anatomy of the gut innervation, although there are several minor variations found. Similarly, most neurotransmitters occur in an identical or similar version in fish as in all vertebrates, but the abundance of nerves expressing a certain transmitter may vary substantially between regions of the gut and between species. Details on receptor types and their molecular identity in fish are very limited so far. In fact, in terms of the distribution and types of nerves innervating the fish gut, it is presently still difficult to determine what constitutes an evolutionary trend and what constitutes a species-specific adaptation to lifestyle and habitat.

See also: **Gut Anatomy and Morphology**: Gut Anatomy. **Integrated Function and Control of the Gut**: Gut Motility; Intestinal Absorption. **The Gut:** The Gastrointestinal Tract: An Introduction.

Further Reading

Burnstock G (1969) Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacological Review* 21: 247–324.

Conlon JM and Larhammar D (2005) The evolution of neuroendocrine peptides. *General and Comparative Endocrinology* 142: 53–59.

Holmgren S and Jensen J (2001) Evolution of vertebrate neuropeptides. *Brain Research Bulletin* 55: 723–735.

Hoyle CH (1998) Neuropeptide families: Evolutionary perspectives. *Regulatory Peptides* 73: 1–33.

Gibbins I (1994) Comparative anatomy and evolution of the autonomic nervous system canal. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 1–67. Chur, Switzerland: Harwood.

Jensen J and Holmgren S (1994) The gastrointestinal canal. In: Nilsson S and Holmgren S (eds.) *Comparative Physiology and Evolution of the Autonomic Nervous System*, pp. 119–167. Chur, Switzerland: Harwood.

Kirtisinghe P (1940) The myenteric nerve plexus in some lower chordates. *Quarterly Journal of Microscopical Science* 81: 521–539.

Nilsson S (1983) *Autonomic Nerve Function in the Vertebrates*. Berlin: Springer.

Olsson C (2009) Autonomic innervation of the fish gut. *Acta Histochemica* 111: 185–195.

Olsson C and Holmgren S (1997) Nitric oxide in the fish gut. *Comparative Biochemistry and Physiology* 118A: 959–964 (minireview).

Olsson C and Holmgren S (2001) The control of gut motility. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 128: 481–503.

Holmgren S and Olsson C (2009) The neuronal and endocrine regulation of gut function. In: Bernier NJ, Van Der Kraak G, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 28*: Fish Neuroendocrinology, pp. 467–512. Burlington: Academic Press.

Stevens CE and Hume ID (1995) *Comparative Physiology of the Vertebrate Digestive System*. Cambridge: Cambridge University Press.

Sundström G, Larsson TA, Brenner S, Venkatesh B, and Larhammar D (2008) Evolution of the neuropeptide Y family: New genes by chromosome duplications in early vertebrates and in teleost fishes. *General and Comparative Endocrinology* 155: 705–716.

Young JZ (1933) The autonomic nervous system of selachians. *Quarterly Journal of Microscopical Science* 75: 571–624.

Endocrine Systems of the Gut

E Jönsson and S Holmgren, University of Gothenburg, Gothenburg, Sweden

© 2011 Elsevier Inc. All rights reserved.

Introduction
Gastrin, Histamine, and Somatostatin Interact in the Control of Gastric Acid Secretion
CCK Affects Appetite and Digestion
Serotonin – Hormone or Transmitter?
Glucagon-Like Peptide-1 Has a Deviant Effect in Fish
Ghrelin Influences Appetite and Growth-Hormone Secretion
Leptin's Role in the Gut Is Not Yet Known in Fish
Concluding Remarks
Further Reading

Glossary

Anorexigenic Inhibiting appetite.

Closed-type endocrine cell Intraepithelial endocrine cell without luminal contact.

Enteroendocrine cell Endocrine cell in the epithelium of the gut mucosa.

Open-type endocrine cell Intraepithelial endocrine cell with luminal contact.

Orthologs Genes, or peptides expressed by genes, in different species that have evolved from a common ancestral gene.

Paracrine effect When a hormone from an endocrine cell affects neighboring cells.

Postprandial Period after ingesting a meal.

Proximal intestine First part of the intestine, equivalent to duodenum in mammals.

Vagal afferents Sensory nerves, for example, from the gut, running in the vagus nerve.

Vagal innervation Nerve fibers reaching the gut via vagus nerves.

Introduction

The endocrine cells of the gut are an important source of hormones involved in the control of appetite, digestive processes, and metabolism. They are mainly present in two locations, either scattered in the gut mucosal epithelium or in more concentrated clusters, islets, in the pancreas. The mucosal cells, also called intraepithelial endocrine or enteroendocrine cells, are usually of the open type. These cells have a cone-like appearance, with a broader part containing the nucleus near the basal membrane, and a tapering process toward the lumen (apical side) (**Figure 1**). Closed-type endocrine cells, without the apical process, are less common. The endocrine cells are found all along the gut mucosa, with the highest concentrations in the stomach and proximal intestine. However, when single signal substances are considered, populations may vary from an even distribution all along the gut for some, to populations concentrated to a specific part of the gut such as the cardiac stomach, proximal intestine, or the most distal part of the intestine for others.

The endocrine cells produce a large number of different signal substances. The signal substances are released into the blood circulation, and are thus hormones by definition, but local paracrine effects are also important. Release of hormone from an endocrine cell may be initiated, increased, or inhibited by a large number of factors including food components, mechanical distortion, other hormones or paracrines, and nerves innervating the mucosa. Many gut hormones, such as cholecystokinin (CCK), serotonin, tachykinins, and vasoactive intestinal polypeptide (VIP), are identical in molecular structure or at least closely related to neurotransmitters in the same species. In contrast, others such as gastrin, glucagon, and insulin are more common or exclusive to endocrine cells. For a detailed summary of the occurrence of signal substances in endocrine cells and/or nerves in the gut of different fish species, see **Appendix A** (pp. 1232–1267).

This article presents the hormones of the enteroendocrine cells in a functional (physiological) context, while the pancreatic endocrine system is described in Pancreas **Gut Anatomy and Morphology**: Pancreas. All of the hormones synthesized and produced in the pancreas, that is, insulin, glucagon, somatostatin (SST), and pancreatic polypeptide, also exist in populations of endocrine cells in the gut wall. These may be remnants of a more primitive stage when a separate pancreas was not present.

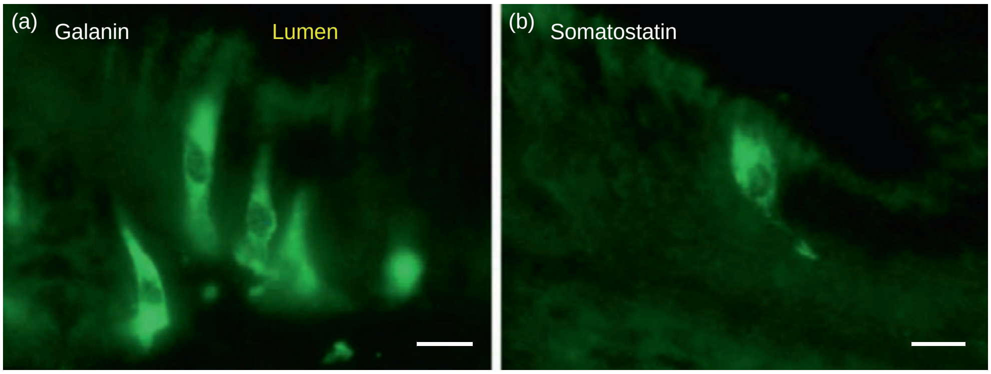


Figure 1 Immunohistochemistry is the primary tool to illustrate the presence of hormones in enteroendocrine cells of the gut, here exemplified by (a) galanin immunoreactive cells in the intestinal mucosa and (b) a somatostatin (SST) immunoreactive cell in the gastric mucosa of shorthorn sculpin (*Myoxocephalus scorpius*). Notice also the cone-shaped structure of the four open-type endocrine cells in (a), with a broad base toward the basal membrane and an apex protruding toward the gut lumen. Photographs courtesy of Mrs. Christina Hagström.

With the exception of SST, they are not further discussed here.

In addition to the slightly better investigated endocrine systems described below, there are more or less occasional findings of, for example, bombesin/gastrin-releasing peptide (GRP)-, calcitonin gene-related peptide (CGRP)-, enkephalin-, FRMRFamide-, galanin- (**Figure 1**), gastric inhibitory peptide (GIP)-, neurotensin-, and VIP/pituitary adenylate cyclase-activating peptide (PACAP)-like peptides in enteroendocrine cells of different fish species (see **Appendix A** (pp. 1232–1267)).

Gastrin, Histamine, and Somatostatin Interact in the Control of Gastric Acid Secretion

Enteroendocrine cells in the stomach mucosa interact with each other and with autonomic enteric nerves, in the control of gastric acid secretion. Among the released hormones (or paracrines), gastrin, histamine, and SST take a prominent position, and their involvement in acid secretion in fish is outlined below.

Gastrin

Gastrins are a group of peptides that belong to a large family of signal peptides also including several cholecystokinins (CCKs). The two peptide groups are very closely related with four amino acids common in the C-terminal end. Unfortunately, most studies in vertebrates investigating the presence of gastrin have used immunocytochemical methods with antibodies that cannot distinguish between gastrins and CCKs, because they are directed toward the common C-terminal. These studies report endocrine cells containing gastrin/CCK-like material in both stomach and intestine in many fish (see **Appendix A** (pp. 1232–1267)). However, judging from the general vertebrate situation, it is most likely that gastric cells contain gastrin, while intestinal endocrine cells and gut nerves contain CCKs.

Histamine

Histamine is an amine formed from the amino acid histidine. In fish, histamine is present in mucosal endocrine cells of the stomach, often called enterochromaffin-like (ECL) cells, due to their staining reaction. Similar cells are found in all vertebrates. In contrast, the presence of histamine-containing mast cells (common in reptiles, birds, and mammals) in the fish gut wall appears more unusual – so far mast cells are found only in the most advanced teleosts, for example, in perch-like fish, and in lungfish. Histamine from mast cells is believed to be involved in inflammatory responses. In rainbow trout (*Oncorhynchus mykiss*), histamine also can produce irregular contractions of preparations of intestinal smooth muscle, possibly mimicking effects of histamine released from infectious bacteria in the live fish.

Somatostatin

Several different SSTs occur in fish (**Figure 2**). They are usually either 14 or about 28 amino acids long. One of the short forms, SST-14-(I), is highly conserved among vertebrates, and is identical in, for example, hagfish (*Myxine glutinosa*), lungfish (*Protopterus annectens*), rainbow trout, and mammals. Other forms vary between species, and there are even those that are found only in fish.

SST is well recognized as a pancreatic hormone in fish as in other vertebrates (**see also Gut Anatomy and Morphology**: Pancreas), but it is also found in endocrine cells of the gut mucosa of most fish species. Typically, SST inhibits the release of gastrointestinal hormones

Somatostatin

From preprosomatostatin I				
SST14-I (all vertebrates !)		AGCKN	FFWKT	FTSC
Pro^5-SST14 (sturgeon)		-P---	-----	----
Ser^5-SST14 (ratfish)		----S	-----	----
Ser^{12}-SST (lamprey)		-----	-----	-S--
SST26 (bowfin)	SAN• •PALA PRERK	-----	-----	----
From preprosomatostatin II				
Anglerfish SST28	-VDS TNN-P -----	-----	-Y--G	----
European eel SST25	-VD• ••NLP -----	-----	-Y--G	----

Figure 2 The amino-acid sequences of SST from some fish species. Dash denotes the same amino acid in this position as in the variant of SST14 that is common to all vertebrates, SST14-(I). Note the similarities between the peptides although they come from two different preprosomatostatins.

from other endocrine cells, and also decreases smooth muscle contractions in the gut (**see also Integrated Function and Control of the Gut**: Gut Motility), which may lead to a reduced rate of gastric emptying.

Gastric Acid Secretion

In most fish species, as in many other nonmammalian vertebrates, gastric acid is secreted from so-called oxynticopeptic cells. The cells are usually gathered in gastric glands in the stomach mucosa, and they secrete both gastric acid and pepsinogen. They have a basal secretory activity when the stomach is empty of food, and the secretion increases dramatically after food intake. An important stimulus for this is the distension of the gut wall caused by the presence of food, which in turn triggers endocrine cells in the stomach mucosa to release their hormones, but several nervous reflexes are also involved. Thus, the hormones gastrin, histamine, and SST play central roles in the control of acid secretion (**Figure 3**) together with the neurotransmitter acetylcholine, in fish as in mammals.

Histamine and acetylcholine stimulate acid secretion in several elasmobranch and teleost species, but the most comprehensive studies have been performed in Atlantic cod (*Gadus morhua*) and rainbow trout. Acetylcholine is released from vagal (and probably enteric) nerve fibers and acts via muscarinic receptors, which appear to be present both on the oxynticopeptic cells and on the histamine cells, stimulating the release of histamine. The effect of histamine is mediated via receptors of the H2-type. Histamine-induced acid secretion is inhibited by SST and the neuropeptide VIP in the Atlantic cod.

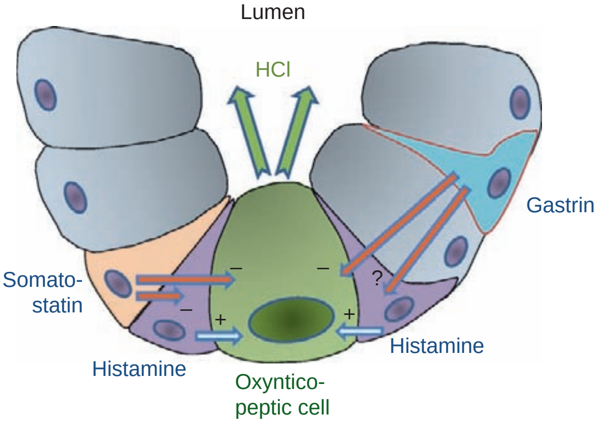


Figure 3 The hormones gastrin, histamine, and somatostatin exert important local control of gastric-acid secretion in fish. In addition (not shown), hormones from other parts of the gut, such as cholecystokinin and serotonin, and transmitters, such as acetylcholine and vasoactive intestinal polypeptide (VIP) released from neurons, are essential in regulating acid synthesis and release.

The role of gastrin in fish is not so clear. It has an unusual effect in Atlantic cod, where it inhibits acid secretion, but stimulates secretion in the common stingray (*Dasyatis pastinaca*), like in mammals. There are too few studies in fish, involving very few species, to show whether these differences are due to evolutionary trends, species differences, or methodological problems caused by using mammalian gastrins.

In addition, hormones released from the intestinal mucosa that reach the stomach via the circulation may affect acid secretion. In Atlantic cod, for example, CCK inhibits secretion. Serotonin, on the other hand, shows a dose dependency: low doses stimulate secretion, while high doses inhibit secretion.

CCK Affects Appetite and Digestion

CCK-like material is found in endocrine cells of the fish intestine in representatives of all fish groups except lungfish (see **Appendix A** (pp. 1232–1267)). As mentioned above, CCKs are closely related to gastrins, but it is likely that the material present in endocrine cells in the mucosa of the proximal intestine is predominantly CCK related. CCK, either hormonal or from enteric nerves, affects many gastrointestinal functions. For example, it controls gut motility and gastric emptying (**see also Integrated Function and Control of the Gut**: Gut Motility), and release of pancreatic hormones (**see also Gut Anatomy and Morphology**: Pancreas). It also affects appetite and gallbladder emptying. A CCK/gastrin receptor, named CCK-X, has been found in fish, and it is suggested that this represents an ancestral common form that has evolved into CCK-A and CCK-B receptors in mammals.

CCK and Gallbladder Contraction

The very first established effect of CCK is emptying of the gallbladder (**Figure 4**). Arrival of acid, fat, and amino acids in the proximal intestine of fish presumably releases CCK from local endocrine cells; CCK in turn contracts the gallbladder. The ability of food components such as fats and certain amino acids and/or CCK to contract the gallbladder has been shown in teleost, elasmobranch, and holostean species.

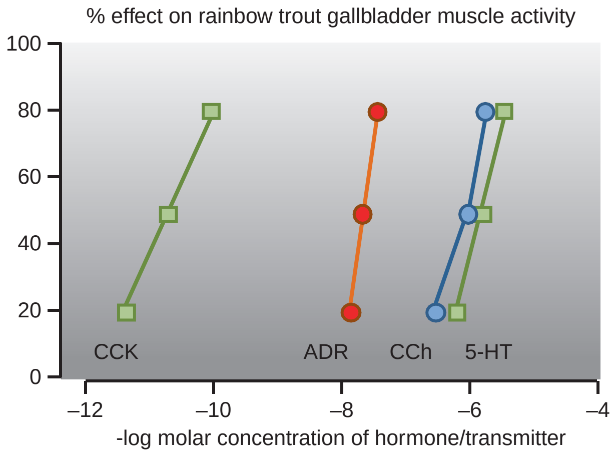


Figure 4 The potency of some hormones and neurotransmitters that affect motility of the gallbladder. The more potent a substance is, the less is needed to cause an effect and the more to the left the curve lies in the diagram. Note how much more effective CCK is than the other hormones and transmitters. ADR, adrenaline, mainly neuronal, inhibitory; CCh, carbachol, enzyme-resistant cholinergic drug, mimics neuronal acetylcholine, stimulatory; CCK, cholecystokinin, mainly hormonal, stimulatory; 5-HT, 5-hydroxytryptamine = serotonin, mainly hormonal. Modified from Aldman G and Holmgren S (1987) Control of gallbladder motility in the rainbow trout, *Salmo gairdneri*. *Fish Physiology and Biochemistry* 4: 143–155.

CCK and Appetite Regulation

CCK is also a classical so-called satiety factor and the first to be defined as such in 1973. This means that as the stomach becomes filled with food during a meal, intestinal CCK is released and signals to the brain to reduce further food consumption. One of the most extreme examples of this is the Burmese python (*Python molurus*), where CCK levels in plasma can increase 25-fold after a meal. The anorexigenic function of CCK is evolutionarily well conserved and has been confirmed across vertebrates including different teleosts such as goldfish (*Carassius auratus*), sea bass (*Dicentrarcus labrax*), rainbow trout, and coho salmon (*Oncorhynchus kisutch*). It is believed that gut-derived CCK induces the sensation of satiety mainly by stimulating CCK receptors on vagal sensory nerves, which then transmit the signal to the brain.

Due to difficulties in measuring CCK in fish plasma, it is still not established exactly which nutrients in the food regulate the endocrine release of CCK from the gut, but a recent study on rainbow trout showed that it was influenced by the dietary protein-to-lipid ratio. The postprandial increase in plasma CCK in rainbow trout was less pronounced and slower than in mammals.

Another approach is to measure mRNA levels. In the yellowtail (*Seriola quinqueradiata*), CCK mRNA levels increased after a meal. In line with this, in the more long term, CCK mRNA expression was lower during fasting in yellowtail and winter flounder (*Pseudopleuronectes americanus*), when the gut is empty of food. But different species react differently; for example, gut CCK mRNA expression increases in coho salmon during winter when appetite is suppressed, although the stomach is empty. In the elasmobranch winter skate (*Leucoraja* (*Raja*) *ocellata*), CCK mRNA levels also increase during fasting, whereas they remain unchanged in fasted Atlantic salmon (*Salmo salar*).

Serotonin – Hormone or Transmitter?

Many of the gut hormones are also expressed in an identical or very similar form in gut nerves. This often occurs in the same species (see **Appendix A** (pp. 1232–1267)), but in some cases a substance is expressed exclusively in endocrine cells in some species or groups, and in nerves only in others. Serotonin (5-hydroxytryptamine, 5-HT) represents an interesting case in this context, with different distributions in nerves and/or endocrine cells, sometimes even between closely related species (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut). Based on this, a theory has been put forward that endocrine cells express serotonin only when there is no serotonergic innervation of the mucosa. Pharmacological experiments in fish demonstrate that serotonin may stimulate pepsinogen secretion from oxynticopeptic cells and increase gut and gallbladder (**Figure 4**) contractions.

Glucagon-Like Peptide-1 Has a Deviant Effect in Fish

In addition to glucagon, the intestinal proglucagon gene generates two other peptides through alternative splicing, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). These hormones have been described across vertebrate species. In teleosts, GLP-1 is produced both in the intestine and in the pancreas (**see also Gut Anatomy and Morphology**: Pancreas), whereas GLP-2 is produced only in the intestine. So far, a function for GLP-2 is only known for mammals.

GLP-1 is important for the maintenance of glucose levels in the blood, but interestingly it has opposite effects in mammals and fish. This may be related to differences between these vertebrate groups in the importance of glucose as a nutrient and how they regulate a glucose load. In mammals, GLP-1 is released from the intestine during feeding even before blood glucose levels are elevated. It inhibits gastric emptying and thus slows down nutrient absorption. At the same time, GLP-1 stimulates insulin release (this is called the incretin effect), which in turn lowers blood glucose levels. In fish, on the other hand, GLP-1 has glucagon-like effects; it increases blood glucose levels through the stimulation of liver glycogenolysis and gluconeogenesis. GLP-1 also increases the capacity of enterocytes from black bullhead catfish (*Ictalurus melas*) to transport glucose *in vitro*. This indicates that GLP-1 is involved in the not-so-well-known local hormonal regulation of intestinal nutrient absorption in fish.

Ghrelin Influences Appetite and Growth-Hormone Secretion

Ghrelin is produced in all vertebrates, predominantly in endocrine cells in the stomach epithelium. An exception in stomach-less species, such as goldfish, where ghrelin-expressing cells are especially abundant at the base of the mucosal folds in the proximal intestine (intestinal bulb) (see **Appendix A** (pp. 1232–1267)). Ghrelin is a 12–26-amino-acid-long peptide in fish, and varies between 25 and 28 amino acids in other vertebrates (**Figure 5**).

In mammals, most ghrelin is produced in the acid-secreting mucosa in the so-called X/A-like cells, now often referred to as ghrelin cells. Similar to this, the ghrelin immunoreactive or mRNA-expressing cells are located in the gastric glands or stomach mucosal folds in, for example, Japanese eel (*Anguilla japonica*), summer flounder (*Paralichtys dentatus*), Atlantic halibut (*Hippoglossus hippoglossus*), sea bass, and rainbow trout. At least in rainbow trout and Atlantic halibut, these cells are both of open- and closed-type. Ghrelin also is present in a species-specific manner in several other tissues, for example, in endocrine cells in the intestine, pancreas, and lungs, as well as in the brain and heart.

Ghrelin was discovered as an endogenous stimulator of growth hormone (GH) release, acting through the GH-secretagogue receptor (GHS-R) in the pituitary. One by one, the list of ghrelin's physiological functions has expanded, and perhaps the most well known is its instrumental role in appetite control. In mammals, ghrelin stimulates hunger, increases food consumption, and the deposition of fat. In birds, on the other hand, the effect of ghrelin on appetite depends on dose and site of administration. The detailed roles of ghrelin in fish are not clear-cut, but a generalized overview is given in **Figure 6**. Ghrelin increases food intake in tilapia (*Oreochromis mossambicus*) and goldfish, but decreases or has no effect on food intake in rainbow trout. In goldfish, ghrelin stimulates appetite by acting on vagal afferents, similar to its mechanism of action in rats. Plasma ghrelin levels as well as gut ghrelin mRNA expression fluctuate with feeding status in fish, but again the patterns vary between studies and species. The detailed nutritional regulation of ghrelin release/production is little explored in fish. One study on tilapia, however, showed that glucose increased stomach ghrelin mRNA expression, which is in contrast to what occurs in mammals.

In line with the original description of ghrelin's function in mammals, it also has direct *in vitro* and *in vivo*

Ghrelin

Zebrafish	GTSFL	SPT•Q	KPQ••	•••G•	RRPPR	VG•
Goldfish	–––––	––•A–	–––••	•••–•	–––––	M–•
Rainbow trout 1	–S–––	––•S–	–––••	VRQ–K	GK–––	––•
Hammerhead shark	–V––•	H–RLK	EKD•D	NSS–N	S–KSK	NP•
Pig	–S–––	––EH–	–V–QR	KESKK	PAAKL	KPR

Figure 5 The amino-acid sequences of ghrelin from some fish species and from a mammal (pig). Dash denotes the same amino acid in this position as in ghrelin from zebrafish (*Danio rerio*); dots denote presumed amino-acid deletions in the peptide sequence during evolution. Note the large differences between teleost, elasmobranch, and mammalian sequences.

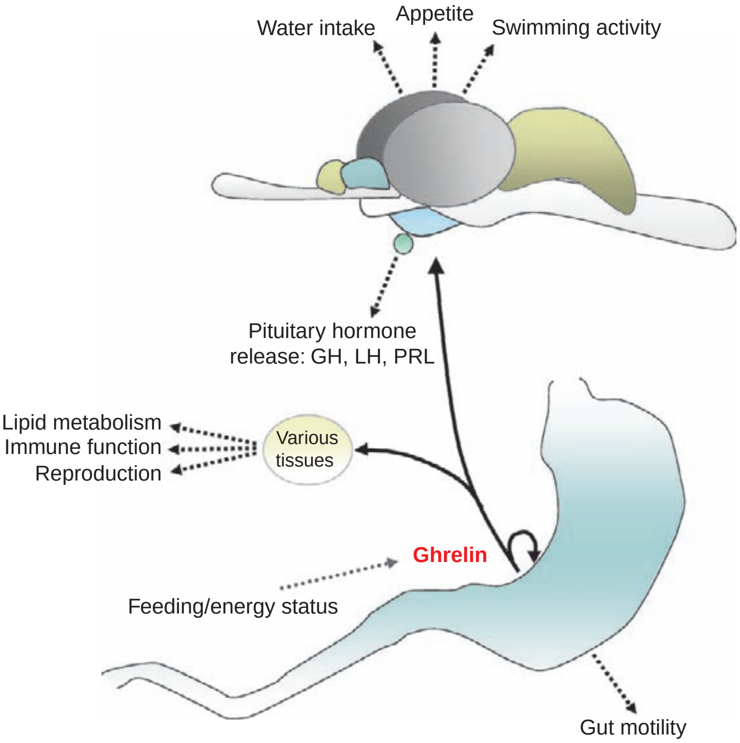


Figure 6 Overview of the effects of ghrelin (hatched black arrows) in fish. Ghrelin released into the circulation potentially acts on various target tissues (black solid lines). The functions of ghrelin as a growth-hormone secretagog and a modulator of food intake are so far the most well studied, but ghrelin may also play a role in several other processes. Both short- and long-term energy status influence plasma ghrelin levels and gut ghrelin mRNA expression (dotted gray arrow).

systemic effects on pituitary GH release in fish. In some fish species, ghrelin also stimulates prolactin and luteinizing-hormone (LH) secretion. Less is known about the effects of ghrelin on metabolism in fish but, as in humans and rodents, it inhibits the breakdown of fat and stimulates lipogenesis in tilapia. It also stimulates intestinal motility in zebrafish. This is consistent with the distribution of ghrelin receptor-like immunoreactivity in the gut muscle layers and with results from other vertebrates. Ghrelin also is implicated having a role in many other processes in fish such as in the control of immune function, reproduction, and water intake.

Des-acyl ghrelin, ghrelin without the fatty acid modification of the third amino acid (**Figure 5**), also is present in the circulation of mammals and has some biological actions. Des-acyl ghrelin has not been characterized in fish but inhibits ghrelin-induced food intake in goldfish.

In 2005, a new putative hormone obestatin, encoded by the ghrelin gene, was found in rat. An ortholog of the mammalian obestatin gene exists in sea bream, but the obestatin protein has not yet been isolated in a nonmammalian vertebrate. Ghrelin also has structural resemblance to motilin, another gastric peptide, and these two hormones may have evolved from a common ancestral peptide. Although motilin stimulates gastrointestinal motility in zebrafish, only a few motilin-immunoreactive cells are present in the gut (**see also Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism).

Leptin's Role in the Gut Is Not Yet Known in Fish

It was not until 2005, more than ten years after its discovery in obese mice, that leptin was characterized in a fish species, the pufferfish (*Takifugu rupripes*). Leptin plays a key appetite-inhibiting role in the control of food intake, but it is also involved in the regulation of gut proliferation, nutrient absorption, and gut motility in mammals.

Leptin is primarily produced by adipocytes in mammals, and in minor amounts in other tissues. The primary source of circulating leptin in fish is not clarified, and it is likely that it differs from mammals since fish store lipids to a larger extent in several tissues, but its presence in gut endocrine cells has been reported in representatives from all vertebrate groups. Leptin release is affected by nutritional status and CCK. One problem in fish studies is that, generally, antisera raised against mammalian leptin have been used to detect fish leptin. In light of the fact that the amino-acid-sequence similarity between human and different teleost leptin is only 16–25%, the presence of leptin-immunoreactive cells in different tissues should be verified using species-specific antibodies. Leptin mRNA expression has been demonstrated in the gut of zebrafish and pufferfish, but attempts to detect it in rainbow trout gut have so far failed. What type of gut cells in addition to endocrine cells express leptin remains to be clarified, together with its gastrointestinal functions in fish, which are still virtually unknown.

Concluding Remarks

There is a rich diversity of gut hormones, often with profound systemic effects in the whole fish as well as having important roles in local control of gastrointestinal functions. Often the identity, function, and distribution of the gut hormones are conserved among vertebrates, but comparative research also highlights that there are differences between fish and especially mammals, and also among fish. For example, gastrin stimulates gastric-acid secretion in most investigated vertebrates, but in Atlantic cod, gastrin inhibits acid secretion. Similarly, GLP-1 has opposite effects on blood glucose levels in fish and mammals. Ghrelin stimulates appetite in goldfish and tilapia, but suppresses appetite in rainbow trout, and it has species-dependent effects on the release of various pituitary hormones. Furthermore, both ghrelin and CCK levels show variable species-dependent responses to food deprivation. This reflects the great diversity in this vertebrate group and the complex interactions between environmental/seasonal, nutritional, and hormonal factors.

See also: **Gut Anatomy and Morphology**: Gut Anatomy; Pancreas. **Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism. **Integrated Function and Control of the Gut**: Gut Motility; Intestinal Absorption; Nervous System of the Gut. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Aldman G and Holmgren S (1995) Intraduodenal fat and amino acids activate gallbladder motility in the rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology* 100: 27–32.

Anderson C and Campbell G (1988) Immunohistochemical study of 5-HT-containing neurons in the teleost intestine: Relationship to the presence of enterochromaffin cells. *Cell and Tissue Research* 254: 553–559.

Bomgren P and Jönsson AC (1996) Basal, H2-receptor stimulated and pH-dependent gastric acid secretion from an isolated stomach mucosa preparation of the Atlantic cod, *Gadus morhua*, studied using a modified pH-static titration method. *Fish Physiology and Biochemistry* 15: 275–285.

Conlon JM and Larhammar D (2005) The evolution of neuroendocrine peptides. *General and Comparative Endocrinology* 142: 53–59.

Holmgren S and Holmberg A (2005) Control of gut motility and secretion in fasting and fed nonmammalian vertebrates. In: Starck JM and Wang T (eds.) *Physiological and Ecological Adaptations to Feeding in Vertebrates*, ch.13, pp. 325–355. Enfield, NH: Science Publishers.

Holmgren S and Olsson C (2009) The neuronal and endocrine regulation of gut function. In: Bernier NJ, Van Der Kraak G, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 28: Fish Neuroendocrinology*, pp. 467–512. Burlington: Academic Press.

Holstein B (1982) Inhibition of gastric acid secretion in the Atlantic cod, *Gadus morhua*, by sulphated and desulphated gastrin, caerulein, and CCK-octapeptide. *Acta Physiologica Scandinavica* 114: 453–459.

Jönsson E, Forsman A, Einarsdottir IE, Egnér B, Ruohonen K, and Björnsson BTh (2006) Circulating levels of cholesystokinin and gastrin-releasing peptide in rainbow trout fed different diets. *General and Comparative Endocrinology* 148: 187–194.

Kaiya H, Miyazato M, Kangawa K, Peter RE, and Unniappan S (2008) Ghrelin: A multifunctional hormone in non-mammalian vertebrates. *Comparative Biochemistry and Physiology* 149A: 109–128.

Mulero I, Pilar Sepulcre M, Meseguer J, García-Ayala A, and Mulero V (2007) Histamine is stored in mast cells of most evolutionarily advanced fish and regulates the fish inflammatory response. *Proceedings of the National Academy of Sciences* 104: 19434–19439.

Murashita K, Kurokawa T, Nilsen TO, and Rønnestad I (2009) Ghrelin, cholecystokinin and peptide YY in Atlantic salmon (*Salmo salar*): Molecular cloning and tissue expression. *General and Comparative Endocrinology* 160: 223–235.

Volkoff H, Unniappan S, and Kelly SP (2009) The endocrine regulation of food intake. In: Bernier NJ, Van Der Kraak G, Farrell AP, and Brauner CJ (eds.) *Fish Physiology, Vol. 28: Fish Neuroendocrinology*, pp. 422–467. Burlington: Academic Press.

THE GUT

Osmotic, Ionic and Nitrogenous–Waste Balance

Contents

Osmoregulation in Fishes: An Introduction
Mechanisms of Gill Salt Secretion in Marine Teleosts
Mechanisms of Ion Transport in Freshwater Fishes
Water Balance and Aquaporin
Osmosensing

Osmoregulation in Fishes: An Introduction

DH Evans, University of Florida, Gainesville, FL, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Paleoecology of Fishes
Osmoregulation in Marine Fishes
Osmoregulation in Freshwater Fishes
The Origin of Ionic Uptake Systems in the Early Vertebrates
Why Aren't All Fishes Euryhaline?
Further Reading

Glossary

Diffusion Net movement of a solute from an area of higher concentration to an area of lower concentration.

Glomerulus Tuft of capillaries surrounded by the Bowman's capsule of the vertebrate kidney where blood is filtered to produce urine in the proximal tubule.

Hyperosmotic Containing a greater concentration of solutes than another solution. If the solutions are separated by a permeable membrane (such as gill epithelium), solute will diffuse from a hyperosmotic to a hyposmotic solution and water will move from the hyposmotic to hyperosmotic solution.

Hyposmotic Containing a lesser concentration of solutes than another solution.

Isosmotic Containing the same total solute concentration as another solution. If the solutions are separated by a permeable membrane, there will be no net movement of either solute or water from one solution to the other.

Osmoregulation The maintenance of consistent cellular or organismal fluid composition and volume.

Osmosis Essentially diffusion for water molecules. As with diffusion for dissolved solutes, water moves due to random thermal oscillations, in a net sense, from where there is a higher water concentration (amount per unit volume) to where there is a lower water concentration. This is generally inversely related to the situation for dissolved solutes, as higher levels of these occupy space leaving less space for water and reducing its concentration. Water therefore (again in a net sense) moves from where the osmolality is lower to where it is higher (the opposite of what occurs for the diffusion of solutes).

Introduction

Osmoregulation is a fundamental process of living systems, equivalent in importance to respiration, digestion, or reproduction. Osmoregulatory processes are those that enable a fish to maintain its cellular fluid composition and volume. This is of critical importance because the protein-based processes of cells are sensitive to cytoplasmic ionic concentrations, and cell membranes tolerate relatively small deviations in cell volume (they will either collapse or explode). While terrestrial organisms are continually faced with the problem of dehydration, fishes are surrounded by water, and the water can tend to either dehydrate (in seawater, SW) or hydrate them (in freshwater, FW).

The differences in the ionic and total osmotic concentrations of the water and those of the fish body fluids determine the magnitude and the direction of ion (diffusion) and water (osmosis) movements across the fish gill epithelium. Fishes can limit ion and water exchanges by creating barriers at the skin surface (e.g., scales and mucus layers) that limit permeability. However, they still possess gills that are specialized for gas exchange (large surface area, thin, highly vascularized), which remains an excellent site for osmotic movement of ions and water. In fact, when oxygen and carbon dioxide diffusion across the gills increases during activity, ion and water diffusion increases too. This creates a problem for a fish called the osmorespiratory compromise (**see also Role of the Gills**: The Osmorespiratory Compromise). Therefore, post-exercise fish not only have to rectify the depletion of oxygen stores, but they must also deal with ions and water they may have lost or gained as a result of breathing more! When terrestrial animals exercise, they only lose water via their respiratory surface.

With the exception of the hagfishes, the ionic concentrations of all fish plasma are intermediate between SW and FW levels (**Table 1**). Consequently, excluding hagfishes, all fishes must osmoregulate because they usually live in either FW (hyposmotic to fish plasma) or SW (hyperosmotic to fish plasma). Hagfishes are exceptional because their plasma has similar ionic and osmotic concentrations as the SW environment (termed isosmotic), and osmoregulation is not necessary. Animals that maintain their blood plasma isosmotic with the environment are able to save metabolic energy, because life in nonisosmotic solutions requires compensatory transport of solutes and water by cell membranes, epithelial tissues, or specific organs and these processes are energy demanding (variously estimated at 2–8% of resting metabolic rate).

The differences in the ionic (and total osmotic) concentrations between SW and FW are extreme (1000 times in most cases), by comparison with the difference in plasma ionic concentrations among fish (only ~3 times). Most fishes are adapted to either FW or SW, not both. Fishes that are only able to osmoregulate over a very narrow range of salinities are termed ‘stenohaline’, those that can tolerate a wide range of salinities (e.g., both FW and SW) are termed ‘euryhaline’. Freshwater fishes can often tolerate salinities up to isosmolarity and marine species can often tolerate salinities down to isosmolarity. Euryhaline species can osmoregulate on both sides of the isosmolarity point. Estuarine environments provide a full range of salinities in which many fish live; isosmolar habitats may exist here too. Very few fish species are adapted to hypersaline conditions. (**see also Intertidal Fishes**: Intertidal Habitats).

Table 1 Ionic compositions of plasma in fishes in SW and FW

Salinity or species	*Total osmolarity*[a]	Na^{+}[b]	Cl^{-}	K^{+}	Ca^{2+}	Mg^{2+}	SO$_4^{2-}$	Urea	TMAO
Seawater	1050	439	513	9.3	9.6	50	26		
Hagfish	1035	486	508	8.2	5.1	12	3		
Anadromous lamprey	333	156	159	5.6	3.5	7.0			
Euryhaline bull shark	1067	289	296	5.8	4.4	1.8		370	47
Dogfish shark	1118	255	241	6.0	5.0	3.0	0.5	441	72
Euryhaline Atlantic stingray	953	319	295					330	
Euryhaline steelhead trout	325	153	135	4.0	1.4	0.65			
Euryhaline puffer	363	179	144	2.8	3.1	1.8			
Freshwater (soft)	1.0	0.25	0.23	0.01	0.07	0.04	0.05		
Anadromous lamprey		112	99.6	2.3	1.8	1.5			
Landlocked lamprey	272	120	104	3.9	2.5	2.0			
Euryhaline bull shark	595	221	220	4.2	3.0	1.3		151	19
Euryhaline Atlantic stingray	621	212	209					196	
Freshwater stingray	319	178	146					1.22	
Gar		159	133	4.2	6.1	0.3			
Bowfin	279	133	110	1.5					
Carp	274	130	125	2.9	2.1	1.2			
Euryhaline steelhead trout	260	153	133	3.8	1.4	0.5			
Euryhaline puffer	346	166	128	3.0	3.5	1.3			

[a] mOsm.l^{-1}.

[b] mM.l^{-1} or mM.kg^{-1}.

Modified from Evans DH and Claiborne JB (2009) Osmotic and ionic regulation in fishes. In: Evans DH (ed.) Osmotic and Ionic Regulation: *Cells and Animals*, pp. 295–366. Boca Raton, FL: CRC Press.

Over 95% of the total solute concentration (32–35 parts per thousand, ‰) of SW is due to the salts listed in **Table 1**, but over 80 other elements and ions are present in measurable quantities. The same elements predominate in FW but at much lower concentrations and more variable compositions because of differences in input from the terrestrial substrates (**Table 1**). One of the more important variables in FW is the calcium concentration, which, although lower than that in SW, may affect the permeabilities (salt and water) of biological membranes, thereby indirectly influencing osmoregulation (see below).

Paleoecology of Fishes

Much can be learned about the possible evolutionary course of osmoregulatory strategies and mechanisms by comparing the plasma ionic compositions and habitats among the major phylogenetic fish groups. The finding that the plasma ionic concentration of all fishes (except for the hagfishes) is 30–40% that of SW has intrigued physiologists for over a century. Based upon the fossil record and the fact that the most primitive bony fishes (sturgeons, gar and bowfin, and bichirs) are FW fishes, and the fact that euryhaline marine species reduce their plasma salt concentrations as they enter lower salinities, it is generally agreed that ancestral fish species entered FW over 500 million year ago from a marine origin. Subsequent evolution of modern marine fishes (both bony and cartilaginous), as well as tetrapods (amphibians, reptiles, birds, and mammals), took place after this FW invasion. Extant lampreys, which are either FW or euryhaline migrants (FW to SW to FW) as they mature and reproduce (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and **Toxicology**: The Effects of Toxicants on Olfaction in Fishes), are probably modern descendents of these ancestral fish species. The stenohaline, marine hagfishes are thought to be remnants of the ancestral, marine pre-vertebrates, largely because, like marine invertebrates and unlike any other chordates, they are ionically isosmotic to SW.

Osmoregulation in Marine Fishes

Hagfishes

Although hagfish plasma is isosmotic to SW, there are ionic differences, most notably greater Na$^+$ but lesser Cl$^-$, Mg^{2+}, and SO$_4{}^{2-}$ concentrations (**Table 1**). The mechanisms maintaining these ionic gradients are largely unknown, although the copious slime production by hagfishes may be an excretory pathway for at least the divalent ions (**see also Hagfishes and Lamprey**: Hagfish and Hagfish Slime). The slightly greater plasma Na$^+$, and lesser Cl$^-$ concentration may be secondary to the presence of gill Na$^+$/H$^+$ and Cl$^-$/HCO$_3^-$ exchangers used in net acid secretion for acid–base regulation or because of the charge distribution secondary to plasma organics or excretion of unwanted divalent ions (e.g., Ca^{2+} or Mg^{2+}), but these propositions are largely untested.

Teleosts

Marine teleosts face dehydration and net ionic loading because they are hyposmotic to SW (**Table 1**). In the 1930s, two pioneers in fish osmoregulation (Homer Smith and Ancel Keys) demonstrated that teleosts ingest SW to offset the osmotic loss of water and excrete the excess NaCl, using the gills, rather than the kidneys (as mammals and birds do). Specialized 'chloride' cells (now termed mitochondrion-rich cells, MRCs) were described in the marine fish gill by Keys, and substantial evidence now exists that MRCs are the site of NaCl extrusion by the marine teleost gill epithelium (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). The ingested divalent ions (Ca^{2+}, Mg^{2+}, and SO$_4{}^{2-}$) are excreted by the intestine and kidneys (**Figure 1**). In fact, the ingested

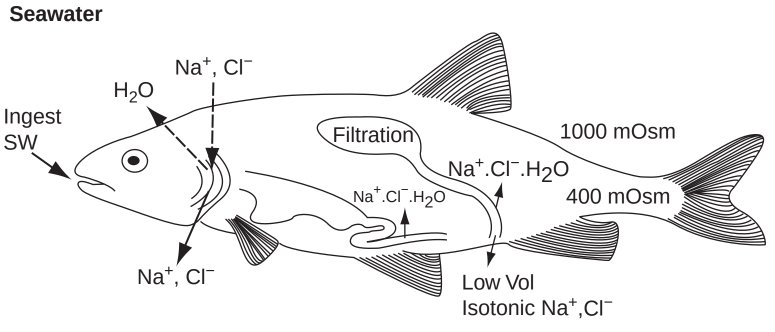


Figure 1 General pattern for SW teleost osmoregulation. From Evans DH (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys? *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 295: R704–R713.

Ca^{2+} is precipitated out as a bicarbonate salt, which is excreted and actually appears to play a significant role in marine carbon cycling (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation). The urine flows of marine teleosts are quite low, and there are some marine teleosts that have secondarily 'lost' the blood-filtering renal glomerulus and produce urine by ionic secretion in the proximal tubule (**see also Role of the Kidneys**: The Kidney). Indeed, it was investigations of these aglomerular species that provided direct evidence for proximal tubular ionic secretion in the vertebrate kidney (including mammals), which was controversial until the aglomerular teleost species were discovered.

Elasmobranchs

The marine sharks, skates, and rays have evolved very different mechanisms of osmoregulation, and it is not clear why there is such disparity between the teleost and elasmobranch mode of marine osmoregulation. This is a good case of 'divergent evolution'. Elasmobranch plasma contains exceedingly high concentrations of the organic solutes urea and trimethylamine oxide (TMAO; **Table 1**). Indeed, the urea concentration in these fishes is 5–10 times higher than coma-producing levels in mammals. Importantly, it is the relatively high TMAO concentrations that counteract the toxic effects of the extraordinarily high urea concentrations in elasmobranchs (**see also Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations).

Elasmobranchs do not ingest appreciable volumes of SW because they are slightly hyperosmotic to SW, so there is no osmotic loss of water across the gills. In fact, the osmotic uptake of water is balanced by proportional urine excretion in elasmobranchs, produced by glomerular filtration and, relatively unstudied, tubular secretory and reabsorptive processes in the exceedingly complex elasmobranch renal nephrons. There are also ionic gradients producing a net, diffusional gain of NaCl, which is offset by extra-renal and extra-branchial salt secretion by a specialized rectal gland that secretes a hypersaline solution into the cloaca (**see also Role of the Gut**: Dogfish Rectal Gland). The rectal gland has extremely high concentrations of the ubiquitous ion transport enzyme, Na^+/K^+-activated ATPase, which plays a central role in the mechanisms of NaCl secretion by this gland, as well as by the teleost gill epithelium (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). In fact, the transporting cell in the shark rectal gland looks very much like the teleost gill MRC. MRCs are also found in the elasmobranch gill epithelium, but there is no evidence that they function in net salt secretion. It is apparent that the elasmobranch gill MRC function in acid–base regulation by secreting variable amounts of H^+ versus HCO_3^-.

Lampreys

Lampreys are anadromous species that breed in FW; the resulting larval forms (ammocoetes) metamorphose after a few years into juveniles that migrate downstream and live most of their adult life in the marine environment (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development). Because the marine adults are so rarely captured, and the upstream migrating, premating adults have lost the ability to osmoregulate in SW, little is known about the mechanisms for osmoregulation in SW lampreys (**see also Hagfishes and Lamprey**: Lampreys: Environmental Physiology). Their plasma is hyposmotic to SW (**Table 1**), so studies have found that, like teleosts, they ingest SW, and presumably excrete the excess NaCl across the gill, because the kidney cannot produce urine that has greater NaCl concentrations than the plasma. The branchial epithelium has MRCs, but no functional or molecular studies have been published to support the logical suggestion that the pathways and mechanisms for gill salt extrusion are the same as have been well documented in teleosts.

Osmoregulation in Freshwater Fishes

Teleosts

Freshwater teleosts are hyperosmotic to their environment (**Table 1**) and therefore face a net influx of water and loss of NaCl across their branchial epithelium. They balance the osmotic influx by producing a much larger volume of urine than their marine relatives, with a much lesser ionic concentrations to conserve salt. Nevertheless, renal loss of salt adds to the diffusional loss, and must be offset by some uptake mechanism (**Figure 2**). Some salt may be gained by ingestion of food, but this has rarely been quantified and would limit osmoregulation during periods of low food availability. In the 1930s, August Krogh provided direct evidence for the independent uptake of Na^+ and Cl^- by a variety of freshwater animals, including fishes. He also proposed that, in order to avoid bioelectrical problems, it was likely that the uptake of each ion was in exchange for a counter-ion that was relatively abundant in the plasma: H^+ (or NH_4^+) and HCO_3^-, respectively. Subsequent studies have confirmed this proposition, although the actual cell and pathways are still under debate, and may be species specific. It is probable that MRCs are involved, and most species that have been studied possess MRC subtypes that may have separate functions (**Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes). The current debate centers on whether the

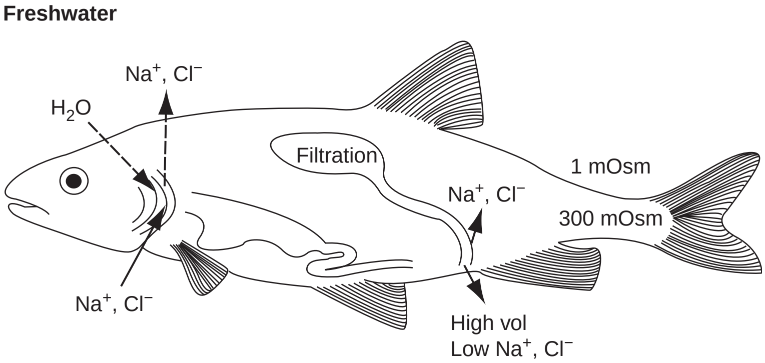


Figure 2 FW teleost osmoregulation.. From Evans DH (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys? *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 295: R704–R713.

Na^+/H^+ exchange is via an electroneutral chemical linkage (e.g., via a class of proteins termed Na-H exchangers (NHEs), which mediate this exchange in other tissues) or via an electrogenic proton pump (H-ATPase) which produces a cytoplasmic negativity that draws in Na^+ from the freshwater via a Na^+ channel. There is also emerging evidence that a coupled transport of Na and Cl may play some role in this uptake process.

Elasmobranchs

Freshwater elasmobranchs are rare; the most notable example is the euryhaline bull shark (*Carcharhinus leucus*) that can migrate into freshwater in Central America, Australia, and India (and even Illinois, where one was captured in the Mississippi River 3800 km from the Gulf of Mexico). Another example is the Atlantic stingray (*Dasyatis sabina*) that has a resident population in lakes in Central Florida. The only truly freshwater stingray, *Potamotrygon* sp., is found in tributaries of the Amazon and Orinoco Rivers in South America. The euryhaline species all display reduced plasma NaCl and urea levels when captured in lowered salinities, and *Potamotrygon* plasma contains nearly no urea (**Table 1**). Indeed, *Potamotrygon* can neither increase the plasma urea concentrations nor tolerate substantial increases in salinity. The rates of urea synthesis are much lower than in marine elasmobranchs, and the inability to increase plasma urea concentrations may also be associated with the loss of specific tubules in the kidney. These tubules have been implicated in renal reabsorption of urea filtered from the plasma. As might be expected, the rectal gland is much reduced in freshwater elasmobranchs, and the osmotic uptake of water is balanced by excretion of extremely high volumes of dilute urine. The diffusional, renal, and small rectal gland loss of salts must be balanced by branchial uptake. At least in *D. sabina*, the extraction of NaCl from the hyposmotic environment is mediated by two cells that have the apical proteins necessary for independent Na^+ and Cl^- uptake.

Lampreys

Larval, juvenile, and adult lampreys in freshwater show much reduced plasma ionic concentrations compared to the marine adults (**Table 1**). The osmotic uptake of water is balanced by production of large volumes of very dilute urine. The renal and diffusional loss of ions is offset by active branchial epithelial uptake via what appears to be two types of freshwater MRCs that disappear as the lamprey migrates into SW and reappear when the adults return to FW. These cells are thought to extract needed NaCl via a combination of the ionic transporters proposed for freshwater teleosts (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes, **Cellular, Molecular, Genomics, and Biomedical Approaches**: Physiology of Triploid Fish; Mechanisms of Ion Transport in Freshwater Fishes; **Hagfishes and Lamprey**: Lampreys: Environmental Physiology).

The Origin of Ionic Uptake Systems in the Early Vertebrates

Despite the uncertainty of the specific transport pathways that mediate NaCl uptake in freshwater fishes, it is clear that the evolution of this system was critical for the entry of primitive marine fishes into the freshwater environment nearly 500 million years ago. Where did it come from? Unlike mammals, fishes cannot substantially alter their blood pH by changes in renal secretion of H^+ or HCO_3^- or changes in respiratory excretion of CO_2. Thus, it has been found that acid–base regulation in fishes is mediated by differential excretion of H^+ or HCO_3^- by the gill epithelium (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation). The demonstration that marine teleosts, elasmobranchs, and even hagfishes use these gill transport mechanisms for acid–base regulation led to the proposition that the evolution of ionic exchange systems that could excrete blood H^+ and HCO_3^- in exchange for external Na^+ and Cl^- predated the evolutionary entrance into freshwater. Thus, the origin of ionic exchange mechanisms for acid–base regulation presumably afforded a pre-adaptation for ionic regulation in reduced salinities.

Why Aren't All Fishes Euryhaline?

If the gill epithelium of all fishes, including hagfishes, possess the ionic exchange systems (for acid–base regulation) that potentially can be used for ionic uptake in freshwater, why aren't all fishes euryhaline? One might propose that intestinal and renal functions are limiting, but these are usually quantitative rather than qualitative differences. Presence of a putative ionic uptake system is qualitative; without it, freshwater osmoregulation is not possible. It appears that the limiting factor for euryhalinity is the efficiency of the ionic uptake systems versus the ionic permeability of the branchial epithelium itself. The best example of this relationship is the finding, nearly 80 years ago, that normally stenohaline marine reef fishes (e.g., angel fish, puffer, gray snapper, and horse-jack) are found in freshwater lakes in the Bahamas and springs in western Florida (e.g., Homosassa Springs). Because these water bodies have relatively high concentrations of Ca^{2+} (studies have shown that it can decrease the passive loss of ions from fishes), it has been suggested that one of the primary limiting factors for invasion into freshwater is the water calcium concentrations and the overall permeability of the gill.

See also: **Hagfishes and Lamprey**: Hagfish; Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes; Mechanisms of Gill Salt Secretion in Marine Teleosts. **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations. **Role of the Gills**: The Osmorespiratory Compromise. **Role of the Gut**: Dogfish Rectal Gland; Gut Ion, Osmotic and Acid-Base Regulation. **Role of the Kidneys**: The Kidney. **The Skin**: Hagfish Slime. **Toxicology**: The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Bartels H and Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462.

Beyenbach KW (2004) Kidneys sans glomeruli. *American Journal of Physiology, Renal Physiology* 286: F811–F827.

Carrier JC and Evans DH (1976) The role of environmental calcium in freshwater survival of the marine teleost, *Lagodon rhomboides*. *Journal of Experimental Biology* 65: 529–538.

Claiborne JB, Edwards SL, and Morrison-Shetlar AI (2002) Acid–base regulation in fishes: Cellular and molecular mechanisms. *Journal of Experimental Zoology* 293: 302–319.

Evans DH (1984) Gill Na/H and Cl/HCO_3 exchange systems evolved before the vertebrates entered fresh water. *Journal of Experimental Biology* 113: 464–470.

Evans DH (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys? *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 295: R704–R713.

Evans DH and Claiborne JB (2009) Osmotic and ionic regulation in fishes. In: Evans DH (ed.) *Osmotic and Ionic Regulation: Cells and Animals*, pp. 295–366. Boca Raton, FL: CRC Press.

Evans DH, Piermarini PM, and Choe KP (2004) Homeostasis: Osmoregulation, pH regulation, and nitrogen excretion. In: Carrier J, Musick J, and Heithaus J (eds.) *Biology of Sharks and Their Relatives*, pp. 447–475. Boca Raton, FL: CRC Press.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Marshall WS and Grosell M (2006) Ion transport, osmoregulation and acid–base balance. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, pp. 177–230. Boca Raton, FL: CRC Press.

Olson KR (1999) Rectal gland and volume homeostasis. In: Hamlett WC (ed.) *Sharks, Skates, and Rays*, pp. 329–352. Baltimore, MD: Johns Hopkins University Press.

Parks SK, Tresguerres M, and Goss GG (2007) Blood and gill responses to HCl infusions in the Pacific hagfish (*Eptatretus stoutii*). *Canadian Journal of Zoology* 85: 855–862.

Silva P, Solomon RJ, and Epstein FH (1996) The rectal gland of *Squalus acanthias*: A model for the transport of chloride. *Kidney International* 49: 1552–1556.

Wilson RW, Millero FJ, Taylor JR, *et al.* (2009) Contribution of fish to marine inorganic carbon cycle. *Science* 323: 359–362.

Mechanisms of Gill Salt Secretion in Marine Teleosts

DH Evans, University of Florida, Gainesville, FL, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Mitochondrion-Rich Cell
Mechanisms of NaCl Transport across the MRC
Molecular Evidence for Routes of NaCl Transport
Further Reading

Glossary

Cystic fibrosis transmembrane conductance regulator (CFTR) The chloride channel that allows the passive movement of Cl^- down its electrochemical gradient from the cytoplasm to the outside of a variety of cells. Mutations in the gene for CFTR produce the human disease called cystic fibrosis.

Electrical gradient The gradient of electrical charge across a plasma or epithelial membrane. Positive ions will move from areas of relative positivity to areas of relative negativity; negative ions will move in the opposite direction.

Electrochemical gradient A difference in concentration and charge between two solutions separated by a semi-permeable membrane, including a plasma epithelial membrane, that produces a net movement of an ion by diffusion.

Epithelium A relatively flat tissue (composed of one or a few cells layers) that lines cavities and surfaces in multicellular organisms.

Immunohistochemistry Cellular or subcellular localization of specific molecules by staining them with antibodies labeled with a fluorescent or pigmented material.

Messenger RNA (mRNA) transcripts Messenger RNA sequences coding for proteins specific to a given cell or tissue.

Microvilli Plasma-membrane-bound, cellular extensions that often characterize epithelial cells. They provide for an elaboration of the surface, plasma membrane, and are common, for instance, in intestinal cells that absorb nutrients.

Mitochondrion A cellular organelle that acts as the 'powerhouse' of the cell and produces the chemical energy source adenosine triphosphate, using oxygen as the final electron acceptor.

Mitochondrion-rich cell Cell in the gill epithelium of fishes that has many mitochrondria on the extensive basolateral membrane and expresses relatively high levels of the transport enzyme Na^+–K^+-activated ATPase.

NKCC An ion-translocating enzyme that utilizes a sodium gradient to transport Na^+, K^+, and Cl^- into the cell.

Operculum The covering over the gill chamber of a fish. In most species, movement of the operculum is part of the respiratory cycle.

Osmotic or ionic gradient The concentration gradient of either water or a specific ion. Both water and ions usually flow down their respective gradients.

Introduction

The NaCl and total salt concentration of the plasma of marine teleosts are distinctly below that measured in seawater (specifically table 1 from **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). This generates both osmotic and ionic gradients across the permeable gill epithelium, leading to potential salt loading and dehydration of the fish. The osmotic loss of water is balanced by oral ingestion of seawater, but this adds to the salt loading of the fish and the need to excrete even more salt. Contrary to mammals, marine teleosts are unable to produce urine that is more salty than the blood. Instead, the pathways for salt secretion that have evolved in fishes are extra-renal. In teleost fishes, these pathways are located in the epithelium of the gills (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy and **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics), which contains the requisite cell membrane transport proteins and channels that are remarkably similar to those found in a variety of transporting tissues throughout the animal kingdom.

The Mitochondrion-Rich Cell

The teleost gill epithelium is composed of a variety of specialized cells, including pavement cells (PVCs), which predominate (~90%), and a smaller population (~10%) of cells that contain extraordinary numbers of mitochondria and are therefore termed 'mitochondrion-rich cells' or MRCs. Next to the MRCs are accessory cells (ACs), which may be immature MRCs. In the seawater epithelium, the MRCs and adjacent ACs usually form clusters that open into an apical crypt, overlain by surrounding PVCs (**Figures 1**(**a**) and **1**(**c**)). This is to be contrasted with the gill epithelium of freshwater teleosts, which is characterized by the lack of apical crypts, and MRC microvilli protruding between the overlying PVCs (**Figures 1**(**b**) and **1**(**d**)). The MRCs are usually on the trailing edge of gill filaments in both marine and freshwater teleosts, and also may appear on the gill lamellae in some freshwater species.

Fortunately for physiologists, the skin lining the inner surface of the operculum in a few species, most notably the killifish, *Fundulus heteroclitus*, contains about 60% MRCs on a flat epithelium. This opercular epithelium has provided the tissue that has been used to delineate much of what we know about the actual pathways for NaCl secretion by the fish gill, which is a much more structurally complex tissue (**see also Role of the Gills**: Morphology of Branchial Ionocytes). Using the opercular epithelium, research has made clear that the MRCs are the transporting cells because their number correlates well with the rate of transport across the epithelium, and microprobe studies have shown that the current of transport is directly over the MRC. An electron micrograph of the MRC (**Figure 2**) clearly shows why they are termed mitochondrion rich.

Numerous mitochondria line the tubules formed by invagination of the basolateral membrane. In fact, there is very little open cytoplasm in these cells, which has precluded some of the electrophysiological techniques that have been used to delineate the ionic transport steps in other epithelia, such as the vertebrate kidney, bladder, or intestine. For clarity, a single MRC is diagramed in **Figure 3**.

Mechanisms of NaCl Transport across the MRC

When the ubiquitous transport enzyme, $Na^{+}+K^{+}$-activated adenosine triphosphatase (NKA) was first described in the fish gill, it was assumed that it was probably on the apical surface of the MRC, and merely extruded cellular Na^{+} in exchange for seawater K^{+}. However, it was soon found that the NKA was on the basolateral surface, making it unable to directly excrete Na^{+} from the cell.

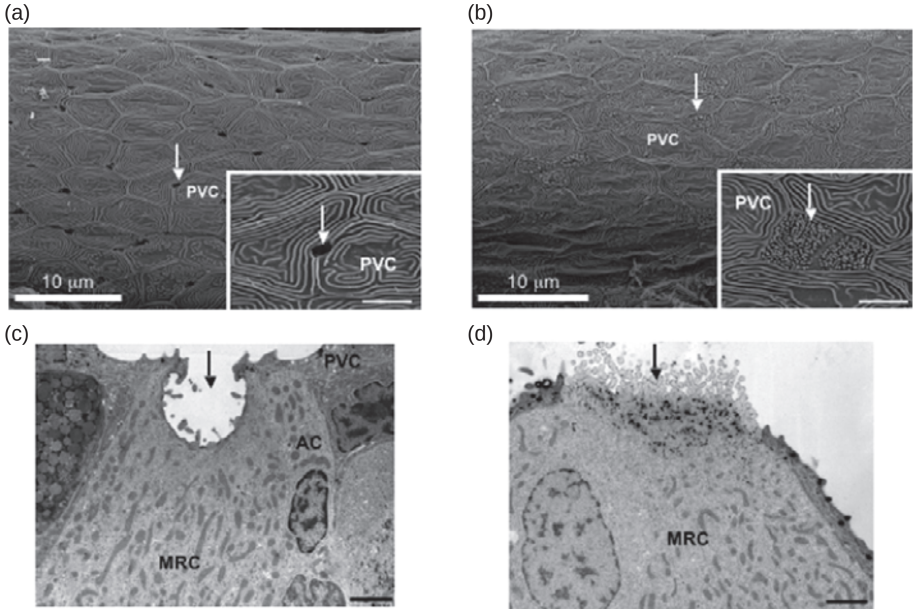


Figure 1 Scanning electron and transmission electron micrographs of gill filaments from killifish in seawater ((a) and (c)) and killifish transferred from seawater to freshwater after 30 days ((b) and (d)). Note transformation of the apical region of MRC (arrows) from a smooth, concave crypt that is recessed below the pavement cells (PVCs) ((a) and (c)) to a convex surface studded with microvilli that extend above the surrounding PVC ((b) and (d)). Also note that an accessory cell (AC) is not associated with the MRC from freshwater-acclimated killifish ((c) and (d)) and that the distinct, whorl-like microridges on the surfaces of PVC do not change with salinity ((a) and (c)). Scale = 1 um, in (c) and (d). From Katoh F and Kaneko T (2003) Short-term transformation and long-term replacement of branchial chloride cells in killifish transferred from seawater to freshwater, revealed by morphofunctional observations and a newly established 'time-differential double fluorescent staining' technique. *Journal of Experimental Biology* 206: 4113–4123; and modified from Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

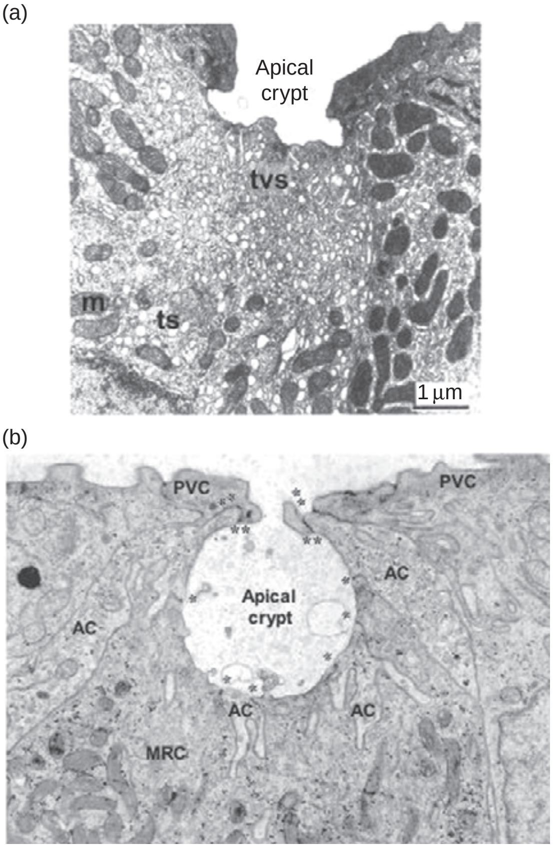


Figure 2 Transmission electron micrographs of MRC from the gills of seawater teleosts. (a) MRC from the sole (*Solea solea*) containing numerous mitochondria (m), a tubular system (ts), a subapical tubulovesicular system (tvs), and an apical crypt. ((b) Apical region of an MRC from the killifish. This MRC forms deep tight junctions (**) with surrounding PVC and shallow tight junctions (*) with surrounding accessory cells (AC), which share an apical crypt with the MRC. (a) From Evans (1993) *The Physiology of Fishes*. (b) Modified from Evans DH, Piermarini PM, and Potts WTW (1999) Ionic transport in the fish gill epithelium. *Journal of Experimental Zoology* 283: 641–652; and from Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Subsequent studies demonstrated that the extrusion of Na^+ and Cl^- was linked and became reduced by applying inhibitors of either NKA or the coupled transport of NaCl (via the co-transporter NKCC) to the basolateral side of the opercular epithelium. With the knowledge that both NKA and coupled NaCl transport were on the basolateral membrane, a model for the transepithelial transport of NaCl was proposed that involved basolateral uptake of NaCl, followed by apical extrusion of Cl^- through the same type of Cl^- channel (cystic fibrosis transmembrane conductance regulator (CFTR)) that is defective in the human disease – cystic fibrosis. Sodium is recycled back across the basolateral membrane and then moves down its electrochemical gradient between adjacent MRCs (through relatively leaky cell-to-cell protein strands) into seawater. Even though there is significantly more Na^+ in seawater than in fish extracellular fluids, there is an electrochemical gradient produced by the electrical potential (*c.* +20 mV, extracellular fluids vs. seawater) across the gill epithelium. The net result is the transepithelial secretion of NaCl (**Figure 4**). The K^+ that enters the MRC via the basolateral NKCC appears to be recycled back into the extracellular fluids via a K^+ channel that has recently been described.

Molecular Evidence for Routes of NaCl Transport

The revolution in the technologies of molecular biology has produced a variety of data supporting this model for NaCl extrusion. For instance, the messenger RNA (mRNA) transcripts for all the relevant transport proteins can be extracted from fish gill tissue, and antibodies produced against the proteins can be used in immunohistochemistry to localize CFTR to the apical membrane and NKA and NKCC to the basolateral membrane of the MRC. Moreover, as a fish acclimates to seawater, both the

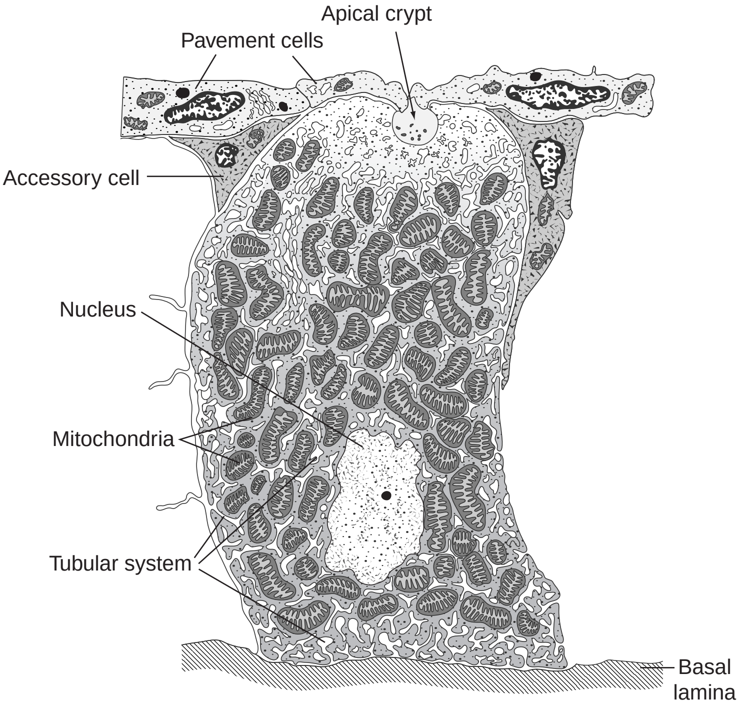


Figure 3 Diagram of an MRC from the opercular epithelium of the killifish. Note the numerous mitochrondria, extensive tubular system, apical crypt, PVC, and AC. From Degnan KJ, Karnaky KJ, Jr., and Zadunaisky J (1977) Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *Journal of Physiology, London* 271: 155–191.

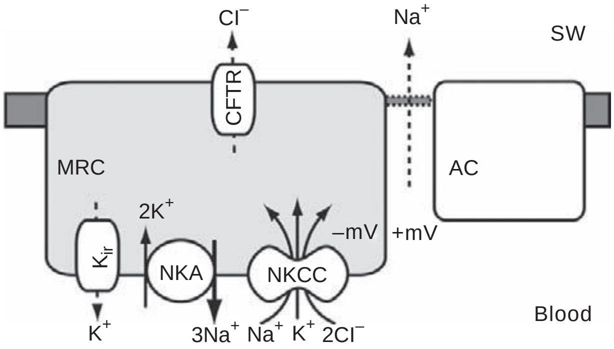


Figure 4 Current model for the transport of NaCl across the teleost MRC. See text for details. MRC cytoplasm negative to fish blood. mV, millivolt, From Evans DH and Claiborne JB (2009) Osmotic and ionic regulation in fishes. In: Evans DH (ed.) *Osmotic and Ionic Regulation: Cells and Animals*, pp. 295–366. Boca Raton, FL: CRC Press.

mRNA and protein for these transport proteins increase, and CFTR moves to the apical membrane. In some cases, the total NKA does not appear to increase, but this has been shown to be caused by the differential decrease of one isoform of the enzyme, which offsets the increase of another isoform. In fact, recent evidence suggests that there are distinct seawater versus freshwater forms of NKA, which may exist in different MRCs.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction. **Role of the Gills**: Morphology of Branchial Ionocytes. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Degnan KJ, Karnaky KJ, Jr., and Zadunaisky J (1977) Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *Journal of Physiology, London* 271: 155–191.

Evans DH (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys? *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 295: R704–R713.

Evans DH and Claiborne JB (2009) Osmotic and ionic regulation in fishes. In: Evans DH (ed.) *Osmotic and Ionic Regulation: Cells and Animals*, pp. 295–366. Boca Raton, FL: CRC Press.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Evans DH, Piermarini PM, and Potts WTW (1999) Ionic transport in the fish gill epithelium. *Journal of Experimental Zoology* 283: 641–652.

Hirose S, Kaneko T, Naito N, and Takei Y (2003) Molecular biology of major components of chloride cells. *Comparative Biochemistry and Physiology – Part B: Biochemical and Molecular Biology* 136: 593–620.

Karnaky KJ, Jr., Degnan KJ, Garretson LT, and Zadunaisky JA (1984) Identification and quantification of mitochondria-rich cells in transporting epithelia. *American Journal of Physiology* 246: R770–R775.

Katoh F and Kaneko T (2003) Short-term transformation and long-term replacement of branchial chloride cells in killifish transferred from seawater to freshwater, revealed by morphofunctional observations and a newly established 'time-differential double fluorescent staining' technique. *Journal of Experimental Biology* 206: 4113–4123.

Marshall WS and Grosell M (2006) Ion transport, osmoregulation and acid-base balance. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, pp. 177–230. Boca Raton, FL: CRC Press.

McCormick SD, Regish AM, and Christensen AK (2009) Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. *Journal of Experimental Biology* 212: 3994–4001.

Mechanisms of Ion Transport in Freshwater Fishes

P-P Hwang, Academia Sinica, Taipei, Taiwan, China

© 2011 Elsevier Inc. All rights reserved.

Introduction
Na$^+$ Uptake/Acid Secretion
Cl$^-$ Uptake/Base Secretion
Ca^{2+} Uptake
Different Subtypes of Ionocytes
Functional Regulation to Cope with Fluctuating Environments
Summary
Further Reading

Glossary

Co-transporter A transporter that is involved in simultaneous transport of two or more different molecules or ions across the cell membrane in the same direction.

Electrogenic (or electroneutral) transporter A transporter for which the transport pathway causes a change (or no change) in the electrical charge. In the case of passive diffusion, the transport is driven down an electrical (or chemical) gradient across the cell membrane.

Exchanger A transporter that is involved in counter-transport of two or more different molecules or ions across the cell membrane in the reverse directions.

Influx (or efflux) The movement of solute or solvent into (or out of) a cell across the cell membrane.

Ionocytes (mitochondrion-rich cells) A function-specific type of epithelial cell, which expresses ion transporters or enzymes and is responsible for transepithelial transport functions of specific ions. In fish gills, ionocytes responsible for ion regulation are named mitochondrion-rich cells based on the characteristic of abundant mitochondria.

Loss of function A gene mutation approach through which the product of the target gene has less or no function, compared to that of the wild type without mutation. In the present article, loss-of-function experiments were conducted by injecting fertilized zebrafish eggs with antisense morpholino oligomers. The morpholinos were designed and synthesized to knock down the function of a specific gene by blocking the protein translation of the target gene.

Morpholino A molecule used to knock down gene translation. This is achieved by blocking access of ribosomal initiation complex to a messenger RNA (mRNA) with modified antisense oligomers, and thus preventing from making a targeted protein.

Orthologs Genes, or peptides expressed by genes, in different species that have evolved from a common ancestral gene.

Scanning ion-selective electrode technique (SIET) A noninvasive electrophysiological approach, which uses ion-selective microelectrodes to measure the strength of specific ions on the surface of an intact organism (or cell). After taking SIET measurements, the intact organism can be subjected to subsequent analyses. SIET is a powerful *in vivo* approach for analyzing ion transport.

Transporter (or enzyme) isoforms Different forms of the same protein transporter (or enzyme), produced either from related genes, or from the same gene by alternative splicing.

Introduction

Freshwater (FW) fish inhabit aquatic environments that vary greatly in their ionic composition and pH (**Table 1**), but uniformly these environments have much lower concentrations of total salts than the fish's body fluids. As a result, all FW fishes are continuously subject to water gain and ion loss due to passive diffusion down osmotic and ionic gradients between the body fluids and FW environment (**Figure 1**). Without ion/osmoregulation, fish in FW would simply expand in volume and die.

Instead, to cope with living in a dilute environment, FW fishes have evolved highly efficient ion/osmoregulatory mechanisms for maintaining a constant internal ionic environment. In addition, their water content varies very little. They compensate for passive water uptake by not drinking water (or drinking very little), while the kidneys produce copious amounts of dilute urine that involves salt reabsorption. They compensate for ion loss by actively

Table 1 Major solutes and osmolarity of different freshwaters and carp plasma

	Na^+ (mM)	*Cl^- (mM)*	*K^+ (mM)*	*Mg^{2+} (mM)*	*Ca^{2+} (mM)*	*SO_4^{2-} (mM)*	*HCO_3^- (mM)*	*Osmolarity (mOsm)*
Soft lake water [a]	0.17	0.03	-	0.15	0.22	0.09	0.43	<1[d]
River water [b]	0.39	0.23	0.04	0.21	0.52	0.21	1.11	<3[d]
Hard river water [c]	6.13	13.44	0.11	0.66	5.01	1.40	1.39	<30[d]
Freshwater carp plasma [e]	130	125	2.9	1.2	2.1	-	-	274

[a] Lake Nipissing, Ontario.

[b] Mean composition of North American rivers.

[c] Tuscarawas River, Ohio.

[d] Sum of all solutes.

[a,b,c] Data from Schnidt-Nielsen K (1993) *Animal Physiology: Adaptation and Environment*, 4th edn. New York: Cambridge University Press.

[e] Data from Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiology Review* 85: 97–177.

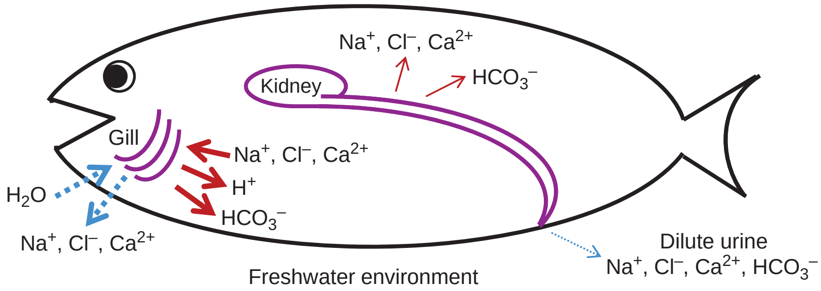

Na^+, Cl^-, Ca^{2+}
HCO_3^-
Kidney
Gill
Na^+, Cl^-, Ca^{2+}
H^+
HCO_3^-
H_2O
Na^+, Cl^-, Ca^{2+}
Freshwater environment
Dilute urine
Na^+, Cl^-, Ca^{2+}, HCO_3^-

Figure 1 Ion uptake and acid–base regulation mechanisms in freshwater fishes (for details refer to text). Passive water gain and ion loss (bold dashed arrow) mainly occur in the gills. For compensation, fishes produce dilute urine by salt reabsorption (thin solid arrow) in the kidneys to minimize ion loss (thin dashed arrow) and extrude excess water, while actively absorbing salt (bold solid arrow) from the environment through the gills. Internal pH homeostasis is mainly achieved by differentially regulating H^+ and HCO_3^- effluxes across the gills. The kidneys also contribute to the regulation of plasma HCO_3^- levels by HCO_3^- reabsorption (thin dashed arrow). Bold or thin arrow reflects the relative fluxes of ions.

extracting salts from external water across the gill epithelium (**Figure 1**). The gills are responsible for the majority of ion (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, etc.) uptake into the animal, which is mainly achieved through active transport by specific ion-transporting cell types, termed 'ionocytes'. The main ionocyte in the gill of the FW fish is the mitochondrion-rich (MR) cell, formerly called chloride cells (**Figure 2**), and there are several distinct types of these cells. This article reviews what is currently known about the mechanism of Na^+, Cl^-, and Ca^{2+} uptake at the gill and the specific ion transporter proteins or enzymes that move these ions from the environment to the internal body fluids (**Figure 2**).

In FW fish, the biochemical process involved in ionregulaiton (i.e., the maintenance of internal Na^+, Cl^-, Ca^{2+}, and Mg^{2+} levels) cannot be separated from the process of acid–base or pH regulation, because the two processes occur at the same site (the gill) and share common pathways. This is unlike the situation in air-breathing tetrapods, which regulate their internal acid–base status primarily through adjustments to ventilation (respiratory compensation) and urinary acid excretion (metabolic compensation). In contrast, the majority of fishes achieve internal pH balance by adjusting plasma HCO_3^- levels by varying the excretion (efflux) of H^+ and HCO_3^- across the gills (**Figure 1**). Moreover, H^+ and HCO_3^- efflux is mechanistically coupled to the influx of Na^+ and Cl^-, respectively, at the fish gill (**Figure 2**). The kidney of FW fish plays a minor role in maintaining whole-body pH balance relative to the gill.

In order to move charged ions from the water into the fish (or vice versa in the case of acid), the gills use a variety of transport proteins embedded within the membranes of gill ionocytes. These transport proteins first move ions from the fluid on one side of the cell, across a membrane into the cell, and then another set of proteins move the ions across the other membrane into the fluid on the other side of the cell. The cell membrane separating the inside of the gill from the water environment is called the 'apical membrane' and the cell membrane separating the cell from the blood or other cells inside the body of the fish is called the 'basolateral membrane'. The gill cells, like other animal cells, have a negative membrane potential (extracellular medium reference). In addition, the transepithelial potential difference (TEP), which is the voltage across an epithelial cell layer, is

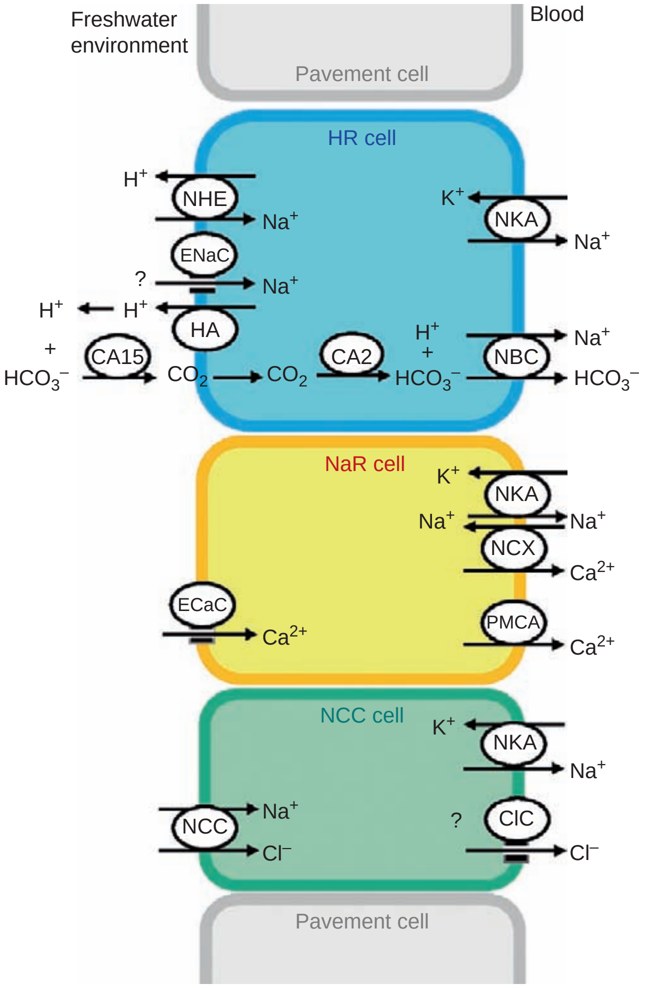


Figure 2 Model of the ion uptake and acid–base regulation mechanisms in zebrafish skin/gill ionocytes. Three types of ionocytes, H^+–ATPase-rich (HR) cells, Na^+–K^+–ATPase-rich (NaR) cells, and Na^+–Cl^- co-transporter (NCC) cells, express different sets of ion transporters and/or enzymes, and are respectively responsible for acid secretion/Na^+ uptake, Ca^{2+} uptake, and Cl^- uptake (for details refer to text). A question mark indicates an unidentified pathway.

generally between −5 and −40 mV (outside FW reference) for FW fish gill epithelia. The gill TEP is sensitive to environmental Ca^{2+} levels (+5 to +10 mV in hard FW) and this switch in polarity has substantial effects on the ion-regulatory pathways in the gill epithelia. These voltage gradients, like concentration gradients, influence how ions are transported across the gill epithelia.

Table 1 shows the considerable variation in the ion composition among different FW environments; pH of FW also varies due to acid rain, pollution, or the accumulation of naturally occurring tannins, especially in waters from peat bogs. Soft water, waters with poor buffering capacity, is especially vulnerable to acidification. The wide variation in ion composition of FW means that FW fish must be able to differentially modulate the uptake mechanisms for each ion to maintain plasma osmolarity and acid–base homeostasis. This is probably one of the reasons why the mechanisms of ion regulation in FW fish appear more intricate than those in saltwater fish and why there has been decades of debate on the precise nature of FW ion regulation (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). The following describes our current understanding.

Na^+ Uptake/Acid Secretion

Ionic gradients between FW environment and FW fish blood favor passive efflux of Na^+ (and other ions) down a concentration gradient, but typically opposed by the TEP. In fact, to maintain internal ionic homeostasis, FW fish gill ionocytes have to actively absorb Na^+ (and other ions) from the water against, in the case of Na, an ~1800-fold concentration gradient (calculated from **Table 1**). At these gill ionocytes, Na^+ uptake from the water is coupled to H^+ efflux from the blood. The gill ionocytes can secrete H^+ from the blood, which becomes acidified due to the metabolic/respiratory acidosis or the impacts of environmental pH fluctuations.

Early studies suggested two models for the apical transport of Na^+ and H^+ in fish ionocytes (**Figure 2**). The first model proposed that protons were actively pumped from the cell into the environment by an apical V-type of H^+-adenosine triphosphatase (ATPase) and that Na uptake in the opposite direction was accomplished by electrically linking the H^+ movement to Na^+ absorption through the apical membrane Na^+ channel (ENaC). The second model proposed an electroneutral exchange of Na^+ and H^+ via an apical Na/H exchange (NHE) protein. There has been much debate, which is far from over, about which model applies to different fish species and various experimental water conditions. According to most models, an apical membrane H^+-ATPase actively pumps H^+ from the gill ionocytes (efflux), which in turn generates a negative intracellular electrical potential. This negative intracellular electrical potential is a driving force to allow Na^+ entry from the water down an electrochemical gradient through the apical ENaC. Molecular physiological and pharmacological evidence supports the role of H^+-ATPases in these pathways; however, so far, ENaC-like proteins have not been identified in fish. On the other hand, theoretical calculation of the ratios of intracellular/environmental concentrations of Na^+ and H^+ suggests unfavorable thermodynamic conditions for the operation of an electroneutral apical NHE in most FW environments. However, other molecular and physiological studies suggest analogous Na^+ uptake/acid secretion mechanisms (**Figure 2**) to those in the mammalian kidney proximal tubular cells, which involve H^+-ATPase and NHE, rather than ENaC as suggested above. In this model, a water-facing membrane-bound carbonic anhydrase (CA) catalyzes the rapid condensation of HCO_3^- from the environment with H^+ secreted by the apical NHE or V-type H^+-ATPase, forming CO_2 and H_2O outside of the apical membrane of gill ionocytes. This mechanism facilitates the passive diffusion of CO_2 into ionocytes. Then, cytosolic CA hydrates CO_2 to form HCO_3^- and H^+. Protons are recycled back into the ambient environment by NHE and H^+-ATPase. This recycling mechanism serves to keep HCO_3^- high in the cell to assist with basolateral Na^+ transport. Basolateral Na^+/K^+-ATPase (NKA) pumps cytosolic Na^+ across the basolateral membrane and provides a negative intracellular potential to drive the electrogenic Na^+ transport of the basolateral Na^+–HCO_3^--co-transporter (NBC), a major basolateral transporter in mammalian proximal tubular cells.

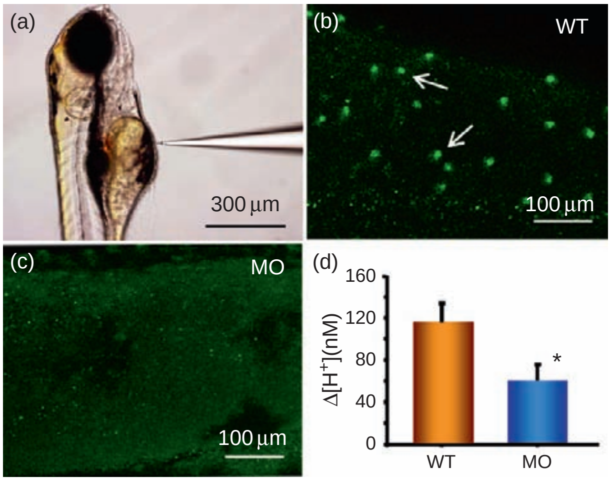


Figure 3 Loss-of-function experiments on zebrafish embryos injected with morpholino oligomers (MO) specific for H^+–ATPase. (a) An ion-selective microelectrode (SIET) approaches an intact embryo for SIET measurement of acid secretion function. (b, c) Compared to wild-type (WT) embryos, embryos injected with MO lost the protein signals of H^+–ATPase (arrow), indicating the effect of translational knockdown of the gene. (d) Knockdown of H^+–ATPase impaired the acid secretion function ($\Delta[H^+]$, difference from the background; measured by SIET, for details refer to text) in zebrafish skin. Mean ± SD ($n = 10$). *Significant difference (unpaired *t*-test, $p \leq 0.05$).

Recent studies in zebrafish (*Danio rerio*) provide the most convincing evidence to support the proximal tubular-like model of Na^+ uptake/acid secretion pathways in FW fish gills (**Figure 2**). These studies used a noninvasive scanning ion-selective electrode technique (SIET) (**Figure 3(a)**) *in vivo* to identify specific ionocytes, which expresses H^+-ATPase on the apical membrane of embryonic skin and secrete H^+ (these ionocytes are called H^+-ATPase-rich cells, or HR cells). In very young zebrafish embryos, the skin serves as the major site of ion uptake and acid–base regulation, because the gills are not fully developed. Loss-of-function experiments using specific morpholinos have shown that knockdown of H^+-ATPase or CA protein amount impacts Na^+ uptake/H^+ secretion in zebrafish embryos, suggesting roles in Na^+ uptake/H^+ secretion mechanisms (**Figures 3(b)–3(d)**). Only HR cells in zebrafish embryonic skin, but not other cell types, accumulated sodium green fluorescence (an *in vivo* marker of Na^+) (**Figure 4**), which was blocked by an NHE-specific inhibitor. Moreover, HR cells co-expressed all the transport proteins essential for this mechanism to work, including NHE3b, membrane-bound CA15a, cytosolic CA2-like a, and NKA.

Cl^- Uptake/Base Secretion

FW fish gill ionocytes can actively absorb Cl^- from the water against a ~4000-fold concentration gradient (calculated from **Table 1**) and secrete HCO_3^-, which

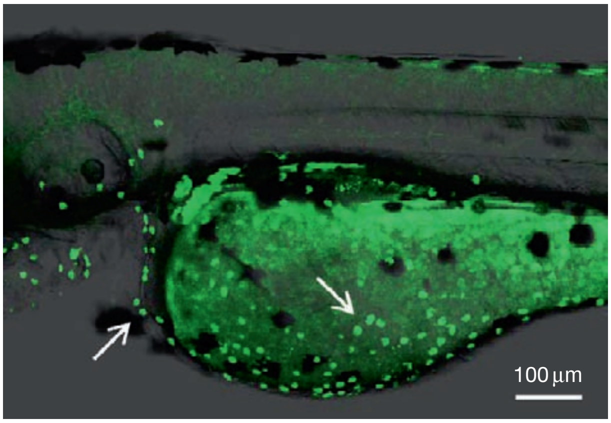


Figure 4 *In vivo* evidence for the Na^+ uptake function in zebrafish skin ionocytes. Accumulation of sodium green fluorescence (arrow) occurs in a group of ionocytes (HR cells; for details refer to the text) in zebrafish embryonic skin.

accumulates in blood due to metabolic processes (CO_2 production) and changes in environmental pH or CO_2. It has long been proposed that Cl^- uptake is closely linked with base (HCO_3^-) secretion; however, compared to our understanding of the mechanisms of Na^+ uptake/acid secretion in fish, even less is known about Cl^- uptake/base secretion.

The central mechanism for Cl^- uptake/base secretion involves a direct exchange of Cl^- with HCO_3^- via an apical anion exchanger (AE). There are at least two different candidate proteins that could play this role in the apical membrane of the gill. They are AE1 (a solute carrier from a group of proteins called SLC4) and pendrin (a SLC26 member). Some physiological and pharmacological data support the possible role of AE1 in the apical transport of Cl^- and HCO_3^-; however, there is still no convincing evidence to demonstrate that an AE is actually expressed in the apical membrane of fish gill ionocytes and functions in apical Cl^- uptake. On the other hand, some other types of Cl^-/HCO_3^- exchangers, several members of the SLC26 family including pendrin, have recently been implicated in Cl^- uptake/base secretion. Recent studies using loss-of-function experiments in zebrafish showed that knockdown of several SLC26 members, namely a3, a4, and a6, impairs Cl^- uptake and HCO_3^- secretion in embryos, strongly suggesting a role in Cl^- uptake/HCO_3^- secretion. However, evidence to demonstrate co-localization of these SLC26 members with basolateral H^+-ATPase or Cl^- channels in the same ionocytes is lacking, and this type of co-localization is necessary for transepithelial base secretion and Cl^- uptake. As a result, this transporter is not included in **Figure 2**.

The Na^+–Cl^- co-transporter (NCC, a member of SLC12) was first discovered in the urinary bladder of a flounder (*Pseudopleuronectes americanus*), and then was demonstrated to be important in the Na^+/Cl^- reabsorption mechanism in the distal convoluted tubular (DCT) cells of the mammalian kidney. Recent studies suggest that an NCC-like protein (termed ortholog) may be involved in Cl^- uptake in fish skin and gills (**Figure 2**). NCC-like protein resides on the apical membrane of specific ionocytes in gills and embryonic skin of tilapia (*Oreochromis mossambicus*) and zebrafish. Loss-of-function studies with NCC-like protein reduced both Cl^- uptake and whole-body Cl^- levels in zebrafish embryos, implicating an NCC-like mechanism in Cl^- uptake. Similar to mammalian DCT cells, Cl^- movement across the basolateral membrane is thought to involve a basolateral Cl^- channel and this channel, along with NCC, has been proposed as one of the mechanisms responsible for transepithelial Cl^- uptake in NCC-expressing ionocytes.

Both the Cl^-/HCO_3^- exchanger and NCC are likely electroneutral; however, importantly, the ion (Na^+, Cl^-, and HCO_3^-) gradients across the apical membranes of gill ionocytes do not appear to favor the operation of the two transporters in FW environments and experiments to resolve these issues are still lacking.

Ca^{2+} Uptake

Unlike terrestrial vertebrates, FW fishes obtain the majority of their calcium via absorption from the aquatic environment rather than through their diet. Gills are responsible for >97% of the whole-body Ca^{2+} uptake and MR cells have long been thought to be the main site for transepithelial Ca^{2+} transport in fish gills. According to the current model for mammals, active transepithelial Ca^{2+} uptake is carried out through the operation of apical epithelial Ca^{2+} channel (ECaC), the basolateral Na^+/Ca^{2+} exchanger (NCX), and plasma membrane Ca^{2+}-ATPase (PMCA). A similar model for Ca uptake has been proposed for fish gills (**Figure 2**). The intracellular Ca^{2+} concentration of gill ionocytes is presumably $<1 \times 10^{-6}$ M, and therefore the gradient appears to favor diffusional Ca^{2+} uptake via an ECaC even from soft FW with $\sim 1 \times 10^{-5}$ M Ca^{2+}. Following the diffusion of Ca^{2+} from the water into the gill ionocyte, cytosolic Ca^{2+} probably diffuses via shuttles (e.g., Ca^{2+}-binding proteins) to the basolateral membrane where Ca^{2+} is extruded out of the cell via the NCX (operating down the Na^+ gradient created by NKA) and PMCA. Only recently has ECaC been identified in fish gill ionocytes and its expression is positively associated with Ca^{2+} uptake in fish. In zebrafish, ECaC and two specific isoforms of NCX and PMCA (NCX1b and PMCA2) are co-expressed in the same ionocytes, providing molecular evidence to support the role of these Ca^{2+} transporters in fish gill Ca^{2+} uptake mechanisms. Among these three Ca^{2+} transporters, ECaC is thought to be the rate-limiting step in transepithelial Ca^{2+} uptake; therefore, its regulation is likely important when coping with environmental changes.

Different Subtypes of Ionocytes

In mammalian kidneys, different types of ionocytes variously express specific ion transporters and enzymes within kidney segments, which each have their own functions. As described earlier, ion uptake and acid–base regulatory mechanisms of FW fish gills are, in some respects (transporter expression patterns and ion-transport pathways), analogous to those of mammalian kidneys. From an evolutionary standpoint, it is reasonable to generalize that FW fish gills also possess similar types of ionocytes (or MR cells) to achieve a variety of distinct ion-transport functions.

The early literature on this subject proposed functional subtypes of MR cells mainly based on cell morphologies, expression of NKA, and/or related ion fluxes. Direct evidence to support the notion of ionocyte heterogeneity emerged only recently with the adoption of molecular/cellular physiological approaches in zebrafish, rainbow trout (*Oncorhynchus mykiss*), and tilapia. In the zebrafish (**Figure 2**), three types of gill ionocytes, HR cells, NKA-rich (NaR) cells, and NCC cells, have been identified mainly based on evidence from double/triple immunocytochemistry/*in situ* hybridization and loss-of-function studies. HR, NCC, and NaR cells were deemed involved in Na^+ uptake/acid secretion, Ca^{2+} uptake, and Cl^- uptake, respectively, based on the specific expression and functioning of distinct sets of ion transporters and enzymes as described earlier.

In rainbow trout, two subtypes of MR cells have been identified based on their ability to bind peanut lectin agglutinin and are termed PNA^- and PNA^+ MR cells, which show high expression of H^+-ATPase and NKA, respectively. A series of *in vitro* physiological and pharmacological studies demonstrated the distinct ion-transport functions of the two MR cell types: H^+-ATPase-dependent Na^+-uptake by PNA^- cells and Ca^{2+} uptake (via the ECaC) by PNA^+ cells. However, convincing data to support the existence and function of other transporters or ion channels in MR cell types are still lacking. Using immunocytochemistry, MR cells in tilapia skin/gills were classified into four types: type I which shows only basolateral NKA staining, type II which shows basolateral NKA and apical NCC staining, type III which shows basolateral NKA/$Na^+-K^+-2Cl^-$ co-transporter (NKCC1a) and apical NHE3 staining, and type IV which shows basolateral NKA/NKCC1a and apical cystic fibrosis transmembrane conductance regulator/NHE3 staining. Based on the distinct expressions of NHE3 and NCC, zebrafish HR and NCC cells are likely respectively analogous to tilapia type II and III MR cells. However, there is no direct physiological data to demonstrate the functions of these subtypes of MR cells in tilapia so far.

Functional Regulation to Cope with Fluctuating Environments

As suggested above, fish regulate transport functions and capacities of gill ionocytes and their proteins to maintain ion and acid–base homeostasis in fluctuating FW environments. Many studies report that acclimation to low-Na^+ (or -Cl^-, -Ca^{2+}) or low-pH FW can lead to an increase in Na^+ (or Cl^- and Ca^{2+}) uptake or proton secretion in gills (or embryonic skin) as a compensation. Upon exposure to a new environment, gill ionocytes must be able to adjust their transporter activity and function quickly in order to prevent large changes in blood ion and acid–base composition. In tilapia embryos, acute exposure to low-Cl^- FW converted inactive to active ionocytes after only 4 h and stimulated Cl^- uptake within 24 h. After 48 h, the number of ionocytes increased. Similarly in zebrafish, embryonic skin HR cells altered apical surface structure and acid secretion within 10–120 min after acute transfer from pH 7 to pH 4. These rapid changes appear to involve the trafficking of transporter proteins between intracellular compartments and apical cell membranes, as reported for ionocytes of the mammalian kidney. A recent loss-of-function study in zebrafish embryos indicated that blocking the function of Nherf1, a regulator of apical trafficking of ion transporters that includes NHEs, impaired the Na^+ uptake function of HR cells.

With longer acclimation (days to weeks) to ion-deficient or acidic FW, numerous studies involving a variety of fish species report increases in the number and/or activity of ionocytes in gills. Whether the increase in the number of cells is mediated by the proliferation and differentiation of newly recruited ionocytes is an important, but unresolved issue. In tilapia embryos, most (75–90%) skin ionocytes survived 24–96 h after transfer to low-Cl^- FW, indicating little ionocyte turnover and only <10% of ionocytes are new recruits, presumably originating from undifferentiated cells. However, recent studies on zebrafish shed new light on the mediation of cell proliferation and differentiation in the functional regulation of ionocytes during acclimation to fluctuating environments. In zebrafish, acclimation to acidic (pH 4) FW for 3–4 days induced a compensatory enhancement in acid secretion, which was achieved not only by increasing the number of HR cells but also by increasing the acid-secreting function of individual cells. Examination of expression of p63 (a marker for the epithelial stem cells) and GCM2 (a transcription factor regulating HR cell differentiation) further demonstrated that additional HR cells in acid water had differentiated from ionocyte precursor cells and also from newly proliferating epithelial stem cells.

Summary

Taken together, fish appear to cope with harsh environments with different compensatory strategies. These include: (1) acute modulation of existing mechanisms (transporters and ionocytes) and, for long-term acclimation, (2) increasing the number of ionocytes (through cell proliferation and/or differentiation) and synthesizing new transporters, resulting in an enhancement of the overall ion-regulatory function.

See also: **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts.

Further Reading

Evans DH (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 295: R704–R713.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiology Review* 85: 97–177.

Gilmour KM and Perry SF (2009) Carbonic anhydrase and acid–base regulation in fish. *Journal of Experimental Biology* 212: 1647–1661.

Hwang PP (2009) Ion uptake and acid secretion in zebrafish (*Danio rerio*). *Journal of Experimental Biology* 212: 1745–1752.

Hwang PP and Lee TH (2007) New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 148: 479–497.

Hwang PP and Perry SF (2010) Ionic and acid–base regulation. In: Perry SF, Ekker M, Farrell AP and Brauner CJ (eds.) *Fish Physiology*, vol 29, pp. 311–343. San Diego, CA: Elsevier.

Lin LY, Horng JL, Kunkel JG, and Hwang PP (2006) Proton pump-rich cell secretes acid in skin of zebrafish larvae. *American Journal of Physiology – Cell Physiology* 290: C371–C378.

Marshall WS and Grosell M (2006) Ion transport, osmoregulation and acid–base balance. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp.177–230. Boca Raton, FL: CRC Press.

Parks SK, Tresguerres M, and Goss GG (2008) Theoretical considerations underlying Na^+ uptake mechanisms in freshwater fishes. *Comparative Biochemistry and Physiology. Part C, Toxicology and Pharmacology* 148: 411–418.

Schnidt-Nielsen K (1993) *Animal Physiology: Adaptation and Environment,* 4th edn. New York: Cambridge University Press.

Water Balance and Aquaporin

CP Cutler, Georgia Southern University, Statesboro, GA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Aspects of Osmosis
Gill Water Transport
Gastrointestinal Water Transport
Renal Water Flows
Urinary Bladder
Further Reading

Glossary

Basolateral tubular network A network of membranous tubules that are thought to be an internal extension of the basal plasma membrane of chloride cells of the gill. The network runs throughout much of the basolateral (inside facing) portion of the cell and there is speculation that tubules may be permanently or temporarily connected to lateral plasma membranes of chloride cells and consequently be connected to the paracellular space (between cells).

Mitochondrion-rich cells This is often synonymous with branchial chloride cells but is a broader category, because not all mitochondria-rich cells are necessarily chloride cells (engaged in chloride transport). As the name suggests, mitochondrion-rich cells are recognizable due to their preponderance of mitochondria, which suggests that are metabolically very active and may potentially be involved in active ion transport.

Osmoregulation/osmotic homeostasis Osmoregulation is, generally speaking, the regulation of the internal osmotic environment. In fish, this has many physiological and/or biochemical components. Physiologically, this would include the control of (1) the permeability of the gills to ions and water, (2) the rate of drinking and water and ion intake from the diet, and (3) the loss of ions and or water from urine production. These together control the osmolality (osmotic concentration) and overall volume of body fluids and produce osmotic homeostasis (maintaining the osmolality of body fluids at a constant level). Biochemically, this would include the net effect of water gain or loss and ion excretion or absorption processes across the gills. Ion absorption in the esophagus. Ion and water absorption or secretion in the intestine and kidney, and water absorption in the urinary bladder.

Osmolality This as a simple definition is the number of moles of dissolved particles per liter of solvent (usually water). However, in reality the osmotic effect of dissolved substances varies from one compound/molecule to another (due to differences in hydration shells and, for larger molecules, surface area to volume ratios) and would therefore need to be calculated taking into account osmotic coefficients of the individual component solutes of a solution. In practice, it is usually measured empirically.

Osmosis Essentially diffusion for water molecules. As with diffusion for dissolved solutes, water moves due to random thermal oscillations, in a net sense, from where there is a higher water concentration (amount per unit volume) to where there is a lower water concentration. This is generally inversely related to the situation for dissolved solutes, as higher levels of these occupy space leaving less space for water and reducing its concentration. Water therefore (again in a net sense) moves from where the osmolality is lower to where it is higher (the opposite of what occurs for the diffusion of solutes).

Paracellular/transcellular pathways/transport Transport of solutes or water across an epithelial cell layer involves two different pathways, either transcellular (through the cell, i.e., crossing the basolateral and apical plasma membranes usually through transporter proteins) or paracellular (between the cells where there is a small paracellular space and transport would involve diffusion, osmosis, or bulk flow, but this is crucially determined by the permeability properties of the tight junctions that seal off the basolateral side (internal environment side) of epithelial cell layers from the apical side (external environment)).

Introduction

Water homeostasis in fish can be broken down into different components. Overall, the total body fluid volume is a key parameter; however, this is made up of intracellular (cell) volume and extracellular fluid volume. As the water transport performed by cells and tissues is thought to be a passive process, this is driven by or considerably impacted by ion and osmolyte movement. Teleost and other fish species occupy a variety of osmotic environments and the nature of the fish's environment determines the osmoregulatory mechanisms that are employed to achieve both ion and water homeostasis.

In freshwater (FW) teleost fish, the main osmotic problem is the continuous ingress of water due to osmosis, as FW, while variable, is of low osmotic strength, whereas a fish's body fluids are of much higher osmolality. Consequently, there is a net influx of water across permeable body surfaces, particularly the gills. FW teleost fish deal with the continuous inflow of water in a similar way to mammals, by producing large quantities of dilute urine to eradicate the water from the body. Some additional ions may be reabsorbed by the urinary bladder. However, as these mechanisms cannot produce a urine output of pure water, some ions are lost in the process and these have to be regained by specialized ion transporting cells of the gill, called 'chloride cells' or 'mitochondria-rich cells', or through the diet (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes).

Marine teleost fish have the opposite osmotic problem with their FW counterparts, in that seawater (SW) is of considerably higher osmotic strength than that of the body fluids; therefore, fish tend to lose water to the external environment across permeable body surfaces. The fish counteracts this in a manner that might not be anticipated; they drink SW to regain the lost water. Of course, this is somewhat counterintuitive as it also results in the ingestion of a considerable salt load. The imbibed SW is first desalinated down to approximately isosmotic levels before passage through the stomach where little change in osmolality of the fluid is thought to occur. When the fluid reaches the intestine, further salts and water are absorbed into body fluids, resulting in around 90% of the imbibed fluid being absorbed and the equivalent of 10% then being excreted rectally. The salts absorbed from intestinal fluid are then excreted across the surface of the gills by mitochondria-rich cells (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). This achieves an uptake of fluid that counteracts dehydration.

In addition to stenohaline FW or marine fish species, there are also some (euryhaline) fish species capable of survival in a wide variety of osmotic environments, including FW up to many times the osmotic strength of SW. Euryhaline species have been widely used to study osmoregulation and water homeostasis, as differences in the mechanisms operating in different osmotic environments can easily be identified and studied. However, euryhalinity has evolved independently numerous times in teleosts; thus, the precise natures of the mechanisms differ between species.

Overall, the general mechanisms of ion and water homeostasis in diverse fish species have been known for some time and a great deal is known about the molecular aspect of ion transport in many of the major osmoregulatory organs (gill, esophagus, kidney, intestive, and urinary bladder). However, much less is known of the molecular aspects of water transport, which is the focus of this article.

General Aspects of Osmosis

One of the important factors concerning water homeostasis, particularly in external environments that are not in osmotic equilibrium with the fish's internal fluids, is that all tissues/organs interacting with the external milieu have epithelial barriers composed of cells. These epithelial barriers control the flow of substances into and out of the body and its composition and ability to control the flow of water is crucial to water homeostasis. All the major osmoregulatory organs, including the skin or integument, have an epithelial barrier composed of cells. Between these cells occur tight junction complexes that seal off one side of the epithelium from the other. Conventionally, the part of the cell facing the outside world is the apical plasma membrane domain and that facing the inside of the body are the basolateral membrane domains. The tight junctions, as well as sealing off one side of the cell layer from the other, also prevent movement of plasma membrane components from one membrane domain to the other. An additional consequence is that the fluid composition on either side of the epithelial layer can be different, creating gradients for the various dissolved substances. Osmotic gradients can also exist on either side of the epithelial and these may drive the flow of water from one side of the epithelium to the other depending on the permeability of the epithelium to water.

As a consequence of the composition of the epithelial barrier, water can really only flow across these barriers via two main routes – either through the cell (transcellular) or, between the cells, through the tight junctions (paracellular). Concerning the transcellular route, it is generally considered that small amounts of water can pass between temporary gaps between phospholipids of the plasma membranes of cells, allowing some water to move into or out of the cell. Otherwise water must go through some kind of transporter or channel in the membrane and this usually involves transmembrane proteins (**Figure 1**). By far, the most widely studied water transport membrane proteins are the aquaporin (AQP) water channels discovered by Preston and Agre in 1991, for which Peter Agre received the Nobel prize for chemistry in 2003. As the name suggests, these allow water molecular to cross the plasma membrane passively (i.e. depending on the prevailing osmotic gradients) through

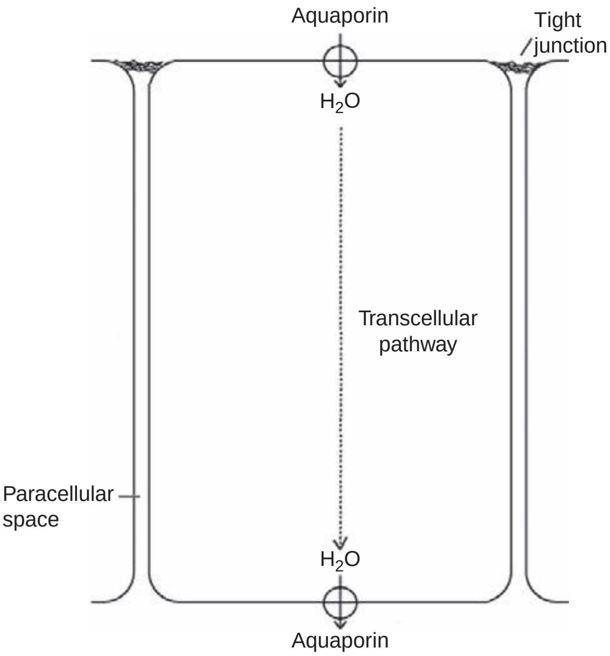


Figure 1 Cartoon of the transcellular water transport pathway.

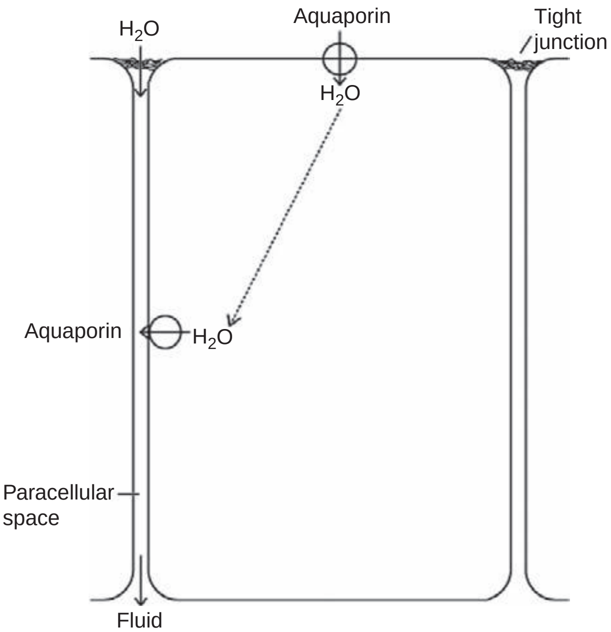


Figure 2 Cartoon of possible routes for paracellular water transport.

(often exclusively) water-selective channels they form in the plasma membrane.

Concerning the paracellular route for water movement across an epithelium (**Figure 2**), water can move by osmosis between the cells depending on the water permeability of the tight junctions. Tight junctions are composed of various proteins of which the main two are occludins and claudins. The exact type, or combination of types of claudins (in particular) that are present, seems to determine the overall water and ion permeability characteristics of tight junctions. Due to the theory concerning osmosis, it is generally the case that water flows down osmotic gradients toward solutions with a higher osmotic strength. However, there may well be situations where water can be transported isosmotically or even against an osmotic gradient as suggested by Diamond in the 1960s. What is necessary for this transport to occur is a locally occluded area that has a standing gradient of solutes and can attract water locally even when the overall osmotic gradient would otherwise be unfavorable for net water transport. Such areas could potentially be created inside cells (if the plasma membrane is invaginated) or between cells (i.e., in the paracellular space). In fish osmoregulation, there are at least two places where this may be relevant. First, this may occur in mitochondria-rich cells of the gill that have an extensive internal invagination of the basolateral plasma membrane. Second, the surface epithelial cells of the intestine may utilize such a mechanism in their paracellular spaces.

Water absorption against an apparent osmotic gradient has been shown to occur in the eel intestine and this may at least partly be explained by the actions of Na^+/K^+-ATPase, an enzyme that is thought to be predominantly laterally located and would continuously transport sodium ions into the paracellular space, elevating the local osmotic concentration. Water may then flow from the intestinal lumen (space in the center of the intestine) across permeable tight junctions or transcellularly through water transporting membrane proteins such as AQPs located in the apical and lateral plasma membranes. The arrival of water by osmosis creates an expanded volume and/or hydrostatic pressure in the paracellular space that would tend to force fluid to flow out of the space to the basolateral side of the epithelium.

The final considerations necessary, when thinking about water transport, are the hydration shells of solutes, solvent drag, and bulk fluid flow. All dissolved solutes have a hydration shell of water molecules but this varies depending on the ions or molecules concerned. In general, for small molecules and ions, it is reasonable to think that the osmotic effects of 1 mol of a dissolved substance are similar to 1 mol of another dissolved substance (e.g., 1 mol sucrose compared to 1 mol of sodium ions); however, this is not the case with large macromolecules such as proteins. Consequently, it may be necessary to consider the osmotic coefficients of dissolved substances to fully understand a solute's osmotic effect. These coefficients take into account the moles of water temporarily attached in a hydration shells per mole of solute. When solutes are moved across membranes by transport proteins, it is not yet clear whether or to what extent the hydration shells are removed during the transport process. If hydration shell removal is not complete, some water will be transported along with the solute and this process is known as solvent drag. Furthermore, when fluid is transported in a mechanical fashion (e.g., due to force from hydrostatic pressure), which is known as bulk flow, this occurs irrespective of the solute content of the fluid and consequently dissolved substances can potentially be transported against their gradients.

Gill Water Transport

Due to the large surface area (roughly 70% of the total body surface) of the gill required for gas exchange, the gill represents the main site of water movement and therefore is an important barrier to examine. Although it is difficult to measure accurately, gill osmotic water permeability (net gain/loss of water) is thought to be greater in stenohaline FW teleosts than in marine teleosts and increases with increasing size of the fish and with increasing temperature. Some euryhaline teleosts such as eels also show a similar trend having greater gill osmotic water permeability in FW, whereas other species such as trout and flounder have gill permeabilities that are the same or greater in SW than in FW.

There are three main routes for water movement across the gill cell membranes and each route can probably be regulated to a degree. The first route involves water moving directly through the plasma membrane lipids. There is some evidence that cholesterol in the apical membrane of gill cells may decrease the permeability, and therefore changes in the amount of cholesterol present may change the permeability of the gill cell membranes.

The second pathway for water movement across gills involves the paracellular system and/or the basolateral tubular system of mitochondria-rich cells. The characteristics of the gill cell tight junctions differ between FW and SW acclimated fish, particularly between mitochondria-rich cells and their adjacent cells. In FW fish, tight junctions are deeper with increased numbers of strands, whereas the tight junctions in SW-acclimated fish are thought to be shallow (i.e., with lower numbers of strands). Recent research suggests that a number of claudin tight junction protein isoforms may be differentially expressed and regulated in FW compared to SW acclimated fish.

There are around 56 claudin isoforms (numbered 1–33 with additional duplicate copies of most types) in the pufferfish (genus *Fugu*) genome, and there are probably more present in other fish species. Studies suggest that gill claudins 3(a,c), 4, 8(d), and 30 show gill mRNA and/or protein expression at higher levels in FW-acclimated compared with SW-acclimated euryhaline fish. By contrast, claudin 10(e) shows increased mRNA expression in SW-acclimated fish gill relative to the FW gill. Clearly, differences in the makeup of tight junction proteins in different environments could affect the permeability characteristic of the tight junctions and affect water flow through the paracellular system. However, it is not yet clear what combinations of claudins in the tight junctions lead to what permeability characteristics.

Depending on the nature of the basolateral tubular network in mitochondria-rich cells and how this system interacts with the paracellular system, it may be involved in generating standing osmotic gradients within the tubular system lumen and possibly by extension in the paracellular system (**Figure 3**). If this were

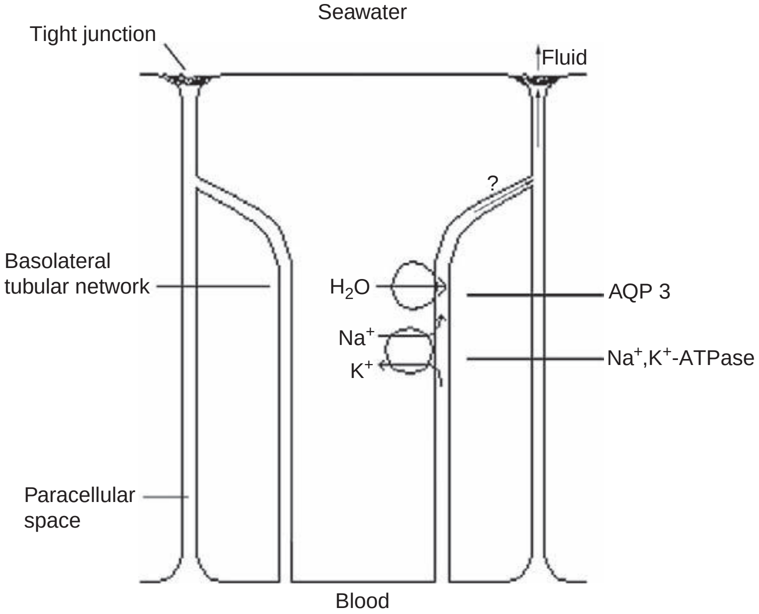


Figure 3 Cartoon diagram of possible gill mitochondria-rich cell fluid transport.

true, it would impact water flows through the paracellular system either through osmosis or possibly even through some kind of bulk fluid flow mechanism. However, this is as yet unproven and is consequently purely speculative.

The third potential route for gill water flow involves a transcellular route through the cell via AQP water channels in the cell membrane. In mammals, AQP isoforms are numbered from 0 to 12 and where the counterpart genes have been identified in fish, similar nomenclature has been used. Some fish AQPs are not clearly identifiable as counterparts of mammalian AQPs or are duplicate copies, and have been named differently. Many studies have shown that there is up to a 97% increase in mRNA and protein levels of AQP3 in the gills of euryhaline teleosts when acclimated from FW to SW. Furthermore, various studies have shown that AQP3 is expressed in mitochondria-rich cells and sometimes in surface pavement or respiratory cells, especially in epithelial cells covering the branchial arch of the gill. In the mitochondria-rich cells, AQP3 expression is localized to the basolateral tubular network but there is also a pool of AQP3 protein located in the apical pole of these cells but not in the apical plasma membrane itself.

The precise function of AQP3 in the SW mitochondria-rich cells is unknown; however, several hypotheses exist. First, AQP3 may be necessary to provide water as a substrate for the enzyme carbonic anhydrase in the cell. Second, AQP3 may play an important role in osmosensing (sensing the osmotic concentration as part of a regulatory response to changes in osmolallity; **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmosensing). Third, it is present in the cell as an exit pathway for water that is likely to inundate the cell when the fish experiences an FW environment. Finally, it may provide a flow of water to the basolateral tubular network, if this generates an osmotic standing gradient that produces fluid flow due to hydrostatic pressure. All these roles and other possibilities have some merit but again are purely speculative. Infusions of the hormone cortisol have been shown to downregulate gill AQP3 mRNA expression in FW fish. This suggests that cortisol, which is elevated during SW acclimation, may be at least partially responsible for the decreased expression of AQP3 in SW-acclimated fish (**see also Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish).

Gastrointestinal Water Transport

In SW, teleost fish drink to counteract the effects of dehydration due to water loss across the gills. Imbibed water is first desalinated in the esophagus and this is thought to occur due to ion absorption via secondary active (ion exchangers) rather than active transport (directly due to Na^+,K^+-ATPase or V-type H^+-ATPase ion pumps). It has long been thought that there is very little net water movement across this epithelium and as a consequence the epithelium has been labeled as 'tight' with low permeability. Despite this notion, the small amount of data available suggest that the unidirectional water fluxes (flows in each individual direction) may be quite high. High unidirectional fluxes however may still yield low net fluxes if the inward flux is of similar magnitude as the outward flux, consequently canceling each other out yield low net flux measurements. Of course, the ion absorption/desalination is a dynamic process beginning at the mouth, and water transport studies usually use the whole esophagus, so it is possible that at the anterior (head) end there may be a net loss of water to the imbibed fluid in the lumen due to osmosis, cancelled out by a matching net water absorption toward the posterior end of the esophagus, where the osmotic concentration of fluid would be much more favorable for this to occur. Unfortunately, there are no data available to know whether these hypotheses are correct. One thing that is becoming apparent is that the esophagus expresses a number of different AQP isoforms. AQP mRNA expression, including AQP3, AQP1 and its duplicate copy (ADP1dup), and other AQP transcripts, have been shown to be present in the eel and may play a role in protecting surface cells from the dehydrating effect of the SW in the esophageal lumen. However, the actual role these AQPs play is yet to be determined.

Following desalination of the imbibed SW in the esophagus, it then passes into the stomach where it is generally thought that there is no further change in the osmolality or fluid volume. Once the desalinated fluid reaches the intestine, further salts are absorbed together with water. The water absorption is tightly coupled to salt absorption. Divalent cations (e.g., Mg^{2+}) precipitate with carbonates in the lumen, taking these ions out of the osmotic equation and ultimately allowing more water to be absorbed. What is not clear is how the water is absorbed from the lumen. Water absorption could occur via a paracellular system or through a transcellular route, probably involving AQPs, or water uptake could involve a mixture of the two processes (**Figure 4**). Part of the reason why salt and water absorption are tightly coupled is due to osmosis, where water follows salts that have elevated the osmotic concentration. It is also possible that standing gradients are generated in the paracellular spaces due to the action of Na^+/K^+-ATPase located on the lateral side surfaces of the tall columnar epithelial cells that are the predominant cell type lining the intestinal lumen. This would lead to the continuous elevation of the paracellular sodium concentration and this region of elevated osmotic concentration would draw in water from the intestine lumen. There are two possibilities of

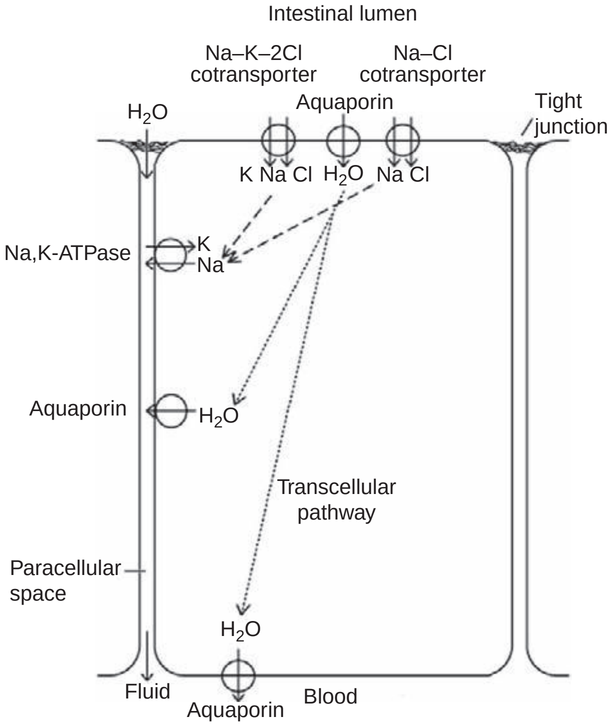


Figure 4 Cartoon of an intestinal enterocyte and possible routes for water transport.

where the water could come from. First, across the tight junctions, if these are permeable to water, and/or second from any AQP water channels located in the lateral membranes of the cells. Either way, the expansion of fluid volume in the space would produce hydrostatic pressure to push fluid out at the open end of the paracellular space on the basolateral side of the cell layer. Currently, there is only limited evidence for the lateral localization of AQPs on this cell type in some teleosts. However, there is indirect evidence suggesting that standing gradients may exist, as fish such as eels have been shown to be able to absorb water against an apparent osmotic gradient.

Recent evidence suggests that different portions of the intestine may rely on different modes of water transport. In the puffer fish (genus *Tetraodon*), there are data suggesting that the depth or number of strands in the intestinal epithelium tight junctions increases toward the posterior end of the tract leading to a decreased permeability. Overall, this suggests that at least in the puffer fish, there is the potential to absorb less water toward the posterior segments of the gut such as the rectum. However, it may also suggest a switch to a more transcellular path for water absorption, possibly involving AQPs toward the end of the intestine/rectum. There are also changes in the distribution of tight junction (claudin 3) isoform proteins along the intestine, and the expression of claudin 3 isoforms also changes with acclimation of fish from FW to SW. Several AQP isoforms are expressed in the intestine. AQP3 is thought to be located in macrophages in the intestine and mucus cells in the rectum and probably plays no role in water uptake. Likewise, AQPe/SbAQP (possible mammalian AQP10 counterpart) is expressed in intestine but may be located in tissues underlying the epithelium; thus, it is unlikely to be involved in water uptake. More interesting is the potential role of AQP1 and AQP1dup. In eels, AQP1 is expressed in the apical (brush border) membranes of intestinal tall columnar surface epithelial cells and in blood vessel endothelium. In FW-acclimated eels and sea bass, AQP1 protein and mRNA expression levels are higher toward the posterior end of the gut, and when fish are acclimated to SW eel, mRNA levels increase by up to 25-fold higher than in FW. These elevated levels found in SW fish mimic the infusion of cortisol into FW fish, suggesting that cortisol contributes to the regulation of AQP1 mRNA. Localization studies suggest that eel AQP1 protein is expressed in surface epithelial cells and mainly found near the sphincter delineating the posterior intestine from the rectum. Clearly, this protein is in a location that suggests that it may be involved in water uptake but that is yet to be demonstrated directly. The situation for AQP1dup is more variable between species, showing only sporadic expression in eel intestine. AQP1dup counterparts (AQP1o/AQP1b) are expressed in the intestine of sea bream but not in sole or zebrafish. In sea bream the expression pattern is somewhat different, where AQP1a is expressed in apical (brush border) and lateral membranes of tall columnar surface epithelial cells in the duodenum and hindgut, whereas AQP1b, not AQP1a, is expressed in rectum. Sea bream AQP1a protein expression was downregulated in all gut segments with SW to FW acclimation, whereas AQP1b protein levels only decreased in the rectum and this response was quite variable between individual fish.

Renal Water Flows

One of the most important facts to realize about the teleost kidney is that some fish are aglomerular, which means that they have renal tubules without a glomeruli that filters fluid from the blood into renal tubules (**see also Role of the Kidneys**: The Kidney). Consequently, some fish species have renal tubules that produce urine entirely due to secretion (transcellular fluid movement from renal tissue fluid) into the tubule lumen. In all probability, all fish and possibly all animals have the possibility to secrete fluid into the renal tubule. In marine fish, fluid secretion probably occurs to enhance divalent cation secretion since fish cannot make significantly concentrated urine. As this would contribute to water loss, urine production tends to be the minimum needed for this purpose. In FW fish, fluid secretion may be a mechanism used to increase renal (fluid, i.e., water) output, and while salts are transported into the tubule lumen along with water, these salts can mostly be reabsorbed further down the renal tubule (in more distal segments) probably due to the action of NaCl cotransporters (NCC and NKCC2). The consequence of this from a water homeostasis point of view is that there are two (in some ways opposing) water transport mechanisms occurring in the renal tubule. First, the proximal tubule secretion and second, in the distal segments, there is a mechanism for water reabsorption. Mammals are able to produce hyperosmotic concentrated urine due to water reabsorption using AQP2. Teleost fish do not possess an AQP2 homolog and this may be the main reason why fish cannot concentrate urine. Other AQP isoforms are however present. AQP3 is expressed in the renal tubule but its location is unknown. By analogy to mammals, it is most likely located in a distal segment and may be involved in the process of reabsorption. AQP1 and AQP1dup are also expressed in the kidney and their mRNA levels have been shown to decrease when eels are transferred from FW to SW. This is the opposite of what might be expected if fish renal tubules were only engaged in reabsorption and it consequently suggests that AQP1 and AQP1dup may be involved in the fluid secretion mechanism.

Urinary Bladder

The urinary bladder of SW teleosts can also reabsorb water (and monovalent salts) and this further concentrates urine divalent cations. The mechanism of salt reabsorption varies from species to species, probably involving cotransporters (NCC in flounder; NKCC2 in eel) or ion exchangers (a Na^+/H^+ exchanger and Cl^-/HCO_3^- exchanger in trout). The purpose of the NaCl reabsorption in the urinary bladder is essentially to allow further reabsorption of water (to reduce dehydration) and this has the secondary consequence of concentrating divalent cations. The mechanism of water reabsorption, transcellular or paracellular, is currently unknown.

See also: **Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmosensing. **Role of the Kidneys**: The Kidney.

Further Reading

Ando M, Mukuda T, and Kozaka T (2003) Water metabolism in the eel acclimated to sea water: From mouth to intestine. *Comparative Biochemistry and Physiology* 136: 621–633.

Baldisseroto B, Mancera Romero JM, and Kapoor BG (2007) *Fish Osmoregulation*. Enfield, NH: Science Publishers.

Cutler CP, Brezillion S, Bekir S, Sterling R, and Cramb G (2009) The molecular basis of osmoregulation, and ion and water transport in teleostean fish. In: Handy R, Bury N, and Flik G (eds.) *Osmoregulation and Ion Transport: Integrating Physiology, Molecular and Environmental Aspects,* Essential Reviews in Experimental Biology, ch. 3, vol. 1, pp. 63–108. London: Society for Experimental Biology Press.

Cutler CP, Martinez A-S, and Cramb G (2007) The role of aquaporin 3 in fish. *Comparative Biochemistry and Physiology* 148: 82–91.

Evans DH and Claiborne JB (2008) Fish osmotic and ionic regulation. In: Evans DH (ed.) *Osmotic and Ionic Regulation: Cells and Animals*, ch. 8, pp. 295–366. Boca Raton, FL: CRC Press.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Review* 85: 97–177.

Hirose S, Kaneko T, Naito N, and Takei Y (2003) Molecular biology of major components of chloride cells. *Comparative Biochemistry and Physiology* 136: 593–620.

Hwang P-P and Lee TH (2007) New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology A* 148: 479–497.

Marshall WS and Grosell M (2006) Ion transport, osmoregulation and acid–base balance. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., ch. 6, pp. 177–230. Boca Raton, FL: CRC Press.

McCormick SD and Bradshaw D (2006) Hormonal control of salt and water balance in vertebrates. *General and Comparative Endocrinology* 147: 3–8.

Osmosensing

D Kültz, University of California, Davis, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Why Do Fishes Need to Sense Environmental Salinity?
Basic Principles of Osmosensory Signal Transduction
Molecular Osmosensors
The Ion–Damage–Cytokine Model of Osmosensing
Cellular Osmosensing and Chloride Cell Differentiation
Role of Osmosensing for Fish Sperm Motility
Further Reading

Glossary

Cytokine A biologically active peptide or small protein that mediates systemic and cellular signaling.

Euryhalinity The ability of an organism to tolerate a wide range of environmental salinity.

Hormone Signaling molecule released by a cell that either stimulates the cell of origin (autocrine), nearby cells (paracrine), or travels via the bloodstream to distant cells (endocrine).

Hyposmotic Containing a lesser concentration of solutes than another solution.

Hyperosmotic Containing a greater concentration of solutes than another solution. If the solutions are separated by a permeable membrane (such as gill epithelium), solute will diffuse from a hyperosmotic to a hyposmotic solution and water will move from the hyposmotic to hyperosmotic solution.

Mechanosensory The ability of cells and organisms to recognize and measure mechanical forces.

Signal transduction A process by which an extracellular signal (e.g., a hormone, the first messenger) initiates intracellular processes; generally involves a receptor, linking proteins, an enzyme that generates a second messenger (e.g., cAMP), that activates downstream protein kinases which alters regulator-phosphorylation patterns.

Why Do Fishes Need to Sense Environmental Salinity?

Fish inhabit all but the most extreme aquatic habitats, ranging from freshwater (FW) to hypersaline. Some species can even survive extended periods in completely dried up riverbeds and ponds. Many of these habitats are also characterized by greatly fluctuating salinity. Examples include desert salt lakes, the marine intertidal zone, estuaries, and salt marshes (**see also Intertidal Fishes**: Intertidal Habitats). In addition, global climate change is predicted to cause an overall decrease in ocean salinity and a rise in sea level that could greatly alter the salinity in large areas of low-altitude estuarine areas. Because of these environmental salinity fluctuations that occur in many habitats, it is important to understand how fishes sense changes in the salinity of their environment to trigger appropriate acclimatory responses. Moreover, since fishes constitute by far the most diverse group of vertebrates with greatly differing abilities to tolerate salinity fluctuations, they provide a great system for understanding how euryhalinity (the ability to tolerate a wide range of environmental salinity) has evolved. Evolvability (the rate of evolutionary change) of salinity tolerance depends on many variables, including the number and properties of key genes involved in salinity tolerance and osmosensing.

Of the three main taxa of fishes (Agnatha, Chondrichthyii, and Actinopterygii), only the ray-finned fishes (actinopterygii, largest group = bony fishes, teleosts) are osmoregulators that maintain a constant osmotic concentration in their extracellular body fluids (blood plasma, interstitial fluid, etc.) when the environment changes, much like the other vertebrate classes. The osmolality of teleost blood is approximately 300–350 mosmol kg^{-1}, corresponding to a salinity of about 10 parts per thousand (ppt) or roughly one-third of that of the oceans (35 ppt). FW fishes have plasma that is hyposmotic to the environment (FW < 0.4 ppt), whereas seawater (SW) fishes have plasma that is hyperosmotic to the environment (SW = 35 ppt). Nevertheless, the osmolality of extracellular body fluids such as plasma is roughly equal in both FW and SW fish (*c.* 300–350 mosmol kg^{-1}) and the mechanisms of salt movement involved in maintaining this osmolality in these different environments is outlined elsewhere (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**:

Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes).

To activate the appropriate mechanisms of salt transport (secretion or absorption), fish must be able to sense the salinity (osmotic concentration) of their environment. In particular, euryhaline fish that move between hyposmotic and hyperosmotic environments need to accurately monitor environmental salinity to activate either salt secretion or salt absorption to the appropriate degree that is necessary to offset passive ion movements between extracellular fluid and the external milieu. Therefore, osmosensing mechanisms are required to monitor environmental salinity and control signaling networks, which are responsible for tuning the direction and the mechanisms of active, transepithelial salt transport in fish. Despite the clear importance of osmosensing in fish, the entire field is underdeveloped relative to other aspects of ionregulation in fish (e.g., the mechanisms for ion transport; (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). The exception is the knowledge of the actions of osmoregulatory hormones and the regulation of short-circuit current and ion transport across opercular epithelium, which is an experimental model with ion transport properties similar as gill epithelium.

Basic Principles of Osmosensory Signal Transduction

It has become clear during the past decade that the vertebrate (including fish) osmosensor systems are quite different and much more complex compared with those of unicellular organisms and invertebrates. This is true even though the molecular and cellular triggers are similar and both groups use similar proteins for osmosensing. The main difference is due to increased complexity, functional differentiation, and more elaborate signaling contingencies in vertebrates. The once-prevailing notion of a single or a few specific osmosensor(s) connected to a handful of osmosensory pathways that control the multitude of osmoregulatory effector mechanisms is now obsolete.

Instead, it is likely that osmosensory input at the cellular level of vertebrates is integrated from a large number of molecular osmosensors, each of which monitors a different event associated with a change in environmental salinity. Such osmosensors may include specific ion sensors, membrane stretch-activated channels activated, molecular chaperones that monitor protein folding efficiency, DNA damage sensors, and cytoskeleton-associated proteins. At the organismal level, coordination of acclimatory responses to salinity change is achieved via cytokines, hormones, and neurotransmitters. There is evidence for the existence of both central (e.g., systemic osmoreceptors) and local osmosensors. Central osmosensors sense changes in plasma osmolality, whereas local osmosensors have the potential to sense direct external osmolality changes (e.g., gill epithelial cells) in addition to plasma osmolality changes. Experiments with isolated opercular membrane preparations of killifish (*Fundulus heteroclitus*) indicate that a reduction of Cl^- secretion can be rapidly initiated via alpha2-adrenergic receptors and inositol-3-phosphate (IP3), suggesting an involvement of the sympathetic nervous system (SNS) in osmosensing. Central osmosensors of fishes also include systemic osmoreceptors and baroreceptors that monitor plasma osmolality, extracellular fluid volume, and blood pressure. They appear to be located primarily in gills and pituitary gland of fishes.

The operating range of various osmosensors differs considerably and it is likely that information about the particular magnitude of environmental salinity change can be derived through integration of signaling input from multiple osmosensors. For instance, cell volume, cytoskelelatal strain, and macromolecular damage sensors respond to more severe osmotic stress compared to the activation of calcium-sensing receptors (CaSRs) or transient receptor potential (TRP) ion channels. Another benefit of integrating input from multiple osmosensors is that the specific combination of sensory input can be highly stressor specific without the need for having any single stressor-specific osmosensor. Different and unique combinations of input from a steady set of sensors could be utilized for sensing different environmental stimuli (**Figure 1**). This strategy seems to be what has evolved in multicellular organisms such as fishes that have a great need for multitasking with the limited set of proteins they can accommodate in the crowded cellular space. As a result of this strategy, the complexity of osmosensory (and other types of) signal transduction in fishes and other vertebrates is tremendous. Consequently, tight linkages and interactions between mechanisms and protein elements of osmosensory signal transduction networks and other vital physiological functions exist, including immunity, apoptotic volume decrease, cell proliferation and differentiation, and development.

Molecular Osmosensors

Calcium as a Central Stimulus for Osmosensing

Calcium is a ubiquitous second messenger of intracellular signal transduction pathways. This divalent cation has been identified as a central element in osmosensory signal transduction networks of tilapia (*Oreochromis* sp.) and other fishes and plays a critical role in acclimation to salinity change. Calcium is necessary for organic osmolyte release and cell volume regulation during hyposmotic stress in gill cells of sea bass (*Dicentrarchus labrax*), but not in renal proximal

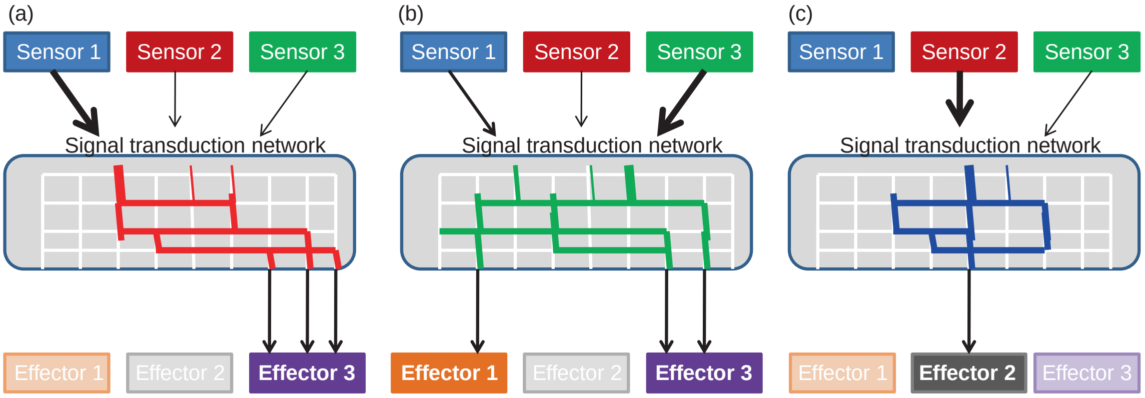


Figure 1 Schematic illustration of a strategy for utilizing a conserved set of proteins for sensing and transducing information about specific environmental parameters, including salinity. Osmosensory signaling networks are controlled by multiple sensors that monitor changes in salinity/osmolality and the particular combination of osmosensors as well as their relative activity control the information flow through the signaling network. (a) Moderate osmotic stress activates sensor 1 strongly and sensors 2 and 3 weakly, which is integrated into a distinct pattern of information flow through the signaling network resulting in activation of a specific set of effectors (effector 3). (b) Severe osmotic stress results in a different pattern of osmosensor activation leading to modification of information flow through the signaling network and allowing assessment of osmotic stress severity. (c) During environmental stresses other than osmotic stress, many (but not all) of the same proteins involved in osmosensing and signaling are utilized but the different activity pattern of the combined set of sensors allows for a stressor-specific outcome.

tubule cells of rainbow trout (*Oncorhynchus mykiss*). Thus, effects of calcium on osmosensory signal transduction mechanisms may depend on species and/or type of tissue/cell. Intracellular calcium levels are modified by multiple mechanisms during salinity stress, including activation of voltage-gated calcium channels, regulation of release from intracellular calcium stores, and systemic factors such as cortisol and angiotensin II (ANGII).

Extracellular calcium concentration is sensed by the large membrane-bound glycoprotein CaSR. The CaSR is a dimeric, seven-transmembrane-domain, G-protein-coupled receptor that senses the concentration of extracellular calcium. It is conserved throughout the Chordata. CaSR is expressed in the pituitary gland, corpuscles of Stannius, olfactory epithelium, and in ion-transporting tissues such as kidney, intestine, gills, and shark rectal gland. In these tissues is it thought that CaSR may have direct effects on monovalent and divalent ion transport that are independent of systemic factors. CaSR recognizes multiple types of ligands, including calcium and magnesium ions, other polyvalent cations, and amino acids. It senses calcium concentration in the millimolar range, which is appropriate in particular for marine fishes. CaSR is thought to sense extracellular calcium as a surrogate measure of environmental salinity in marine and brackish environments. Interestingly, monovalent cations shield CaSR-binding sites in a competitive manner and therefore may influence the osmosensory function of CaSR.

CaSR has been identified in phylogenetically diverse groups of fishes, where it is thought to be the main osmosensor. In addition to sensing extracellular osmolality directly, CaSR stimulates stanniocalcin secretion in salmon and may, therefore, also modulate calcium-regulated osmotic stress signaling more indirectly. Teleost CaSR has been shown to activate downstream intracellular signaling pathways, a function that is essential for any physiological osmosensor. Signaling pathways activated by CaSR include phospholipase C regulated pathways and mitogen-activated protein kinase (MAPK) pathways.

Cytoskeletal Strain as an Osmosensory Signal

Cytoskeletal strain and membrane stretch can result from severe osmotic stress such as that generated during acute transfer between salinities that differ by more than 10 ppt. In such a scenario, it is possible that changes in cell volume occur in gill and other epithelial cells that are directly exposed to the external milieu or (less likely) even in internal cells as a result of significant changes in plasma osmolality. Multiple proteins have been identified in mediating the immediate osmotic stress response in tilapia exposed to acute salinity increases. Several of these proteins regulate the actin-based cytoskeleton and may be involved in sensing changes in actin cytoskeleton organization during salinity stress. These proteins include annexin 11A (ANX11A), gelsolin, and galectin 4. All three of these proteins represent functional links between calcium signaling, discussed above, and cytoskeleton dynamics. ANX11A is a member of the Ca^{2+}-dependent, phospholipids-binding protein family. It is important for organizing the interface between plasma membrane and cytoskeleton due to its ability to bind the negatively charged phospholipids of the inner leaflet of the plasma membrane and the cytoskeleton. Annexins are also involved in phospholipase inhibition, intracellular signaling, anticoagulation, and membrane fusion. Gelsolin is a Ca^{2+}-regulated and polyphosphoinositide 4,5-bisphosphate-regulated actin filament severing and capping protein that promotes actin remodeling. Galectins also regulate interactions between the cell membrane and the cytoskeleton, which are critical targets of osmotic stress-induced cell volume changes. Galectin-4, in particular, organizes lipid rafts and membrane microdomains consisting mainly of glycosphingolipids and cholesterol in the outer leaflet of the plasma membrane lipid bilayer. Such remodeling may also support changes in tight junction structure during salinity acclimation. Collectively, these data support the notion that acute osmotic stress causes cytoskeletal strain that acts as an osmosensory signal.

The Rho-GTPase pathway is also a promising candidate for an early transducer or even a sensor of cytoskeletal strain. This pathway is overrepresented in the osmoregulatory tissues of sharks and represents an integral part of osmotic stress response networks identified in sharks and teleosts. In particular, Rho guanine exchange factors (Rho-GEFs) and Rho-GTPase seem to play a role for osmosensory signal transduction in fish. Rho is a small G protein that interacts with the cytoskeleton and is capable of activating MAPK pathways in response to cytoskeletal strain. Ezrin/moesin, an actin-interacting protein, may also be involved in transducing cytoskeletal strain to downstream signaling pathways. This protein has been identified in gill tissue of tilapia and sharks using proteomics and represents a major target of Rho kinase.

Stretch-activated/mechanosensory ion channels have been implicated as potential osmosensors in fish and are thought to be responsible for triggering signal transduction that controls prolactin (PRL) secretion from the pituitary. However, unlike in PRL cells of the fish pituitary, membrane stretch may not occur in epithelial cells that are extensively folded at both the basolateral and apical membranes. Instead, relaxation or compression of microridges and other membrane infoldings as a result of cell volume changes are more likely to modulate the underlying cytoskeleton. Thus, molecular sensors triggered by cytoskeletal strain are more likely to function as osmosensors during acute osmotic stress in epithelial cells than stretch-activated ion channels. Macromolecular crowding and dilution are also signals that result from cell volume changes. Such signals could affect essentially any protein in the cell and are virtually impossible to experimentally separate from other triggers such as cytoskeletal strain and changes in membrane folding.

Membrane Protein Osmosensors

In addition to CaSR, several other membrane proteins, including TRP channels, aquaporin (AQP), and adenylate cyclase (AC), have been implicated as osmosensors of fish. Transient receptor potential vanilloid receptor 4 (TRPV4) senses salinity changes in diverse animals and has been cloned from zebrafish (*Danio rerio*), although there is no direct evidence for TRP being an osmosensor in fish. Consistent with the model shown in **Figure 1**, TRP channels are activated by multiple stimuli, not just osmotic stress. AQPs are important effector proteins of osmoregulation in fish (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin) and play a role in altering the water permeability of plasma membranes in response to salinity changes. Moreover, AQPs are known to function as osmosensors in other vertebrates. AC is known to be an osmosensor in bacteria and there is mounting evidence that it may play a similar role in fish. In many euryhaline fishes, AC controls chloride secretion across gill epithelium, PRL, and growth hormone (GH) secretion from pituitary gland cells in response to salinity stress. In addition, forskolin (an activator of AC) stimulates chloride secretion across killifish opercular epithelium.

The Ion–Damage–Cytokine Model of Osmosensing

Cells sense environmental stress using two avenues. One is relatively stimulus specific and termed the cellular homeostasis response (CHR) and the second is a nonspecific response to damage, termed the cellular stress response (CSR). During osmotic stress, the CHR, on the one hand, is responsible for conferring specificity to osmosensory signal transduction and is presumably based on sensing concentrations of specific cations such as calcium, sodium, and potassium in intra- and/or extracellular fluid and on sensing intracellular ionic strength. The CSR, on the other hand, is important for counteracting damage and strain on macromolecules caused by osmotic stress and is responsible for the cross-tolerance phenomena. In addition to these two cellular modes of osmosensing, fishes have systemic osmoreceptors that coordinate osmosensory signal transduction in different tissues and organs in an effort to synchronize osmoregulation at the whole organism level.

Osmoregulatory hormones and more broadly cytokines are important mediators of this whole organism synchronization. The interplay of ion concentrations, macromolecular damage, and cytokines/hormones during osmosensing is schematically summarized in **Figure 2**. Like cytokines, systemic regulation mediated via neurotransmitters may also serve to synchronize and

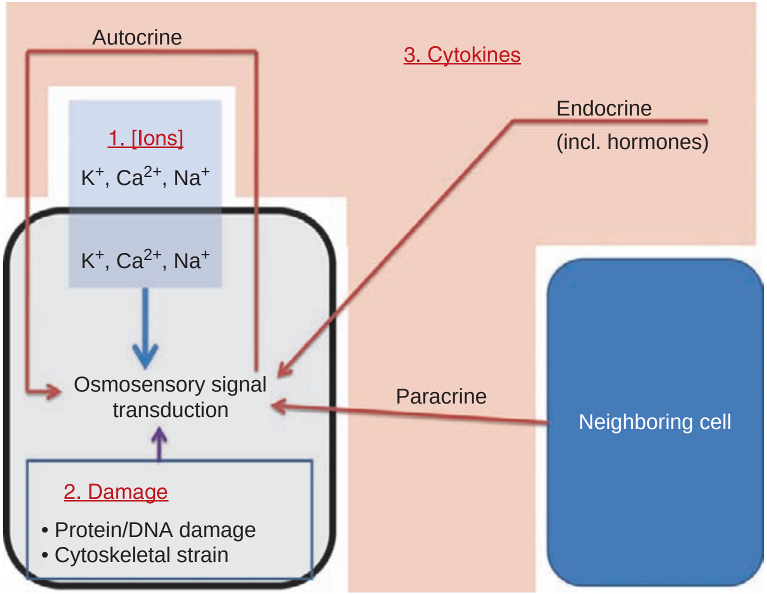


Figure 2 The ion–damage–cytokine model of osmosensing. The first two elements of osmosensing (ions and macromolecular damage) are cell autonomous and do not depend on systemic factors. The third element (cytokines, including peptide hormones and other regulatory peptides) coordinate osmosensory signal transduction at the whole organism level and act in autocrine, paracrine, and endocrine fashion. In addition to cytokines/peptide hormones, some steroid hormones and neurotransmitters also contribute to systemic osmoregulation. Components 2 and 3 are also involved in other types of environmental stress responses and responsible for cross-tolerance phenomena, whereas component 1 (ions) confers stressor specificity.

fine-tune osmoregulatory responses at the whole organism level. The overly simplistic 'one sensor–one pathway–one specific response' concept that prevailed through much of the twentieth century has been replaced by the realization that environmental stress signaling networks in multicellular animals, in particular vertebrates, are much more complex and interdependent than in unicellular organisms. These highly differentiated animals need to make repeated use of each protein present in the limited set of proteins encoded by their genome. In fishes and other vertebrates, almost every protein is likely used for many different functions depending on the developmental, environmental, and life history context of organisms. Which function of a particular protein dominates depends on its interaction with other proteins and its intracellular compartmentalization and localization, which in turn are regulated via posttranslational protein modifications (PTMs).

Macromolecular Damage Contributes to Osmosensing

An important avenue of osmosensory input is macromolecular damage. This signal is presumably relatively nonspecific and responsible for cross-tolerance to other types of environmental stressors. However, if integrated with additional ion-sensory input signals, the signaling pathways controlled by protein and DNA damage sensors are modulated in specific ways that enable activation of an appropriate array of effector mechanisms (**Figure 1**). In addition, such signal integration and amplification is likely also responsible for accurate quantification of the severity of osmotic stress and an appropriate degree of activation of effector mechanisms. Very little is known on how the severity or extent of osmotic stress is measured in cells and this is an important area for future studies. Although most macromolecular damage may be nonspecific, the possibility that certain types of DNA or protein damage are unique during osmotic stress cannot be excluded. Preferential degradation of particular proteins in response to osmotic stress may serve as an osmosensory signal. In agreement with this notion, several specific proteins involved in DNA, RNA, protein stabilization, repair, and degradation are rapidly induced in gill of teleosts and rectal gland of elasmobranchs exposed to hyperosmotic stress. In addition, key signaling proteins of DNA and protein damage response pathways are integral parts of osmosensory signaling networks in teleosts and elasmobranchs. Moreover, osmoregulatory actions of cytokines/peptide hormones and osmotic stress signaling proteins are controlled by proteolysis events mediated by peptidases and proteases. Differences in peptidase activity in osmoregulatory tissues of teleosts have been attributed to their different osmoregulatory mechanisms in FW versus SW.

Cytokines Coordinate Osmosensory Signal Transduction

The term 'cytokine' is used as a common denominator of a diverse array of soluble proteins and peptides that regulate cell and tissue functions via humoral actions at low concentrations. Many of them (like growth factors) regulate cell survival, differentiation, and apoptosis. Cytokines are secreted proteins/peptides and several are synthesized as procytokines and activated by proteolytic cleavage, which sometimes coincides with shedding of a membrane-bound form, from the cell membrane, into the extracellular fluid (e.g., tumor necrosis factor alpha (TNF-α)). Most cytokines are glycoproteins that are secreted via classical secretory pathways from cells. They are not usually stored in cells but there are exceptions that are relevant to osmosensing, for example, TGF-β and TNF-α procytokines. In general, differences between classical hormones and cytokines are: (1) cytokines are not necessarily produced by specialized glands or tissues; (2) all cytokines are peptides/proteins encoded in the genome (unlike steroid hormones); and (3) cytokines have a generally wider spectrum of target tissues/cells. However, there are exceptions to these general rules, and the distinction between cytokines and hormones is blurred. Classical peptide hormones and growth factors that are important for teleost osmoregulation are also considered to be cytokines in the mammalian literature (e.g., GH, insulin-like growth factor 1 (IGF-I), atrial natriuretic peptide (ANF), and PRL (**Figure 3**)).

TNF-α and interleukins

We have recently performed several transcriptomic and proteomic screens for osmotic stress signaling proteins in teleosts and elasmobranchs. The results of these studies and bioinformatic pathway modeling showed that classical cytokines, including TNF-α and several interleukins (ILs), constitute conserved elements of osmosensory signal transduction networks in fishes. These classical cytokines are also critical in many environmental contexts, including pathogen invasion stress, and for mounting an appropriate immune response. Their involvement in osmosensing may explain why abiotic environmental stresses, such as osmotic stress, modulate the immune system and change the ability of fish to ward off infections by bacteria and viruses. TNF-α and ILs are cytokines that can act in autocrine and paracrine fashions and, therefore, reinforce and coordinate epithelial responses to salinity change. In tilapia, TNF-α amd IL signaling during osmotic stress is in part mediated by the MAPK and toll-like receptor pathways. From work on other vertebrates, it is known that transforming growth factor beta-activated kinase 4 binding protein 2 (TAB2; an MAP3K interacting protein) is stimulated by IL-1 and activates the MAP3K TAK1. TAK1 interacts with TNF receptor-associated factor 6 (TRAF6), which is activated by TNF-α and controls downstream signaling pathways for cell differentiation, proliferation, apoptosis, and stress-responsive gene expression.

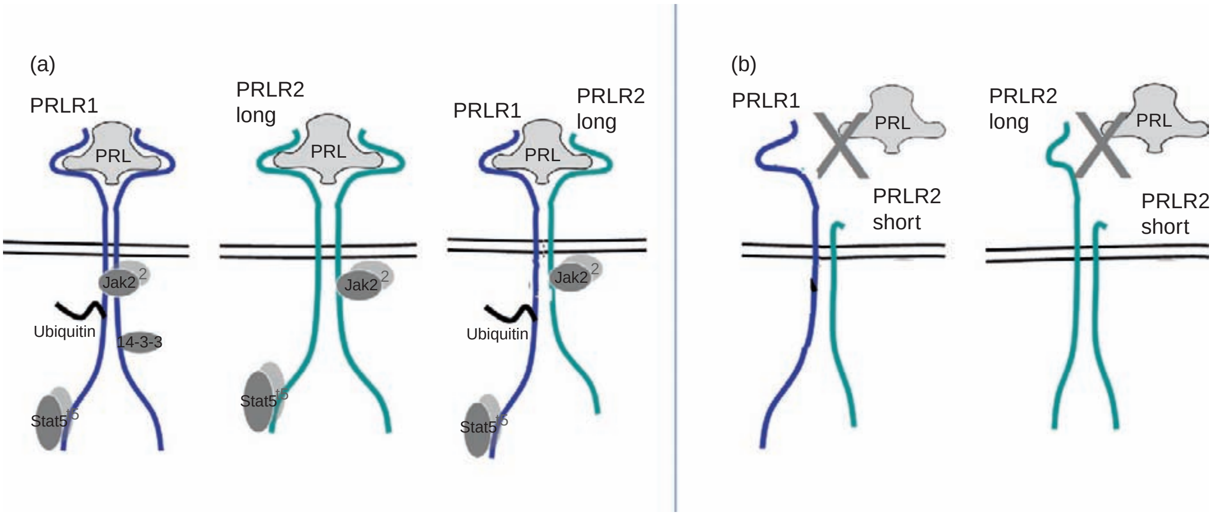


Figure 3 Model for prolactin receptor (PRLR) regulation during salinity stress. (a) In FW long splice variants of both PRLRs are expressed that homo- or hetrodimerize and effectively bind PRL, which is present in high concentration in plasma. On ligand-binding, PRLR dimers activate intracellular signaling pathways, including the Jak2/ Stat5 pathway. (b) In SW expression of PRLR2 is induced and the abundance of a short splice variant of PRLR2 increases. The short PRLR2 variant lacks much of the extracellular domain and dimerizes with long PRLR variants to turn them into inactive decoy receptors. Together with a decrease in plasma prolactin, this mechanism turns off intracellular PRL signaling in target cells. Modified from Fiol DF *et al*. (2009) A novel tilapia prolactin receptor is functionally distinct from its paralog. *Journal of Experimental Biology* 212: 2006–2014.

Cellular Osmosensing and Chloride Cell Differentiation

Chloride cells (or more general mitochondrion-rich cells, MRCs) are the main cellular engines of transepithelial ion transport in euryhaline teleost gill and shark rectal gland. MRCs undergo extensive re-differentiation in response to salinity change and these events are controlled by osmosensing and osmotic stress signaling mechanisms. MRC in preparations of sealed tilapia yolk sac membranes undergo the same transformation (increase in size, formation of multicellular complexes, ion transporter compartmentation, etc.) as those observed in yolk sac and gill epithelium of intact animals but administration of cortisol (0.1–10 $\mu g\,mL^{-1}$) had no effect on MRC differentiation. Thus, major parts of MRC reprogramming in response to salinity change can proceed independent of cortisol or other systemic (neuroendocrine) signals. Likewise, expression of osmotic stress transcription factor 1 (OSTF1) in primary cultures of tilapia gill epithelial cells is independent of cortisol or other systemic signals indicating that expression of (at least some) osmotic stress signaling genes can be regulated autonomously at the cellular/epithelial level. The lack of dependence of OSTF1 expression on cortisol was a surprise because of known corticosteroid regulation of the OSTF1 ortholog glucocorticoid-induced leucine zipper (GILZ) in mammalian cells. Even though major MRC reorganization events triggered by salinity change appear to be autonomous, presently it is still unclear to what degree salinity-dependent epithelial reorganization can be completed in the absence of systemic signals (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts, Mechanisms of Ion Transport in Freshwater Fishes, Osmoregulation in Fishes: An Introduction, and The **Hormonal Control of Metabolism and Ionic Regulation**: Hormonal Control of Osmoregulation in Teleost Fish).

According to elegant *in vivo* sequential observations on chloride cells in the yolk sac membrane of tilapia, single FW-type MRCs are transformed into multicellular SW-type MRCs in response to SW transfer, suggesting plasticity in the ion-transporting functions and the directionality of active NaCl transport of MRCs. Individual MRC observed sequentially over time increased in size in response to SW transfer but not in FW controls in tilapia yolk sac preparations. Moreover, the compartmentalization of key ion transporters changed dynamically in tilapia yolk sac membranes during transfer from FW to SW. Three types of FW MRCs with either basolateral NKA, basolateral sodium–potassium–adenosine triphosphate (NKA), and apical $Na^+/K^+/2Cl^-$co-transporter (NKCC), or basolateral NKA and basolateral NKCC compartmentation transform into SW MRCs having basolateral NKA, basolateral NKCC, and apical cystic fibrosis transmembrane receptor. These studies suggest that existing MRC can re-differentiate into a different phenotype depending on environmental salinity. Other studies show that MRC turnover, programmed cell death of MRC, and differentiation of ionocyte precursor cells are influenced by environmental salinity implying that FW-type MRCs are replaced by SW-type MRCs during salinity change. It appears likely that both of these general mechanisms contribute to MRC reprogramming in response to osmotic stress in fish gills, perhaps with different time courses.

In stenohaline zebrafish the transcription factor glial cell missing 2 (Gcm2) is highly expressed in gills and necessary for development of H^+-ATPase-rich, mitochondrion-rich cells (HR-MRCs) that promote acid secretion and Na^+ absorption in FW. The expression of Gcm2 is subject to positive feedback from the forkhead transcription factor Foxi3a and vice versa. Foxi3a is also necessary for the development of HR-MRCs. In the zebrafish mutant mind bomb characterized by impaired Notch function Foxi3a expression is increased and differentiation of precursor cells is greatly shifted toward the HR-MRC cell fate. Conversely, overexpression of Notch drives precursor cell differentiation toward the keratinocyte cell fate. Collectively, these experiments indicate that Delta C and Foxi3a signaling pathways promote differentiation into HR-MRC, whereas Notch promotes the alternative keratinocyte cell fate. Thus, determination of ionocyte precursor cell differentiation into either HR-MRC or keratinocytes depends on Delta–Notch inhibition and paracrine signaling that precludes HR-MRC neighboring cells from differentiating into the same cell type (**Figure 4**). Analysis of another zebrafish mutant (*quadro*) that is deficient in Foxi1 expression revealed that Foxi1 is also essential for HR-MRC differentiation and positioned upstream of Delta C, Foxi3a, and Gcm2 in the pathway that promotes HR-MRC differentiation. Although these studies were done in stenohaline zebrafish larvae in a developmental rather than salinity acclimation context, they provide groundbreaking insight into signaling mechanisms that may control the epithelial and MRC reorganization during salinity stress in euryhaline teleosts. Future study of the role of Foxi1, Foxi3a, and Gcm2 transcription factors as well as Delta–Notch signaling for osmosensory signal transduction in euryhaline fish is warranted.

In the context of the findings on the regulation of zebrafish HR-MRC differentiation, it is noteworthy to point out a signaling link to bone morphogenetic proteins (BMPs) that may be central for osmosensory signal transduction in euryhaline teleosts. Vertebrate BMP2 and BMP7 are downregulated in Delta C (also known as Jagged) mutants likely as a result of negative feedback, and it has been proposed that BMPs control the Delta–Notch signaling pathway and HR-MRC differentiation in

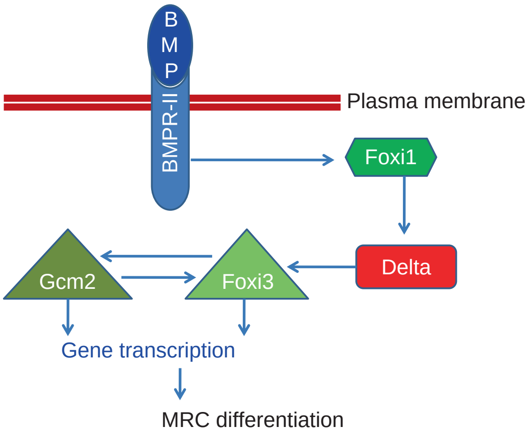


Figure 4 Model for the regulation of MRC differentiation by bone morphogenetic proteins (BMPs), which represent a subfamily of cytokines. During hyperosmotic stress BMP receptors (BMPRII) are upregulated in gill epithelium leading to the activation of the Delta C pathway that controls MRC differentiation. Key components of the BMP–Delta C pathway are shown. Foxi3 and Gcm2 are transcription factors. Modified from Esaki M *et al*. (2009) Mechanism of development of ionocytes rich in vacuolar-type $H^{(+)}$-ATPase in the skin of zebrafish larvae. *Developmental Biology* 329: 116–129.

zebrafish. In addition, we have previously identified a receptor for BMPs (BMPRII) as an immediate early gene whose expression is induced very rapidly in response to salinity stress in tilapia gill epithelium resulting in enhanced BMP signaling. Therefore, an increase in BMPRII during hyperosmotic salinity stress may change the balance between Delta–Notch signaling in any given cell, which could explain alteration of MRC differentiation and multicellular MRC complex formation when euryhaline fish transition between plasma-hyposmotic and plasma-hyperosmotic environments (**Figure 4**).

Role of Osmosensing for Fish Sperm Motility

Fish sperm motility is controlled tightly by osmolality and the concentration of cations. Therefore, fish sperm represents an excellent model for studying general mechanisms of cellular osmosensing in fish. Depending on habitat salinity and osmoregulatory strategy, sperm motility is either activated or inhibited by increased osmolality. In marine teleosts, sperm motility increases when released into a plasmahyperosmotic environment (SW), whereas in FW teleosts it increases upon release into a plasmahypoosmotic environment (FW). In striped bass sperm motility is promoted by Ca^{2+}-free solutions and suppressed when $[Ca^{2+}]$ exceeds 10 mM. The effect of $[Ca^{2+}]$ is transient and irreversible; once sperm motility has been activated, it cannot be suppressed by high $[Ca^{2+}]$ (80 mM) and reactivation by Ca^{2+}-free medium after suppression is also not possible. Verapamil, a Ca^{2+} channel inhibitor, and cAMP had no effect on sperm motility in striped bass suggesting that Ca^{2+} acts through other pathways. These data reinforce the notion that calcium is of central importance for osmosensing (see above). Because of the rapid time course of initiation of sperm motility, the study of sensory and signaling mechanisms underlying this event in marine versus FW fishes may provide critical clues about mechanisms of osmosensing in fish. In addition to calcium, intracellular $[K^{+}]$ has also been shown to be critical for the regulation of sperm motility in FW (zebrafish) and marine (pufferfish) species. Thus, intracellular inorganic cation concentrations are critical signals for osmosensory signal transduction in fish sperm.

See also: **Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish. **Intertidal Fishes**: Intertidal Habitats. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction; Water Balance and Aquaporin.

Further Reading

Alavi SMH and Cosson J (2006) Sperm motility in fishes. (II) Effects of ions and osmolality: A review. *Cell Biology International* 30: 1–14.

Esaki M *et al.* (2009) Mechanism of development of ionocytes rich in vacuolar-type $H^{(+)}$-ATPase in the skin of zebrafish larvae. *Developmental Biology* 329: 116–129.

Fiol DF *et al.* (2009) A novel tilapia prolactin receptor is functionally distinct from its paralog. *Journal of Experimental Biology* 212: 2006–2014.

Fiol DF and Kültz D (2007) Osmotic stress sensing and signaling in fishes. *FEBS Journal* 274: 5790.

Kültz D (2005) Molecular and evolutionary basis of the cellular stress response. *Annual Review of Physiology* 67: 225–257.

Kültz D and Burg M (1998) Evolution of osmotic stress signaling via MAP kinase cascades. *Journal of Experimental Biology* 201, 3015–3021.

Loretz CA (2008) Extracellular calcium-sensing receptors in fishes. *Comparative Biochemistry and Physiology Part A – Molecular and Integrative Physiology* 149: 225–245.

Relevant Websites

http://kueltzlab.ucdavis.edu– Environmental Stress Response · Kültz Lab · Evolutionary Proteomics.

THE GUT

Role of the Gills

Contents

Morphology of Branchial Ionocytes
The Osmorespiratory Compromise

Morphology of Branchial Ionocytes

JM Wilson, Centre for Interdisciplinary Marine and Environmental Research (CIIMAR), Porto, Portugal

© 2011 Elsevier Inc. All rights reserved.

Introduction
Pavement Cells
MRCs or Chloride Cells
MCR Identification
Further Reading

Glossary

Basal labyrinth The elaborate infoldings of the basolateral plasma membrane increasing surface area.

Freeze-fracture electron microscopy A technique used to visualize the inside (p-face) and outside (e-face) leaflet of lipid bilayer of cellular membranes; cells are rapidly frozen and fractured, which exposes the bilayer surfaces *en face*. A replica is created and viewed by scanning electron microscope. This technique reveals the pattern of integral membrane proteins.

Ionocytes (mitochondrion-rich cells) A function-specific type of epithelial cell, which expresses ion transporters or enzymes and is responsible for trans-epithelial transport functions of specific ions. In fish gills, ionocytes responsible for ion regulation are named mitochondrion-rich cells based on the characteristic of abundant mitochondria.

Interlamellar space (ILS) The part of the filament epithelium found between the bases of the gill lamellae.

Lamellae Also known as secondary lamellae, these are attached in rows to the gill filaments. They are the primary sites for gas exchange in fish gills. Each lamella is made up of two epithelial layers separated by pillar cells. Oxygen is taken up by erythrocytes flowing inside the lamellae from water flowing between the lamellae.

Tubular system A network of regularly anastomosing tubules of uniform diameter that is continuous with the basolateral plasma membrane, characteristic of chloride cells.

Tubulovesicular system A collection of subapical irregular tubules and vesicles.

Introduction

Aristotle noted in his work *Historia Animalia* (345 BC) that the fish gill was indeed a special organ. However, it was not until the pioneering work of Homer Smith in the 1930s that an ionoregulatory function could be ascribed to the gills (**see also Osmotic, Ionic and Nitrogenous-Waste Balance:** Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). Since Keys and Willmer's identification in 1932 of presumptive chloride-secreting cells, with their greater affinity for eosin, ovoid shape, and granular cytoplasm, much effort has been put into the study of branchial ionocytes. Branchial ionocytes are found in all taxa of fish from the osmoconforming hagfish to the air-breathing lungfish. Light and electron microscopic descriptions have been complemented by enzyme and immunohistochemistry to characterize the expression of ion transport proteins that make these cells ionocytes (**see also Osmotic, Ionic and Nitrogenous-Waste Balance:** Mechanisms of Gill Salt Secretion in

Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). Two cell types potentially function as ionocytes in the fish gill, the pavement cell (PVC) and mitochondrion-rich cell (MRC), and their morphological attributes form the subject of this article. Functional consideration of these cell types in ion and acid–base regulation are detailed in **Osmotic, Ionic and Nitrogenous-Waste Balance:** Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes.

Pavement Cells

PVCs are the squamous to cuboidal cells that cover much of the gill (>90% of the surface area; **Figures 1**(**a**) and **1**(**b**); **see also Design and Physiology of Arteries and Veins:** Branchial Anatomy). The apical surface of PVCs is usually large and polygonal and may be smooth or have microvilli (finger-like projections) or microridges, which typically form concentric rings (referred to microplicae when viewed in cross section). A double ridge generally marks the border of neighboring PVCs. The types of apical features present show variation between species and also within and between the different gill epithelia (lamellar vs. filament). PVCs typically have low mitochondrial densities and unelaborated basolateral membranes (**Figure 2**(**a**)). The nucleus in squamous PVCs is compressed, while it is rounded in cuboidal PVCs. PVCs have the typical intracellular organelles, rough and smooth endoplasmic reticulum (ER), Golgi apparatus, lysosomes, and vesicles with contents of various electron densities. In the nonteleost fishes, PVCs display abundant mucous secretory granules in the apical cytoplasm (**Figure 3**). PVCs are joined by desmosomes and the peripheral cytoplasm contains microfilaments. The tight junctions associated with PVCs have multiple strands and are not characterized as leaky, minimizing paracellular fluxes of ions (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes). In the hagfish and lamprey, communicating gap junctions have been found between neighboring PVCs, and PVCs and undifferentiated cells, however, not with MRCs. The presence of communicating gap junctions is significant because they allow the electrochemical coupling of the cells of the epithelium, which is important in coordinating cell growth, differentiation, and function. No studies have been undertaken in other fishes to specifically determine the presence of gap junctions.

PVCs have been shown to be involved in the covering and uncovering of MRCs during acid–base and ionoregulatory disturbances, but the role of the PVC in the process is somewhat secondary to that of the MRC in the functional response. Generally, PVCs themselves have been shown to be morphologically unresponsive to changes in environmental conditions. However, part of the problem stems from the fact that the MRCs are generally the focus of attention in morphological studies and the changes in PVCs may have been overlooked. It also might be difficult to recognize these changes if they only occur in a subpopulation of PVCs, since there are proportionally many more PVCs (90% of epithelial cells).

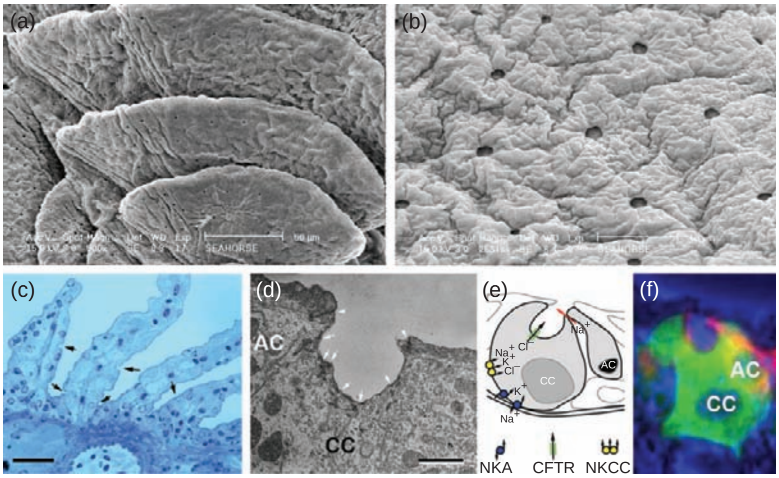


Figure 1 Characterization of chloride cell (CC) and accessory cell (AC) apical crypts in marine teleost fishes. (a, b) SEM images of a seahorse (*Hippocampus kuda*) gill filament lamellae. Note that the surface of the lamellae is pitted with CC apical crypts. (c) A thick (0.5μm) section stained with toluidine blue of afferent edge lamellae showing CC apical crypt (arrows) corresponding to pitting. (d) TEM sections through the apical crypt showing clear evidence of sharing by more than one cell (CC and AC) as is evident from the appearance of multiple shallow tight junction complexes (arrows) in contrast to the CC–PVC junctions (arrowheads). (e) Schematic illustrating the relative locations of the ion transport proteins involved in NaCl excretion. (f) Representative IHC staining of CC (Na^+/K^+-ATPase Ab α5, green)–AC (NHE2 Ab 597 red) complex in seawater adapted tilapia (*Oreochromis mossambicus*). Scale = (a) 50, (b) 10, (c) 25, and (d) 1 μm. From Wilson, JM, unpublished data.

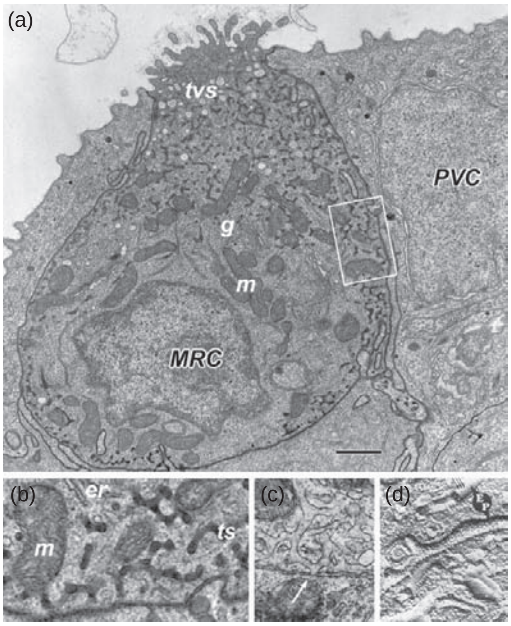


Figure 2 (a, b) TEMs of the MRC of the freshwater chum salmon larvae (*Oncorhynchus keta*) using the potassium ferrocyanide-reduced osmium stain that preferentially stains the tubular system (ts) and the plasma membrane while other membrane systems remain weakly stained. (c) TEM showing the connections of the tubular system with the basolateral membrane (indicated by arrows) in the neon tetra (*Paracheirodon innesi*) MRC using conventional heavy metal staining. (d) Freeze-fracture replicas of the seawater mullet MRC showing studded appearance of the tubular elements. Scale = (a) 1 ìm; (b, c) 0.5 ìm. (a–c) Adapted from figure 8 in Wilson JM and Laurent P (2002) Fish gill morphology: Inside out. *Journal of Experimental Zoology* 293: 192–213. (d) Adapted from figure 4 in Sardet C (1980) Freeze fracture of the gill epithelium of euryhaline teleost fish. *American Journal of Physiology* (*Regulatory, Integrative and Comparative Physiology*) 238: R207–R212, used with permission of American Physiological Society.

With that said, there have been a few studies that have shown that PVCs increase apical surface area (densities of microvilli and microplicae) in response to hypercapnia and alkaline exposure which induce acid–base disturbances. It has also been found that there are studded subapical vesicles that resemble the proton pump containing vesicles from other animals. These morphological characteristics of PVCs help support the hypothesis that they are the site of proton-pump-driven sodium uptake at least in some species (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts).

MRCs or Chloride Cells

The descriptive morphological study of the gill has been dominated by the mitochondrion-rich chloride cell (CC) and its subtypes (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). In general, the numerous mitochondria in these cells are thought to supply the adenosine triphosphate (ATP) for ion transport proteins to drive the vectorial transport of ions as part of ion and acid–base regulation.

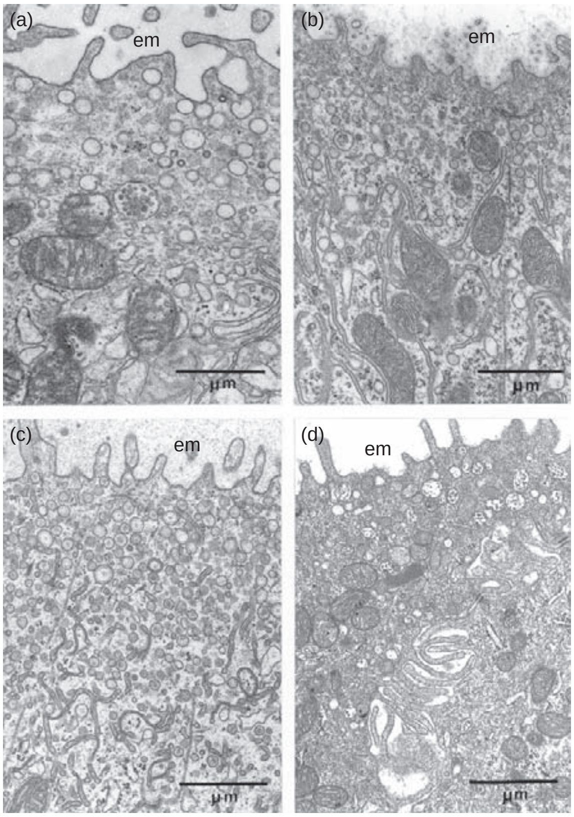


Figure 3 TEMs of the apical regions of the MRCs of (a) *Raja erinacei*, (b) Acipenser beari, (c) *Perca fluviatilis*, and (d) *Protopterus annectens* showing the tubulovesicular system. Scale = 1 μm. Reproduced from figure 9 in Wilson JM and Laurent P (2002) Fish gill morphology: Inside out. *Journal of Experimental Zoology* 293: 192–213.

MRCs tend to be concentrated in the afferent region of the filament epithelium and have an intimate association with the arteriovenous circulation, notably the central venous sinus, although in the interlamellar region MRCs also are associated with the basal channels of the lamellar arterioarterial circulation (**see also Design and Physiology of Arteries and Veins:** Branchial Anatomy 2000). The defining morphological feature of this cell type is a high mitochondrial density. However, MRCs also generally have an amplification of their basolateral membrane through either folding forming a basal labyrinth or the presence of a tubular system (**Figures 2, 4, 5,** and **7**). Although basolateral amplification can be less developed as in lamprey intercalated cell (IC)-type MRCs (**Figure 6**) and lacking in mitochondrion-rich PVCs. Associated with the basolateral membrane

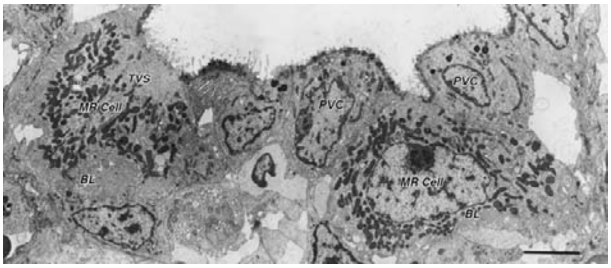


Figure 4 TEM micrograph montage of the interlamellar region of the primary filament from the gill of a dogfish *Squalus acanthias*. Note the mitochondria-rich (MR) cells identified by their ovoid shape, numerous mitochondria, dense subapical tubulovesicular system (TVS), and extensive basal labyrinth (BL). The apical surface is covered with microvilli. Scale = 5 μm. Reproduced from figure 1 in Wilson JM, Morgan JD, and Randall DJ (2002). Role of mitochondria-rich cells in the gills of an elasmobranch fish *Squalus acanthias*. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 132(2): 365–374, with permission from Elsevier.

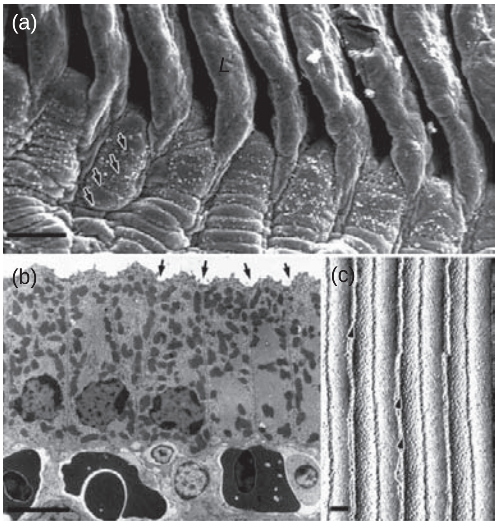


Figure 5 Chloride cells in the gill epithelium of an adult *Geotria australis* in seawater. (a) SEM view of the filament epithelium afferent side. Arrows indicate the borders of CC in a row extending beyond the interlamellar space. (b) Cross section of the filament, showing a row of chloride cells on each side of the central blood space. (c) Freeze fracture of a bundle of parallel membranous tubules, showing the helicoidally arranged particles (arrowheads). Scale = (a) 25 μm, (b) 5 μm, and (c) 0.1 μm. Adapted with permission from figure 7 in Bartels H and Potter C (2004) Cellular composition and ultrastrucutre of the gill epithelium of larval and adult lampreys. Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462 (doi: 10.1242/jeb0115); and from figure 5 in Bartels H, Moldenhauer A, and Potter IC (1996) Changes in the apical surface of chloride cells following acclimation of lampreys to seawater. *American Journal of Physiology* 270: R125–R133, used with permission of the American Physiological Society.

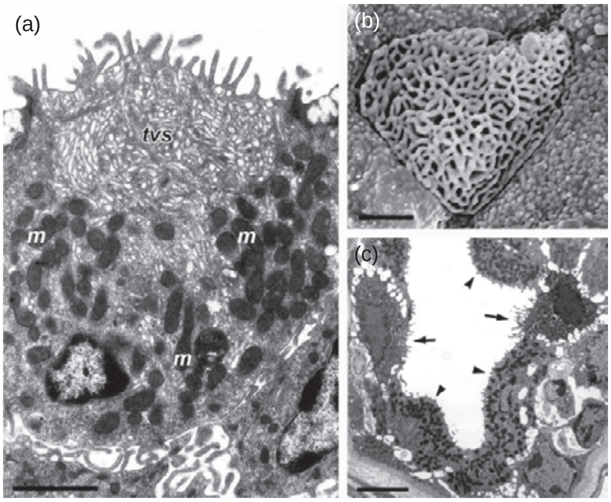


Figure 6 MRCs in the lamprey *Geotria australis* gill epithelium. (a) Intercalated MRC between two pavement cells in the gill epithelium of a downstream migrant (young adult). The apical surface is enlarged by slender microplicae and the apical cytoplasm contains large numbers of membranous tubules and vesicles. (b) Scanning electron micrograph of an intercalated MRC surrounded by ammocoete MRCs in the gill epithelium of a larva. (c) Gill epithelium in the interlamellar region of the filament and at the bases of the lamellae, showing intercalated MRCs (arrows) intercalated between ammocoete MRCs (arrowheads) in ammocoetes maintained in ion-poor water. Scale = (a, b) 2 μm and (c) 5 μm. (a, b) Adapted with permission from figures 2 and 3 in Bartels H and Potter C (2004) Cellular composition and ultrastrucutre of the gill epithelium of larval and adult lampreys. Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462 (doi: 10.1242/jeb001157). (c) From figure 1 in Bartels H, Schmiedl A, Rosenbruch J, and Potter IC (2009) exposure of the gill epithelial cells of larval lampreys to an ion-deficient environment: A stereological study. *Journal of Electron Microscopy* 58(4): 253–260 by permission of Oxford University Press.

amplification is the important ionoregulatory enzyme Na^+/K^+-ATPase. The presence of Na^+/K^+-ATPase has been observed by freeze-fracture electron microscopy as dense studding of the tubular elements (**Figure 2**(**d**)). In the apical region of MRCs is a collection of tubules and vesicles (tubulovesicular system), which is distinct from the tubular system (**Figure 3**). The apical membrane can be quite variable in appearance ranging from concave to convex, sometimes forming deep crypts. In addition, the surface topography has been shown to be smooth, or having microvilli of varying densities and lengths, as well as being highly branched to give a sponge-like appearance (**Figures 1**(**d**), **2**(**a**), and **3**–**7**). The use of morphological features has led to the classification of different types of MRCs.

The term chloride cell relates to the function of the MRC in Cl^- elimination. In seawater teleosts, the MRCs have quite convincingly been shown to be sites of active Cl^- elimination and hence the name is fitting (**see also Osmotic, Ionic and Nitrogenous-Waste**

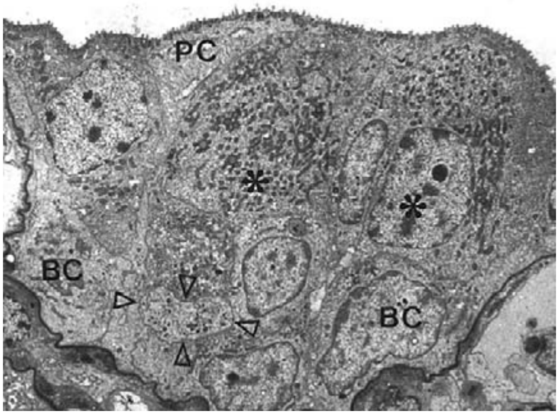


Figure 7 Epithelium covering a gill lamella of the Atlantic hagfish *Myxine glutinosa*. The following cell types can be distinguished: pavement cells (PC), mitochondrion-rich cells (asterisks), basal cells (BC), small granule cells (indicated by open arrowheads), and granulocytes (G). The arrow points to the microvilli-bordered apical surface of an MRC. Magnification ×2000. Reproduced from figure 1 in Bartels H (1984) Orthogonal arrays of particles in the gill epithelium of the Atlantic hagfish, *Myxine glutinosa*. *Cell and Tissue Research* 238: 657–659 with kind permission of Springer Science + Business Media.

Balance: Mechanisms of Ion Transport in Freshwater Fishes). In the agnathans, chondrichthyans, chondrosteans, holosteans, and freshwater teleosts, the evidence that MRCs are involved in Cl^- fluxes is limited to the few teleosts and elasmobranchs. Therefore, in order to avoid any confusion on the matter, we will only use the term chloride cell when referring to the seawater teleost MRC, unless otherwise noted.

The teleost MRC is characterized by its elaborate intracellular system of branching tubules that is continuous with the basolateral membrane (tubular system; **Figures 2**(**a**)–**2**(**d**)). This network of anastomosing tubules is closely associated with the mitochondria and is only excluded from the area of the Golgi apparatus and the band immediately beneath the apical membrane. The elements of the tubular system can be found alongside the ER, making identification somewhat problematic. Extracellular space markers such as horseradish peroxidase and ruthenium red have been used to clearly establish the basolateral continuity of the tubular system and its separation from the ER. The Karnovsky potassium ferrocyanide-reduced osmium stain has also been used to unambiguously differentiate the membrane elements of the tubular system from the ER without the need of extracellular markers. The selective heavy staining of the tubular system contrasted markedly with the poorly stained ER (**Figures 2**(**a**) and **2**(**b**)). Environment ionic conditions have a marked effect on the morphology of the teleost MRCs, which of course relates to the role of these cells in active ion transport (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). In seawater, the gill is involved in active ion elimination, while in freshwater active ion uptake takes place.

The mitochondrion-rich CC and accessory cell (AC) are two types of MRCs that are universally expressed in seawater teleosts (**Figures 1**(**d**)–**1**(**f**)). These cells form multicellular complexes in the filament epithelium. The larger chloride cell may be elongate, ovoid, or cuboidal in shape, which depends upon the species. The tubular and the tubulovesicular systems are highly developed and the cell is packed with mitochondria. High densities of Na^+/K^+-ATPase are associated with the CC tubular system. The apical membrane of a CC is recessed from the surface of neighboring PVCs and may be further deepened through invagination (**Figures 1**(**b**)–**1**(**d**). The smaller AC is superficially located, semi-lunar or pear-shaped, and generally has a less extensive tubular system and a poorly developed tubulovesicular system. Notably, they have low levels of Na^+/K^+-ATPase, unlike the CCs. The AC sends cytoplasmic processes into the larger CC which emerge at the apical surface to form a complex mosaic. Notably, the tight junctions found between CC and AC contain fewer strands than do tight junctions formed with PVCs. Therefore, the AC interdigitations within the CC apical membrane would greatly increase the linear distance of leaky tight junction, which is functionally important for the paracellular sodium efflux. There is also ample evidence that Cl^- efflux occurs transcellularly through the CC (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts).

In freshwater teleost fishes, there is less consensus about MRC subtypes, which might relate to the instability and variability of the freshwater environment itself. Pisam and co-workers, however, have described α and β subtypes of MRCs with electron-lucent (light or pale) and electron-dense (dark) cytoplasms, respectively, in a number of species. It should be noted that this naming system is unrelated to that used to describe acid- and base-secreting cells in other epithelia (kidney collecting duct and bladder). In addition to a pale cytoplasm, α-cells were found at the edges of the interlamellar space (ILS) in contact with the basal lamina opposite the arterioarterial circulation. The β-cells, on the other hand, were generally observed in the ILS in contact with basal lamina associated with the arteriovenous circulation. Besides, a manganese–lead (Mn–Pb) stain was found that selectively stained the contents of a population of variably sized membrane bound bodies in the apical cytoplasm of only the β-cell type. The material in the apical vesicles of the β-cell has been shown to include carbohydrate material, and binding of the lectin wheat germ agglutinin (WGA: sialic acid and *N*-acetylglucosaminyl residues) to the apical vesicles of a subpopulation of MRCs has also been found in tilapia (presumably β-cells). In the guppy (*Poecilia reticulatus*), it was shown that the α-cell has high levels of Na^+,K^+-ATPase immunoreactivity, while the β-cell has only weak labeling. In studies with rainbow trout (*O. mykiss*) and Atlantic salmon, differentiation of these two subtypes was not made.

Transfer to seawater of euryhaline freshwater fishes results in the degeneration of the α-cell through apoptosis and hypertrophy of the β-cell. The β-cell is transformed into what is recognized as the seawater CC. The transformation consisted of an increase in the size of the β-cell, density of the cytoplasm, number of mitochondria, the tubular system network with a tighter polygonal mesh size, and the apical tubulovesicular system. A mitochondrion-poor cell type has been described that possesses a well-developed tubular system but has relatively few mitochondria. This cell increases in frequency following seawater acclimation and infrequently contacts the water. No function was suggested for this cell type.

Although typically associated with marine fishes, the accessory cell type has also been described in some freshwater euryhaline species. These cells are associated with the α-cell type in tilapia and Atlantic salmon but β-cell type in brown trout (*Salmo trutta*), and are similar to the AC described in the seawater fish gill, but they are less common and do not send cytoplasmic processes into the apical cytoplasm of the CC. Upon transfer to seawater or in anticipation of transfer (smolting), their numbers increase and interdigitations develop. The ACs are presumably present in fishes living in freshwater in order to facilitate the rapid switch to NaCl elimination. In a tilapia species (*Alcolapia grahami*) found in the extremely alkaline (pH 10) brackish water of Lake Magadi in Kenya, seawater ACs associated with larger CC-type MRCs have also been found. The lake water has very high HCO_3^- and CO_3^{2-} concentrations of 40 and 265 mEq l^{-1}, respectively, with Na^+ and Cl^- at 342 and 108 mEq l^{-1}, respectively. One model proposes that the CC–AC complex facilitates paracellular Na^+ efflux via the leaky tight junction, while HCO_3^- is pumped out by the CC.

In freshwater tilapia (*O. mossambicus*), three MRC types have been classified according to the apical membrane appearance by scanning electron microscopy (SEM), including wavy-convex, shallow-basin, or deep-hole types. The wavy-convex type has a convex apically exposed area with variable ornamentation with microvilli and a relatively large two-dimensional area. The surface of the shallow-basin-type MRCs is flat but recessed below neighboring PVCs. The density of the wavy-convex-type MRCs increases in low NaCl and the shallow-basin-type MRCs increases in low Ca^{2+} water, suggesting different roles in Na^+, Cl^-, and Ca^{2+} uptake, respectively. Results have been corroborated by immunohistochemistry (IHC; **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes). The deep-hole MRCs are also recessed between PVCs, like the shallow-basin type, but they form a deeper apical crypt characteristic of seawater CCs. In the fathead minnow (*Pimephales promelas*), the deep-hole MRCs are associated with exposure to acid conditions, suggesting a role in acid excretion. However, in other species, like the rainbow trout, this system of classification is not applicable, and an alternative system using lectin histochemistry has arisen, using peanut agglutinin (PNA; terminal β-galactose residues) ± staining to identify functionally different MRC subtypes in trout. Lectins bind to specific glycoproteins and differential staining patterns have been used to identify subtypes with concanvalin A (Con-A; α-mannopyranosyl, and α-glucopyranosyl glycoprotein residues), and WGA (*N*-acetylglucosamine and *N*-acetylneuraminic acid (sialic acid) residues) as well.

Although MRCs tend not to be as frequently found in the lamellae as in the filament epithelium in freshwater and especially seawater fishes, acclimation of rainbow trout to ion-poor (<0.01 mM $[Na^+]$) conditions elicits a massive proliferation of MRCs in the lamellar epithelium. The number and area of exposed surface of these cells both increase dramatically. The effects of ion-poor water on MRC proliferation can also be reproduced using hormone treatments with cortisol and growth hormone (**see also Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish). Since MRCs are generally quite large, proliferation on the lamellae results in the thickening of the epithelium and, consequently, the blood to water diffusion distance is increased, leading to an osmo-respiratory compromise (for more detail, **see also Role of the Gills**: The Osmorespiratory Compromise).

In the other Osteichthyans, which includes the Chondrosteans (e.g., sturgeon and paddlefish), Holosteans (e.g., *Amia* and gars), Dipnoans (lungfishes), and Coelacanth, MRCs have all been described (see **Figure 3**). However, in marine or euryhaline forms, evidence for AC–CC complexes is still lacking.

The chondrichthyan (elasmobranchs and chimaeras) salt-secreting rectal gland replaces the gill as the dominant salt-secretory organ. Yet, branchial MRCs remain in significant numbers. Even so, the important ionoregulatory enzymes Na^+/K^+-ATPase and H^+-ATPase are associated with distinct subpopulations of these cells and have been implicated in acid–base regulation rather than NaCl secretion (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). Branchial MRCs are generally found singly in the filament epithelium and are cuboidal or ovoid in shape with a basally located nucleus (**Figure 4**). In addition to their high mitochondrial densities, they have a basolateral membrane amplified through heavy folding into basal labyrinth, and a densely packed subapical tubulo-vesicular system. The tight junctions between MRCs and neighboring PVCs consist of many strands and are not considered leaky. The apical surfaces of elasmobranch MRCs range from being deeply invaginated to convex and are covered by microvilli of varying densities.

The agnathans or jawless fishes represent the most primitive group of fishes, consisting of the osmoconforming marine hagfishes and the osmoregulating lampreys. These differences in osmoregulatory abilities are reflected in the differences in MRC morphology among the agnathans. In freshwater lamprey, three different types of MRC have been described, one of which is associated with the preadaptation to seawater and is the dominant type found in the seawater-adapted lamprey (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and Lampreys: Environmental Physiology). The seawater-type MRC (swMRC) is commonly referred to as the chloride cell due to its morphological similarities with the teleost chloride cell (**Figure 5**). In seaward-migrating freshwater lamprey, the swMRCs are found in a continuous row along the length of the ILS of the filament epithelium. The swMRCs are disk-shaped and covered over by neighboring PVCs with the apically exposed surface containing microvilli. Following acclimation to seawater, the overlying PVCs retract and the apical surface increases to extend the full width of the ILS. The surface bulges slightly, and is relatively smooth with few microvilli. The number of strands in the tight junction between neighboring swMRCs also decreases following seawater transfer. The shallow tight junction is an important morphological characteristic that gives a good indication of the cell's function, namely NaCl elimination.

In the freshwater lamprey, the MRC types are referred to as IC MRCs, and one of the noticeable differences to the swMRC is the lack of a tubular system (**Figure 6**). One type is only present in the larval ammocoete and is characterized by electron-dense mitochondria, small secretory mucus granules, short microvilli and/or microplicae, and the presence of globular particles in its apical membrane (as shown by freeze fracture). They are found in both the lamellar and filament epithelia, and comprise approximately 60% of the total epithelium. The function of this ammocoete MRC type is unknown, but it disappears following metamorphosis and does not appear to be involved in ion regulation. The second IC type is generally found singly or in pairs in the interlamellar region and at the base of the lamellae, where it intercalates with neighboring PVCs and/or other MRC types. It is characterized by an apical membrane with elaborate microplicae, the absence of secretory mucous granules, the presence of numerous small vesicles and membranous tubules, and the presence of rod-shaped particles in either the apical or lateral membranes (as determined by freeze-fracture electron microscopy). These rod-shaped particles are similar to those described in the mitochondrion-rich ICs of other acid-secreting epithelia. The plasma membrane domains having dense rod-shaped particle aggregates also show strong immunoreactivity for the proton pump (vacuolar type H^+-ATPase), which has been demonstrated by IHC. When the proton pumps are in the apical location, these cells function in acid excretion while in cells in which the particles have a basal lateral location, the function is for base excretion.

In the hagfish, MRCs are restricted to the lateral wall of gill pouches and the lateral half of gill folds. All MRCs occur singly, being separated by PVCs, and lack leaky tight junctions. The hagfish MRCs have abundant, large mitochondria and a tubular system continuous with the basolateral membrane along with a subapical vesiculotubular system (**Figure 7**). These cells also have perinuclear glycogen and high levels of carbonic anhydase, H^+-ATPase, and Na^+/K^+-ATPase expression. Since hagfish are osmoconformers and therefore lack active NaCl regulation, their presence is linked to acid–base regulation. Communicating gap junctions are not found between hagfish MRCs and its neighbors (PVCs and nondifferentiated cells). Their apical membrane contains microvilli and is usually convex and deep apical crypts are not common. Freeze-fracture electron microscopy has shown the presence of rod-shaped particles in the apical membrane which represent proton pumps as confirmed by IHC.

MCR Identification

In addition to direct morphological analysis, MRCs have been identified by their affinity for eosin, and acid Fuschin stain, associated with abundant mitochondria and Champy–Maillet solution ($ZnI–OsO_4$), which reacts specifically with membrane and thus the reaction with the abundant membrane systems of the MRC results in its selective blackening. Additional techniques are directed at the detection of the abundant mitochondria which results in a stronger signal than background. The mitochondrial vital stains dimethylaminostyrylmethylpyridiniumiodide (DASPMI), dimethylaminostyrylethylpyridiniumiodide (DASPEI), and Mitotracker^{TM} (Molecular Probes), as well as immunohistochemical techniques directed against mitochondria-specific proteins have been used to this end.

Another approach has been to target the enzyme Na^+/K^+-ATPase, which has been shown to be expressed in high concentrations in MRCs. Ouabain specifically binds and inhibits the Na^+/K^+-ATPase and both ^{3}[H] ouabain autoradiography and anthroylouabain fluorescence have been found to localize to MRCs. Enzyme histochemical and immunohistochemical methods have been used in the past to localize Na^+/K^+-ATPase to MRCs. The use of antibodies directed against the Na^+/K^+-ATPase have proven very useful as is evident in the large number of studies published recently using this approach (**Figure 1**(**f**)). Practically all of these studies make use of either the mouse monoclonal or rabbit polyclonal antibodies directed against conserved epitopes or regions of the Na^+/K^+-ATPase α-subunit. The further study of MRC functional subtypes through the use of multiple immunohistochemical labeling with additional ion transporters is addressed in the articles **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes .

See also: **Hagfishes and Lamprey**: Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Role of the Gills**: The Osmorespiratory Compromise.

Further Reading

Bartels H and Potter C (2004) Cellular composition and ultrastrucutre of the gill epithelium of larval and adult lampreys. Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Keys A and Willmer EN (1932) ''Chloride secreting cells'' in the gills of fishes, with special reference to the common eel. *Journal of Physiology* 76: 368–378.

Laurent P (1984) Gill internal morphology. In: Hoar W and Randall DJ (eds.) *Fish Physiology*, p. 73. New York: Academic Press.

Perry SF (1997) The chloride cell: Structure and function in the gills of freshwater fishes. *Annual Review of Physiology* 59: 325–347.

Pisam M and Rambourg A (1991) Mitochondria-rich cells in the gill epithelium of teleost fishes: An ultrastructural approach. *International Review of Cytology* 130: 191–232.

Wilson JM and Laurent P (2002) Fish gill morphology: Inside out. *Journal of Experimental Zoology* 293: 192–213.

The Osmorespiratory Compromise

RJ Gonzalez, University of San Diego, San Diego, CA, USA

© 2011 Elsevier Inc. All rights reserved.

What Is the Osmorespiratory Compromise?
The Effect of Changes in Branchial Surface Area
The Effect of Changes in Branchial Diffusion Distance
Effect of Branchial Remodeling
Further Reading

Glossary

Acclimation Physiological and/or biochemistry adjustments made by an organism in response to a prolonged change in their environment, e.g., temperature.

Catecholamine Any of a class of amines derived from a catechol that acts as chemical messengers (a hormone or neurotransmitter). The fish's main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Critical swimming speed (U_{crit}) The U_{crit} is determined in a swim tunnel where the fish are forced to swim against a current. The current velocity is increased in steps and each step is maintained for a specified time (usually 10 min to 1 h) or until the fish fatigues.

Diffusion Net movement of a solute from an area of higher concentration to an area of lower concentration.

Epinephrine Catecholamine hormone secreted by the adrenal gland.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Norepinephrine Catecholamine hormone secreted by the adrenal gland.

Normoxia Normal levels of oxygen in water or arterial blood.

Oxygen consumption ($\dot{M}O_2$) Amount of oxygen consumed by an animal per unit time.

P_{crit} The environmental O_2 tension below which an animal cannot sustain a routine rate of O_2 uptake independent of environmental O_2 tensions. The lower a fishes P_{crit}, the better able it is to maintain normal levels of activity and therefore fish with lower P_{crit}'s are belived to be more hypoxia tolerant than those with higher P_{crit} values.

Trans-epithelial potential The electrical potential across an epithelium as a function of concentration differences and relative permeabilities of all salts.

What Is the Osmorespiratory Compromise?

The gill epithelium is the primary site of both gas exchange and ion regulation in fish and the term 'osmorespiratory compromise' refers to the functional clash that can exist between these two processes. The basis for the osmorespiratory compromise is that diffusion is involved in movement of both gases and salts (primarily Na^+ and Cl^-) across the branchial epithelium. Diffusion of O_2 across the gill is a function of surface area, diffusion distance, mean water-to-blood P_{O_2} difference, and O_2 permeability of the epithelium. Diffusion of Na^+ and Cl^- across the gill depends upon surface area, diffusion distance, mean blood-to-water concentration differences, epithelial permeability to salts, and trans-epithelial potential (TEP). Thus, both gill surface area and water-to-blood diffusion distance are common to the diffusion of gases and ion. In freshwater, any increase in surface area or reduction in diffusion distance to promote gas diffusion will also elevate diffusive ion loss, which must be replaced actively. Conversely, any reduction in surface area and/or increase in diffusion distance to limit ion losses could restrict O_2 uptake. The term 'respiratory/osmoregulatory compromise' coined in early studies eventually morphed into the slightly more user-friendly osmorespiratory compromise in the early 1990s, by which it is generally known today. The first studies of the osmorespiratory compromise used exercise to induce changes in branchial surface area and monitored its effects on gas and/or ion transfer. Subsequent studies have used acclimation to a variety of water conditions to induce changes in branchial diffusion distance and monitored the consequences for gas and ion movement.

The Effect of Changes in Branchial Surface Area

Exercise

The first researchers to examine the osmorespiratory compromise argued that when fish begin to swim faster, a suite of cardiovascular and respiratory changes, mediated by catecholamine release, alter branchial blood flow in order to promote O_2 uptake. Changes include decreased vascular resistance, increased blood pressure, and re-direction of blood from more central paths to lamellar circuits, which presumably would increase exchange area (**see also Gas Exchange**: Respiration: An Introduction). Indeed, resting rainbow trout (*Oncorhynchus mykiss*) perfuse less than 60% of their gill lamellae but when given a dose of epinephrine or exposed to hypoxia, the number rises to about 75%. The implication is that during exercise, when O_2 demand rises, fish increase the 'functional surface area' of the gills to promote O_2 uptake. Such an increase in surface area, it was hypothesized, would lead to elevated rates of ion loss (primarily Na^+ and Cl^-). Sure enough, when rainbow trout are forced to swim by occasional prodding or electric shock, rates of appearance of radioactive ^{22}Na in surrounding media are higher than in resting fish. Similarly, fish injected with the catecholamine norepinephrine also had higher rates of ^{22}Na loss relative to controls or sham injected fish. Ensuing studies measured actual rates of Na^+ efflux while distinguishing between short, exhaustive exercise and extended, more aerobic swimming. When fish are forced to swim exhaustively for short periods, their rates of Na^+ efflux rise 70% above resting levels, confirming the previous findings.

When both Na^+ efflux and O_2 uptake are measured simultaneously, additional factors are revealed to be involved in the initial high rates of ion loss. With exercise, increased O_2 uptake is driven not only by the increase in functional surface area, but also by greater water-to-blood P_{O_2} difference mainly due to higher rates of O_2 consumption in muscle tissue. The increased functional surface area to promote O_2 uptake would lead to elevated rates of ion loss, but since there is no change in the blood-to-water concentration difference corresponding to the P_{O_2} difference, one would expect a smaller rise in ion loss than O_2 uptake. In fact, at the onset of exercise the opposite is true. Immediately after being chased for 5 min or during the first 30 min of chronic swimming at 85% U_{crit} (about 4.5 body lengths s^{-1}) O_2 consumption in rainbow trout rises 2.8–3.4 times, but Na^+ efflux jumps 4.5- to 5.2-fold (U_{crit} – maximum prolonged swimming speed; **Table 1**). To put it another way, the ion to gas flux ratio rises by almost 65%. The cause of the disproportionate rise in Na^+ efflux appears to be increased epithelial permeability to salts brought about by a catecholamine-induced increase in intra-lamellar pressure at the beginning of exercise, which stretches paracellular tight junctions (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes).

Table 1 Na^+ efflux (J_{out}^{Na}), rate of O_2 consumption ($\dot{M}_{O_2}$) and ion to gas flux ratio (IGR) for rainbow trout (*Oncorhynchus mykiss*) at rest and immediately after exhaustive exercise

Treatment	J_{out}^{Na}	$\dot{M}_{O_2}$	*IGR*
At rest	8.8±0.8	72.2±2.2	122.2
Post-exercise	39.7±2.3	200.7±5.4	200.0±12.3

Units for J_{out}^{Na} and $\dot{M}_{O_2}$ are nmol g^{-1} min^{-1}, and units for IGR are pmol Na^+/nmol O_2.
Values are means ± SE.

While the marked increase in branchial permeability at the onset of swimming is striking, it does not last long. During recovery from exhaustive exercise, Na^+ efflux falls to within 10% of resting rates in trout while at the same time O_2 consumption returns to a resting rate. Part of the reduction in ion permeability can be ascribed to lower intra-lamellar pressure due to declining catecholamine levels and/or downregulation in catecholamine receptor density, but it appears that in addition there is a significant level of control of epithelial ion permeability applied at the tight junctions. This marked ability to regulate paracellular ion permeability raises the possibility that during chronic swimming any increase in ion loss due to increased functional surface area could be negated by reduced permeability. Indeed, at moderate swimming speeds of 2 body lengths s^{-1} Na^+ efflux falls to resting levels within an hour and it falls well below resting levels within 5 h. At a higher swimming speed, 4.5 body lengths s^{-1}, Na^+ efflux remains elevated well above resting rates for the duration of the exercise, although they drop from higher initial rates. It appears that vigorous swimming may interfere in some way with the control of ion permeability. Such high rates of Na^+ efflux with vigorous exercise lead to high rates of net ion loss, which cannot be sustained for prolonged periods since they would lead to a significant depletion of internal stores and initiate a cascade of cardiovascular disturbances. The key adjustment in this case is not a control of Na^+ efflux, but rather a rapid up to threefold stimulation of ion transport. The rise in active uptake allows the fish to maintain internal levels despite the higher loss rates.

All work on the effects of exercise on the osmorespiratory compromise to this point had been performed on rainbow trout, which is an active, coldwater species. When other species that differ in energetic lifestyle and temperature preferences, but had similar gill sizes, were examined, significant differences were found. Resting rates of O_2 consumption vary more than threefold among the species (**Table 2**), and the likely physiological basis for such a range is differences in branchial O_2 permeability. Further, after exhaustive exercise elevation of O_2 uptake for the less active species is associated with much greater rates of ion loss. For instance, after exercise banded sunfish (*Enneacanthus obesus*) have a rate of O_2 consumption less than half that of common shiners (*Notropis cornutus*), but its rate of Na^+ loss is more than 50% higher. Consequently, while ion to gas flux ratio for the shiner rises only 25% immediately after exercise, it is more than quadruples for the sunfish. Since shiners have

Table 2 Na^+ efflux (J_{out}^{Na}), rate of O_2 consumption ($\dot{M}_{O_2}$) and ion to gas flux ratio (IGR) for common shiners (*Notropis cornutus*), golden shiners (*Notemigonus crysoleucas*), smallmouth bass (*Micropterus dolomieui*), yellow perch (*Perca flavescens*), and banded sunfish (*Enneacanthus obesus*) at rest and immediately after exhaustive exercise

	At rest			*Post-exercise*		
Species	J_{out}^{Na}	$\dot{M}_{O_2}$	*IGR*	J_{out}^{Na}	$\dot{M}_{O_2}$	*IGR*
Common shiner	4.8±0.5	118.6±13.5	40.5	9.3±0.8	191.8±15.0	50.3±6.8
Golden shiner	12.7±1.3	141.0±16.6	90.1	18.1±1.2	192.6±10.4	94.9±8.0
Smallmouth bass	3.5±0.3	74.7±8.6	46.9	21.3±2.3	152.0±4.6	141.0±14.8
Yellow perch	4.2±0.4	72.7±5.7	57.8	21.9±1.5	133.4±4.6	164.2±10.2
Banded sunfish	3.0±0.3	41.3±3.4	72.6	28.9±3.7	90.1±3.6	317.6±35.7

Units for J_{out}^{Na} and $\dot{M}_{O_2}$ are nmol g^{-1} min^{-1}, and units for IGR are pmol Na^+/nmol O_2.
Values are means ± SE.

higher branchial O_2 permeability, they can achieve higher rates of O_2 uptake without the need for big changes in surface area brought about by high intra-lamellar pressures that stimulate ion losses. Sunfish, in contrast, require much greater increases in surface area, brought about by the elevated pressure that promotes ion loss. Interestingly, species such as smallmouth bass (*Micropterus dolomieu*) and yellow perch (*Perca flavescens*), that are intermediate in resting O_2 consumption, experience intermediate elevations in ion loss. These findings indicate that less active species such as banded sunfish, that inhabit densely vegetated coastal swamps, place less emphasis on conserving ions with activity since they probably rarely experience prolonged bouts of activity in their native habitats. The more active a fish is, the greater the potential osmotic costs. Very active species, such as the stream dwelling common (*N. cornutus*) and golden shiners (*Notemigonus crysoleucas*), avoid exorbitant osmotic costs of activity by virtue of their high branchial O_2 permeability.

Studies of the osmorespiratory compromise in exercising fish seem to confirm, in general terms, the existence of a conflict between ion and gas flux across the gills of fish based upon surface area. Such conflict is most notable when exercise is commencing and catecholamine-induced cardiovascular adjustments are occurring to enhance O_2 pickup. Under those circumstances, ion loss increases more than predicted based only on functional surface area considerations due to additional elevation of branchial ion permeability. The rise in permeability is transitory and disappears within an hour or less as catecholamines dissipate from circulation. Further, fish show considerable ability to regulate anion and cation permeability independently and can significantly lower branchial ion permeability well below resting levels and upregulate transport. Studies with a range of species indicate that branchial gas permeability also varies among species and this influences the severity of the impact of increased gas uptake on ion loss. Fish with high O_2 permeability can take up more additional O_2 during exercise without much ion loss, while fish with low O_2 permeability incur much higher rates of ion loss even with lower absolute rates of O_2 uptake.

Osmorespiratory Compromise in Seawater

The basis of the osmorespiratory compromise in seawater is essentially the same as in freshwater except that the concentration differences for Na^+ and Cl^- are reversed (lower internally). For example, a freshwater fish typically has a Na^+ concentration difference across the gill epithelium of around 149 mmol L^{-1} (inside higher), while in seawater it is over 350 mmol L^{-1} (outside higher), which means that increases in surface area with activity could potentially be more costly in seawater than in freshwater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). The issue is complicated, however, by the role TEP plays in salt excretion at the gills. The general model uses a positive TEP (blood relative to water) to excrete Na^+ across the gills. Despite the concentration difference, Na^+ would not enter across the gills because of the opposing TEP. Further complicating the issue is the fact that the gut absorbs salts in order to take up water to replace what is lost. In fact, elevated water loss with exercise is likely a bigger problem due to the ion load incurred in order to replace it.

Few studies have examined the osmorespiratory compromise in seawater probably because of the technical difficulty of measuring ion fluxes in saline waters. For this reason, the few studies available have used indirect measures of ion regulation such as gill and gut Na^+/K^+-ATPase activity and have tended to compare species that vary in energetic lifestyle and branchial surface area. More active fish have larger gills (with greater surface area) and thus should incur a greater osmoregulatory cost. Indeed, branchial Na^+/K^+-ATPase activities of some active epipelagic species are higher than in sluggish meso-pelagic fishes. It was argued that the sluggish fish had lower metabolic rates and thus reduced osmoregulatory costs. In contrast, branchial Na^+/K^+-ATPase activities are not higher in the epipelagic yellowfin tuna *(Thunnus albacares)* or skipjack tuna *(Katsuwonus pelamis)*, which are among the most aerobically active of all fish and have very large branchial surface areas, compared to less active species; it was concluded that osmoregulatory costs are not elevated in tunas. Based on this diversity of results and paucity of studies, it is difficult to reach any conclusions of the osmorespiratory compromise in seawater fish.

The Effect of Changes in Branchial Diffusion Distance

Acclimation to Soft Water

For many freshwater fish, exposure to soft water (water low in Na^+ and/or Ca^{2+}) disrupts the ability to regulate internal salt levels. Exposure to low $[Ca^{2+}]$ water leaches Ca^{2+} from paracellular tight junctions and promotes diffusive ion loss, while exposure to low $[Na^+]$ water reduces concentration-dependent active Na^+ uptake. Extended time in either or both water conditions can lead to significant depletion of internal ion levels. One way to restore ion balance is to upregulate ion transport, which will either compensate for the scarcity of Na^+ in the surrounding water, or counteract the higher rates of ion loss in low Ca^{2+} water. Upregulation of ion transport is typically associated with a proliferation of mitochondrion-rich cells (MRCs) in the branchial epithelium. In ion-rich waters, MRCs, which are much larger than the surrounding pavement cells, are primarily found on filaments and filling-in the intra-lamellar spaces; they make up less than 10% of the surface area of the gills. As they proliferate, MRCs appear on the lamellar epithelium, which acts to raise the average water-to-blood diffusion distance for gases. Thus, acclimation to restore ion balance in soft water could, in theory, act to impede gas exchange. Whether or not this happens in reality depends upon the degree of MRC proliferation, its effect on average water-to-blood diffusion distance, and the presence of any compensatory adjustments.

When rainbow trout are exposed to soft water or treated with a combination of cortisol and ovine growth hormone, MRC density more than doubles and fractional area rises from about 5% to 20%. Visual inspection alone is enough to illustrate the impact (**Figure 1**). Measurements of average water-to-blood diffusion distance along the lamellae show that it almost doubles following MRC proliferation. This appears to be a general consequence of soft water acclimation, because similar results were obtained from the tropical *Hoplias malabaricus*.

The proliferation of MRCs and associated increase in diffusion distance across the branchial epithelium suggests the potential for impairment of O_2 uptake, but in normoxic water results vary. Some studies show a lower arterial P_{O_2} in trout undergoing MRC proliferation, but some do not. The uptake of O_2 by resting fish in normoxic waters is thought to be perfusion limited and may not be influenced by the reduction in diffusion potential. Alternatively, a compensatory elevation in ventilation frequency may be important in maintaining arterial P_{O_2}. Thickening of branchial epithelium should also act to impair diffusion of CO_2, and since CO_2 excretion is thought to be diffusion limited, one would expect to see the effects of MRC proliferation in normoxic waters. Again results vary among studies. One study reported elevated arterial P_{CO_2}, while two did not.

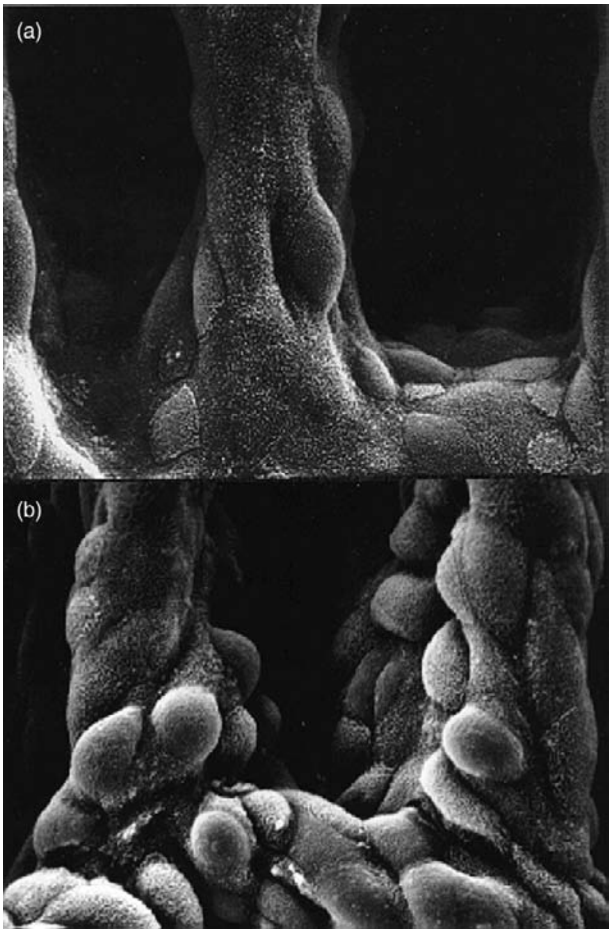


Figure 1 Scanning electron micrograph of gills of rainbow trout in ion-rich water (a) and after acclimation to soft water (b). Photos by permission of S. Perry.

While the effects of lamellar thickening on gas exchange in normoxic waters are not completely apparent, what is clear is that thickening does have a significant effect on gas exchange when fish are challenged with hypoxia. Researchers observe reduced arterial P_{O_2} in fish with thickened epithelia during exposure to hypoxia. Likewise, during exercise when O_2 demand is elevated, soft water-held trout have lower U_{crit} values and rely more heavily on anaerobic metabolism while swimming. It appears that as water-to-blood P_{O_2} difference falls or O_2 demand is elevated with exercise, then O_2 uptake becomes diffusion limited and lamellar thickening becomes important.

Acclimation to soft water does not fit the original model of the osmorespiratory compromise based upon changes in functional surface area, but it does highlight other possibilities for conflict between these two processes. In the original scenario, alterations to enhance diffusion of O_2 for uptake also enhance diffusive ion loss. Here we see that proliferation of MRCs, to upregulate active ion transport, causes a thickening of lamella that affects O_2 and CO_2 diffusion. An acclimatory response to restore or defend internal ion levels is achieved at the cost of gas exchange.

Acclimation to Changing Salinity

Movement between freshwater and seawater requires a fish to make a host of osmoregulatory adjustments to successfully regulate internal ions. For instance, movement from seawater to freshwater requires a hyporegulating teleost that actively excretes ions and drinks seawater (incurring an additional salt load to absorb the water) to suddenly stop drinking, start actively taking up salts, and reduce branchial ion losses. Morphological adjustments made to assist maintenance of ion balance could in theory influence gas exchange (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

Prickly sculpins (*Cottus asper*), primarily a freshwater species, can still successfully and tightly regulate Na^+ and Cl^- in full seawater. In contrast, closely related Pacific staghorn sculpins (*Leptocottus armatus*) are marine and less tolerant of freshwater. Prickly sculpins have smaller gills (on a mass specific basis) than Pacific staghorn sculpins, which allows for lower ion permeability during exposure to freshwater. Further, during exposure to freshwater, prickly sculpins increase the thickness of their branchial epithelium by about 40%, which raises the water-to-blood diffusion distance. This means that prickly sculpins drop an already low branchial ion permeability even further when in freshwater, thereby controlling diffusive ion loss and maintain internal levels. However, the adjustment in diffusion distance to help conserve salts comes with a price for the prickly sculpin. Measurements of critical oxygen tensions (P_{crit}) show a marked $\sim$15 torr increase in freshwater-held prickly sculpins compared to those in seawater. In other words, the increase in epithelial thickness to limit ion losses hinders O_2 uptake at low environmental P_{O_2} levels. As with acclimation to soft water, adjustments made to aid ion regulation, in turn, impede gas exchange. The morphological adjustments were different in the two cases – one a proliferation of MRC and the other a general thickening of the epithelium – both increased the water-to-blood diffusion distance for O_2. In each case, differences were not obvious in normoxic waters, but became evident during exposure to hypoxic waters.

Effect of Branchial Remodeling

Gills of crucian carp (*Carassius carassius*) undergo a striking morphological transformation in normoxic waters at low temperature when metabolic O_2 demand is low (**see also Bony Fishes**: Crucian Carp). Cells proliferate between branchial lamella filling in the space creating an intra-lamellar cell mass (ILCM) and greatly reducing surface area and increasing average diffusion distance With exposure to hypoxia or high temperatures, when O_2 demand is elevated, these ILCMs disappear revealing the underlying lamella, restoring the higher respiratory area and reducing diffusion distance. A similar response is seen in the goldfish (*Carassius auratus*) and in the Lake Qinghai scaleless carp (*Gymnocypris przewalskii*) native to a high-altitude lake in central China. Similarly, the osteoglossid (*Arapaima gigas*) from the Amazon River actually loses its lamellae during ontogenetic transition from obligate water breathing at hatching to obligate air breathing (**Figure 2**). In this case, however, the change is irreversible. A very similar response is reported for

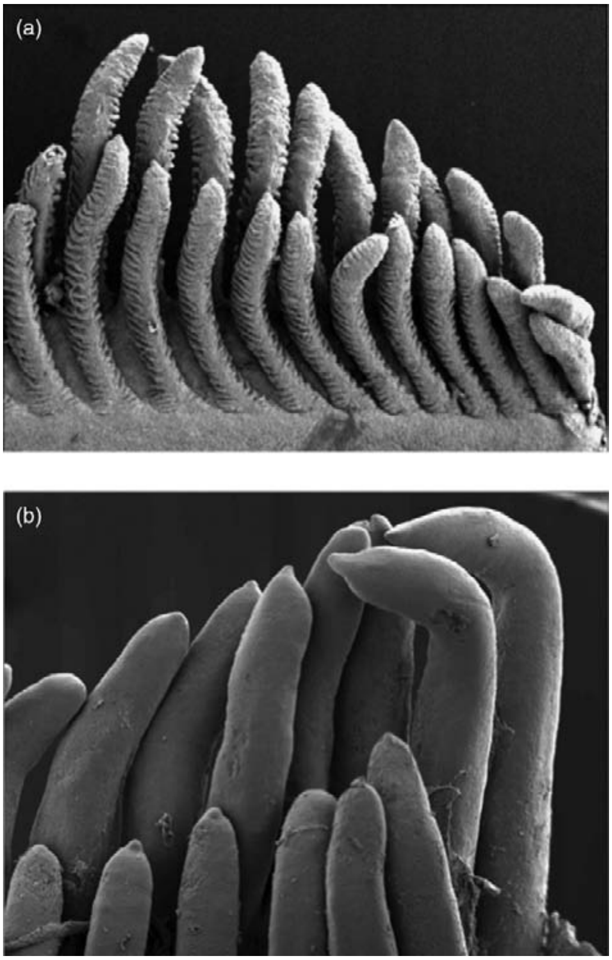


Figure 2 Scanning electron micrographs of gills of (a) 10 g *A. gigas* and (b) 1000 g *A. gigas*. Photos by permission of C. Brauner.

overwintering large mouth bass (*Micropterus salmoides*) and for fathead minnows (*Pimephales promelas*) exposed to toxic water left over from refining oil sands. The explanation generally offered for such morphological changes is that a reduction in surface area and an increase in diffusion distance when O_2 supplies are ample to meet demands lower osmotic costs.

Results generally support the osmotic cost hypothesis. Goldfish with ILCM at low temperature have lower Cl^- and polyethylene glycol (indicator of paracellular diffusion) fluxes than goldfish at higher temperature without ILCMs. Similarly, Lake Qinghai scaleless carp that lose ILCMs in hypoxic waters experience a 10–15% drop in plasma Na^+ and Cl^- concentrations, suggesting higher rates of diffusive loss. In contrast, larger *A. gigas* that have lamellae-less gills and a fourfold greater water-to-blood diffusion distance actually have diffusive ion loss rates 3 times higher than smaller fish with lamellae-bearing gills. The likely difference here is probably reduced branchial ion permeability in smaller fish that more than compensates for higher surface area and shorter diffusion distance.

In stark contrast to the response to hypoxia we see in Crucian carp, goldfish, and Lake Qinghai scaleless carp is the response observed in the Amazonian oscar (*Astronotus ocellatus*) – an extremely hypoxia-tolerant fish that can tolerate complete anoxia for up to 6 h and severe hypoxia for 1–2 days. During exposure to severe hypoxia (10–20 torr), oscars show no increase in functional gill surface area and/or reduction in diffusion distance. In fact, Na^+ efflux actually drops significantly, which acts to preserve ion balance since Na^+ uptake also falls due to the lack of O_2 and downregulation of Na^+/K^+-ATPase. The drop in Na^+ efflux appears to be a result of pavement cells quickly covering over MRCs, thus markedly reducing their surface area and lowering branchial permeability. This is a very different take on the osmorespiratory compromise where adjustments are made to defend ion balance while not interfering with gas exchange. Perhaps conflict avoidance is only possible in such a strikingly hypoxia tolerant fish.

See also: **Bony Fishes**: Crucian Carp. **Gas Exchange**: Respiration: An Introduction. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction; Mechanisms of Gill Salt Secretion in Marine Teleosts.

Further Reading

Booth JL (1979) The effects of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *Journal of Experimental Biology* 83: 31–39.

Brill R, Swimmer Y, Taxboel C, Cousins K, and Lowe T (2001) Gill and intestinal Na^+–K^+ ATPase activity, and estimated maximal osmoregulatory costs, in three high-energy teleosts: Yellowfin tuna *(Thunnus albacares)*, skipjack tuna *(Katsuwonus pelamis)*, and dolphin fish *(Coryphaena hippuus)*. *Marine Biology* 138: 935–944.

Farrell AP, Kennedy CJ, and Kolok A (2004) Effects of wastewater from an oil-sand-refining operation on survival, hematology, gill histology, and swimming of fathead minnows. *Canadian Journal of Zoology* 82: 1519–1527.

Gonzalez RJ and McDonald DG (1992) The relationship between oxygen consumption and ion loss in a freshwater fish. *Journal of Experimental Biology* 163: 317–332.

Gonzalez RJ and McDonald DG (1994) The relationship between oxygen uptake and ion loss in fish from diverse habitats. *Journal of Experimental Biology* 190: 95–108.

Henriksson P, Mandic M, and Richards JG (2008) The osmorespiratory compromise in sculpins: Impaired gas exchange is associated with freshwater tolerance. *Physiological and Biochemical Zoology* 81: 310–319.

Nilsson GE (2007) Gill remodeling in fish – a new fashion or an ancient secret?. *Journal of Experimental Biology* 210: 2403–2409.

Perry SF (1998) Relationships between branchial chloride cells and gas transfer in freshwater fish. *Comparative Biochemistry and Physiology A* 119: 9–16.

Randall DJ, Baumgarten D, and Malyusz M (1972) The relationship between gas and ion transfer across the gills of fishes. *Comparative Biochemistry and Physiology A* 41: 629–637.

Wood CM, Iftikar FI, Scott GR, *et al.* (2009) Regulation of gill transcellular permeability and renal function during acute hypoxia in the Amazonian oscar *(Astronotus ocellatus)*. *New angles on the osmorespiratory compromise. Journal of Experimental Biology* 212: 1949–1964.

Wood CM and Randall DJ (1973) The influence of swimming activity on sodium balance in the rainbow trout *(Salmo gairdneri)*. *Journal of Comparative Physiology* 82: 207–233.

Wood CM and Randall DJ (1973) Sodium balance in the rainbow trout *(Salmo gairdneri)* during extended exercise. *Journal of Comparative Physiology* 82: 235–256.

THE GUT

Role of the Kidneys

Contents
Histology of the Kidney
The Kidney

Histology of the Kidney

DB McMillan, University of Western Ontario, London, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Evolution of the Kidney
Pronephros or Head Kidney
Opisthonephros
Further Reading

Glossary

Ammocoete Alternate name for the larval stage of the lamprey life cycle.

Amniotes Vertebrates whose embryonic development takes place in a fluid-filled sac called amnion that is responsible for keeping the embryo moist. Reptiles, birds, and mammals are amniotes.

Basal lamina Extracellular supporting layer found beneath all epithelia.

Brush border Closely packed microvilli on the apical surface of cells.

Coelom Continuous sheet of peritoneum entirely enclosing the body cavity – visceral peritoneum over the organs and parietal peritoneum lining the cavity.

Endothelium A thin layer of thin, flat cells forming the lining of all blood and lymph spaces.

Erythrocyte The most abundant cell found in the blood of nearly all fish. It contains the respiratory pigment hemoglobin and is commonly called a red blood cell because of its color.

Fenestrated Having openings in the wall.

Freeze-fracture Tissues are deeply frozen and split with a small blade, much like an axe splitting wood. Contours remaining on the split surface are coated with a thin layer of carbon. The tissue is removed with acid and the remaining carbon impression examined with a transmission electron microscope. Most of the splits occur within the hydrophobic region of cell membranes, thereby revealing details of the membrane, especially junctions.

Hemopoiesis The formation of blood cells.

Juxtaglomerular cells Modified smooth muscle cells in the afferent arteriole of a glomerulus; said to produce the enzyme renin.

Mesangium Space between the loops of glomerular capillaries.

Parietal peritoneum The lining of the coelom.

Renin An enzyme involved in the control of glomerular filtration rate.

Rete mirabile A capillary bed occurring within a small artery or vein so that blood from the heart passes through two capillary beds before returning.

Simple squamous epithelium A single layer of flat cells covering a surface.

Visceral peritoneum A layer enclosing the viscera located within the coelom.

Introduction

Excretion involves the separation and elimination of metabolic waste products from the body. Various organs are involved in this process: the lungs, gills, skin, etc. The kidneys and their ducts are the major full-time excretory organs and comprise the excretory system.

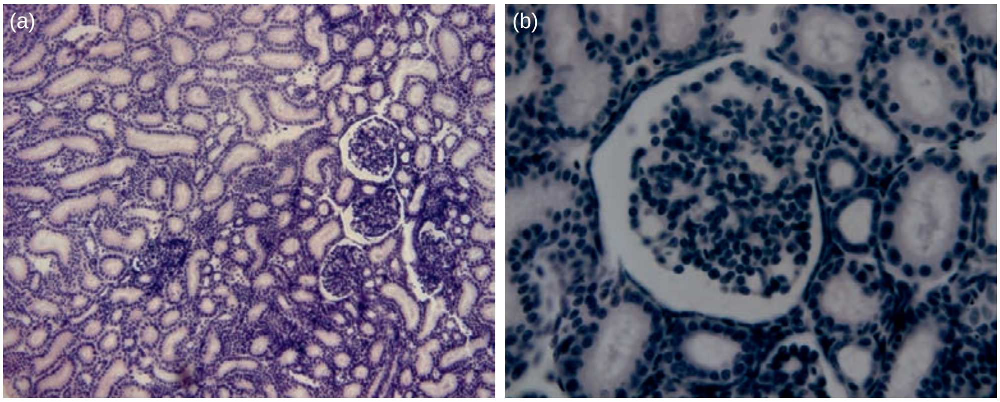


Figure 1 Photomicrographs of a section of the kidney of a spiny dogfish (*Squalus acanthias*). (a) Three glomeruli (singular, glomerulus) at the right are surrounded by masses of renal tubules. Delicate capillaries (not seen) invade the loose connective tissue between the tubules. 10×. (b) One of the glomeruli from (a) is surrounded by a nephric capsule (Bowman's capsule). The capsule consists of a simple squamous epithelium and resembles a balloon which has been pushed in by the glomerulus so that each capillary is covered by the visceral layer of the nephric capsule and the space is enclosed by the parietal layer. Masses of renal tubules fill the remaining field. Occasional capillaries appear between the tubules 40×.

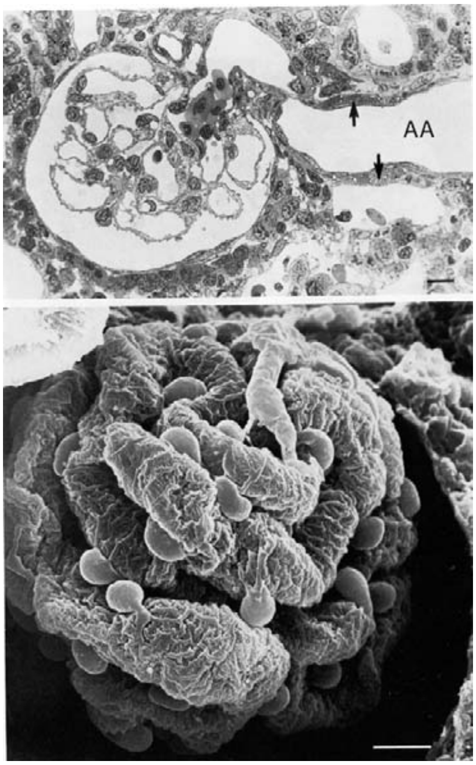


Figure 2 Micrographs to demonstrate the capillary loops in renal corpuscles of a freshwater teleost fish (*Carassius auratus gibelio*) Top: Photomicrograph of a section showing the well-developed knot of capillaries with many loops and widely patent lumina. AA, afferent arteriole with juxtaglomerular cells (arrows). Scale = 10 μm. Bottom: Scanning electron micrograph of the surface of the capillary loops sporting globular podocytes from which extend primary and secondary foot processes. Scale = 10 μm. From Elger M and Hentschel H (1981) The glomerulus of a stentohaline fresh-water teleost, *Carassius auratus gibelio*, adapted to saline water. A scanning and transmission electron-microscopic study. *Cell and Tissue Research* 220: 73–85.

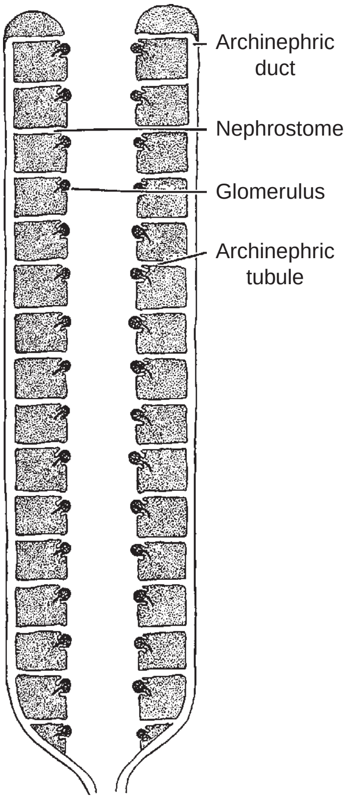


Figure 3 Diagram showing the hypothetical structure of the archinephros. A series of paired segmental tubules opens into the coelom by funnel-shaped nephrostomes that are associated with knots of blood vessels, the glomeruli. From Weichert CK (1965) *Anatomy of the Chordates*, 3rd edn., New York: McGraw-Hill Book Company, figure 7.2.

In addition to the elimination of metabolic wastes, the excretory system functions in the maintenance of a proper water balance in the body: an equilibrium of water, inorganic salts, and other substances in the internal environment of the organism. There is a great difference in the problems of water balance encountered by marine, freshwater, and terrestrial vertebrates, and it is remarkable that their kidneys are as much alike as they are.

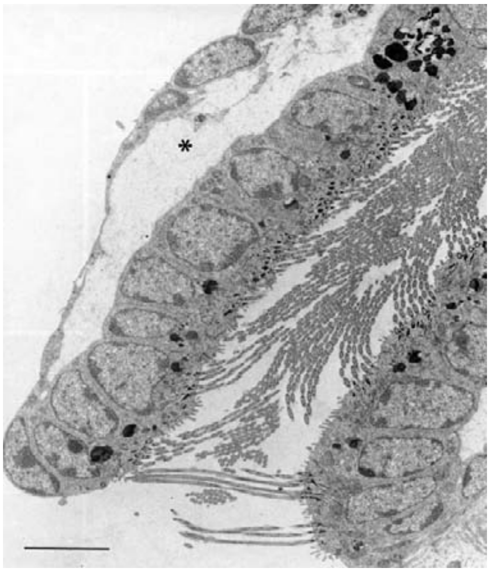


Figure 4 Transmission electron micrograph of a longitudinal section of the nephrostome from the pronephros of an early ammocoete (*Petromyzon marinus*). Both cilia and microvilli extend into the lumen. There is continuity between the peritoneal and tubular epithelium at the lower left. Loose connective tissue (*) lies between the two types of epithelia. Scale = 5 μm; 3700×. From Kluge B and Fischer A (1991) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petromyzontes): Fine structure of the renal tubules. *Cell and Tissue Research* 263: 515–528, figure 9.

The kidney of all vertebrates consists of knots of blood vessels, either glomeruli or glomera, closely associated with masses of kidney tubules (**Figures 1**(**a**), **1**(**b**), and **2**). A single tubule, with its associated blood vessels, is a nephron.

Evolution of the Kidney

It is believed that the kidney of the vertebrate ancestor was an archinephros (i.e., first kidney; **Figure 3**), a type found in larval hagfish. It consists of a series of several tubules, one per segment, on each side, that connect to a pair of longitudinal ducts, the archinephric ducts, running along the dorsal wall of the coelom. Each tubule opens into the coelom by a ciliated, funnel-shaped nephrostome (**Figure 4**) and is associated with a knot of blood vessels, the glomerulus (**Figure 5**) (plural: glomeruli).

The knot of blood vessels may protrude into the coelom as an external glomerulus (**Figure 6**). From it a protein-free filtrate of the blood plasma passes through the parietal peritoneum and into the coelom to be picked up by a nephrostome. Instead, the glomerulus may push against one side of a tubule and become almost completely surrounded (like a fist pushed into a balloon) by the wall of the tubule (**Figure 7**). This internal glomerulus passes the plasma filtrate through its wall into the tubule. Sometimes several glomeruli unite to form a larger knot of blood vessels, the glomus (plural: glomera).

The glomerulus and its surrounding tubule is the renal corpuscle (**Figure 8**); in the corpuscle fluids are removed from the blood. These fluids pass down tubules, richly entwined with blood vessels (**Figure 9**), where occurs a selective reabsorption

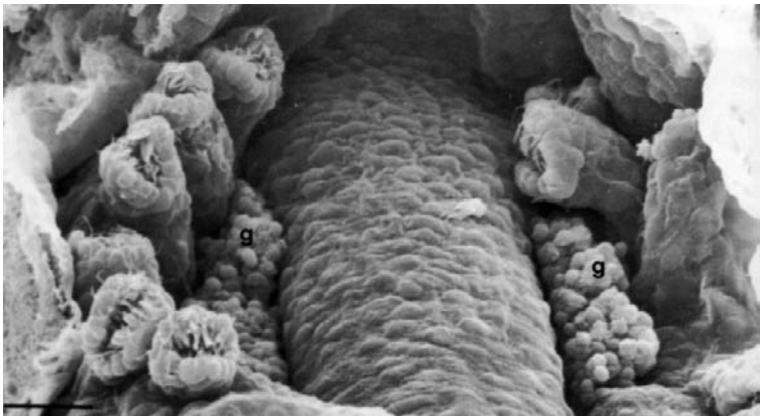


Figure 5 Scanning electron micrograph of the pronephric region of an early ammocoete (*Lampetra fluviatilis*). Two nephrostomes, with oval openings, protrude into the body cavity. There are two glomera (g) on each side of the esophagus. Scale = 20 μm; 170×. From Kluge B and Fischer A (1991) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petromyzontes): fine structure of the renal tubules. *Cell and Tissue Research* 263: 515–528, figure 7.

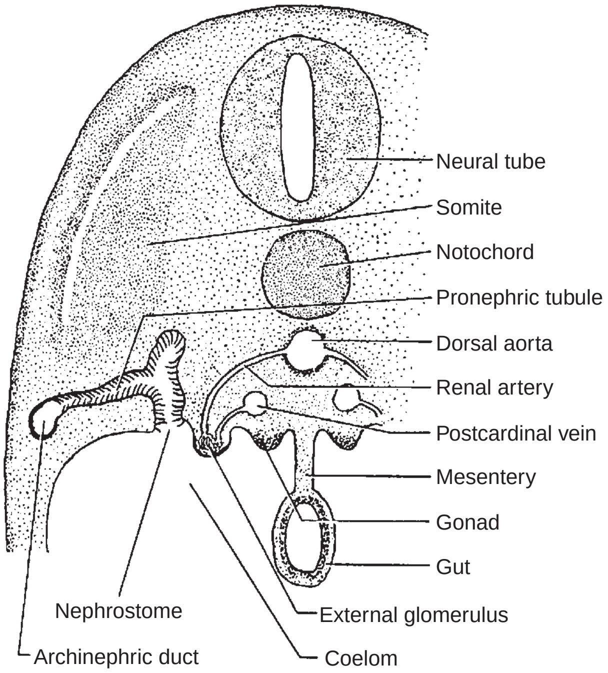


Figure 6 Diagram of a section through a vertebrate embryo showing the relation of an external glomerulus to the pronephric tubule and nephrostome. A protein-free filtrate of the blood plasma passes through the parietal peritoneum into the coelom to be picked up by the nephrostome. From Weichert CK (1965) *Anatomy of the Chordates*, 3rd edn. New York: McGraw-Hill figure 7.4.

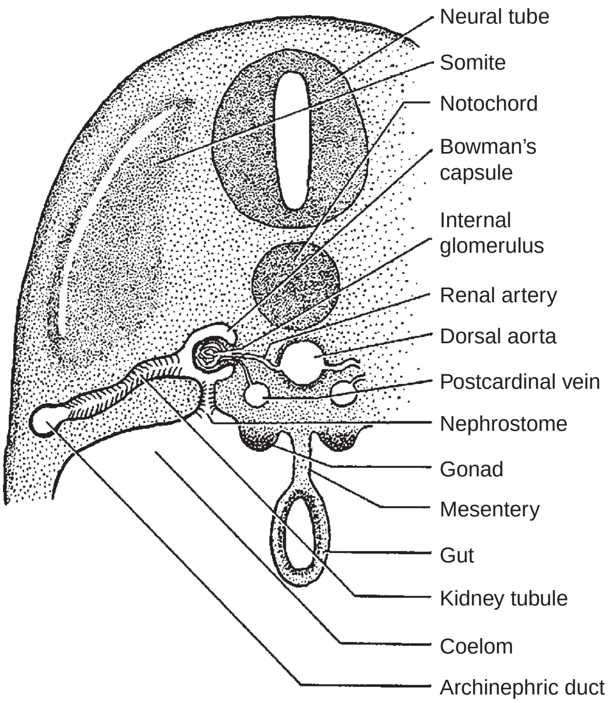


Figure 7 Diagram of a section through a vertebrate embryo showing the relation of the internal glomerulus to the kidney tubule and archinephric duct. The glomerulus has become almost completely surrounded by the wall of the tubule and a plasma filtrate passes through its wall directly into the tubule. From Weichert CK (1965) *Anatomy of the Chordates*, 3rd edn. New York: McGraw-Hill, figure 7.5.

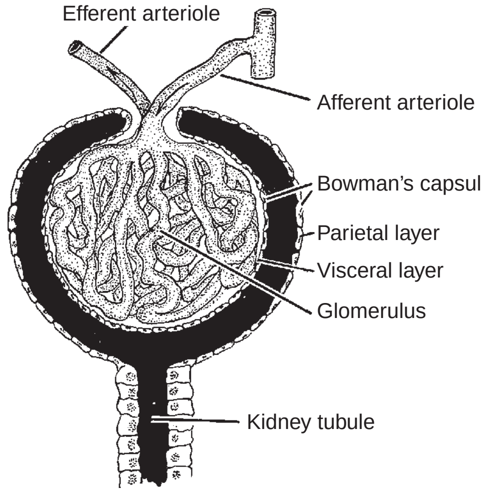


Figure 8 Diagram of a renal corpuscle. The expanded end of the kidney tubule, made up of simple squamous epithelium, becomes invaginated by the glomerulus, thereby forming a visceral layer over the vessels and an outer parietal layer. Fluids removed from the blood plasma in the nephric capsule (Bowman's capsule) pass into the tubules, which are richly entwined by blood vessels. There is a selective reabsorption of much of the water and other valuable constituents. From Weichert CK (1965) *Anatomy of the Chordates*, 3rd ed. New York: McGraw-Hill, figure 7.3.

into the blood of much of the water and other constituents so that a concentrated urine enters the excretory duct. In marine and terrestrial vertebrates, where conservation of water is essential, the glomerulus may be either lost or reduced (the aglomerular kidney of some marine fish). Freshwater fishes, on the other hand, are constantly threatened with becoming water-logged and have well-developed renal corpuscles that allow large amounts of water to escape. The Chondrichthyes appear to contradict these generalizations for, although they live in saltwater, they have large glomeruli and a kidney similar to that of freshwater fish. Excessive water loss is prevented by the retention in the blood of large amounts of urea, thereby raising the internal osmotic pressure to that of the surroundings. The archinephric duct persists in cyclostomes, elasmobranchs, and teleosts.

The most anterior portion of the archinephros, the pronephros or head kidney, appears first in developing vertebrates but is a transitory structure in the embryos of most (**Figure 3**). The remainder of the kidney posterior to the pronephros persists as the opisthonephros (i.e., back kidney) and is the functional kidney of most adult fishes. Only the posterior, unsegmented portions of the kidney tissue are retained in amniotes and these constitute the metanephros (i.e., after kidney).

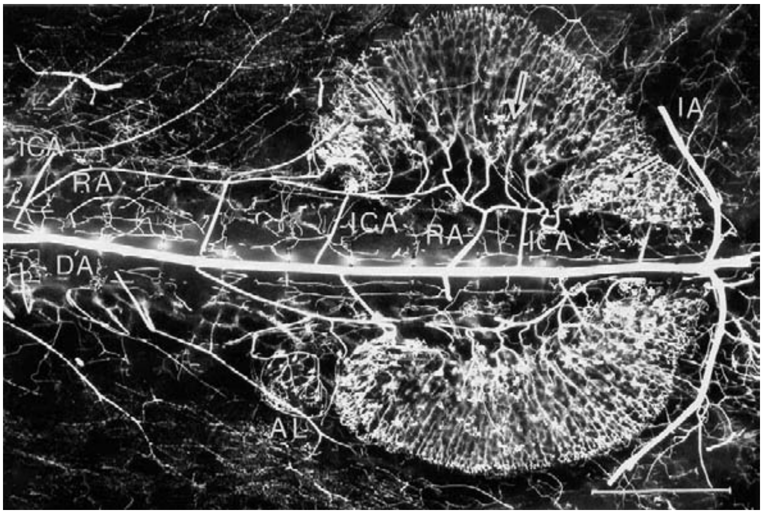


Figure 9 Photomicrograph of a corrosion preparation of the vascular system of a marine skate (*Raja erinacea*) demonstrated by the injection of silicone rubber into the blood vessels and the subsequent dissolution of all tissues. Clumps of the injection mass indicate glomeruli; masses of capillaries entwining the tubules indicate their elaborate vascularization. AL, anterior lobule of the right kidney; DA, dorsal aorta; IA, iliac artery; ICA, intercostal arteries; RA, renal arteries. Scale = 5 mm. From Hentschel H (1988) Renal blood vascular system in the elasmobranch, *Raja erinacea* Mitchill, in relation to kidney zones. *American Journal of Anatomy* 183: 130–147, figure 1.

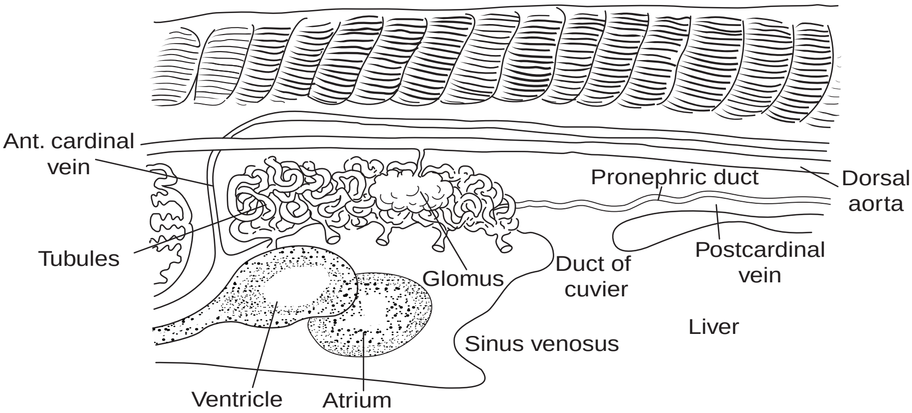


Figure 10 Diagrammatic view of the pronephros in a sagittal section of the cardiac region of a first-year ammocoete; several glomeruli have come together to form a glomus. From Bowen PC (1969) The Cytology of the Pronephros of Lampreys, PhD Thesis, University of Oxford text – figure 1.

Pronephros or Head Kidney

The pronephros, a functioning excretory organ in developing vertebrates, persists only in adult cyclostomes, a few teleosts, and lungfishes (**Figure 10**). Although the pronephros may not always function in excretion, it often supports hemopoiesis (blood cell formation) in the loose vascular connective tissue between its tubules (**Figure 11**). In Chondrichthyes, the pronephros is embryologically short-lived and never survives in the adult.

Near the mouth of each nephrostome may be an external glomerulus, a capillary net branching off the dorsal aorta (**Figures 5** and **6**). Capillaries of the glomerulus are formed of fenestrated squamous epithelial cells, which permit the leakage of fluids into the coelom. These delicate cells are reinforced by the embrace of finger-like pedicels of elaborate podocytes (**Figures 12** and **13**). Each coiled pronephric tubule begins as a ciliated funnel or nephrostome (**Figure 14**) whose flattened epithelium is continuous with that of the peritoneum. Cilia of the nephrostome move fluids into the pronephric tubule, a convoluted tubule, lined with simple cuboidal epithelial cells that are richly provided with an apical microvillous border characteristic of fluid transport; it is likely that these cells retain ions and water.

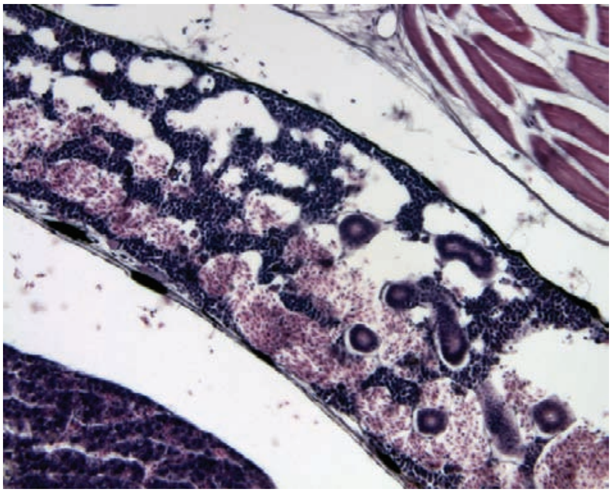


Figure 11 Photomicrograph of a section of the pronephros in a darter. Hemopoietic tissue invades the loose connective tissue between the few vestiges of kidney tubules remaining. 20×.

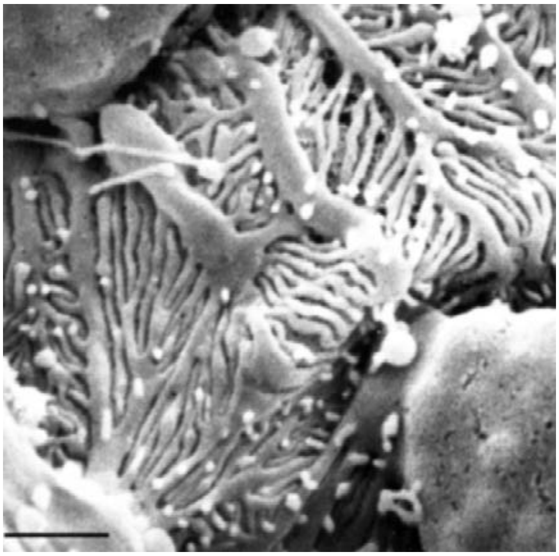


Figure 12 Scanning electron micrograph showing the interdigitation between pedicels of neighboring podocytes embracing a capillary in the glomus of an early ammocoete (*Lampetra fluviatilis*). Scale = 5 μm; 3200×. From Kluge B and Fischer A (1990) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petromyzontes): Fine structure of the external glomus. *Cell and Tissue Research* 260: 249–259, figure 13.

Opisthonephros

In most adult fishes, the pronephros disappears and the kidney is an opisthonephros. This kidney is more compact than its predecessor. It is a mass of tubules and blood vessels supported on a framework of loose fibrous connective tissue and contained within a capsule of connective tissue. Often, hemopoietic activity can be seen in the connective tissue between the tubules. The segmental arrangement of tubules is lost in the opisthonephros and several tubules may lie within a single segment.

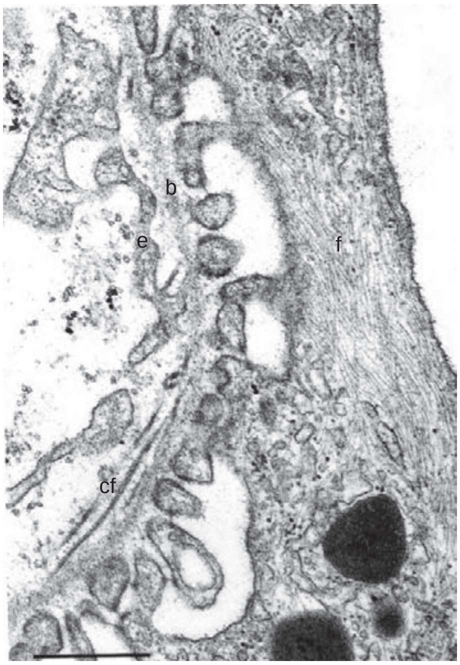


Figure 13 Transmission electron micrograph of a section through the glomus of an early ammocete (*Petromyzon marinus*). Bundles of filaments (f) extend into a large process of a podocyte. Foot processes make intimate contact with the basal lamina (b). Collagen fibers (cf) are interspersed between the basal lamina and the fenestrated endothelial cells (e) of the capillary. Scale = 0.5 μm; 44 000×. From Kluge B and Fischer A (1990) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petromyzontes): fine structure of the external glomus. *Cell and Tissue Research* 260: 249–259, figure 8.

The glomerulus is enclosed by a double capsule of tubular tissue, the glomerular capsule (Bowman's capsule) (**Figure 15**). The inner visceral layer of the capsule is closely applied to the blood vessels of the glomerulus and is separated from the outer parietal layer by the lumen or capsular space; at the arterial pole, or point of entrance of the blood vessels, the two layers are continuous. Fluid from the blood is filtered directly into the capsular space without first passing into the coelomic fluid. The filter consists of the endothelium of the capillary, the visceral layer of the glomerular capsule, and their combined basal laminas (**Figure 16**).

The blood vessels of the glomerulus are large capillaries that are interposed in the course of an arteriole; as such, they form a rete mirabile (**Figure 17**). Blood enters the glomerulus by way of an afferent arteriole and leaves by way of an efferent arteriole. From the efferent arteriole, it breaks up into true capillaries that twine around the tubules (**Figure 9**).

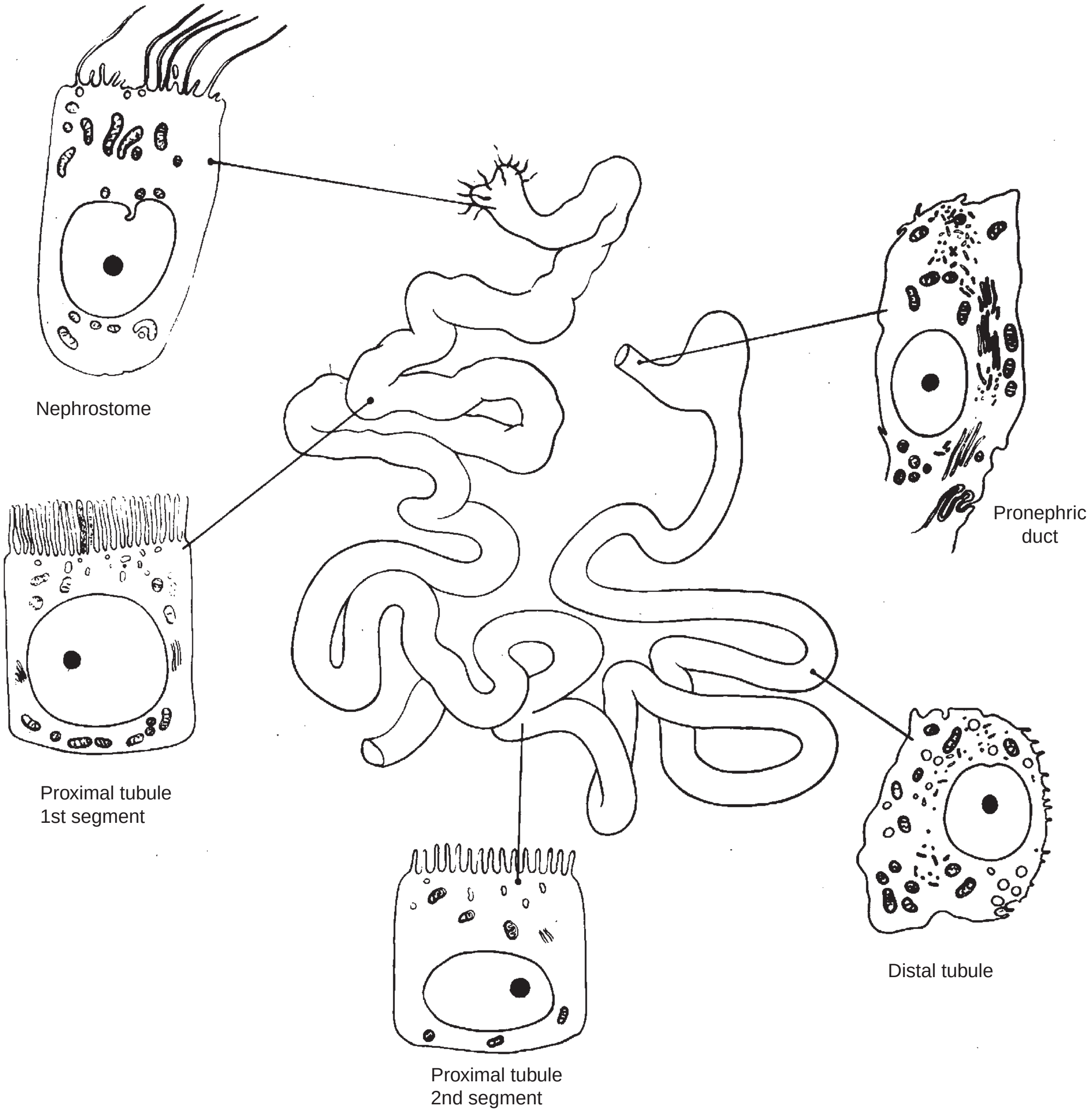


Figure 14 Diagram of a single pronephric tubule from a first-year ammocoete. The various regions of the tubule and their constituent cell types are shown. From Bowen PC (1969) The Cytology of the Pronephros of Lampreys, PhD Thesis, University of Oxford, text – figure 2.

Granules occur within epithelioid cells in the wall of the afferent arterioles in the glomeruli of elasmobranchs and teleosts (**Figure 18**). These cells have much in common with the juxtaglomerular cells of higher vertebrates and are presumed to release renin which is involved in the control of glomerular filtration rate (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange).

The visceral epithelium of the glomerular capsule is made up of elaborate cells, the podocytes, whose processes embrace the capillary loops (**Figure 19**). The extreme complexity of these cells, which resemble a basket starfish, can be best appreciated in scanning electron micrographs. The cell body or perikaryon has several branching arms fringed with foot-like processes, the pedicels; these interdigitate with pedicels from other podocytes to surround the glomerular capillaries. A single podocyte may extend processes to embrace several adjacent capillaries. The relationship of podocytes to the glomerular endothelium is seen in transmission electron micrographs (**Figure 16**). The endothelium is thin and pierced by many large fenestrations. It is sheathed by a well-defined, continuous basal lamina on which

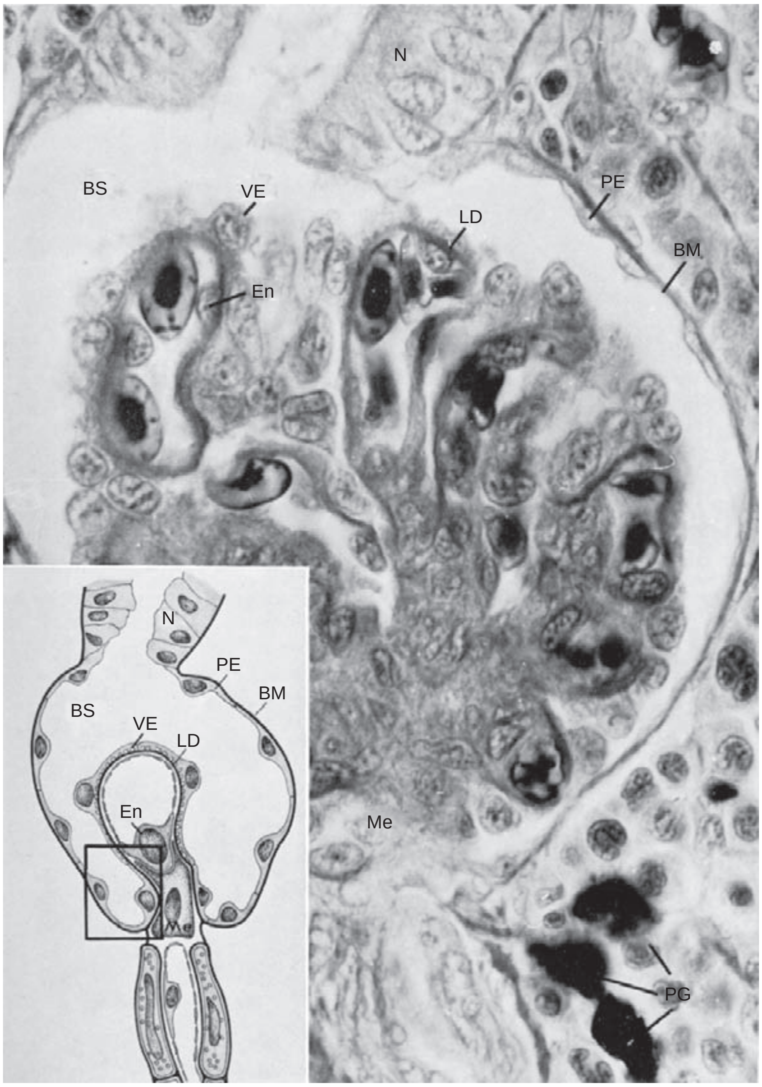


Figure 15 Photomicrograph of a section of the renal corpuscle of a trout. 1540×. Inset: Schematic drawing of the glomerulus of a freshwater trout. Abbreviations: BM, basal lamina of the parietal epithelium; BS, nephric space; En, endothelial cell of a glomerular capillary; Me, mesangial cell; N, neck segment; PE and VE, parietal and visceral epithelium of the nephric capsule; LD, basal lamina between the endothelium of the capillary and the visceral epithelium; PG, pigment granules. From Anderson BG and Loewen RD (1975) Renal morphology of freshwater trout. *American Journal of Anatomy* 143: 93–114 figures 2 and 3.

are implanted the pedicels of the podocytes. Filtration slits between adjacent pedicels open into the capsular space. A thin slit membrane spans the slit and joins adjacent pedicels, probably functioning as a spacer. Podocytes reinforce the delicate filter against the pressure of blood within the capillaries. Occasional stellate mesangial cells may be seen between the endothelial cells and the basal lamina, especially where the capillaries branch (**Figure 15**). These wandering cells likely remove fragments of degenerating materials.

The glomerular capsule is lined with a simple squamous epithelium and empties into a long, tortuous tubule that can be divided into four parts (**Figure 20**): a short ciliated neck segment; the proximal convoluted tubule of cells with a brush border; the intermediate segment, often ciliated; and the distal convoluted tubule, smaller than the proximal tubules and with a larger lumen. Each distal convoluted tubule empties directly into the archinephric duct (Wolffian duct).

The epithelial cells of the kidney tubule use energy in moving substances across a biological gradient and salvage valuable substances that have leaked into the nephric capsule; this is active transport. These epithelial cells demonstrate several characteristic morphological features: a brush border (**Figure 21**),

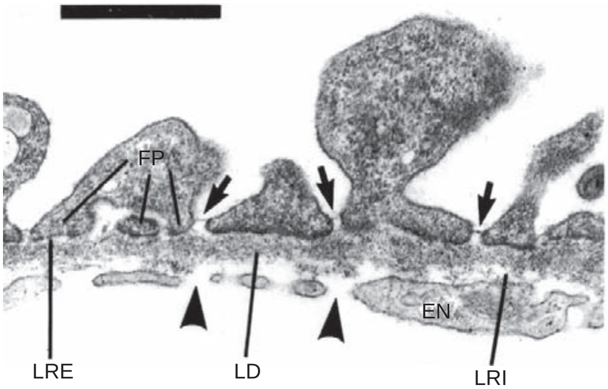


Figure 16 Transmission electron micrograph of a section through the filtration barrier in the glomerulus of a rainbow trout (*Salmo gairdneri*). The basal lamina occurs in three layers: the lamina densa (LD) between the lamina rara externa and lamina rara interna (LRE, LRI). Foot processes (FP) from podocytes reinforce the delicate filter by embracing the basal lamina. The endothelium (EN) of the capillaries is perforated by fenestrations (arrowheads). Note that the spaces between the pedicels (arrows) are spanned by delicate membranes that probably act as spacers. Scale = 500 nm; 46 000×. From Gray CJ and Brown JA (1987) Glomerular ultrastructure of the trout, *Salmo gairdneri*: effects of angiotensin II and adaptation to seawater. *Cell and Tissue Research* 249: 437–442, figure 5.

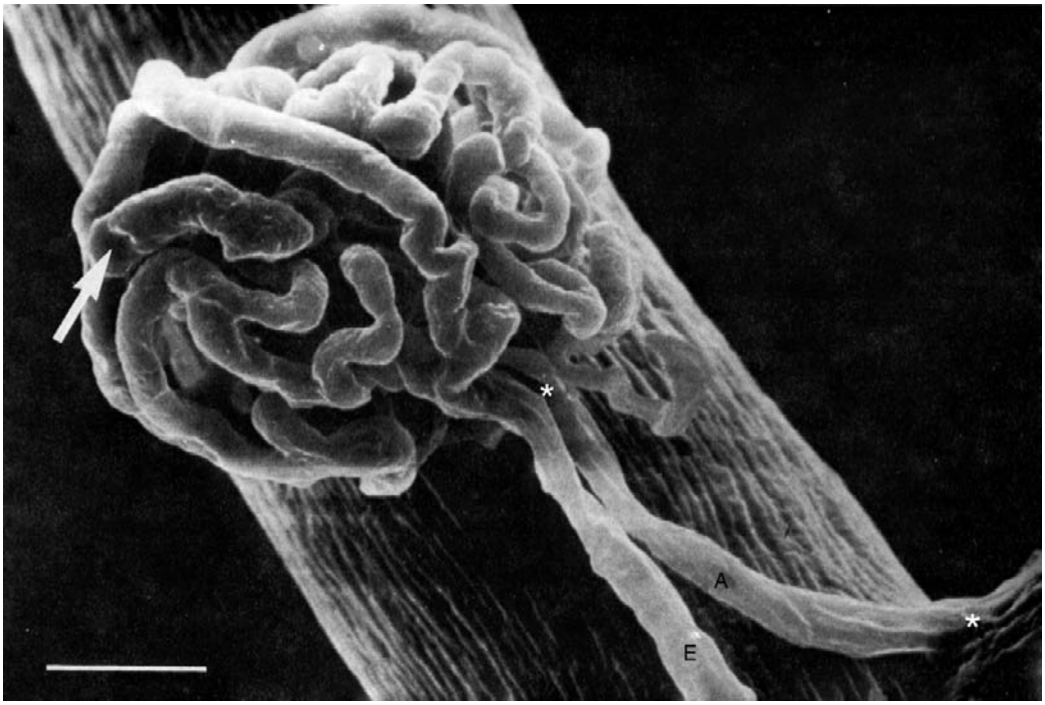


Figure 17 Scanning electron micrograph of a corrosion cast of the glomerulus of a rainbow trout; an arteriole lies behind. A, afferent arteriole; E, efferent arteriole; arrow, indentation from an endothelial cell. Scale = 20 μm. From Brown JA (1985) Renal microvasculature of the rainbow trout, *Salmo gairdneri*: Scanning electron microscopy of corrosion casts of glomeruli. *Anatomical Record* 213: 505–523, figure 1.

apical tight junctions (**Figures 22** and **23**), interdigitations between adjacent cells (**Figure 24**), basal infoldings of the plasmalemma (**Figure 25**), cellular projections that interpose themselves under neighboring cells (**Figures 25** and **26**), and abundant mitochondria (**Figures 21**, **22**, **24**, and **25**).

Part of the kidney of the male fish is used for the passage of sperm, which may be found filling many of

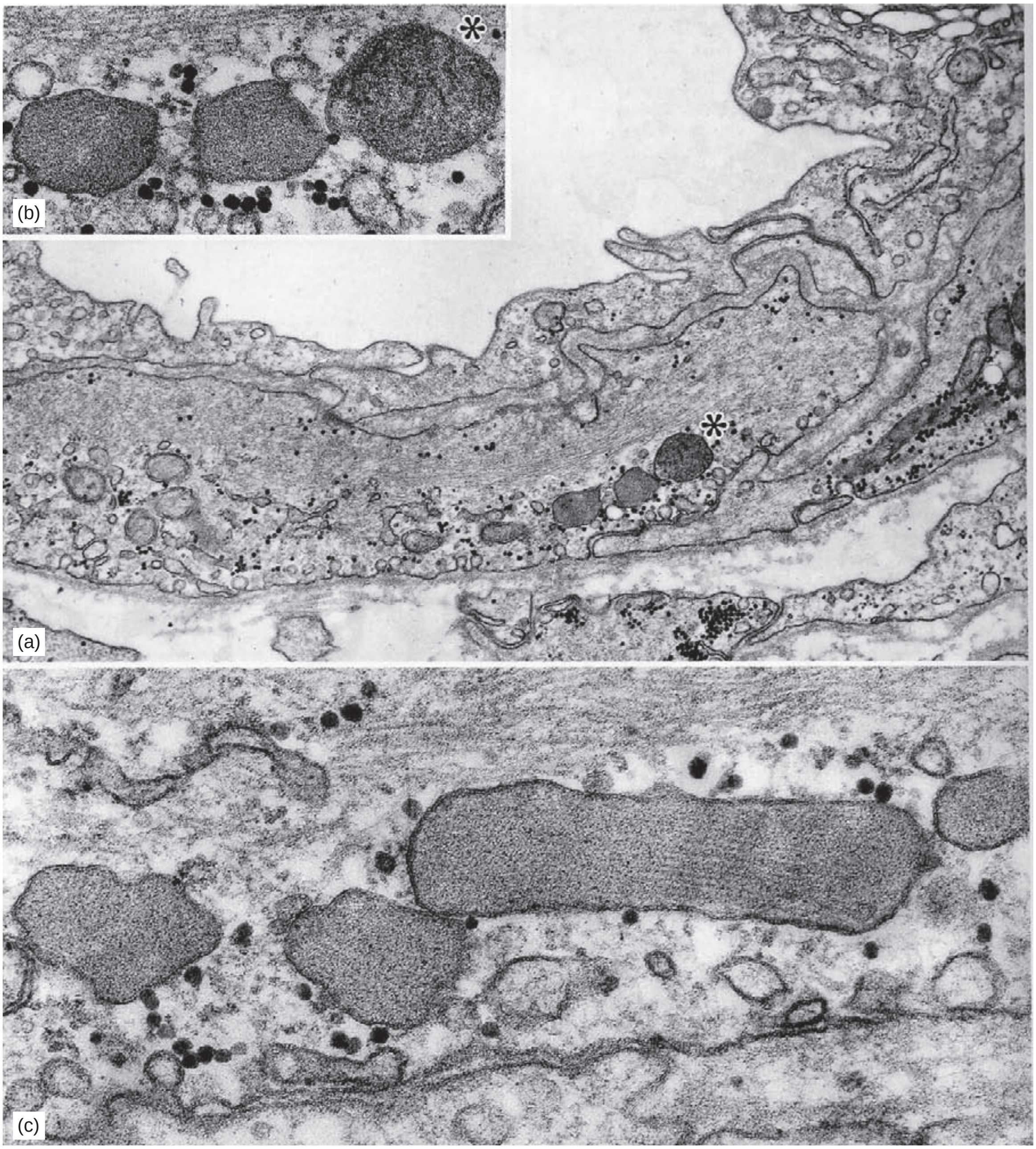


Figure 18 Transmission electron micrograph of a section of the glomerulus of the spiny dogfish (*Squalus acanthias*). The afferent arteriole (a) contains membrane-bound granules within the smooth muscle in the wall of the arteriole. The same granules (*) are shown in (b). Granules in another smooth muscle cell (c) display a paracrystalline structure. (a) 20 500×; (b) 60 000×; (c) 83 000×. From Lacy ER and Reale E (1990) The presence of a juxtaglomerular apparatus in elasmobranch fish. *Anatomy and Embryology* 182: 249–262, figure 8.

the tubules during the breeding season. Glomeruli are absent or few in marine fish, thereby reducing water loss.

The unusual opisthonephros of an adult lamprey is roughly the shape of a loaf of French bread with a longitudinal trench hollowed out of the medial side. The trench contains a single complex renal corpuscle that consists of a number of lobed glomeruli lying one behind the other to form an elongate glomus. Nephric

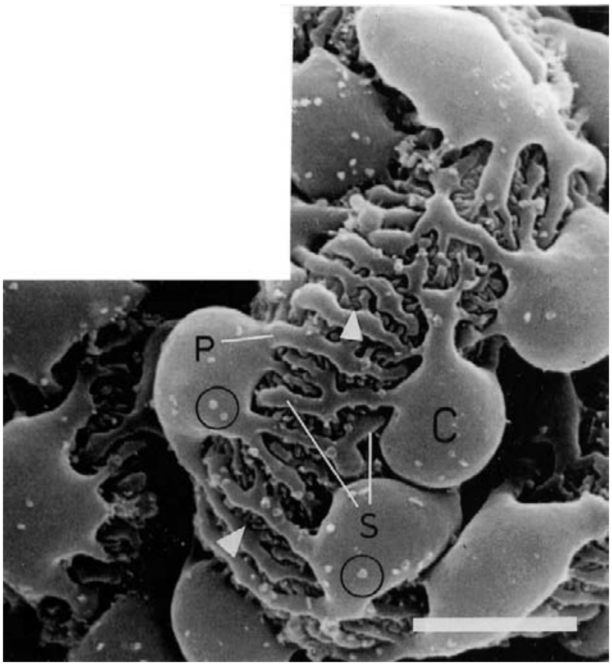


Figure 19 Scanning electron micrograph of podocytes from the glomeruli of rainbow trout (*Salmo gairdneri*). Podocytes embracing the capillaries have rounded cell bodies (C), primary processes (P) branching to secondary processes (S) and tertiary or foot processes (arrowhead). Scale = 2.5 μm. From Gray CJ and Brown JA (1987) Glomerular ultrastructure of the trout, *Salmo gairdneri:* effects of angiotensin II and adaptation to seawater. *Cell and Tissue Research* 249: 437–442, figure 1.

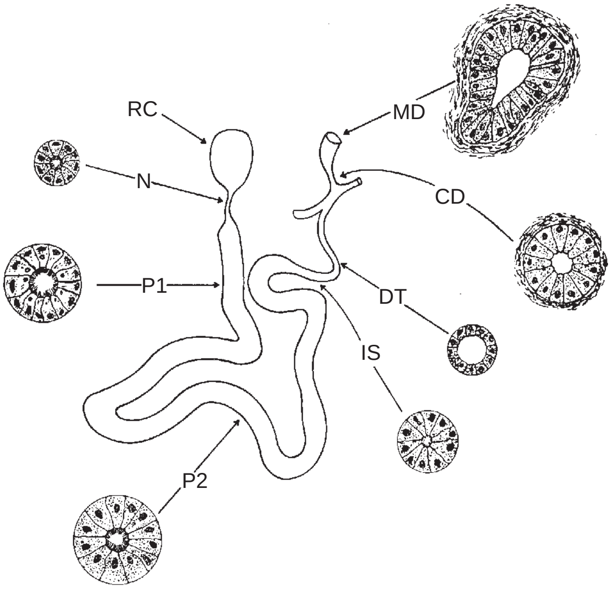


Figure 20 Schematic drawing of the nephric tubule and duct system in trout. CD, collecting duct; DT, distal tubule; IS, intermediate segment; MD, archinephric duct; N, neck segment; P_1 and P_2, first and second portions of the proximal convoluted tubule; RC, renal corpuscle. From Anderson BG and Loewen RD (1975) Renal morphology of freshwater trout. *American Journal of Anatomy* 143: 93–114, figure 1.

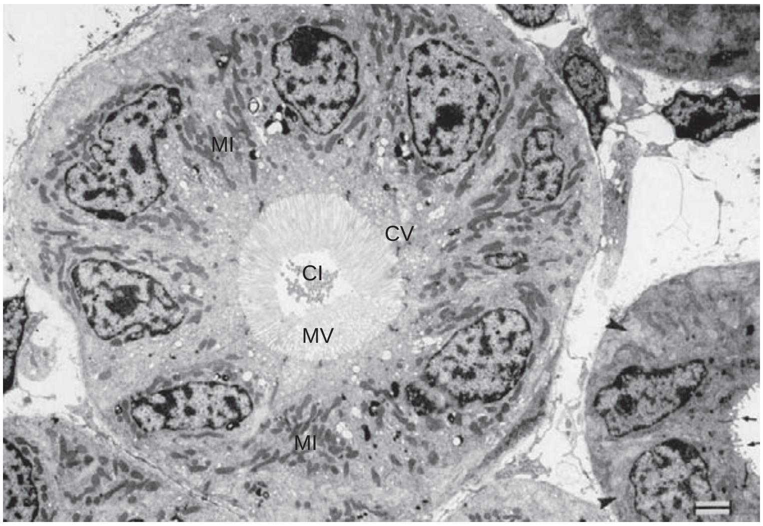


Figure 21 Transmission electron micrograph of a section through the proximal tubule of an adolescent dogfish (*Scyliorhinus caniculus*). The epithelial cells have a high brush border consisting of densely packed, long microvilli (MV). A large number of densely staining mitochondria occupy the perinuclear region. The apical cytoplasm is filled with numerous small clear vesicles (CVs). Cilia (CI) from other epithelial cells lie in the lumen. A distal tubule (arrowheads) is shown at the right bottom. Scale = 1 μm. From Hentschel H (1991) Developing nephrons in adolescent dogfish, *Scyliorhinus caniculus* (L.), with reference to ultrastructure of early stages, histogenesis of the renal countercurrent systems, and nephron segmentation in marine elasmobranchs. *American Journal of Anatomy* 190: 309–333, figure 14.

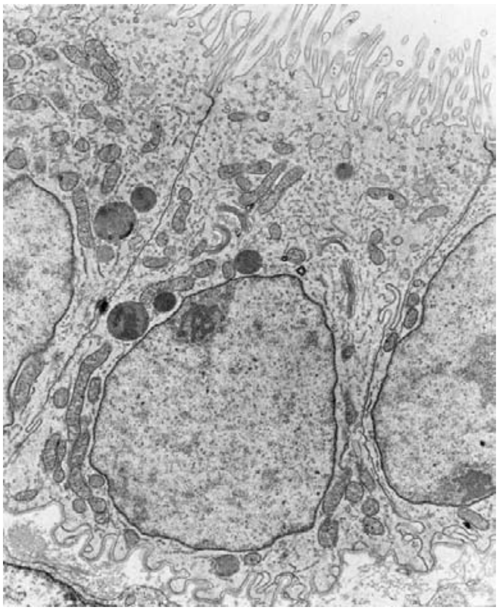


Figure 22 Transmission electron micrograph of a section of a proximal convoluted tubule of a skate (*Raja erinacea*). Tight junctions seal the apical borders between these epithelial cells. 12 000×. From Lacy ER and Reale E (1991) Fine structure of the elasmobranch renal tubule: Neck and proximal segments of the little skate. *American Journal of Anatomy* 190: 118–132, figure 5.

capsules are not invaginated to form the usual egg cup-shaped structure but are compressed between these glomeruli so that their simple squamous epithelium envelops the loops of capillaries. The capsules are drained by conspicuous, ciliated neck segments (**Figure 27**). The glomus is surrounded by tubules of the nephron that are basically similar to those seen in other kidneys.

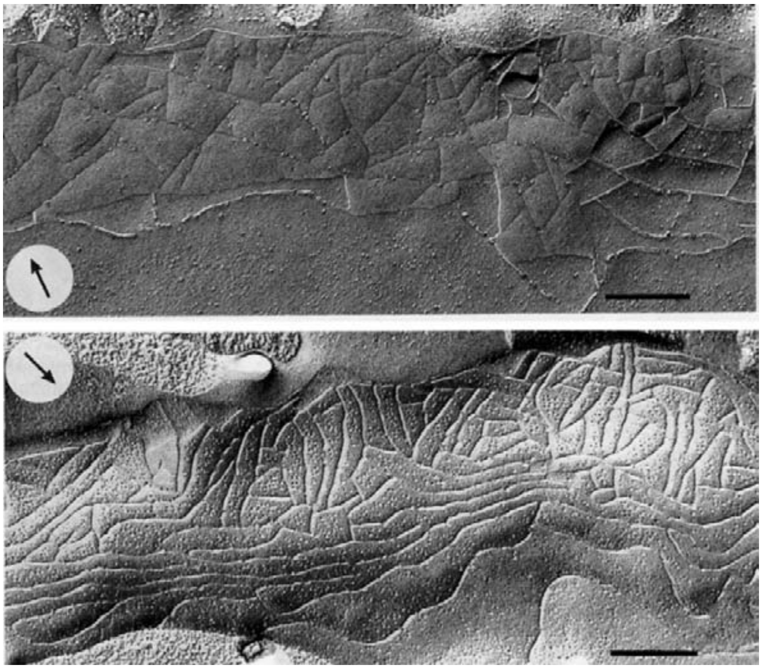


Figure 23 Electron micrographs of freeze-fracture replicas demonstrating apical tight junctions between membranes of adjacent epithelial cells of the proximal tubule (top) and distal tubule (bottom) of the dogfish (*Scyliorhinus caniculus*). The junction is much more elaborate between the cells of the distal tubule, probably indicating that fluids are transported against a steeper gradient than in the proximal tubule. From Hentschel H, *et al.* (1993) Renal tubule of dogfish, *Scyliorhinus caniculus*: A comprehensive study of structure with emphasis on intramembrane particles and immunoreactivity for H^+–K^+-aenosine triphosphatase. *Anatomical Record* 235: 511–532, figure 7.

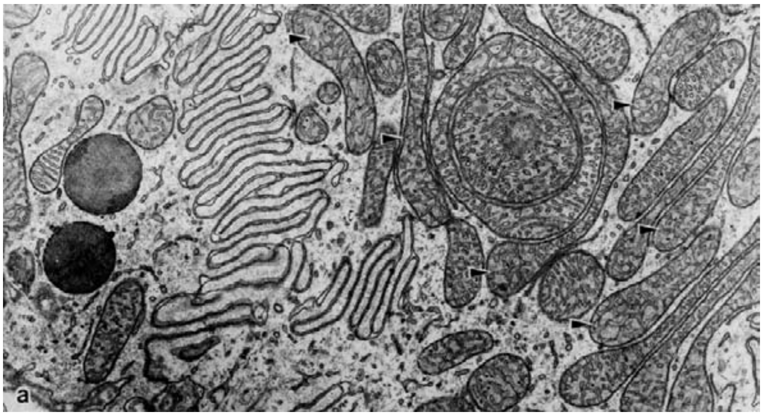


Figure 24 Transmission electron micrograph of a section through the intermediate segment of the renal tubule in the kidney of the skate (*Raja erinacea*). Elaborate infoldings occur between adjacent epithelial cells. Mitochondria (arrowheads) are abundant. From Lacy ER and Reale E (1991) The fine structure of the elasmobranch renal tubule: Intermediate, distal, and collecting duct segments of the little skate. *American Journal of Anatomy* 192: 478–497, figure 5a.

The kidney of the hagfish is an opisthonephros of almost diagrammatic simplicity (**Figure 28**). Each of the segmentally arranged nephrons consists of a renal corpuscle, comprising a glomerulus and glomerular capsule, and a short tubule that drains into the archinephric duct. Each renal corpuscle is supplied with blood from

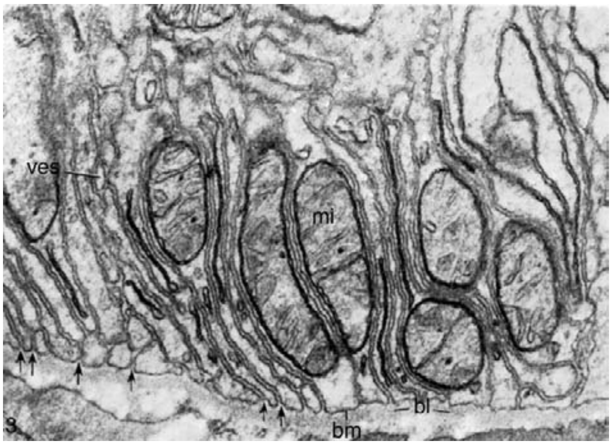


Figure 25 Transmission electron micrograph of a proximal tubule in the kidney of the stickleback (*Gasterosteus aculeatus*) showing the infoldings of the basal plasma membrane (bm) that constitute the basal labyrinth. Most of the folds of membrane are continuous with the outer cell membrane (arrows). bl, basal lamina; mi, mitochondrion; ves, vesicular membrane structure confined within the folds of membrane. 60 000×. From Wendelaar Bonga SE and Veenhuis M (1974) The membranes of the basal labyrinth in kidney cells of the stickleback, *Gasterosteus aculeatus*, studied in ultrathin sections and freeze-etch replicas. *Journal of Cell Science* 14: 587–609, figure 3.

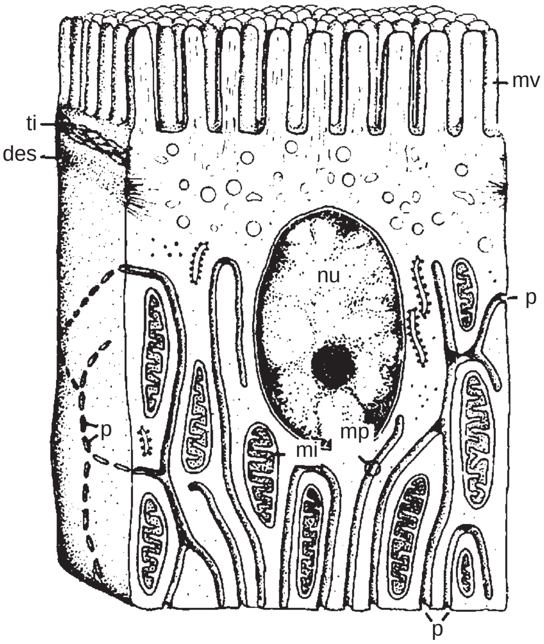


Figure 26 Diagram of a cell from the proximal tubule of the stickleback to illustrate the basal labyrinth. The interiors of the folds are continuous with the extracellular space by slit-like pores (p). des, desmosome; mi, mitochondrion; mp, membrane pairs of an infolding; mv, microvilli; tj, tight junction. From Wendelaar Bonga SE and Veenhuis M (1974) The membranes of the basal labyrinth in kidney cells of the stickleback, *Gasterosteus aculeatus*, studied in ultrathin sections and freeze-etch replicas. *Journal of Cell Science* 14: 587–609, figure 2B.

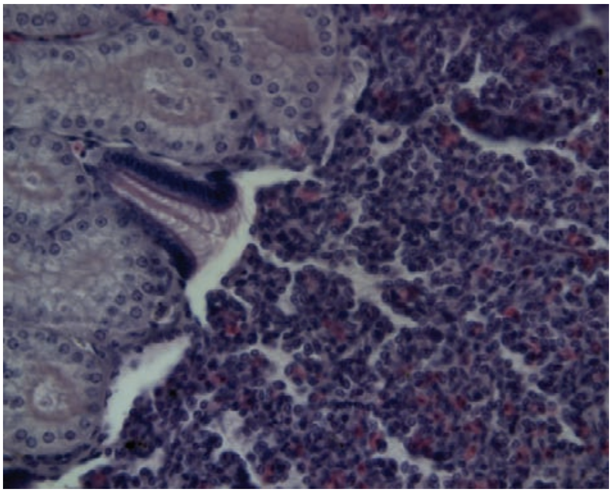


Figure 27 Photomicrograph of a section through the opisthonephros of a larval lamprey (ammocoete, *Petromyzon marinus*). All of the glomeruli are grouped together to form one large glomus (right); the bright pink flashes are erythrocytes in its capillaries. At the left is a mass of kidney tubules; a ciliated neck segment is interposed between individual glomeruli and draws fluid from the renal capsule and delivers it to the tubules. The epithelium of the ciliated neck segment is continuous with the simple squamous epithelium of the renal corpuscle investing the loops of blood vessels of each glomerulus. Capillaries invade the loose connective tissue between the tubules; pink erythrocytes can be seen in the capillary at the extreme upper left. 40×.

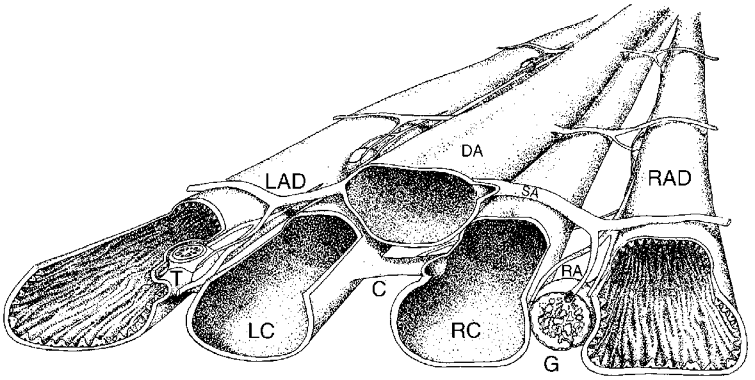


Figure 28 Schematic drawing of a portion of the corpuscular region of the hagfish kidney (*Myxine glutinosa*). The dorsal aorta (DA) gives off segmental arteries (SAs) that give rise to renal arteries (RAs). Glomeruli (G), located within each segment, receive blood from the renal arteries. The glomeruli are closely associated with the archinephric ducts on each side (LAD and RAD). C, cardinal anastomosis; LC and RC, left and right posterior cardinal veins; T, tubule. From Heath-Eves MJ and McMillan DB (1974) The morphology of the kidney of the Atlantic hagfish, *Myxine glutinosa* (L.). *American Journal of Anatomy* 139: 309–334, figure 1.

one afferent arteriole but is drained by several efferent arterioles that in turn send branches over the tubules and the archinephric duct. The tubules are not differentiated into regions and, like the archinephric duct, are lined by a simple columnar epithelium with a brush border.

See also: **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange.

Further Reading

Anderson BG and Loewen RD (1975) Renal morphology of freshwater trout. *American Journal of Anatomy* 143: 93–114.

Bowen PC (1969) The Cytology of the Pronephros of Lampreys, PhD Thesis, University of Oxford.

Brown JA (1985) Renal microvasculature of the rainbow trout, *Salmo gairdneri*: scanning electron microscopy of corrosion casts of glomeruli. *Anatomical Record* 213: 505–523.

Christensen JA, Mikeler E, and Bohle A (1989) Granular epithelioid cells of the kidneys in salmon adapted to fresh- and seawater. *Anatomical Record* 223: 21–26.

Elger M and Hentschel H (1981) The glomerulus of a stentohaline freshwater teleost, *Carassius auratus gibelio*, adapted to saline water. A scanning and transmission electron-microscopic study. *Cell and Tissue Research* 220: 73–85.

Gray CJ and Brown JA (1987) Glomerular ultrastructure of the trout, *Salmo gairdneri*: Effects of angiotensin II and adaptation to seawater. *Cell and Tissue Research* 249: 437–442.

Heath-Eves MJ and McMillan DB (1974) The morphology of the kidney of the Atlantic hagfish, *Myxine glutinosa* (L.). *American Journal of Anatomy* 139: 309–334.

Hentschel H (1988) Renal blood vascular system in the elasmobranch, *Raja erinacea* Mitchill, in relation to kidney zones. *American Journal of Anatomy* 183: 130–147.

Hentschel H (1991) Developing nephrons in adolescent dogfish, *Scyliorhinus caniculus* (L.), with reference to ultrastructure of early stages, histogenesis of the renal countercurrent systems, and nephron segmentation in marine elasmobranchs. *American Journal of Anatomy* 190: 309–333.

Hentschel H, Mähler S, Herter P, and Elger M (1993) Renal tubule of dogfish, *Scyliorhinus caniculus*: a comprehensive study of structure with emphasis on intramembrane particles and immunoreactivity for H^+–K^+–adenosine triphosphatase. *Anatomical Record* 235: 511–532.

Kluge B and Fischer A (1990) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petronyzontes): Fine structure of the external glomus. *Cell and Tissue Research* 260: 249–259.

Kluge B and Fischer A (1991) The pronephros of the early ammocoete larva of lampreys (Cyclostomata, Petromyzontes): Fine structure of the renal tubules. *Cell and Tissue Research* 263: 515–528.

Lacy ER and Reale E (1990) The presence of a juxtaglomerular apparatus in elasmobranch fish. *Anatomy and Embryology* 182: 249–262.

Lacy ER and Reale E (1991) Fine structure of the elasmobranch renal tubule: Neck and proximal segments of the little skate. *American Journal of Anatomy* 190: 118–132.

Weichert CK (1965) *Anatomy of the Chordates*, 3rd edn. New York: McGraw-Hill.

Wendelaar Bonga SE and Veenhuis M (1974) The membranes of the basal labyrinth in kidney cells of the stickleback, *Gasterosteus aculeatus*, studied in ultrathin sections and freeze-etch replicas. *Journal of Cell Science* 14: 587–609.

Youson JH (1981) The kidneys. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys,* vol. 3 pp 191pp–261. London: Academic Press.

Youson JH and McMillan DB (1970) The opisthonephric kidney of the sea lamprey of the Great Lakes, *Petromyzon marinus* L. I. The renal corpuscle. *American Journal of Anatomy* 127: 297–332.

Zuasti A and Ferrer C (1989) Haemopoiesis in the head kidney of *Sparus auratus*. *Archives of Histology and Cytology* 52: 249–255.

The Kidney

SF Perry, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Kidney Structure
Urine Formation
Acid–Base Balance by the Kidney
Nitrogen Excretion by the Kidney
Further Reading

Glossary

Acid–base balance The regulation of constant pH in the body compartments usually achieved by adjustments of PCO_2 and/or HCO_3^- levels.

Glomerular ultrafiltration The process whereby blood plasma is filtered at the glomerulus to produce the primary urine which is subsequently modified by secretion and reabsorption.

Glucosuria The presence of abnormally elevated levels of glucose in the urine.

Hypercapnia High levels of carbon dioxide in blood.

Mesangial cell A renal cell type that contacts the endothelial cells of the glomerulus and thus constitutes a portion of the filtration barrier.

Pedicel The extended projections arising from podocytes that interdigitate to form filtration slits.

Podocyte Cells of the renal visceral epithelium that form a critical component of the glomerular filtration barrier, contributing size selectivity and maintaining a large filtration surface.

Tubular transport maximum The maximal rate at which a substance can be reabsorbed (or secreted) by the kidney.

Introduction

The predominant function of the kidney is excretion, which is broadly defined as the removal of materials from the body. The broad category of excretion can be subdivided into several more well-defined physiological processes including nitrogenous waste elimination, ionic and osmotic regulation, acid–base balance, and the control of extracellular fluid volume.

The excretory process, while universal among fish, can vary tremendously with respect to the volume and chemical composition of the excretory fluid (urine) produced, as well as the mechanisms leading to urine formation. For example, fish inhabiting a freshwater (FW) environment produce large volumes of dilute urine to rid the body of water absorbed at the gill without unduly exacerbating net salt loss. On the other hand, seawater (SW) teleost fish produce small quantities of isosmotic urine to conserve water and to eliminate salts in the face of continual branchial passive water loss and salt gain. In most fish, urine formation is the net result of glomerular filtration, fluid and solute reabsorption, and secretion. However, a number of SW species possess aglomerular kidneys and thus produce urine exclusively by tubular secretion. This is but one example of the enormous functional and phenotypic diversity surrounding the fish kidney that make it such an excellent model for studying structure–function relationships in piscine physiological systems.

Kidney Structure

Aglomerular Kidneys

Only 30 species of fish are known to possess aglomerular (lacking glomeruli) kidneys representing a mere 0.1% of extant fish species. Three distinct lineages evolved from glomerular ancestors on separate occasions 120, 65, and 23 million years ago. Examples of fish with aglomerular kidneys are toadfish (family Batrachoididae), goosefish and anglerfish (family Lophiidae), pipefish and seahorses (family Syngnathidae), and some pufferfish (family Tetraodontidae). Not only do the kidneys of these fish lack glomeruli, they also do not possess distal tubules. Thus, the basic structure of the aglomerular nephron consists of a proximal portion resembling the second proximal segment of the teleost glomerular kidney (see below) and a collecting duct system. As the distal tubule is crucial for the production of dilute urine, the aglomerular kidney is limited in its capacity to accommodate fluid loads because the lower limit of urine osmolarity is believed to be approximately 200 mOsm L^{-1}. Thus, the renal excretion of a large fluid load would also be accompanied by significant loss of salt. It is probably for this reason, rather than the absence of glomeruli, *per se*, that aglomerular fish are restricted to the SW or estuarine environment.

Glomerular Kidneys

Majority of fishes have what is termed a glomerular kidney. Glomerular kidneys share the common function of producing urine through a first step of ultrafiltration at the glomerulus (a network of capillaries contained within Bowman's capsule). Beyond this one common feature, however, glomerular kidneys exhibit substantial anatomical variation among the fish groups (**see also Role of the Kidneys**: Histology of the Kidney). Adult hagfish possess two discrete kidneys (pronephric and mesonephric); however, only the posterior mesonephric kidney, a paired elongated structure lying on either side of the midline, is believed to be functional. Each side of the hagfish mesonephric kidney contains about 40 nephrons that drain into paired mesonephric ducts (ureters). The ultrafiltrate produced by glomerular filtration within Bowman's capsule enters a short neck segment prior to delivery into the mesonephric duct. The epithelial cells lining the mesonephric duct resemble the cells of the first proximal segment of the teleost nephron (see below).

The kidney of cyclostomes (lampreys), as in hagfish, is a paired, elongated structure. However, in most other respects, the cyclostome kidney bears little resemblance to the hagfish nephron but more closely resembles the kidneys of the more advanced teleosts. Thus, the glomerulus is followed by a short neck segment, a proximal tubule, an intermediate segment, a distal tubule, and a collecting duct which empties into a mesonephric duct.

The elasmobranch nephron is by far the most elaborate within the various fish taxa and, in some respects, is similar to the mammalian kidney because of the occurrence of countercurrent tubular flow. The tubules form two loops with limbs that descend and ascend in countercurrent parallel pathways through two discrete zones, a bundle zone and a sinus zone. The bundle zone is encapsulated by a peritubular sheath that may allow control over the chemical composition of the interstitial fluid and possibly assist urea and trimethylamine oxide (TMAO) reabsorption via countercurrent multiplication.

The teleost kidney contains two sections: a head kidney, which comprises about 20% of the anterior portion of the kidney and the trunk kidney which comprises the other 80% and extends along the dorsal wall of the body cavity. The head kidney is largely composed of steroidogenic interrenal cells, chromaffin cells, and hematopoietic tissue while the trunk kidney contains the vast majority of filtering nephrons in addition to hematopoietic and pigmented cells.

The teleost kidney is typically grouped into three functional categories (FW, SW, and euryhaline) and into five anatomical classes (types I–V). The distinction of the five classes is based largely on the extent of separation between the trunk and head kidney and the degree of fusion of the left and right kidneys. For example, nearly all marine species are grouped within category III in which only the posterior portion of the kidney is fused and the anterior region is composed of two slender branches. There are relatively few anatomical differences in the tubular composition of FW, SW, and euryhaline fishes with all of them possessing a neck segment exiting Bowman's capsule, two segments of proximal tubule, and a collecting duct. The collecting ducts drain into two mesonephric ducts, which fuse to form a common archinephric duct (bladder). The FW nephron, which is designed to produce large volumes of dilute urine, has large glomeruli and a distal tubule which is essential for NaCl reabsorption and the production of dilute urine. The FW nephron also contains an intermediate segment positioned between the proximal and distal tubules; the function of the intermediate segment is unknown. The nephron of euryhaline species also contains a distal tubule, but its role in NaCl reabsorption varies according to salinity. The marine nephron which is designed for water conservation possesses smaller glomeruli and lacks the diluting distal tubule (**Figure 1**).

Urine Formation

In the glomerular kidney, urine is formed through the interactive effects of ultrafiltration, water and solute reabsorption, and secretion. This strategy allows great flexibility in the chemical composition of the final urine that is excreted because the primary urine (ultrafiltrate) can be extensively and variably modified by reabsorption and secretion (**Figure 2**).

Ultrafiltration

Ultrafiltration is the process whereby blood plasma is selectively filtered at the glomerulus to produce the primary urine in an epithelial-lined tubule. The process of glomerular ultrafiltration is driven by pressure gradients across a filtration barrier within the glomerulus, which is composed of the capillary endothelium, a matrix of mesangial cells, a basement membrane, and a visceral epithelium. The visceral epithelium is composed of podocytes that give rise to cellular projections termed pedicels, which can be arranged to form slits through which filtration occurs. Unlike in mammals, the glomerular capillaries of fish lack fenestrations (pores) and this difference presumably reduces hydraulic conductivity of the filtration barrier in fish, all other things being equal.

As in other kidneys, two types of pressure determine the net filtration pressure (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange). A positive

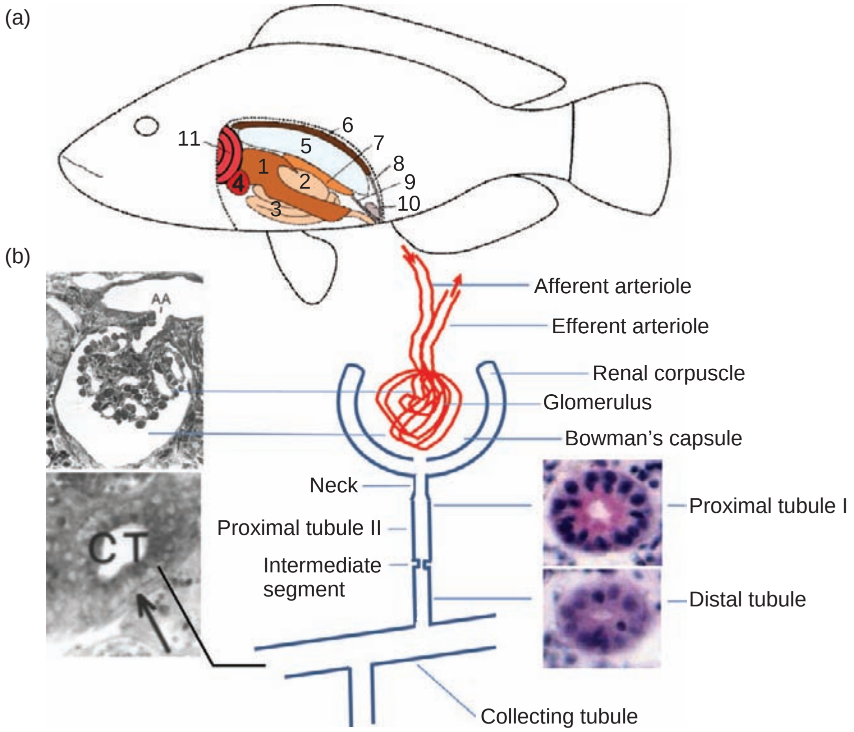


Figure 1 (a) A schematic drawing of inner anatomy of a teleost fish. 1, liver; 2, stomach; 3, intestine; 4, heart; 5, swimbladder; 6, kidney; 7, testicle; 8 ureter (mesonephric duct); 9, efferent duct; 10, urinary bladder (archinephric duct); 11, gills. (b) A montage illustrating the major components of the teleost glomerular nephron. (a) Reproduced with permission (original author Uwe Gille) under a free documentation license (http://commons.wikimedia.org/wiki/Commons:GNU_Free_Documentation_License). (b) Photomicrographs reproduced from Tsuneki K, Kobayashi H, and Pang OK (1984) Electron-microscopic study of innervation of smooth muscle cells surrounding collecting tubules of the fish kidney. *Cell and Tissue Research* 238(2):307–312; Elger M, Wahlqvist I, and Hentschel H (1984) Ultrastructure and adrenergic-innervation of preglomerular arterioles in the euryhaline teleost, *Salmo gairdneri*. *Cell and Tissue Research* 237(3): 451–458; and Georgalis T, Yorston J, Gilmour KM, and Perry SF (2006) The roles of cytosolic and membrane bound carbonic anhydrase in the renal control of acid–base balance in rainbow trout, *Oncorhynchus mykiss*. *American Journal of Physiology* 291: F407–F421.

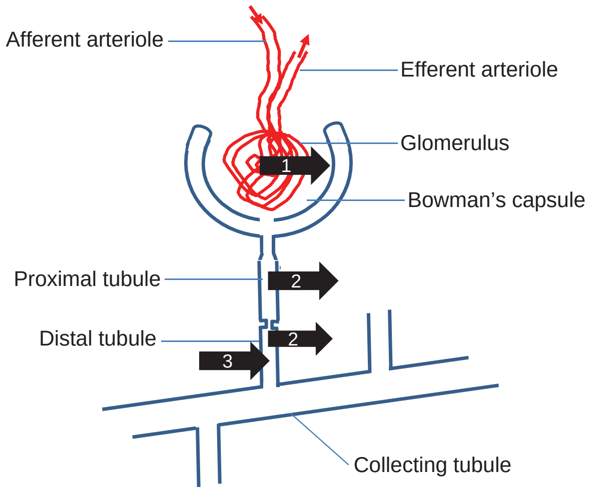


Figure 2 A schematic representation of a teleost glomerular nephron depicting the three main processes underlying urine formation: ultrafiltration (1), secretion (3), and reabsorption (2).

hydrostatic pressure difference between glomerular capillaries and the lumen of Bowman's capsule generates filtration across the filtration barrier. A counteracting negative colloid osmotic pressure difference opposes filtration. The hydrostatic pressure in the glomerular capillaries is largely dictated by the blood pressure in the glomerular capillaries, which is greatly influenced by variations in cardiac output and the vascular resistance in the afferent and efferent renal arterioles. The colloid osmotic pressure difference arises because the filtrate formed within Bowman's capsule is largely devoid of protein. Net filtration along the length of glomerular capillaries persists as long as hydrostatic pressure exceeds plasma colloid osmotic pressure. However, as fluid is lost from glomerular capillaries by filtration, colloid osmotic pressure increases and hydrostatic pressure decreases (very slightly) until net filtration pressure falls to zero. Consequently, the process of ultrafiltration produces primary urine which, except for its lack of protein, has a chemical composition similar to plasma (**Figure 3**).

Within any given nephron, the rate at which the ultrafiltrate is formed is termed the single nephron glomerular filtration rate (SNGFR), which is determined by pressure gradients, hydraulic conductivity, and the surface area over which filtration is occurring. The glomerular filtration rate (GFR) for the entire kidney is set by the number

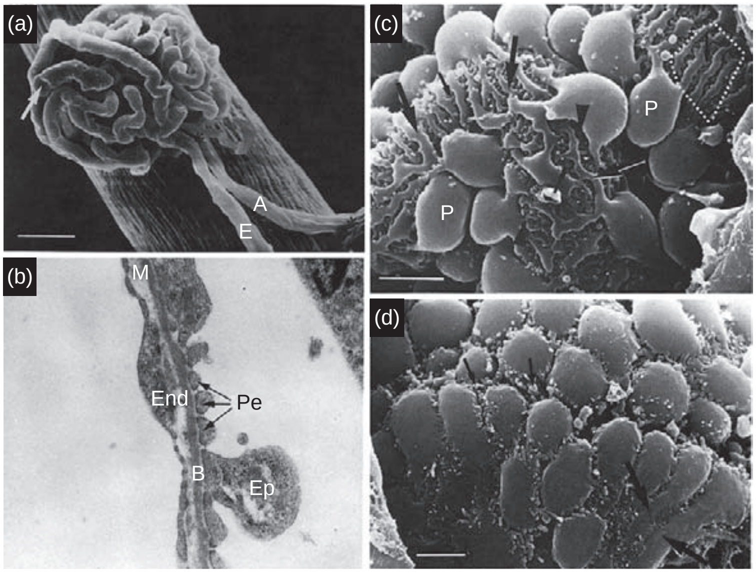


Figure 3 Structure of the teleost glomerular kidney. (a) A corrosion cast of a glomerulus with single afferent (A) and efferent (E) arterioles. Arrow indicates endothelial cell indentations. Asterisks show positions of origin and end of afferent arteriole used to measure the length of the vessel. (b) A transmission electron micrograph of the glomerular capillary wall in an FW trout; capillary endothelium (End), mesangial matrix (M), basement membrane (B), visceral epithelium (Ep). Epithelial pedicels (Pe) are connected by slit diaphragms. (c) Visceral epithelium of glomerulus of an FW trout showing rounded epithelial podocytes (P) with primary processes, sometimes branching into secondary and tertiary processes(large arrows) and terminating in pedicels (small arrows). An area of regular interdigitation of pedicels is enclosed by dotted outline. Some pedicels arise directly from podocytes (arrowheads). Few cytoplasmic microprojections present on podocyte and its processes. (d) Seawater adapted fish. Flattened podocytes and broad, flat primary processes (large arrows) with little visible interdigitation of pedicels. Many cytoplasmic microprojections (small arrows). Bar = (a) 20 μm; (b) ~ 1 μm; (c) ~ 4 μm; and (d) 4 μm. (a) Reproduced from Brown JA (1985) Renal microvasculature of the rainbow trout, *Salmo gairdneri* – scanning electron-microscopy of corrosion casts of glomeruli. *Anatomical Record* 213(4): 505–513. (b)–(d) Reproduced from Brown JA, Taylor SM, and Gray CJ (1983) Glomerular ultrastructure of the trout, *Salmo gairdneri* – glomerular capillary epithelium and the effects of environmental salinity. *Cell and Tissue Research* 230(1): 205–218.

of filtering nephrons and their SNGFRs. Urine flow rate, which can be highly variable in FW fish, is always less than GFR because of tubular reabsorption; but since fractional tubular reabsorption tends to be held constant, GFR and urine flow rate tend to be proportional. Typically, a small fraction of nephrons are unperfused and thus do not contribute to urine formation. Moreover, not all of the perfused nephrons are actually filtering at any given time. Thus, GFR can be rapidly modified by glomerular recruitment (or de-recruitment), whereby the numbers of filtering nephrons are adjusted.

The principal factors contributing to glomerular recruitment are changes in arterial blood pressure and renal blood flow. Factors contributing to increased systemic arterial blood pressure, such as circulating catecholamines or activation of sympathetic nerve fibers, will promote glomerular recruitment, besides increasing SNGFR (assuming no changes in renal blood-flow patterns). Angiotensin II, the product of the renin–angiotensin-system (RAS), while promoting an elevation of systemic blood pressure (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels), actually acts as an antidiuretic (reduces urine formation) in fish because of its vasoconstrictory actions on the afferent renal vasculature.

The lower urine flow rates in SW fish or in euryhaline fish experiencing increasing environmental salinity are predominantly related to marked reductions in GFR. In SW fish, the reduced GFR primarily reflects anatomical changes – fewer and smaller glomeruli as well as alterations in their morphology that promote a reduced hydraulic permeability. For example, the pedicels, which are arranged to form leaky filtration slits in FW fish, are either poorly developed or tightly packed together to limit filtration. In addition, the podocytes overlying the glomerular capillaries are more sparsely distributed in FW fish, while they tend to be more abundant and flattened in SW fish to further limit filtration.

Reabsorption

Depending on species and environmental salinity, fractional reabsorption may be entirely absent (hagfish) or as high as 97% (FW teleosts). While impressive in FW teleosts, the capacity of the fish kidney to reabsorb filtered NaCl remains considerably less than in the mammalian nephron where ~99% of the filtered NaCl is reabsorbed. Moreover, unlike in mammals where the bulk of NaCl reabsorption occurs at the proximal tubule, fish appear to rely at least equally on segments distal to the proximal tubule. For example, in the lamprey (*Lampreta fluviatilis*) only 10% of filtered NaCl is reabsorbed at the proximal tubule, while 80% is reabsorbed at the collecting duct.

In teleosts, the predominant solutes reabsorbed from the primary urine are Na^+, Cl^-, Ca^{2+}, and glucose. For NaCl, the greatest rates of reabsorption are found in FW species because of their need to produce dilute urine (i.e., retain salt but lose excess water). Correspondingly, relatively little water is reabsorbed. Typically, less than 50% and occasionally as little as 5% of the filtered water is reabsorbed.

The mechanisms of Na^+ and Cl^- reabsorption are not well understood. Na^+ uptake from the filtrate probably involves electroneutral Na^+/H^+ exchange via one or more members of the SLC9 family of Na^+/H^+ exchangers (e.g., NHE3 or NHE2), while Cl^- uptake may be achieved by members of the SLC4 (e.g., SLC4A2) or SLC26 (e.g., SLC26A4 and SLC26A6) anion transporter gene families. An essential driving force for trans-tubular Na^+ uptake is basolateral Na^+/K^+-ATPase (NKA), which facilitates the final re-entry step of Na^+ into the blood. The tubular reabsorption of Ca^{2+} is thought to involve its passive entry across the luminal (mucosal) membrane through an epithelial Ca^{2+} channel (ECaC) followed by active transport across the serosal membrane via Ca^{2+}-ATPase (PMCA).

The teleost nephron, like the mammalian kidney, has an excellent capacity to reabsorb dissolved glucose lost to the filtrate; virtually all glucose is removed from the filtrate under normal conditions. In rainbow trout, the predominant mechanism of glucose reabsorption is via coupled Na^+–glucose transporters. The tubular transport maximum for glucose (TmG) in the trout kidney is about $150\,\mu mol\ kg^{-1}\ h^{-1}$, which corresponds to plasma glucose levels exceeding 20 mM. Thus, it is unlikely that significant glucose will be lost in the urine (a condition termed glucosuria) except after acute dietary carbohydrate loading.

Secretion

In addition to ultrafiltration, solutes can be removed from the blood and they can enter the urine by secretion; examples of secreted solutes include Ca^{2+}, Mg^{2+}, NH_4^+, H^+, K^+, SO_4^{2-}, and PO_4^{2-}. To determine whether or not a solute is undergoing net secretion, the renal clearance ratio (RCR) for that solute must be determined. The RCR is the ratio of the amount of solute excreted in the urine (UFR × urine [solute]) to the amount filtered (GFR × plasma [solute]). If the RCR exceeds 1 (excretion exceeds filtration), this indicates net secretion into the urine whereas a RCR less than 1 indicates net reabsorption. It is important to emphasize that the RCR provides data only on the net tubular processes. RCR only reveals which processes dominate in cases where solutes are reabsorbed as well as secreted (e.g., K^+ and Ca^{2+}).

In FW fish, the extent of tubular secretion for any given secreted ion essentially reflects the blood chemistry, which in turn is largely controlled by dietary loading and acid–base status. For example, dietary ingestion of Mg^{2+} and SO_4^{2-} will promote increased secretion of these solutes, while catabolism of ingested protein to ammonia will promote NH_4^+ secretion. Acidification of the blood may be associated with increased secretion of H^+ (see below), which in turn may be associated with markedly increased secretion rates of PO_4^{2-} and NH_4^+. SW fish secrete significant quantities of divalent ions which are largely derived from ingested seawater (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation). The nature of divalent ion secretion is poorly understood but may involve Mg^{2+}/Na^+ exchange, Mg^{2+}/H^+ exchange, and anion/SO_4^{2-} exchanges (possibly via SLC26A1).

Acid–Base Balance by the Kidney

Although the gill is the predominant site of acid–base regulation in fish, the kidney also plays an essential role that should not be underestimated. Fish regulate blood pH through modulations of plasma HCO_3^- levels, which in turn are dictated by adjustments of branchial acid excretion (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). For example, the regulation of respiratory acidosis is associated with marked increases in plasma HCO_3^- levels which may routinely exceed 20 mM and even reach values exceeding 70 mM (e.g., in European eel, (*Anguilla anguilla*) exposed to an external P_{CO_2} of 45 mmHg). Under such conditions, it is essential that the kidney is able to reabsorb the filtered HCO_3^-. Essentially, branchial acid excretion would become a futile exercise in the absence of a renal mechanism for the retention of accumulated HCO_3^- ions. Thus, for every additional mol of HCO_3^- appearing in the glomerular filtrate, the kidney must secrete an additional mol of H^+ to ensure complete HCO_3^- reabsorption. As increases in renal acid secretion are effectively used to reabsorb excess filtered HCO_3^-, it is rare that net acid excretion by the kidney will ever match the markedly elevated rates of acid excretion occurring at the gill during compensation of acidosis. On the other hand, the rates of renal acid secretion required to sustain a condition of fully compensated respiratory acidosis (i.e., elevated HCO_3^- to achieve normal pH at high P_{CO_2}) are likely to approach the rates of peak acid excretion at the gill. It is important to emphasize that under conditions of chronic hypercapnia, high rates of renal acid secretion must be sustained whereas the elevation of branchial acid excretion need last only as long as it takes to accumulate sufficient HCO_3^- to raise pH back to normal.

The potential for renal mechanisms to contribute to acid–base regulation appears to be highest in FW fish, where large volumes of urine are produced (see above). In SW fishes, the pH-regulating capacity of the kidney is limited by low urine flow rates and an apparent lack of responsiveness to systemic acid–base disturbances. By contrast, the responses of the FW teleost kidney to acid–base disturbances are comparable in pattern and flexibility to those of the mammalian kidney, although quantitatively subservient to those of the gill (although see above).

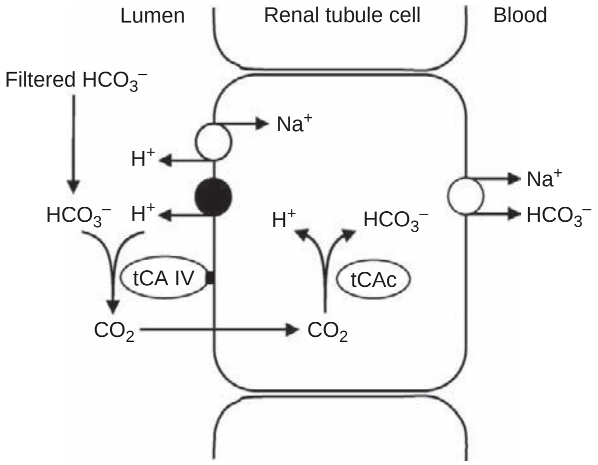


Figure 4 A model depicting the proposed mechanism of renal HCO_3^- reabsorption in fish. Acid added to the filtrate by the V-type H^+-ATPase and NHE3 titrate-filtered HCO_3^- in the tubule lumen in a reaction catalyzed by membrane-bound CA IV. The resultant CO_2 enters the renal epithelial cell by diffusion and is hydrated to H^+ and HCO_3^- in the presence of a high activity cytoplasmic CA isoform (tCAc). Finally, HCO_3^- ions are moved across the basolateral membrane by NBC1, while protons are recycled into the tubule lumen. Energy-consuming transporters are indicated by filled circles.Reproduced from Perry SF and Gilmour KM (2006) Acid–base balance and CO_2 excretion in fish: Unanswered questions and emerging models. *Respiratory Physiology and Neurobiology* 154:199–215.

Recent work suggests that the mechanism of renal HCO_3^- reabsorption in rainbow trout in many ways parallels that of the mammalian proximal tubule, the site responsible for 80–90% of renal HCO_3^- reabsorption in mammals (**Figure 4**).

Acid secretion into the filtrate across the luminal membrane is achieved via two mechanisms: electroneutral Na^+/H^+ exchange and active H^+ pumping by V-type H^+-ATPase. In rainbow trout, the specific Na^+/H^+ exchanger thought to be involved in renal acid secretion is NHE3 or SLC9A3. H^+ ions secreted into the filtrate combine with HCO_3^- to form CO_2, a reaction catalyzed by membrane-associated carbonic anhydrase isoform IV (tCA IV). The CO_2 diffuses into the tubule cells where it is dehydrated to HCO_3^- and H^+ in the presence of cytosolic carbonic anhydrase (tCAc). The H^+ ions thus formed re-enter the filtrate via NHE3 or H^+-ATPase while the HCO_3^- is reabsorbed into the plasma via $Na^+-HCO_3^-$ co-transporter isoform 1 (NBC1) (**Figures 5–7**).

Nitrogen Excretion by the Kidney

The two predominant forms of nitrogenous waste excreted in the urine are ammonia and urea with negligible contributions from other N_2-containing substances such as uric acid, creatine, and creatinine. It is important to point out that the rates of renal ammonia and/or urea excretion, while substantial, comprise only a small fraction (<5%) of whole-body nitrogen excretion. Although increasing the rates of renal ammonia excretion will not markedly affect whole-body N_2 balance, it can have a significant consequence on renal acid–base balance

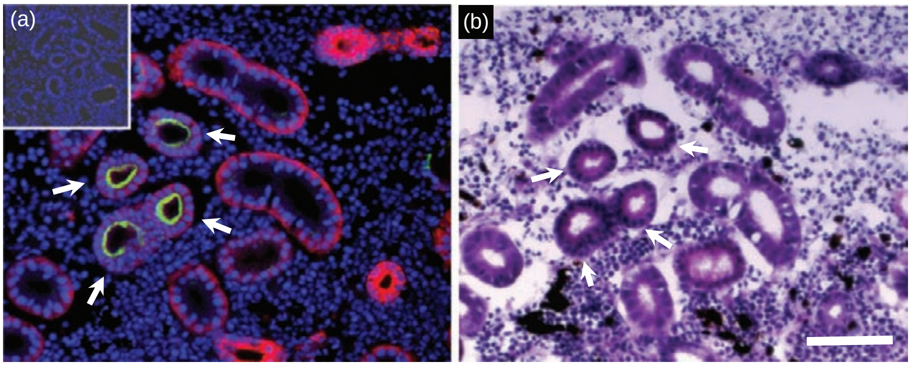


Figure 5 Localization of NHE3 protein to apical regions of proximal renal tubules in rainbow trout (*Oncorhynchus mykiss*) kidney using immunocytochemistry. (a) Apical NHE3 (stained green; indicated by white arrows) was localized to cells containing basolateral $Na^+–K^+$-ATPase (stained red); nuclei are stained blue (4,6-diamidino-2-phenylindole). (b) Periodic acid Schiff (PAS) staining of the same section demonstrated that NHE3 was specifically localized to the brush border of proximal tubule cells. The NHE3 and $Na^+–K^+$-ATPase immunofluorescence were absent with omission of primary antibodies (inset in (a)) when image was acquired under the same exposure as in (a). Bar = 50 μm. Reproduced from Ivanis G, Braun M, and Perry SF (2008) Renal expression and localization of SLC9A3 sodium hydrogen exchanger (NHE3) and its possible role in acid–base regulation in freshwater rainbow trout *(Oncorhynchus mykiss). American Journal of Physiology* 295: R971–R978.

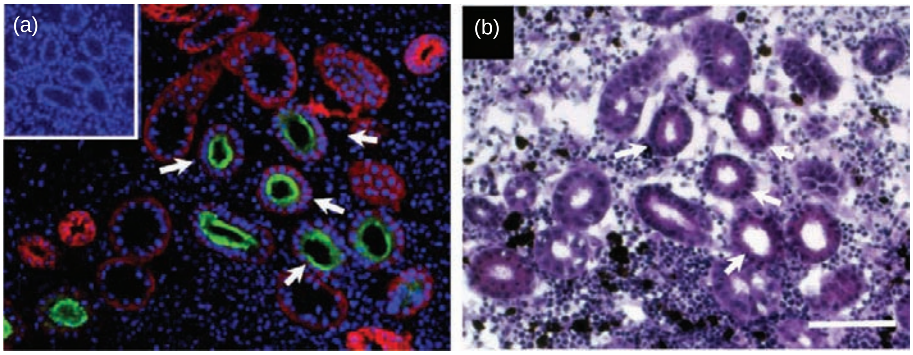


Figure 6 Localization of vacuolar-type H$^+$-ATPase (V-ATPase) protein to apical regions of proximal renal tubules in rainbow trout (*Oncorhynchus mykiss*) kidney using immunocytochemistry. (a) Apical V-ATPase (stained green) was localized in cells containing basolateral Na$^+$–K$^+$-ATPase (stained red). (b) The identity of the V-ATPase-enriched tubules (white arrows) as proximal tubules was based on the presence of a prominent brush border (stained pink using PAS on the same section). The V-ATPase and Na$^+$–K$^+$-ATPase immunofluorescence were absent with omission of primary antibodies (inset in (a)) when image was acquired under the same exposure as in (a). Bar = 40 μm. Reproduced from Ivanis G, Braun M, and Perry SF (2008) Renal expression and localization of SLC9A3 sodium hydrogen exchanger (NHE3) and its possible role in acid–base regulation in freshwater rainbow trout (Oncorhynchus mykiss). *American Journal of Physiology* 295: R971–R978.

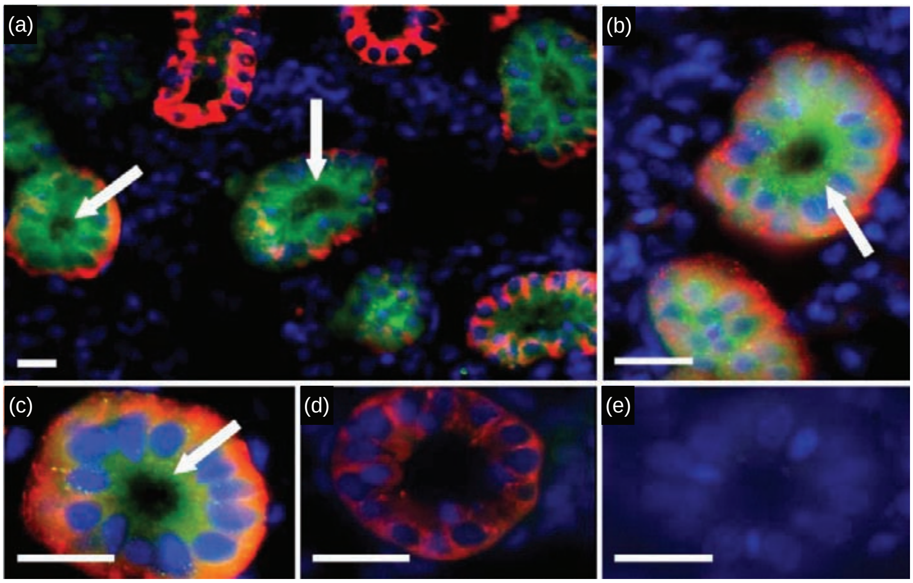


Figure 7 Representative fluorescence micrographs revealing the presence of CA IV protein in rainbow trout (*Oncorhynchus mykiss*) renal tubules by immunocytochemistry. Green color indicates CA IV, red color indicates Na$^+$–K$^+$-ATPase, and nuclei are stained blue (DAPI). (a–c) Different tubules viewed at ×40, 63, and 100 magnifications, respectively. In cells co-expressing the two proteins, CA IV was localized to the apical regions of tubules (arrows) or co-localized with Na$^+$–K$^+$-ATPase (yellow/orange) in basolateral regions. In some tubules not expressing CA IV, Na$^+$–K$^+$-ATPase was broadly distributed. Specific fluorescence was prevented by pre-absorption of the CA IV antibody with immunizing peptide (d) or omission of both primary antibodies (e). Scale bars = 25 μm. Reproduced from Georgalis T, Yorston J, Gilmour KM, and Perry SF (2006) The roles of cytosolic and membrane bound carbonic anhydrase in the renal control of acid–base balance in rainbow trout, *Oncorhynchus mykiss. American Journal of Physiology* 291: F407–F421. (2005).

because ammonia is an important urinary buffer. Ultimately, the capacity of the urine to excrete acid is related to the presence of buffers such as ammonia and phosphate. In the ureogenic marine elasmobranchs that accumulate high levels of urea in the blood as an osmolyte, the role of the kidney is to ensure that filtered urea is effectively reabsorbed. The mechanism of reabsorption of urea by the elasmobranch kidney likely involves a facilitated urea transporter which has been cloned and characterized in dogfish.

See also: **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange. **Osmotic, Ionic and Nitrogenous-Waste Balance**:

Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **Role of the Kidneys**: Histology of the Kidney.

Further Reading

Beyenbach KW (2004) Kidneys sans glomeruli. *American Journal of Physiology* 286: F811–F827.

Brown JA (1985) Renal microvasculature of the rainbow trout, *Salmo gairdneri* – scanning electron-microscopy of corrosion casts of glomeruli. *Anatomical Record* 213(4): 505–513.

Brown JA, Taylor SM, and Gray CJ (1983) Glomerular ultrastructure of the trout, *Salmo gairdneri* – glomerular capillary epithelium and the effects of environmental salinity. *Cell and Tissue Research* 230(1): 205–218.

Elger M, Wahlqvist I, and Hentschel H (1984) Ultrastructure and adrenergic-innervation of preglomerular arterioles in the euryhaline teleost, *Salmo gairdneri*. *Cell and Tissue Research* 237(3): 451–458.

Georgalis T, Yorston J, Gilmour KM, and Perry SF (2006) The roles of cytosolic and membrane bound carbonic anhydrase in the renal control of acid–base balance in rainbow trout, *Oncorhynchus mykiss*. *American Journal of Physiology* 291: F407–F421.

Hickman CP and Trump BF (1969) The kidney. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, pp. 91–239. New York: Academic Press.

Ivanis G, Braun M, and Perry SF (2008) Renal expression and localization of SLC9A3 sodium hydrogen exchanger (NHE3) and its possible role in acid–base regulation in freshwater rainbow trout (*Oncorhynchus mykiss*). *American Journal of Physiology* 295: R971–R978.

Lacy ER and Reale E (1985) The elasmobranch kidney. I. Gross anatomy and general distribution of the nephrons. *Anatomy and Embryology (Berlin)* 173: 23–34.

Pelis RM and Renfro JL (2004) Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion. *American Journal of Physiology* 287: R491–R501.

Perry SF and Gilmour KM (2006) Acid–base balance and CO_2 excretion in fish: Unanswered questions and emerging models. *Respiratory Physiology and Neurobiology* 154: 199–215.

Tsuneki K, Kobayashi H, and Pang OK (1984) Electron-microscopic study of innervation of smooth muscle cells surrounding collecting tubules of the fish kidney. *Cell and Tissue Research* 238(2): 307–312.

THE GUT

Role of the Gut

Contents
Gut Ion, Osmotic and Acid-Base Regulation
Dogfish Rectal Gland

Gut Ion, Osmotic and Acid-Base Regulation

RW Wilson, University of Exeter, Exeter, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Gut Anatomy
Ion and Osmotic Regulation Functions of the Gut in Nonfeeding Teleost Fish
Intestinal Cl^- and Water Absorption Directly Linked to HCO_3^- Secretion
Intestinal Water Absorption Indirectly Linked to HCO_3^- Secretion via Alkaline $CaCO_3$ Precipitation
Ion Regulation in the Gut of Freshwater Fish
Ion and Osmotic Regulation by the Gut of Feeding Freshwater Teleost Fish
Ion and Osmotic Regulation by the Gut of Feeding Marine Teleost Fish
The Role of the Gut in Acid–Base Regulation
Further Reading

Glossary

Aquaporins Membrane proteins that act as water channels (discovered by Peter Agre for which he won the Nobel prize in 2003).

Metabolon A structural–functional relationship between enzymes or membrane transporters that have linked functions.

Paracellular Refers to the route between cells of an epithelium, usually through tight junctions.

Serosal Refers to the side of an epithelium normally bathed by blood or extracellular fluid.

Transcellular transport Refers to the transfer of substances through the cell, i.e., across both the basolateral and apical membranes.

Introduction

The gut (**see also The Gut**: The Gastrointestinal Tract: An Introduction) is well known for processing the dissolution products from the digestion of food, which obviously includes large quantities of organic and inorganic ions. However, the majority of our knowledge of the ionic and osmoregulatory roles of the gut in fish comes from nonfeeding animals and, in particular, the central role the gut plays in the hypoosmoregulatory strategy of marine teleosts. This article therefore begins by describing the ion and water transport processes involved in the gut of fish under nonfeeding conditions, followed by the lesser-known ionoregulatory responses of the gut to feeding. In both cases, the discussion includes some of the inevitable interactions with acid–base regulation. This is not only because acid–base relevant ions (e.g., H^+, HCO_3^-, and NH_4^+) are often involved in ion transport processes generally, but particularly in the gut where conspicuously high rates of gastric acid secretion and pancreatic bicarbonate base secretion are well known in all vertebrates. Furthermore, very high rates of net base secretion occur in the intestine of marine fish as part of their osmoregulatory strategy.

Gut Anatomy

The gut of all animals is best known for its role in feeding, digestion, and assimilation of nutrients. However, within fish the gut plays a number of alternative roles, including respiratory gas exchange, ion and osmotic regulation, nitrogenous waste excretion, and acid–base balance. Not surprisingly, this multi-functionality is associated with highly varied gut morphology within the fish world (**see also The Gut**: The Gastrointestinal Tract: An Introduction and **Food Acquisition and Digestion**: Digestive Efficiency). However, for the purposes of the present article, it is sufficient to appreciate three main features of the gastro-intestinal tracts in fish.

First, within genetically related species, quality of diet influences relative gut length (in order from longest to shortest; detritivores and coralivores, herbivores, omnivores, and carnivores). Second, many fish (~25% of known taxa) are completely agastric (no stomach), and others can have a stomach but lack typical gastric acid secretion (**see also Integrated Function and Control of the Gut**: Barrier Function of the Gut and Intestinal Absorption, **Gut Anatomy and Morphology**: Development of Fish Gut). Third, many fish species have pyloric ceca, blind-ended sacs at the anterior end of the intestine, but the number can vary from zero to several hundred. The function of these pyloric ceca is still debated but they certainly enhance the intestinal surface area and are involved in the secretion of some digestive enzymes and bicarbonate, as well as absorption of nutrients and water.

Ion and Osmotic Regulation Functions of the Gut in Nonfeeding Teleost Fish

Much more is known about ion regulation in the gut of marine fish than freshwater fish, and more about teleosts than either elasmobranchs (**see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays) or agnathans (**see also Hagfishes and Lamprey**: Hagfishes, Lampreys: Energetics and Development, and Lampreys: Environmental Physiology). For this reason, we first describe our current knowledge for marine teleost fish.

Like most vertebrates, marine teleosts have body fluids with an osmolality and Na$^+$ and Cl$^-$ concentrations that are roughly one-third of seawater (~1050 mOsm kg^{-1} and ~150 mM, respectively; **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). This generates a strongly dehydrating osmotic driving force for water loss and salt gain across their outer body surface (mainly via the large area and thin diffusion distance of the gills). To compensate for the osmotic loss, marine teleosts therefore are obliged to continuously drink large amounts of the external medium equivalent to 2.5–12% of their body mass per day (1–5 ml kg^{-1} h^{-1}).

Drinking of seawater occurs steadily in marine teleosts, with small volumes being ingested very regularly. The imbibed seawater is 3 times more concentrated than blood and is dominated by the monovalent Na$^+$ and Cl$^-$ ions (**Table 1**). Yet, by the time the remaining fluid reaches the distal end of the intestine, it has been reduced greatly in volume (50–85% of the water has been absorbed) and its chemistry is very different from the original ingested seawater, instead being dominated by Mg^{2+}, SO$_4^{2-}$, and HCO$_3^-$ ions (**Table 1**)

During passage through the gut, most of the imbibed Na$^+$ and Cl$^-$ are absorbed in order to drive the essential uptake of water that compensates for living in the dehydrating marine environment. The Mg^{2+} and SO$_4^{2-}$ become more concentrated mainly due to the low permeability of

Table 1 The chemistry of blood plasma and rectal fluid in teleost fish, and in seawater and different types of freshwater.

	Seawater,	*Plasma*	*Rectal fluid*	*Typical freshwater*	*Ion-poor freshwater*
Osm	1050	320	~300	<10	<1
Na$^+$	470	140–180	40–100	0.35	0.009
Mg^{2+}	53	1	100–150	0.21	0.002
Ca^{2+}	10	2	2–5	0.75	0.001
K$^+$	10	2–4	5	0.08	0.004
Cl$^-$	548	130–170	30–100	0.23	0.031
SO$_4^{2-}$	28	<1	~100	0.21	-
HCO$_3^-$	2	5–10	50–100	1.11	0.036

Ion concentrations are in mmol l^{-1} and osmolality is in mOsm kg^{-1}.

Typical freshwater values represent averages for worldwide rivers (Bayly and Williams, 1973). Ion-poor freshwater values represent averages for forest streams in the Amazon (Furch, 1984). Seawater values represent those for a salinity of 34 (Barnes, 1954)

Bayly IAE and Williams WD (1973) *Inland Waters and Their Ecology*, 316p. Melbourne: Longmanp; Barnes H (1954) Some tables for the ionic composition of sea water. *Journal of Experimental Biology* 31: 582–588; and Furch K (1984) Water chemistry of the Amazon basin: The distribution of chemical elements among freshwaters. In: Sioli H (ed.) *The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and Its Basin*, pp.167–199. Dordrecht: Junk.

the intestine for these ions and the progressive absorption of water. By contrast, the high levels of bicarbonate arise from its net secretion from enterocytes (intestinal epithelial cells). In turn, this causes further modification of the gut fluid chemistry through the chemical precipitation of most of the imbibed calcium and some magnesium as their insoluble carbonates ($CaCO_3$ and $MgCO_3$). The production of these chalky precipitates is now acknowledged to play an important role in offsetting the potentially detrimental accumulation of unabsorbed divalent cations (see later). Details of the precise mechanisms involved in the above processing of imbibed seawater within each section of the gut are given in the following.

Esophagus and Stomach

Relatively few studies have focused on the role of the esophagus, but it is clearly important in marine teleosts for large absorptive fluxes of Na^+ and Cl^-. The imbibed fluid entering the stomach therefore is somewhat desalinated and much closer to the osmolality of blood than the original seawater ingested. This ion transport role of the esophagus is very specific to Na^+ and Cl^-, with little permeability to other ions or water. The NaCl absorption is thought to be driven by both active and passive mechanisms, with basolateral Na^+K^+-ATPase (NKA) providing the driving force. The stomach may be the site of some further changes, including slight dilution of luminal fluid due to net secretion of water, but generally the modified seawater enters the intestine slightly hyperosmotic to blood and still dominated by Na^+ and Cl^- ions.

Intestinal NaCl Co-Transport and Water Absorption

Upon entering the intestine, active absorption of Na^+ and Cl^- is essential to drive the uptake of water into the blood. The co-transport of Na^+ and Cl^-, and the fluid absorption this generates, is ultimately driven by the electrogenic basolateral NKA (**Figure 1**). It is noteworthy that the activity of this key-energizing, ion-transporting enzyme is higher in the intestine than in the other two major ionoregulatory organs in fish (the gills and kidney). For every two K^+ ions pumped into the cell by the NKA, three Na^+ ions are pumped out of the basolateral side of the cell and into the lateral intercellular space (LIS). This generates a low intracellular Na^+ concentration, as well as a negative membrane potential (intracellular relative to extracellular). Collectively, these provide an electrochemical Na^+ gradient that facilitates coupled uptake of both Na^+ and Cl^- across the apical membrane via two transport mechanisms: Na^+:Cl^-

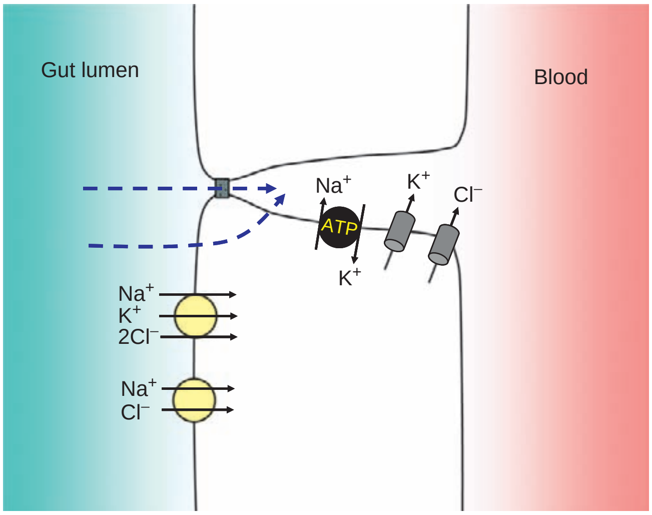


Figure 1 The mechanism of NaCl co-transport and solute-linked water transport in the intestine of marine teleosts. Basolateral NKA uses the energy in ATP to pump K^+ into the cell and Na^+ into the LIS. The latter generates a low intracellular Na^+ concentration which in turn provides the electrochemical gradient for apical Na^+ uptake from the gut lumen into the cell. This apical Na^+ uptake occurs via co-transporters, which use the prevailing Na^+ gradient to simultaneously drag Cl^- ions (NC) or Cl^- and K^+ ions (NKCC2) into the cell. Chloride ions subsequently exit the cell on the basolateral membrane via chloride channels, down their electrochemical gradient. Potassium ions accumulating in the cell from both basolateral NKA and apical NKCC then recycle out of the cell via K^+ channels. Blue dashed arrows represent potential routes for water movement that follow the net transport of solutes (primarily Na^+ and Cl^-) in the apical-to-basolateral direction. The accumulation of both Na^+ and Cl^- in the LIS generates a slightly hyper-osmotic environment which will draw water by osmosis from the gut lumen. The pathway for this osmotic flow of water is not yet known, but could involve paracellular movement via the tight junction, or transcellular via water channels (aquaporins) on the apical and basolateral membrane. Water and NaCl ultimately enter the gut vasculature, and the excess Na^+ and Cl^- ions are then actively excreted via mitochondria-rich cells in the gills.

(NC) and Na^+:K^+:2Cl^- (NKCC) co-transporters. Note that the apical entry of Cl^- is uphill (i.e., against the prevailing electrochemical gradient for Cl^-) and driven by the downhill electrochemical gradient for Na^+ ions. The apical co-transport of Na^+ and Cl^- ions therefore is secondary active transport, ultimately dependent on the energy consumed by the primary active NKA on the basolateral membrane. Subsequently, the apical entry of $Cl^-$is followed by Cl^- exit at the basolateral membrane, via either electrochemical diffusion through channels or K^+Cl^- co-transport. For all these processes to continue, it is essential that K^+ ions are recycled via channels to prevent its intracellular accumulation.

All of the above transport processes ultimately serve to drive the net absorption of both Na^+ and Cl^- (the main solutes in seawater), which subsequently provides an appropriate transepithelial osmotic gradient to drive net water absorption by the gut. This is often described as solute-linked or solute-coupled water flow, which is clearly secondary to salt transfer in the same direction. However, despite decades of research, the precise mechanism and route of the water absorption is still uncertain.

Although a number of aquaporins (water channels; **see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin) have been identified for potentially osmoregulatory roles in the marine teleosts intestine (in particular, AQP-8b), there is no direct evidence that transepithelial water absorption actually occurs via such channels. Indeed, it is not even certain that net transepithelial water movement is transcellular (i.e., through both the apical and basolateral membranes). Most favored models explain net water absorption as occurring via the paracellular pathway in the gut, drawn by osmosis across the tight junctions into a slightly hyperosmotic LIS environment (**Figure 1**). Further intrigue on the issue of water absorption arises from the concept of the simultaneous translocation of a fixed number of water molecules within the tertiary structure of various ion co-transporters (termed 'molecular water pumps' by Zeuthen and co-workers). For example, the Na^+/glucose co-transporter (SGLT1) is proposed to carry two Na^+, one glucose, and 264 water molecules simultaneously. Despite our recent advances in molecular physiology, precise details of the mechanism of water transport across the gut remain elusive and continue to be a topic of important research activity.

Intestinal Cl^- and Water Absorption Directly Linked to HCO_3^- Secretion

The principles behind apical NaCl co-transport, fueled by basolateral NKA, ultimately providing the driving force for water absorption in the gut are well established. However, this is not the only major driving force for water absorption in the marine teleosts intestine in particular. A hint of this is indicated by the fact that the rate of Cl^- ion absorption is always much higher than Na^+ ion absorption. Some of this can be accounted for by NaCl co-transport being at least partially mediated via the NKCC (i.e., two Cl^- ions for each Na^+ ion and K^+ ion). However, the early work of Homer Smith (1930) demonstrated that the intestinal fluid of marine teleosts differs greatly from other vertebrates in being very alkaline pH (8.2–9.2) and having high levels of bicarbonate (typically 50–100 mM; **Table 1**). This unusual gut fluid chemistry results from apical anion exchange that involves the net secretion of HCO_3^- ions in return for uptake of Cl^- ions. In some cases, apical Cl^-/HCO_3^- exchange can account for up to 70% of the total Cl^- absorption in the marine teleost intestine. The molecular identity of this apical anion exchanger (AE) is likely to be SLC26a6 (one of a large family of solute carrier group of membrane transport proteins), which is electrogenic exporting more than one HCO_3^- ion for each Cl^- ion imported (although the precise ratio is uncertain, hence it is denoted as $n\mathrm{HCO}_3^-/\mathrm{Cl}^-$ exchange). This is important because it means that the uphill Cl^- absorption mechanism is part of a net export of negative charge, which is supported by the apical membrane potential (i.e., inside negative by about −100 mV relative to outside).

The source of intracellular bicarbonate ions in most species is probably a flexible combination of the hydration of metabolic CO_2 (catalyzed by intracellular carbonic anhydrase; **see also Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory Systems) and basolateral uptake of HCO_3^- from the blood via a Na^+:HCO_3^- co-transporter (NBC1) (**Figure 2**). In the latter case, basolateral uptake of bicarbonate from the blood simply provides the intracellular bicarbonate for subsequent apical secretion via Cl^-/HCO_3^- exchange. However, in the former situation, hydration of CO_2 generates intracellular protons as well as bicarbonate ions. Therefore, to maintain cellular pH balance and to ensure a continued supply of bicarbonate for apical exchange with chloride, these protons must be readily extruded from the basolateral side of enterocytes.

The precise molecular identity of this acid export is not yet known, but there is functional evidence for basolateral Na^+/H^+ exchanger (NHE) in toadfish and molecular evidence for abundant basolateral proton pumps (H^+-V-type ATPase) in rainbow trout. Whatever transporters are involved, and whether bicarbonate secretion is transepithelial or is fueled by intracellular CO_2 hydration, the net effect is the same: net base secretion to the gut lumen and net acid absorption into the blood (because bicarbonate uptake into the cell from the blood is equivalent to acid moving in the opposite direction in acid–base terms). Therefore, this has acid–base repercussions for the whole animal, which are discussed later.

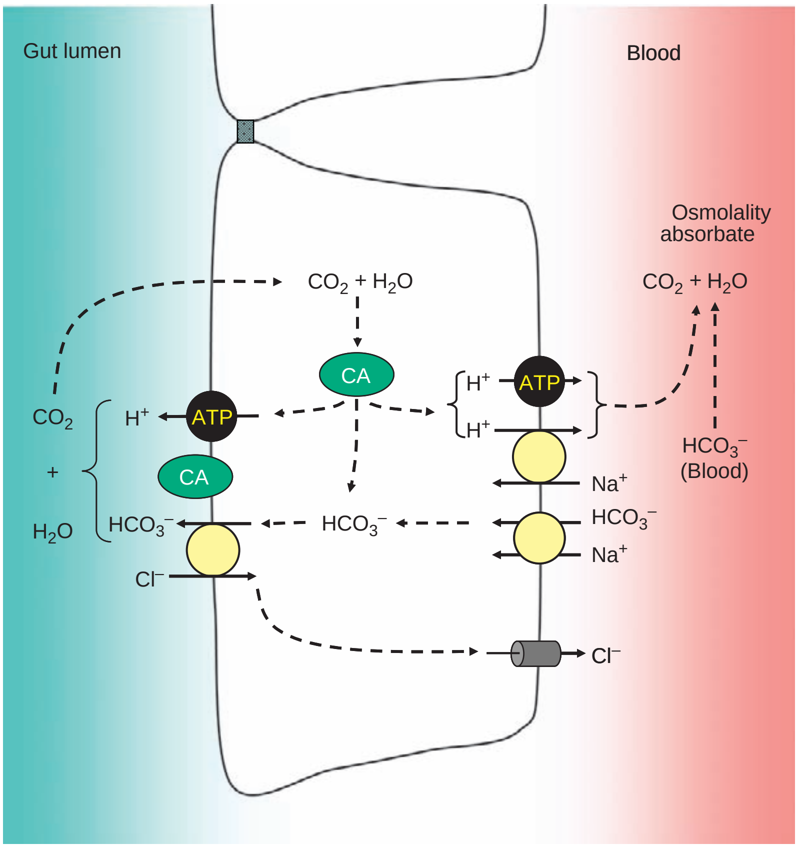


Figure 2 Direct role of HCO_3^- secretion in water absorption by the intestine of marine teleosts. The figure combines elements of models presented by both Grosell et al. (2009) and Whittamore et al. (2010) that relate to the role of epithelial bicarbonate secretion and the associated mechanisms that generate a driving force to support net water absorption. Bicarbonate ions are generated inside the enterocyte either from the hydration of metabolic CO_2 (catalyzed by cytosolic carbonic anhydrase) or from the basolateral uptake from the blood side via Na^+:HCO_3^- co-transporters (NBC1). The HCO_3^- ions are then exported into the gut lumen via apical Cl^-/HCO_3^- exchangers (SLC26a6). Chloride ions taken up across the apical membrane ultimately reach the blood side of the cell via basolateral Cl^- channels (the same as proposed in **Figure 1**). The intracellular hydration of CO_2 also generates protons (H^+). To maintain cellular pH balance, these H^+ ions are normally removed by basolateral proton pumps and/or NHEs. Basolaterally absorbed protons ultimately are buffered by blood-derived HCO_3^- in the subepithelial space, producing gaseous CO_2 from these dissolved ions. This conversion helps in reducing the osmolality of basolateral fluid which would otherwise become hyperosmotic as a result of the net absorption of HCl and NaCl. When luminal Ca^{2+} concentrations are low, this limits the potential for apical HCO_3^- removal via $CaCO_3$ precipitation in the gut lumen. Under these circumstances, a second mechanism operates whereby apical proton pumps export some of the intracellular H^+ ions. This appears to be coupled to the apical anion exchange of Cl^-/HCO_3^- in a functional metabolon. Co-localized carbonic anhydrase enzymes present on the apical membrane (CA-IV) promote the rapid conversion of the luminal osmolytes (HCO_3^- and H^+) to CO_2 gas. This reduces the luminal osmolality, thus promoting apical water absorption. It also encourages further apical Cl^-/HCO_3^- exchange by enhancing the apical HCO_3^- ion gradient. The resulting Cl^- absorption also helps drive water absorption. Intriguingly, the apically generated molecular CO_2 probably diffuses back into the cell, thus regenerating the intracellular source of HCO_3^- ions.

So how is water transport linked to this process of bicarbonate secretion? At face value, the apical exchange of intracellular HCO_3^- for luminal Cl^- should at best be neutral in terms of osmolyte movement (if the exchange ratio is 1:1). However, if the intracellular bicarbonate is derived from CO_2, then effectively the whole process results in a CO_2 gas molecule (which is osmotically neutral at cellular CO_2 concentrations) being replaced by a Cl^- ion, which is a strong osmolyte. This effect alone will tend to draw water across the apical membrane and, provided the Cl^- ultimately is absorbed in the blood, an osmotic movement of water across the whole epithelium will result. Thus, bicarbonate secretion can be directly responsible for a portion of water absorption in the intestine. The entire length of the intestine (anterior, mid-, and posterior sections) is capable of substantial bicarbonate secretion, which should support this mechanism of water absorption. It is also worth noting that for the fish species that have pyloric ceca, these are capable of strong bicarbonate secretion and water absorption, and these anatomical features clearly offer additional functional area for these processes.

A further aspect of bicarbonate secretion may be important in water absorption, especially when luminal Ca^{2+} concentrations are low (**see** section Intestinal Water

Absorption Indirectly Linked to HCO_3^- Secretion via Alkaline $CaCO_3$ Precipitation). This involves the combined action of Cl^-/HCO_3^- exchange and proton pumps on the apical membrane, in a functional metabolon, whose simultaneous action at first seems counter-intuitive for a base-secreting epithelium (**Figure 2**). However, the rate of apical acid secretion is much less than the rate of apical bicarbonate secretion, so net base secretion still ensues. The benefit of this metabolon for Cl^- ion and water absorption works in three ways. First, the limiting factor within the cell is provision of HCO_3^- ions to the Cl^-/HCO_3^- exchanger (identified as SLC26a6 in toadfish). Intracellular accumulation of protons near this site would reverse the CO_2 hydration reaction and inhibit further HCO_3^- ion secretion, so co-localized proton pumps offer an effective way to remove the accumulating protons and maintain the local supply of HCO_3^- ions. Second, the resulting supply of protons to the mucosal boundary layer will titrate a portion of the secreted HCO_3^- to CO_2, thus locally reducing the outward HCO_3^- gradient. This has the double benefit of (1) assisting uphill Cl^- uptake via Cl^-/HCO_3^- exchange (thus promoting solute-linked water absorption) and (2) reducing the osmolality within the mucosal boundary layer due to the conversion of osmotically active HCO_3^- and H^+ ions to osmotically inert CO_2 gas, thus further promoting the transmembrane osmotic gradient for water absorption. Third, the gaseous CO_2 may be recycled (by simple diffusion across the apical membrane) back into the cell to help fuel a continued supply of H^+ and HCO_3^- for further apical export. Thus, many processes involved in HCO_3^- ion secretion are directly involved in driving water absorption, which is a vital role of the intestine in marine teleosts fish.

One puzzling aspect of water absorption driven by the HCO_3^- ion secretion mechanism is that numerous studies and models indicate that the associated fluid absorbed (absorbate) must be strongly hyperosmotic to the blood, as well as being highly acidic containing predominantly HCl. In contrast, studies in which intestinal bicarbonate secretion is experimentally enhanced show that blood plasma osmolality is actually reduced compared to normal, and any blood pH disturbances are small. The gills (**see also Role of the Gills**: Morphology of Branchial Ionocytes) and kidney (**see also Role of the Kidneys**: The Kidney) undoubtedly act in concert with the gut for osmoregulation in marine fish, and may well remove most of the excess salts from any hyperosmotic intestinal absorbates. However, a model has also been proposed which involves only the intestine and helps explain this discrepancy. The absorbate should not be considered as an isolated volume of fluid absorbed by the intestine. In reality, it rapidly becomes mixed with a supply of extracellular fluid within the subepithelial space. Here the acidic absorbate will almost immediately be buffered by the continuous supply of HCO_3^- ions from the blood, which will neutralize the pH and increase the circulating level of CO_2. In addition, the neutralization reaction will simultaneously reduce the osmolality by converting osmotically active HCO_3^- and H^+ ions to CO_2 gas. This neutralization within the gut vasculature is estimated to effectively make the absorbate hypoosmotic to blood, which is the ideal scenario for marine teleosts living in the dehydrating seawater environment.

Intestinal Water Absorption Indirectly Linked to HCO_3^- Secretion via Alkaline $CaCO_3$ Precipitation

Besides the direct roles in water absorption described above, bicarbonate secretion plays a highly novel and indirect role in water absorption. The high pH (8.2–9.2) and bicarbonate concentrations (50–100 mM) reached in the intestinal fluids are far above those resulting from pancreatic secretions in mammals, and occur whether post-prandial or inter-digestive. So they do not serve purely to neutralize the acidic stomach contents that enter the intestine, and they are clearly associated with the particular osmoregulatory needs of marine teleosts. It is this unusual gut fluid chemistry that contributes a further important driving force for water absorption. The alkaline, HCO_3^--rich conditions cause unabsorbed Ca^{2+} from imbibed seawater to precipitate as insoluble calcium carbonate ($CaCO_3$, and, to a lesser extent, Mg^{2+} as $MgCO_3$). The resulting solid carbonate crystals become wrapped in mucus and are periodically excreted along with rectal fluids. Remarkably, the amount of $CaCO_3$ excreted by marine teleosts has recently been shown to make a significant and previously unrecognized contribution to the global inorganic carbon cycle. In teleosts, the secretion of HCO_3^- and subsequent precipitation of $CaCO_3$ in the intestine is dependent upon ambient salinity and the requirement to drink and thus a fundamental role in osmotic regulation has been proposed.

Water absorption by any mechanism means that imbibed solutes that are not absorbed become increasingly concentrated along the intestine. The divalent ions Ca^{2+}, Mg^{2+}, and SO_4^{2-} are quite abundant in seawater (10, 53, and 28 mM, respectively) and are generally poorly absorbed compared to monovalent ions. This may simply be due to a very low permeability of the epithelium to these divalent ions. Conversely, there is some evidence of a very effective active secretion mechanism (at least for SO_4^{2-} ions in exchange for Cl^- uptake), which serves to counteract most of the passive absorption that occurs. Regardless of the explanation for the impermeance of these divalent ions, if 85% of the imbibed fluid is absorbed (as for seawater rainbow trout), any unabsorbed ions that remain in solution would be left behind in 15% of the original fluid volume (i.e., concentrated by 6.7-fold). For Ca^{2+}, Mg^{2+}, and SO_4^{2-}, this would result in their accumulation to over 67, 353, and 187 mM, respectively. Collectively, these represent >600 mM of unabsorbed solutes in the fluid of the distal intestine, which would massively oppose water absorption. Despite low permeation of the intestine by these divalent ions, a small portion is inevitably absorbed given the large gradients (and these divalent ions are subsequently excreted by the kidney in marine fish). However, the chemical precipitation of Ca^{2+} and Mg^{2+} by reaction with the secreted bicarbonate removes a large portion of these ions from solution, thus avoiding a detrimental accumulation (**Figure 3**). The carbonate precipitation reduces luminal (mucosal) osmolality and enhances the transepithelial osmotic gradient for water absorption, providing a further important mechanism of facilitating water transport. The importance of this mechanism to osmoregulation is highlighted by the acute sensitivity of intestinal bicarbonate secretion to the luminal calcium concentration both *in vivo* and *in vitro*. The intestinal epithelium appears to be highly tuned to remove imbibed calcium (and to a lesser extent Mg^{2+}) at whatever rate it enters the intestine.

Ion Regulation in the Gut of Freshwater Fish

Freshwater fish face the opposite osmotic problem to marine fish (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes); rather than continually combating dehydration, they suffer from a continual osmotic gain of water. This is mainly via the large surface area and thin diffusion distance of the gills. Freshwater fish therefore have little need to drink for osmoregulatory purposes, and typically have drinking rates 10- to 50-fold lower than marine teleosts. In addition, any fresh water that is imbibed typically has very low ion concentrations compared

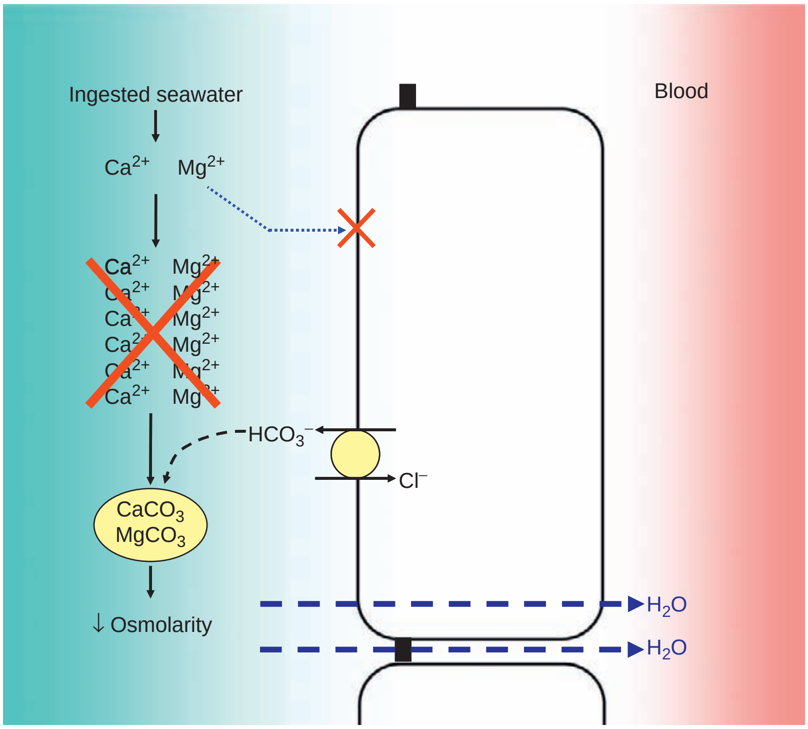


Figure 3 Indirect role of alkaline precipitation of calcium and magnesium carbonates in water absorption by the intestine of marine teleosts. Ingested seawater contains relatively high concentrations of Ca^{2+} (10 mM) and Mg^{2+} (53 mM). These divalent cations are poorly absorbed during their passage along the intestine and so theoretically their concentrations will increase as water is absorbed via the direct mechanisms described in **Figures 1** and **2**. For example, if 85% of the ingested fluid volume is absorbed, this would leave behind 67 and 353 mM of Ca^{2+} and Mg^{2+}, respectively. If allowed to occur unchecked, this would represent a substantial increase in the osmolality of fluid in the gut lumen, which in turn would retard net water absorption. However, the net secretion of bicarbonate ions (see **Figure 2**) results in the removal of almost all of the accumulated Ca^{2+} and a smaller fraction of the accumulated Mg^{2+}, by precipitating them as their insoluble carbonate salts ($CaCO_3$ and $MgCO_3$). The resulting removal of these dissolved solutes reduces the osmolality of fluid in the gut lumen which improves the apical to basolateral osmotic gradient to facilitate further net water absorption.

to either body fluids or seawater. As a result, there is little scope for the gut to play a major role in ion regulation in freshwater fish under nonfeeding conditions. However, in freshwater fish in particular, the diet can provide a vital source of many ions, as well as a net sink for endogenous water.

Ion and Osmotic Regulation by the Gut of Feeding Freshwater Teleost Fish

For many freshwater fish, at least for the best-studied ions Na^+, Cl^-, and Ca^{2+}, the active transport processes in the gills are capable of satisfying the ion uptake requirements. In other words, the gill is sufficient to balance any obligatory ion losses via urine flow and skin or gill diffusion. In these examples, stable whole-body ion balance can be achieved for long periods in the absence of feeding and involvement of the gut. However, for other species gill ion uptake may be insufficient to maintain whole-body ion balance in the absence of dietary intake. Examples include Amazonian fish that live in extremely ion-poor water that limits uptake ability, and also a range of species such as eels, killifish, and bluegill that have minimal capacity for active gill Cl^- transport. For these fish, therefore, the gut is an essential ionoregulatory organ. In most cases though, the need to understand gut ion regulation during feeding comes from an aquaculture perspective, that is, appreciating the limiting and loading factors in commercial diets, and the influence that the ion content of food has on various aspects of the physiology of fish. In what follows, we describe our limited current knowledge on the handling of ions and water by the gut of freshwater fish.

During feeding some water is gained as part of the diet itself (animals and plants contain generally 60–80% water). Furthermore, when farmed fish are fed on commercial feeds, which are typically very dry pellets (~10% water; **see also Energetic Models**: Bioenergetics in Aquaculture Settings), the dietary water can be supplemented by larger amounts of imbibed water (e.g., 1.6 ml water for every 1 g of food), which is important to improve the motility of ingested food within the gut. Nevertheless, the gut is actually the site of net water loss during feeding in freshwater fish for two separate reasons. First, the active secretion of digestive juices in the stomach and intestine contributes to net fluid export. Second, even if the fluid component of the undigested meal begins as relatively iso-osmotic to fish blood, the digestion process results in dissolution of cellular and hard materials (e.g., bone) that yields very high salt concentrations and therefore high osmolality in the digested contents of the stomach (called chyme) and anterior intestine, which induces a net osmotic movement of water from the body fluids into the gut. Although the more distal sections of the intestine may be the sites of water absorption, as a whole the gut is responsible for net fluid loss during feeding.

For monogastric animals, like humans, the stomach is usually considered to be purely an organ of secretion and digestion. However, a surprising discovery from the limited studies on freshwater fish is that the stomach performs substantial absorption of many ions. Indeed, the stomach of trout absorbs the majority of Na^+, K^+, and Mg^{2+}, and 50% of the Ca^{2+} present in a commercial pellet diet. As expected, the trout stomach is the site of net secretion of H^+ and Cl^-. However, this simple summary obscures some interesting temporal changes in the fluxes of these various ions. Almost all the Cl^- secretion occurs during the first half of the food's residence within the stomach, whereas almost all the K^+ absorption occurs during the second half of this gastric digestion phase. This probably reflects the established mechanism of gastric acid secretion in vertebrates generally, and putitatively in teleosts, which involves an apical H^+/K^+-ATPase. During stimulation of net acid secretion, ATP is consumed to drive the secretion of H^+ and uptake of K^+, but to maintain the functioning of this pump the K^+ ions must be recycled back to the stomach lumen via K^+ channels. Presumably, only once gastric acidity has reached its target pH and acid secretion is downregulated will the K^+ recycling pathway also be shutdown allowing net K^+ absorption to proceed. However, the actual transport mechanisms for gastric absorption of K^+ (and Na^+, Ca^{2+}, and Mg^{2+}), and their molecular identities, remain unknown in fish.

Although the stomach is capable of surprisingly high rates of absorption of these four ions, it would appear that the intestine is responsible for substantial net secretion, at least for Na^+, Ca^{2+}, and Mg^{2+}. Whereas virtually 100% of the dietary K^+ is assimilated by the gut as a whole, for dietary Ca^{2+} and Mg^{2+} the overall assimilation is only ~30% and ~60%, and for Na^+ the common situation seems to be zero net absorption or secretion by the gut overall due to intestinal secretion exactly counterbalancing gastric uptake.

Ion and Osmotic Regulation by the Gut of Feeding Marine Teleost Fish

In seawater trout fed on a similar commercial diet, the gut processing of the meal results in a similar net loss of water as that found in freshwater fish. However, this obviously compounds the existing osmoregulatory problems experienced under nonfeeding conditions, representing a significant additional physiological challenge for digestion in marine teleosts. Unlike freshwater fish, most of the dietary ions are not assimilated by the gut as a whole, which is appropriate given the additional salt load it would create on top of the diffusive influx caused by breathing seawater.

The Role of the Gut in Acid–Base Regulation

There has been surprisingly little study of the possible role of the gastro-intestinal tract of fish in acid–base regulation. This is despite its good candidacy given the well-known capacity for acid secretion by the stomach, and base secretion by the pancreatic tissue and intestine itself (for marine teleosts at least, see above). Despite this potential, the gut is generally considered to be a minor player relative to the gills and kidney. Although the gills are typically considered to perform the vast majority of any acid–base regulatory work in fish, the kidney has recently been re-evaluated for its energetic importance in maintaining blood acid–base status in fish, in particular the high re-absorption rates for filtered HCO_3^- ions (**see also Role of the Kidneys**: The Kidney). We will now discuss some of the more recent work on the role of the gut in acid–base regulation.

It was clear from the early work on intestinal bicarbonate secretion in marine teleosts that the continual production of alkaline- and HCO_3^--rich gut fluid together with carbonate precipitates represented a significant route of base excretion from the body. The majority of this base stems from the HCO_3^--rich rectal fluids rather than the carbonate precipitates, and although the release of rectal fluid is quite intermittent, it occurs at sufficiently high rates overall (0.5–1.1 ml kg^{-1} h^{-1}) to generate base excretion rates in the range of 100–150 μEq kg^{-1} h^{-1}. Under resting conditions, this clearly contributes to the whole animal acid–base balance when compared against the acids and bases excreted via the gills and kidney, that is, roughly one-half to one-third of the combined ammonia or bicarbonate excreted by these other organs. Thus, there appears to be a degree of anatomical separation for net base fluxes (a large fraction via gut) and acid fluxes (primarily via the gills) in resting marine teleosts. However, the importance of the intestine in true acid–base regulation following an acid–base disturbance is less clear.

The most natural situation for the intestine to assist whole-body acid–base regulation would be during the post-prandial metabolic alkalosis commonly known as the alkaline tide. It is well known that following a meal, the stomach of vertebrates secretes large quantities of isotonic HCl for gastric digestion (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). It is less well known that for every molecule of acid secreted by the gastric oxyntic cells in mammals (or oxyntopeptic cells in nonmammalian vertebrates), an equal amount of bicarbonate base must be exported into the blood to maintain cellular acid–base neutrality. This compensatory transport generates the alkaline tide phenomenon – a rapid increase in blood HCO_3^- concentration and pH in the minutes/hours after a meal. This is well characterized in mammals and ectothermic terrestrial vertebrates in which the initial blood alkalosis is rapidly compensated by reduced ventilation (by raising internal levels of CO_2) which helps by offsetting the initial rise in blood pH, followed by a more gradually excretion of the excess plasma HCO_3^- via the kidney. The alkaline tide has now been demonstrated in both freshwater and seawater fish. Unlike air-breathing vertebrates, fish do not compensate the initial alkalosis by any hypoventilatory rise in blood CO_2. However, the intestinal mechanism for bicarbonate secretion (which already functions in osmoregulation within marine teleosts) appears to play a secondary role in sequestering at least some of the excess plasma HCO_3^- arising from the alkaline tide. Interestingly, this seems to be more prevalent in freshwater rather than seawater trout, even though the latter should be better prepared with respect to the intestinal HCO_3^- transport mechanism. This suggests that freshwater trout already have the cellular machinery present to carry out effective intestinal HCO_3^- secretion and can use this to help offload some of the excess HCO_3^- accumulated in the blood after a meal. By contrast, seawater trout appear to excrete most of the excess plasma HCO_3^- via the gills. This surprising difference between freshwater and seawater trout may reflect the availability of counter-ions to the gills for driving these ion-transport mechanisms in the two media.

Under less natural conditions, when seawater teleosts were subjected to a metabolic alkalosis (due intra-vascular infusion of excess $NaHCO_3$ at ~500 μEq kg^{-1} h^{-1}), this treatment failed to produce any upregulation of rectal fluid base excretion. Instead, the nonintestinal sites (gills, skin, and kidney) excreted almost all of the exogenous base load. By contrast, a respiratory acidosis induced by exposure to hypercapnia (raised ambient CO_2) causes a twofold increase both in intestinal $CaCO_3$ excretion rate, and in the rectal fluid concentration of dissolved HCO_3^-. Therefore, under certain exogenous acid–base disturbances, the intestine can respond to alter its base excretion rates. However, it is worth noting that the response to hypercapnic acidosis is actually counter-productive in terms of restoring normal internal acid–base balance, as the enhancement of rectal base excretion would simply exacerbate the blood acidosis, providing the gills and kidney with even more acid–base regulatory work.

So although the intestine is a relatively important factor when considering acid–base homeostasis under nonfeeding conditions, it appears to play little positive role in true acid–base regulation during recovery from either metabolic alkalosis or respiratory acidosis.

See also: **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Detection and Generation of Electric Signals**: Physiology of Tuberous Electrosensory

Systems. **Energetic Models**: Bioenergetics in Aquaculture Settings. **Food Acquisition and Digestion**: Digestive Efficiency. **Gut Anatomy and Morphology**: Development of Fish Gut. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Integrated Function and Control of the Gut**: Barrier Function of the Gut; Intestinal Absorption; Gut Secretion and Digestion. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction. Water Balance and Aquaporin. **Role of the Kidneys**: The Kidney. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

Barnes H (1954) Some tables for the ionic composition of *sea water. Journal of Experimental Biology* 31: 582–588.

Bayly IAE and Williams WD (1973) *Inland Waters and Their Ecology*, 316p. Melbourne: Longmanp.

Furch K (1984) Water chemistry of the Amazon basin: The distribution of chemical elements among freshwaters. In: Sioli H (ed.) *The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and Its Basin,* pp.167–199. Dordrecht: Junk.

Grosell M (2006) Intestinal anion exchange in marine fish osmoregulation. *Journal of Experimental Biology* 209: 2813–2827.

Grosell M, Mager EM, Williams C, and Taylor JR (2009) High rates of HCO_3^- secretion and Cl^- absorption against adverse gradients in the marine teleost intestine: The involvement of an electrogenic anion exchanger and H^+-pump metabolon? *Journal of Experimental Biology* 212: 1684–1696.

Kurita Y, Nakada T, Kato A, *et al.* (2008) Identification of intestinal bicarbonate transporters involved in formation of carbonate precipitates to stimulate water absorption in marine teleost fish. *AJP – Regulatory, Integrative and Comparative Physiology* 294: R1402–R1412.

Loretz CA (2001) Drinking and alimentary transport in teleost osmoregulation. In: Goos HJTh, Rastogi RK, Vaudry H, and Pierantoni R (eds.) *Perspective in Comparative Endocrinology: Unity and Diversity*, pp.723–732. Bologna: Monduzzi Editore.

Pelis RM and Renfro JL (2003) Active sulfate secretion by the intestine of winter flounder is through exchange for luminal chloride. *AJP – Regulatory, Integrative and Comparative Physiology* 284: R380–R388.

Whittamore JM, Cooper CA, and Wilson RW (2010) HCO_3^- secretion and $CaCO_3$ precipitation play major roles in intestinal water absorption in marine teleost fish *in vivo. AJP – Regulatory, Integrative and Comparative Physiology* 298: R877–R886.

Wilson RW, Millero FJ, Taylor JR, *et al.* (2009) Contribution of fish to the marine inorganic carbon cycle. *Science* 323(5912): 359–362.

Dogfish Rectal Gland

WW Dowd, Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Anatomy
Studying Rectal Gland Secretory Function
Mechanism of Salt Transport
Controls of Rectal Gland Function
Conclusion
Further Reading

Glossary

Active ion transport The process of using cellular energy (usually in the form of ATP) to move ions across a membrane, typically against their concentration gradient. This process can be further divided into primary (direct action of an ion transporter that hydrolyzes ATP) and secondary (ions move down an electrochemical gradient created by other primary processes) active transport. The rectal gland Na^+/K^+-ATPase moves Na^+ and K^+ ions across the cell membrane in an example of primary active transport. Secretion of Cl^- by the gland qualifies as secondary active transport because it relies on the Na^+ gradient established by Na^+/K^+-ATPase.

Alkaline tide The systemic increase in blood pH (reduced H^+ ion concentration or alkalosis) that follows a meal. As acid (HCl) is secreted into the stomach for digestion of a meal, the blood plasma is left with an excess of base (bicarbonate ion, HCO_3^-), leading to a systemic rise in pH.

Apical membrane The side of an epithelial cell facing the outer world; for the gut and rectal gland, this is the lumen.

Basolateral membrane In rectal gland secretory cells, the basolateral membrane faces the interior of the animal and is exposed to humoral factors. It is extensively infolded and is separated from the apical membrane region by tight junctions between adjacent cells.

Concentration gradient A difference in the concentration of a substance (e.g., ions in the body fluids) across some spatial scale. In the context of rectal gland, concentration gradients most often refer to differences in ion concentrations across the cell membrane or across the secretory epithelium.

Cytoskeleton A network of tubules and filaments that provides structural support to cells. Major cytoskeletal proteins include tubulins (in microtubules), actins, and myosins. These cytoskeletal elements also provide anchor points or scaffolding for cellular organelles, membranes, and proteins. Changes in cytoskeletal composition and structure, known as cytoskeletal reorganization, lead to changes in cell morphology.

Endocrine/paracrine/autocrine factors A diverse class of molecules that exert specific downstream effects on target tissues and cells, including activation or inhibition of specific pathways or processes. Depending on the point of origin, these molecules are classified as endocrine (usually originating from one or more glands remote from the point of action and circulating in the blood), paracrine (originating from tissues or cells neighboring the point of action on a different cell type), or autocrine (originating from and acting on the same tissue or cell type).

Euryhaline Animals that physiologically adjust to a wide range of water salinity are considered euryhaline (*eury*, broad; *haline*, salt) in contrast to stenohaline animals.

Secretagog Collective term for substances that can stimulate secretion; for the rectal gland these include forskolin, CNP, VIP, and theophylline.

Stenohaline Describes an organism that cannot live in environments that undergo large changes in salinity (steno 'narrow').

Introduction

Regulation of the body's salt and water content is a central physiological challenge for all animals. Marine fishes, including the elasmobranchs (the sharks, skates, and rays), maintain their body fluid ionic (especially Na^+ and Cl^-) concentrations below that of the surrounding seawater (**Table 1**). Elasmobranchs exhibit a peculiar osmoregulatory strategy, in which they retain large quantities of the nitrogenous waste product urea as an organic

Table 1 Representative composition of the surrounding seawater, the secreted rectal gland fluid, and the blood plasma for an elasmobranch (*Squalus acanthias*) from two different experiments

	Concentration (mmol L^{-1})				
Fluid	*Na^+*	*Cl^-*	*K^+*	Urea	*Osmolality (mOsm L^{-1})*
Rectal gland fluid[a]	517	527	4.5	7–28.8	–
Plasma	278	275	3.2	379	893
Rectal gland fluid[b]	540	533	7.1	14.5	1018
Plasma	286	246	–	351	1018
Seawater[b]	440	496	9.1	~0	930

[a] Rectal gland secretion was simulated by infusing 500 mM NaCl for 6 h. Plasma values are pre-infusion. From Wood CM, Munger RS, Thompson J, and Shuttleworth TJ (2007). Control of rectal gland secretion by blood acid–base status in the intact dogfish shark (*Squalus acanthias*). *Respiratory Physiology & Neurobiology* 156: 220–228; and Walsh, PJ, Kajimura M, Mommsen TP, and Wood CM (2006) Metabolic organization and effects of feeding on enzyme activities of the dogfish shark (*Squalus acanthias*) rectal gland. *Journal of Experimental Biology* 209: 2929–2938.

[b] From Burger JW and Hess WN (1960) Function of the rectal gland in the spiny dogfish. *Science* 131: 670–671.

osmolyte; they also accumulate trimethylamine oxide (TMAO), which counteracts urea's destabilizing effects on proteins. This urea-based strategy maintains elasmobranch body fluids slightly hyperosmotic to seawater, eliminating the need for constant water absorption via drinking. However, elasmobranchs still face a constant influx of salts, driven by the inwardly directed ion gradients. Furthermore, the act of feeding expands the intravascular fluid volume and introduces salts into the body fluids both directly by ingestion of seawater and food items, and indirectly as a consequence of acid–base adjustments associated with digestion.

While the gill handles regulation of the major monovalent ions (Na^+ and Cl^-) in bony fishes, the elasmobranchs (and coelacanth) possess a small, highly specialized structure known as the rectal gland (also known as the digitiform gland) that carries out the majority of salt secretion. This gland, located near the posterior section of the intestine, secretes a concentrated solution of NaCl (~500 mM) that is twice the ionic concentration of the plasma, but that is nearly iso-osmotic with seawater and the body fluids. Although the rectal gland was described by scientists as early as in the 1600s, its salt-secreting function was not demonstrated until the 1950s, by Burger. In addition to its role in ion regulation, the gland also functions to regulate intravascular volume because water follows osmotically into the secretion fluid. In fact, several authors have suggested that vascular fluid volume regulation is the gland's primary function, but the distinction seems unnecessary given the intimate relationship between water and salt movements in the gland, the kidney's major role in elasmobranch volume regulation, and the likely co-occurrence of salt and volume loading after a meal in the whole organism. The bulk of available evidence indicates that the rectal gland acts as a fine-tuning mechanism for regulating both intravascular volume and body salt content, necessitated by the elasmobranch kidney's inability to secrete urine with a higher salt concentration than the vascular fluid. Interestingly, some elasmobranchs can maintain their body-fluid ion concentrations when the rectal gland is removed or ligated.

The rectal gland has for decades been a productive model for studying both the mechanisms and the controls of salt secretion in vertebrates due to its highly specialized function, its cells rich in the enzymes and transporters that drive salt transport, and its experimentally convenient anatomical features. The long history of rectal gland studies at multiple levels of organization – from molecular biology to individual cells to the *in vivo* gland – also clearly demonstrates the iterative path of scientific inquiry. These studies have revealed the surprisingly complex and dynamic anatomy and physiology of what at first glance appears to be a simple tissue.

Anatomy

The anatomy of the rectal gland has been thoroughly studied, and it is only described briefly here. Readers are referred to Olson's work (see section 'Further reading') and references therein for an excellent, detailed review.

Gross Morphology

The rectal gland's name derives from its location in the dorsal mesentery above the posterior end of the spiral intestine (i.e., near the rectum) (**Figure 1**(**a**)). Rectal gland weights per unit of body mass range from 100–600 mg kg^{-1} body mass in marine and brackish species to 15–60 mg kg^{-1} body mass in euryhaline and freshwater species. The smallest and least complex (fewest tubules) rectal glands are found in Amazonian freshwater stingrays of the family Potamotrygonidae, in which the need for salt secretion is negligible in an ion-poor habitat.

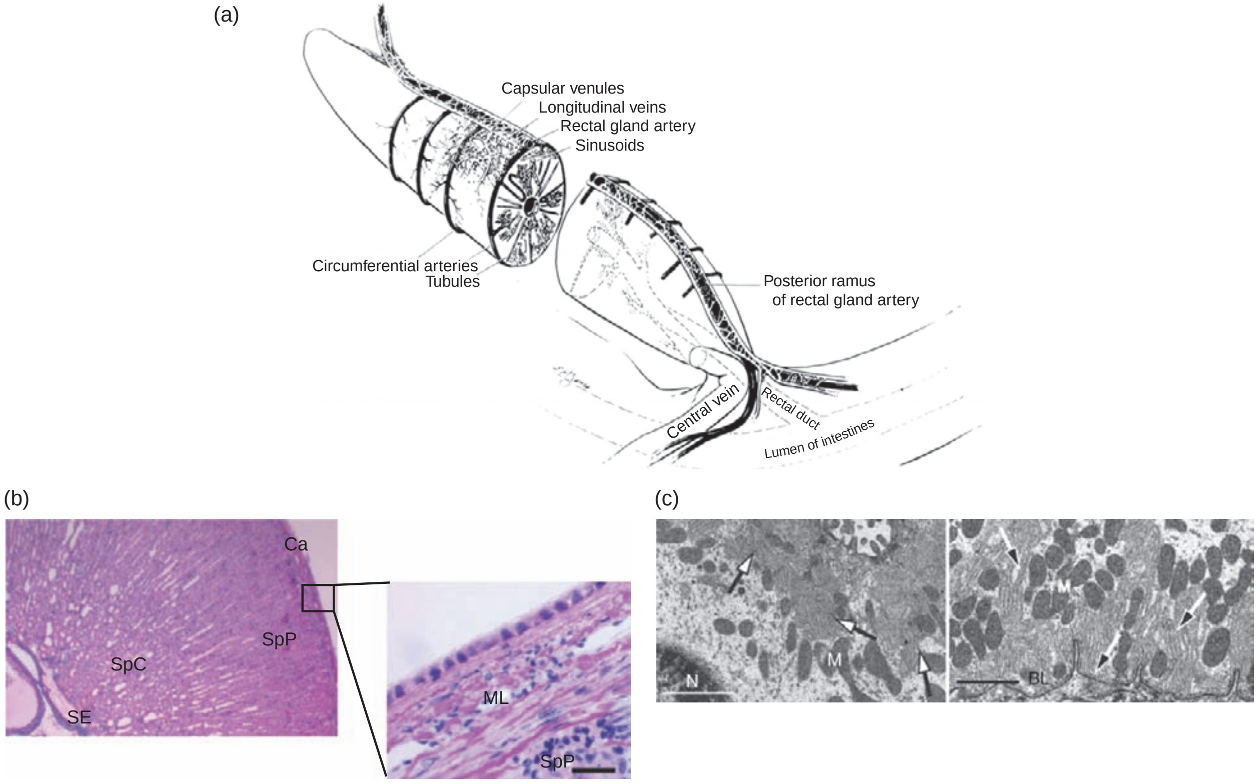


Figure 1 (a) Diagram of the gross morphology and vascular circulation of the rectal gland. (b) Histological cross section of the rectal gland of *Squalus acanthias*, demonstrating the three layers: outer capsule (Ca), middle secretory parenchyma (peripheral SpP and central SpC zones), and stratified epithelium (SE). The rectal gland lumen appears at the bottom left. The inset magnifies the outer capsule and reveals the ring of smooth muscle layer (ML); Scale bar = 20 μm. (c) Transmission electron micrographs of the apical (left) and basolateral (right) regions of rectal gland tubular secretory cells 20 h after a meal. Extensive lateral membrane infoldings are indicated by white-headed arrows. Note the clusters of mitochondria between interdigitations of the basolateral membranes (black-headed arrows). BL, basolateral membrane; M, mitochondria; N, nucleus. Scale bar (c) = 2 μm. (a) Reproduced from Kent B and Olson KR (1982). Blood flow in the rectal gland of *Squalus acanthias*. *American Journal of Physiology* 243: R296–R303; and (b) and (c) Reproduced from Matey V, Wood CM, Dowd WW, Kültz D, and Walsh PJ (2009) Morphology of the rectal gland of the spiny dogfish (*Squalus acanthias*) shark in response to feeding. *Canadian Journal of Zoology* 87: 440–452.

The gland consists of three tissue layers: an outer muscular capsule, a middle secretory parenchyma, and an inner stratified epithelium that lines a central lumen (**Figure 1**(**b**)). The capsular, smooth muscle ring that surrounds the entire gland contracts in response to secretagogs and appears to play a role in changing the gland's overall morphology during intermittent gland secretion. The central lumen is fed by a radiating network of densely packed, blind secretory tubules that come in small and large diameters and that extend through the parenchyma. Nerve fibers, which contain the secretagog vasoactive intestinal peptide (VIP), are interspersed among the tubular secretory cells.

Vasculature

The rectal gland is a heavily vascularized tissue (**Figure 1**(**a**)). A single devoted vessel, the posterior mesenteric artery (PMA), supplies the gland with blood and the humoral factors that regulate gland activity (see below). The anterior branch of the PMA divides first into circumferential arteries, followed by a capsular web of arterioles, and then into an extensive network of radial capillaries that associate closely with the secretory cells of the tubules in the parenchyma. In the middle parenchyma, larger venous sinuses are sometimes associated with the secretory tubules. Downstream, these vessels coalesce into luminal veins, and ultimately into a single central vein that drains into the vasculature of the intestine. The posterior branch of the PMA runs along the dorsal surface of the gland and bypasses its circulation altogether. A third vascular pathway functions as a shunt, allowing blood to bypass the tubular capillaries while circulating through the capsule. Constrictions in the arteriovenous anastomoses that lead to this bypass system, as well as rectal gland vasoconstriction in response to norepinephrine treatment (which is abolished by the secretagog VIP), suggest that blood perfusion through the gland is dynamically regulated. However, definitive studies of the regulation and function of these two bypass pathways are yet to be performed. Notably, perfusion and secretory activity of the rectal gland are both highly variable in time, although a direct correlation between perfusion rate and salt secretion is not always found.

Secretory Cell Morphology

The mechanisms of salt transport in the rectal gland are intimately tied to its epithelial and cellular morphology. The rectal gland tubule walls are composed almost entirely of a single layer of columnar secretory cells. These secretory cells exhibit highly infolded basolateral membranes, which enhance the cell-membrane surface area to accommodate the numerous and varied ion transporters that reside there. Dense aggregations of mitochondria are closely associated with the basolateral – but less so with the apical – membrane regions of the cell, supplying aerobically derived adenosine triphospate (ATP) to fuel active ion transport (**Figure 1**(**c**)). The apical membrane includes a number of microvilli extending into the tubule lumen. Neighboring secretory cells also exhibit extensive lateral interdigitation that has been referred to as jigsaw-puzzle-like and that enhances the extent of the paracellular pathway for sodium exit (see below). Shallow (~20–60 nm compared to hundreds of nm in other epithelia), leaky tight junctions that selectively permit Na^+ efflux join these interdigitated cell membranes.

Studying Rectal Gland Secretory Function

The fluid secreted in the rectal gland lumen drains via a single duct into the intestine, from where it is excreted with the feces. This feature, in concert with the simple vascular-supply structure, makes the intact gland an almost ideal study system for controlled perfusion studies. By cannulating the rectal gland duct, PMA, and central vein (but not the posterior bypass of the PMA, as noted by Olson), researchers can manipulate the ionic concentrations, hormone levels, pH, perfusion pressure, and other variables of the perfusing humoral fluid and then analyze the excurrent humoral fluid as well as the quantity and composition of the fluid secreted by the gland. Such studies may be performed *in vivo* (within the anesthetized or unrestrained animal), *in situ* (within pithed animals, where the central nervous system is destroyed), or *in vitro* (outside of the animal; literally, in glass). Researchers have also isolated individual rectal gland tubules and cultured the secretory epithelium of a single tubule for more focused biochemical and physiological studies. Different experimental preparations have their advantages and disadvantages. Isolated gland or tubule preparations, in particular, have allowed for the experimental manipulation of individual variables and have led to many of the major advances in rectal gland physiology. Building on these successes, recent *in vivo* studies have refocused on the rectal gland as a dynamic tissue with important organismal function(s) particularly in the context of feeding and the associated physiological perturbations. The *in vivo* preparation using unrestrained animals allows for perhaps the most physiologically realistic measurements; however, this comes at the cost of limited ability to manipulate the experimental parameters. Finally, the availability of secretagogs, specific activators and inhibitors of the various constituents of the secretory apparatus, and, more recently, cloned sequences for these constituents, has allowed for detailed characterization of the mechanisms and controls of rectal gland function.

Mechanism of Salt Transport

The molecular mechanisms of NaCl transport in the rectal gland are very similar to those used in other salt-transporting epithelia, including bony fish gills and the human kidney. In fact, many of the details of ion transport in these other tissues were first worked out in the spiny dogfish (*Squalus acanthias*) rectal gland.

Transcellular, Secondary Active Transport of Cl^- is Coupled to Paracellular Na^+ Efflux

The current model for ion transport in the rectal gland is summarized in **Figure 2**(**a**). The energizing step for transcellular, secondary active chloride-ion transport occurs at the basolateral membrane of secretory cells, where high densities of Na^+/K^+-ATPase pump three Na^+ ions out of the cell for every two K^+ ions that are pumped in. This pump creates both a strong inwardly directed gradient for Na^+ and a net negative charge inside the cells (i.e., an electrochemical gradient for Na^+ to reenter the cells). The Na^+ ions move down this electrochemical gradient into the cell via the passive transporter sodium–potassium two-chloride cotransporter (NKCC), bringing along two Cl^- ions (against the transmembrane Cl^- gradient) and a K^+ ion. K^+ ions pass back out of the cell through a passive K^+ channel to avoid an intracellular excess. The Na^+ ions are then actively re-transported out of the cells via the Na^+/K^+-ATPase pump. This process yields an intracellular Cl^- ion excess relative to the tubule lumen. The Cl^- ions then follow their transmembrane gradient into the tubule lumen via an apical passive chloride channel, which shares structural and biochemical properties with the human cystic fibrosis transmembrane conductance regulator (CFTR). The Na^+ ions enter the tubule lumen via a paracellular route through the leaky tight junctions between secretory cells; efflux of Na^+ is driven

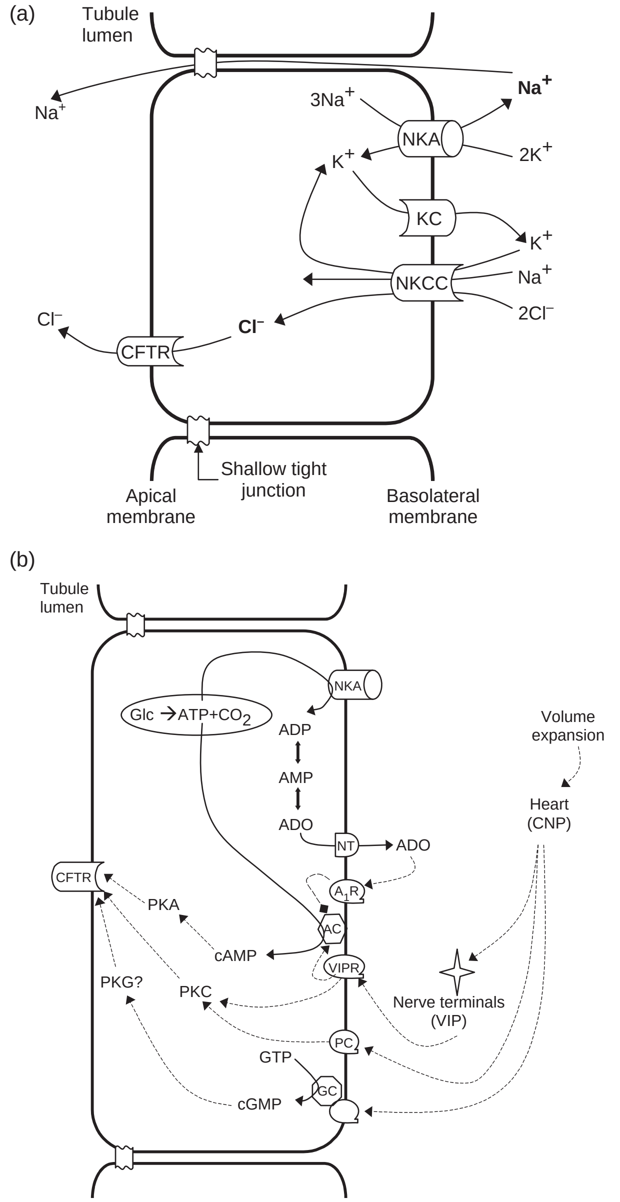


Figure 2 (a) Schematic of the current model of sodium (Na$^+$) and chloride (Cl$^-$) ion transport in secretory cells of rectal gland tubules. Bold symbols indicate intracellular and extracellular accumulations of chloride and sodium, respectively. NKA, Na$^+$/K$^+$-ATPase; KC, potassium channel; CFTR, cystic fibrosis transmembrane conductance regulator chloride channel; NKCC, sodium–potassium two-chloride cotransporter. (b) Schematic of some of the consensus controls of rectal gland salt secretion, including volume expansion via the effects of CNP and negative feedback via endogenous production of adenosine. Other proposed control mechanisms are described in the text. Dashed lines with arrowheads indicate activation; long-dashed lines with diamond heads indicate inhibition. A$_1$R, inhibitory adenosine A$_1$ receptor; AC, adenylyl cyclase; ADO, adenosine; cGMP, cyclic guanosine monophosphate; GC, guanylyl cyclase; Glc, glucose; NT, nucleoside transporter; PC, receptor linked to phospholipase C and the phosphoinositide system; VIPR, VIP receptor.

by the net electrical gradient established by the buildup of Cl$^-$ in the lumen. Thus, neither Cl$^-$ nor Na$^+$ secretion into the tubule lumen is achieved directly by primary active transport processes.

Water follows the secreted ions into the tubule lumen down an osmotic gradient, either across the secretory cells or through the tight junctions between cells. The secreted rectal gland fluid drains into the central lumen and subsequently exits via a lone duct into the posterior intestine. Although it was proposed by Burger in the 1960s that the inner stratified epithelium of the central lumen further modifies the secreted fluid, definitive studies of this function have not been conducted.

Energetic Requirements for Salt Transport

The high density and activity of Na$^+$/K$^+$-ATPase in the basolateral membrane of secretory cells means that these cells have high energetic (i.e., ATP) requirements. Dense aggregations of mitochondria, the aerobic power plants of cells, reside in close proximity to the basolateral membrane and supply ATP for active ion transport. Consequently, the rectal gland has an extremely high aerobic metabolic rate, and it extracts ~95% of the blood oxygen that enters its circulation in both the basal and stimulated states. The enzyme carbonic anhydrase, probably located in the extracellular space, converts the aerobic end product CO$_2$ to carbonic acid. This reaction prevents buildup of CO$_2$ in metabolically active secretory cells and thus facilitates their continued activity.

What are the metabolic fuels that support the secretory activity of the rectal gland? In many elasmobranch tissues, ketone bodies appear to be the dominant aerobic metabolic fuels. Somewhat surprisingly, recent work demonstrated that glucose is necessary to support rectal gland salt transport in the spiny dogfish and that ketones only supplement glucose-fueled secretion. Notably, these and other studies are yet to study the role of fatty-acid fuels in rectal gland function.

Several observations suggest that vascular oxygen supply and/or aerobic ATP production cannot always support secretion in the gland. First, biochemical measures indicate a high glycolytic (anaerobic) capacity in the rectal gland, suggesting that gland activity may be intermittently driven by less efficient anaerobic pathways. Consistent with this theory, volume loading enhances activity of the anaerobic pathway enzyme lactate dehydrogenase in the gland. Intermittent reliance on glycolytic pathways may help to explain the absolute requirement in the rectal gland for glucose as a metabolic fuel. Second, another enzyme, creatine kinase, is spatially tightly coupled to Na$^+$/K$^+$-ATPase. Creatine kinase transfers phosphate groups between the phosphagen creatine and adenosine diphosphate (ADP), allowing for rapid restoration of localized ATP levels for cellular work and also possibly buffering secretory cells against variations in metabolic fuel or oxygen availability. Third, several authors have noted that low perfusion rates *in vivo* may limit the delivery of oxygen and/or the export of CO_2 from active secretory cells, and this, in turn, would necessitate a switch to anaerobic metabolism. Thus, anaerobic support of rectal gland secretion may be more important in the intact animal than previously thought, although this remains to be experimentally demonstrated.

Other Rectal Gland Functions

Given the urea-based osmoregulatory strategy of the elasmobranchs, it is striking that rectal gland fluid contains very little urea (~20 mM in the rectal gland fluid vs. ~400 mM in vascular fluid, **Table 1**). Recent work showed that the basolateral and, especially, the apical membrane of rectal gland secretory cells both exhibit low permeabilities to urea. Consistent with this idea, a shark-specific facilitated urea transporter has been putatively identified in the rectal gland, although its location within the cell and its function remain to be demonstrated.

Another set of experiments revealed xenobiotic transport function in the rectal gland via multidrug-resistance proteins, but the physiological significance of this function is unclear.

Controls of Rectal Gland Function

Temporal variation in the rate of rectal gland secretion has been recognized for a long time. Baseline secretion rates are low or negligible; in fact, some *in vitro* preparations require pharmacologic or hormonal stimulation just to establish a baseline rate for comparison with the experimental conditions. Numerous studies, many of them conducted on *Squalus acanthias*, have demonstrated a large and interacting suite of factors that stimulate or inhibit rectal gland secretion. Early studies of rectal gland secretion demonstrated that it was activated by injecting into the animal, solutions of salt and sugar, or just water alone. A series of experiments by Solomon and colleagues suggested that intravascular volume expansion is the dominant stimulus, at least in *Squalus acanthias*. Recent studies on the gland *in vivo* offer further insight, suggesting that gland activation is a graded and temporally dynamic process, with salt transport (and activation of associated enzymes and transporters) peaking ~6–20 h after a meal/infusion. Importantly, the act of feeding and its associated physiological consequences (including, but not limited to, the alkaline tide and intravascular volume loading) elicit biochemical and morphological changes in the gland that cannot be reproduced entirely by modification of intravascular volume, pH, or salt alone. Thus, the *in vivo* control of rectal gland physiology appears extraordinarily complex (see the section 'Further reading' for details)

Endocrine, Paracrine, and Autocrine Regulation of Secretion

The search for the humoral (i.e., hormonal) factor(s) that stimulate rectal gland secretion resulted from the early discovery that adenosine 3,5-cyclic monophosphate (cAMP) (or activation of adenylyl cyclase, the enzyme that produces cAMP) is a potent stimulus for secretion; cAMP is an important second messenger in receptor-mediated hormone signaling cascades. The first hormone shown to stimulate rectal gland secretion was VIP, although blood levels of this peptide did not change coincident with gland activation. The recent consensus model posits that blood-volume expansion (e.g., after feeding) causes release of the hormone C-type natriuretic peptide (CNP) from the elasmobranch heart. At the rectal gland, CNP exerts its effects directly on secretory cells through a protein kinase C (PKC)-dependent pathway and indirectly through stimulation of VIP release from nerve terminals within the gland. Thus, VIP functions as a neurotransmitter in this scenario, at least in *S. acanthias*, and it activates adenylyl cyclase and PKC, complementing and magnifying the direct effect of CNP on secretory cells. CNP also contributes to vasodilation in the gland and thus regulates its perfusion. Overall, it appears that this CNP pathway functions in a negative feedback loop to fine-tune intravascular volume by enhancing rectal gland secretion. The related hormones atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) may also play a more limited role.

Other hormones may also contribute to the control of rectal gland secretion. Of particular relevance in the context of feeding is a gut hormone known as rectin (also known as scyliorhinin II). Rectin has a stimulatory effect on the secretion rate, possibly representing a communication link between digestive activity and the rectal gland. The parenchyma also hosts binding sites for the unique elasmobranch hormone 1α-hydroxycorticosterone, but its physiological significance remains unexplored in this context.

Further complicating the picture, the rectal gland is also subject to autocrine regulation. Specifically, adenosine, a product of ATP hydrolysis, accumulates in stimulated rectal gland cells. After moving across the basolateral membrane, adenosine at nanomolar concentrations inhibits rectal gland activity by binding to an inhibitory adenosine A_1 receptor.

Nervous Control of Secretion

Studies of the hormonal control of rectal gland secretion have dominated much of the literature. However, given the extensive innervation of the rectal gland and the known role for nerve-derived VIP in gland activation in *S. acanthias*, it is not surprising that other neurotransmitters also affect secretion rate. In particular, neuropeptide Y (downstream of cAMP formation) and somatostatin (upstream of cAMP formation) both inhibit rectal gland secretion. Somatostatin has been localized to nerve terminals that also contain VIP. Thus, regulation of these and other nervous signals appears quite complex, and their *in vivo* physiological significance remains elusive, at least in part due to the heavy reliance on *in vitro* experimental rectal gland preparations that have severed the nerve connections.

Extracellular pH and Ionic Strength Affect Secretion

In addition to the hormonal and the nervous controls of rectal gland secretion, changes in the hydrogen ion concentration (i.e., pH) and ionic strength of the vascular and extracellular fluids directly influence rectal gland activity. The act of digestion involves the secretion of acid into the gut lumen, culminating in an increase in pH (i.e., an alkalosis) of the vascular fluids. This alkaline tide has been the subject of much recent attention with reference to its impact on the rectal gland, again largely in *S. acanthias*. These studies demonstrate that extracellular pH, rather than either the partial pressure of CO_2 or the concentration of bicarbonate base, is the dominant signal of the alkaline tide involved in rectal gland activation. Accordingly, alkalosis stimulates Na^+/K^+-ATPase activity more than volume expansion alone. The recent characterization of the basolateral K^+ conductance in *S. acanthias* as a two-pore-domain K^+ channel (TASK-1) that is sensitive to pH offers one possible mechanism for the effect of pH on rectal gland activity, but other pathways are likely to be involved.

The presence of a calcium-sensing receptor (CaSR, a G-protein-coupled receptor) in rectal gland artery and tubules supports a direct link between the ionic strength of the vascular fluids and rectal gland secretion. The CaSR's activity decreases in response to increased ionic strength. A recent model proposes that the CaSR constitutively inhibits cAMP formation under basal conditions, while increased ionic strength (e.g., following a meal) releases cAMP formation from the inhibitory effects of CaSR and leads to the activation of ion transport. CaSR also influences cAMP formation in the rectal gland artery, suggesting a complementary role in control of rectal gland perfusion.

Molecular Mechanisms of Secretion Control

Downstream of hormonal or other modes of stimulation or inhibition, salt-transport processes in the rectal gland are modulated at the molecular level. Many of the enzymes and transporters involved in salt secretion are subject to post-translational modifications, including (de)phosphorylation, that either activate or inhibit their activity. For example, upon stimulation by factors that increase intracellular cAMP, protein kinase A phosphorylates the R domain of CFTR, leading to opening of this apical Cl^- channel. Activation of basolateral NKCC by phosphorylation lags behind CFTR; this event coincides with decreases in intracellular Cl^- as it leaves through the apical CFTR, but it appears to be triggered by transient cell shrinkage. Other modes of regulation have been postulated, including the possible existence of discrete pools of Na^+/K^+-ATPase with different sensitivities to cAMP-dependent stimulation.

Given the dramatic difference in salt-transport rate between basal and activated states in the gland, it is not surprising that recent biochemical and proteomics studies also demonstrate regulation of the activities and abundances of energy-producing enzymes following feeding (increase) or dilution of the surrounding seawater medium (decrease). However, the underlying molecular-signaling mechanisms are yet to be determined.

Morphological Contributions to Rectal Gland Activation

The rectal gland's morphology was recently shown to be highly plastic at both the gland and cellular levels, particularly in response to feeding. Shortly following a meal, the diameters of rectal gland tubules increase, while the smooth muscle ring surrounding the gland becomes compressed. The morphology of individual secretory cells also shifts after a meal, including expansion of the apical surface area via disaggregation of the apical microvilli (**Figure 3**). Actin filaments and actin-bundling proteins, such as transgelin, probably play a significant role in rectal gland activation, although the specific mechanism and the stimulus for cytoskeletal reorganization following feeding both are yet to be defined. In accord with this actin hypothesis, rectal gland activation by CNP (but not VIP) can be reversed by inhibitors of actin cytoskeleton organization, and the actin cytoskeleton reorganizes following K^+ manipulation or hypotonic exposure of rectal gland secretory cells. Whether these cytoskeletal events somehow regulate the localization and/or activity of the molecular constituents of the salt-transport apparatus remains to be seen. For example, cytoskeletal elements may play a role in sensing cell shrinkage and activating NKCC.

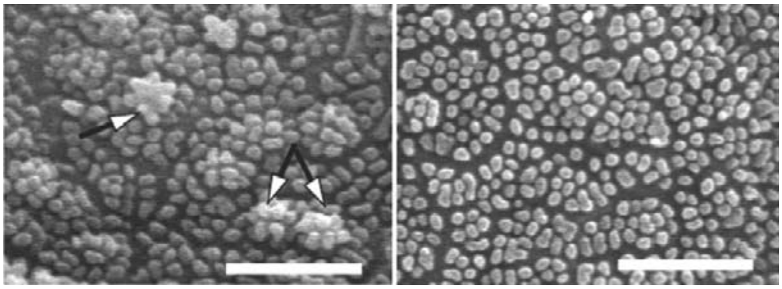


Figure 3 Scanning electron micrograph of the apical (luminal) surface of rectal gland tubular secretory cells of the spiny dogfish (*Squalus acanthias*) before (left) and 6 h after (right) a meal. Note the fusion of apical microvilli into flower-like structures in the fasted gland. These structures disaggregate at 6 h after feeding, when the microvilli are regularly spaced. Scale bars = 2 μm. Reproduced from Matey V, Wood CM, Dowd WW, Kültz D, and Walsh PJ (2009) Morphology of the rectal gland of the spiny dogfish (*Squalus acanthias*) shark in response to feeding. *Canadian Journal of Zoology* 87: 440–452.

Conclusion

Few animal tissues have received as much attention in the physiological and biomedical literature as the elasmobranch rectal gland. Despite decades of research and numerous advances at the molecular, cellular, and tissue levels, a comprehensive picture of the *in vivo* controls of rectal gland function is yet to be elucidated. In particular, it remains extremely challenging to assess the spatial and temporal integration of the numerous signals (endocrine, paracrine, autocrine, neural, etc.) that are known to influence gland secretion. Significantly, there are exceedingly few studies of how the controls of rectal gland function vary among species (e.g., in euryhaline vs. stenohaline species), in different environmental conditions (e.g., in low vs. high salinity), or across life-history stages. Therefore, a number of opportunities exist for researchers to analyze this intriguing and complex gland from new perspectives.

See also: **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation.

Further Reading

Burger JW and Hess WN (1960) Function of the rectal gland in the spiny dogfish. *Science* 131: 670–671.

Dowd WW, Harris BN, Cech JJ, Jr., and Kültz D (2010) Proteomic and physiological responses of leopard sharks (*Triakis semifasciata*) to salinity change. *Journal of Experimental Biology* 213: 210–224.

Dowd WW, Wood CM, Kajimura M, Walsh PJ, and Kültz D (2008) Natural feeding influences protein expression in the dogfish shark rectal gland: A proteomic analysis. *Comparative Biochemistry and Physiology Part D* 3: 118–127.

Evans DH, Piermarini PM, and Choe KP (2004) Homeostasis: Osmoregulation, pH regulation, and nitrogen excretion. In: Carrier JC, Musick JA, and Heithaus MR (eds.) *Biology of Sharks and Their Relatives*, pp. 247–268. Boca Raton, FL: CRC Press.

Fellner SK and Parker L (2002) A Ca^{2+}-sensing receptor modulates shark rectal gland function. *Journal of Experimental Biology* 205: 1889–1897.

Forrest JN, Jr. (1996) Cellular and molecular biology of chloride secretion in the shark rectal gland: Regulation by adenosine receptors. *Kidney International* 49: 1557–1562.

Henson JH, Roesener CD, Gaetano CJ, *et al.* (1997) Confocal microscopic observation of cytoskeletal reorganizations in cultured shark rectal gland cells following treatment with hypotonic shock and high external K^+. *Journal of Experimental Zoology* 279: 415–424.

Kelley GG, Aassar OS, and Forrest JN, Jr. (1991) Endogenous adenosine is an autacoid feedback inhibitor of chloride transport in the shark rectal gland. *Journal of Clinical Investigation* 88: 1933–1939.

Matey V, Wood CM, Dowd WW, Kültz D, and Walsh PJ (2009) Morphology of the rectal gland of the spiny dogfish (*Squalus acanthias*) shark in response to feeding. *Canadian Journal of Zoology* 87: 440–452.

Olson KR (1999) Rectal gland and volume homeostasis. In: Hamlett WC (ed.) *Sharks, Skates, and Rays*, pp. 329–352. Baltimore, MD: Johns Hopkins University Press.

Silva P and Epstein FH (2002) Role of the cytoskeleton in secretion of chloride by shark rectal gland. *Journal of Comparative Physiology B* 172: 719–723.

Silva P, Solomon RJ, and Epstein FH (1996) The rectal gland of *Squalus acanthias*: A model for the transport of chloride. *Kidney International* 49: 1552–1556.

Silva P, Solomon RJ, and Epstein FH (1999) Mode of activation of salt secretion by C-type natriuretic peptide in the shark rectal gland. *American Journal of Physiology* 277: R1725–R1732.

Silva P, Stoff J, Field M, *et al.* (1977) Mechanism of active chloride secretion by shark rectal gland – role of Na-K-ATPase in chloride transport. *American Journal of Physiology* 233: F298–F306.

Solomon RJ, Taylor M, Rosa R, Silva P, and Epstein FH (1984) *In vivo* effect of volume expansion on rectal gland function. II. Hemodynamic factors. *American Journal of Physiology* 246: R67–R71.

Solomon RJ, Taylor M, Sheth S, Silva P, and Epstein FH (1985) Primary role of volume expansion in stimulation of rectal gland function. *American Journal of Physiology* 248: R638–R640.

Solomon RJ, Taylor M, Stoff JS, Silva P, and Epstein FH (1984) *In vivo* effect of volume expansion on rectal gland function I. Humoral factors. *American Journal of Physiology* 246: R63–R66.

Walsh PJ, Kajimura M, Mommsen TP, and Wood CM (2006) Metabolic organization and effects of feeding on enzyme activities of the dogfish shark (*Squalus acanthias*) rectal gland. *Journal of Experimental Biology* 209: 2929–2938.

Wood CM, Kajimura M, Mommsen TP, and Walsh PJ (2008) Is the alkaline tide a signal to activate metabolic or ionoregulatory enzymes in the dogfish shark (*Squalus acanthias*)? *Physiological and Biochemical Zoology* 81: 278–287.

Wood CM, Munger RS, Thompson J, and Shuttleworth TJ (2007) Control of rectal gland secretion by blood acid–base status in the intact dogfish shark (*Squalus acanthias*). *Respiratory Physiology and Neurobiology* 156: 220–228.

THE GUT

Nitrogenous-Waste Balance

Contents

Excretion of Ammonia
Ureotelism

Excretion of Ammonia

DJ Randall, City University of Hong Kong, Hong Kong, China

© 2011 Elsevier Inc. All rights reserved.

Introduction
Ammonia Gas, Ammonium Ions, and pH
Ammonia Excretion and pH
Ammonia Transfer across Cell Membranes
Ammonia and Gill Ventilation
Ammonia Volatilization
Ammonia Toxicity
Ureogenesis
Feeding and Exercise
Further Reading

Glossary

Ammoniotelic An animal that predominantly excretes ammonia.

Deamination The release of amino groups ($-NH_2$) from amino acids.

Diffusion limited Transfer limited by diffusion processes at an exchange site.

Lipophilic Having an affinity for lipids.

Na^+/K^+-ATPase This is a major ATP-consuming ion pump that, directly or indirectly, drives many ion-regulatory processes, including maintaining sodium and ion gradients across cell plasma membranes and being present at high levels in ionocytes.

NE-2 A plasma protein that is an electrically neutral Na^+/H^+ antiporter.

NMDA receptor *N*-methyl-D-aspartate receptor in the vertebrate brain.

Ornithine–urea cycle Cyclic succession of reactions that produce urea from ammonia in livers of ureotelic animals.

Perfusion limited Transfer limited by rate of perfusion of an exchange site.

Rhesus (Rh) glycoprotein Membrane protein that facilitates ammonia transfer.

Transamination The transfer of amino groups ($-NH_2$) between amino acids.

Ureotelic An animal that predominantly excretes urea.

Uricolysis The breakdown of uric acid producing urea.

V-type proton ATPase Vacuolar-type ATPase that hydrolyzes ATP to generate a H^+ gradient.

Introduction

When amino acids are catabolized, the amino group ($-NH_2$) is released (deamination) or transferred (transammination) to another molecule for removal or reuse. If amino groups are not salvaged for resynthesis of amino acids, they must be dissolved in water and excreted to avoid a toxic rise in the plasma concentration of nitrogenous wastes. Elevated levels of these wastes can cause convulsions, coma, and eventually death. Excess nitrogenous wastes are excreted by teleost fish as ammonia, whereas elasmobranchs and a few teleosts also produce and excrete urea. Lesser quantities of nitrogen are excreted in the form of other compounds, such as creatinine, creatine, or trimethylamine oxide, and, in very small quantities, amino acids, purines, and

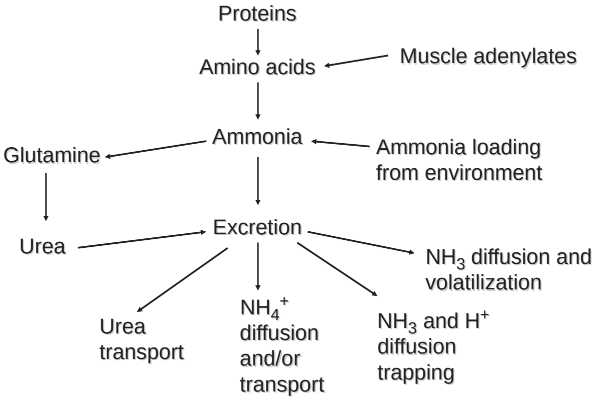

Proteins
Amino acids
Muscle adenylates
Ammonia
Glutamine
Ammonia loading from environment
Urea
Excretion
Urea
NH_3 diffusion and volatilization
Urea transport
NH_4^+ diffusion and/or transport
NH_3 and H^+ diffusion trapping

Figure 1 Sources and sinks of ammonia.

pyrimidines. Rates of ammonia excretion vary with diet and the condition of the fish, and are between 5% and 30% of that for CO_2. In fact, about 40–60% of the nitrogen intake from fish food is excreted as ammonia within 24 h. In fasting fish, muscle proteins are broken down for energy, producing ammonia waste. In addition, ammonia can be produced through the deamination of adenosine monophosphate (AMP) in skeletal muscle during violent exercise. Some fish have been shown to reduce the rate of ammonia production from amino acid catabolism in response to an ammonium load to slow down the buildup of ammonia internally (**Figure 1**).

Ammonia Gas, Ammonium Ions, and pH

Ammonia gas (NH_3) in solution combines with a proton to form ammonium ions (NH_4^+), the pK of the reaction varying between 9 and 9.5. When pH is equal to pK, then the ratio of NH_3 to NH_4^+ is one to one, but at the more physiological pH of 7.5 the ratio is around 1:100, that is, most of the ammonia in fish is in the form of NH_4^+. As pH falls, such as following intense exercises and during severe hypoxia, the proportion of NH_3 will decrease further such that the NH_3 to NH_4^+ ratio will approach 1:1000, that is, most ammonia is present in the fish body is as NH_4^+, with only a small proportion as NH_3.

The pH of living tissues, including those of fish, is such that it is midway between the pK of the ammonia/ammonium reaction and the apparent pK of the CO_2/bicarbonate reaction. As a result, there are adequate levels of both CO_2 and NH_3 for removal by diffusion of the gaseous form of these metabolic products. Ammonia moves between compartments largely as NH_3. If ammonium chloride (NH_4Cl) levels in the extracellular fluid increase, NH_3 enters the cell and the ratio of NH_3 to NH_4^+ within the cell increases. NH_3 combines with hydrogen ions to form NH_4^+ within the cell, thus raising intracellular pH. During NH_4Cl exposure, intracellular pH quickly reaches a maximum and then starts to fall because of the action of somewhat slower acid–base regulating mechanisms in the membrane. A subsequent return of the external NH_4Cl level to the original value results in a sharp fall in intracellular pH as NH_3 diffuses out of the cell leaving behind a proton. Cell pH falls below the initial level and slowly returns to the original value as NH_4^+ ions and protons slowly move out of the cell. Protons and NH_4^+ can be transported across plasma membranes by a variety of passive and active mechanisms that vary from cell to cell and are discussed later.

Ammonia Excretion and pH

Acid waters enhance ammonia excretion, whereas fish have difficulty excreting ammonia in alkaline waters. NH_3 diffusing into an acid environment will combine with a proton and be trapped as NH_4^+, unable to diffuse back into the cell or animal (**Figure 2**). The excretion of protons or acidification due to either CO_2 excretion or bicarbonate uptake will enhance ammonia movement due to NH_4^+ trapping, maintaining the gradient for NH_3 across the gill epithelium. Thus, diffusion of ammonia gas across the gills is enhanced by simultaneous CO_2 and proton excretion. The proton excretion is due to V-type H^+-ATPase activity (ATP, adenosine triphosphate) in the apical membrane, which increases membrane potential; this enhances sodium influx through sodium channels in the gill membrane. There is also sodium/proton exchange across the gills, and the exchange protein (NHE-2) has been located in the gills of several freshwater fish and shown to be involved in sodium uptake. The overall result is that there is often a correlation between sodium uptake and ammonia excretion across the gills.

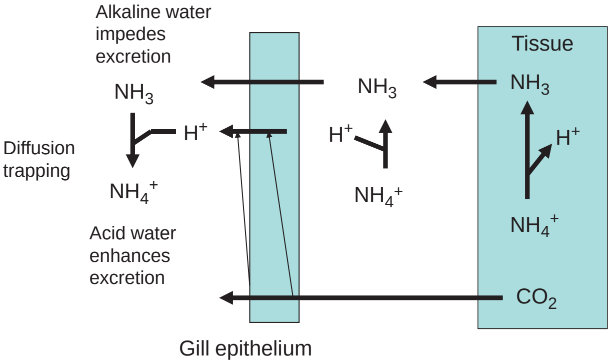


Figure 2 NH_3 excretion acidifies the tissues and is enhanced by diffusion trapping due to the conversion of NH_3 to NH_4^+ in the gill water boundary layer. Proton flux across the apical gill membrane and carbon dioxide excretion enhances this process.

Fish can be trapped in puddles of water for many hours or days; continual excretion of endogenous ammonia into a small volume of external media can lead to high external ammonia concentrations. The associated excretion of CO_2 will reduce water pH. This lowering of environmental pH has advantages for dealing with elevations in environmental ammonia levels by reducing the concentration of NH_3 in the water and, therefore, ammonia levels in the fish constituting environmental ammonia detoxification. Mudskippers build and lay eggs in water-filled burrows in the tidal mud flats. It has been reported that the giant mudskipper (*Periophthalmodon schlosseri*) can decrease the pH of the burrow water by acid, in addition to CO_2 excretion, presumably to protect their embryos against ammonia toxicity. Because ammonia excretion is usually as NH_3, alkaline waters are much more toxic to fish than acid waters as there is no acid trapping of excreted NH_3. Fish accumulate ammonia in waters higher than pH 9 and few fish can survive in such waters. The Lake Magadi tilapia is unusual in that it converts ammonia to urea via the ornithine–urea cycle (OUC) and can survive in these extremely alkaline waters.

Ammonia Transfer across Cell Membranes

Biological membranes are usually much more permeable to NH_3 than NH_4^+. Although NH_3 has a high solubility in water, it is only weakly soluble in lipids. In this respect, NH_3 contrasts strongly with lipophilic molecules such as CO_2; the permeability to NH_3 of nearly all cell membranes is much less than their permeability to other respiratory gases such as CO_2 and O_2. Unlike freshwater fish, seawater fish have shallow tight junctions between mitochondria-rich cells, which increase cation permeability. A significant portion of total ammonia can be excreted by NH_4^+ diffusion through this paracellular route in seawater fish.

Ammonia diffusion across many membranes is enhanced by the presence of Rhesus (Rh) glycoproteins. It has been shown in zebrafish that the transfer of ammonia is correlated to the expression of Rh genes. There are several types of Rh proteins found in a variety of tissues on both baso-lateral and apical regions of cells. These channels have been found in fish gills and a number of other tissues. The overall process is the diffusion of NH_3 through an Rh protein channel in the membrane. The isoforms Rhcg1 and Rhcg2 have been reported to be present and play a role in the transfer of ammonia across the fish gills.

A small amount of NH_4^+ can permeate biomembranes through K^+ channels. Most of the values for P_{NH4}/P_K through K^+ channels in a wide range of animals, including vertebrates and invertebrates, range between 0.1 and 0.3, that is, K^+ channels are more permeable to K^+ than NH_4^+. The hearts of some fish have K^+ channels with a very low NH_4^+ permeability, important in preventing heart attacks following feeding as discussed later. In addition, NH_4^+ can also be actively transported through membranes by substituting for potassium on Na/K-ATPase on the baso-lateral membrane of the gills. The mudskipper (*P. schlosseri*) uses this mechanism to actively excrete ammonium ions, which contributes to its ability to survive concentrations of ambient ammonia that would be toxic to other fishes.

Active excretion of NH_4^+ would be costly if associated with high membrane NH_3 permeability and would result in ammonia recycling and the transfer of protons across the membrane. If the membrane is permeable to NH_4^+ then its distribution will be determined by membrane potential, whereas the NH_3 level in each compartment will be determined by pH. Under certain conditions, an efflux of NH_4^+ will be associated with an influx of NH_3 which will short-circuit membrane potential and the pH gradient and require energy to maintain cell pH and the membrane potential. The mudskipper (*P. schlosseri*) reduces NH_3 membrane permeability by modifying the phospholipid and cholesterol compositions of membranes to reduce such recycling.

Ammonia and Gill Ventilation

Under most conditions, ammonia excretion is probably diffusion rather than perfusion limited. Ammonia transfer across the gill epithelium of rainbow trout has been shown to be linearly related to the NH_3 partial pressure gradient and there are a number of mechanisms to augment ammonia transport across membranes in the face of a relatively low NH_3 membrane permeability.

Ventilation is regulated to meet the requirements for oxygen transfer and will normally be more than sufficient to meet the requirements for the much lower flux of ammonia. Nevertheless, elevated ammonia levels are associated with increases in gill ventilation that are independent of changes in water pH. These increases in gill ventilation, however, have negligible effect on ammonia excretion and probably represent a nonphysiological response on the part of the fish in regard to ammonia excretion. There are large increases in blood ammonia levels following a meal which last several hours. An interesting possibility is that the postprandial increase in ventilation to deliver the increased oxygen uptake required for food intake and digestion is mediated by ammonia-induced increases in ventilation.

Neuroepithelial cells in the fish gill are similar in structure to mammalian oxygen chemoreceptors and are probably involved in the increases in ventilation associated with hypoxia in fish. If the K^+ channels in fish gill neuroepithelial cells are permeable to NH_4^+, then these cells would be depolarized by elevated NH_4^+ levels and the response would be an increase in gill ventilation. Thus, the increase in gill ventilation associated with elevated ammonia may be due to activation of the chemoreceptors in the gills of fish but this probably has little effect on ammonia excretion but does facilitate increased oxygen uptake in the postprandial fish.

Ammonia Volatilization

Many blennies (*Blennius pholis; Alticus kirki*), mangrove killifish (*Rivulus marmoratus*), and the oriental weatherloach (*Misgurnus anguillicaudatus*) are capable of volatilizing significant amounts of ammonia during aerial exposure. High temperatures and humidity, as well as high body ammonia levels, increase the likelihood of the ammonia being volatilized from the film of water covering the body of tropical fishes. These fish are able to tolerate very high tissue ammonia levels, which is an essential prerequisite for high rates of volatilization to occur. In *M. anguillicaudatus*, there appears to be at least two sites of NH_3 volatilization, that is, the skin and the digestive tract, under terrestrial conditions. During emersion, the surface of the skin and the mucosal surface of the anterior portion of the digestive tract become significantly more alkaline. Since the fraction of NH_3 present is strongly influenced by the pH of the medium, the skin and digestive tract of *M. anguillicaudatus* could be a site of NH_3 volatilization. Although both the skin and the digestive tract may be involved in NH_3 volatilization when the fish is moving on land, excretion of ammonia through the skin would be inhibited when the fish burrows into the mud. When the fish is surrounded by mud, the digestive tract (either through the mouth or through the cloaca) becomes the only avenue for NH_3 volatilization.

Ammonia Toxicity

Release and breakdown of sewage from humans and other animals into water and the subsequent increase of ammonia levels is a particular problem. Elevated levels of ammonia in the environment can reduce excretion or lead to ammonia uptake by the animal. Although low levels of ammonia in the environment can be beneficial and have been shown to promote growth in fish, elevated ammonia levels in the environment can decrease exercise performance, growth, and feeding, and inflict gill damage. Acute ammonia toxicity results in excessive activation of brain *N*-methyl-D-aspartate (NMDA) receptors, leading to convulsions and death in all vertebrates.

While ammonia can be converted to less toxic urea, urea synthesis consumes ATP (see below) and energy can be saved if enough water is available to support the excretion of nitrogenous waste as dilute ammonia. When ammonia cannot be excreted effectively, fish may reduce ammonia production. In addition, some fish species prevent the buildup of internal ammonia by converting ammonia to less toxic forms, namely glutamine and urea. A number of fish species convert ammonia to glutamine for storage until the ammonia levels in the body have returned to normal, for example, following feeding (see below). Glutamine is much less toxic than ammonia but is not normally excreted in significant quantities. The brains of many fish are protected, to a certain extent, by detoxifying ammonia to glutamine. Increased levels of cellular glutamine, however, can increase cell osmolarity, and the subsequent water influx could cause cell swelling. This swelling can create severe problems in the mammalian brain which is tightly constrained in the skull but seems to be less of a problem in fish with brains in a cavity larger than the brain itself. An increase in Gulf toadfish brain glutamine levels is not associated with any change in brain water content indicating that glutamine formation was not causing cerebral swelling in toadfish. In addition, the brain is more loosely held in the brain case of many fish, and inhibition of glutamine synthetase (GS) is not protective in fish. Thus, unlike mammals, glutamine synthesis and accumulation in the brain does not appear to be a major cause of death following ammonia intoxication in fish. In fact, ammonia tolerance in fish may be related to the capacity for producing glutamine to detoxify ammonia in the brain.

Glutamine is produced from glutamate and NH_4^+, the reaction catalyzed by GS. Glutamate may, in turn, be produced from either α-ketoglutarate (α-KG) and NH_4^+ catalyzed by glutamate dehydrogenase (GDH) in the mitochondrial matrix, or α-KG and other amino acids catalyzed by various transaminases (**Figure 3**). Whether the synthesis of glutamine begins with glutamate or α-KG is determined by the distribution of GS within the cell,

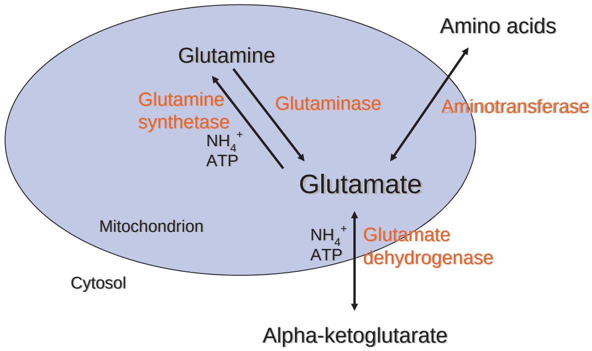


Figure 3 The conversion of alpha-ketogluterate to glutamine removes two NH_4^+ ions.

and multiple GS genes have been reported for the rainbow trout and the Gulf toadfish.

The blood–brain barrier of fish is apparently permeable to NH_3, because the brain ammonia content of some fish species can build up to very high levels. The mechanisms involved in ammonia tolerance in the brain cells of these ammonia-tolerant fish are presently uncertain. The brain is often the organ undergoing the largest increase in glutamine content following elevations of blood ammonia levels and it appears that fish brains detoxify the permeated ammonia to glutamine. Although glutamine accumulation and the consequent astrocyte swelling is known to be one of the reasons behind hepatic encephalopathy in mammals, it does not seem to be a problem in fish. The GS inhibitor methionine sulfoximine (MSOX), at a dosage protective for mammals, does not protect mudskippers or the oriental weather loach against acute ammonia toxicity. It has been demonstrated that the increase in brain glutamine was not associated with any changes in brain water in the Gulf toadfish, *Opsanus beta*, indicating that glutamine formation was not causing cerebral swelling in toadfish. This suggests that detoxification of ammonia to glutamine may be important to ammonia tolerance but, unlike mammals, glutamine synthesis and accumulation in the brain does not appear to be a major cause of death following ammonia intoxication in fish. Some tropical air-breathing fishes can detoxify endogenous ammonia to glutamine in noncerebral tissues as well as the brain. Sleepers (the marble goby, *Oxyeleostris marmoratus*, and four-eyed sleeper, *Bostrichthys sinensis*) and the swamp eel, *Monopterus albus*, are exceptional because they can detoxify ammonia to glutamine in their livers and muscles during aerial exposure and ammonia loading. The Gulf toadfish *O. beta* detoxifies endogenous ammonia to glutamine to suppress ammonia excretion during confinement stress.

Excessive activation of NMDA receptors is a key event in the mechanism of ammonia toxicity in mammals. It was thought that high extracellular glutamate levels caused excessive NMDA receptor activation, but it was observed that activation of NMDA receptor precedes elevation of extracellular glutamate upon ammonia intoxication. Depolarization of postsynaptic neurons will increase the possibility of glutamate activation of NMDA receptors, even in the absence of high levels of glutamate. It is possible that ammonia activates NMDA receptors by depolarization of neuron cell membranes through the background K^+ channels, resulting in excessive activation of NMDA receptors and eventual neuronal death. Ammonia is known to cause depolarization of cell membranes activating NMDA receptors. Injection of the NMDA receptor antagonist (5R, 10S)-(+)-methyl-10,11-dihydro-5H-dibenzo[a, d]cyclohepten-5, 10-imine hydrogen maleate (MK-801) at a dosage of $2\,\mu g\ g^{-1}$ fish prior to ammonium acetate injection significantly reduced mortality rate in the weatherloach, but had no protective effect on either *P. schlosseri* or *Baleophthalmus boddaerti*. Interestingly, fish that are hypoxia tolerant also seem to be ammonia tolerant. The exact nature of ammonia toxicity in fish is not known.

Ureogenesis

In ureogenic fishes, the OUC consists of the enzyme CPS III and four other enzymes (plus GS) located in the matrix of liver mitochondria, utilizing glutamine as a substrate. Thus, ureogenic fishes possess high levels of GS in liver mitochondria. Because ammonia is already detoxified to glutamine, which is proton neutral and will not disrupt oxidative phosphorylation, the formation of carbamoyl phosphate via CPS III from glutamine in the liver mitochondrial matrix cannot be regarded as an ammonia-detoxifying system. This is different from terrestrial vertebrates, whose OUC involves carbamoyl phosphate synthetase I which utilizes NH_3 directly as a substrate. In the case of ureogenic and ureotelic marine elasmobranchs, two GS isozymes exist separately in the brain and the liver, localized in the cytosol and the mitochondria, respectively. GS has also been detected from mitochondria of the elasmobranch kidney, which may function as part of a substrate cycle for ammonia excretion during acidosis.

Urea is accumulated as an osmolyte in elasmobranchs, holocephans, and coelacanths. Marine elasmobranchs are ureogenic and they possess a functional OUC in mitochondria of their livers and muscles. They are also ureotelic, that is, urea is the primary product (>50%) of nitrogen excretion. Elasmobranchs have high concentrations of urea in their bodies, which is prevented from leaking into the environment by the very low permeability to urea of their gill epithelia. This low permeability is due to the high cholesterol composition of the gill membrane, coupled to a urea/sodium antiporter in the basolateral membrane that transports urea back into the blood. The elasmobranch kidney also functions to conserve urea but this pathway is less significant because of low rates of urine flow in elasmobranchs.

For those fishes that are ureogenic but nonureosmotic, the OUC in the liver can apparently detoxify ammonia, which is produced at high rates mainly in the mitochondrial matrix through catabolism of excess amino acids from food. There are increases in rates of urea synthesis and excretion in the giant mudskipper *P. schlosseri* and the slender lungfish *Protopterus dolloi* within 24 h after feeding. Recent work on marine sharks and freshwater stingray confirms that ureogenic elasmobranchs also increase the rate of urea synthesis upon feeding, but urea can be retained for

Table 1 Ammonia toxicity in resting and swimming rainbow trout: USA Environmental Protection Agency (EPA) criteria protect resting but not swimming fish

Condition	*Total Ammonia (mg l^{-1})*
96h LC_{50} resting	207
EPA salmonid acute value	~100 decreasing with increasing pH
EPA salmonid criterion maximum concentration	~40 decreasing with increasing pH
96h LC_{50} swimming (60% U_{crit})	32

the purpose of osmoregulation, instead of being excreted.

Only a few teleosts are ureotelic in water (e.g., the gulf toadfish) or able to detoxify a minor quantity of ammonia to urea during ammonia loading (*Mugilogobius abei*). The majority of tropical teleosts studied so far does not use ureogenesis as a major strategy to detoxify ammonia. Ureogenesis in fishes is energetically expensive. For teleosts and elasmobranchs, a total of 5 mol of ATP is hydrolyzed for each mole of urea synthesized, equivalent to 2.5 mol ATP used for each mole of nitrogen assimilated. It is probably because of this that ureogenesis is not commonly adopted as a single major strategy to handle ammonia toxicity when air-breathing tropical teleosts are exposed to terrestrial conditions or environmental ammonia.

To date, the only teleost for which unequivocal evidence is available for the OUC to function primarily for the purpose of ammonia detoxification is the tilapia *Alcolapia grahami* in highly alkaline lakes (pH 10) in Kenya. This fish has a high capacity to detoxify ammonia to urea via the OUC and is the first known example of complete ureotely in an entirely aquatic teleost fish. The majority of the urea synthesized in *A. grahami* is excreted through the gills. By contrast, African lungfishes synthesize and accumulate urea during emersion and estivation on land; they are ureogenic and possess a full complement of OUC enzymes.

Feeding and Exercise

Fish exhibit a postprandial elevation of blood ammonia levels lasting up to several hours. The increase in blood NH_4^+ levels could be expected to cause cardiac membrane depolarization and a heart attack, but the fish cardiac potassium channels have a very low permeability to NH_4^+ and the heart is protected. The postprandial ammonia levels in the blood, however, can be similar to those that kill fasting fish, presumably due to action on the brain. Why feeding fish are less sensitive to ammonia than fasting fish is not entirely clear. It appears that feeding fish increase GS activity in the brain, increasing brain glutamine production and protecting the brain against ammonia toxicity. Rhesus glycoprotein channels (Rhcg) are upregulated in the gills to augment ammonia excretion following feeding. Acid excretion is also enhanced by upregulation of sodium/proton and V-type H^+ATPase activity. The overall result is that fed fish can more readily excrete ammonia following feeding and have a greater capacity to produce glutamine. Similar responses have been documented following exposure to elevated environmental ammonia.

Exercising fish are very sensitive to environmental ammonia. Fish produce ammonia through the deamination of AMP in skeletal muscle during violent exercise but appear not to invoke protective mechanisms. As a result, swimming fish are much more sensitive to elevations in environmental ammonia than resting fish (**Table 1**).

In summary, ammonia is both a substrate and product of metabolism and, if allowed to accumulate in the body, is toxic. There are postprandial ammonia pulses that would kill a starving fish. Fish, however, have evolved mechanisms to deal with this situation some of which are useful in dealing with elevated levels of environmental ammonia. This is not the case for swimming salmonids who are very sensitive to increases in environmental ammonia.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Evolution of Fish Genomes; Fish as Model Organisms for Medical Research; Transgenesis and Chromosome Manipulation in Fish.

Further Reading

Binstock L and Lecar H (1969) Ammonium ion currents in the squid giant axon. *Journal of General Physiology* 53: 342–361.

Brusilow SW (2002) Hyperammoniemic encephalopathy. *Medicine* 81: 240–249.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Hermenegildo C, Monfor CP, and Felipo V (2000) Activation of *N*-methyl-D-aspartate receptors in rat brain *in vivo* following acute ammonia intoxication: Characterization by *in vivo* brain microdialysis. *Hepatology* 31: 709–715.

Ip YK, Randall DJ, Kok TKT, *et al.* (2004) The mudskipper *Periophthalmodon schlosseri* facilitates actvie NH_4^+ excretion by increasing acid excretion and having a low NH_3 permeability in the skin. *Journal of Experimental Biology* 207: 787–801.

Lande MB, Donovan JM, and Zeidel ML (1995) The relationship between membrane fluidity and permeabilities to water, solute, ammonia, and protons. *Journal of General Physiology* 106: 67–84.

Randall DJ and Ip YK (2006) Ammonia as a respiratory gas in water and air-breathing fishes. *Respiratory Physiology and Neurobiology* 154: 216–225.

Randall DJ and Tsui TKN (2002) Ammonia toxicity in fish. *Marine Pollution Bulletin* 45: 17–23.

Randall DJ, Wilson JM, Peng KW, *et al.* (1999) The mudskipper, *Periophthalmodon schlosseri*, actively transports NH_4^+ against a concentration gradient. *American Journal of Physiology* 46: R1562–R1567.

Randall DJ, Wood CM, Perry SF, *et al.* (1989) Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature* 337: 165–166.

Wicks BJ and Randall DJ (2002) The effect of feeding and fasting on ammonia toxicity in juvenile rainbow trout, Oncorhynchus mykiss. *Aquatic Toxicology* 59: 71–82.

Wright PA and Anderson PM (ed.) (2001) Fish Physiology, Volume 20: Nitrogen Excretion, 358pp. New York: Academic Press.

Ureotelism

PA Wright, University of Guelph, Guelph, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Synthesis of Urea
Retention of Urea in Elasmobranchs and Coelacanths
Urea Excretion
Environmental Impacts on Urea Excretion
Early Life Stages
Further Reading

Glossary

Ammoniotelic An animal that excretes predominantly ammonia.

Antiporter Membrane transporter where two molecules move in opposite directions across the membrane.

Aquaporins Membrane proteins that act as water channels (discovered by Peter Agre for which he won the Nobel prize in 2003).

Branchial Of the gills.

Chorion Proteinaceous coat or egg shell surrounding the embryo.

Convection Bulk movement of a medium, e.g, gas, water or blood.

Cutaneous Of the skin.

Estivation Dormancy in response to the danger of dehydration or high ambient temperatures.

Facilitated transporters Carrier-mediated membrane transporters that enhance the movement of molecules down the gradient.

Glomerulus Tuft of capillaries surrounded by the Bowman's capsule of the vertebrate kidney where blood is filtered to produce urine in the proximal tubule.

Nephron The morphological and functional unit of the vertebrate kidney involved in urine formation.

Osmoconformer An animal that matches its internal osmolarity to the external osmolarity.

Ureotelic An animal that excretes predominantly urea.

Uricolysis The breakdown of uric acid producing urea.

Introduction

In animals, waste products are formed when food is metabolized. Food in the form of protein is hydrolyzed releasing individual amino acids. Catabolism of excess amino acids results in the formation of the waste products, ammonia and carbon dioxide (CO_2). CO_2 can be eliminated by respiration, whereas ammonia must be excreted in solution or converted to other nitrogen products (e.g., urea and uric acid) prior to excretion.

Fish excrete two main nitrogenous end products (ammonia and/or urea) and some minor compounds (such as trimethylamine oxide (TMAO), creatine, creatinine, and amino acids). One major problem that all animals face is the fact that ammonia is a relatively toxic compound when concentrated in body tissues. Ammonia is a major neurotoxin that interferes with cellular and neurotransmitter metabolism, as well as nerve conduction. Therefore, nitrogen excretory strategies must be designed to avoid the toxic accumulation of ammonia. Most fish excrete the majority of their nitrogen wastes as ammonia (ammoniotelic). Ammonia is highly soluble in water, provided water is abundant, then eliminating ammonia is an energetically inexpensive mechanism (it requires $\sim$400 ml water to excrete 1 g of ammonia; but requires no adenosine triphosphate (ATP)). Some amphibious fish in water-restricted habitats, however, may repackage ammonia into urea, a relatively nontoxic end product. The trade-off is that synthesizing urea via the urea cycle is energetically expensive, requiring 5 ATPs per mole of urea produced. Consequently, other amphibious fish have opted for alternative strategies in water-restricted habitats. Some fish eliminate gaseous NH_3 across their skin or through the gut, whereas other species store ammonia in amino acids (e.g., glutamine (GLN)) or reduce ammonia production.

Although water availability is a key factor in the evolution of nitrogen excretion in animals, it is not the whole story. Some teleost fish living in highly alkaline water (pH) excrete no ammonia, only urea (ureotelic). Marine elasmobranch fishes (skates, sharks, and rays) synthesize and retain urea for osmoregulatory purposes. Finally, there is evidence that many teleost fish are ureotelic during early life stages, but switch to ammonotelism as adults. These examples are discussed in more detail in the following.

Synthesis of Urea

In fish there are three possible pathways for urea synthesis: the urea cycle (**Figure 1**), catabolism of dietary arginine by the enzyme arginase (ARG), and/or uricolysis. The urea cycle in fish starts with the fixation of ammonia and glutamate into GLN, catalyzed by the mitochondrial enzyme glutamine synthase (GS). Glutamine (GLN) and HCO_3 are combined to form carbamoyl phosphate, catalyzed by the enzyme carbamoyl phosphate synthetase type III (CPS III). Ornithine transcarbamylase (OTC) is the enzyme that catalyzes the conversion of carbamoyl phosphate to citrulline. In the cytosol, citrulline is converted into arginine in two steps, catalyzed by the enzymes argininosuccinate synthetase (ASS) and lyase (ASL). Finally, arginine is transported back into the mitochondria for final degradation to urea and ornithine, catalyzed by mitochondrial ARG.

When present, the location of urea synthesis is typically in the liver of fishes, where all the necessary enzymes are located. In fish that can complete the urea cycle in the liver, skeletal muscle may also be a secondary site of urea synthesis. Although a functional urea cycle is present also in adult amphibians and mammals, there are several differences in the intracellular location of the enzymes and substrate preferences of CPS.

Most fish have relatively high levels of ARG activity, even if they lack a functional urea cycle. The contribution of dietary arginine catabolism to overall urea excretion in fish is not well understood. In ammoniotelic fish, however, it is generally assumed that a significant portion of urea released is derived from uricolysis. The three enzymes involved in the degradation of uric acid (uricase, allantoinase, and allantoicase) are located in the peroxisomes of liver cells in fish (**Figure 2**). Therefore, in ammoniotelic fish lacking the urea cycle, some nitrogen wastes are excreted as urea and this urea is derived from arginolysis and/or uricolysis.

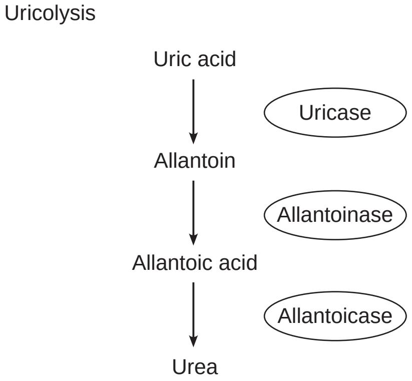


Figure 2 Uricolysis involves the breakdown of uric acid to urea in three steps, catalyzed by the enzymes uricase, allantoinase, and allantoicase.

Retention of Urea in Elasmobranchs and Coelacanths

Marine elasmobranch fishes are osmoconformers and retain urea as an osmolyte to balance internal osmolarity to

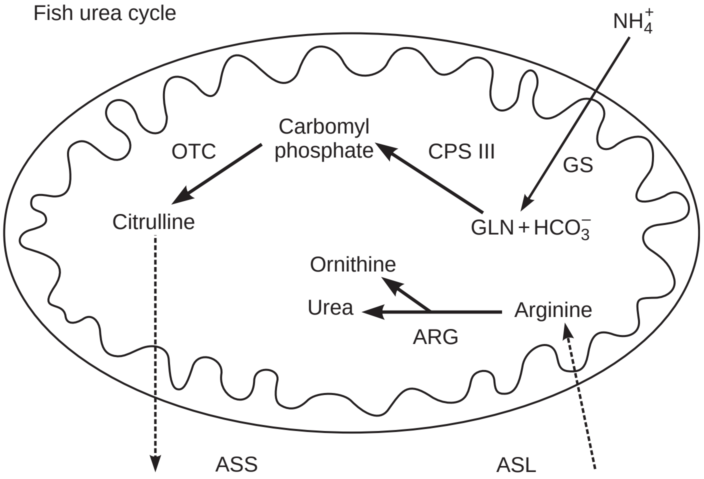


Figure 1 The fish urea cycle consists of five key enzymes (CPS III, carbamoyl phosphate synthetase; OTC, ornithine transcarbamylase; ASS, argininosuccinate synthase; ASL, argininosuccinate lyase; and ARG, arginase) and the accessory enzyme glutamine synthetase (GS) that combines glutamate and $NH4^+$ to produce glutamine (GLN). In fish, four of these six enzymes are positioned within the mitochondria of hepatic and/or skeletal muscle cells. Although elasmobranchs, coelacanths, and lungfish have a functional urea cycle, most teleost fish express the urea cycle during early life stages but not as adults.

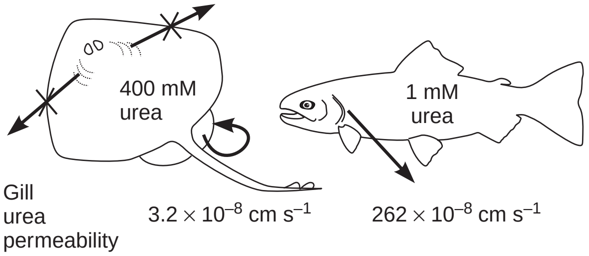


Figure 3 Elasmobranchs are osmoconformers and retain urea as an osmolyte. Elasmobranch gills are relatively impermeable to urea compared to a teleost, such as rainbow trout. Branchial urea permeability is ~80 times greater in a trout relative to an elasmobranch. Urea is reabsorbed by the elasmobranch kidney. These features of the elasmobranch gills and kidney prevent excessive loss of urea to the environment; however, some urea is excreted across the gills.

match the external osmolarity. Thus, urea concentrations are typically between 350 and 450 mM in elasmobranch plasma and tissues, whereas in other fishes urea is considerably lower, typically less than 1/100th of the concentration (**Figure 3**).

Urea at these relatively high concentrations impairs protein function. To combat this, stabilizing solutes (e.g., TMAO, betaine, and taurine) counteract the destabilizing effect of urea. Across many marine elasmobranch species the ratio of urea:stabilizing solutes is about 2:1. Even early embryos use this ureosmotic strategy. For example, urea concentrations are ~400 mM in marine skate embryos (*Raja erinacea*) when they hatch after ~9 months, and the ratio of urea:TMAO and other organic osmolytes is 2:1 as early as 4 months of age.

While elasmobranchs are ureotelic, urea is not a true waste product. Unlike other animals that want to rid themselves of urea, elasmobranchs have evolved mechanisms to prevent excessive urea loss. Why? Foremost, urea is energetically expensive to produce (see above) and excretion would represent a net loss of this energy investment. Second, they require high internal levels to maintain isosmotic balance. Therefore, sharks have evolved mechanisms to retain urea at potential sites for loss.

The two main sites of potential urea loss are the gills and kidney (**Figure 3**), and both have mechanisms to retain urea in the blood. In the kidney, plasma urea moves across the glomerulus into the filtrate, but urea is reabsorbed from the filtrate back into the plasma so that very little urea is lost in the urine. The elasmobranch nephron structure is very complex and the detailed mechanisms involved in renal urea reabsorption are not well understood. In euryhaline elasmobranchs, moving from seawater to dilute seawater initially increases the amount of urea lost in the urine and decreases the plasma osmolarity and urea concentration after a few days. Remarkably, freshwater elasmobranchs from the Amazon River (*Potamotrygon* sp.) lack the urea cycle enzymes, and plasma urea concentrations are as low as in ammonotelic teleosts.

Urea loss across the gills in elasmobranchs is much lower (~80 times lower) relative to teleost fish (**Figure 3**). Two factors help to explain this difference. First, there is an active urea transporter in the gills (see below) that returns urea back to the blood. In addition, the gill cell membranes have an exceptionally high cholesterol content inserted into the membrane phospholipids that impedes passive diffusion of small-molecular-weight solutes, such as urea. Therefore, although urea is lost to seawater at the gills down a diffusion gradient, much less urea is lost given the concentration gradient because of the transport protein and lipid composition of the gill cell membranes.

Compared with elasmobranchs, very little information exists for urea retention in coelacanths. The coelacanth *Latimeria* was first discovered in 1938 off the Comoro Islands, but very few live specimens have since been examined. Plasma and tissue urea levels appear to be similar to marine elasmobranchs (~350 mM) and they also retain TMAO. Presumably, their major nitrogen excretory product is urea and like elasmobranchs they likely have strategies to limit urea loss. This is just one of the many gaps in our knowledge of ureosmotic regulation in coelacanths.

Urea Excretion

In most fish, nitrogen excretion occurs primarily across the gills because of the high surface area for exchange between blood and water, and the relatively high permeability of the gill epithelium to small-molecular-weight solutes such as ammonia and urea. Branchial urea excretion depends on the blood-to-water urea concentration gradient, although water urea concentrations are usually negligible. On average, fully aquatic teleost fish excrete about ~15% of their nitrogen wastes as urea and ~85% as ammonia. A small percentage of urea is excreted in the urine or across the skin in water-breathing fish, but, in amphibious fish on land, more urea may be lost across the posterior end of the fish (i.e., skin and urine).

There are three fundamental ways in which urea may cross the epithelial cells that form the barrier between blood and water: through membrane protein transporters; by lipid-phase permeation; or between cells (paracellular pathway). Evidence suggests that in most cases urea is transported by membrane proteins, through either urea transporters (UTs) or, in some cases, water channels (aquaporins).

The first UT identified in fish was cloned from shark kidney and the gene sequence is similar to mammalian kidney-facilitated UTs. UTs are found in the gills of both marine and freshwater fishes. These UTs are facilitated bidirectional transporters that allow urea to diffuse down the concentration gradient (no ATP required), saturated at higher urea concentrations and inhibited by the drug phloretin. UTs are expressed during early fish development and have been shown in zebrafish larvae to be critical for maintaining urea excretion rates (see below).

Another class of UT proteins is found on the basolateral membrane of shark gill epithelium. These are secondary active urea transporters, where urea is transported against the gradient linked to the downhill movement of Na^+. In other words, some urea diffuses passively from the blood into the gill cell, but the urea/Na^+ antiporter moves urea back to the blood, recovering some of the lost urea. Low Na^+ concentrations are maintained by the Na^+K^+ATPase pump on the basolateral membrane (cost 0.5 ATP/Na^+). Therefore, to save one urea molecule that has cost 5 ATP to be synthesized, just 0.5 ATP are spent. The gene sequence for active UT has not been determined in fish (or mammals).

Environmental Impacts on Urea Excretion

Water availability has probably played an important role in the evolution of ureotelism in fishes. When lungfish (*Protopterus*, *Lepidosiren*) estivate, urea, not ammonia, accumulates in the tissues. Thus, urea synthesis via the urea cycle prevents ammonia accumulation over weeks or months out of water. Elevated urea levels occur only during estivation. When African lungfish (*Protopterus*) return to water after estivation, a large washout of urea occurs immediately during the first day, one that is correlated with the induction of UT messenger RNA (mRNA) levels. Once back in an aquatic habitat lungfish can easily excrete ammonia across the gills and again become ammoniotelic.

Most adult teleost fish do not have the capacity to synthesize urea at high rates. However, a few teleost species have a functional urea cycle that allows them to turn on urea production whenever water is limiting in the environment (e.g., *Heteropneustes fossilis*) or in response to elevated water ammonia levels (e.g., *Mugilogobius abei*). In other cases, fish are ureotelic because the chemical composition of the water prevents efficient ammonia elimination.

The tilapia (*Alcolapia grahami*) of Lake Magadi, Kenya top the list as the most ureotelic of teleosts. They thrive in waters of pH 10, 37 °C with high levels of carbonate salts. Ammonia is converted to urea via the urea cycle and they excrete 100% of their nitrogen wastes as urea, mostly across the gills. In other cases where teleost fishes inhabit somewhat less severe alkaline lakes, fish are primarily ammoniotelic but urea excretion is considerably elevated compared to fish in neutral pH waters. For example, the Lahontan cutthroat trout *Oncorhynchus clarki henshawi* found in Pyramid Lake, Nevada (pH 9.4, 10 °C) excrete ~34% of their nitrogen wastes as urea and the tarek *Chalcalburnus tarichi* of Lake Van, Turkey (pH 9.8, 18 °C) excrete 37% as urea. Thus, fish faced with a chronic high pH environment typically excrete more of their nitrogen wastes as urea. This is most simply explained by the fact that as blood pH increases, an increasing proportion of the total ammonia wastes in fishes exists as the more toxic form NH_3 rather than $NH_4{}^+$.

An interesting teleost that can switch between ammoniotelism and ureotelism and induce urea synthesis via a hepatic urea cycle is the marine gulf toadfish, *Opsanus beta*. Under laboratory conditions, toadfish rapidly switch from ammonia excretion to urea synthesis and excretion during stressful conditions, such as crowding, confinement, elevated environmental ammonia, or air exposure. Stressed toadfish eliminate urea across the gills in pulses over short time intervals (1–3 h) per day. So far, this pattern of pulsatile urea excretion has not been described for any other fish. Researchers were perplexed about the physiological value of excreting urea in pulses. Recent evidence suggests that this unusual pattern of urea excretion may have evolved as a predator-avoidance strategy. When toadfish excrete ammonia and urea together, the urea functions as a cloaking or masking molecule that prevents predators such as the gray snapper (*Lutjanus griseus*) from detecting the ammonia. Ammonia excreted alone may be a strong chemical attractant for some fish predators. These results in toadfish underline the importance of understanding the ecological connections of a particular fish in order to understand the possible adaptive value of ureotelism.

Early Life Stages

Nitrogen metabolism and excretion during early life stages in fish is not simply a replica of adult patterns on a smaller scale. There are three key differences. First, embryos feed endogenously, rapidly consuming yolk amino acids and proteins to provide fuel for growth and development. The potentially toxic ammonia molecule is continuously produced from amino acid catabolism. In adult fish, amino acids are generally spared from catabolic processes in favor of carbohydrates and fats.

Second, fish embryos and early larval stages rely on cutaneous excretion, whereas in most adult fish excretion is largely branchial. Cutaneous exchange is not supported by the convection of water over its surface, or by a dense capillary network to support blood convection. In addition, diffusion distances can be greater, slowing the rate of diffusion of nitrogeneous wastes at a time when they are being produced very fast.

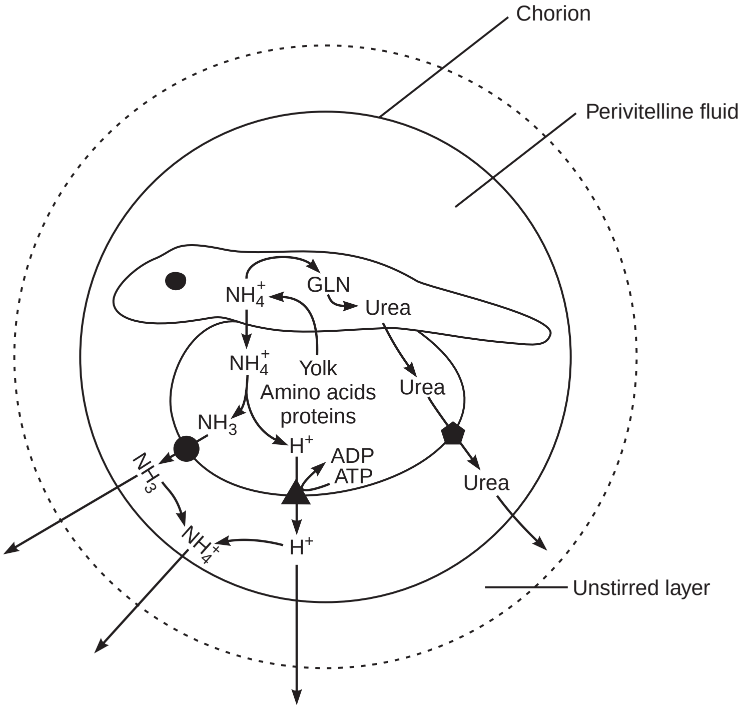


Figure 4 Ammonia is produced by the catabolism of yolk amino acids and proteins in fish embryos. Some ammonia is converted to glutamine (GLN) and urea via the urea cycle. Ammonia and urea are transported across the cutaneous surface by membrane proteins. The solid circle represents the Rhesus (Rh) glycoproteins that transport NH_3. The solid triangle represents the H^+ATPase pump that transports H^+s creating a slightly acidic environment that promotes NH_3 excretion. The solid pentagon represents facilitated urea transporters (UTs) that transport urea down the gradient from embryo to the perivitalline fluid. An unstirred or boundary water layer is present, adjacent to the chorion where molecules move only by diffusion.

Third, developing embryos are encapsulated within a shell or chorion, surrounded by the perivitelline fluid (**Figure 4**). This creates a stagnant layer of water both inside and outside the chorion, which further adds to the diffusion barrier. Nitrogen excretory products are present at a higher concentration in the perivitelline fluid and external unstirred layer compared to the surrounding environment. One might predict that ammonia elimination from the developing embryo would be more efficient in cases where embryos were exposed to high water flows that, in turn, reduce the thickness of the unstirred water layer. Fish embryos convert ammonia to GLN and/or urea via the urea cycle, which may be a strategy to prevent the accumulation of ammonia during critical stages of development.

Rainbow trout embryos provided with radiolabeled bicarbonate were shown to synthesize radiolabeled urea, experiments that provided the first evidence for a functional urea cycle in any teleost fish. Later experiments showed that embryos of several species of teleosts, both freshwater and marine, had the key urea cycle enzyme CPS III (**Figure 1**), as well as other required enzymes for the urea cycle. Overall, activities of the urea cycle enzymes are relatively high during early stages but may be low or absent in adult stages, except in the case of ureotelic species (see above).

The pattern of nitrogen excretion in embryos varies among species. Some embryos are ureotelic, for example, zebrafish *Danio rerio* and Atlantic cod *Gadus morhua* but as adults are ammoniotelic. Rainbow trout *O. mykiss*, on the other hand, are ammoniotelic during all life stages. These differences in relative urea excretion rates may be due to differences between rearing environments or the timing of development stages. In the viviparous blenny *Zoarces viviparous*, for example, synthesis and excretion of urea by embryos may be advantageous in protecting the mother and other embryos from exposure to toxic levels of ammonia.

Urea excretion in early stages depends on the facilitated UT protein, at least in freshwater rainbow trout and zebrafish. Ammonia transporters that belong to the Rhesus (Rh) glycoprotein family facilitate NH_3 diffusion from the embryo. Acid transport of H^+ ions via H^+ATPase ensures an external water environment close to the skin that is sufficiently acidic to maintain the embryo-to-environment NH_3 gradient by rapidly removing excreted NH_3 by converting NH_3 to $NH_4{}^+$ according to the pK for this reaction (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). Most of the urea and ammonia excreted probably exits in the yolk sac membrane where blood vessels lie just under the surface. In addition, the chorion has small pores that allow for the diffusion of small-molecular-weight wastes (urea, ammonia) into the environment. After hatching when the chorion is shed, early larvae still depend on cutaneous nitrogen excretion. However, whole body and pectoral fin movements probably enhance nitrogen excretion by disturbing the unstirred layers and increasing the convection of water over the surface.

In summary, urea is produced by all fish, but only those that excrete at least 50% of their nitrogenous wastes as urea are considered ureotelic. Elasmobranchs, coelacanths, and lungfish use the urea cycle to synthesize urea. Most adult teleost fishes lack a functional urea cycle, yet the pathway is complete in teleost embryos. Under some extreme environmental conditions, urea synthesis may occur in a few rare teleost fishes to prevent the toxic accumulation of ammonia. Urea is transported across cell membranes by UTs, either facilitated or secondary active transporters.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Water Balance and Aquaporin.

Further Reading

Anderson PM (2001) Urea and glutamine synthesis. In: Wright PA and Anderson PM (eds.) *Fish Physiology*, vol. 20, pp. 239–277. New York: Academic Press.

Barimo JF and Walsh PJ (2006) Use of urea as a chemosensory cloaking molecule by a bony fish. *Journal of Experimental Biology* 209: 4254–4261.

Braun MH, Steele SL, and Perry SF (2009) Nitrogen excretion in developing zebrafish (*Danio rerio)*: A role for Rh proteins and urea transporters. *American Journal of Physiology – Renal Physiology* 296: F994–F1005.

Felskie AK, Anderson PM, and Wright PA (1998) Expression and activity of carbamoyl phosphate synthetase III and ornithine urea cycle enzymes in various tissues of four fish species. *Comparative Biochemistry and Physiology* 119B: 355–364.

Fines GA, Ballantyne JS, and Wright PA (2001) Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. *American Journal of Physiology – Integrative and Comparative Physiology* 280: R16–R24.

Iwata K, Kajimura M, and Sakamoto T (2000) Functional ureogenesis in the gobiid fish, *Mugilogobius abei*. *Journal of Experimental Biology* 203: 3703–3715.

Janssens PA and Cohen PP (1966) Ornithine–urea cycle enzymes in the African lungfish, *Protopterus aethiopicus*. *Science* 152: 358–359.

Mistry AC, Honda S, Hirata T, Kato A, and Hirose S (2001) Eel urea transporter is localized to chloride cells and is salinity dependent. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 281: R1594–R1604.

Pilley CM and Wright PA (2000) The mechanisms of urea transport by early life stages of rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 203: 3199–3207.

Randall DJ, Wood CM, Perry SF, *et al.* (1989) Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature* 337: 165–166.

Saha N, Das L, Dutta S, and Goswami UC (2001) Role of ureogenesis in the mud-dwelled Singhi catfish (*Heteropneustes fossilis)* under condition of water shortage. *Comparative Biochemistry and Physiology* 128A: 137–146.

Walsh PJ, Grosell M, Goss GG, *et al.* (2001) Physiological and molecular characterization of urea transport by the gills of the Lake Magadi tilapia (*Alcolapia grahami)*. *Journal of Experimental Biology* 204: 509–520.

Wright PA (1995) Nitrogen excretion: Three end products, many physiological roles. *Journal of Experimental Biology* 198: 273–281.

Wright PA and Fyhn HJ (2001) Ontogeny of nitrogen metabolism and excretion. In: Wright PA and Anderson PM (eds.) *Fish Physiology*, vol. 20, pp. 149–200. New York: Academic Press.

Yancey PH, Clark ME, Hand SC, Bowlus RD, and Somers GN (1982) Living with water stress: Evolution of osmolyte systems. *Science* 217: 1214–1222.

HORMONAL CONTROLS

The Pituitary

Contents

Development of the Hypothalamus-Pituitary-Interrenal Axis
Pituitary Gland or Hypophysis

Development of the Hypothalamus-Pituitary-Interrenal Axis

D Alsop, McMaster University, Hamilton, ON, Canada

N Aluru, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Components of the HPI Axis
Ontogeny of the HPI Axis
HPI Axis Development in Zebrafish
Stress Axis Development in Other Teleosts
Conclusion
Further Reading

Glossary

Adrenocorticotropic hormone (ACTH) A protein hormone primarily produced by the corticotropic cells of the anterior pituitary that causes release of cortisol from the interregnal.

Corticotropin-releasing factor (CRF) A neuropeptide secreted by the paraventricular nucleus of the hypothalamus. CRF stimulates the corticotropic cells of the anterior pituitary to secrete ACTH.

Glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) Ligand-activated, nuclear hormone receptors (subfamily 3, group C) that bind steroids such as cortisol. Upon activation, the receptors bind to glucocorticoid response elements in the genome, upstream of target genes.

Melanocortin receptor 2 (MC2R) A G-protein-coupled receptor belonging to the melanocortin family of receptors. MC2R specifically binds ACTH and is highly expressed in the cellular membrane of the interrenal cells.

Stress The disruption of homeostasis with exposure to a biotic or abiotic factor.

Whole genome duplication The duplication of the entire genome. It is thought that a genome duplication event 350 MYA in an ancient fish led to the massive expansion and diversification of the teleost group, and is also responsible for the 2000–3000 duplicate genes that exist in teleosts today.

Introduction

Stress is the disruption of homeostasis from exposure to a biotic or abiotic stressor. In vertebrates, a highly conserved response to stress is an elevation of plasma corticosteroid levels, which serve to restore homeostasis. The exact nature of this response is variable and dependent on a number of factors, including the type, duration, and intensity of the stressor. However, circulating corticosteroid concentrations have long been considered a reliable indicator of the activation of the stress response.

Cortisol is the primary corticosteroid in teleosts and has been implicated in a wide array of biochemical, physiological, and behavioral functions, including the release of glucose from stored glycogen to provide energy for the stress recovery process (**see also Hormonal Responses to Stress**: Hormone Response to Stress). Beyond metabolism, cortisol action also modulates feeding and growth (**see also Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism), immune responses, osmoregulation (**see also Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of

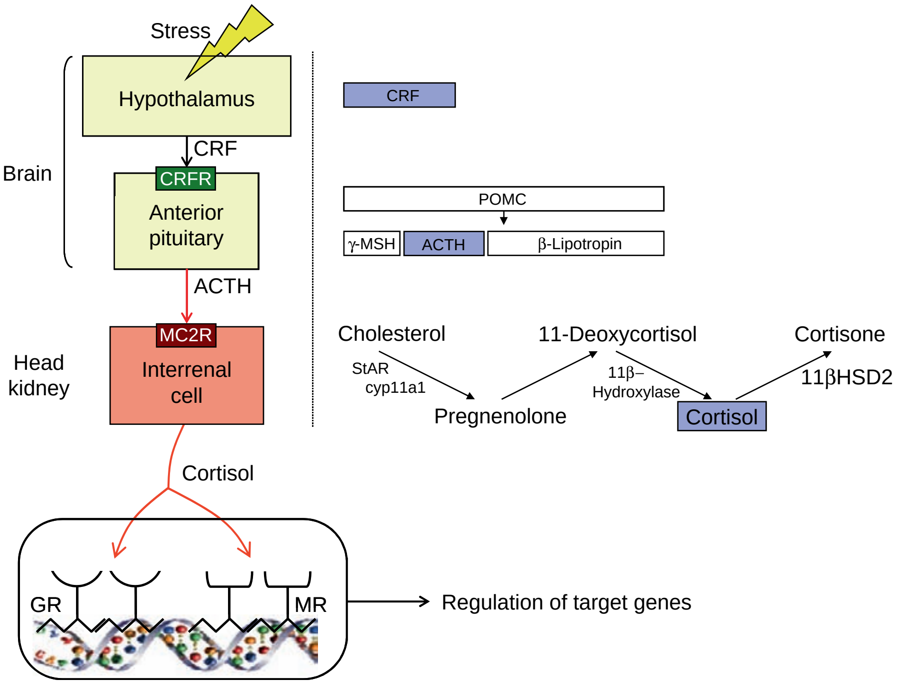


Figure 1 Overview of the hypothalamus–pituitary–interrenal (HPI)-axis anatomy and signaling pathways. Upon exposure to a stressor, the hypothalamus secrets corticotrophin-releasing factor (CRF), which stimulates the pituitary corticotropic cells to release adernocorticotropic hormone (ACTH) to the circulation. ACTH is one of many signaling molecules derived from the proopiomelanocortin (POMC) gene. Circulating ACTH binds specifically the melanocortin receptor type 2 (MC2R) expressed by the interrenal cells of the headkidney. MC2R induces cAMP signaling pathways that stimulate the cortisol synthesis from cholesterol and subsequent secretion to the bloodstream. In turn, circulating cortisol can enter a cell and bind to the glucocorticoid or mineralocorticoid receptors (GR and MR, respectively). These receptors bind to glucocorticoid response element upstream of target genes, thereby up- or downregulating their expression.

Osmoregulation in Teleost Fish) and reproduction (**see also Hormonal Responses to Stress**: Impact of Stress in Health and Reproduction). Cortisol signaling is not only critical in the recovery from stress, but also regulates many of these processes under unstressed conditions.

In teleosts, circulating cortisol levels are regulated by the functioning of the hypothalamus–pituitary–interrenal (HPI) axis (**Figure 1**), which is homologous to the mammalian hypothalamus–pituitary–adrenal (HPA) axis (**see also Hormonal Responses to Stress**: Hormone Response to Stress). While the pathways and functions of the stress axes are highly conserved among vertebrates, some differences do exist between fish and mammals. For instance, fish do not possess a discrete adrenal gland, but instead the steroidogenic cells (interrenal cells) are distributed throughout the head kidney region, primarily along the posterior cardinal veins and their branches. Also, the teleost genome underwent a whole genome duplication event 350 million years ago (MYA), resulting in two copies of a variety of stress axis genes (which have, in some cases, differentiated) compared to other jawed vertebrates. Another major difference is that only teleosts possess a caudal neurosecretory system (CNSS), which produces some of the same stress-related neuropeptides as the hypothalamus.

The functions of the HPI and HPA axes have been extensively studied in juvenile and adult animals, but little information exist on the ontogeny of the cortisol stress response. There is mounting evidence, primarily in birds and mammals, that stressful events occurring during the early life stages can predispose an organism to different pathological states as an adult. This has led to the paradigm "developmental origins of adult disease". For example, prenatal undernutrition in rats and sheep results in cardiovascular alterations such as hypertension. This is thought to be caused by decreased corticosteroid feedback control of the HPA axis that is programmed during fetal development. Very little is known about the long-term effects of stress axis modulation during embryonic and larval development in fish.

This article first reviews the HPI axis and cortisol signaling in teleosts, focusing primarily on molecular pathways. The second section describes the ontogeny of the stress axis, both the molecular pathways and the timing of the functional activation of the cortisol stress response. The subsequent section focuses on zebrafish

(*Danio rerio*), due to the popularity of this species in developmental and molecular studies. Development of the cortisol stress response in other teleosts, including common carp (*Cyprinus carpio*) yellow, perch (*Perca flavescens*) rainbow, trout (*Oncorhynchus mykiss*), and Atlantic salmon (*Salmo salar*), are also addressed.

Components of the HPI Axis

Hypothalamus

The hypothalamus receives inputs from various elements of the central (e.g., cerebral cortex and olfactory system) and peripheral (e.g., viscera) nervous systems. If the hypothalamus interprets an environmental or homeostatic cue as stressful, corticotrophin-releasing factor (CRF; **Figure 1**) is secreted from the nucleus preopticus (NPO) in the hypophysis and plays a key role in coordinating the neuroendocrine, autonomic, and behavioral responses to stress. Axons of the NPO CRF cells project directly into the anterior pituitary gland via synapses, or into the circulation via neurosecretion in the pars nervosa. In addition to CRF, CRF-binding protein (CRFBP) and CRF receptors (CRFR1 and R2) are involved in the regulation of the stress response by modulating CRF action. Several CRF-related neuropeptides such as urotensin I (UI) and urocortin 2 and 3 also control the HPI axis in teleosts through activation of urotensin and CRF receptors, similar to other vertebrates. CRF and UI are not only secreted by the hypothalamus, but are also secreted from the CNSS directly into the circulation in response to stressors to allow direct interaction with interrenal tissue and epithelial tissues.

Pituitary

CRF released to the anterior pituitary binds to CRF receptors (R1 and R2) in the membrane of the corticotropic cells. These are G-protein-coupled receptors and activation stimulates cAMP signaling pathways and the release of adrenocorticotropic hormone (ACTH) to the circulation. ACTH is derived from the proopiomelanocortin (POMC) gene, which is a precursor to a number of smaller signaling peptides (**Figure 1**). However, ACTH is the main product of POMC in the corticotropic cells. All POMC-derived peptides bind to and activate the G-protein-coupled melanocortin receptors in target tissues and ACTH specifically binds to the melanocortin 2 receptor (MC2R) located in target tissues.

Interrenal

ACTH binding to MC2R in the interrenal cells stimulates adenylate cyclase and cAMP-dependent signaling pathways such as protein kinase A, to stimulate cortisol synthesis. The increase in cAMP induced by MC2R is dependent upon receptor interactions with melanocortin receptor accessory proteins (MRAPs). Three of these proteins are found in zebrafish (MRAP1, 2a, and 2b), whereas two have been identified in mammals. MRAPs are also essential for the initial receptor protein folding, glycosylation, and translocation to the cell membrane.

In the interrenal cells, the initial step in steroidogenesis is the transport of cholesterol from the outer to inner mitochondrial membrane. Although the precise mechanism by which this cholesterol transport is accomplished is still uncertain, the steroidogenic acute regulatory protein (StAR) and the peripheral-type benzodiazepine receptor (PBR) are thought to play a key role in this mitochondrial intermembrane shuttling. The initial, rate-limiting enzymatic step is the conversion of cholesterol to pregnenolone, catalyzed by cyp11a1 (also known as cytochrome P450 side-chain cleavage (P450scc); **Figure 1**). Pregnenolone is further metabolized by a series of cytochrome P450 steroid hydroxylases and dehydrogenases leading to the production of cortisol. StAR, PBR, and cyp11a1 are particularly responsive to ACTH signaling (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids).

Nuclear Receptors and Cortisol Signaling

Cortisol signaling in target tissues is mediated by glucocorticoid and mineralocorticoid receptors (GR and MR, respectively), which belong to the nuclear receptor superfamily of ligand-bound transcription factors. GR is ubiquitously expressed, indicating a widespread adaptive role for cortisol. A primary target for cortisol is the GR in the liver, which plays a key metabolic role for providing energy while coping with stressors (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids). The stress-induced disturbance of homeostasis results in the upregulation of energy-demanding pathways, and in fish, cortisol enhances liver gluconeogenic capacity leading to elevated glucose liberation. This response is a key aspect of the stress recovery process, as glucose is the preferred fuel for tissues, in particular the gill and brain. GR activation by cortisol also regulates diverse processes such as osmoregulation, protein metabolism, the immune response, growth, reproduction, and behavior.

Cortisol is a very high affinity ligand for MR; however, 11βHSD2 is often co-expressed with MR in tissues such as the gill and kidney. This enzyme deactivates cortisol by metabolizing it to cortisone, which does not bind to MR. In mammals, this enzyme allows hormones such as aldosterone, which is at a much lower concentration than cortisol, access to MR. Aldosterone does not appear to be synthesized in fish, but 11-deoxycorticosterone (DOC) is instead the leading candidate as the teleost MR-specific ligand. However, a physiological role for DOC signaling via MR activation is still lacking in fish. The current thinking is that cortisol may be playing a key role in MR activation, while the specific effect associated with this receptor activation, including expression of MR-responsive genes, is unknown in fish.

Duplicated HPI Axis Genes

The whole genome duplication event in fish 350 MYA resulted in a number of duplicated genes in the HPI axis. For example, carp possess two genes for CRF, ACTH (POMC) and GR. However, zebrafish are unique among teleosts in that they have returned to single gene systems for CRF, ACTH, and GR (although zebrafish have two genes that encode POMC, the cleavage site for ACTH is mutated in one copy, resulting in ACTH derived from only one gene). Although this duplicate stress gene loss may have simplified studies on the development of the zebrafish stress axis, it also raises some very interesting questions regarding genome evolution and the functions of single versus duplicate gene systems.

Ontogeny of the HPI Axis

From early embryogenesis, components of the stress axis undergo dynamic changes in expression suggesting that they are functional at this time. However, aside from the well-defined role of cortisol and GR in fetal lung maturation in mammals, the specific stress- or basic developmental-related functions of the HPI/HPA components in the embryo are not well understood.

Embryos and larvae of oviparous teleosts have continuous access to cortisol because of a maternal deposit of cortisol in the oocyte. The temporal profile of this steroid during development has been documented in a variety of species (although similar cortisol profiles have yet to be characterized in viviparous and ovoviviparous fish). In all species examined, this deposited cortisol is depleted from the oocyte as embryonic development progresses, reaching a minimum level around hatch, after which time, the larva begins to synthesize cortisol and basal levels increase (**Figure 2**). However, when is the cortisol stress axis first responsive to stress? This section reviews the molecular ontogeny of the HPI axis with reference to the timing of basal and stress-induced cortisol production.

The majority of the work on the temporal expression of signaling molecules, receptors, and enzymes of the HPI axis during teleost development has been performed with zebrafish. The findings from this species are summarized first, followed by information that is available for salmonids, common carp, and yellow perch (primarily cortisol measurements).

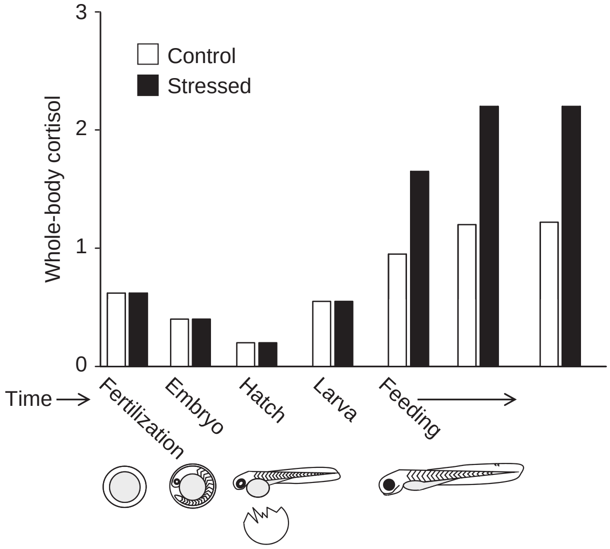


Figure 2 Representative whole-body cortisol levels (arbitrary units) in developing teleost embryos and larvae. Fish oocytes contain a maternal cortisol deposit, and after fertilization basal (open bars) cortisol levels decrease and reach a minimum around the time of hatching (approximately 2–3 dpf for zebrafish, 10 dpf for perch, and 4 wpf for salmonids). The newly hatched larva is able to synthesize cortisol and basal levels begin to rise. In contrast, cortisol levels after a handling stressor (black bars) do not increase above basal levels until after hatch, toward the onset of exogenous feeding. The exception is carp, which display an accelerated HPI-axis development.

HPI Axis Development in Zebrafish

The Mature Oocyte

Maternal factors are essential during early development as the initial steps of embryogenesis are driven solely by maternal mRNAs or proteins that were produced during oogenesis. In most animals, a substantial portion of embryogenesis occurs prior to the activation of zygotic transcription, which normally begins during the mid-blastula transition (MBT; approximately 2.5 hours post-fertilization (hpf) in zebrafish).

In zebrafish, a number of maternal HPI-axis transcripts are expressed in the mature oocyte or zygote. There is an abundance of transcripts for CRF, CRFBP, CRF receptors (CRFR1 andR2), and GR, while there are very few transcripts for StAR, 11β-hydroxylase and 11βHSD2. In addition, there is a maternal deposit of cortisol (4 pg/egg in zebrafish). Although present, the exact roles of maternally derived cortisol or HPI-axis transcripts in the early embryo are unknown.

Embryogenesis

During embryogenesis, there are dramatic changes in the expression of HPI-axis genes. In addition, the maternal cortisol deposit is being continuously depleted, but cortisol levels in the embryo are not responsive to a stressor (**Figure 2**).

CRF signaling system. CRF transcripts are detected throughout zebrafish embryogenesis. CRF signal is observed by 25 hpf in the primordial hypothalamus, which has begun to form as a ventral expansion of the diencephalon. By 48 hpf, CRF-positive cells are observed in bilateral pairs of cells in the preoptic region of the hypothalamus, as well as outside the hypothalamus, in the thalamus and rostral medulla oblongata (**Figure 3**(**b**)). The distribution of CRF expression outside the hypothalamus suggests that CRF may be involved in processes other than HPI-axis signaling, especially since the cortisol stress response is not activated at this time in response to a stressor. Indeed, CRF has been shown to play other roles in mammals, where it induces astrocyte proliferation in the developing rat brain. CRFBP is also expressed in many brain regions (**Figure 3**(**d**)), although the exact role of CRFBP in CRF signaling is unclear. Studies, to date, suggest that CRFBP prevents CRF from binding to its receptors and subsequent signaling.

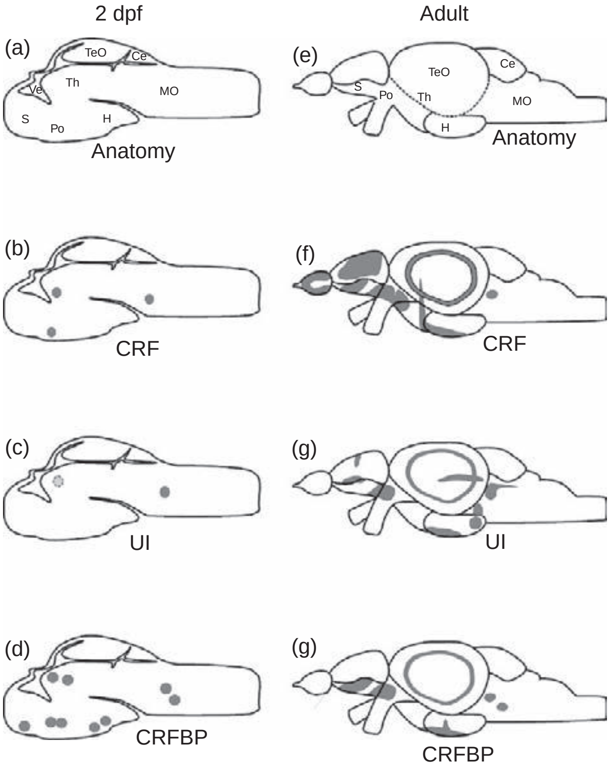


Figure 3 Summary of regional gene expression for corticotrophin-releasing factor (CRF), urotensin I (UI), and CRF-binding protein (CRFBP) in the zebrafish brain at 2 days post-fertilization (dpf; left panel) and in adult male fish (right panel). Schematics of the brain for 2 dpf (a) and adults (e) in rostro-caudal orientation are shown for reference, and brain regions that are identifiable at 2 dpf are indicated. A comparison between 2 dpf and adult regional expression is shown for CRF ((b) and (f), respectively), UI ((c) and (g), respectively), and CRFBP ((d) and (h), respectively). Each gray circle in (b), (c), and (d) represents the approximate location of a pair of cells that expressed the respective gene of interest in all 2 dpf brains analyzed. The open circle in (c) represents the approximate location of a pair of cells that expressed UI in half of the brains analyzed. Ce, cerebellum; H, hypothalamus; MO, medulla oblongata; Po, pre-optic region; S, subpallium; TeO, optic tectum; Th, thalamus; Ve, ventricle. Courtesy of Sarah Alderman and Nicholas Bernier, University of Guelph, Canada.

UI was not detected in the embryonic or larval hypothalamus, although low levels were observed in the thalamus and medulla oblongata (**Figure 3**(**c**)). In contrast, UI is highly expressed in the adult brain (**Figure 3**(**g**)).

CNSS. There is no change in CRF or UI transcript abundance in the tail region during embryonic or larval development, although levels are much higher in the adult. The functional significance or stress responsiveness of the CNSS during development is unknown in fish.

POMC/ACTH. POMC is first detected in the pituitary anlage at 24 hpf and is expressed in an anterior/posterior organization starting around 48 hpf. At this time, the anterior corticotropic cells synthesize the ACTH peptide. It is unknown whether development of corticotropic cells is regulated by the hypothalamus and CRF secretion, as is the exact timing of corticotropic responsiveness to CRF. Regardless, POMC and ACTH are strongly localized in the anterior pituitary prior to hatch, although the stress response is nonfunctional.

Cortisol biosynthesis. One of the earliest molecular markers of the developing interrenal tissue is the localized expression of the fushi tarazu factor 1β (*ff1b*), a transcription factor, and homolog of the mammalian steroidogenic factor 1. The initial expression of *ff1b* occurs at 22 hpf and is required for interrenal cell differentiation. It is also responsible for the initiation of P450scc expression at 24 hpf, which is the rate-limiting enzyme in steroidogenesis. StAR is also initially detected in the primordial interrenals at 24 hpf, followed by 3β-hydroxysteroid dehydrogenase (3β-HSD) (28 hpf) and MC2R (32 hpf). The expression of 11β-hydroxylase, which catalyzes the final step of cortisol synthesis, increases by 48 hpf, around the time of hatch. This is immediately followed by a rise in basal whole-body cortisol levels.

Interrenal development over the first 48 hpf occurs independently from higher signals such as ACTH. This is demonstrated by MC2R knockdown morphants or mutants that lack corticotropic cells (and therefore ACTH), which maintains both normal interrenal tissue development and the expression of steroidogenic enzymes. However, by 5 days post-fertilization (dpf), a lack of ACTH signaling results in the loss of steroid synthesizing capacity as genes involved in steroidogenesis are undetectable at this time. This indicates that

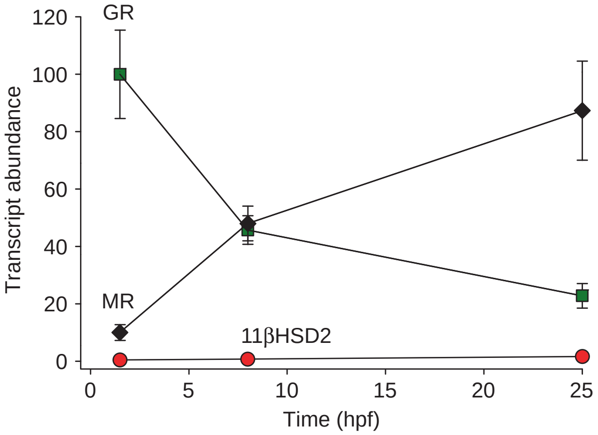


Figure 4 Glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) expression (arbitrary units) in zebrafish embryos. The paucity of 11βHSD2 transcripts suggests that cortisol has access to MR during embryonic development, which is steadily increasing in transcript number.

interrenal function is autonomous over the first 2 dpf, but control is transferred to higher levels by 5 dpf.

ACTH and CRF in the pituitary and hypothalamus, respectively, are expressed by 48 hpf, and the interrenals are beginning to produce cortisol. The brain appears to be poised to activate cortisol production in response to a stress. However, salinity and handling stressors do not stimulate a cortisol stress response at this point in time.

Glucocorticoid and mineralocorticoid receptors. The initial abundance of maternally derived GR transcripts in the mature oocyte is unstable and is rapidly degraded (**Figure 4**). This suggests that GR signaling may not be critical during early embryonic development. By hatch, GR transcript numbers have rebounded and remain elevated. GR signaling may be critical at this time for metabolic energy repartitioning during the onset of feeding and growth.

In contrast to GR, MR transcripts increase steadily from fertilization to feeding (**Figure 4**). In addition, there are very few 11βHSD2 transcripts in the embryo, which would suggest that cortisol is not deactivated and may be a ligand for MR. The embryo has continuous access to cortisol, from the maternal deposit during the early stages, or from *de novo* synthesis beginning around hatch.

The lack of a cortisol stress response during embryogenesis suggests that GR and MR signaling may be regulating basic aspects of development such as metabolism or osmoregulation as in adults. In mammals, GR regulates fetal carbohydrate metabolism, based on observations in GR knockout animals that display altered expression of key gluconeogenic enzymes.

Summary. The HPI axis appears to be fully expressed and the interrenal cells are producing cortisol by hatch. However, there is no cortisol stress response during early embryogenesis. Whether the cortisol response is delayed simply due to developmental constraints or is intentional to protect against elevated cortisol signaling during sensitive stages (similar to the stress hypo-responsive period in postnatal mammals) is unknown.

Hatching – Embryo to Larva Transition

Hatching begins at 48 hpf in zebrafish and this coincides with further increases in the expression of CRF, StAR, 11β-hydroxylase, 11βHSD2, and the rebound of GR. At hatch, whole-body cortisol levels are beginning to rise as synthesis is initiated. In spite of these developments, cortisol levels in zebrafish are unresponsive to salinity or handling stressors. It appears that development of the sensory inputs to the hypothalamus, critical for stress perception and CRF release, is the limiting step delaying the activation of the cortisol stress response during development.

The stress response is first observed at 3 dpf (72 hpf), where cortisol levels in freshwater reared larvae are elevated after exposure to increased salinity (seawater). Although this is the earliest indication of a functional HPI axis, a handling stress (swirling around in a glass vial) at this same stage does not elicit an increase in cortisol. One hypothesis to explain this difference is that handling and osmotic challenges activate different signaling pathways, which have reached different degrees of functional development by 3 dpf. The HPI axis appears to be fully functional and receptive to different types of stressors at 4 dpf (approaching the onset of exogenous feeding). At this time both an osmotic challenge and handling stress elicit an increase in cortisol levels.

Stress Axis Development in Other Teleosts

Common Carp

The common carp and zebrafish are both cyprinids and have similar developmental rates and times to hatch (2–3 dpf). However, the cortisol stress response develops at an earlier stage in carp: basal cortisol and ACTH levels increase well before hatch, and also increase in response to stressor exposure before hatching. The evolutionary pressures that would lead to an accelerated development of HPI axis in carp are unknown.

Rainbow Trout, Atlantic Salmon, and Yellow Perch

Relative to hatching time, the temporal development of basal and stress-induced cortisol levels in rainbow trout, Atlantic salmon, and yellow perch are very similar to zebrafish. Cortisol levels are at a minimum around

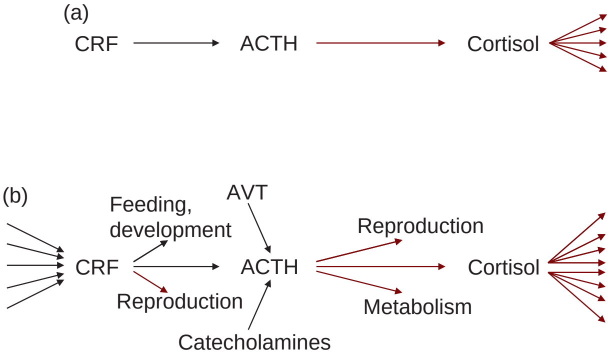


Figure 5 The HPI-axis signaling pathway: (a) simplified and (b) with some of the known signaling pathways, modulators, and functions of the HPI axis.

hatching (hatching times: zebrafish – 2–3 dpf; perch – 10 dpf; salmonids – 4 wpf), and then begin to increase. However, stress-induced elevations of cortisol are not observed until after hatching (1 week post-hatch in perch and 2 weeks post-hatch in salmonids).

Early exposure to stressors can alter the activation of the stress response later on in life. For example, rainbow trout embryos and larvae exposed to a stressor have a depressed cortisol response as juveniles (5 months post-fertilization). This dampening of the stress response is also observed in juvenile rainbow trout that were exposed to waterborne cortisol immediately after fertilization for approximately 2 days. This illustrates that the development and programming of the HPI axis is sensitive to stressor exposure, and long-lasting effects can be imprinted early on during development. However, determining the physiological consequences of these changes in the stress response is still required, and is essential in furthering our understanding of the development of the HPI axis.

Conclusion

There is clearly a good understanding of the molecular ontogeny of the HPI axis in teleosts, or more specifically, the CRF-ACTH-cortisol axis (**Figure 5**(**a**)). However, this is an extremely simplified view of a complex system that possesses multiple paths of regulation and function (**Figure 5**(**b**)). To date, the majority of experiments have focused on the regulation of stress axis genes in terms of transcript abundance under various conditions. However, there are several genetic and epigenetic mechanisms that influence these parameters. Characterizing these molecular mechanisms is useful not only to understand gene regulation but also to identify novel pathways, which can be used as bio-indicators of exposure to stressful stimuli. In addition, perhaps one of the more significant issues that currently remains unresolved is determining the basic functions of the stress axis components such as CRF, ACTH, GR, and MR during embryogenesis, given that there is no active stress response during this time.

Model species such as zebrafish have occupied a pivotal position in the study of physiological and molecular pathways of the HPI axis. However, teleosts are a large group with diverse developmental patterns, so it would be presumptuous to believe that the mechanisms are similar between different species, especially in light of the accelerated HPI-axis development in carp and the unique situation of single gene systems for CRF, ACTH, and GR in zebrafish. Hence, studies conducted in nonmodel species that are of importance to aquaculture, environmental conservation, and management are necessary.

See also: **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids; The Hormonal Control of Osmoregulation in Teleost Fish. **Hormonal Responses to Stress**: Hormone Response to Stress; Stress Effect on Growth and Metabolism; Impact of Stress in Health and Reproduction.

Further Reading

Alsop D and Vijayan MM (2009) The zebrafish stress axis: Molecular fallout from the teleost-specific genome duplication event. *General and Comparative Endocrinology* 161: 62–66.

Alsop D and Vijayan MM (2009) Review: Molecular programming of the corticosteroid stress axis during zebrafish development. *Comparative Biochemistry and Physiology. Part A: Molecular and Integrative Physiology* 153: 49–54.

Aluru N and Vijayan MM (2009) Stress transcriptomics in fish: A role for genomic cortisol signaling. *General and Comparative Endocrinology* 164: 142–150.

Bernier NJ (2006) Review: The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. *General and Comparative Endocrinology* 146: 45–55.

Matthews SG and Phillips DIW (2010) Minireview: Transgenerational inheritance of the stress response: A new frontier in stress research. *Endocrinology* 151: 7–13.

Wintermantel TM, Berger S, Greiner EF, and Schütz G (2005) Evaluation of steroid receptor function by gene targeting in mice. *Journal of Steroid Biochemistry and Molecular Biology* 93: 107–112.

Pituitary Gland or Hypophysis

DB McMillan, University of Western Ontario, London, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Anatomy
Hormones of the Adenohypophysis
Hormones of the Neurohypophysis
Histological Structure of the Adenohypophysis
Further Reading

Glossary

Adenohypophysis The anterior part of the pituitary composed of the pas distalis and pars intermedia, derived from the roof of the embryonic mouth.

Antigen A substance capable of reacting specifically with an antibody to induce an immune response.

Antiserum Serum containing an antibody or antibodies.

Axon The long extension of a nerve cell that carries spikes (action potentials, or neural impulses) to distant target cells away from the cell body where the nucleus lies (also called the nerve fiber).

Bony fish The majority of extant fishes – class Osteichthyes.

Connective tissue This tissue binds and supports the body.

Diencephalon The more caudal of the two divisions of the forebrain. Caudal part of the prosencephalon (forebrain) associated with the third ventricle. The general subdivisions are (from dorsal to ventral): epithalamus, dorsal thalamus, subthalamus, and hypothalamus.

Diverticulum (pl. diverticula) A pouch opening from a hollow organ.

Ectodermal cell Cell of the outermost layer of an early animal embryo.

Electrolyte A solute that has an ionic charge.

Epithelial cells These cells cover a surface or line a cavity.

Hypothalamus Region at the floor of the forebrain (diencephalon) that controls several vegetative physiological processes, including food intake and secretion of hormones from the pituitary gland

Infundibulum The neural stalk connecting the median eminence to the pars nervosa.

Median eminence A downward extension of the hypothalamus.

Melanophores Stellate connective tissue cells containing melanin granules in their cytoplasm.

Mesenchyme Embryonic connective tissue composed of stellate cells, wandering cells, and capillaries in an amorphous intercellular substance.

Negative feedback It tends to stabilize a process by reducing its rate when its effects are too great.

Neurohypophysis The portion of the pituitary that is derived from the downgrowth of nervous tissue from the diencephalon.

Neurosecretion The production of hormones within the body of nerve cells and their release from axon tips.

Pars distalis The anterior endocrine part of the pituitary derived from the ectoderm lining the roof of the mouth; it secretes a number of hormones.

Pars intermedia Part of the pituitary lying between the pars distals and pars nervosa; it is derived from the pars distalis.

Pituicytes Elongated cells of unecertain function in the neurohypophysis; their long processes course between neurosecretory axons.

Portal vessel A blood vessel connecting two capillary beds.

Releasing factor A hypothalamic neurosecretion that stimulates the liberation of a specific hormone from the adenohypophysis.

Saccus vasculosus A thin vesicle of unknown function on the floor of the diencephalon of most fishes.

Septum (pl. septa) A membrane between body spaces.

Sympathetic nervous system The division of the autonomic nervous system involved with the fight-or-flight reaction. Autonomic pathways from the thoracic and lumbar segments of the (mammalian) spinal cord.

Transporting epithelium (pl. epithelia) It a acts as a selective barrier to the diffusion of substances.

Vascular plexus A network of blood vessels.

Anatomy

An intimacy develops among vertebrate embryos between a mass of ectodermal cells that migrate upward from the roof of the mouth and the infundibulum, a stalk of nerve fibers that grows downward from the hypothalamus in the floor of the diencephalon. The tissue mass formed by this union becomes permeated with vascular mesenchyme that develops into a capsule besides providing the vascular connective tissue that supports and nourishes the organ. This is the beginning of the hypophysis or pituitary gland, the master gland of the endocrine system.

The pituitary secretes several hormones that control diverse activities in the body. It is two glands in one: the adenophypopophysis or anterior pituitary, which develops from the cells that budded upward from the roof of the mouth, and the neurohypophysis, or pars nervosa, derived from the bundles of nerve cell processes (axons) that grew down from the hypothalamus. This stalk of nerve fibers persists throughout life and the pituitary gland remains attached to the brain, thereby enabling the nervous system to exert its control over the endocrine system. In elasmobranchs and teleosts, the neurohypophysis also includes an outgrowth of the infundibulum, the nonglandular saccus vasculosus of uncertain function.

The proximity of the neurohypophysis to the adenohypophysis enhances integration of pituitary function by easing the passage of materials between them. This transfer may be facilitated by a portal system involving capillaries draining the neurohypophysis that pass to a capillary bed in the adenohypophysis.

The adenohypophysis of fish is subdivided into two parts: the pars distalis and the pars intermedia. Although there are variations in the arrangement of these parts, the pituitary of the puffer (*Fugu stictonatus*) is a good example of the structure of this gland in bony fish (**Figure 1**). The pars distalis is further subdivided into the anterior rostral pars distalis and the posterior proximal pars distalis, which lie below the neurohypophysis. Groups of cells with a variety of functions comprise the regions of the adenohypophysis. Finger-like ramifications of the pars nervosa invade the adenohypophysis, especially the pars intermedia (**Figure 2**).

The pars nervosa of bony fish is a neurosecretory organ where droplets of secretion, produced in the bodies of nerve cells located within the hypothalamus (**Figures 3** and **4**), travel down inside the axons of these cells (**Figure 5**) to be released into the vascular connective tissue of the pars nervosa; the axon terminals do not penetrate the adenohypophysis. The association between the neurohypophysis and adenohypophysis of teleosts is especially intimate (**Figure 6**) and a portal system is absent in most. Pituicytes, which lurk among the nerve fibers, are probably not secretory and have an unknown function. They may be derived from the cells of the vascular mesenchyme, which invaded the region during its development.

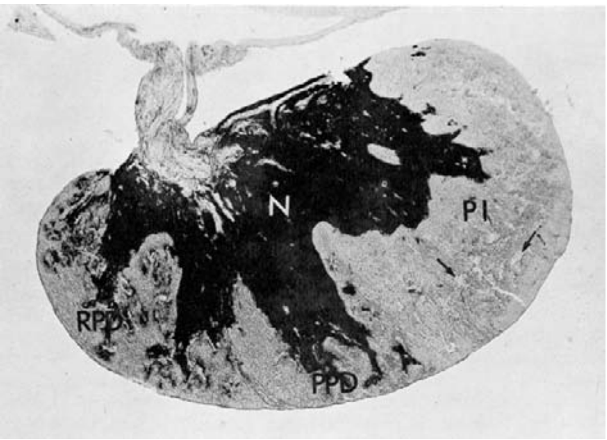


Figure 1 Median sagittal section of the hypophysis of a juvenile puffer, *Fugu stictonotus*, showing the neurohypophysis (N) and three parts of the adenohypophysis: the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). The infundibular stalk suspends the hypophysis from the diencephalon. Anterior to the left. Aldehyde fuchsin – fast green. 125×. From Chiba and Honma (1974) Cell types in the adenohypophysis of the puffer, *Fugu stictonotus*, with special reference to the basophils in the pas distalis. *Archivum Histologicum Japonicum* 36: 291–305, figure 1.

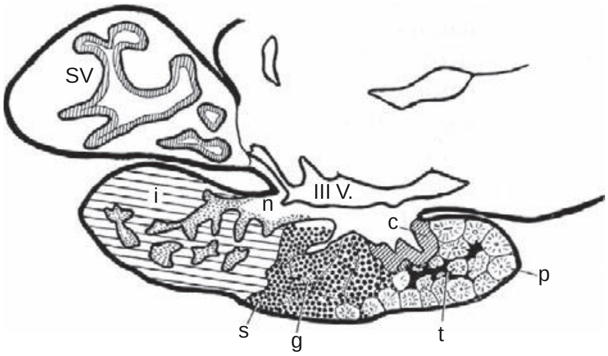


Figure 2 Diagram of a median sagittal section of the hypophysis of a male silver eel (*Anguilla anguilla*); anterior to the right. In the rostral pars distalis: p, prolactin cells (follicles); t, thytropes (black); c, corticotropes (oblique hatching). In the proximal pars distalis: g, gonadotropes (fine stipple); s, somatotropes (heavy stippling). The pars intermedia (i) is deeply invaded by processes of the neurohypophysis (n). The saccus vascuolosus (sv) extends behind the pituitary. IIIv, third ventricle whose floor and sides are the hypothalamus. From Olivereau (1967) Observations sur l''hypophyse de l'Anguille femelle, en particulier lors de la maturation sexuelle. *Zeitschrift für Zellforschung* 80: 286–306, figure 1.

A large pars intermedia in elasmobranchs is surrounded by diverticula of the neurohypophysis to form a neurointermediate lobe (**Figure 7**). The gland is subtended by an elongated dorsal lobe of the pars distalis; the ventral lobe of the pars distalis, which extends ventrally,

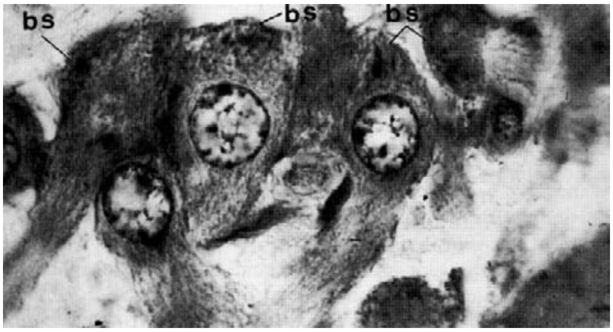


Figure 3 Photomicrograph of neurosecretory cells in the preoptic nucleus of a sturgeon (*Acipenser güidenstädti*). Basophilic material (bs) is abundant in the cytoplasm. 900×. From Polenov and Pavlovíc (1978) The hypothalamo-hypophysial system in Acipenseridae. VII. *Cell and Tissue Research* 186: 559–570, figure 1b.

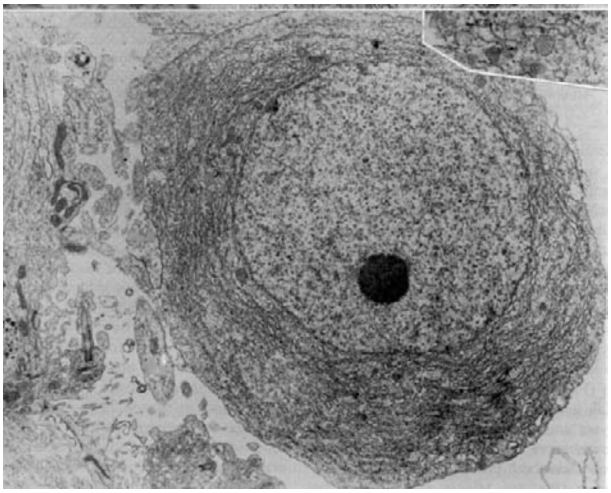


Figure 4 Electron micrograph of the body of a neurosecretory neuron from the brain of a lamprey *Lampetra japonica*. The cytoplasm contains parallel arrays of granular endoplasmic reticulum. 8800×. The inset shows scattered elementary granules and a few mitochondria in the cytoplasm. 23 000×. From Chiba and Honma (1989) The occurance of preoptic neurosecretory cells in the diencephalic ventricle of the Arctic lamprey, *Lampetra japonica*. *Japanese Journal of Ichthyology* 35: 488–492.

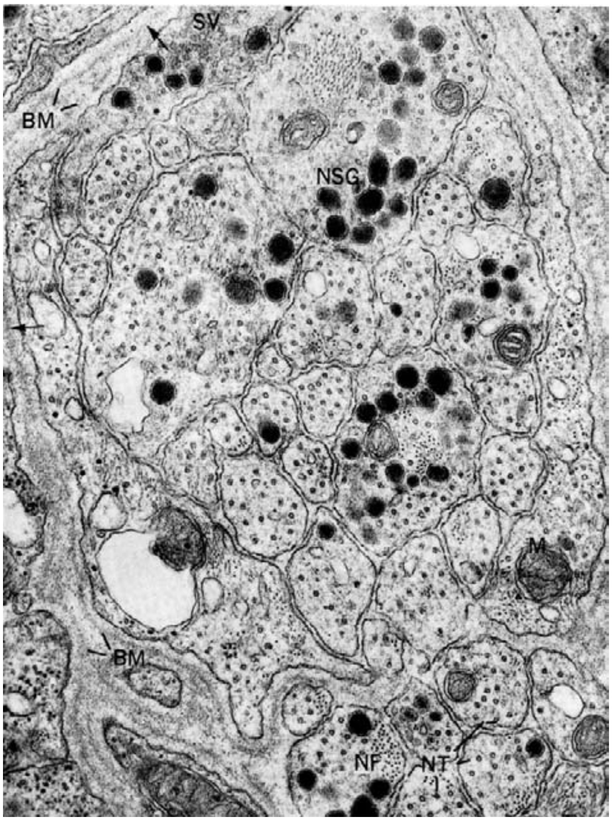


Figure 5 Electron micrograph of a cross section of a trunk of neurosecretory axons in the pars nervosa of the loach (teleost), *Misgurnus fossilis*. BM, basal lamina; M, mitochondria; NF, neurofilaments; NSG, neurosecretory granules to neurosecretory droplets; NT, neurotubules; SV, synaptic vesicles. 21 400×. From Jasinski and Kilarski (1970) Fine structure of pars neuro-intermedia the loach, *Misgurnus fossilis* L. *Zellforschung* 105: 259–275, figure 7.

has been omitted from the diagram. The pars distalis lacks arteries in elasmobranchs and is supplied by a portal system that collects blood from the median eminence and hypothalamus. The diverticula of the pars nervosa have a vessel-free outer surface that contains as much neurosecretory material as the parts facing the pars intermedia.

The pars distalis of the lamprey is embedded in connective tissue which separates it from the pars intermedia and the neurohypophysis (**Figure 8(a)**). The neurohypophysis of lampreys is only a slight thickening of the floor of the third ventricle. A vascular plexus intervenes between the pars intermedia and the neurohypophysis.

The pituitary of the hagfish is a simple strucure (**Figure 8(b)**). Its adenohypophysis shows no clear distinction between a pars distalis and a pars intermedia and consists of follicles and clusters of cells embedded in connective tissue below a hollow evagination of the hypothalamus that constitutes the neurohypophysis. The thin septum of connective tissue separating the neurohypophysis and adenohypophysis may be sufficiently thin to permit diffusion of materials.

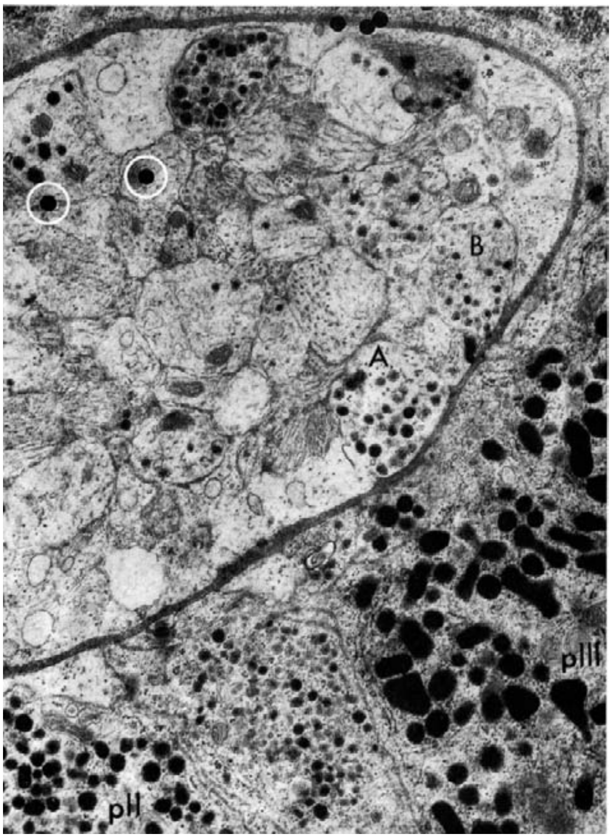


Figure 6 Electron micrograph of an evagination of the neurohypophysis extending between cells of the pars distalis. It contains two types of axons, A and B. Axonal tips (pII and pIII) penetrate between cells of the pars distalis. A limiting membrane separates the two regions. White circles are drawn around grains of latex. 10 900×. From Jasinski (1974) Fine structure of the anterior neurohypophysis of the pond-loach, *Misgurnus fossilis* L., with reference to the neurosecretory innervation of intrinsic cells of the pars distalis. *Acta Anatomica* 87: 193–208, figure 2.

The means by which neurosecretions reach the adenohypophysis are variable in the vertebrates (**Figure 9**). Portal vessels of higher vertebrates collect blood from capillaries in the neurohypophysis and carry it to capillaries in the adenohypophysis. There is no portal system in most teleosts, and extensions of the neurohypophysis intrude upon the adenohypophysis, allowing their neurosecretions to diffuse to the adenohypophysis; direct innervation by processes from the pars nervosa may also take place. A portal system occurs in the hypophysis of elasmobranchs carrying neurosecretions from the neurohypophysis to the adenohypophysis. Simple diffusion of neurosecretory material occurs in the agnatha.

Hormones of the Adenohypophysis

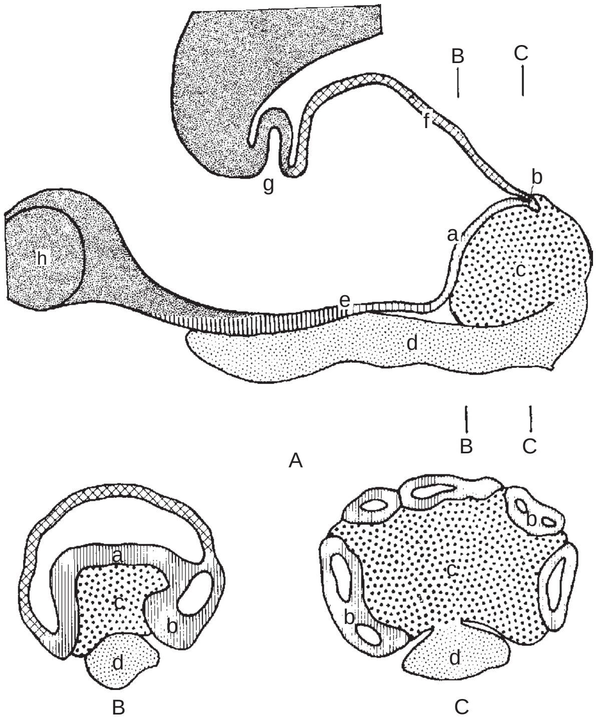


Figure 7 Diagrams of sections of the hypophysis of the dogfish shark, *Etmopterus spinax*. A is a median sagittal section; B and C are transverse sections taken at the levels shown in A. The ventral lobe of the pars distalis, which extends ventrally from the large anterior lobe, has been omitted. a, pars nervosa; b, diverticula of the pars nervosa; c, pars intermedia; d, pars distalis; e, median eminence and infundibular stem; f, saccus vasculosus; g, posterior recess; h, optic chiasma. From Meurling (1963) Nerves of the neuro-intermediate lobe of *Etmopterus spinax* (Elasmobranchi). *Zeitschrift für Zellforschung* 61: 182–201, figure 1.

Immunocytochemistry has been used extensively to determine the distribution of cells in the pituitary gland (**Figure 10**). Antisera to many hormones, often mammalian, have been used to identify and localize the different cell types. Secretory cells of the teleost fish are distributed in well-defined zones. **Figure 11** summarizes these findings for the pituitary of the Atlantic croaker *Micropogonias undulatus* – the literature abounds with similar findings. The rostral pars distalis contains corticotropes (adrenocorticotropic hormone (ACTH) cells) and prolactin (PRL) cells (PRL cells); the proximal pars distalis contains growth hormone cells (GH cells), gonadotropes (gonadotropic hormone (GTH) cells), and thyrotropes (thyroid-stimulating hormone (TSH) cells); there is evidence that cells that stain with lead hematoxylin (PbH+ cells) in the pars intermedia secrete melanophore-stimulating hormone (MSH) and that cells that are positive for the periodic acid-Schiff stain (PAS+ cells) secrete somatolactin (SL), a hormone that particpates in ion regulation.

Heavy metals have been attached to antigens in order to visualize secretory cells with the electron microscope. **Figure 12** is an electron micrograph of a section of the cytoplasm of a prolactin cell from the

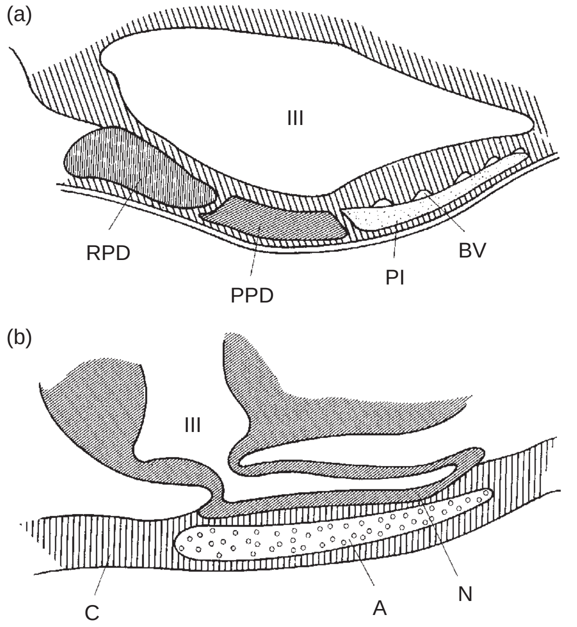


Figure 8 (a) Diagram of a median sagittal section of the pituitary of a lamprey. III, third ventricle; PI, pars intermedia; BV, blood vessels; PPD, proximal pars distalis; RPD, rostral pars distalis. (b) Diagram of a median sagittal section of the pituitary of a hagfish. III, third ventricle; A, adenohypophysis; C, cartilaginous base of the skull in which adenohypophyseal islets are embedded; N, neurohypophysis. From Matty AJ (1985) *Fish Endocrinology*, 267pp. London: Croom Helm., figure 1.3.

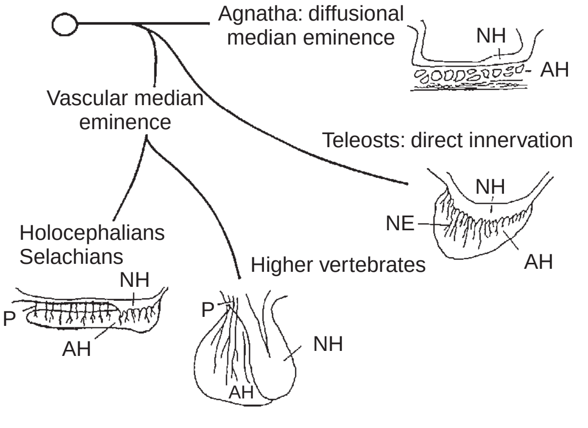

Agnatha: diffusional median eminence
NH
AH
Vascular median eminence
Teleosts: direct innervation
NH
NE
AH
Holocephalians Selachians
NH
P
AH
Higher vertebrates
P
NH
AH

Figure 9 Brain pituitary relations in vertebrates. In agnathans, a thin neurohypophysis lies adjacent to a thin layer of glandular epithelial cells, permitting direct diffusion between these two structures. Many neurohypophysial axonal extensions penetrate the adenohypophysis of teleosts and either directtly innervate the adenohyphysial cells or end near these cells allowing the diffusion of neurosecretions. In higher vertebrates, portal vessels collect blood from capillaries in the neurohypophysis and carry it to the adenohypophysis. Cartilaginous fish (lower left) show many short vessels between the neurohypohysis and adenpohyphysis. AH, adenohypophysis; NH, neurohypophysis; P, portal blood vessel from the vascular median eminence. From Nozaki *et al.* (1994) Diffusion between the neurohypophysis and the adenohypophysis of lampreys, *Petromyzon marinus*. *General and Comparative Endocrinology* 96: 385–391, figure 2.

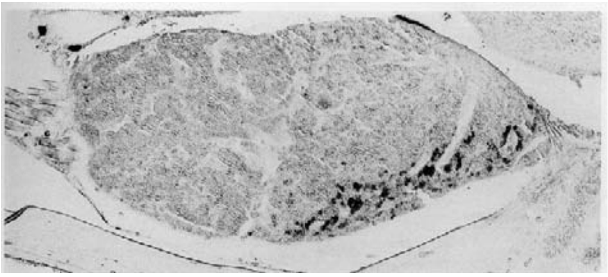


Figure 10 Sagittal section of the pituitary of the barbel, *Barbus barbus,* stained with an antiserum to human ACTH. Cells of the rostral pars distalis are stained. From Toubeau *et al.* (1991) Immunocytochemical study of cell type distribution in the pituitary of *Barbus barbus* (Teleostei, Cyprinidae). *General and Comparative Endocrinology* 83: 35–47, figure 2.

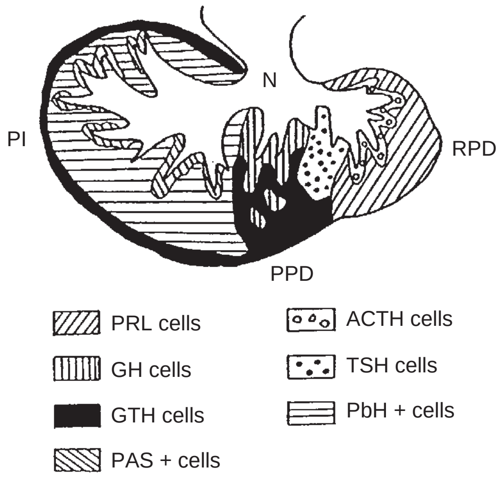


Figure 11 Diagram of a sagittal section of the pituitary of the Atlantic croaker, *Micropogonias undulatus,* showing the distribution of the various cell types. From Yan and Thomas (1991) Histochemical and immunocytochemical identification of the pituitary cell types in three sciaenid fishes: Atlantic croaker (*Micropogonias undulatus*), spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*). *General and Comparative Endocrinology* 84: 389–400, figure 1A.

pituitary of *Tilapia mossambicus* labeled with immunogold. Granules in the cytoplasm containing the hormone prolactin have picked up the labeled antigen and appear as black spots.

By a system of negative feedback in teleosts, ACTH causes the adrenal gland to release corticosteroids. PRL acts on transporting epithelia in the gills, kidney, urinary bladder, skin, and gut, thereby maintaining electrolyte and water balance in the body fluids of freshwater fish; its function in animals in seawater is unclear. GH promotes growth. GTH stimulates maturation of the gonads; two forms may be distinguished:

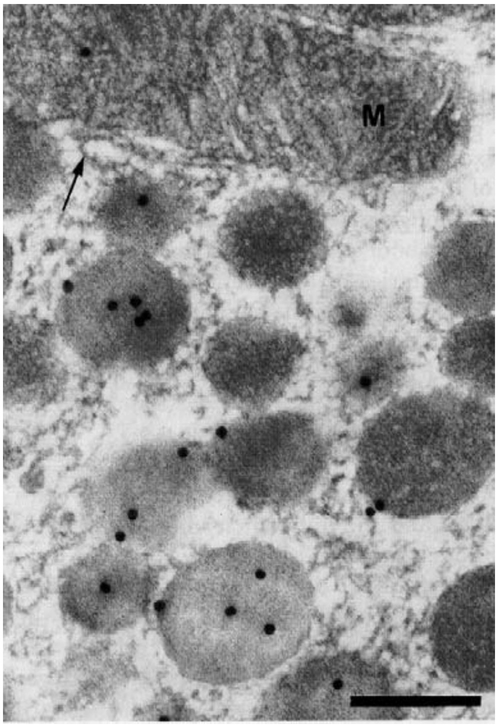


Figure 12 Electron micrograph of a section of the cytoplasm of a prolactin cell from the pituitary of *Tilapia mossambicus* labeled with immunogold. Granules in the cytoplasm containing the hormone prolactin have picked up the labeled antigen which appears as black spots. M, mitochondrion; arrow, endoplasmic reticulum. Bar = 0.25 μm. From Hwang and Wang (1993) Immunoelectron microscopical study of prolactin in pituitary of tilapia (*Oreochromis mossambicus*). *Acta Histochemica et Cytochemica* 26: 203–211, figure 9.

follicle-stimulating hormone (FSH) and luteinizing hormone (LH). TSH regulates thyroid function. MSH brings about color change in the skin of teleosts by affecting distribution of pigment granules in stellate melanophores; this effect is modulated by the sympathetic nervous system.

The distribution of hormone-secreting cells in elasmobranchs is less certain. ACTH and MSH may be produced in the neurointermediate lobe, TSH in the ventral lobe of the pars distalis (which was omitted from **Figure 7**), and PRL in the rostral pars distalis. The origins of GH and the gonadotropes FSH and LH are unclear. Conventional histological staining has produced only tentative speculation as to the nature of hormones produced by the pituitary of cyclostomes. In lampreys, immunocytochemistry has shown the presence of MSH in the pars intermedia and the rostral pars distalis. ACTH was confined largely to the rostral pars distalis. Information is sketchy for the hagfishes. The ultrastructure of pituitary cells suggests somatotropes and corticotropes in the adenohypophysis. In cyclostomes and elasmobranchs, the sympathetic nervous system is not involved in color change.

Hormones of the Neurohypophysis

Two similar polypeptide hormones of nine amino acids each are released at the axon endings in the pars nervosa of teleosts and elasmobranchs. Arginine vasotocin is said to have an effect on osmoregulation and salt/water balance. Oxytocin plays a role in reproduction, especially mating and egg laying. The picture is less clear in cyclostomes where arginine vasotocin may influence salt/water balance.

Histological Structure of the Adenohypophysis

The adenohypophysis consists of cords and clusters of secretory cells supported by a sparse stroma of delicate reticular fibers. It is invaded by fenestrated sinusoidal blood vessels with their accompanying connective tissue. The various secretory cells have been described for several species. Cells of the pituitary of the sailfin molly, *Poecilia latipinna*, will be described here.

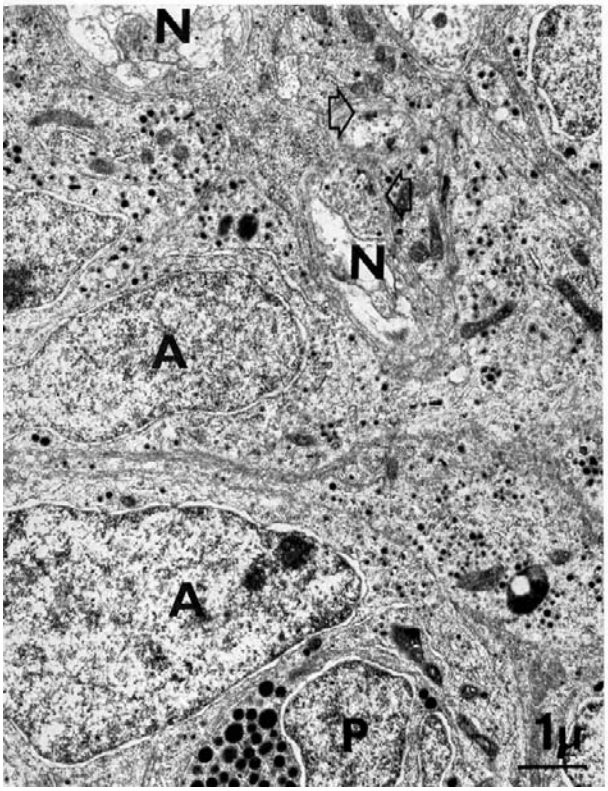


Figure 13 Electron micrograph of a section of the adenohypophysis of the sailfin molly *(Poecilia latipinna)*. ACTH cells (A) lie between prolactin cells (P) and the neurohypophysis (N). Arrows indicate terminations of nerve fibers. 12 000×. From Batten *et al.* (1975) Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna*. *Cell and Tissue Research* 161: 239–261, figure 3.

Cells of the Rostral Pars Distalis

ACTH cells (corticotropes) form a layer between the rostral pars distalis and the neurohypophysis, often only three or four cells deep. Two basal laminae separate the two regions. Nuclei of the corticotropes are often indented and elongated (**Figure 13**). The sparse cytoplasm contains a few round or rod-shaped granules and forms a narrow rim around the nucleus. A Golgi complex and mitochondria are present; granular endoplasmic reticulum is rare but free ribosomes were scattered throughout the cytoplasm.

PRL cells occupy most of the rostral pars distalis (**Figures 13** and **14**). They are usually rounded with a central round nucleus that is often deeply indented and contains one or two small nucleoli; electron-dense secretory granules pack the cytoplasm. The Golgi complex consists of scattered arrays of flattened cisternae and small vesicles; mitochondria are scarce. These features change when the ambient salinity is reduced – in fish adapted to freshwater, the PRL cells appear more active, with less granulation, more mitochondria, prominent Golgi complexes, and extensive granular endoplasmic reticulum.

Stellate cells are nonsecretory interstitial cells (**Figure 14**) with long, narrow processes that extend between the secretory cells to contact processes from other stellate cells, the basal lamina of the neurohypophysis, and the membrane around blood spaces. They may regulate the passage of materials throughout the adenohypophysis by way of intercellular channels. Their nuclei are flattened with an indistinct nucleolus. The pale cytoplasm occasionally contains Golgi-like vesicles, mitochondria, fragments of granular endoplasmic reticulum, and scattered ribosomes.

Cells of the Proximal Pars Distalis

Somatotropes (GH cells) lie in the inner regions of the proximal pars distalis intimately interdigitated with the neurohypophytsis (**Figure 15**). The nucleus is small, often irregular, usually lying at one end of the cell. The dense cytopasm contains abundant round secretory granules.

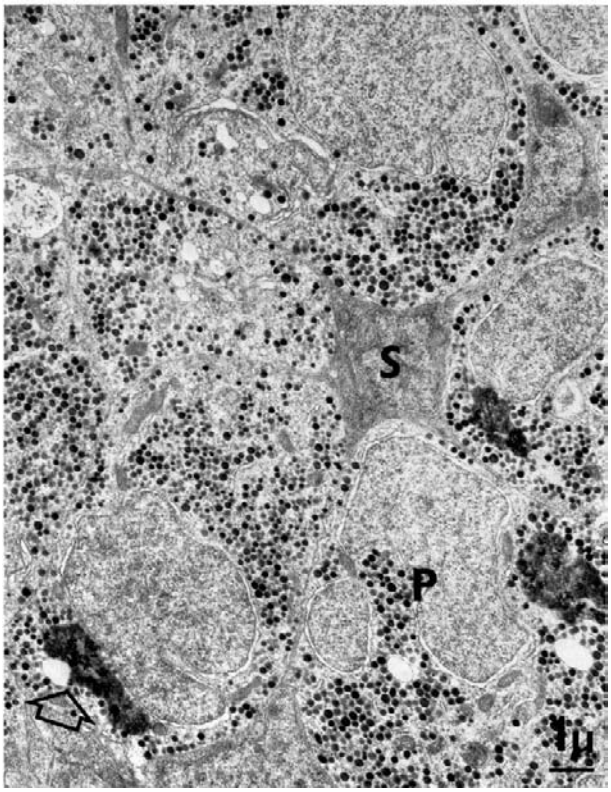


Figure 14 Electron micrograph of a section of the adenohypophysis of the sailfin molly (*Poecilia latipinna*). Prolactin cells packed with granules (P) surround stellate cells (S). 8000×. From Batten *et al.* (1975) Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna. Cell and Tissue Research* 161: 239–261, figure 4.

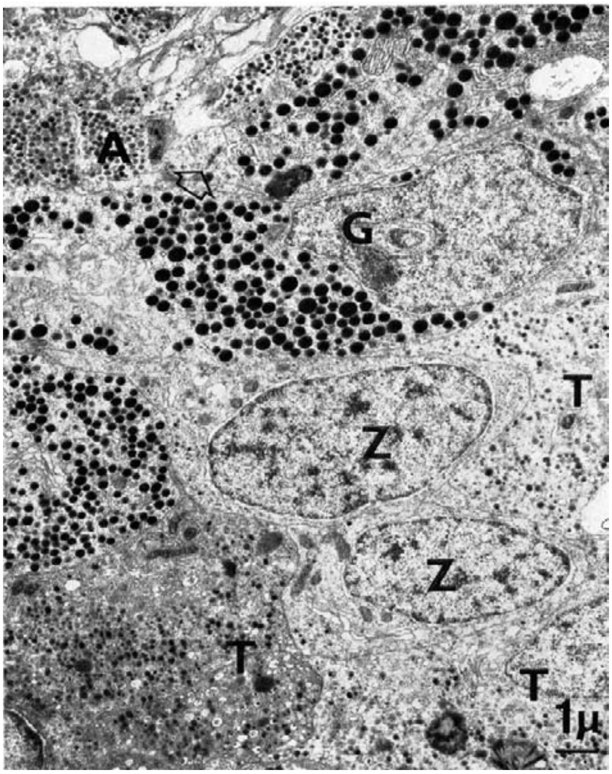


Figure 15 Electron micrograph of a section of the inner part of the proximal pars distalis of the sailfin molly (*Poecilia latipinna*) showing growth hormone cells (G), thyrotropes (T), and cells of uncertain significance (Z). Nerve fibers were noted (A and arrow). 8000×. From Batten *et al.* (1975) Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna. Cell and Tissue Research* 161: 239–261, figure 10.

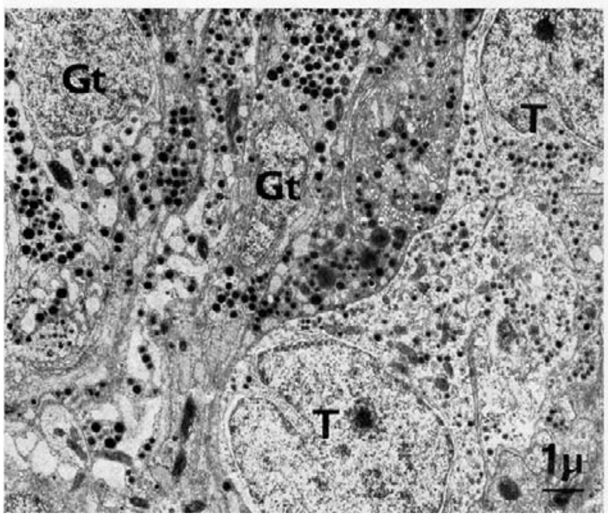


Figure 16 Electron micrograph of a section of the adenohypophysis of the sailfin molly *(Poecilia latipinna)*. Thyrotropes (T) border an area of gonadotropes (Gt). 8000×. From Batten *et al.* (1975) Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna. Cell and Tissue Research* 161: 239–261, figure 7.

Gonadotropes (GtH cells) show great variability depending on the condition of the gonads (**Figure 16**). In a female fish with oocytes in late vitellogenesis, the gonadotropes displayed a central round or oval indented nucleus and an indistinct nucleolus. The secretory granules are less electron dense than those of the somatotropes and prolactin cells. The cytoplasm varies, sometimes containing an extensive Golgi complex, abundant mitochondria, clear vesicles, dilated granular endoplasmic reticulum, free ribosomes, and multivesicular bodies.

Thyrotropes (TSH cells) are mixed with somatotropes. They vary considerably in appearance (**Figure 16**). Usually, they are large, irregular cells with a large, deeply indented nucleus lying at one end of the cell; occasionally, a small nucleolus is present. Mitochondria are abundant, but Golgi complexes are inconspicuous. The granular endoplasmic reticulum exists in scattered fragments throughout the cell. Secretory granules vary in size, shape, and electron density.

Stellate cells are also present in the proximal pars distalis, often lying between the gonadotropes. They send their processes between the secretory cells to approach the neurohypophysis.

Cells of the Pars Intermedia

Two types of secretory cells – both of uncertain function – have been identified in the pars intermedia: lead-hematoxylin cells (PbH+ cells) and periodic acid-Schiff-positive cells (PAS+ cells) (**Figure 17**).

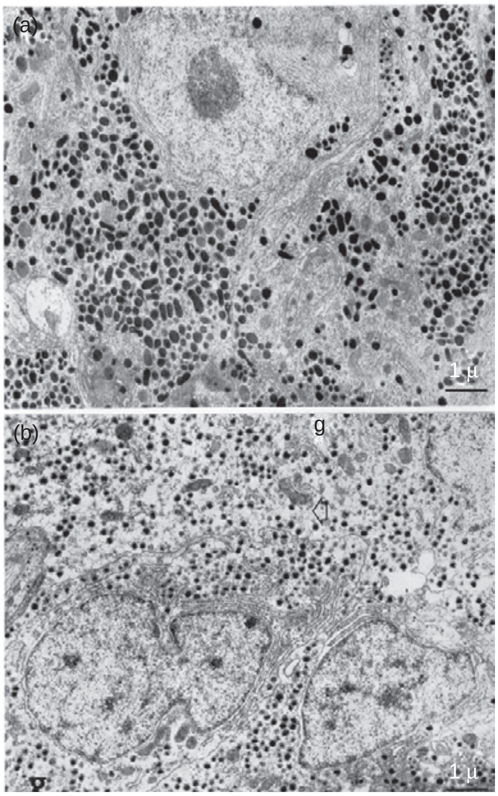


Figure 17 Electron micrographs of secretory cells from the pars intermedia of the sailfin molly *(Poecilia latipinna)*. 10 000×. (a) PAS+ cells showing secretory granules of variable density, shape, and size. (b) PbH+ cells with small, regular haloed granules. Arrow: microtubules; g, Golgi complexes. The irregular nuclei are enclosed by parallel membranes of granular endoplasmic reticulum. From Batten T, Ball JN, and Benjamin M (1975) Ultrastructure of the b0015 adenohypophysis in the teleost Poecilia latipinna. *Cell and Tissue Research* 161: 239-261 figures 11,12.

Further Reading

Acher R (1996) Molecular evolution of fish neurohypophysial hormones: Neural and selective evolutionary mechanisms. *General and Comparative Endocrinology* 102: 157–172.

Ball JN and Baker BA (1969) The pituitary gland: Anatomy and histophysiology. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 2, pp. 1–110. New York: Academic Press.

Batten T, Ball JN, and Benjamin M (1975) Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna*. *Cell and Tissue Research* 161: 239–261.

Chiba A and Honma Y (1974) Cell types in the adenohypophysis of the puffer, *Fugu stictonotus*, with special reference to the basophils in the pas distalis. *Archivum Histologicum Japonicum* 36: 291–305.

Chiba A and Honma Y (1989) The occurrence of the preoptic neurosecretory cells in the diencephalic ventricle of the Arctic lamprey, *Lampetra japonica*. *Japanese Journal of Ichthyology* 35: 488–492.

Hwang PP and Wang S-W (1993) Immunoelectron microscopical study of prolactin in pituitary of tilapia (*Oreochromis mossambicus*). *Acta Histochemica et Cytochemica* 26: 203–211.

Jasinski A (1974) Fine structure of the anterior neurohypophysis of the pond-loach, *Misgurnus fossilis* L., with reference to the neurosecretory innervation of intrinsic cells of the pars distalis. *Acta Anatomica* 87: 193–208.

Jasinski A and Kilarski W (1970) Fine structure of pars neuro-intermedia the loach, *Misgurnus fossilis* L. *Zeitschrift für Zellforschung* 105: 259–275.

Matty AJ (1985) *Fish Endocrinology, 267pp*. London: Croom Helm.

Meurling P (1963) Nerves of the neuro-intermediate lobe of *Etmopterus spinax* (Elasmobranchi). *Zeitschrift für Zellforschung* 61: 183–201.

Meurling P (1967) The vascularization of the pituitary in elasmobranchs. *Sarsia* 28: 1–104.

Mousa MA and Mousa SA (1999) Immunocytochemical study on the localization and distribution of the somatolactin cells in the pituitary gland and the brain of *Oreochromis niloticus* (Teleostei, Cichlidae). *General and Comparative Endocrinology* 113: 197–211.

Nozaki M and Gorbman A (1983) Immunocytoichemical localization of somatostatin and vasotocin in the brain of the Pacific hagfish, *Eptatretus stouti*. *Cell and Tissue Research* 229: 541–550.

Nozaki M, Gorbman A, and Sower SA (1994) Diffusion between the neurohypophysis and the adenohypophysis of lampreys, *Petromyzon marinus*. *General and Comparative Endocrinology* 96: 385–391.

Nozaki M, Ominato K, Takahashi A, Kawauchi H, and Sower SS (1999) Possible gonadotropin cells in the lamprey pituitary: Colocalization of mammalian LH-like immunoreactivity and glycoconjugate in adult sea lampreys (*Petromyzon marinus*). *General and Comparative Endocrinology* 113: 23–31.

Olivereau M (1967) Observations sur l''hypophyse de l'Anguille femelle, en particulier lors de la maturation sexuelle. *Zeitschrift für Zellforschung* 80: 286–306.

Polenov AL and Pavlovic M (1978) The hypothalamo-hypophysial system in Acipenseridae. VII. *Cell and Tissue Research* 186: 559–570.

Rand-Weaver M, Baker BJ, and Kawauchi H (1991) Cellular localization of somatolactin in the pars intermedia of some teleost fishes. *Cell and Tissue Research* 263: 207–215.

Toubeau G, Poilve A, Baras E, *et al.* (1991) Immunocytochemical study of cell type distribution in the pituitary of *Barbus barbus* (Teleostei, Cyprinidae). *General and Comparative Endocrinology* 83: 35–47.

Yan HY and Thomas P (1991) Histochemical and immunocytochemical identification of the pituitary cell types in three sciaenid fishes: Atlantic croaker (*Micropogonias undulatus*), spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellartus*). *General and Comparative Endocrinology* 84: 389–400.

HORMONAL CONTROLS

Hormonal Control of Metabolism and Ionic Regulation

Contents
The Hormonal Control of Osmoregulation in Teleost Fish
Corticosteroids

The Hormonal Control of Osmoregulation in Teleost Fish

S D McCormick, USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, USA

Published by Elsevier Inc.

Introduction: Salt and Water Balance in Fish
Cortisol
Prolactin
Growth Hormone and IGF-I
Thyroid Hormones
The Special Case of Anadromy
Summary
Further Reading

Glossary

Adrenocorticotrophic hormone (ACTH) A protein hormone produced in the pituitary that causes release of cortisol from the interregnal.

Anadromy Life-history strategy entailing reproduction and early rearing in freshwater followed by a significant growth phase in seawater.

Apoptosis The process of programmed cell death.

Cortisol The major corticosteroid of teleosts, produced in the interrenal and acting on metabolism and ion regulation.

Cystic fibrosis transmembrane conductance regulator (CFTR) An apical chloride channel involved in chloride secretion.

Euryhaline Animals that physiologically adjust to a wide range of water salinity are considered euryhaline (eury ‘broad’; haline ‘salt’), in contrast to stenohaline animals.

Growth hormone (GH) A pituitary protein hormone controlling growth, metabolism, and ion regulation.

Hormone Signaling molecule released by a cell that either stimulates the cell of origin (autocrine), nearby cells (paracrine), or travels via the bloodstream to distant cells (endocrine).

Insulin-like growth factor-I (IGF-I) A protein hormone produced in the liver in response to growth hormone and acting on growth and ion regulation.

Na^+,K^+-ATPase (NKA, the sodium pump) A major ATP-consuming ion pump that, directly or indirectly, drives many ion-regulatory processes, including maintaining sodium and ion gradients across cell plasma membranes and being present at high levels in ionocytes.

Na^+,K^+,$2Cl^-$ cotransporter (NKCC) An ion-translocating enzyme that utilizes a sodium gradient to transport Na^+, K^+, and Cl^- into the cell.

Prolactin A protein hormone produced in the pituitary acting on ion balance and reproduction.

Receptor A signal transduction molecule that is activated by a hormone.

Stenohaline Describes an organism that cannot live in environments that undergo large changes in salinity (steno ‘narrow’).

Thyroid hormones Thyroxine and triiodothyronine, produced in the thyroid gland and acting on development and metabolism.

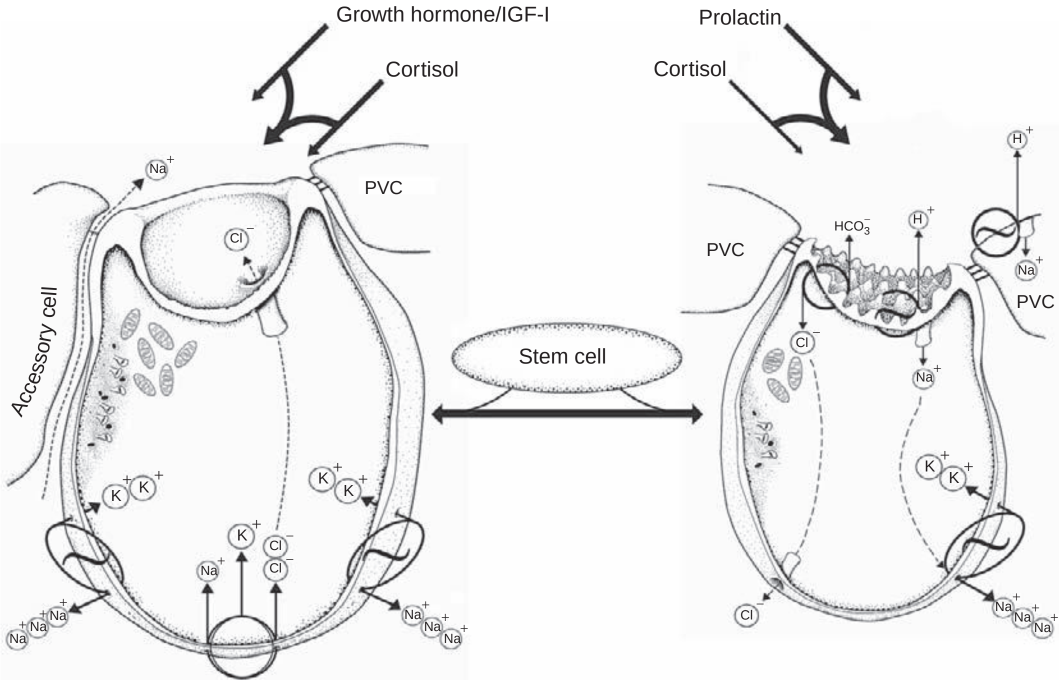
Transactivation Binding of a transcription factor, such as a receptor–ligand complex, to DNA resulting in increased gene transcription.

Introduction: Salt and Water Balance in Fish

Whether in freshwater or seawater, teleost fish maintain their plasma osmotic concentration at about one-third that of seawater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). In freshwater, this requires counteracting the passive gain of water and loss of ions, and is accomplished through the production of large volumes of dilute urine and active uptake of ions across the gills. In seawater, teleosts must counteract the passive gain of ions and loss of water. This is accomplished by drinking seawater, absorbing water and salts across the gut, and excreting monovalent ions across the gills and divalent ions through the kidney. It has been estimated that 95% of teleost species are stenohaline, living wholly in either freshwater or seawater. The remaining 5% are euryhaline, having the capacity to tolerate a wide range of salinities. This trait is widespread among teleost lineages and has apparently evolved many times, and may be one reason that teleosts can be found in almost all aquatic habitats.

As outlined above, the gills are the primary site of sodium and chloride transport, actively taking up salts in freshwater and excreting them in seawater. Most of the recent work on the endocrine control of ion transport in fish has focused on the gill, so this review will necessarily be biased in this direction. It has been known for some time that the mitochondrion-rich cell (also known as the chloride cell) is the site of salt secretion (**see also Role of the Gills**: Morphology of Branchial Ionocytes and **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). There is substantial evidence indicating that the major transporters involved in salt secretion in the gill include basolaterally located Na^+,K^+-ATPase (NKA, the sodium pump) and Na^+,K^+,$2Cl^-$ cotransporter (NKCC), and an apical Cl^- channel that is homologous with the cystic fibrosis transmembrane conductance regulator (CFTR) (**Figure 1**).

The site and mechanisms involved in ion uptake by the gill in freshwater are less certain. Both mitochondrion - rich cells and pavement cells may be involved in sodium and chloride uptake (**see also Osmotic, Ionic and**



Seawater (ion secretion)

Freshwater (ion uptake)

Figure 1 Morphology, transport mechanisms, and hormonal control of gill chloride cells in freshwater and seawater. Chloride cells are characterized by numerous mitochondria and an extensive tubular system that is continuous with the basolateral membrane. In seawater, mitochondrion-rich cells are generally larger and contain a deep apical crypt, whereas in freshwater, there is a broad apical surface containing numerous microvilli. Growth hormone and cortisol can individually promote the differentiation of the seawater chloride cell, and also interact positively to control epithelial transport capacity. Prolactin inhibits the formation of seawater chloride cells and promotes the development of freshwater chloride cells. Cortisol also promotes acclimation to freshwater by maintaining ion transporters and chloride cells, and by interacting to some degree with prolactin PVC, pavement cell. Reproduced from McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. *American Zoologist* 41: 781–794.

Nitrogenous-Waste Balance: Mechanisms of Ion Transport in Freshwater Fishes). Chloride is exchanged for HCO_3^- at the apical surface and leaves at the basolateral membrane moving downhill on an electrical gradient (the chloride cell being more negative than the blood). Sodium may enter the gill epithelia by exchange with H^+, or through an apical Na^+ channel coupled to an apical H^+-ATPase, and pass into the bloodstream at the basolateral gill surface through NKA. Recent work suggests that in some species there is also a Na^+/Cl^- co-transporter on the apical surface that is involved in ion uptake. More direct evidence is needed to be certain of the roles and location of these transporters involved in ion uptake in teleosts.

There are three primary lines of evidence for the involvement of hormones in ion regulation. The first involves the treatment of animals (*in vivo*) or isolated tissues (*in vitro*) with hormones and examining ion regulatory performance and/or the response of ion transport proteins and cells. The second line of evidence comes from changes in circulating hormones when environmental salinity is changed, such as a transfer from freshwater to seawater. Third, the presence and regulation of specific hormone receptors in the organs and cell types responsible for ion regulation is taken as evidence for the involvement of that hormone in salt and water balance.

This article illustrates that there are many common features to the endocrine control of osmoregulation in teleost fish. It should be noted, however, that only a small number of teleosts have been examined and that we know little or nothing about the hormonal control of osmoregulation in the vast majority of fish. Given the great diversity among teleosts and the differing acclimation responses and strategies that have evolved, it should not be surprising to find that not all teleost fishes conform to a single scheme. Indeed, one of our outstanding research challenges is to determine how the hormonal control of osmoregulation differs among teleosts, and to what extent it has been shaped by natural selection.

Cortisol

Cortisol and Seawater Acclimation

Cortisol is the major corticosteroid produced by the interrenal tissue of teleost fish (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids). This hormone has several established physiological roles in osmoregulation, intermediary metabolism, growth, stress, and immune function. It has been shown for many species of euryhaline fish that treatment with cortisol in freshwater improves their subsequent survival and capacity to maintain low levels of plasma ions after exposure to seawater. This effect is due to increases in the size and abundance of gill mitochondrion-rich cells, which has been demonstrated *in vivo* and *in vitro*. Cortisol has also been shown to increase the transcription and abundance of the major transport proteins involved in salt secretion by the gill: NKA, NKCC, and CFTR. The effect of exogenous cortisol generally requires several days to reach its peak, suggesting that cell proliferation and differentiation are required for its complete action. In the intestine, exogenous cortisol stimulates NKA activity, together with ion and water absorption, thus improving acclimation to high environmental salinity. An increased drinking response after transfer to seawater has been observed in salmonids treated with cortisol.

Changes in circulating cortisol in response to increased environmental salinity are reported for many teleost species. The clearance rate (the inverse of the amount of time a compound remains in circulation) of cortisol also increases in seawater, suggesting increased utilization by osmoregulatory target tissues (**see also Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation). The release of cortisol from the interrenal is primarily controlled by the pituitary hormone adrenocorticotrophic hormone (ACTH), though other endocrine factors may also be involved (**see also Hormonal Responses to Stress**: Hormone Response to Stress). Although there is evidence for activation of pituitary ACTH cells, salinity effects on circulating levels of ACTH have not been detected. ACTH production by the isolated pituitary does not appear to be directly responsive to changes in external osmolality. The increase in cortisol during osmotic stress occurs in both stenohaline and euryhaline fishes and may be part of a general stress response. Thus, the presence and regulation of cortisol receptors is also a critical component of osmoregulation in these fishes.

The classical signaling action of steroids begins by transport into the cell, followed by binding to a cytosolic receptor, which is then translocated into the nucleus. There, the steroid binds to specific genes to increase or decrease their expression. A large number of binding studies in fish have found evidence for a single class of corticosteroid receptors (CRs). These receptors are present in high concentrations in the gill, gut, and kidney, and their abundance is often altered by environmental salinity. During exposure to increased salinity, intracellular cortisol and CR levels in the gill shift from the cytosol to the nucleus, indicative of CR binding and translocation. High concentrations of CR have been found in the gill mitochondrion-rich cells.

In the past several years, molecular techniques have demonstrated the presence of genes in several teleost species related to the mammalian glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). Two isoforms of fish GR genes have been found in several teleost species, and these have different activation affinities for cortisol. In addition, an MR-like gene has been characterized in rainbow trout (*Oncorhynchus mykiss*) and the cichlid (*Haplochromis burtoni*). When the rainbow trout MR gene is expressed in a mammalian cell line, it was found to have high transactivation efficiency for both aldosterone and 11-deoxycorticosterone (DOC), similar to the binding characteristics of the mammalian MR. It has been suggested that DOC, present in the plasma of some teleosts at levels that could activate the fish MR, might be a mineralocorticoid in fish. Injection studies indicate, however, that DOC cannot carry out the seawater-adapting functions of cortisol. Thus, the physiological function of DOC and MR in fish remains to be established.

Cortisol and Freshwater Acclimation

As noted above, cortisol has largely been identified as a seawater-adapting hormone in a large number of teleost species. There is an increasing body of evidence demonstrating that cortisol is also involved in ion uptake, thus giving it a dual osmoregulatory function. Cortisol treatment in freshwater fish increases the surface area of gill mitochondrion-rich cells and the influx of sodium and chloride, affecting both renal and branchial functions. Survival and plasma ion levels of freshwater fish that have had their pituitary removed are increased by treatment with adrenocoritcotrophic hormone (ACTH), which can be presumed to be acting through its stimulation of cortisol release from the interrenal. Cortisol is also required to maintain water movement across the gut of freshwater eels. Cortisol treatment significantly increases the ion regulatory capacity of marine fish during exposure to low salinity and the ability of acid-resistant fish to maintain plasma sodium levels after exposure to acidic water.

Changes in circulating levels of cortisol also provide supporting evidence for a role of this hormone in ion uptake. Transfer of euryhaline species from seawater to freshwater results in short-term increases in plasma cortisol. Transfer of several species of marine fish from high to low salinity results in increased circulating cortisol that remains elevated for days to weeks. In Mozambique tilapia (*Oreochromis mossambicus*), transfer from freshwater to distilled water or injection of prolactin (PRL) results in elevated plasma cortisol. These studies on increasing cortisol levels in response to decreases in salinity, along with the cortisol treatment studies described above, provide evidence that, in many teleosts, cortisol has a physiological role in promoting ion uptake. This function of cortisol has not been fully appreciated due to an emphasis on the role of cortisol in salt secretion.

Although there is substantial evidence indicating that cortisol can increase gill and gut NKA, it is unclear whether this is primarily related to cortisol's seawater-adaptive effects, or whether upregulation of the sodium pump is also involved in ion uptake. If maintenance of the sodium pump is indeed integral to ion uptake, then the effect of cortisol on gill NKA could be one mechanism by which cortisol exerts a dual osmoregulatory function. In addition, cortisol also likely promotes the development of the freshwater morphology of chloride cells. Recent evidence indicates that in salmonids there are two salinity-dependent isoforms of gill NKA: one that is more abundant in freshwater and the other more abundant in seawater. Cortisol upregulates both of these isoforms, which is further evidence for a dual osmoregulatory role for this hormone.

Prolactin

By removing the pituitary (hypophysectomy) and then replacing the lost PRL with injections, Grace Pickford conclusively demonstrated 50 years ago that the pituitary hormone PRL was critical for the maintenance of ion balance of teleost fish in freshwater. Since then, evidence for PRL as an important freshwater-adapting hormone in fish comes from studies on exogenous PRL treatment, changes in PRL gene expression and circulating levels in response to salinity change, and the localization and regulation of PRL receptors.

Although PRL has been shown to have sodium- and chloride-retaining activity in a variety of freshwater and euryhaline teleosts, there is surprisingly little information on the cellular and biochemical effectors of the osmoregulatory actions of PRL. Throughout vertebrates, a large proportion of the various actions of PRL seem to be associated directly or indirectly with cell proliferation and/or apoptosis. PRL has been shown to affect chloride cells, both by inhibiting the development of seawater chloride cells and promoting the morphology of ion uptake cells. PRL treatment also reduces ion and water permeability of the esophagus and intestine, a response that normally occurs during acclimation to freshwater. PRL can induce intestinal cell proliferation in some euryhaline fishes. It has recently been shown that the abundance of NaCl co-transporter present on the apical surface of ion uptake cells in the tilapia gill decreases following hypophysectomy and is restored to original abundance with PRL treatment.

Gene expression, synthesis, secretion, and plasma levels of PRL all increase following exposure to freshwater. Metabolic clearance rate of PRL increases following fresh water acclimation of salmonids. In some teleosts such as tilapia, plasma osmolality and cortisol exert direct regulatory actions on PRL secretion. As in mammals, however, a specific hypothalamic PRL-releasing factor, PRL-releasing peptide (PrRP), has recently been identified in teleosts. PrRP promotes PRL transcription and secretion, with the histochemical localization of PrRP neuronal terminals near PRL cells in the pituitary. Moreover, PrRP seems to be an essential stimulator of PRL since antiserum to PrRP decreases circulating PRL levels. In the amphibious euryhaline mudskipper (*Periophthalmus modestus*), the brain-pituitary axis of PrRP-PRL operates during both terrestrial and freshwater acclimation.

PRL receptor transcription and abundance are high in osmoregulatory organs such as the gill, intestine, and kidney. In the gill, the transcription and abundance of PRL receptor are lower in seawater than in freshwater in tilapia and salmonids. High levels of PRL receptor transcription have been found in mitochondrion-rich cells of the gill and enterocytes of the intestine.

There is evidence that both PRL and cortisol are necessary for normal ion regulation in freshwater. The two may even interact to control ion uptake. In hypophysectomized catfish (*Heteropneustes fossilis*), PRL and cortisol together cause a greater elevation of plasma ions than either hormone alone. Also, cortisol and PRL together have a greater effect than either hormone alone in promoting the transepithelial resistance and potential of an *in vitro* gill-cell preparation. In hypophysectomized and/or interrenalectomized fish, both PRL and/or ACTH or cortisol are necessary to completely restore ion and water balance in freshwater. Although an interaction between PRL and cortisol in controlling freshwater acclimation has been proposed, there is as yet little direct evidence for this hypothesis and no mechanism has been proposed.

Growth Hormone and IGF-I

In the 1950s, D.C.W. Smith observed that multiple injections of growth hormone could increase the capacity of brown trout to tolerate exposure to seawater. At first, this was attributed to the growth effect of the hormone because size confers greater salinity tolerance in salmonids. Several decades later, it was found that a single injection of growth hormone in unfed fish was sufficient to increase salinity tolerance, indicating a relatively rapid effect that was independent of body size. Growth hormone has since been shown to increase the number and size of gill mitochondrion-rich cells as well as the abundance of NKA and NKCC (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish).

A major route of the osmoregulatory action of GH is through its capacity to increase circulating levels and local tissue production of insulin-like growth factor-I (IGF-I). Exogenous treatment of IGF-I has been found to increase the salinity tolerance of rainbow trout, Atlantic salmon (*Salmo salar*), and killifish (*Fundulus heteroclitus*). GH cannot directly (in culture) increase gill NKA activity, whereas IGF-I can. The ability of prior GH treatment to increase *in vitro* responsiveness of gill tissue to IGF-I further suggests an indirect action of GH on gill tissue, and a direct action of IGF-I. Levels of IGF-I mRNA in gill and kidney increase following GH injection and exposure to seawater, indicating that local production of IGF-I may act in a paracrine fashion to influence transport capacity of gill and renal epithelia.

In addition to the effects of exogenous hormones, changes in gene expression, secretion, circulating levels, and metabolic clearance rate of growth hormone and IGF-I also provide evidence for the osmoregulatory actions of the GH/IGF-I axis in several euryhaline species. Plasma GH levels have also been found to increase in stenohaline freshwater fish following exposure to brackish water (e.g., 12 ppt). Following exposure of rainbow trout to seawater, there are increases in plasma levels of IGF-I and three of its binding proteins. IGF-I mRNA levels in liver, gill, and kidney increase following growth hormone injection and exposure to seawater, indicating that local production of IGF-I may also act to influence transport capacity of gill and renal epithelia. IGF-I has been found specifically in gill chloride cells whose number and/or size are stimulated by growth hormone.

Growth hormone receptors in high concentrations have been found in the liver, gill, gut, and kidney of euryhaline teleost fish. Occupancy of hepatic growth hormone receptors by growth hormone increased following exposure to seawater. Growth hormone transcription has also been detected in osmoregulatory organs and may be acting in an autocrine or paracrine manner in these tissues. Growth hormone receptor mRNA levels in the gill increase after seawater acclimation of salmonids. Specific high-affinity, low-capacity IGF-I receptors have been found in gill tissue of salmon and tilapia, and have been immunocytochemically localized to gill chloride cells in striped bass (*Morone saxatilis*) and tilapia.

Few teleost species have been examined for the physiological impact of the GH/IGF-I axis on osmoregulation. Exogenous treatments have been found to affect salmonids, tilapia, and killifish. Changes in circulating hormones and production of IGF-I in osmo-regulatory organs in salmonids provide convincing evidence for both endocrine and paracrine actions of the GH/IGF-I axis, but there is relatively little information in this area from other teleosts. There is no apparent osmoregulatory role for GH in sea bream, a marine species with a limited capacity for hyperosmoregulation. Species variation linked to different ion regulatory capacities and/or strategies for ion regulation may determine to what extent the GH/IGF-I axis is involved in osmoregulation. A similar situation may occur for PRL: there may be limited osmoregulatory effects of PRL in stenohaline marine teleosts where any ion uptake may be maladaptive. Expanded research on both euryhaline and stenohaline species will be necessary to determine how widespread the osmoregulatory actions of PRL and the GH/IGF-I axis are among teleosts, and how phyletic histories and natural selection have acted to bring about any observed patterns.

Interaction of the GH/IGF-I Axis with Cortisol

The GH/IGF-I and cortisol axes work together to regulate salt secretion in teleosts. Injection of GH and cortisol together increases gill NKA activity and salinity tolerance in salmonids and killifish to a greater extent than either hormone alone. This effect can be seen in both hypophysectomized and intact fish. Cortisol treatment of freshwater Atlantic salmon causes an increase in both the freshwater and seawater isoforms of NKA, but treatment with GH and cortisol causes the seawater isoform to increase to an even greater extent, and the freshwater isoform to decrease. Much of the interaction of GH and cortisol is through GH's capacity to upregulate the number of gill cortisol receptors, which makes the tissue more responsive to cortisol.

Growth hormone increases mitotic activity in several cell types in the gill of rainbow trout. Cortisol has no effect on mitotic activity but increases the number of chloride cells, suggesting that cortisol acts primarily to promote differentiation of chloride cells. Therefore, another pathway for GH/IGF-I and cortisol interaction is stimulation of stem cell proliferation by GH and/or IGF-I, creating more stem cells that can then be acted on by cortisol. The GH/IGF-I axis and cortisol may also interact at higher regulatory pathways, such as the hypothalamus and pituitary. *In vivo* and *in vitro* exposure to GH increases the sensitivity of interrenal tissue to ACTH, causing increased release of cortisol. Corticotropin-releasing hormone is a potent stimulator of *in vitro* growth hormone release in the European eel (*Anguilla anguilla*).

Thyroid Hormones

Although there is conflicting evidence regarding the role of thyroid hormones in osmoregulation, most studies have found that thyroid hormones, by themselves, cannot increase ion uptake or secretory capacity. Exceptions to this are prolonged thyroxine (T4) treatment that accelerated smolt-related increases in gill mitochondrion-rich cells and NKA activity in Atlantic salmon, and dietary triiodothyronine (T3) treatment that increased the number of gill mitochondrion-rich cells without affecting gill NKA activity. Thyroid hormones play at least a supportive role in seawater acclimation, and may interact with both the GH/IGF-I and cortisol axes. Inhibition of the thyroid axis with thioruea in killifish caused increased plasma ions in seawater but had no effect in freshwater. T4 treatment alone has no effect, but potentiates the action of cortisol on gill NKA activity in tilapia, and the action of GH on gill NKA activity in salmon. Inhibiting the conversion of T4 to T3 interferes with normal and GH-induced seawater acclimation in trout. T3 treatment increases the number of gill cortisol receptors in trout and salmon. Thyroid hormones thus appear to exert their influence on salt secretory mechanisms primarily through an interaction with cortisol and the GH/IGF-I axis.

The Special Case of Anadromy

As part of their normal life history, anadromous fish (such as salmon) must move between freshwater and seawater at least twice during their life history (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). As juveniles in freshwater, salmon have only a limited capacity to enter seawater. At the time of their downstream migration (when they are known as smolts), juvenile salmon develop a very high capacity for salt secretion before they actually arrive in the ocean. The timing of this developmental process differs among salmonids, but is accompanied by changes in the gill (increased mitochondrion-rich cells, NKA, and NKCC), gut, and kidney.

During smolt development, GH, IGF-I, and cortisol increase in response to developmental and/or environmental cues such as photoperiod and temperature (**Figure 2**). PRL can inhibit smolt development, and increases early during smolt development, but then decreases at the peak of smolting. Thyroid hormones also increase during smolting and are thought to play an indirect role in osmoregulatory changes, but a direct role in morphological, metabolic, and behavioral changes. Thus, the same hormones that are altered by exposure to salinity in other species increase prior to seawater exposure in salmon, inducing preparatory changes in salt secretory mechanisms that allow rapid movement into seawater with minimal osmotic perturbations.

The developmental changes that occur during smolting in salmon appear to be similar to other species, such as lamprey and shad that make just one or a few migrations from freshwater to seawater. Species that make more frequent migrations between freshwater and seawater may rely more on rapid regulation ion uptake or salt secretion rather than developmentally cued regulation.

Summary

Cortisol has long been known to have an important role in seawater acclimation of teleost fishes. Current evidence indicates that the GH/IGF-I axis also has a role in seawater acclimation, and that GH, IGF-I, and cortisol interact positively to promote salt secretion and the underlying physiological mechanisms. PRL has a

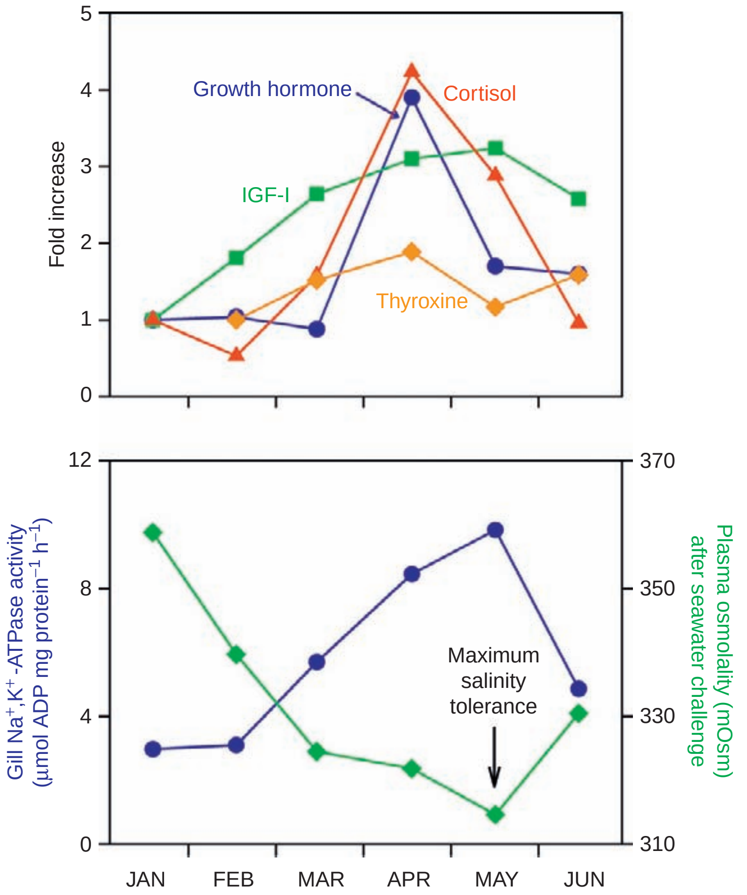


Figure 2 Changes in plasma hormone levels, gill NKA activity, and salinity tolerance during the parr–smolt transformation of Atlantic salmon. Interaction among cortisol, growth hormone, IGF-I, and thyroid hormones results in physiological changes that are preparatory for seawater entry. Adapted from McCormick SD, Lerner DT, Monette MY, Nieves–Puigdoller K, and Björnsson BTh (2009) Taking it with you when you go: How perturbations to the freshwater environment, including temperature, dams and contaminants, affects seawater performance of anadromous fish. In: Haro AJ, Smith KL, Rulifson RA, *et al*. (eds.) *Challenges For Diadromous Fishes in a Dynamic Global Environment.* American Fisheries Society Symposium, vol. 69, pp. 195–214. Bethesda, MD, USA.

well-established role in ion uptake and inhibition of salt secretion. In addition to its role in seawater acclimation, several studies indicate that cortisol is also involved in ion uptake and can interact positively with PRL, indicating that cortisol has a dual osmoregulatory function in teleosts. The action of cortisol in promoting ion uptake or secretion may therefore depend partly on its interaction with growth hormone and PRL: when growth hormone is high and PRL is low, cortisol may act primarily to promote salt secretion. Conversely, when growth hormone is low and PRL is elevated, cortisol will act to promote ion uptake.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish. **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle. **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids. **Hormonal Responses to Stress**: Hormone Response to Stress. **Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction. **Role of the Gills**: Morphology of Branchial Ionocytes.

Further Reading

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97–177.

Hoar WS (1988) The Physiology of Smolting Salmonids. In: Hoar WS and Randall D (eds.) *Fish Physiology,* vol. XIB pp. 275–343. New York: Academic Press.

Marshall WS (2002) Na$^+$, Cl$^-$, Ca^{2+} and Zn^{2+} transport by fish gills: Retrospective review and prospective synthesis. *Journal of Experimental Zoology* 293: 264–283.

McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. *American Zoologist* 41: 781–794.

McCormick SD (2009) Evolution of the hormonal control of animal performance: Insights from the seaward migration of salmon. *Integrative and Comparative Biology* 46: 408–422.

McCormick SD, Lerner DT, Monette MY, Nieves–Puigdoller K, and Björnsson BTh (2009) Taking it with you when you go: How perturbations to the freshwater environment, including temperature, dams and contaminants, affects seawater performance of anadromous fish. In: Haro AJ, Smith KL, Rulifson RA, *et al.* (eds.) *Challenges For Diadromous Fishes in a Dynamic Global Environment* American Fisheries Society Symposium, vol. 69 pp. 195–214. Bethesda, MD, USA.

Sakamoto T and McCormick SD (2006) Role of prolactin and growth hormone in osmoregulation of teleost fish. *General and Comparative Endocrinology* 147: 24–30.

Takei Y and Loretz CW (2006) Endocrinology. In: The Physiology of Fishes, 3rd edn., pp. 271–318. Boca Raton, FL: CRC Press.

Corticosteroids

P Kiilerich and P Prunet, Institut National de la Recherche Agronomique (INRA), Rennes, France

© 2011 Elsevier Inc. All rights reserved.

Introduction
Corticosteroid Synthesis
Central Regulation of Cortisol Release
Corticosteroid Plasma Levels
Molecular Biology of Corticosteroid Receptors
11β-Hydroxysteroid Dehydrogenase Type 2
Molecular Mechanisms of Corticosteroid Signaling
Corticosteroid Target Tissue: Expression of Teleost Corticosteroid Receptors
Regulation of the Corticosteroid Signaling System by Stress Exposure
Evolution of the Teleost Corticosteroid Signaling System: What Is the Role of the Mineralocorticoid-Like Axis?
Further Reading

Glossary

Corticosteroid A collective term for glucocorticoid (such as cortisol and corticosterone) and mineralocorticoid (aldosterone and DOC) hormones.

Corticosteroid receptors Ligand-activated nuclear hormone receptors with the ability to bind corticosteroids, such as GR and MR.

Glucocorticoid response element (GRE) A response element for glucocorticoid hormones, a short sequence of DNA within the promoter of a gene, that specifically binds GRs and MRs to regulate transcription. The consensus sequence is a pair of inverted repeats separated by three nucleotides.

Half-maximal effective concentration (EC_{50}) The concentration of a ligand that induces 50% of the maximum response. It is a measure commonly used to express the potency of a ligand.

Interrenal tissue The fish analog of the mammalian adrenal cortex located in the head kidney region, which produces and releases cortisol upon stimulation.

Ligand A molecule that binds to another molecule, such as an enzyme substrate, cofactor, or signaling molecule (Latin ligare = to bind).

Ligand-activated nuclear hormone receptor A family of cytoplasmic-located proteins, which, upon ligand binding, translocates to the nucleus, where it binds specific response elements to regulate gene transcription.

Polymerase chain reaction (PCR) A method to measure mRNA transcript levels by amplification of specific target sequences.

Teleost fish A large group of modern bony fishes, including the most common fishes, distinct from the group of elasmobranches (sharks and rays).

Trans-activation Stimulation of transcription by a transcription factor binding to DNA. These molecules are said to act in trans, that is, one gene product (a protein) interacts with another gene (promoter).

Introduction

Corticosteroids are a group of steroid hormones derived from cholesterol, which are used as endocrine signaling molecules in all classes of vertebrates. Corticosteroids are traditionally divided in two classes according to their main function. The glucocorticoids (cortisol, cortisone, and corticosterone) are named after their role in the regulation of glucose metabolism (carbohydrate metabolism and gluconeogenesis) and their synthesis takes place in the adrenal cortex. Mineralocorticoids (aldosterone and 11-deoxycorticosterone (DOC)) are named after their function in the regulation of hydromineral balance (water and ions).

The main corticosteroid hormone in teleost fish, cortisol is involved in regulation of many physiological functions besides metabolism, such as growth, immune function, reproduction, development, cardiovascular function, behavior, and hydromineral homeostasis, and is an essential part of the stress response. It is especially interesting to note the osmoregulatory function of cortisol in fish, a function otherwise taken care of by aldosterone in higher vertebrates (amphibians, reptiles, birds, and mammals). Aldosterone cannot be synthesized in fish in the absence of suitable enzymes. However, a mineralocorticoid-like hormone, DOC, has recently been identified in fish and may be an ancestral corticosteroid hormone. Overall, the corticosteroid signaling system in fish shows many similarities with that of higher vertebrates; significant differences also exist. The essentials of the corticosteroid signaling system in fish and the specific differences it exhibits from that of mammals are discussed in this article.

Corticosteroid Synthesis

Corticosteroid synthesis takes place in the steroidogenic cells in the interrenal tissue of the head kidney, which is equivalent to the adrenal cortex in mammals, and released upon endocrine stimuli from the brain (see later). While cortisol is the steroid mainly produced, other steroids such as cortisone, corticosterone, 11-deoxycortisol, and the mineralocorticoid-like DOC are synthesized to a lesser extent. Cortisol is predominantly synthesized by converting cholesterol to pregnenolone, by cytochrome P450 (CYP)11A1, followed by a C-17 hydroxylation by CYP17 to yield 17-hydroxy-pregnenolone, which, in turn, converted to 17-hydroxy-progesterone by 3*β*-hydroxysteroid dehydrogenase (HSD) and further hydroxylated to 11-deoxycortisol by CYP21 before being converted into cortisol by C-11 hydroxylation by CYP11B (**Figure 1**). A minor portion of cortisol synthesis might come from DOC or corticosterone, since the hydroxylation of C-11 and C-21 does not prevent C-17 hydroxylation. Due to the lack of the aldosterone synthetase enzyme (CYP11B2), fish cannot produce the main mammalian mineralocorticoid, aldosterone. However, DOC, which is synthesized from pregnenolone by C-21 hydroxylation of progesterone, could potentially bind and activate the MR in fish (see later). In mammals, increasing evidence suggests that paracrine signaling is possible by extra-adrenal synthesis of corticosteroids, for example, in the brain.

Central Regulation of Cortisol Release

The endocrine control of cortisol release is managed by the hypothalamic–pituitary–interrenal (HPI) axis. The neuropeptide corticotrophin-releasing hormone (CRH) is released from the hypothalamic nucleus preopticus in response to stressors such as handling, hypoxia, exercise, social stress, ambient water temperature, osmolality, infection, and pollutants (**Figure 2**). CRH controls the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary to the blood stream. ACTH, which is the main regulator of cortisol release, then in turn stimulates cortisol release from the interrenal cells in the head kidney. The endocrine control of cortisol release appears very complex since several other neuroendocrine peptides and pituitary hormones affect cortisol release, such as isotocin, vasotocin, dopamine, neuropetide Y, melonophore-concentrating hormone, thyrotropin-releasing hormone, catecholamines, angiotensin II, and melanophore-stimulating hormone. Furthermore, control of cortisol release is autoregulated through negative

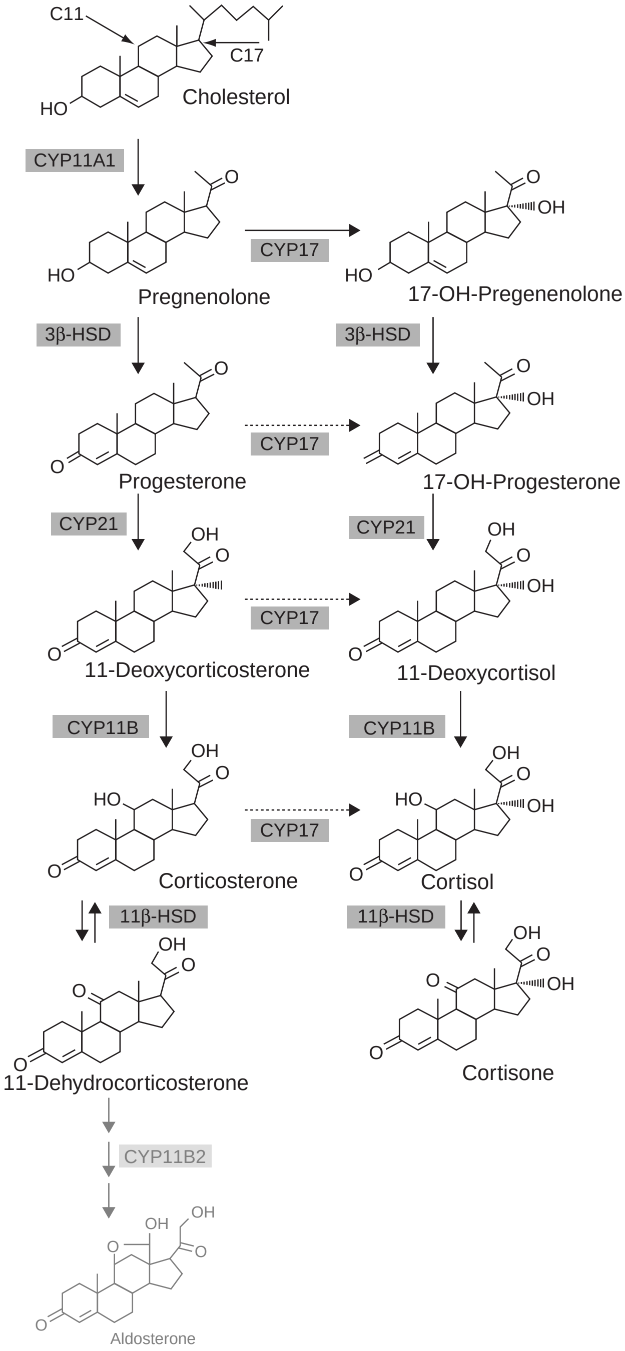


Figure 1 Overview of the synthesis pathway of corticosteroid hormones from cholesterol. CYP denotes cytochrome P450 proteins and HSD denotes the hydroxysteroid dehydrogenases comprising the steroidogenic enzymes. Fish lack CYP11B2 aldosterone synthase and cannot produce aldosterone. Redrawn from Bury NR and Sturm A (2007) Evolution of the corticosteroid receptor signalling pathway in fish. *General and Comparative Endocrinology* 153: 47–56.

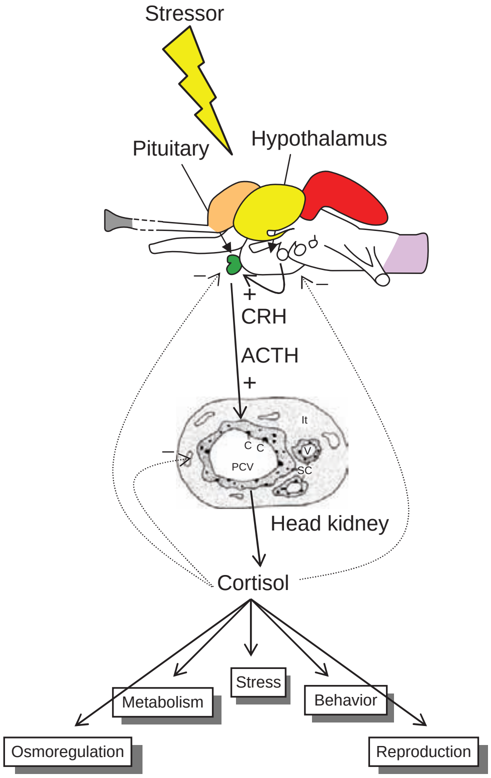


Figure 2 Schematic representation of the hypothalamic–pituitary–interrenal and cortisol-target-tissue axis. Corticosteroid-releasing hormone (CRH) is released from hypothalamus upon stimuli. CRH triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary to the blood stream (denoted by a plus sign). ACTH, then, in turn triggers steroidogenic cells (sc) in the interrenal tissue of the head kidney to release cortisol to the blood stream. Some of the physiological processes regulated by cortisol are boxed. Cortisol exerts negative regulation on release of CRH and ACTH from the hypothalamus and pituitary, respectively, and cortisol release from head kidney (denoted by a minus sign). From HowStuffWorks 2001 © and Wendelaar Bonga SE (1997) The stress response in fish. *Physiological Reviews* 77: 591–625.

feedback of the hormone at all levels of the HPI axis, the preoptic nucleus, the anterior pituitary, and the interrenal (**see also Hormonal Responses to Stress**: Hormone Response to Stress).

Corticosteroid Plasma Levels

Plasma levels of cortisone, corticosterone, 11-deoxycortisol, and DOC are relatively low – around 0.1–1.0 nM. Plasma cortisol in a nonstressed condition is around 5 ng mL^{-1} (10 nM), but can increase up to 10–100-fold (up to 1000 nM) in response to stress. Since only cortisol and DOC function as high-affinity ligands for the corticosteroid receptors, and thus must be expected to have some physiological relevance, these are the only corticosteroids discussed in the remainder of this article.

In mammals, around 90% of the cortisol is bound by corticosteroid-binding globulins (CBGs) in the plasma, which transports glucocorticoids in blood and regulate the bioavailability and access to target cells. The question of CBGs in fish has received incredibly little attention so far. Early investigations suggesting the presence of such proteins in fish have never been confirmed. In fact, CBGs seem to be absent in fish, based on recent database searches for a CBG ortholog in the sequenced fish genomes available.

Molecular Biology of Corticosteroid Receptors

In fish, three different corticosteroid receptors have recently been identified and described: two glucocorticoid receptors (named GR1 and GR2) and one mineralocorticoid receptor (MR). These three receptors show differences in hormone binding, transcriptional activity, and regulation among them, suggesting the presence of three functional receptors in fish compared to only two, one GR and one MR, in mammals. Analogous to mammals, the glucocorticoid receptor operates only as a cortisol receptor, whereas the MR is efficiently activated by cortisol, aldosterone, and DOC alike.

In general, the corticosteroid receptors consist of the GR (NR3C1) and the MR (NR3C2), both belonging to subfamily 3C of the nuclear hormone receptor superfamily, which also consists of the androgen, progesterone, and estrogen receptor. Composition of the fish GRs and MR structure is identical to the well-described mammalian nuclear hormone receptors, with an amino-terminal transactivating domain (the A/B-domain), a central DNA-binding domain (DBD), a hinge region, and a carboxy-terminal ligand-binding domain (LBD) (**Figure 3**). The highest degree of sequence conservation between the corticosteroid receptors (CRs) is found in the DBD, which consists of two zinc-finger-binding motifs, which recognize and bind specific DNA sequences, called response elements, in the target-gene promoters. As a result, all three CRs bind the same DNA sequence and are potentially able to regulate transcription from the same target genes. The differences in the LBDs provide different ligand-binding properties, where MR binds both cortisol and DOC, whereas GRs bind only cortisol. These differences may also contribute to the different cofactor-binding capacities and possibly to dimerization efficiency/ability, thus yielding an important diversity in the signaling potential. The A/B domain is the most divergent among the nuclear hormone receptors and contains a ligand-independent activation function.

Although carried out in mammalian cell lines using simplified model promoters, transient transfection analysis of hormone binding and transactivation properties of

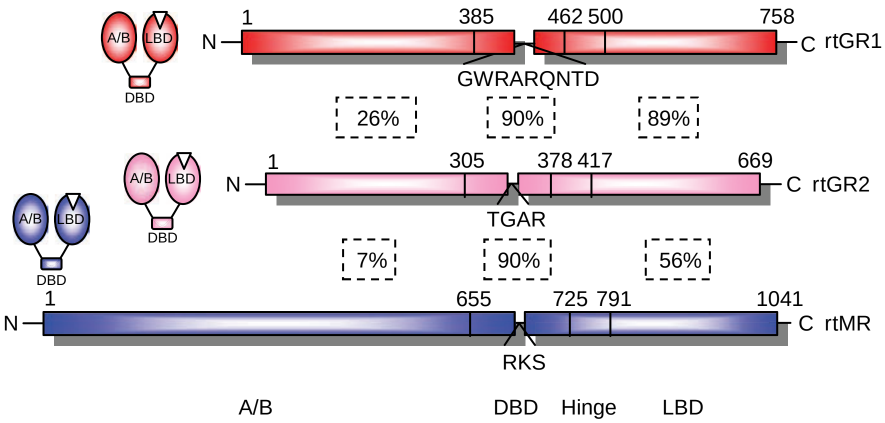


Figure 3 Corticosteroid receptors identified in rainbow trout (rt GR1, rtGR2, and rtMR). Numbering of amino acids is placed above the receptors. The amino- and carboxy-terminal ends are marked with N and C, respectively. Numbers in punctuated boxes between two receptors denote amino acid identity to rtGR1. The different steroid receptor domains are denoted A/B regulatory domain, DNA-binding domain (DBD, C-domain), hinge (D-domain), and ligand-binding domain (LBD, E-domain). Amino acid inserts between the two DBD zinc fingers in CR splice variants are shown below the receptor.

the fish CRs have proven interesting. As in mammals, cortisol and dexamethasone (a synthetic glucocorticoid widely used in studies of glucocorticoid effects) are the most effective steroids stimulating fish GR1 and GR2 transactivation, when measured as the half-maximal transcriptional activity induced by the hormone (EC_{50}). While GR1 and GR2 have the same binding affinity for glucocorticoids (dexamethasone), GR2 transactivation is induced at far lower concentrations of dexamethasone and cortisol compared to GR1. MR has the same sensitivity to cortisol when compared to GR2 but is around 50 times more sensitive to cortisol than GR1 (**Figure 4**). Interestingly, aldosterone and DOC are about 10 times more potent activators of MR than cortisol, which suggests that DOC could be a physiological ligand of MR in fish.

By and large, and even with some profound homologies with the mammalian corticosteroid signaling system (including structure, hormone, and DNA-binding abilities), several unique features have been identified in the teleost corticosteroid signaling system. Nevertheless, detailed knowledge of the functional and physiological significance of these features is still lacking.

11β-Hydroxysteroid Dehydrogenase Type 2

With cortisol plasma levels about 10–100-fold higher than DOC, glucocorticoid signaling through MR is apparently favored over mineralocorticoid signaling. Thus, although the EC_{50} value is 10 times lower for DOC than cortisol, the MR is expected to be occupied by cortisol even at its resting levels. This resembles the situation in mammals where co-localization of MR with the 11βHSD2 enzyme that converts cortisol to cortisone, which does not activate MR, seems to be a prerequisite to ensure specific aldosterone signaling. By comparison, co-localization of

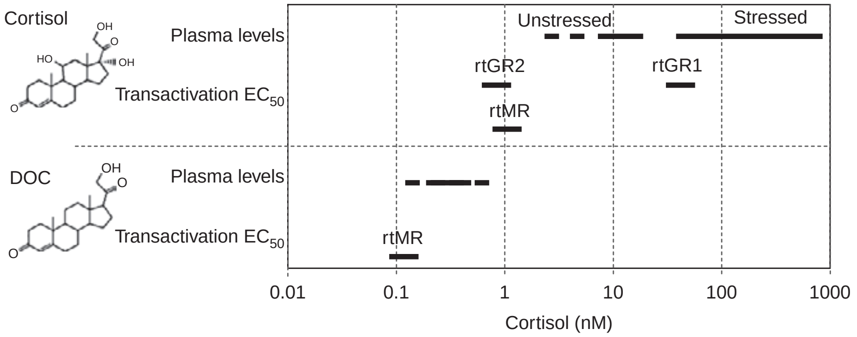


Figure 4 Comparison of the cortisol half-maximal effective concentration (EC_{50}) value of the corticosteroid receptors and plasma cortisol levels in unstressed and stressed rainbow trout. Cortisol resting levels are normally in the range of 10 nM – enough to activate MR and GR2, but not GR1. Stress induces an increase in cortisol plasma levels up to 100–1000 nM.

11βHSD2 with MR is probably equally essential to ensure the proposed DOC signaling in teleosts. Recently, the presence of a functional 11βHSD2 enzyme, located in many different organs and cell types, was described in fish. Regulation of 11βHSD2 mRNA abundance in salmon gill after osmotic stress (when cortisol levels increase) suggests a physiological significance for this enzyme. However, details are scarce at this point.

Molecular Mechanisms of Corticosteroid Signaling

In the absence of hormone, the corticosteroid receptors are located in the cytosol, chaperoned by heat shock proteins (hsp) and immunophilins (**Figure 5**). Upon hormone binding, the receptor translocates to the nucleus, dimerizes, and binds to specific DNA sequences in target-gene promoters called glucocorticoid response element (GRE, three-basepair spaced, imperfect, inverted hexamer palindromes).

Due to the high sequence similarity in the DBD, all three CRs may bind the GREs. However, small differences in the DBD among the receptors may affect GRE recognition and binding affinity. Furthermore, splice variants of GR1 and MR encompassing nine and three amino acids inserted between the zinc fingers, respectively, may further confer regulatory specificity of the fish CRs.

When bound to DNA, the corticosteroid receptors modulate corticosteroid target-gene transcription by inducing or inhibiting transcriptional activity. Furthermore, these receptors are also extensively involved in regulation of target genes for other signaling pathways, through DNA-dependent as well as independent mechanisms by interaction with other transcription factors. A whole regulatory network where GR interacts with a diversity of transcription and cofactors (such as NF-κB, AP-1, Stat5, FoxA, HNF6, PPARγ, Ets, YY1, Oct-1, and C/EBPβ) and participates in regulation of a vast variety of genes has been described in mammals. Even though such information is still lacking in fish, a similar situation is expected.

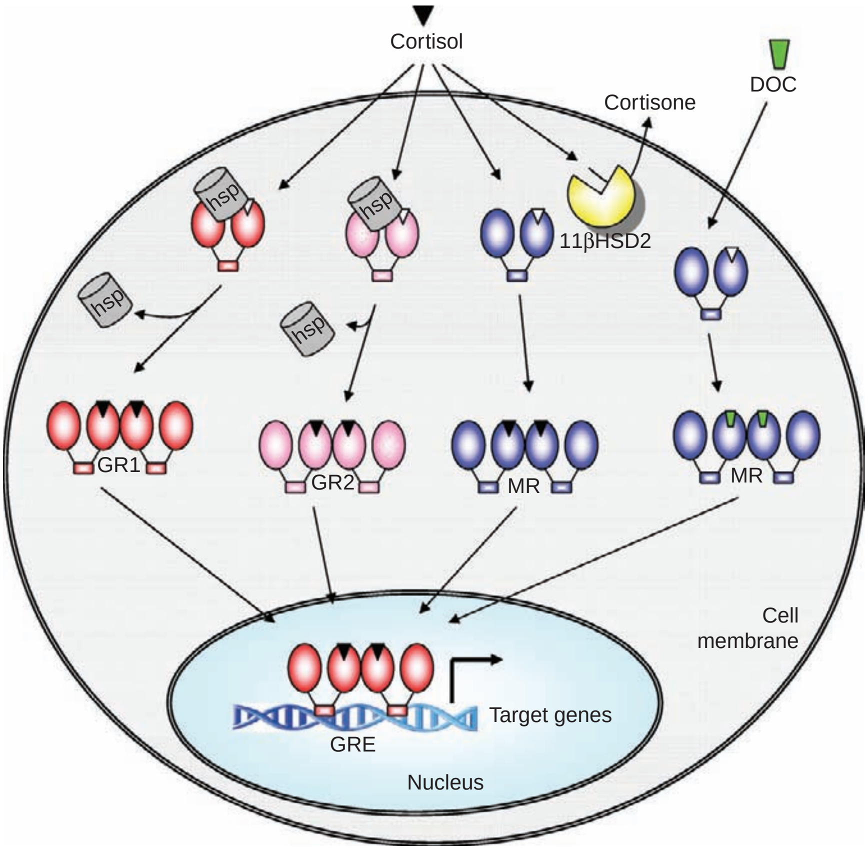


Figure 5 Simplified molecular mechanism of corticosteroid hormone receptor activation. Cortisol and DOC are transported in the blood where they enter the target-cell cytoplasm. Cortisol binds to the cytoplasmic-located GR1, GR2, or MR, whereas DOC binds only the MR. In cells containing 11βHSD2, cortisol is converted to the receptor-inactive cortisone to facilitate DOC-binding to MR. In a nonligand state, the corticosteroid receptors are bound by heat shock proteins (hsp's), but dimerize and translocate to the nucleus upon hormone binding. Specific glucocorticoid response elements (GREs) in target-gene promoters are recognized and bound by the receptor dimers to alter mRNA transcription.

Corticosteroid Target Tissue: Expression of Teleost Corticosteroid Receptors

All three corticosteroid receptors are widely expressed in fish tissue representing all major tissue groups such as osmoregulatory tissue (gills, kidney, and intestine), neurological tissue (brain and pituitary), metabolic tissue (liver and muscle), cardiovascular tissue, reproductive organs, and immune tissue (head kidney and spleen). Consequently, all tissue types and organs are potential targets for corticosteroid regulation. The level of GR1 transcript seems to be fairly equal among the tissues investigated, whereas GR2 and MR levels seem to be highest in the spleen and brain, respectively. Whether this has significance for the physiological roles of these receptors is still unknown.

With reference to the stress response, the major cortisol target tissue is depicted in **Figure 6**. In the stress response, cortisol serves to mobilize energy for an acute increase in energy consumption of specific tissues, such as increased activity in muscles, brain, liver, heart (cardiac activity), and gills (oxygen uptake). Increase in glycolysis, gluconeogenesis, lipolysis, and proteolysis in liver and proteolysis in muscle provide substrates for increased hepatic gluconeogenesis leading to hyperglycemia. Among particular cortisol target genes coding for proteins related to metabolism in these tissues are aminotransferases for tyrosine, alanine, and aspartate, glutamate dehydrogenase and synthetase, glucose 6-phosphatase, glucose 6-phosphatase dehydrogenase, phosphoenolpyruvate carboxykinase, glycerol kinase, glycogen phophorylase, and 3-hydroxyl-coenzyme A dehydrogenase.

The increase in energy demand in the organism is distributed to organs with essential functions in the stress response, including brain, muscle (increased activity), liver, heart, and gills (increased oxygen uptake). This reallocation of energy is carried out at the expense of energy consumption of gills (osmoregulation), muscle (growth), head kidney, and gonadal tissue, thus impairing hydromineral balance and acid–base regulation, growth, immune function, and reproduction, respectively. Thus, cortisol differentially activates or inhibits specific functions in the same organ to cope with a stressor. If the stress becomes chronic, the stress response eventually becomes unfavorable and results in disadvantages on growth, immune function, and reproduction (**see also Hormonal Responses to Stress**: Hormone Response to Stress).

Regulation of the Corticosteroid Signaling System by Stress Exposure

Autoregulation of GR levels by cortisol is well known in mammals – this is especially important in ensuring the functionality of a negative feedback loop to efficiently

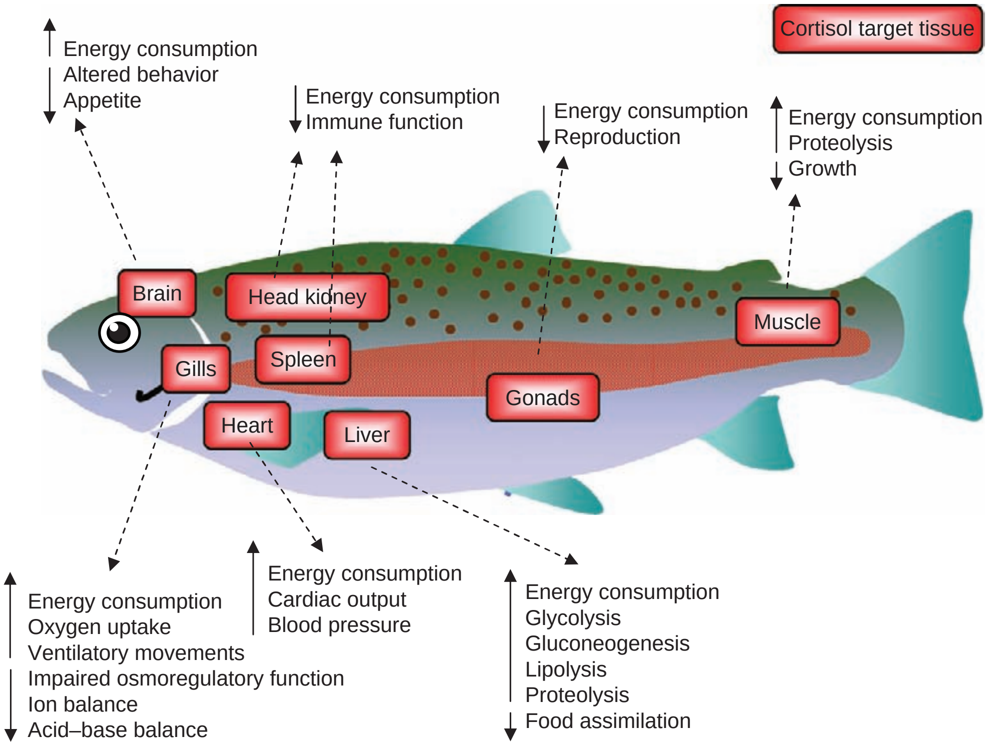


Figure 6 Summary of the main cortisol target tissue in the stress response and the major physiological processes regulated. An upward or downward arrow indicates an increase or decrease, respectively, of the physiological process. Trout drawing by G. Yoshizaki.

regulate release of cortisol along the HPI axis (**Figure 1**). In parallel, binding studies using radiolabeled ligands have demonstrated an inverse correlation between plasma cortisol levels and GR numbers in several different tissues (such as gill, liver, peripheral blood leukocytes, and brain), thus confirming autoregulation of GRs in fish as well. On the other hand, an elevation of plasma cortisol after osmotic stress or during smoltification (pre-preparatory development of saltwater tolerance in salmonids) has been correlated with an increase in gill GR mRNA transcript levels. Differences in the methods used to measure the GR level, whether measuring the total number of cortisol-binding proteins (GR1, GR2, and MR) using binding studies or measuring a specific corticosteroid receptor type (GR1, GR2, or MR) at the mRNA level using PCR methods, might account for some of these discrepancies.

However, the effect of cortisol on GR levels is not straightforward. It is certainly dependent on the type and duration of the stressor (physical, acute, or chronic), target tissue, developmental stage, and previous corticosteroid history of the fish. Thus, due to the recent identification of two GR subtypes and a MR, any stress-related differential regulation among these receptors is at present largely unknown.

Evolution of the Teleost Corticosteroid Signaling System: What Is the Role of the Mineralocorticoid-Like Axis?

The two teleost glucocorticoid receptors (GR1 and GR2) we know today were generated by a genome duplication (2R) specific for the teleost lineage early in the evolution of teleost fish. A notable exception is the zebrafish *(Danio rerio)*, where only one GR subtype has been identified in the fully sequenced genome. Differences in the regulation of receptor levels and ligand-induced transactivation, together with the preservation of two GR genes in most fish species despite the high evolutionary pressure in fish, suggest that the two GRs have different functions (neofunctionalism). However, this is still to be confirmed. As a result of the teleost-specific genome duplication, two MR isoforms should theoretically exist, but one copy seems to have been lost during evolution, since only one MR isoform has been found in teleosts so far.

The contribution of glucocorticoids in hydromineral regulation has changed during evolution from a predominant osmoregulatory corticosteroid in fish and anuran amphibians to shared osmoregulatory roles in terrestrial amphibians and reptiles, and ultimately fully to aldosterone and MR in birds and mammals. The ability of MR to bind aldosterone is an inherited trait from the ancient CR, from which MR and GR both evolved. The emergence of steroid hormone receptors can be narrowed down to the beginning at the vertebrate lineage around Agnathans (lamprey and hagfishes), where only one CR is present. This ancient receptor, which possessed the ability to bind cortisol, DOC, and aldosterone with high affinity, is considered the ancestral receptor from which the modern GR and MR evolved. A genome expansion (1R) after the divergence of jawed vertebrates from cyclostomes, but before the split between bony and cartilaginous vertebrates, gave rise to two CR genes (**Figure 7**). One CR gene – the MR – retained the ancient hormone-binding abilities with affinity for cortisol and DOC (and aldosterone), and the other CR – the GR – through only two mutations in the hormone-binding pocket of the receptor changed the hormone-binding ability from DOC, aldosterone, and cortisol to cortisol only. Thus, through evolution, MR retained the ancient hormone-binding abilities whereas GR evolved a restricted hormone-binding ability, being able to bind only cortisol.

Consequently, the osmoregulatory role of cortisol in fish essentially questions the physiological role and regulation by the mineralocorticoid-like signaling axis, MR-DOC. MR could be a third cortisol receptor in teleosts, conferring the corticosteroid signaling system with more diversity by mediating the cortisol signal. However, the recent discovery of the MR-specific agonist DOC suggests that MR has functions other than the cortisol signaling pathway. DOC was present in lamprey, apparently, before the emergence of GR and MR from CR (**Figure 7**), suggesting that DOC might be an ancient corticosteroid agonist. The presence of 11βHSD2 enzyme in teleost fish further suggests MR-specific signaling. It was suggested that 11βHSD2 emerged during evolution sometime between the invertebrate urochordates and the vertebrate actinopterygii (ray-finned fish), but a more exact appearance is unknown.

Taking a look at the evolution of the 11βHSD family of enzymes may be helpful in attaining a better understanding of the corticosteroid signaling system in fish. At this point, three 11βHSD enzymes have been identified: 11βHSD1, -2, and -3. 11βHSD1 catalyzes the conversion of CR inactive cortisone to cortisol; 11βHSD2 catalyzes the opposite reaction as mentioned above; and the enzymatic function of 11βHSD3 is still unknown. Evolutionarily speaking, 11βHSD3 seems to be the ancestral 11βHSD since it arose in invertebrates (long before evolution of CRs) long before 11βHSD1 and -2, which were first described in shark. While all three 11βHSDs are found in terrestrial vertebrates, teleost fish seem to have lost 11βHSD1 during evolution and possess only 11βHSD2 and -3. Some species (zebrafish and fathead minnow (*Pimephales promelas*)) even have two 11βHSD3 isoforms, 3A and B. Yet, while neither 11βHSD1, -2, nor -3 are found in the lamprey genome, the evolution of the 11βHSDs is still not fully understood. Furthermore, since

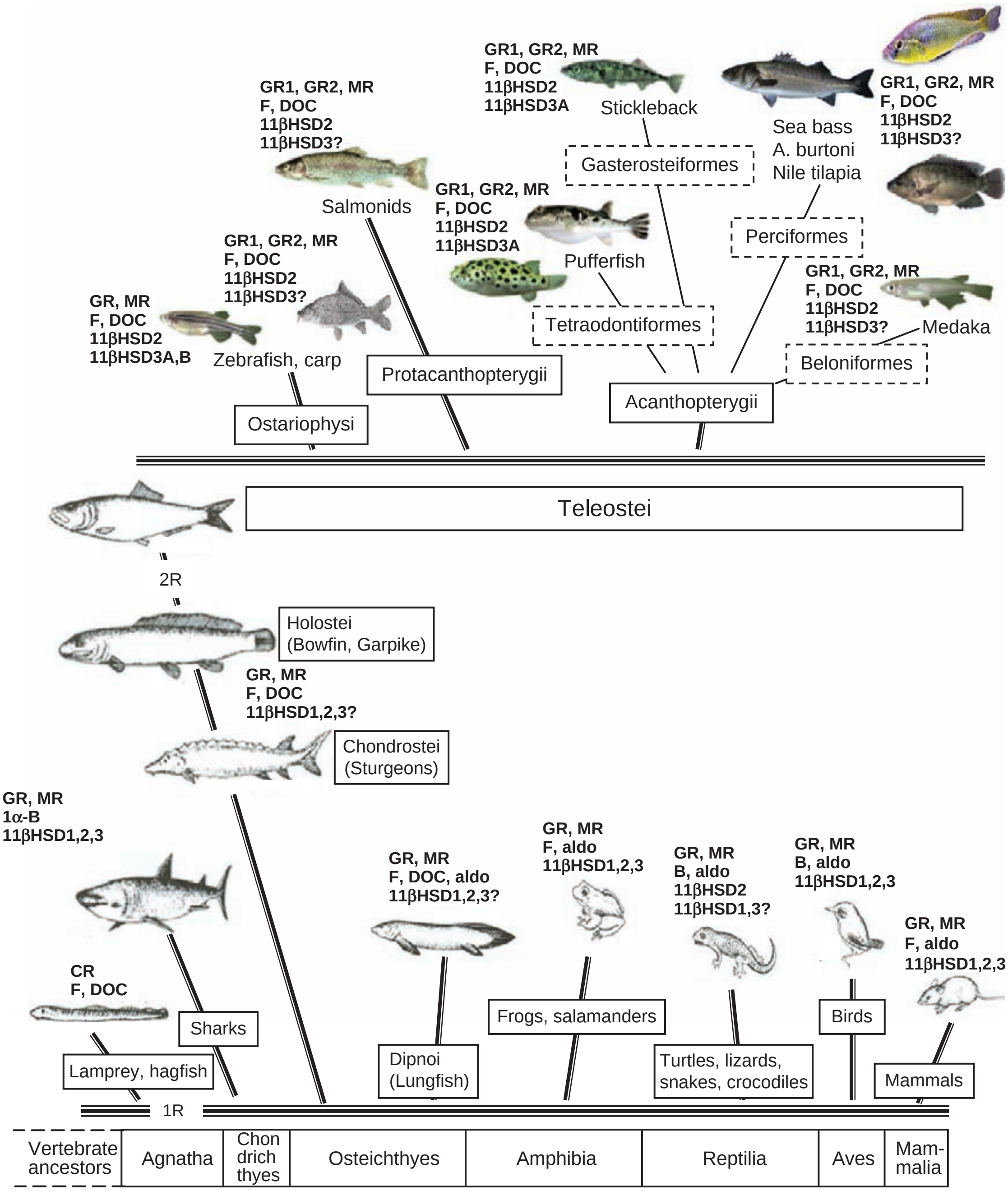


Figure 7 Schematic overview of evolution of corticosteroid signaling system in vertebrates. In the ancestral agnathan vertebrate lineage, only one corticosteroid receptor (CR) is present together with the hormones cortisol (F) and 11-deoxycorticosterone (DOC). A whole genome duplication (1R) before the split of cartilaginous and bony fish created two corticosteroid receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). A teleost-specific second genome duplication (2R) gave rise to two GR subtypes, which are present in three large teleost suborders. The aldosterone-synthesizing ability first arose in lungfish, comprising an evolutionary transition between water and air breathing, explaining the lack of aldosterone (aldo) in teleosts. The corticosteroid signaling system in teleosts thus consists of MR, GR1, GR2, cortisol, and DOC, whereas the tetrapod similarly consists of MR, GR, cortisol, and aldosterone. For comparison, the evolution of 11β hydroxysteroid dehydrogenase (11βHSD) enzymes is included. A question mark denotes the lack of data on the presence of 11βHSDs in that species or animal group. The 11βHSD ancestor, 11βHSD3, arose in invertebrates (not depicted here), whereas 11βHSD1 and -2 seem to have arisen sometime in evolution between agnatha (lamprey and hagfish) and chondrichthyes (sharks). 1α-B denotes 1α-hydroxycorticosterone (only found in sharks and rays). Common names and Latin names for the fishes in the figure are: Zebrafish, *Danio rerio*; Common carp, *Cyprinus carpio*; Salmonids, *Salmoniformes*; Pufferfish, *Tetraodontiformes*; Three-spined Stickleback, *Gasterosteus aculeatus*; European Sea bass, *Dicentrarchus labrax*; Burtons Mouthbrooder, *Astatotilapia burtoni*; Nile tilapia, *Oreochromis niloticus*; Medaka, *Oryzias latipes*. Modified from Bentley PJ (1998) *Comparative Vertebrate Endocrinology*, 3rd edn. Cambridge, UK: Cambridge University Press; Volff JN (2005) Genome evolution and biodiversity in teleost fish. *Heredity* 94(3): 280–294 (Review); Bury NR and Sturm A (2007) Evolution of the corticosteroid receptor signalling pathway in fish. *General and Comparative Endocrinology* 153: 47–56; and Baker ME (2010). Evolution of 11beta-hydroxysteroid dehydrogenase-type 1 and 11beta-hydroxysteroid dehydrogenase-type 3. *FEBS Letters* [Epub ahead of print].

the exact enzymatic function, tissue distribution, and regulation of 11βHSD3 are still unknown, it is too soon to reach any conclusion on the significance of the presence or absence of the 11βHSDs for the corticosteroid signaling system in fish.

See also: **Hormonal Responses to Stress**: Hormone Response to Stress.

Further Reading

Baker ME (2010) Evolution of 11beta-hydroxysteroid dehydrogenase-type 1 and 11beta-hydroxysteroid dehydrogenase-type 3. *FEBS Letters* 584(11): 2279–2284.

Bentley PJ (1998) *Comparative Vertebrate Endocrinology*, 3rd edn. Cambridge: Cambridge University Press.

Bridgham JT, Carroll SM, and Thornton JW (2006) Evolution of hormone-receptor complexity by molecular exploitation. *Science* 312: 97–101.

Bury NR and Sturm A (2007) Evolution of the corticosteroid receptor signalling pathway in fish. *General and Comparative Endocrinology* 153: 47–56.

Colombo L, Dalla VL, Fiore C, Armanini D, and Belvedere P (2006) Aldosterone and the conquest of land. *Journal of Endocrinological Investigation* 29: 373–379.

Lin HY, Muller YA, and Hammond GL (2010) Molecular and structural basis of steroid hormone binding and release from corticosteroid-binding globulin. *Molecular and Cellular Endocrinology* 316(1): 3–12.

Mommsen TP, Vijayan MM, and Moon TW (1999) Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* 9: 211–268.

Phuc Le P, Friedman JR, Schug J, *et al.* (2005) Glucocorticoid receptor-dependent gene regulatory networks. *PLoS Genetics* 1(2): e16.

Prunet P, Sturm A, and Milla S (2006) Multiple corticosteroid receptors in fish: From old ideas to new concepts. *General and Comparative Endocrinology* 147: 17–23.

Volff JN (2005) Genome evolution and biodiversity in teleost fish. *Heredity* 94(3): 280–294 (Review).

Wendelaar Bonga SE (1997) The stress response in fish. *Physiological Reviews* 77: 591–625.

HORMONAL CONTROLS

Hormonal Control of Reproduction and Growth

Contents

Endocrinology of Fish Growth
Endocrine Control of Sex Differentiation in Fish
Endocrine Regulation of Fish Reproduction
Control of Appetite in Fish

Endocrinology of Fish Growth

MA Sheridan, North Dakota State University, Fargo, ND, USA

© 2011 Elsevier Inc. All rights reserved.

Food Intake and Growth Are Coordinated
General Features of Appetite Regulation
Appetite Stimulation
Appetite Suppression
Environmental Influences on Food Intake
Patterns of Growth in Fish
The GH–Insulin-Like Growth Factor System
Other Growth-Regulating Hormones
Control of Growth Hormone Production and Release
Peripheral Control of Growth
Environmental Influences on Growth
Further Reading

Glossary

Anorexigenic complex A collection of brain regions that inhibit food intake.

Brockmann body Large aggregate of endocrine pancreatic tissue found especially within teleost fish.

Chondrocyte A cell type in cartilage that produces extracellular cartilage matrix.

Circadian rhythm An internal rhythm, free running under constant conditions with a period slightly differing from the environmental 24 h cycle.

Compensatory growth A period of accelerated growth following a period of stress, such as food deprivation.

Euryhaline fish A species of fish that can survive in water of wide-ranging salinity.

Hyperplasia Growth by increase in the number of cells of an organ or tissue.

Hypertrophy Growth by increase in the cell size of an organ or tissue.

Hypoosmotic regulation The ability of an organism to survive in an environment of greater osmotic concentration than its own body fluids by effectively regulating ion and water movement across its surfaces.

Hypothalamus A region at the floor of the brain that controls several physiological processes, including food intake and secretion of hormones from the pituitary gland.

Myoblast A type of progenitor cell that can differentiate into skeletal muscle cells.

Neurosecretory cell A neuron that is specialized in secreting hormones into blood.

Orexigenic complex A collection of brain regions that promote food intake.

Sexual maturation A process an organism undergoes that enables it to reproduce.

Smoltification A collection of physiological, morphological, and behavioral processes that a juvenile salmonid fish undergoes in preparation for the transition from freshwater to seawater.

Food Intake and Growth Are Coordinated

Fish, like all animals, must obtain matter and energy from the environment in order to support their life processes. Energy and matter are taken up in the form of food; once ingested, the food is digested into absorbable units and assimilated into tissues (**Figure 1**). Energy is partitioned into maintenance metabolism, growth, reproduction, and storage. Some energy is lost to the environment during transformation reactions, and metabolic by-products are eliminated as waste. The flow of nutrients, from their uptake to their partitioning, is tightly regulated and elaborate mechanisms involving many hormonal systems serve to coordinate and integrate energy allocation. Prioritization of energy allocation is not fixed; adjustments are made as an animal moves through its life history. For example, as an animal undergoes sexual maturation, more resources are devoted to reproduction while fewer are devoted toward growth. As fish occur in a diverse range of aquatic habitats, they have evolved elegant and often elaborate life-history patterns so as to adjust to the conditions present in their environments. Consequently, the energy-allocation strategies and regulatory mechanisms of fish vary somewhat with their life-history pattern, and they are strongly influenced by environmental factors such as temperature, photoperiod, and salinity.

The effective coordination of nutrient allocation results from linkages between and among regulatory systems. As fish growth cannot occur in the absence of nutrients (i.e., food-deprived individuals do not grow), the regulatory systems that serve to control food intake and growth are tightly linked. This article describes basic aspects of feeding and growth, as well as the hormonal systems that serve to regulate them.

General Features of Appetite Regulation

The control of food intake involves interactions between the brain and signals from the peripheral environment, including sensory information as well as signals conveyed via the blood (e.g., hormones and nutrient molecules). The main region of the brain that regulates food intake is the inferior lobe of the hypothalamus. Two distinct collections of neurons in the hypothalamus govern appetite and feeding: one that stimulates food intake (orexigenic complex) and one that inhibits food intake (anorexigenic complex). Other regions of the fish brain are also involved in appetite control. For example, electrical stimulation of the ventral telencephalon or optic tectum stimulates food intake, whereas lesion of the olfactory tract inhibits food intake. The hypothalamic centers integrate information from these other neural circuits in the brain as well as sensory information conveyed by peripheral nerves. Hormones produced by peripheral tissues and organs such as the gut, pancreas, interrenal gland (homologous to the adrenal cortex of mammals), and adipose, as well as the energy state of the animal as reflected by metabolites in the blood, all affect the activity of neurons in the orexigenic and anorexigenic complexes to influence food intake. A model illustrating the brain (central) and peripheral factors that influence feeding is shown in **Figure 2** (**see also Integrated Function and Control of the Gut**: Nervous System of the Gut).

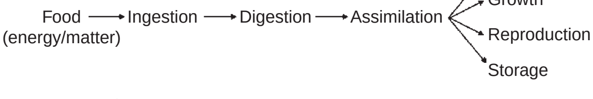


Figure 1 Schematic of the processing of energy and matter in fish. Matter and energy derived from food are assimilated into the organism and partitioned into four basic processes: (1) maintenance metabolism, (2) growth, (3) reproduction, and (4) storage.

Appetite Stimulation

Several compounds produced by different neurons in the hypothalamus and other regions of the brain have been found to stimulate food intake. Neuropeptide Y (NPY), galanin, orexins (includes orexin A and orexin B), and ghrelin are produced by different neurons and stimulate food intake. For example, injection of NPY, galanin, orexins, or ghrelin into the ventricles of the brain (referred to as intracerebroventricular (ICV) injection) stimulates food consumption. The orexigenic action of these factors is supported by the observation that their expression increases during periods of food deprivation. Interactions between and among some of the central orexigenic factors also take place. For example, galanin acts synergistically with NPY and orexins. Similarly, NPY augments the actions of galanin and orexins, and orexins promote the action of NPY.

Ghrelin is also produced in the stomach/gut of fish; therefore, ghrelin acts systemically via the blood. Peripheral injection of ghrelin increases food intake in a manner similar to ICV injection. Immediately after feeding, ghrelin expression in the hypothalamus and gut declines in concert with reduced plasma levels of ghrelin. Ghrelin also stimulates the release of growth hormone (GH) from the pituitary gland, an action that links appetite regulation with growth control. Notably, GH treatment of fish (via intraperitoneal injection (IP) or implantation) stimulates feeding behavior (**see also Integrated Function and Control of the Gut**: Endocrine Systems of the Gut).

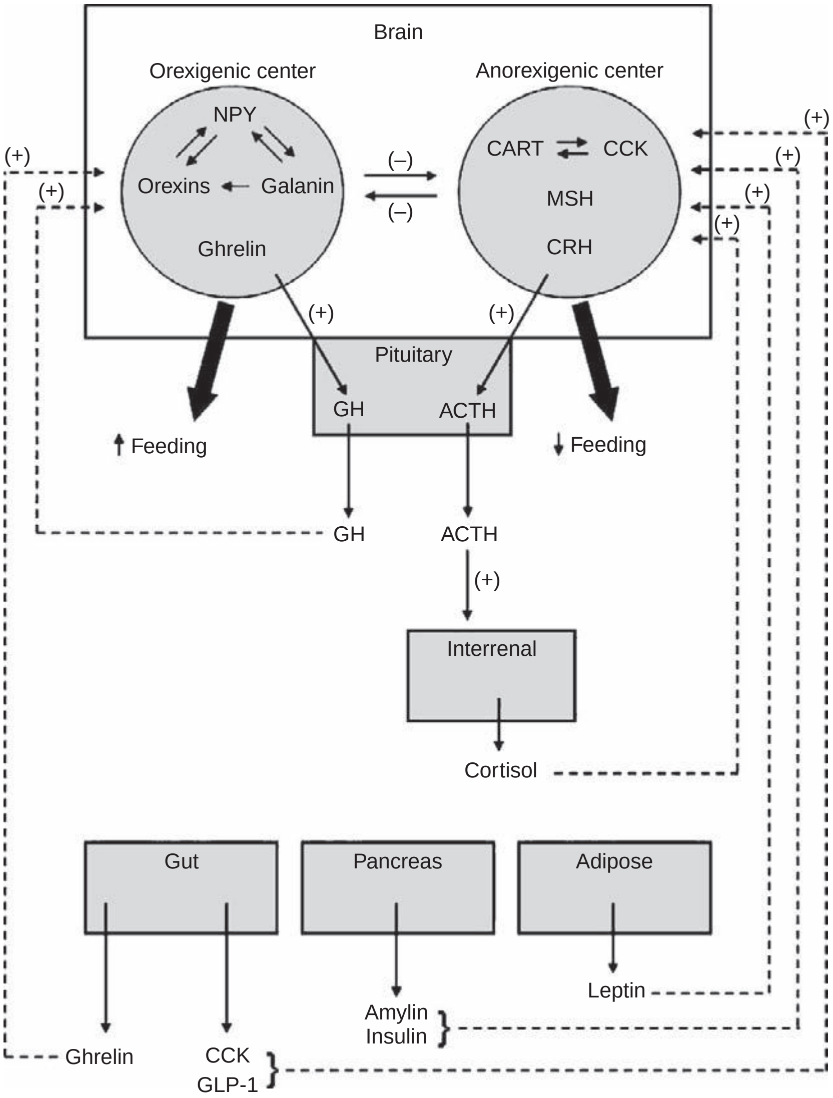


Figure 2 Model of appetite regulation in fish. The orexigenic and anorexigenic centers of the brain integrate sensory information from the external environment as well as information from the periphery of the animal to control feeding. Solid lines indicate direct actions; dashed lines indicate feedback actions.

Appetite Suppression

The brain also produces a number of compounds that suppress appetite and reduce food intake. Cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH), cholecystokinin (CCK), and melanocyte-stimulating hormone (MSH) are produced by different neurons in various regions of the brain and are part of the anorexigenic complex. ICV injection of CART, CRH, CCK, or MSH inhibits food intake. Hypothalamic levels of CART messenger (mRNA) increase immediately after eating and decline in association with fasting. In addition, CCK antagonists stimulate food intake. Similar to the situation for the orexigenic complex, there are interactions between components of the anorexigenic complex. For example, CCK and CART augment each other's actions. In addition, there is reciprocal inhibition between the orexigenic and anorexigenic complexes. Notably, CART inhibits both NPY- and orexin-stimulated food intake, whereas NPY blocks CART- and CRH-inhibited food intake.

Several hormones produced by peripheral organs/tissues suppress food intake by stimulating the anorexigenic complex in the brain. Leptin and leptin-like material have been isolated from adipose tissue from several species of fish. Although leptin has been shown to inhibit food intake in mammals, studies in fish have been hampered by the lack of available fish leptin; therefore, most studies in fish have been conducted with mammalian hormone and the results are mixed. For example, mammalian leptin has no effect on food intake in salmon or catfish, but ICV or IP injection of mammalian leptin significantly decreases food intake of goldfish. In addition, ICV injection of mammalian leptin augments the anorexigenic actions of CART and CCK and inhibits NPY- and orexin-induced food intake in goldfish. Recent work in trout showed that recombinant trout leptin suppresses food intake and reduces NPY mRNA expression in the brain. The gastrointestinal tract produces CCK (which is in addition to that produced by the brain) and glucagon-like peptide-1 (GLP-1) (derived from the same precursor as that giving rise to glucagon). Plasma levels of CCK and GLP-1 increase following a meal, and peripheral injection of either peptide suppresses food intake. Insulin and amylin are produced by the endocrine portion of the pancreas of fish (referred to as the Brockmann body) and elsewhere. ICV injection of insulin or amylin can inhibit food intake. Cortisol is produced by the interrenal tissue of fish (homologous to the cortex of the mammalian adrenal gland) under the influence of adrenocorticotropic hormone (ACTH) from the pituitary and, ultimately, CRH from the hypothalamus. Cortisol has many functions including mediation of organismal stress responses (e.g., mobilization of stored energy reserves and suppression of immune system), and in some species of fish, cortisol inhibits food intake, which may explain, at least in part, stress-induced appetite suppression.

Environmental Influences on Food Intake

Several intrinsic (i.e., nutritional status; and stage of life history, including sexual maturity) and extrinsic (i.e., temperature, photoperiod, salinity, etc.) environmental factors can inhibit or stimulate food intake of fish. The presence of nutrients in the blood, especially glucose or amino acids, reduces food intake; the higher the concentration of glucose in the blood, the longer the time to the next feeding. Nutrients may affect feeding directly by altering metabolic processes of neurons in the orexigenic and anorexigenic complexes and indirectly by acting on peripheral tissues/organs. For example, the effects of amino acids could be explained, in part, by stimulating insulin release from the pancreas, and the insulin, in turn, stimulating the anorexigenic complex to suppress food intake. The profile of many of the appetite-regulating hormones varies over the course of development, with clear species differences observed in the profiles. In some cases, factors appear early in development and perhaps play a role in embryogenesis or nutrient-absorption acquisition in larvae, while in other cases, factors appear later in development and perhaps play a role in metamorphosis or shift in feeding strategy. Sex-specific differences in the distribution and expression of appetite-regulating factors such as galanin and ghrelin have been observed. The seasonal changes in NPY are correlated with gonadal cycles. The role of sex steroids on feeding behavior, however, remains to be clarified.

The influence of temperature on feeding is complex; generally food consumption increases with increasing temperature, but at extreme temperatures (either low or high), feeding is suppressed. The mechanisms that underlie temperature-associated changes in food intake are not fully known, but hypothalamic CART (a component of the anorexigenic complex) expression is higher in fish held in cold water compared with fish held in warmer water (**see also Food Acquisition and Digestion:** Cost of Digestion and Assimilation).

Food intake is also influenced by photoperiod in some species of fish but not others. In trout and salmon, for example, food intake (and growth, as is discussed next) is stimulated by a longer daily-light regime. Many hormones may be responsible for such effects, including GH and melatonin, the latter of which is produced in a circadian rhythm by the pineal organ on the dorsal surface of the brain and exhibits peak levels during periods of darkness. Recent studies indicate that IP injection of melatonin inhibits food intake.

Patterns of Growth in Fish

Organismal growth integrates a host of biological processes that lead to a fertilized egg developing into an adult and to the maintenance and modulation of adult structures appropriate to their function. Animal growth is influenced by genetic, environmental, and nutritional factors. Extrinsic factors are particularly important in the growth of poikilothermic vertebrates, such as teleost fish, which rely on temperature, photoperiod, and food availability to trigger developmental processes such as hatching, metamorphosis, smoltification, sexual maturation, and spawning. Integration of external and internal cues leads to the coordination of animal growth via the interplay of numerous hormones.

Fish are unique among vertebrates in that most species have the capacity to grow continuously throughout their life. Such indeterminate growth is in contrast to determinate growth, in which an increase in body size ceases, usually after sexual maturation. Typically, however, the rate of body growth for fish slows over time, especially just prior to sexual maturity when energy is channeled to gonad growth and gamete production.

The GH–Insulin-Like Growth Factor System

The primary control of growth of vertebrates including fish is through the GH–insulin-like growth factor (IGF) system. GH, the initial hormone in this system, has been isolated from the pituitary gland from representatives in every extant class of vertebrate. GH results in increased amino acid uptake, increased RNA synthesis, increased protein synthesis, increased muscle growth, and increased cartilage synthesis. Many of the growth-promoting actions of GH in fish and other animals are mediated by IGF-1, so named because of it structural similarity to insulin (INS). Circulating GH stimulates the synthesis and secretion of IGF-1 from liver and other sites (e.g., gill and muscle) and IGF-1, in turn, binds to IGF-1 receptors (IGFR-1s) on target tissues, which are distinct from INS receptors, resulting in growth promotion and other biological actions (e.g., energy metabolism and osmoregulation). GH has also been shown to stimulate differentiation of chondrocyte stem cells into cells which respond to and/or produce IGF-1. The availability and actions of GH and IGF-1 are influenced by GH-binding proteins and IGF-1-binding proteins (IGFBPs), respectively. IGFBPs also have direct, non-IGF-1-dependent effects on growth. The GH–IGF system of teleost fish is more complex as a result of a genome duplication associated with their evolution, and multiple forms of GH, GH receptors (GHRs), and IGFR-1s have been described. An overview of the GH–IGF system is depicted in **Figure 3**.

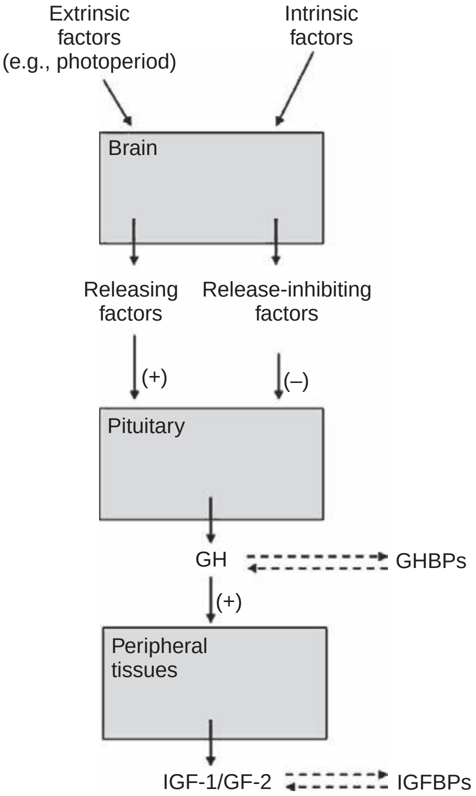


Figure 3 Schematic of the growth hormone (GH)–insulin-like growth factor (IGF) system in fish. External and internal environmental information is integrated by the brain and results in the secretion of either GH-releasing- or GH-release-inhibiting factors from the hypothalamus. With a preponderance of GH-releasing factors, the pituitary secretes GH which, in turn, stimulates the secretion of IGF-1 and IGF-2 from the liver and other tissues. The distribution and action of GH and IGF-1/IGF-2 are influenced by GH-binding proteins (GHBPs) and IGF-binding proteins (IGFBPs), respectively.

Other Growth-Regulating Hormones

Numerous other hormones are known to influence the growth of fish. Many of these growth-promoting actions result from modulation of some aspect of the GH–IGF system. INS is required for normal growth of fish and INS deficiency results in growth retardation. INS appears to act synergistically with GH to promote IGF-1 production, especially in liver. Thyroid hormones (e.g., thyroxin, T4) are also necessary for normal growth and development of fish. Experimentally induced hypothyroidism (using propylthiouricil) reduces body growth and food conversion, effects that likely result from an attending deficiency in plasma GH. The steroid hormones, cortisol and 17β-estradiol (E2) (the major estrogen in fish), also promote growth by stimulating GH (which is discussed in more detail, next). It should be noted, however, that the actions of E2 are especially complex, sometimes promoting growth while at others inhibiting growth – disparate actions that may be related to the stage of sexual maturation.

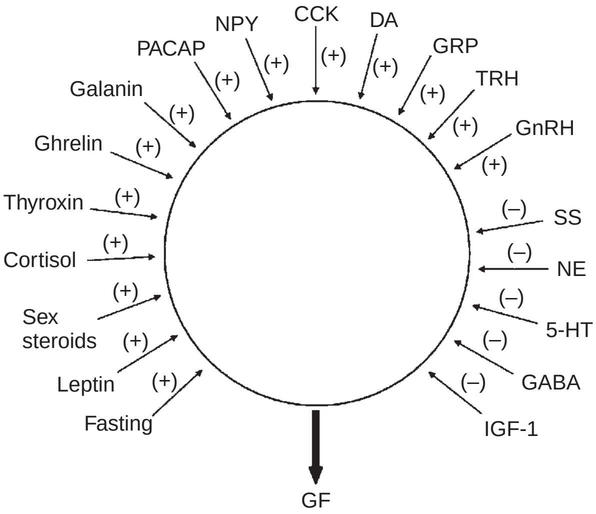


Figure 4 Schematic of the control of growth hormone (GH) release from the pituitary gland of fish. Numerous factors from the hypothalamus of the brain or from the periphery influence, either positively or negatively, the secretion of GH.

Control of Growth Hormone Production and Release

Much of the research on the regulation of animal growth has focused on the production and release of GH (**Figure 4**). In most species studied, including mammals and fish, pituitary GH release is under dual antagonistic control from the hypothalamus of the brain. Neurosecretory cells originating in the hypothalamus release amines and peptides that either stimulate or inhibit the production/release of GH. The major inhibitors of GH release are somatostatins (SSs), a structurally and functionally diverse family of peptide hormones. In fish, the structural heterogeneity arises from the tissue-specific processing of a larger precursor molecule (preprosomatostatin (PPSS)) as well as from the existence of multiple PPSS-encoding genes. SSs are not equipotent in their ability to inhibit GH. For example, SS-14, a SS form that appears highly conserved in vertebrates, from fish to mammals, is very effective in inhibiting GH from isolated goldfish pituitary fragments, but teleost SS-25, a form containing Tyr^7, Gly^{10} amino-acid substitutions in its *C*-terminus and found only in teleost fish, has almost no effect. Norepinephrine (NE), 5-hydroxytryptamine (5-HT; or sometimes called serotonin) and γ-aminobutyric acid (GABA) also inhibit secretion of GH from fish pituitary. Numerous neurosecretory factors stimulate GH release, including GH-releasing hormone (GHRH), pituitary adenylate cyclase-activating peptide (PACAP), dopamine, gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), gastrin-releasing peptide (GRP), galanin, CCK, ghrelin, and NPY. The efficacy of these various factors to promote GH release varies among species.

Factors produced by peripheral organs and delivered to the pituitary through the systemic circulation also affect GH release. In addition to cortisol, T4, and E2, leptin, which is expressed in adipose tissue and liver of fish, can stimulate GH in fish just as in mammals. By contrast, IGF-1 (predominately expressed in liver) inhibits GH release, completing a negative feedback loop for growth control. It should be noted that some factors produced by neurosecretory cells in the hypothalamus are also produced by epithelial cells in peripheral organs; as a result, such factors affect GH release by two routes. One example is SS, which in addition to the hypothalamus is also produced by epithelial cells in the gut and by δ-cells of the endocrine pancreas (**see also Gut Anatomy and Morphology**: Pancreas). A second example is ghrelin, which is also produced by epithelial cells in the lining of the stomach and the upper intestine of fish. A third example is NE, which is also produced by dispersed chromaffin tissue (equivalent to the medulla of the adrenal gland of mammals). High circulating levels of NE (as well as epinephrine) are observed under stressful conditions, which may contribute to stress-associated suppression of both feeding and growth. Given the complexity of the GH–IGF system and the observation that there are tissue-specific differences in growth, it is increasingly clear that the regulation of growth also occurs at many levels outside the pituitary (**see also Integrated Function and Control of the Gut:** Nervous System of the Gut).

Peripheral Control of Growth

Increasing research indicates that growth of fish is also regulated by adjusting peripheral sensitivity to GH and IGF-1, as well as by modulating GH and IGF-1 actions in target cells. In keeping with its moniker as 'the great inhibitor', SS has effects on several levels of the GH–IGF system outside the pituitary, where it inhibits GH release. For example, SS reduces peripheral sensitivity to GH in liver cells by inducing the internalization of surface-associated GHR and by decreasing the biosynthesis of new GHRs. SS also inhibits IGF-1 biosynthesis in, and secretion from, liver cells. Furthermore, the sensitivity to IGF-1 is reduced by SS in target cells, such as those found in the gill, via inhibition of mRNA and functional expression of IGFR1s. SS may also influence growth through its actions on other hormonal systems. In particular, SS inhibits INS secretion from the endocrine pancreas of fish, and plasma levels of SS are inversely correlated with plasma levels of T4. Other hormones also appear to affect peripheral components of the GH–IGF system. INS and androgens such as testosterone may heighten sensitivity to GH by increasing expression of GHR mRNAs, particularly in liver. Hepatic IGF-1 mRNA expression is stimulated by androgen and inhibited by E2. A negative feedback is provided by IGF-1 by inhibiting expression of IGFR-1 mRNAs in muscle, including cardiac muscle (**see also Cellular, Molecular, Genomics, and Biomedical Approaches:** Growth Hormone Overexpression in Transgenic Fish).

Environmental Influences on Growth

As for feeding, several intrinsic (i.e., nutritional status; and stage of life history, including sexual maturity) and extrinsic (i.e., temperature, photoperiod, salinity, etc.) environmental factors affect the growth of fish. The quantity and quality of food are the most important regulators of growth. Fasting and reduced ration result in decreased growth. Carnivorous fish fed a high carbohydrate diet also display reduced growth. Several nutrient–hormone interactions may explain these observations. In particular, fasting reduces INS secretion and increases SS secretion, alterations that together would lead to reduced growth as discussed above. Moreover, INS-secreting cells of carnivorous fish are more sensitive to amino acids than to glucose; therefore, such fish fed a high carbohydrate diet have a reduced INS response compared to when fed a lower carbohydrate and higher protein-content diet.

Refeeding fish after a period of food deprivation leads to a period of compensatory growth during which the rate of growth is accelerated. During compensatory growth, fish display increased food intake and increased food conversion compared with periods of normal growth. The hormonal mechanisms that underlie compensatory growth are not fully known, but they may be related to IGF-1 kinetics and interaction with the orexigenic complex.

Generally, increased temperature increases growth, within the temperature-tolerance range of the fish, a response that appears to be related to enhanced IGF-1 production at high temperatures.

Photoperiod has varying effects on the growth of fish, depending on the species. Some species (e.g., Atlantic salmon, Atlantic halibut, and Atlantic cod) display higher growth rates when reared under extended photoperiod conditions, whereas the growth of other species (e.g., black porgy and sunshine bass) does not appear to be affected by extended daylength. Extended photoperiod, which elevates plasma GH, is used to advance the timing of smoltification and sexual maturation in the salmon aquaculture industry.

The influence of salinity is complex and depends on other variables (temperature and oxygen level). Generally, marine fish have higher growth rates in reduced salinity (optimal growth in the range of 12–19 parts per thousand (ppth), where seawater is typically 35 ppth), whereas freshwater fish have higher growth rates in water of elevated salinity (2 ppth). The argument advanced for these observations surrounds reduced costs of osmoregulation, enabling animals to channel more energy into growth. Both GH and IGF-1 stimulate hypoosmotic regulatory ability, and transfer of euryhaline fish from freshwater to seawater is accompanied by transient increases in plasma levels of GH and IGF-1 as well as an increase in GHR and IGFR1 expression. The extent to which such changes in the components of the GH–IGF system may underlie salinity-associated growth is not known.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Gut Anatomy and Morphology**: Pancreas. **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut; Nervous System of the Gut.

Further Reading

Beckman BR, Shimizu M, Gadberry BA, and Cooper KA (2004) Response of the somatotropic axis of juvenile coho salmon to alterations in the plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41 kDa IGFBP. *General and Comparative Endocrinology* 135: 334–344.

Bjornsson BTh, Johansson V, Benedet S, *et al.* (2004) Growth hormone endocrinology of salmonids: Regulatory mechanisms and mode of action. *Fish Physiology and Biochemistry* 27: 227–242.

Boeuf G and Payan P (2001) How should salinity influence fish growth? *Comparative Biochemistry and Physiology* 130C: 411–423.

Canosa LF, Chang JP, and Peter RE (2007) Neuroendocrine control of growth hormone in fish. *General and Comparative Endocrinology* 151: 1–26.

Chang JP and Wong AOL (2009) Growth hormone regulation in fish: A multifactorial model with hypothalamic, peripheral and local autocrine/paracrine signals. In: Bernier N, Van Der Kraak G, Ferrell A, and Brauner C (eds.) *Fish Physiology*, vol. 28, pp. 151–195. New York: Academic Press.

Gabillard JC, Weil C, Rescan PY, *et al.* (2003) Effects of environmental temperature on IGF1, IGF2, and IGF type 1 receptor expression in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* 133: 233–242.

Gorissen MHAG, Flik G, and Huising MO (2006) Peptides and proteins regulating food intake: A comparative view. *Animal Biology* 56: 447–473.

Klein SE and Sheridan MA (2008) Somatostatin signaling and the regulation of growth and metabolism in fish. *Molecular and Cellular Endocrinology* 286: 148–154.

Leggatt RA, Raven PA, Mommsen TP, *et al.* (2009) Growth hormone transgenesis influences carbohydrate, lipid and protein metabolism capacity for energy production in coho salmon (*Oncorhynchus kisutch*). *Comparative Biochemistry and Physiology* 154B: 121–133.

Mommsen TP (2001) Paradigms of fish growth. *Comparative Biochemistry and Physiology* 129B: 207–219.

Nelson LE and Sheridan MA (2006) Gastroenteropancreatic hormones and metabolism in fish. *General and Comparative Endocrinology* 148: 116–124.

Picha ME, Turano MJ, Tipsmark CK, and Borski RJ (2008) Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* X *Morone saxatilis*). *Journal of Endocrinology* 199: 81–94.

Poppinga J, Kittilson JD, McCormick SD, and Sheridan MA (2007) Effects of somatostatin on the growth hormone–insulin-like growth factor axis and seawater adaptation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 273: 312–319.

Reinecke M, Björnsson BTh, Dickhoff WW, *et al.* (2005) Growth hormone and insulin-like growth factors in fish: Where we are and where to go. *General and Comparative Endocrinology* 142: 20–24.

Sheridan MA and Hagemeister AL (2010) Somatostatin and somatostatin receptors in fish growth. *General and Comparative Endocrinology* 167: 360–365.

Shimizu M, Fukada H, Hara A, and Dickhoff WW (2007) Response of the salmon somatotropic axis to growth hormone administration under two different salinities. *Aquaculture* 273: 320–328.

Silverstein JT, Shearer KD, Dickhoff WW, and Plisetskaya EM (1999) Regulation of nutrient intake and energy balance in salmon. *Aquaculture* 177: 161–169.

Volkoff H, Unniappan S, and Kelly SP (2009) The endocrine regulation of food intake. In: Bernier N, Van Der Kraak G, Ferrell A, and Brauner C (eds.) *Fish Physiology*, vol. 28, pp. 421–465. New York: Academic Press.

Wood AW, Duan C, and Bern HA (2005) Insulin-like growth factor signaling in fish. *International Review of Cytology* 243: 215–285.

Endocrine Control of Sex Differentiation in Fish

F Piferrer, Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain

© 2011 Elsevier Inc. All rights reserved.

Introduction
Morphological Aspects
Endocrine Control of Sex Differentiation
Environmental Influences
Concluding Remarks
Further Reading

Glossary

False hermaphroditism Sometimes called juvenile hermaphroditism, this refers to gonochoristic species of the undifferentiated type at the stage of development when the gonads contain both ovarian and testicular tissues.

Genotypic sex Also known as genetic sex. Sex given by the genotype of an individual, usually established shortly after fertilization during the formation of the zygote. Except in species with simple (monofactorial) sex determining systems or with well defined molecular markers, the genotypic sex usually cannot be distinguished. In species with strong environmental sex determination, genotypic sex proper does not exist or is irrelevant, because the sex of the individual depends on the environmental conditions experienced during specific windows of development.

Intersex Individual with both testis and ovary or with gonads with male and female germ cells that occupy different areas of the gonads, or are intermixed. Thus, strictly speaking, all hermaphrodites are intersex at one time point, but not all intersexes are hermaphrodites. This definition usually is applied to gonochoristic species to describe those individuals that are not normal for the species. Many intersexes are the result of exposure to chemicals present in the aquatic environment.

Phenotypic sex Also known as gonadal sex. Sex defined according to the gonads of an individual. Sperm producers are males and egg producers are females. Gonadal sex usually (but not always) is established by the process of sex differentiation in accordance with the mechanism of sex determination. Sex differentiation is very labile in fish and easily influenced by external factors. Thus, under some circumstances, phenotypic sex does not necessarily coincide with genotypic sex.

Sex change A process present in sequential hermaphrodites whereby animals reorganize their gonads to produce gametes different from those produced during the last sexual maturation. According to the polarity of change, species are classified as protandrous (male → female), protogynous (female → male), or bidirectional (male ↔ female).

Sex determination A genetic or environmental process whereby the gender (male or female) is established in an individual. In most species, sex determination depends on the genotype (genotypic sex determination, GSD), but in some it depends on the value of some environmental variables during a specific developmental stage (environmental sex determination, ESD), with temperature being the most common one (temperature-dependent sex determination, TSD). Even in species with ESD, there always is a genetic component controlling sex determination.

Sex differentiation A process whereby a sexually undifferentiated gonad transforms into a testis or an ovary, according to genetic information, the influence of the environment, or both. Sex differentiation results in the gonadal or phenotypic sex.

Sex ratio The relative number of males and females in a population. Primary, secondary, tertiary, and operational sex ratio refer to the sex ratios of a population right after fertilization, at hatching, during adulthood, and of the part of the population that actually reproduces, respectively.

Sexuality type Fish exhibit three major types of sexuality: gonochorism or separate sexes, by far the most frequent type, in which each individual functions as either a male or a female; hermaphroditism, in which all or most individuals produce both types of gametes during their lifetime, either simultaneously or sequentially; and unisexuality, in which all individuals are females that reproduce by parthenogenesis, either gynogenesis or hybridogenesis.

Testis–ova Also known as ovotestis, in medicine, it refers to a true hermaphroditic gonad with both male and female tissues. In fish, this term usually indicates males of gonochoristic species whose testes, because of certain circumstances (exposure to hormones, pollutants, or high temperatures), contain female germ cells, usually previtellogenic oocytes. Testis–ova are a particular case of intersex.

Introduction

With almost 30 000 species, fish are by far the largest and most diverse group of vertebrates. Furthermore, fish inhabit a wide range of habitats, from shallow waters to the deep sea and have also a wide range of life-cycle strategies. Thus, it is not surprising that they also vary in their reproductive styles.

Fish exhibit all the sexuality types known among vertebrates: gonochorism, hermaphroditism, and unisexuality (**Table 1**). Gonochoristic species (separate sexes) are by far the most abundant – each animal functions either as a male (sperm producer) or as a female (egg producer).

In hermaphroditic species (~500 species), all or most individuals act both as males and females during their lifetime. Hermaphrodites can function as males and females in a sequential (consecutive) or in a simultaneous manner. Sequential hermaphroditic species are grouped according to which sex matures first. In protogynous species, the most abundant type of hermaphrodites, fish mature first as females and then change to males and continue maturing as such. In protandrous species, it is the other way around. Recently, some members of the family Gobiidae have been found to be able to sex change more than once and are referred to as both-way or bidirectional sequential hermaphrodites. In contrast, simultaneous hermaphrodites, such as some members of the family Serranidae, can mature as males and females at the same time, thus producing sperm and eggs. To avoid self-fertilization, usually cross-fertilization takes place. However, in the mangrove rivulus *Kryptolebias* (formerly *Rivulus*) *marmoratus*, self-fertilization can occur. Finally, unisexual species (~100 species) reproduce by parthenogenesis, including hybridogenesis and gynogenesis.

Whether an animal becomes male or female depends on the combined processes of sex determination and sex differentiation (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular and Chromosomal Aspects of Sex Determination). Sex differentiation is the development of an undifferentiated gonad and its transformation into either a testis or an ovary. This produces an individual's phenotypic sex, which contributes to the sex ratio of the population. The processes involved in gonad differentiation are quite plastic in many fish species and thus sex ratios vary with environmental conditions.

Knowledge of the mechanisms leading to a particular sex ratio is of both basic and applied importance. From a basic point of view, it is important to understand the evolution of sex-determining mechanisms. Studying sex determination and differentiation is also important to understand sex allocation, that is, the distribution of resources to each sex, and for understanding the evolution of mating strategies. Recently, it has become evident that the lability of sex determination and differentiation in fish may contribute to the process of speciation. As the sex ratio strongly influences the reproductive capacity of populations, knowledge of sex differentiation is relevant from an applied point of view as well – for example, to manage the sex ratio in aquaculture and population-control programs. It is also important to understand the effects of chemical pollution and climate change, especially for species with environmental sex determination.

Morphological Aspects

The gross morphological aspects of fish gonadal development have been known for some time, but only for a

Table 1 Sexuality types in fish with some representative examples

Sexuality type	*Subtype*		*Representative species*
Gonochorism[a]	Differentiated		Atlantic salmon (*Salmo salar*)
			European sea bass (*Dicentrarchus labrax*)
			Medaka (*Oryzias latipes*)
			Nile tilapia (*Oreochromis niloticus*)
			Platyfish (*Xiphophorus maculatus*)
			Rainbow trout (*Oncorhynchus mykiss*)
	Undifferentiated[b]		European eel (*Anguilla anguilla*)
			Guppy (*Poecilia reticulata*)
			Zebrafish (*Danio rerio*)
Hermaphroditism	Sequential	Proterandry	Gilthead seabream (*Sparus aurata*)
		Proterogyny	Common pandora (*Pagellus erythrinus*)
		Bidirectional	Okinawa rubble goby (*Trimma okinawae*)
	Simultaneous		Blackspot seabream (*Pagellus bogaraveo*)
Unisexuality	Gynogenesis		Amazon molly (*Poecilia formosa*)
			Prussian carp (*Carassius auratus gibelio*)
	Hybridogenesis		Guatopote (*Poeciliopsis monacha-lucida*)

[a] Sometimes referred to as separate sexes.

[b] Sometimes referred to as false hermaphroditism or (incorrectly) as juvenile hermaphroditsim.

fraction of the total number of fishes. In general, fish gonads are paired organs located in the dorsal side of the body cavity in close vicinity to the mesonephros and between the celomic epithelium and the gut. In some species, however, such as some agnathans and some viviparous teleosts, the gonads form a single, centrally fused lobe. In species with swimbladder, gonads are usually attached to it (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis, Regulation of Spermatogenesis, and **Role of the Kidneys**: Histology of the Kidney).

Patterns of Gonadal Development

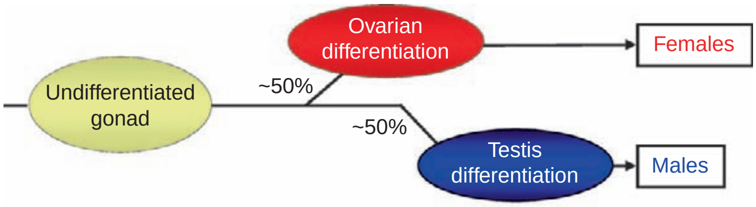
Most fish species (more than 90%) are gonochoristic and two patterns of gonadal development are recognized (**Figure 1**). Gonochoristic differentiated species display one pattern. These species are those in which the formed, but sexually undifferentiated gonad directly differentiates into either ovary or testis. Usually, ovarian differentiation precedes testicular differentiation so that there is a period of time when sexually undifferentiated fish coexist with fish starting ovarian differentiation. Examples include the common carp (*Cyprinus carpio*), trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), medaka (*Oryzias latipes*), and the European sea bass (*Dicentrarchus labrax*).

Gonochoristic undifferentiated species display the other pattern. These species all begin differentiation toward an ovarian-like structure, complete with developing oocytes. Later, about half of the individuals proceed with ovarian differentiation to become females, while the other half stop ovarian differentiation, regress the ovarian-like structures formed so far, and then testicular differentiation takes place. This regression possibly involves programmed cell death (apoptosis), although this process has not been studied in a sufficiently large number of species to allow firm generalizations. Examples include lampreys (*Lampetra* sp.), hagfish (*Myxine* sp.), guppy (*Poecilia reticulata*), European eel (*Anguilla anguilla*), and zebrafish (*Danio rerio*).

Usually, gonadal development in fish depends more on size than on age and thus growth rates can influence its course in species in which there is a genotype–environment interaction in sex determination in such a way that growth rates above average usually cause a higher incidence of females. This phenomenon is known as 'growth-dependent sex differentiation'. Fish arriving at a certain critical developmental stage develop as one sex or the other depending upon their size. Although theoretically larger animals can develop as either sex, in fish that exhibit this growth-dependent sex differentiation, larger individuals at the critical time usually develop as females, whereas the smaller ones develop as males.

From a morphological point of view, the process of sex differentiation in fish can be divided into two major periods regardless of reproductive strategy: the undifferentiated period and the gonadal differentiation period. In turn, the first one is subdivided into the pregonadal stage, in which the primordial germ cells (PGCs) appear early during embryogenesis, migrate along the dorsal mesentery, and concentrate along the peritoneal wall where the future gonads are going to develop; and the gonad formation stage, in which first epithelial cells proliferate and form the gonadal ridges, which are then colonized by the

(a) Gonochoristic differentiated species



(b) Gonochoristic undifferentiated species

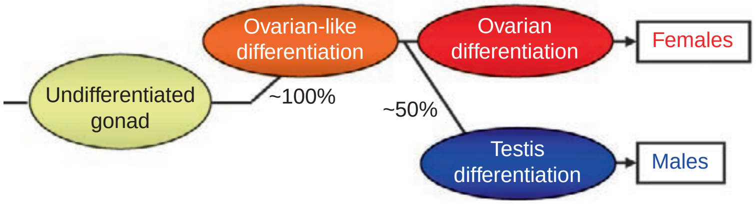


Figure 1 Patterns of sex differentiation in gonochoristic species. (a) Differentiated species. About 50% of the animals differentiate as females while the rest remain undifferentiated and later differentiate as males. The degree of overlap in male vs. female sex differentiation may be species specific and may depend on environmental conditions affecting growth rates. (b) Undifferentiated species. Initially, all fish start developing as females. Later, about 50% stop female differentiation and differentiate as males.

PGCs and somatic cells derived from the mesenchyme. At this point, the animal has sexually undifferentiated gonads (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Germ Cell Migration and Trans Sex).

Sexual differentiation proper takes place during the gonad differentiation period when ovarian and testicular differentiations occur. Usually, ovarian differentiation precedes testicular differentiation. The first histological signs of ovarian differentiation are the elongation of gonad ridges by active mitosis, refolding, and formation of the future ovarian cavity. Cytologically, the first signs of female differentiation are marked by the entry of some oogonia into meiosis to become primary oocytes.

The process of sex differentiation can take a cephalocaudal (head to tail) or caudocephalic (tail to head) gradient depending upon the species. For example, in salmonids such as the coho salmon, *Oncorhynchus kisutch*, the gonads develop in a cephalocaudal fashion, whereas in the European sea bass, it is the other way around. The process of sex differentiation can be completed in a matter of a few weeks, as in medaka or zebrafish, or take many months, as in turbot, *Scophthalmus maximus*, or Atlantic cod, *Gadus morhua*. The process of sex differentiation in the European sea bass is illustrated in **Figure 2**.

Endocrine Control of Sex Differentiation

Fish gonads, like other vertebrate gonads, have two major functions: the production of gametes (gametogenesis) and the production of sex steroids (steroidogenesis) (**see also The Reproductive Organs**

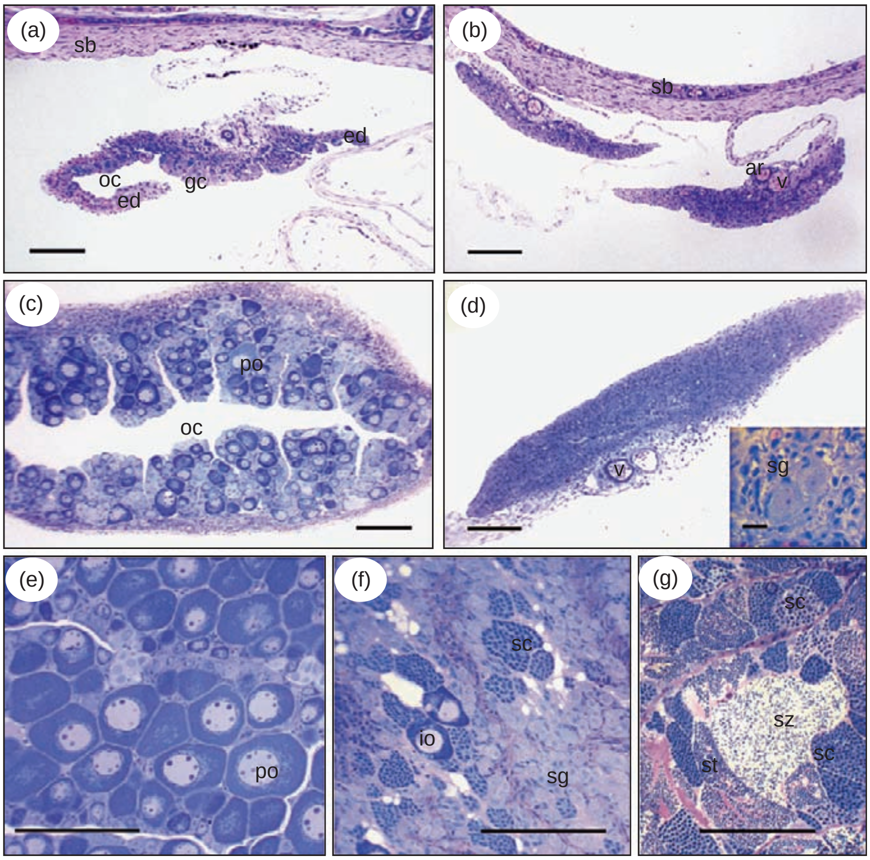


Figure 2 Histological sex differentiation in the European sea bass (*Dicentrarchus labrax*), a gonochoristic differentiated teleost. (a) Differentiating ovary attached to the swimbladder (sb) at 150 days post-hatching (dph). The two edges (ed) of the gonad join to form the ovarian cavity (oc). Clutches of germ cells (gc) are present. (b) Undifferentiated presumptive male gonads at 150 dph attached to the swimbladder (sb), with the vein (v) and artery (ar) visible. (c) Ovary at 200 dph, with primary oocytes (po) and a well-developed ovarian cavity (oc). (d) Testis at 200 dph with spermatogonia (sg, insert) dispersed among somatic cells (v, vein). (e) Completely differentiated ovary at 250 dph. The ovarian lamellae are filled with primary oocytes (po). (f) Testes at 250 dph, showing cysts filled with spermatogonia (sg) as well as spermatocytes (sc). Some isolated intratesticular oocytes (io) are also present. (g) Maturing testis at 300 dph undergoing spermatogenesis, containing all types of germ cells: spermatocytes I and II (sc), spermatids (st), and spermatozoa (sz). Scale = 100 μm except (d) insert where the bar = 10 μm. Reproduced from Papadaki M, Piferrer F, Zanuy S, *et al*. (2005) Growth, sex differentiation and gonad plasma levels of sex steroids in male- and female-dominant populations of *Dicentrarchus labrax* obtained through repeated size grading. *Journal of Fish Biology,* 66: 938–956.

and Processes; Anatomy and Histology of Fish Testis, Regulation of Spermatogenesis).

Some species, including the medaka, rainbow trout, and Nile tilapia, have a chromosomal system of sex that allows the production of fish of a particular genotype after crossing normal individuals with sex-reversed individuals. As sex is known well before gonadal differentiation takes place, it is possible to ascribe changes in the expression of genes encoding key steroidogenic enzymes, receptors, and transcription factors to the developmental events of a given genotype.

Sex Steroids

The sex or gonadal steroids are the sex hormones; the major product secreted by the gonads. However, other organs such as interrenal glands, liver, brain, or adipose tissue also can synthesize sex steroids. Besides sex differentiation, the sex steroids also are implicated in the regulation of the onset of puberty in both sexes, oogenesis in females and spermatogenesis in males during the adult reproductive cycle, and with sexual behavior.

Sex steroids are synthesized from cholesterol first by the action of P450 cholesterol side-chain cleavage, a steroidogenic enzyme localized in the inner membrane of mitochondria. Subsequently, several other steroidogenic enzymes are involved (**Figure 3**). These enzymes are located in special somatic cells in the gonads, globally referred to as steroidogenic cells. In males, they primarily are the Leydig cells, located in the interstitial space surrounding the seminiferous tubules, whereas in females they are the follicular cells, granulose and theca cells that surround the oocytes.

The two main classes of sex steroids are androgens, with a molecular structure consisting of 19 carbon atoms, and estrogens, with 18 carbon atoms. Progestagens (21 carbon atoms) are the third class of sex steroids, distinct from androgens and estrogens. In fish, progestagens are important for gamete maturation but have no known relevance in sex differentiation. Although all types of sex steroids are present in both sexes, albeit at different levels, in general, androgens are considered as male sex hormones, because they have masculinizing effects, while estrogens and progestagens are considered as female sex hormones. Thus, treatment of sexually undifferentiated fish with androgens or estrogens usually results in the production of males and females, respectively, regardless of genotypic sex.

The sex steroids, secreted in small amounts and exerting an autocrine or paracrine effect in the sexually undifferentiated gonad, are also responsible for gonadal sex differentiation. Thus, the major product of secretion of the gonads contributes to their differentiation during the early stages. Estrogens, specifically estradiol-17β (E_2), are essential for female development. Regarding androgens, fish gonads secrete testosterone (T) but 11-oxygenated androgens, such as 11-ketotestosterone (11-KT), 11β-hydroxyandrostenedione (11β-OHΔ^4), and 11β-hydroxytestosterone (11β-OHT), are in fact the androgens linked with male sex differentiation.

Steroidogenic Enzymes

In contrast to other reproductive hormones (e.g., gonadotropins) sex steroids once synthesized are not stored. Thus, the expression and activity of the steroidogenic enzymes actively contribute to the regulation of the relative abundance of the different types of sex steroids. This is reflected in differences between sexes in steroidogenic cell activity and sex-steroid content in the gonads prior to and during sex differentiation.

Cytochrome P450 aromatase (cyp19) is the enzyme that irreversibly catalyzes the conversion of androgens into estrogens. As all estrogens derive from androgens and since cyp19 is at the end of the steroidogenic pathway, cyp19 determines the balance between 11-oxygenated androgens and estrogens. This balance at a critical period during development, rather than their absolute amounts, determines the course of sex differentiation.

In contrast to tetrapods, where there is only a single copy of cyp19, teleost fish have two isoforms of cyp19. One isoform is found primarily in the gonad and referred to as gonadal aromatase, cyp19a, cyp19a1, or P450aromA, and the other localized primarily in the brain and referred to as brain or neural aromatase, cyp19b, cyp19a2, or P450aromB.

Several lines of evidence indicate that cyp19a is of central importance for ovarian differentiation in fish. Changes in cyp19a messenger (mRNA) levels during early development are linked with female sex differentiation. Suppression of cyp19a gene expression, inhibition of aromatase activity, or blocking the effects of estrogen at the receptor level during the key period of sex differentiation invariably leads to genotypic females developing as phenotypic males. This emphasizes the importance of cyp19a and of the presence of estrogens for proper ovarian differentiation in fish. In species in which temperature can affect sex differentiation, cyp19a is present at feminizing temperatures but suppressed at masculinizing temperatures. Cyp19a is also essential to control gonadal reorganization during sex reversal of hermaphrodites, with cyp19a mRNA levels increasing during protandrous sex change and decreasing during protogynous sex change. In hermaphrodites, sex change depends on the antagonistic functions of E_2 and 11-KT signaling. In fact, the evidence demonstrating that cyp19a is a key and central element in fish sex differentiation is so compelling that it is now possible to use cyp19a as an early marker of ovarian differentiation.

The regulation of cyp19a transcription is relevant for controlling cyp19a enzyme abundance. The promoter regions of piscine cyp19 genes of both gonochoristic and hermaphrodite species with different sex-changing

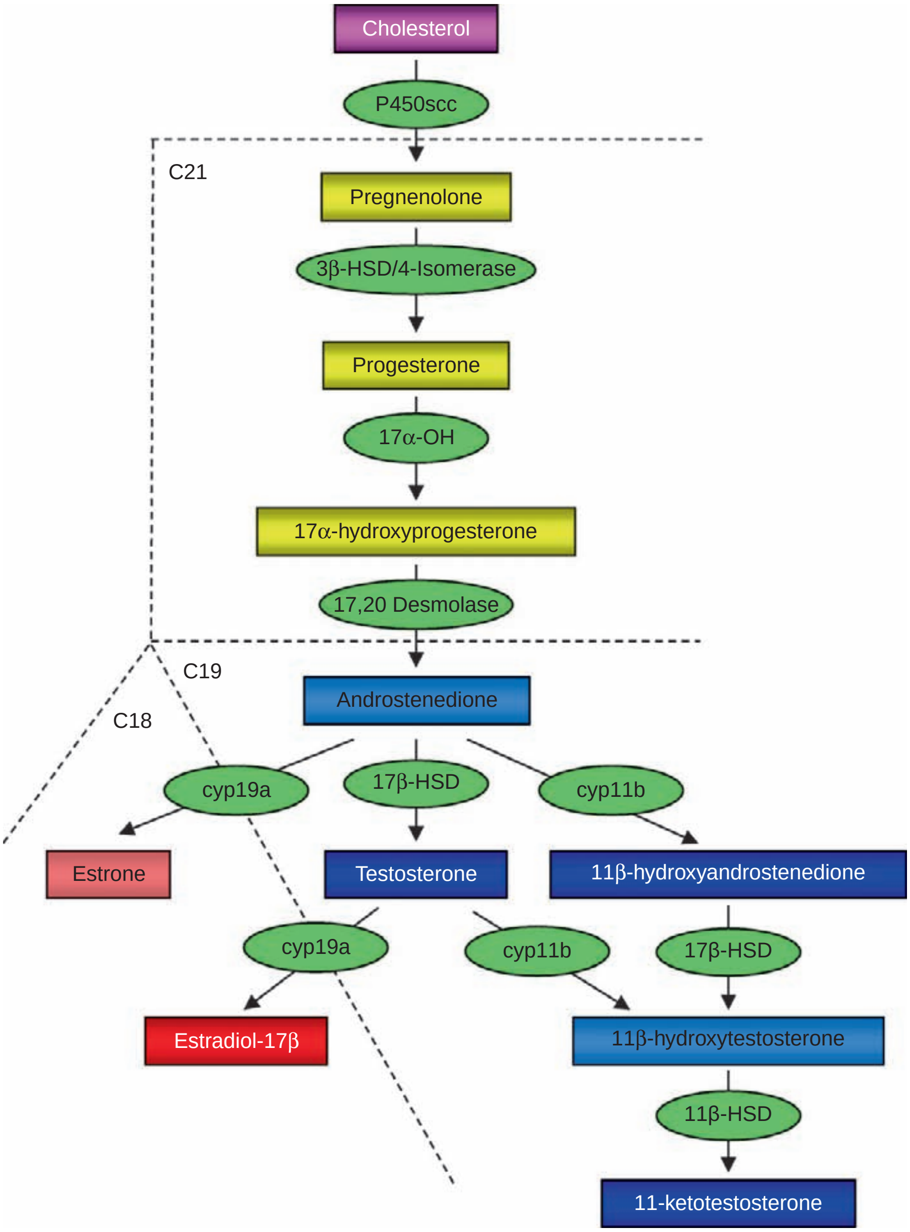


Figure 3 Simplified steroidogenic pathway from cholesterol to the main sex steroids involved in gonadal sex differentiation. The main steroidogenic enzymes are drawn in green. Progestagens, sex steroids with 21 carbon atoms (C21), are shown in yellow, the androgens (19 carbon atoms, C19) in blue, and the estrogens (18 carbon atoms, C18) in red. Darker blue and darker red indicate higher relative androgenic and estrogenic potencies, respectively. Notice that the relative abundance of androgens vs. estrogens essentially depends on the activity of the enzyme aromatase (cyp19a). Abbreviations: P450scc, cytochrome P450 side-chain cleavage; 3β-HSD/4-Isomerase, 3β-hydroxysteroid-dehydrogenase/4-isomerase; 17α-OH, 17α-hydroxylase; 17β-HSD, 17β-hydroxysteroid-dehydrogenase; cyp19a, aromatase; cyp11b, 11β-hydroxylase; 11β-HSD, 11β-hydroxy-steroid-dehydrogenase.

strategies contain a similar repertoire of potential *cis*-acting elements, notably binding sites for transcription factors that exhibit sex-specific changes during early sex differentiation (see below).

The neural form of aromatase, cyp19b, is one of the earliest transcribed genes in developing fish. Thus, the teleost brain is the first aromatization site because the brain develops earlier than the gonads. Further, the teleost brain is characterized by having a remarkably high abundance of cyp19b, which is thought to be related to the capacity of this organ for continued growth through life. Thus, a recurrent question in the study of fish sex differentiation has been whether changes in cyp19b levels somehow dictate gonadal sex differentiation in gonochoristic fish. In fact, in hermaphroditic species changes in some neurotransmitters, gonadotropin-releasing hormone (GnRH), or gonadotropins are correlated with the process of sex change. So far, there is no convincing evidence to support this; however, the brain may signal the gonad to differentiate via pituitary follicle-stimulating hormone (FSH) after stimulation by GnRH.

Besides cyp19a, the other steroidogenic enzyme with a clear sex-specific expression is cytochrome P450 11β-hydroxylase (cyp11b). This enzyme catalyzes the conversion of androstenedione and T into 11-oxygenated androgens such as 11β-OHΔ^4, 11β-OHT, and the precursors for 11-KT synthesis. In fish, cyp11b mRNA also occurs in the head kidney. As opposed to cyp19a, cyp11b is regarded as a marker for testis differentiation.

Sex Steroid Receptors

The sex steroids exert their action by interacting with nuclear hormone receptors which, when bound and activated, act as transcription factors and elicit slow genomic responses. The two major types concerned with fish sexual differentiation are the androgen receptor (AR) and the estrogen receptor (ER). In fish, there are at least two subtypes of ARs, named arα and arβ. Likewise, fish possess three ERs, named erα, erβ1, and erβ2, most likely the products of gene duplications. These ERs are characterized by different tissue distribution and expression patterns, regulation, and nucleotide sequence, and thus different ligand-binding properties.

The presence of ARs and ERs in developing gonads is concordant with the evidence of the effect of sex steroids in sexual differentiation. ARs have been identified in several species of fish but a cause–effect relationship with male development has not been established. Rather, ARs correlate with male sex differentiation only when this differentiation is underway. Thus, in the European sea bass, for example, AR mRNA levels are essentially the same in developing gonads and only higher in males that have initiated active spermatogenesis.

On the other hand, the levels of ERs are positively influenced by estrogens in a positive feedback loop (**Figure 4**). This is important in the context of sex differentiation because it means that basal amounts of estrogen

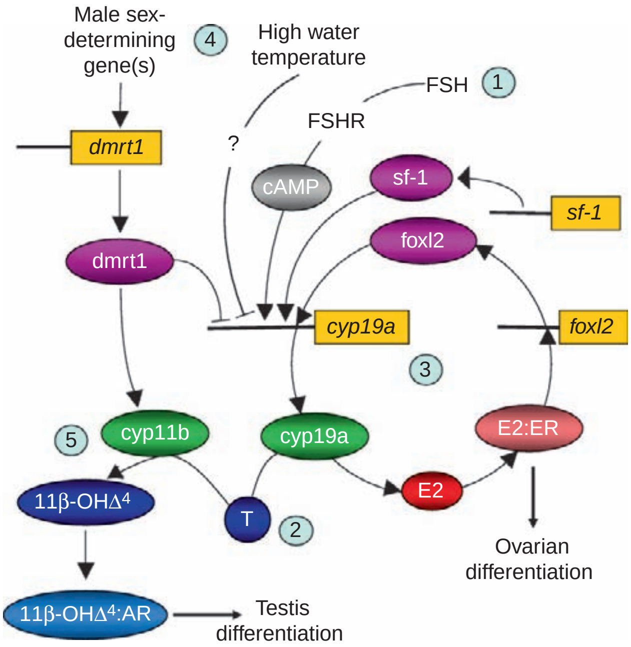


Figure 4 Endocrine control of sex differentiation. Diagram illustrating some of the core players involved, without relating to any particular species. A simplified pathway from either a major or a set of sex-determining genes or an environmental factor to downstream genes is presented but only a subset of the involved genes and regulatory pathways is included. In future females, the ovarian aromatase gene (*cyp19a*) is basally expressed or stimulated by the brain through follicle-stimulating hormone (FSH) signaling on its receptor (FSHR) and the cAMP pathway (1). This results in aromatase enzyme (cyp19a) being synthesized, which converts testosterone (T) into estradiol (E_2) (2). This sets a positive feedback loop (3) that involves the estrogen receptor (ER) and the transcription factor foxl2. Another transcription factor, sf-1, is likely to contribute. In future males – or females exposed to high water temperature (4) – the transcription factor dmrt1 or a still unidentified mechanism (?) inhibits *cyp19a* transcription, resulting in less cyp19a enzyme. This, along with a possible direct stimulation of 11β-hydroxylase (cyp11b), results in more T being converted to 11β-hydroxyandrostenedione (11β-OHΔ^4), which acts through the androgen receptor (AR) or is eventually converted to 11-ketotestosterone, the two major active androgens in fish. The lack of estrogen, essential for female sex differentiation, allows male sex differentiation (5). Thus, the regulation of the steroidogenic enzyme cyp19a and the transcription factor dmrt1 occupy a central position in sex differentiation in fish, which seems to be conserved across species. In females, estrogen is essential for ovarian differentiation, whereas in males, androgens may be a product of testis differentiation.

may be sufficient to start further production of cyp19a, aromatase activity, and estrogen production. However, no major differences in ER levels occur at the beginning of sex differentiation. However, the observation that erα and erβ appear even before the steroidogenic enzymes suggests an important role for estrogen receptors in sexual differentiation. The observation that transgenic medaka undergo normal gonadal differentiation while overexpressing ER, and show the same degree of sex reversal as wild-type nontransgenic fish after treatments with estrogen or androgen, is concordant with this view.

Activated nuclear sex steroid receptors regulate gene expression directly by binding to specific response elements in the promoter of target genes or indirectly by interacting with transcription factors regulating relevant genes. Steroids also can elicit fast, nongenomic responses by interacting with membrane-bound specific receptors. However, because these membrane receptors elicit biological responses minutes or hours after being activated, as opposed to the days or weeks of the classic nuclear hormone receptors, they probably are not involved in sex differentiation of the gonads, a comparatively much slower process.

Transcription Factors

In the process of sex differentiation, from the early stage of germ cell migration and gonadal ridge formation to the final stages of gonad formation, many transcription factors are involved. As many of these are common to all vertebrates, relevant differences are observed not only in their sequence, but also in their sex specificity and temporal expression between species. Of particular importance are those regulating the expression of steroidogenic enzymes. The most studied ones are those that bind cyp19a given its central role in the regulation of fish sex differentiation.

The forkhead box transcription factor l2 (foxl2) is a member of the Winged helix/forkhead group. Foxl2 is one of the earliest known sex dimorphic markers of ovarian differentiation and duplicated copies may occur in fishes due to genome duplications. Foxl2 is expressed mainly in the granulosa cells of the developing ovary and its expression may precede that of cyp19a. Both *in vivo* and *in vitro* studies show that foxl2 acts as a potent transcriptional activator of cyp19a and thus it is important for female sex differentiation. Foxl2 expression can be induced by estrogen treatment and downregulated by exposure to androgen, aromatase inhibitors, or high water temperature. Disruption of endogenous foxl2 in genetically female tilapia results in varying degrees of testicular development, with occasional female-to-male reversal. Together, these observations emphasize the decisive role of foxl2 in ovarian differentiation in fish.

Double-sex and Mab-3 related transcription factor 1 (Dmrt1) is present in both invertebrates and vertebrates and always is associated with the development of the male reproductive system. In fact, dmrt1 is thought to be the male sex-determining gene in birds. A duplication of dmrt1, named dmrt1bY or DMY, is the sex-determining gene in medaka. Dmrt1 is involved in normal testis differentiation in both gonochoristic and hermaphrodite fish. Further, after induced masculinization by treatment with a synthetic androgen, such as 17α-methyltestosterone (MT), or after treatment with an aromatase inhibitor, dmrt1 increases. Dmrt1 may act as a transcription repressor of cyp19a, resulting in suppressed E_2 synthesis, impaired ovarian differentiation, and thus male sex differentiation.

There are many other transcription factors involved in gonadal sex differentiation. Some of the ones that have received more attention include several members of the SRY-related high-mobility group (HMG) containing box (Sox) genes, the dosage-sensitive sex-reversing adrenal hypoplasia congenital critical region on the X (Dax1 or Nr0b1), the Wilm's tumor-suppressor-1 gene (Wt1), and the steroidogenic factor-1 (Sf1), also named adrenal-4-binding protein (Ad4bp) and fushi tarazu factor-1 (Ftz-f1). This nuclear receptor product of the Nr5a1 gene is involved in the expression of steroidogenic enzymes. Notably, a potent interaction between sf1 and cyp19a1 has been demonstrated in several teleosts.

Growth Factors and Other Elements

Several growth factors are also involved in the regulation of sex differentiation. Among them, the anti-Müllerian hormone (amh; also known as Müllerian inhibiting hormone) has been the most studied. Amh is a member of the transforming growth factor β (Tgf-β) superfamily of growth and differentiation factors. Usually, amh expression is detected in undifferentiated gonads and further expressed in juvenile testis but not juvenile ovaries. Other elements for which there is less evidence regarding their involvement in fish gonadal differentiation include some proteins associated with entry into meiosis.

Environmental Influences

As mentioned earlier, one of the characteristics of fish sex differentiation is its lability when compared with other vertebrates. Thus, environmental (biotic or abiotic) influences can easily alter or determine the course of sex differentiation. Here, however, it is important to distinguish between gonochoristic species versus hermaphroditic species. In the latter, it is well established that the process of sex change, which at the gonadal level can be regarded as a sort of a second sexual differentiation toward the opposite sex, is controlled by social stimuli that are integrated by the brain. The brain, then, with its secretion of neurotransmitters is able to change secretion of gonadotropins and contribute to gonadal sex change.

There is no strong evidence showing the influence of social stimuli in the gonadal development of gonochoristic species, although some early studies pointed in this direction (**see also Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes). In these species, the environmental influences on sex determination are abiotic. Here, again, we must distinguish the normal condition from effects of fish under exceptional conditions. In species with environmental sex determination (ESD), the abiotic conditions experienced during specific windows in early development determine the sex of the individual. Among the abiotic factors, temperature is by far the most studied (temperature-dependent sex determination, TSD). Water pH has been shown to affect sex ratios in some South American cichlids of the genus *Apistogramma*. Clearly, more research is needed to learn what environmental factors can serve as a cue for sex differentiation and the species differences. Likewise, it is of central relevance to learn how environmental signals are transduced in morphological changes, that is, the molecular basis underlying cellular responses to a changing environment.

Sex differentiation also can be influenced by the presence of endocrine-disrupting chemicals in their aquatic environment. These chemicals cause altered sex ratios mainly by their ability to act as substances with androgenic or, more often, estrogenic capacity, by binding to estrogen receptors. This does not represent any sort of natural condition; it is only due to the contamination of many water bodies throughout the world.

Concluding Remarks

Despite the large number of fish species and the diversity of their sexuality types, certain general principles emerge in light of recent research. Thus, there is a certain relationship between the patterns of morphological gonadal development and the underlying molecular mechanisms controlling sex determination and differentiation, in such a way that the former reflect the actions of the latter. The presence of a male sex-determining gene or set of genes acting early during gonadal development is reflected in the pattern seen in gonochoristic differentiated species, whereas male sex-determining mechanism that acts relatively later accounts for the pattern observed in gonochoristic undifferentiated species. In any case, the balance between androgens and estrogens in the early stages of gonadal formation accounts for the course of sex differentiation toward ovarian development, in those cases where the self-perpetuating female pathway is not interrupted, or toward the male pathway, when the female pathway is braked by the presence of a natural inhibitor of estrogen synthesis acting on aromatase activity (**Figure 4**). Moreover, new technologies such as the analysis of the transcriptome by using microarrays will undoubtedly contribute toward a better understanding of the genes implicated and how they interact with one another, allowing the emergence of a set of genes with conserved roles or interactions across species. Of particular importance is the elucidation of the molecular mechanisms underlying the response to varying environmental conditions in the context of environmental sex determination in gonochoristic species. Also relevant is the question regarding how social influences controlling sex change in hermaphroditic species are transduced. Finally, cell-fate studies concentrating on the development of gonadal cells starting from precursor cells will be important as will be those studies aimed at elucidating cell–cell interaction during gonadal formation and sex differentiation, particularly concerning the interaction of somatic cells with germ cells.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Germ Cell Migration and Trans Sex; Molecular and Chromosomal Aspects of Sex Determination. **Role of the Kidneys**: Histology of the Kidney. **Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes. **The Pituitary**: Pituitary Gland or Hypophysis. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis; Regulation of Spermatogenesis.

Further Reading

Baroiller JF, D′Cotta H, and Saillant E (2009) Environmental effects on fish sex determination and differentiation. *Sexual Development* 3: 118–135.

Borg B (2004) Androgens in teleost fishes. *Comparative Biochemistry and Physiology* 109C: 219–245.

Cavileer T, Hunter S, Okutsu T, Yoshizaki G, and Nagler JJ (2009) Identification of novel genes associated with molecular sex differentiation in the embryonic gonads of rainbow trout (*Oncorhynchus mykiss*). *Sexual Development* 3: 214–224.

Devlin RH and Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191–364.

Fernandino JI, Hattori RS, Shinoda T, *et al.* (2008) Dimorphic expression of dmrt1 and cyp19a1 (ovarian aromatase) during early gonadal development in pejerrey. *Odontesthes bonariensis. Sexual Development* 2: 316–324.

Guerrero-Estévez S and Moreno-Mendoza N (2009) Sexual determination and differentiation in teleost fish. *Reviews in Fish Biology and Fisheries,* doi:10.1007/s11160-009-9123-4.

Guiguen Y, Fostier A, Piferrer F, and Chang CF (2010) Ovarian aromatase and estrogens: A pivotal role for gonadal sex differentiation and sex change in fish. *General and Comparative Endocrinology* 165: 352–366.

Herpin A, Schindler D, Kraiss A, *et al.* (2007) Inhibition of primordial germ cell proliferation by the medaka male determining gene DmrtlbY. *BMC Developmental Biology* 7. http://www.biomedcentral.com/1471-213X/7/99 (accessed February 2010).

Ijiri S, Kaneko H, Kobayashi T, *et al.* (2008) Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the

Nile tilapia *Oreochromis niloticus. Biology of Reproduction* 78: 333–341.

Kobayashi T and Nagahama Y (2009) Molecular aspects of gonadal differentiation in a teleost fish, the Nile tilapia. *Sexual Development* 3: 108–117.

Kobayashi Y, Nakamura M, Sunobe T, *et al.* (2009) Sex change in the gobiid fish is mediated through rapid switching of gonadotropin receptors from ovarian to testicular portion or vice versa. *Endocrinology* 150: 1503–1511.

Orban L, Sreenivasan R, and Olsson PE (2009) Long and winding roads: Testis differentiation in zebrafish. *Molecular and Cellular Endocrinology* 312: 35–41.

Ospina-Álvarez N and Piferrer F (2008) Temperature dependent sex determination in fish revisited: Prevalence, a single sex ratio response pattern, and possible effects of climate change. *PLoS ONE* 3(7): e2837 (doi:10.1371/journal.pone.0002837).

Penman DJ and Piferrer F (2008) Fish gonadogenesis. Part 1. Genetic and environmental mechanisms of sex determination. *Reviews in Fisheries Science* 16(supplement 1): 16–34.

Piferrer F and Blázquez M (2005) Aromatase distribution and regulation in fish. *Fish Physiology and Biochemistry* 31: 215–226.

Piferrer F and Guiguen Y (2008) Fish gonadogenesis. Part II: Molecular biology and genomics of sex differentiation. *Reviews in Fisheries Science* 16(supplement 1): 35–55.

Saito D and Tanaka M (2009) Comparative aspects of gonadal sex differentiation in medaka: A conserved role of developing oocytes in sexual canalization. *Sexual Development* 3: 99–107.

Siegfried KR and Nusslein-Volhard C (2008) Germ line control of female sex determination in zebrafish. *Developmental Biology* 324: 277–287.

Vizziano D, Randuineau G, Baron D, Cauty C, and Guiguen Y (2007) Characterization of early molecular sex differentiation in rainbow trout, *Oncorhynchus mykiss*. *Developmental Dynamics* 236: 2198–2206.

Wang DS, Kobayashi T, Zhou LY, *et al.* (2007) Foxl2 up-regulates aromatase gene transcription in a female-specific manner by binding to the promoter as well as interacting with Ad4 binding protein/steroidogenic factor 1. *Molecular Endocrinology* 21: 712–725.

Endocrine Regulation of Fish Reproduction

Z Yaron, Tel-Aviv University, Tel Aviv, Israel

B Levavi-Sivan, Hebrew University of Jerusalem, Rehovot, Israel

© 2011 Elsevier Inc. All rights reserved.

Introduction – Overview of the Brain–Hypophyseal–Gonadal Axis
Oogenesis
Spermatogenesis
Gonadal Steroids
Gonadotropins and Their Regulation
Further Reading

Glossary

Follicle-stimulating hormone (FSH) A glycoprotein hormone secreted by the pituitary; stimulates early phases of gametogenesis: spermatogenesis in males and early development of oocytes in females.

Gonadotropin-releasing hormone Neuropeptide hormone produced in the hypothalamus, released at the anterior pituitary gland to regulate gonadotropin secretion and is a key regulator of reproduction.

Luteinizing hormone (LH) A dimeric glycoprotein hormone released from the anterior pituitary gland that acts through gonadal membrane receptors to stimulate steroidogenesis and gametogenesis. LH is a gonadotropin.

Oogonia Female germ cells at the beginning of their development toward becoming an oocyte and egg prior to meiotic division.

Spermatogonia Male germ cells at the beginning of their development toward becoming spermatozoa and prior to meiotic division.

Introduction – Overview of the Brain–Hypophyseal–Gonadal Axis

Environmental signals, in conjunction with social cues, are conveyed by sensory signals to various brain centers culminating in the hypothalamus. In the absence of hypophyseal portal system, hypothalamic nerve fibers branch throughout the adenohypophysis to regulate the synthesis and release of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Dopamine secreted by the hypothalamus may inhibit LH release. FSH and LH, in turn, bind to their respective receptors in the gonads (FSH-R and LH-R, respectively).

The gonads respond by secretion of sex steroid hormones; estradiol 17β (E2) in females that promotes oogonial proliferation and vitellogenesis, and progestogens such as 17α,20β dihydroxy-4-pregnen-3-one (DHP) that promotes initiation of germ cell meiosis and follicular maturation and ovulation (**Figure 1**(**a**)). In males, these are androgens, mainly 11 ketotestosterone (11-KT), that regulate spermatogenesis and spermiogenesis, as well as DHP that initiates the meiotic division of spermatogonia and controls the spermatozoa maturation and spermiation. DHP in its free or conjugated forms serve as pheromones as well. Generally, fertilization occurs in the water, either in specialized nests guarded by one or both parents or in the open water. Several teleost fish such as the guppy (*Poecilia reticulata*) are viviparous.

Oogenesis

Oogonial Proliferation and Meiosis

Oogonia proliferate mitotically under the stimulation of E2 (**Figure 2**). Meiotic division commences in a subpopulation of the oogonia that can be visualized by the presence of synaptonemal complexes (a hallmark feature of homologous chromosomal synapses). Another marker of meiosis is Spo11, a protein involved in the creation of double-stranded breaks in the DNA at early stages of meiotic recombination. Addition of DHP to the medium of cultured ovarian fragments results in increased abundance of synaptonemal complexes and increased expression of Spo11, indicating that DHP acts to induce meiosis in the oogonia of teleost ovary (**Figure 2**).

Oocyte Growth

As the oocyte starts growing during fish puberty, it is still arrested at the prophase of the first meiotic division

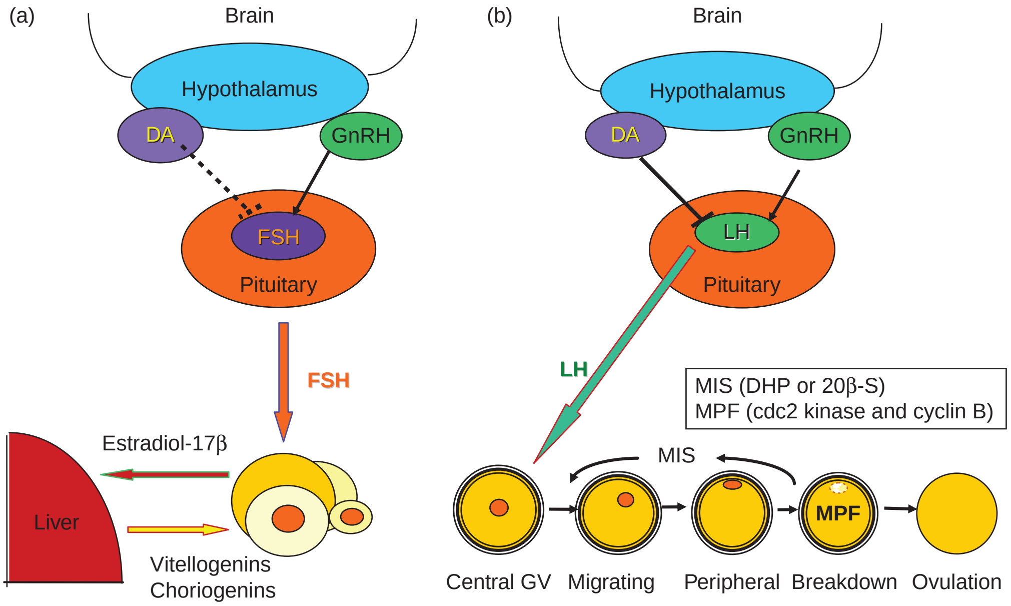


Figure 1 (a) An overview of the endocrine chain, brain–pituitary–gonadal axis (BPG axis) in model female fish during the vitellogenic phase. (b) An overview of the BPG axis during final oocyte maturation and ovulation.

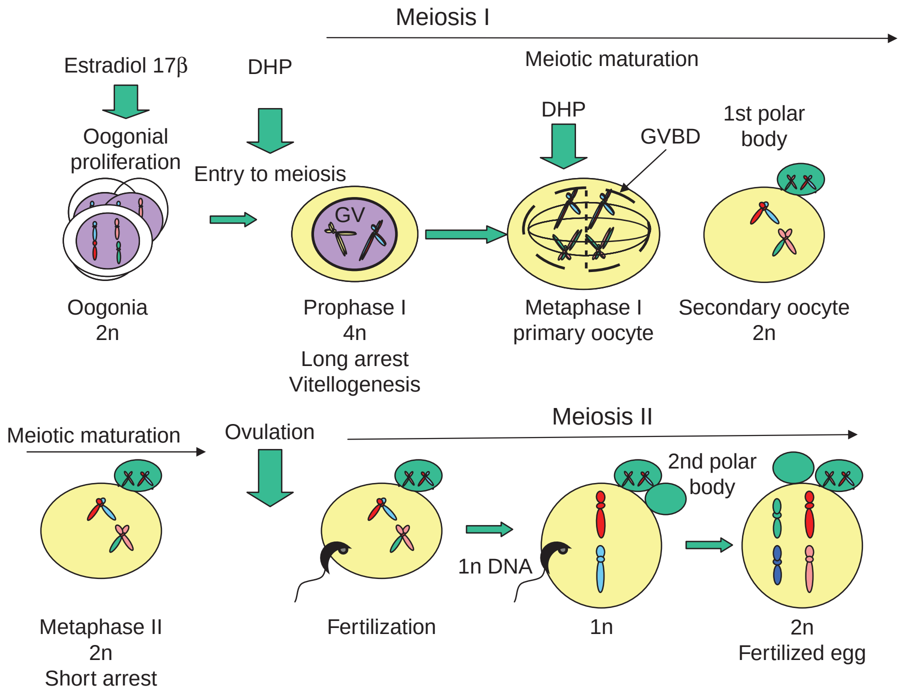


Figure 2 Stages of fish oogenesis and their endocrine regulation.

(**Figure 2**). At this phase, it sequesters yolk precursors (vitellogenins) and chorionic proteins (choriogenins) mainly produced by the liver under estrogenic stimulation. The yolk accumulates in the oocyte which increases in diameter. FSH regulates both the secretion of estradiol and the incorporation of vitellogenins into the oocytes (**Figure 1**(**a**)).

Mutual Regulation of the Follicle Cells and the Oocyte

The granulosa cells surrounding the oocyte and the oocyte itself maintain a mutual communication system by which molecules of the epithelial growth factor

(EGF) family secreted by the oocyte affect the adjacent granulosa cells. Activin, a major mediator of fish pituitary gonadotropins, is released by the somatic cells of the follicle and affects the oocyte through activin receptors present in this germ cell.

Oocyte Maturation

The post-vitellogenic oocyte may remain quiescent for several months but following environmental, social, or pheromonal cues, it begins the process of final maturation, that is, the resumption of meiosis (**Figure 2**). This will commence with a surge in gonadotropin-releasing hormone (GnRH), with or without a decrease in dopaminergic inhibition, followed by a rise in circulating LH. Upon binding of LH to its receptors on the granulosa cells, the ovarian follicle starts the process of maturation, beginning with the production of the maturation-inducing steroid (maturation-inducing steroid (MIS) such as DHP or 17α-20β,21 Trihydroxy-4-pregnen-3-one (20β-S)). Binding of the MIS to its receptors on the oocyte plasma membrane is followed by activation of the maturation-promoting factor (MPF), a complex consisting of existing cdc2-kinase and newly synthesized cyclin B. (**Figure 1**(**b**)). The process of oocyte maturation is reflected morphologically by the migration of the germinal vesicle (GV) toward the animal pole (GV migration) and the disintegration of its membrane, a stage known as GV breakdown (GVBD) (**Figures 1**(**b**) and **2**). The chromosomes then condense, a spindle is formed, and the first polar body is extruded which marks the end of the first meiotic division (**Figure 2**). At this stage the oocytes absorb water and inflate; this is especially pronounced in marine fish with pelagic eggs, the pressure within the follicle increases, the follicular wall is ruptured, and the oocyte is released (ovulated) into the ovarian lumen or to the coelomic cavity. The meiosis is arrested again at metaphase II. Completion of the second meiotic division and extrusion of the second polar body are further delayed and will proceed only if the egg is fertilized (**Figure 2**).

Spermatogenesis

Endocrine Regulation of Spermatogenesis

Spermatogenesis consists of several successive processes, each regulated by a distinct set of hormones. Mitotic divisions of spermatogonia that lead to germ cell renewal are regulated by E2 secreted by the interstitial Leydig cells under gonadotropic stimulation, mainly FSH. Upon binding of E2 to their receptors in the Sertoli cells, the latter secrete the spermatogonial stem-cell renewal factor (possibly gonadal soma-derived growth factor (GSDF)) that stimulates spermatogonial mitotic proliferation (**Figure 3**).

Experimental increase or decrease in the FSH and FSH-Rs expression in the testis of African catfish (*Clarias gariepinus*) resulted in respective changes in Sertoli cell proliferation and testicular growth, suggesting that in fish, as in mammals, FSH in cooperation with androgens is probably the regulator of Sertoli cell number.

Gonadotropic stimulation in the Japanese eel (*Anguilla japonica*) shifts a subpopulation of spermatogonial germ cells from a renewal line to a proliferation line ensuing by meiosis. Gonadotropins (mainly FSH) elicit a surge in the secretion of 11-KT from Leydig cells to stimulate in Sertoli cells the production of several mediators such as activin B, insulin-like growth factor I (IGF-I), and anti-Müllerian hormone (AMH). The action of 11-KT on this spermatogonial line is positively mediated by activin B and IGF-I, and negatively by AMH (**Figure 3**). However, the initiation of meiotic division leading to the formation of spermatids is induced by DHP, which is produced by the germ cells themselves, exerting their effect on the germ cells in a paracrine or autocrine manner (**Figure 3**). The need for DHP to initiate meiosis was demonstrated by 11-KT stimulating DNA replication and meiosis in organ culture of eel testes and anti-DHP serum blocking this effect. Moreover, addition of DHP resulted in the expression of two meiosis-specific markers DMC1 and Spo11, and the appearance of synaptonemal complexes in testicular sections.

Immature spermatozoa undergo sperm maturation, which is also regulated by DHP acting directly on spermatozoa to activate carbonic anhydrase. This enzymatic activation is followed by an increase in the seminal plasma pH, which augments intrasperm cAMP levels to enable their motility (**Figure 3**).

Gonadal Steroids

Formation of Gonadal Steroids in Fish

Fish gonadal steroids are mainly synthesized through pathways similar to those in other vertebrates with a few exceptions (**Figure 4**). Testosterone in fish testis is hydroxylated at carbon 11 by P450-11β and, after oxidation by 11β- hydroxysteroid dehydrogenase (11β-HSD), is converted into 11-KT that is the potent androgen of teleost fish. In certain fish, testosterone hydroxylation occurs in an extra-testicular tissue, the liver. The conversion of 17-hydroxyprogesterone by 20β-HSD produces DHP that acts as the MIS in many fishes. However, in a number of marine perciform fish, the MIS is 17α,20β,21-trihydroxy-4-pregnen-3-one (21β−S). This steroid too derives from

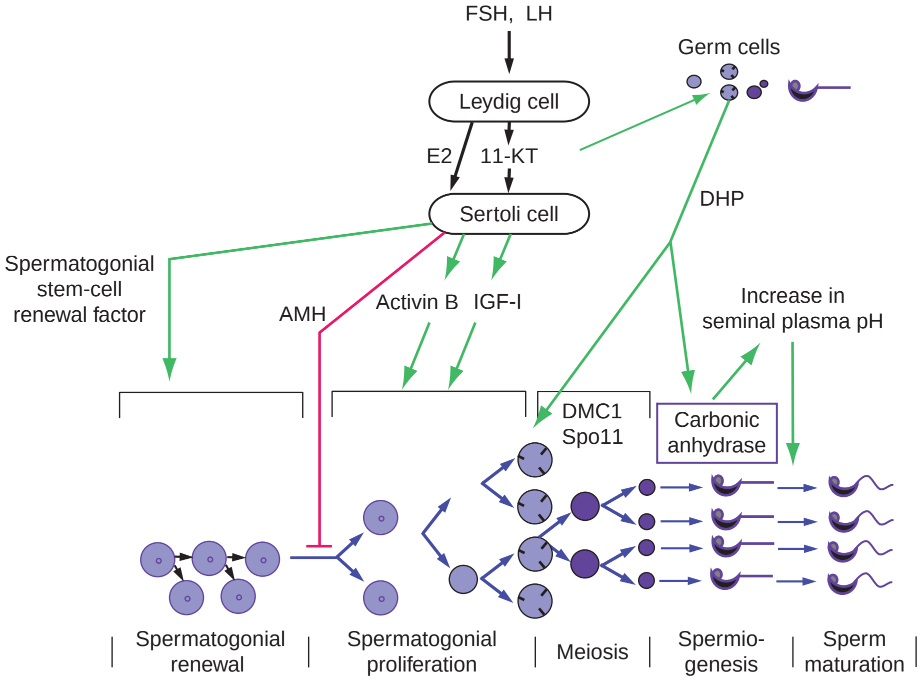


Figure 3 Endocrine mechanisms regulating spermatogenesis in the Japanese eel (*Anguilla japonica*). 11-KT, 11-ketotestosterone; AMH, a peptide homologous to anti-Müllerian hormone; DHP, 17α,20β-dihydroxy-4-pregnen-3-one; DMC1 and Spo11, markers specific to early phases of meiosis; IGF-I, insulin-like growth factor I. Cells at the upper right corner depict the germ cells as the source of DHP acting upon themselves in a paracrine or autocrine manner. Modified from Miura T and Miura C (2001) Japanese eel: Model for analysis of spermatogenesis. *Zoological Science* 18: 1055–1063 and from Miura T and Miura C (2003) Molecular control mechanisms of fish spermatogenesis. *Fish Physiology and Biochemistry* 28: 181–186.

17-hydroxyprogesterone that is hydroxylated at carbon 21 by 21 hydroxylase (P450c21) to form 11-deoxycortisol. The latter is oxidized by 20β-HSD to form 20β–S (**Figure 4**).

The Shift from Estradiol to Progestogens

At the onset of the periovulatory phase, the LH surge is followed by a dramatic shift in the steroidogenic pattern. There is an increase in the formation of DHP concurrently with a decline in E2 level. The shift in the steroidogenic pattern from the formation of C18 and C19 steroids (estrogens and androgens, respectively) toward the formation of C21 steroids (progestogens or corticosteroids) involves a decrease in 17–20 lyase activity and a rise in that of 20β-HSD.

Two genes encoding 17-hydroxylase (P450c17) occur in fish. One is P450c17-I which is similar to that in tetrapod gonads and displays lyase activity resulting in C19 steroids that serve as precursors for androgens and estrogens. P450c17-I is expressed in the ovarian granulosa cells during the vitellogenic phase. The other gene, P450c17-II, encodes a 17-hydroxylase that is devoid of lyase activity and is fully expressed in the oocytes of the Nile tilapia (*Oreochromis niloticus*) only during final oocyte maturation, increasing the production of progestogens instead of androgens and estrogens (**Figure 4**).

Irrespective of the mechanism leading to the steroidogenic shift, estradiol and its seven-transmembrane receptor (GPR30) in the oocyte maintain meiotic arrest. Therefore, the decrease in estradiol is essential for the resumption of meiosis during oocyte maturation (**Figures 1** and **2**).

In addition to the aforementioned free steroid compounds, teleost gonads produce a number of reduced and conjugated steroids, especially in the periovulatory phase. The glucuronidated testosterone and sulfated or glucuronidated progestogens, when released into the water, may act as sex pheromones.

The formation of sex steroids in the vitellogenic ovary of the salmon and probably in other fish as well is a two-step process in which the steroidogenic conversions from cholesterol to testosterone are carried out in the theca cells. The androgen diffuses into the surrounding and is taken up by the granulosa cells possessing P450arom that aromatizes testosterone to form E2 (**Figure 4**).

In a parallel manner, in the ovary approaching final oocyte maturation, all the steroid conversions from cholesterol to 17-hydroxyprogesterone is carried out in the theca of the post-vitellogenic ovary while the conversion

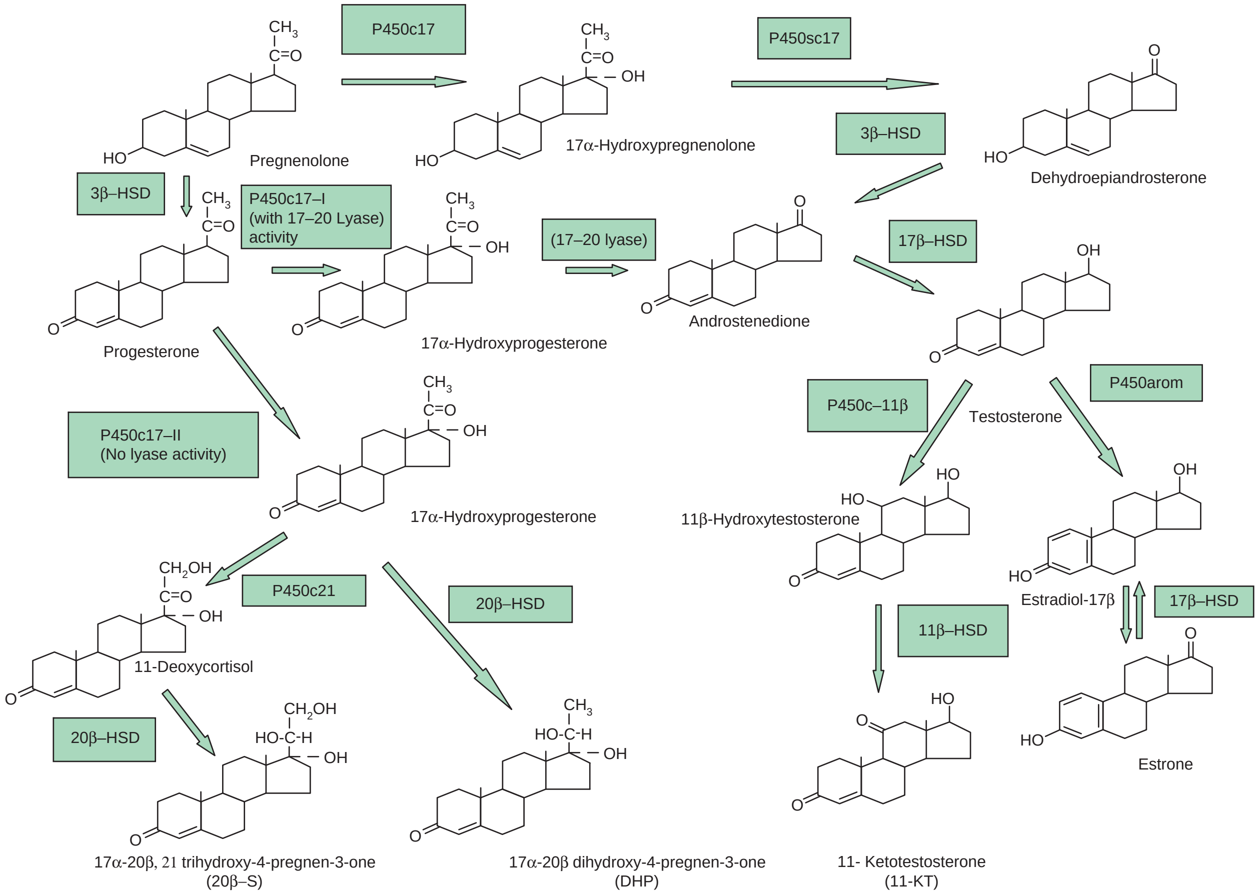


Figure 4 The main pathways in the formation of free gonadal steroids in fish, downstream from the pregnenolone step. Upstream steps are similar to those in mammals and are not shown here. The gonadal steroids specific to teleost fish are 11-KT, the most effective androgen, and DHP, a C21 steroid acting as maturation-inducing steroid (MIS); In several marine teleosts, the MIS activity is carried out by another progestogen, 17α,20β,21-trihydroxy-4-pregnen-3-one (20β–S), produced through oxidation of 11-deoxycortisol by 20β-hydroxysteroid dehydrogenase.

to DHP is performed by the granulosa cells possessing 20β-HSD (**Figure 4**).

Gonadotropins and Their Regulation

The fish pituitary produces and secretes two gonadotropins: FSH and LH. Both are heterodimeric glycoproteins formed by a shared α-subunit (GPα) noncovalently linked to a specific β-subunit. Each subunit, α, FSHβ, and LHβ, is encoded by a distinct gene.

Molecular cloning techniques allowed so far the isolation of the genes encoding the gonadotropin subunits for 56 fish species belonging to 14 teleost orders. The phylogenetic relationships of fish gonadotropins, together with their structural and biological characteristics, provided the evidence for their classification as the piscine counterparts of the mammalian FSH and LH.

The α-subunit is the most conserved among fish species and contains two potential sites for N-glycosylation and 10 conserved cysteines. LHβ and FSHβ subunits each contain 12 conserved cysteines linked by six disulfide bridges. This structure is conserved in fish LHβ subunits, but not in FSHβ, which is the least conserved gene.

All LHβ gene promoters isolated from fish contain putative response elements for Sf-1 and Pitx1, but unlike their mammalian counterparts, the teleost proximal promoters do not contain an early growth response factor 1 (Egr-1). The latter appears to have been replaced by the estrogen receptor (ER). Comparison of FSHβ5′ flanking region in the fish studies so far revealed that they all share several putative response elements, such as GSE, CRE, half sites of ERE, and activating protein 1 response element (AP1), the recognition site for the Fos and Jun transcription factors.

Recombinant Gonadotropins

Fish gonadotropin research has traditionally relied on the laborious isolation and incomplete purification of the native hormones extracted from thousands of fish pituitary glands. The isolation and cloning of cDNAs encoding piscine gonadotropin subunits enabled the production of recombinant hormones (rFSH and rLH) through their expression in heterologous systems. However, to endow the products with the correct biological activity, they have to be glycosylated, folded, and assembled as a heterodimers. All recombinant LH and/or FSH produced so far in teleosts can bind and activate the respective receptors, and stimulate sex-steroid output from isolated gonadal tissue. The lack of cross-contamination of these recombinant hormones enables the study of LH and FSH differential functions. In addition, it is anticipated that recombinant gonadotropins will find use in spawning induction in aquaculture.

Regulation of Gonadotropin Synthesis and Secretion

The hypothalamus regulates synthesis and release of gonadotropins through multiple neurohormones. The major ones are GnRH in its various forms, kisspeptins and dopamine, and potentially other hormones such as γ-aminobutyric acid (GABA), pituitary adenylate cyclase-activating peptide (PACAP), norepinephrine, neuropeptide Y (NPY), serotonin, secretoneurin, ghrelin, leptin, and glutamate (**Figure 5**). Of special interest are hormones associated with growth and metabolism (IGF-1, glutamate, leptin, and ghrelin), which signifies a relationship between reproduction, energy reserves, and body mass of the fish (**Figure 5**).

Gonadotropin-releasing hormone

GnRH stimulates the production and release of gonadotropins from the pituitary of teleosts as in other vertebrates. It increases the amounts of mRNA encoding gonadotropin subunits in the pituitary. The response of GPα and LHβ mRNAs to GnRH implantation in maturing females or males is higher than that of FSHβ, indicating differences in the hypothalamic regulation of the two gonadotropins.

GnRH effects vary with a fish's reproductive stage. In maturing salmonids sGnRH elevates pituitary mRNAs encoding for GPα and FSHβ, but not that of LHβ. The response of the gonadotropin subunit mRNAs to GnRH in common carp (*Cyprinus carpio*) too is differential and depends on the gender and reproductive stage. Thus, information regarding the reproductive stage is potentially conveyed via the steroid hormone profile that can modulate the response and make it specific to each phase (**Figures 1** and **5**). The three forms of GnRH present in the brain of gilthead seabream (*Sparus aurata*) and Nile tilapia (sbGnRH = GnRH1; cGnRHII = GnRH2; sGnRH = GnRH3) all have LH-stimulating activity in mature females. However, the form most abundant in the pituitary of mature fish, GnRH1, is the least potent in inducing LH release.

Dopaminergic inhibition of gonadotropins

Dopamine inhibits both basal and GnRH-stimulated LH secretion (**Figure 5**). *In vitro* experiments, as validated by molecular studies, show that dopamine D$_2$-like, but not D$_1$-like, receptors inhibit gonadotropin secretion directly in the pituitary. In fact, cyprinid spawning induction in aquaculture uses dopamine D$_2$ antagonists, such as domperidone, pimozide, or metoclopramide, to facilitate the stimulation by GnRH of LH release and ovulation. Nevertheless, the dopaminergic inhibition does not operate in all fish and is lacking altogether in

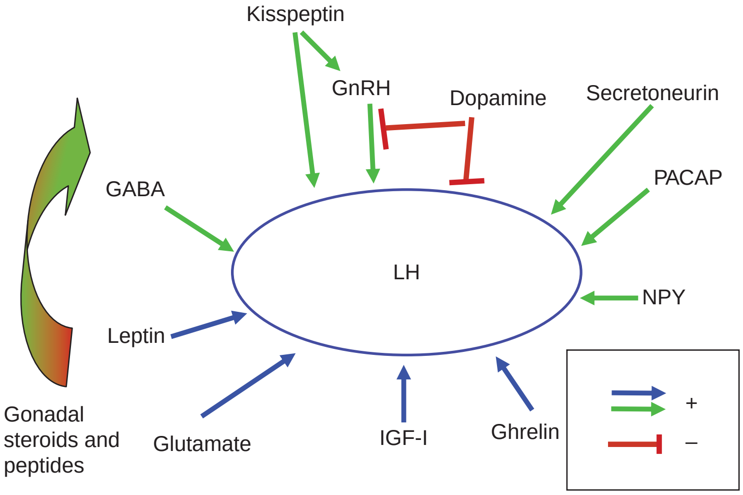


Figure 5 Regulation of LH secretion. Schematic presentation of brain and gonadal regulation of LH secretion. Hormones related to metabolism and growth are indicated by blue arrows.

Atlantic croaker (*Micropogonias undulatus*) and gilthead sea bream.

Kisspeptin regulation of gonadotropins

Kisspeptin, a member of the RF amide peptide family, has important roles in mammals in timing of puberty, maintaining gonadal functions, photoperiod control of seasonal breeding, metabolic gating of fertility, and insulin secretion. Kisspeptin has been proposed to be a novel gatekeeper for the gonadotropic axis of fish mainly due to its potent stimulation of gonadotropin secretion (**Figure 5**).

Kiss1 was cloned in several fish species and its involvement in gonadotropin release and signaling of sexual maturation/puberty has been confirmed so far in medaka (*Oryzias latipes*), zebrafish (*Danio rerio*) and the European sea bass (*Dicentrarchus labrax*). In addition, a second Kiss gene, namely Kiss2, exists in teleost fish. Apparently, the two Kiss genes arose by gene duplication early in vertebrate evolution, and Kiss2 gene might have been lost in the mammalian lineage (**Figure 6**). Kiss1 and Kiss2 in zebrafish are regionally expressed within the hypothalamus, and may exert differential or distinct effects on LH and FSH expression and secretion.

Fish possess two Kiss receptors: kiss1ra and kiss1rb. Sequence identity and genome synteny analyses indicate that zebrafish kiss1ra is a human KISS1R ortholog, whereas kiss1rb is a specific fish form. Both kisspeptins and their receptors are abundantly expressed in the brain, notably in the hypothalamus, suggesting that these ligand–receptor pairs have neuroendocrine and neuromodulatory roles. Fish kisspeptins differ in their signal transduction pathways, tissue distribution, and their gene expression pattern toward puberty.

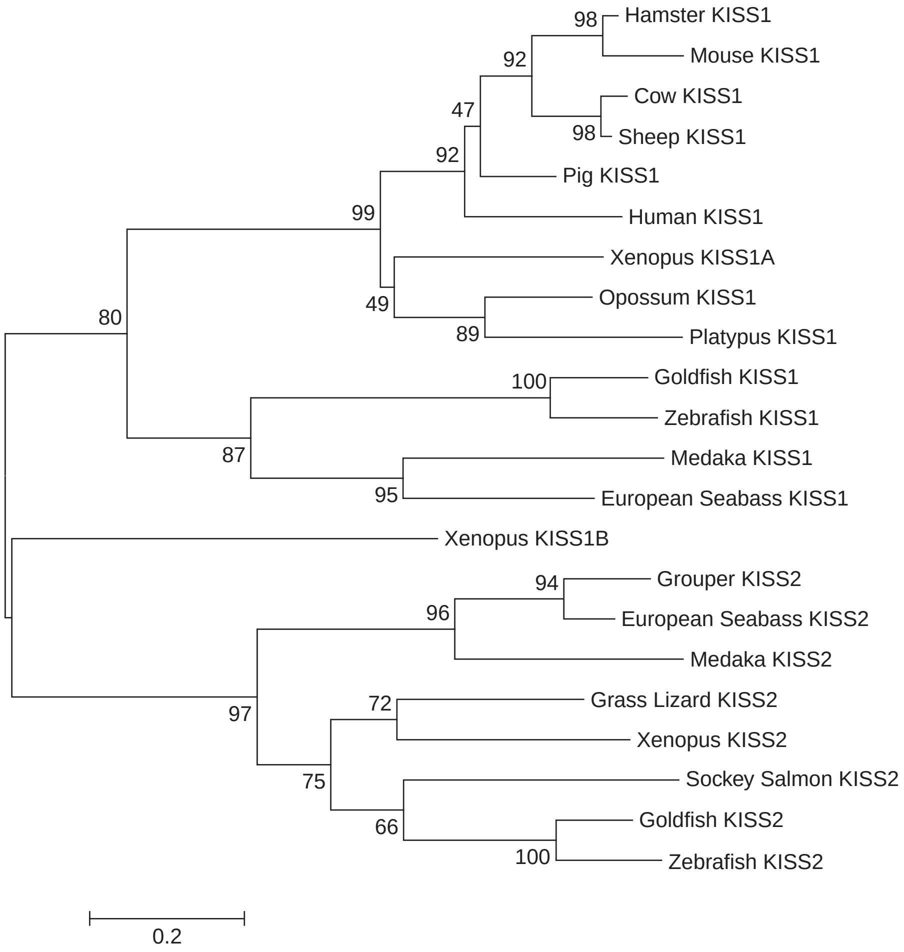


Figure 6 Phylogenetic analysis of kisspeptins. Unrooted phylogenetic trees of kisspeptin sequences generated with a MEGA version 4. Numbers at nodes indicate the bootstrap values as percentages obtained from 1000 replicates. Scale bar indicates the substitution rate per amino acid. Hamster – Siberian hamster (*Phodopus sungarus*); mouse – House mouse (*Mus musculus*); cow (*Bos taurus*); sheep (*Ovis aries*); human (*Homo sapiens*); xenopus (*Xenopus tropicalis*); opossum – gray short-tail opossum (*Monodelphis domestica*); platypus (*Ornithorhynchus anatinus*); goldfish (*Carassius auratus*); zebrafish (*Danio rerio*); medaka (*Oryzias latipes*); European sea bass (*Dicentrarchus labrax*); grouper – orange-spotted grouper (*Epinephelus coioides*); grass lizard – Japanese grass lizard (*Takydromus tachydromoides*); sockeye salmon – sockeye salmon (*Oncorhynchus nerka*).

Regulation by gonadal steroids

Steroids released from gonads can feed back and regulate the production and release of FSH and LH. This type of feedback effect (positive or negative) varies with the gonadal phase of reproductive development. Positive or negative feedback mechanisms operate either indirectly through certain hypothalamic nuclei, or directly on the pituitary cells. Negative feedback of gonadal steroids on gonadotropin secretion was demonstrated using gonadectomy and steroid-replacement protocols in certain teleost species. In rainbow trout (*Oncorhynchus mykiss*), the negative feedback of estrogens on the pituitary is direct or indirect through GnRH inhibition, and potentially by E2 upregulation of dopamine receptor mRNA. Nevertheless, a positive feedback of gonadal steroid also operates in fish, but only early in gonadal development. Estrogens and aromatizable androgens can stimulate LHβ-subunit gene expression and increase pituitary LH protein levels in juveniles of several fish species.

Regulation by gonadal peptides

Activins are peptides composed of two β-subunits that form either hetero- or homodimers: activin A (βa + βa), activin BA (βa + βb), and activin B (βb + βb). Activins A and B increase FSHβ and reduce LHβ mRNA levels in cultured pituitary cells. Furthermore, follistatin, an activin-binding protein, can reverse the effects of exogenous activin on FSHβ and LHβ expression, and also stimulate basal expression of FSHβ gene. In goldfish (*Carassius auratus*), Smad3 is likely the principal signal transducing molecule involved in activin stimulation of FSHβ expression. While activin was first identified as an FSH stimulator and its effect on FSH biosynthesis has been well studied in a variety of fish, the details of its action mechanism are poorly understood.

Gonadotropin Receptors

In line with the gonadotropic duality, two distinct receptors occur in fish gonads: the FSH-R and the LH-R. Both show different expression profiles during follicular development. Expression of FSH-R is associated predominantly with vitellogenesis, while the LH-R is prevalent during oocyte maturation and ovulation.

Studies on gonadotropin receptors selectivity in representatives of three teleost orders (salmoniformes, siluriformes, and cypriniformes) indicated that FSH-Rs not only show a preference for FSH but also respond to LH, whereas LH-Rs specifically respond to LH only. Amago salmon (*Oncorhynchus rhodurus*) is the only known exception to this scheme.

The FSH-Rs and LH-Rs are G-protein-coupled receptors (GPCRs) whose large extracellular domain contributes to the recognition and specific binding of the hormone. The transmembrane domain is the most conserved part of these receptors, both in structure and amino acid sequence. It has seven α-helices composed of 22–28 hydrophobic amino acids, with each crossing the lipid bilayer and being connected by three intracellular and three extracellular loops. The transmembrane domain is responsible for receptor activation and signal transduction of the hormone.

Generally, the cellular localization of the gonadotropin receptors agrees with their steroidogenic biopotencies in mature fish. *In situ* hybridization studies in African catfish revealed FSH-R expression in Sertoli cells, but in contrast to other vertebrates, FSH-R is expressed in the interstitial Leydig cells too, together with LH-R. In European sea bass, FSH-R transcripts are detected in the follicular cells surrounding the pre- and early vitellogenic oocytes but not on fully grown oocytes, indicating the involvement of FSH-R in the recruitment of new oocyte generation and initiation of vitellogenesis.

FSH-R was localized in the theca layer and intensely on granulosa cells of vitellogenic ovaries of coho salmon (*Oncorhynchus kisutch*), whereas in the preovulatory follicle, it occurred in the theca and interstitial connective tissue, but not in the granulosa. At all stages of oogenesis, only granulosa cells of the preovulatory follicle exhibited LH-R, which is in line with LH function in oocyte maturation.

The expression of LH-R in males is consistently correlated with spermiation and spawning in all fish species studied so far.

Unorthodox Sites for Kiss, GnRH, and Gonadotropins

Recently, evidence is accumulating that the gonads produce and release hormones, and contain receptors that originally were thought to be restricted to the hypothalamus and pituitary. Oocytes of the gilthead sea bream produce and release GtHs, processes that can be enhanced by GnRH and reduced by an GnRH antagonist. In the African catfish, GnRH-1 and GnRH-2 mRNAs are expressed in the testis, and GnRH-2 mRNA in the ovary. In addition, kiss1 and kiss1r occur in the ovary and testes of various fish. These local regulatory axes could finely tune the activity of hypothalamic–pituitary–gonadal axis. Nevertheless, further research is still required before an updated comprehensive model for this endocrine regulation of fish reproduction can be formulated.

See also: **Hormones in Communication**: Hormonal Pheromones. **The Pituitary**: Pituitary Gland or Hypophysis. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis; Regulation of Spermatogenesis; Vitellogenesis in Fishes.

Further Reading

Aizen J, Kasuto H, Golan M, *et al.* (2007) Tilapia follicle-stimulating hormone (FSH): Immunochemistry, stimulation by gonadotropin-releasing hormone, and effect of biologically active recombinant FSH on steroid secretion. *Biology of Reproduction* 76: 692–700.

Devlin RH and Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191–364.

Ge E (2005) Intrafollicular paracrine communication in the zebrafish ovary: The state of the art of an emerging model for the study of vertebrate folliculogenesis. *Molecular and Cellular Endocrinology* 237: 1–10.

Levavi-Sivan B, Bogerd J, Mañañós EL, Gómez A, and Lareyre JJ (2010) Perspectives on fish gonadotropins and their receptors. *General and Comparative Endocrinology* 165: 412–437.

Lubzens E, Young G, Bobe J, and Cerdà J (2010) Oogenesis in teleosts: How fish eggs are formed. *General and Comparative Endocrinology* 165: 367–389.

Miura T and Miura C (2001) Japanese eel: Model for analysis of spermatogenesis. *Zoological Science* 18: 1055–1063.

Miura T and Miura C (2003) Molecular control mechanisms of fish spermatogenesis. *Fish Physiology and Biochemistry* 28: 181–186.

Nagahama Y and Yamashita M (2008) Regulation of oocyte maturation in fish. *Development Growth and Differentiation* 50: S195–S219.

Schulz RW, França LR, Lareyre JJ, *et al.* (2010) Spermatogenesis in fish. *General and Comparative Endocrinology* 165: 390–411.

Wong TT and Zohar Y (2004) Novel expression of gonadotropin subunit genes in oocytes of the gilthead seabream (*Sparus aurata)*. *Endocrinology* 145: 5210–5220.

Yaron Z and Levavi-Sivan B (2006) Reproduction. In: Evans DH and Claibourne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp.343–386. Boca Raton, FL: CRC Press/Taylor and Francis.

Zhou L-Y, Wang D-S, Kobayashi T, *et al.* (2007) A novel type of P450c17 lacking the lyase activity responsible for C21-steroid biosynthesis in the fish ovary and head kidney. *Endocrinology* 148: 4282–4291.

Control of Appetite in Fish

H Volkoff, Memorial University of Newfoundland, St. John's, NL, Canada

© 2011 Elsevier Inc. All rights reserved.

How Is Appetite Regulated in Vertebrates?
Fish as a Model to Study the Mechanisms Regulating Appetite
Studying Appetite in Fish: Methods and Approaches
Hormones and Appetite in Fish
Influence of Feeding Status and Circulating Metabolite Levels on Appetite
Influence of Gender, Reproductive Status, and Genetics on Appetite
Environmental Control of Appetite
Further Reading

Glossary

Anorexigenic	Inhibiting appetite.
Gene expression levels	Abundance of messenger RNA (mRNA: an RNA produced by transcription that carries the code for a particular protein from the nuclear DNA to a ribosome in the cytoplasm and acts as a template for the formation of that protein) within a specific cell or tissue.
Hypothalamus	Region at the floor of the forebrain (diencephalon) that controls several vegetative physiological processes, including food intake and secretion of hormones from the pituitary gland.
Intracerebroventricular	Situated within or administered into ventricles (cavities in the brain that are continuous with the spinal cord, and that contain fluid) of the brain.
Intraperitoneal	Situated within or administered by entering the peritoneum (abdominal cavity).
Neurohormone	Hormone produced by the brain that influences neural activity or functioning.
Orexigenic	That stimulates feeding.
Pituitary gland	Small endocrine gland located at the base of the brain and attached to the hypothalamus of the brain, which is also called hypophysis.
Poikilotherm	An animal whose body temperature fluctuates with that of the environment. The opposite of homeotherm, an organism, such as a mammal or bird, capable of maintaining its body temperature within a narrow range, usually above that of its surroundings despite large variations in environmental temperature.
Satiety	The quality or state of being fed to or beyond capacity.
Vagus nerve	Cranial nerve that travels from the brainstem through organs in the neck, thorax, and abdomen. In the abdomen, branches of the vagal nerve enter the stomach, pancreas, and the intestine.

How Is Appetite Regulated in Vertebrates?

In all vertebrates, survival depends on a balance between energy intake and energy expenditure. Energy intake is dependent on food intake, whereas energy expenditure is related to metabolic rate and physical activity. The brain, in particular the hypothalamus, plays a key role in monitoring energy status. Destruction of some hypothalamic areas is associated with increased food consumption and obesity, whereas destruction of other hypothalamic areas results in anorexia and weight loss. Such ablation experiments clearly demonstrate that the hypothalamus plays a role in the regulation of feeding and energy balance. These specific areas of the hypothalamus are often referred to as feeding centers.

The hypothalamus ultimately controls energy intake and expenditure by receiving and integrating sensory signals from the brain, the peripheral circulation, and the gastrointestinal (GI) tract via the vagus nerve (**Figure 1**). Although differences exist, fish brains follow the same basic plan as in other vertebrates (**see also Brain and Nervous System**: Functional Morphology of the Brains of Agnathans, Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria, Functional Morphology of the Brains of Ray-Finned Fishes, Functional Morphology of the Brains of Cartilaginous Fishes, and Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern). Fish brains can be roughly subdivided into (1) hindbrain, which contains the brainstem and receives inputs from the vagus nerve; (2) midbrain, which contains the

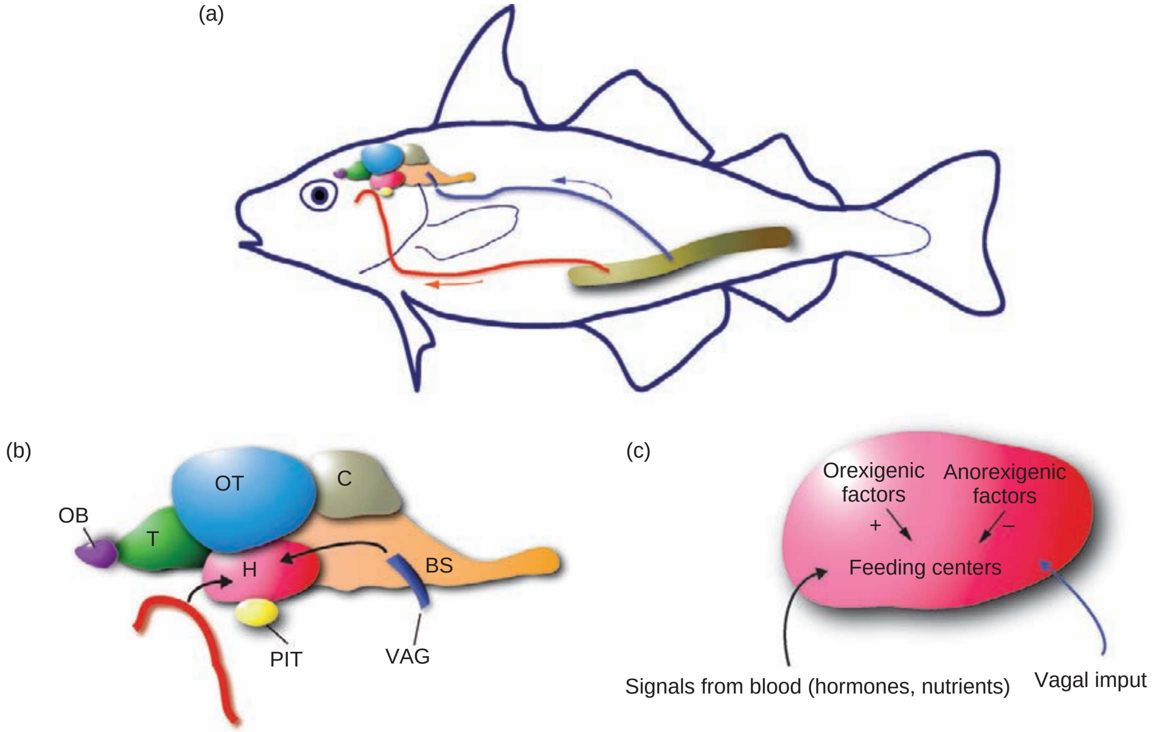


Figure 1 (a) Appetite is controlled by the brain, which integrates information on nutritional status relayed by the blood or by the vagus nerve. (b) Fish brains can be subdivided into hindbrain (which contains the brainstem (BS) and receives inputs from the vagus nerve (VAG)), the midbrain (which contains the cerebellum (C), optic tectum (OT), and hypothalamus (H)), and the forebrain, which is composed of the telencephalon (T) and olfactory bulbs (OB). A pituitary gland (PIT, small endocrine gland) is located at the base of the brain and attached to the hypothalamus. (c) The hypothalamus contains feeding centers that are influenced by hypothalamic orexigenic and anorexigenic factors. These centers are also influenced by neural projections from the vagus nerve as well as by blood-borne nutrients and hormones.

cerebellum, optic tectum, and hypothalamus; and (3) forebrain, which is composed of the telencephalon and olfactory bulbs (**Figure 1**(**b**)).

Signals from the brain consist largely of neurohormones secreted by the hypothalamus. When energy intake exceeds expenditure, anorexigenic (appetite-inhibiting) neurohormones are secreted, whereas when energy expenditure exceeds intake, orexigenic (appetite-stimulating) neurohormones are secreted (**Figure 1**(**c**)). Signals from the peripheral circulation consist of factors released in the blood stream that enter the hypothalamus and bind to hypothalamic receptors, thus acting directly on hypothalamic feeding centers. These factors consist of either nutrients, such as amino acids and glucose, or hormones released by the GI tract during a meal.

Nervous signals arrive to the hypothalamus via innervation from the brainstem, which is a relay center for visceral sensory information carried by the vagus nerve originating from the GI tract. This information consists of both mechanical and hormonal signals (**see also Integrated Function and Control of the Gut**: Endocrine Systems of the Gut, **Gut Anatomy and Morphology**: Gut Anatomy, and **The Gut**: The Gastrointestinal Tract: An Introduction). When the GI tract wall is distended by the presence of food, it activates stretch receptors, which transmit that information to the brain via the vagus nerve. The presence of food also triggers the release of a number of gut hormones, which can either be released in the blood stream (as described above) or bind to receptors on the vagus nerve.

Appetite regulation might be divided into short-term regulation, which pertains to the size and duration of a meal, and long-term regulation, which pertains to weight regulation. Most short-term regulators are hormones released from the GI tract during or after a meal. These so-called satiety factors indicate fullness (or satiety) and terminate a meal. They include cholecystokinin (CCK) and gastrin-releasing peptide (GRP). Long-term regulators, such as leptin and insulin, have levels that vary with adiposity and relay information about long-term energy stores.

Fish as a Model to Study the Mechanisms Regulating Appetite

Besides the same basic brain organization, fish have the same basic mechanisms controlling feeding behavior as other vertebrates, making them a valuable model to study the mechanisms regulating appetite in vertebrates in general.

However, fish also present unique characteristics. Perhaps more than any other vertebrate group, they display a great diversity in feeding habits and consume a large variety of foods (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics). Depending on the species considered, fish might eat plants (herbivores such as grass carp, *Ctenopharyngodon idella* or tilapia, *Oreochromis niloticus*), fish and large invertebrates (carnivores such as cichlids, trouts, flatfish, or sharks), or both plants and animals and occasional detritus (omnivores, such as common carp, *Cyprinus carpio* and goldfish, *Carassius auratus*). Different feeding habits can be found within one family of fish, as among cyprinidae (carps and goldfish) or bleniidae (blennies). These different feeding habits often translate into morphological (in particular, in the structure of the GI tract and the brain) and behavioral specializations for a particular type of food. For example, carnivorous predators usually have a shorter intestine than herbivores and might have higher development of areas of the brain needed for finding preys. However, a characteristic and originality of these animals are that many fish switch their feeding habits throughout life and most fish can adapt their diet to the available food supply. Feeding habits in fish can thus change with locality, age, sex, and season.

Overall food demand is a direct function of metabolic rate. Fish are poikilothermic (cold-blooded) animals, whose body temperature reflects the ambient temperature. As such, they do not expend energy to maintain a constant body temperature, and usually have lesser metabolic rates and needs – thus less feeding requirements – than homeotherms (warm-blooded animals such as mammals). However, as opposed to most mammals, which have a finite growth, most fish have an indeterminate growth, which means they continue to grow throughout their lives. Fish thus feed not only to maintain their basal metabolism but also to build energy stores to sustain both adequate growth and reproduction throughout their lives. The strategies for energy storage vary among species. For example, carp and salmon store excess energy as lipids in the muscle and as visceral fat, while Atlantic cod and sharks store excess energy in the liver.

Fish also experience a number of environmental changes, and often undergo daily and seasonal cycles in appetite that are entrained by the environment (see the next section).

Studying Appetite in Fish: Methods and Approaches

Several methods and approaches can be used to assess appetite in fish. In wild animals, the choice of techniques is rather limited and food intake is usually estimated by examining stomach or gut contents. In contrast, in laboratory conditions, food intake may be assessed accurately by quantifying the amount of food eaten by individual fish. This can de done directly by visually observing the fish and counting the number of pellets eaten by each individual. Appetite can also be measured by weighting the fish before and after feeding, by measuring the radioactivity of fish fed pellets or prey labeled with radioactive material, or by using X-ray to count pellets in stomachs of fish fed food labeled with opaque beads.

Very often, however, the assessment of appetite for individual captive fish is very difficult or impossible. For example, social and schooling species can become stressed as individuals or in small groups, or conversely in high-intensity aquaculture conditions. For groups of fish, feeding of individual fish can be inferred simply by dividing the amount of food eaten by a group of fish by the number of fish and the average weight of fish. To obtain more accurate quantifications of food intake, ingenious devices have been developed by some researchers. One might either continuously collect and detect uneaten food pellets by underwater infrared sensors, or use acoustic methods to detect sounds emitted by fish during feeding. Some fish species (e.g., rainbow trout, *Oncorhynchus mykiss* and sea bream, *Sparus aurata*) can also be trained to eat on demand using self-feeders. These devices consist of detectors that deliver food when activated by a fish, and allow for the assessment of appetite by counting the number of feed demands.

In order to assess the role of specific appetite-regulating hormones, several methods can be used. A direct method consists of submitting fish to hormone treatments. The hormone can be delivered via several routes, including peripheral (intraperitoneal (IP), or in the body cavity) or brain injections (intracerebroventricular (ICV), or in the brain ventricle system), intraperitoneally implanted pellets or osmotic mini-pumps – which deliver the hormone continuously for several days or weeks – and via oral administration. Food intake is then assessed during and/or following treatment and compared to that of control saline-injected fish to detect a possible orexigenic or anorexigenic effect of the hormone (see **Video Clip 1**).

In vivo injections and feeding behavior observations are not always feasible, as some fish species cannot withstand the stress of the procedure or are too large for injections. In these situations, the role of hormones can be assessed indirectly by examining the effects of feeding conditions (e.g., starvation or lipid-rich diet) on blood or protein/gene expression levels. The assumption is that if, for a given hormone, these levels vary between fish feeding different diets (or not feeding), then the hormone most likely has some role in appetite regulation.

The advent of new technologies such as transgenic animals (that carry a foreign gene, which has been deliberately inserted into their genome) or knockdowns (in which expression of a gene is inactivated) might provide promising new data on the genes involved in the regulation of appetite in fish.

Hormones and Appetite in Fish

A number of hormones have been identified as appetite regulators in fish. **Table 1** summarizes those hormones known to regulate appetite in fish to date. A few major hormones known to regulate appetite are described below.

Hormones from the Brain and Pituitary

Neuropeptide Y (NPY), orexins (orexin A and B), and galanin are three of the major brain hormones that stimulate appetite in fish. As in mammals, orexins appear to increase alertness and locomotor activity in fish. It has been suggested that orexins, rather than stimulating feeding, might promote arousal responses that would allow food seeking/foraging behavior. Corticotropin-releasing factor (CRF) (and a related hormone called urotensin) and the cocaine- and amphetamine-regulated transcript (CART) peptide are the two major hypothalamic appetite inhibitors. In fish, as in all vertebrates, CRF also has an important role in the stress response. All these hormones, although produced mostly by the brain, are similarly produced but in lesser amounts by peripheral tissues such as the gut and the gonads.

Growth hormone (GH) is a hormone secreted by the pituitary gland that stimulates growth. GH appears to be involved in the regulation of feeding in fish as both fish treated with GH and GH transgenic fish (in which the GH gene has been added) display high feeding levels.

Peripheral Hormones

Most of the peripheral appetite-regulating hormones both act as a satiety factor and have aid in digestive processes. Most of these gut peripheral hormones are also produced in lesser amounts by the brain and are therefore often referred to as brain–gut hormones.

In fish, as in mammals, CCK is a major satiety factor produced by the intestine. During a meal, CCK is released from the small intestine into the circulation and stimulates digestion (by decreasing gastric emptying as well as stimulating gallbladder contraction and secretions from the pancreatic and gastric) while suppressing food intake. The actions of CCK are mediated by binding to both hypothalamic receptors and receptors on the vagus nerve.

Table 1 Major hormones known to regulate appetite in fish

Hormone	*Origin (P: peripheral C: central)*	*Major producing site*	*Effect on feeding (+: stimulation, –: inhibition)*
Amylin	P	Brain/GI tract	+
Apelin	P	GI tract/brain	–
Calcitonin gene-related peptide (CGRP)	C	Brain	–
Cholecystokinin (CCK)	P	GI tract	–
Cocaine- and amphetamine-regulated transcript (CART)	C	Brain	–
Corticotropin-releasing factor (CRF)	C	Brain	–
Cortisol	P	Interrenal	–
Galanin (GAL)	C	Brain	+
Gastrin-releasing peptide (GRP)	P	GI tract	–
Ghrelin	P	GI tract	+
Glucagon-like peptide -1 (GLP-1)	P	GI tract	–
Gonadotropin-releasing hormone (GnRH)	C	Brain	–
Growth hormone (GH)	C	Pituitary	+
Insulin	P	Pancreas	–
Intermedin (IM)	C	Brain/pituitary	–
Leptin	P	Liver	–
Melanin-concentrating hormone (MCH)	C	Brain	–
Melatonin	C	Pineal	–
Neuromedin U (NMU)	C	Brain	–
Neuropeptide Y (NPY)	C	Brain	+
Orexins (OX)	C	Brain	+
Pituitary adenylate cyclase-activating polypeptide (PACAP)	C	Brain	–
Prolactin-releasing peptide (PrRP)	C	Brain	–
Urotensin I (UI)	C	Brain	–

Like CCK, GRP is produced by the GI tract and acts as a satiety factor. GRP also regulates gastric functions such as acid secretion and gastric motility.

Amylin is produced by the pancreas. It suppresses nutrient-stimulated glucagon secretion, slows down gastric emptying, and reduces food intake and body weight.

First discovered in mammals, leptin is produced by adipose tissue (fat) and it regulates food intake, energy balance, and reproduction. Although mammalian leptin was first isolated in 1994, the structures of leptin and its receptor in fish were only established recently. Very few similarities are seen between the leptin protein structures of fish and those of mammals. In addition, in contrast to mammals, the liver appears to be the major site for leptin expression in fish, unlike adipose tissue in mammals. Fish leptin is also produced by brain, GI tract, muscle, and fat. To date, the role of leptin in fish appears to be controversial. Leptin appears to have a role in regulation of feeding and metabolism in fish. While treatment with mammalian leptin does not affect appetite in some fish species (coho salmon, *Oncorhynchus kisutch* or channel catfish, *Ictalurus punctatus*), it does decrease food intake in goldfish. Peripheral injections of trout leptin in rainbow trout suppress food intake.

To date, only two peripheral hormones have been shown to increase appetite, ghrelin and apelin, in vertebrates. In fish, as in mammals, ghrelin is predominantly secreted by the stomach (and also by the brain) and stimulates GH release. In mammals, ghrelin stimulates appetite, but its role in the regulation of feeding of fish is still unclear. Indeed, injections of ghrelin stimulate appetite in some fish species but do not in others. Apelin is produced by several tissues, including brain and adipose tissues. Both peripheral and brain injections of apelin increase feeding in goldfish. Apelin also regulates cardiovascular functions in both mammals and fish.

Influence of Feeding Status and Circulating Metabolite Levels on Appetite

As a general rule, states of hunger or nutrient deficiency induce an increase in gene expression and plasma levels of orexigenic hormones, while a well-fed satiated state increases levels of anorexigenic hormones. In fish, the brain expression levels of NPY, orexins, and galanin all increase in hungry fish, both immediately before a meal and during periods of food deprivation. Similarly, fasted fish display higher ghrelin plasma levels, higher stomach and brain gene expression levels of ghrelin, and higher apelin brain gene expression levels than fed fish. Conversely, CART and CCK gene expression levels increase following a meal and decrease following fasting. Fasting apparently induces a decrease in liver and plasma leptin levels in some fish species. Starvation also induces changes in both pituitary GH gene expression and plasma GH levels.

Administration of glucose or amino acids decreases appetite and fat fish appear to eat less than lean fish, suggesting that circulating metabolite levels alter food intake in fish.

Influence of Gender, Reproductive Status, and Genetics on Appetite

Individual differences exist for foraging behavior and growth that are related to gender, sexual maturity, and genetic background. In some fish species, gender-specific differences in the presence and distribution of appetite regulators exist (e.g., galanin and ghrelin). Appetite also varies according to the reproductive state. Appetite is suppressed in some species during the reproductive season. This is especially the case during spawning migrations (e.g., salmonids), courtship, spawning, or nest guarding. Males and females have different profiles of reproductive hormones, in particular gonadal sex steroids (androgens and estrogens), and during a fish reproductive cycle, major changes in the levels of these hormones occur. It has thus been hypothesized that gonadal steroids might influence appetite and the expression levels of appetite regulators.

The hypothalamic hormone gonadotropin-releasing hormone (GnRH) is a major reproductive hormone in vertebrate. It stimulates the pituitary to secrete gonadotropins, which in turn acts on the gonads to stimulate production of gonadal steroids and gametes. In goldfish, brain injections with GnRH decrease appetite, in part by decreasing brain orexin gene expression. Conversely, brain injections of orexin inhibit spawning behavior, in part, by decreasing hypothalamic GnRH gene expression levels. These data suggest that the actions of reproductive hormones and appetite regulators are closely related.

Food intake has been shown to vary among fish strains of different genetic backgrounds. Different strains display variations in feeding activities and patterns, nutrient utilization, and growth, which might relate to genetic variations in appetite-regulating hormones. Recent studies also show that GH transgenesis (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish) induces modifications in feeding-related behaviors in both carp and salmon, where transgenic fish display an increase in both food consumption and in time spent foraging in the feeding areas.

Environmental Control of Appetite

In the wild, environmental factors such as temperature and photoperiod can have a dramatic effect on appetite in certain fish species. These environmental factors often act as cues that entrain a seasonal cycle in feeding and reproduction. For example, some temperate fish, such as winter flounder, *Pseudopleuronectes americanus* or Arctic charr, *Salvelinus alpinus*, display a decrease in appetite during winter, when days are short and temperatures are low. Generally, fish tend to increase their appetite and growth rates with rising temperatures. Fish species also vary in their natural daily feeding rhythms, some species being diurnal feeders (Atlantic salmon, *Salmo salar*) and others being nocturnal feeders (channel catfish). Little is known about the hormonal mechanisms regulating these environment-induced changes in feeding, but some data cue to the involvement of appetite-regulating hormones. For example, in Atlantic cod, *Gadus morhua*, brain CART gene expression levels are higher in fish held at 2 °C than in fish held at 15 °C, and in ornate wrasse, *Halichoeres ornatissimus*, fish exposed to constant light conditions display higher orexin receptor hypothalamic expression levels than fish exposed to natural photoperiod. In addition, in Atlantic cod and goldfish, the hypothalamic expression of orexin appears to show daily fluctuations in phase with feeding rhythms.

In captive fish, appetite is influenced by stocking density, in particular in species in which dominant individuals monopolize the available food (e.g., in salmonids; **see also Social and Reproductive Behaviors**: Dominance Behaviors). Tank color also influences feeding and growth. Flounders grow faster in white tanks than in the black tanks.

Most fish eat less and grow slower if stressed by their environment. These situations include drastic changes in temperature, photoperiod, salinity, or low oxygen levels (hypoxia). Excessively high temperature can suppress feeding and can cause severe environment hypoxia, both of which lead to suboptimal growth. Contaminants and disease agents also usually decrease appetite.

See also: **Brain and Nervous System**: Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern; Functional Morphology of the Brains of Agnathans; Functional Morphology of the Brains of Cartilaginous Fishes; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria. **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish. **Gut Anatomy and Morphology**: Gut Anatomy. **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut. **Social and Reproductive Behaviors**: Dominance Behaviors. **The Gut**: The Gastrointestinal Tract: An Introduction.

Further Reading

De Pedro N and Bjornsson B (2007) Regulation of food intake by neuropeptides and hormones. In: Houlihan D, Boujard T, and Jobling M (eds.) *Food Intake in Fish*, pp. 269–296. London: Wiley-Blackwell.

Dockray GJ (2009) The versatility of the vagus. *Physiology and Behavior* 97: 531–536.

Hart P, Webster M, and Ward A (2008) Fish foraging behaviour in theory and practice. In: Magnhagen C, Braithwaite V, Forsgren E, and Kapoor B (eds.) *Fish Behaviour*, pp. 235–268. Enfield, NH: Science Publishers.

Hoskins LJ, Xu MY, and Volkoff H (2008) Interactions between gonadotropin-releasing hormone (GnRH) and orexin in the regulation of feeding and reproduction in goldfish (*Carassius auratus*). *Hormones and Behavior* 54: 379–385.

Jensen J (2001) Regulatory peptides and control of food intake in non-mammalian vertebrates. *Comparative Biochemistry and Physiology – Part A* 128: 471–479.

Magni P, Dozio E, Ruscica M, *et al.* (2009) Feeding behavior in mammals including humans. *Annals of the New York Academy of Sciences* 1163: 221–232.

Peter RE (1979) The brain and feeding behavior. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology, Volume VIII,* pp. 121–159. New York, NY: Academic Press.

Shioda S, Takenoya F, Yagi M, *et al.* (2008) Neural networks of several novel neuropeptides involved in feeding regulation. *Nutrition* 24: 848–853.

Valassi E, Scacchi M, and Cavagnini F (2008) Neuroendocrine control of food intake. *Nutrition Metabolism and Cardiovascular Diseases* 18: 158–168.

Volkoff H and Peter RE (2006) Feeding behavior of fish and its control. *Zebrafish* 3: 131–140.

Volkoff H, Unniappan S, and Kelly SP (2009) The endocrine regulation of food intake. In: Bernier N, Kraak GVD, Farrell A, and Brauner C (eds.) *Fish Physiology, Volume 28,* pp. 421–465. Burlington, MA: Academic Press.

Volkoff H, Xu M, MacDonald E, and Hoskins L (2009) Aspects of the hormonal regulation of appetite in fish with emphasis on goldfish, Atlantic cod and winter flounder: Notes on actions and responses to nutritional, environmental and reproductive changes. *Comparative Biochemistry and Physiology – Part A* 153: 8–12.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

HORMONAL CONTROLS

Hormonal Responses to Stress

Contents

Hormone Response to Stress
Catecholamines
Stress Effect on Growth and Metabolism
Impact of Stress in Health and Reproduction

Hormone Response to Stress

SE Wendelaar Bonga, Radboud University Heiweg 8, EB Malden, The Netherlands

© 2011 Elsevier Inc. All rights reserved.

Hormones, Stress and Stressors
Factors Modifying the Stress Response
Concluding Remarks
Further Reading

Glossary

Autocrine signaling Receptor-mediated transfer of information to a cell by a chemical messenger produced and released by the same cell.

Coping A behavioral response that aims at reduction of the impact of aversive stimuli on physiological homeostasis.

Endocrine signaling Receptor-mediated transfer of information to cells by a chemical messenger (hormone) produced and released by distant cells, and transported via the blood circulation.

Heat shock protein A protein that interacts with other proteins, promoting productive folding pathways to ultimately stabilize cellular proteins and membranes.

Homeostasis The equilibrium or stability of the internal environment of organisms in a changing external environment, maintained by many physiological adjustment mechanisms.

Hormone Signaling molecule released by a cell that either stimulates the cell of origin (autocrine), nearby cells (paracrine), or travels via the bloodstream to distant cells (endocrine).

Metallotheionein A small protein of a group of cysteine-rich proteins with an ability to bind heavy metals.

Neurohormone Hormone produced by the brain that influences neural activity or functioning.

Paracrine signaling Receptor-mediated transfer of information by a chemical messenger produced by a cell toward neighboring cells.

Stress State which extends the adaptive responses developed of an animal beyond of the normal range or the normal functioning as a result of a challenge that attempt to reestablish the physiological equilibrium.

Stressor Any agent generating a situation of stress and inducing a coordinated response involving a transient modification of the physiological regulation.

Urophysis The storage organ of the axon endings of a group of neuroendocrine cells at the distal end of the spinal cord of fishes, releasing the hormones urotensin I and II.

Hormones, Stress and Stressors

Teleost fishes, like many other animals, respond to changes in the external and internal environments by the release of hormones into the blood. Rhythmic changes, such as the daily light–dark cycle and minor changes in the daily routine, are met with adjustments that maintain the dynamic physiological equilibrium of an organism called homeostasis. The hormones involved are dependent on the type of change. In addition to such changes, every animal also meets unexpected and major changes that are threatening its well-being and even survival. Such changes vary from a sudden temperature drop, an encounter with a predator, or a dominant member of the social group, to pollutants such as agrichemicals.

The condition of disturbed homeostasis is defined as stress. If this happens, most vertebrates, including fishes, show a marked, more or less uniform endocrine response, largely independent of the type of stressor. This is the generalized or organismal stress response in which many organs and tissues are involved. It is an adaptive response that, if successful, leads to not only recovery, that is, restoration of homeostasis, but also increased resistance to the same or related types of stressor. The most important hormones and their functions involved in this process are described below. This is done in a generalized way, ignoring many species-specific differences that are found among teleost fishes, a very large group of animals that have colonized all possible aquatic niches.

The term stress is not restricted to the organismal level. Physical and chemical stressors can also act directly at the cellular level, disturbing the cellular physiology. The cellular stress responses, which are manifold and largely stressor specific, include stimulated or induced expression of proteins, such as heat shock proteins, metallotheioneins, and antioxidant enzymes. They occur in all cells, even bacteria, and thus have a much longer evolutionary history than the organismal stress response of the vertebrates. The cellular stress responses are independent of the organismal stress response in the vertebrates, and high levels of, for instance, heat shock proteins cannot be used as reliable indicators of stress at the organismal level.

In the organismal stress response, classically three categories are distinguished: the primary, secondary, and tertiary stress responses. These concern different levels of organization. We will follow this tradition. The primary response results in an often dramatic and therefore easily noticeable increase of the blood levels of the primary stress hormones, the catecholamines (CAs), adrenaline and noradrenaline (**see also Hormonal Responses to Stress**: Catecholamines), and the corticosteroid cortisol (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids). The secondary response comprises the physiological effects of these hormones. The tertiary response includes the physiological and behavioral changes occurring at the whole animal level, which can be restorative, adaptive, as well as pathological. Only the initiation of the primary, secondary, and tertiary responses is sequential in time because the duration of each response is such that it creates temporal overlap once the stress response is underway.

Primary Stress Response

The primary response to the perception of a stressor includes the state of alarm in the brain leading to the release of the primary stress hormones. Key brain areas in the integration of the response are the pallial areas of the telencephalon that are considered the teleost homologs of the mammalian hippocampus, and the brain stem (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes). Central brain monoamines are implicated with a prominent role for serotonin. The messages resulting in the release of CAs and cortisol leave the brain via two major routes (axes; **Figure 1**).

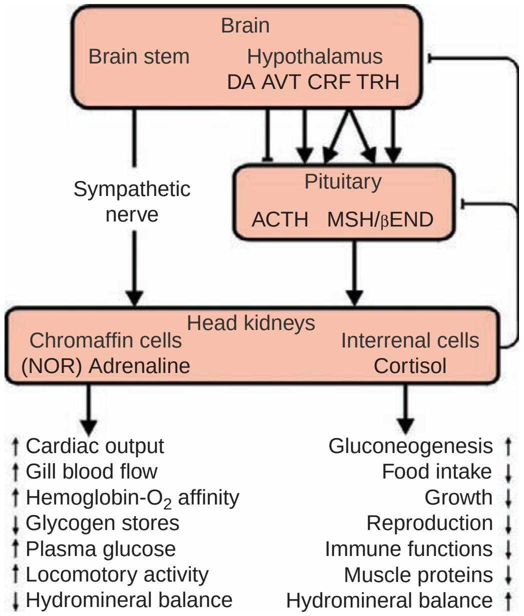


Figure 1 Diagram of the BSC and HPI axes, showing the main neurohormonal and hormonal messengers involved in the organismal stress response, and the major effects of the most important messengers of these axes, adrenaline and cortisol; arrows pointing upward: stimulation; arrows pointing downward: inhibition. AVT, arginine vasotocin; DA, dopamine; betaEND, beta-endorphin

The Brain–Sympathetic–Chromaffin Cell (BSC) Axis

The rise in the blood levels of the CAs starts a few seconds after confrontation with an acute stressor. In fish, adrenaline dominates, with plasma levels up to 10 times higher than those of adrenaline. The CAs are produced and released by the chromaffin cells, which normally are clustered in small groups within the layers of cortisol-producing interrenal cells surrounding the walls of the posterior cardinal veins and their branches (**Figure 2**). Together with the surrounding lymphoid tissue, they form the interrenal glands or head kidneys, the homologs of the mammalian adrenal glands. The chromaffin cells also release other substances, for example, dopamine, enkephalin, and natriuretic peptide, which may have functions related to the release or potentiation

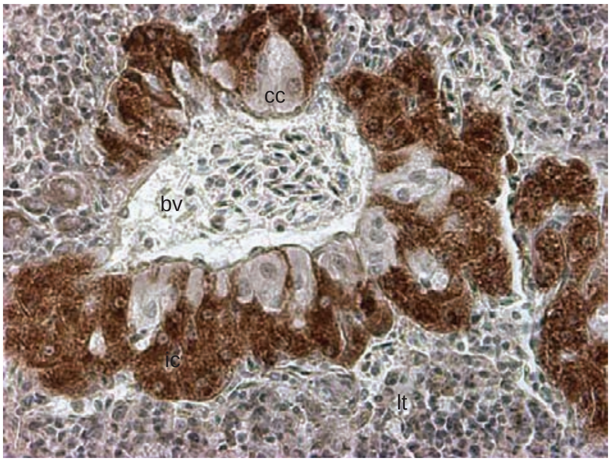


Figure 2 Light microscopic picture of head kidney tissue of carp (*Cyprinus carpio*), showing a blood vessel (bv) with erythrocytes, surrounded by a ring of interrenal cells (ic) immunocytochemically stained with an antibody raised against cortisol (brown deposits in the cytoplasm). Between these cells small numbers of nonstained chromaffin cells (cc) are present. The interrenal cells are surrounded by lymphoid tissue (lt). Courtesy Dr. Gert Flik.

of the CAs. Basal plasma levels of adrenaline are, in general, below 5 nmol. Peak values more than 50 times higher have been reported after acute strong stressors (**Figure 3**). The CA levels rapidly drop after peak levels have been reached, as a result of lowering of the release and a high turnover rate (< 10 min). When the increased CA release continues during chronic stress, the blood levels usually stabilize well below 50 nmol. The CA release is mainly controlled via preganglionic cholinergic fibers of sympathetic nerves terminating around the chromaffin cells. Autocrine and paracrine factors as well as hormones including cortisol may play a modulating role in CA release (**see also Hormonal Responses to Stress**: Catecholamines).

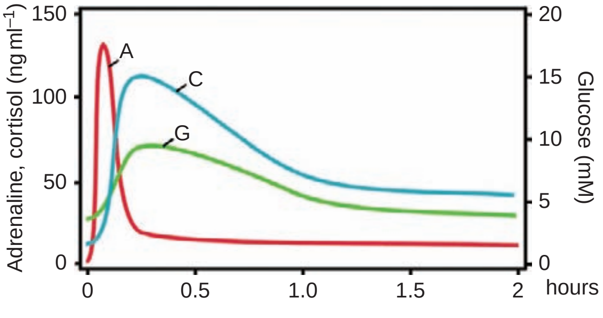


Figure 3 Generalized time course of the blood plasma levels of adrenaline (A), cortisol (C), and glucose (G) following confrontation with an acute stressor (based on observations on carp, *Cyprinus carpio*).

The Hypothalamic–Pituitary–Interrenal Axis

Cortisol is the final messenger of the hypothalamic–pituitary–interrenal (HPI) axis (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids). A key messenger between the brain and the endocrine system during the stress response is corticotropin-releasing factor (CRF), produced by neurons of the preoptic nuclei in the hypothalamus. These CRF neurons project to the pituitary gland and stimulate the release of the adrenocorticotropin hormone (ACTH) from cells in the distal part of the pituitary gland. Arginine vasotocin is also produced in the CRF cells and potentiates the CRF stimulation of the ACTH cells. ACTH is the major stimulant during stress of the interrenal cells for the synthesis and release of cortisol. The tissues producing these messengers are called the HPI axis. CRF is further produced in other areas of the brain, for instance, in the telencephalon and the tectum opticum, where it acts as a neurotransmitter involved in many functions (**see also Brain and Nervous System**: Functional Morphology of the Brains of Agnathans, Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria, Functional Morphology of the Brains of Ray-Finned Fishes, and Functional Morphology of the Brains of Cartilaginous Fishes). It is also released into the circulation where it has effects on many organs including the heart (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels and **Design and Physiology of the Heart**: Physiology of Cardiac Pumping) and the immune system. This endocrine function is the reason that CRF is also called the corticotropin-releasing hormone (CRH). The precise roles of CRF in the body have to be defined. CRF signaling is mediated through at least two types of CRF receptors, R1 and R2, with several subtypes. CRFR1 is the principal receptor for the HPI axis. CRFR2 signaling occurs in many organs including the brain. Thyroid-releasing hormone, another hypothalamic neurohormone, has ACTH-stimulating properties in fishes. Apart from ACTH, cortisol is also stimulated by hormones such as urotensin I released by the urophysis and atrial natriuretic peptide.

Following an acute stressor, cortisol release starts after about 5 min (**Figure 3**). This lag time contrasts with the immediate release following the stimulation of the CA-producing cells. The lag time in cortisol release, the rather slow building up of the peak level (up to 1 h), the low turnover rate (cortisol is converted to cortisone; half life is about 1 h), and the rather simple assay procedures make cortisol blood levels the most frequently used stress parameter in fishes. Cortisol values vary from basal levels between 5 and 30 nmol to peak levels of more than 400 nmol during severe acute stress. During chronic stress, cortisol levels usually stabilize at 2–5 times the basal concentration. Up to 80% of the hormone in circulation is protein bound and therefore biologically inert, although a protein specific for cortisol binding in fish plasma is still debatable.

Other Pituitary Hormones Activated during the Primary Response

In addition to the ACTH release, the release of several other pituitary hormones is stimulated by hypothalamic messengers during the primary stress response. In fishes, CRF also stimulates the release of thyroid-stimulating hormone (TSH), leading to elevated thyroid hormone levels during stress. CRF further activates the cells producing alpha-melanophore-stimulating hormone (alpha-MSH) and beta-endorphin. In some, but not all, species investigated, alpha-MSH and beta-endorphin stimulate cortisol release. The sensitivity of the ACTH and MSH cells is modulated by dopamine (ACTH) and endorphin (MSH) from hypothalamic origin. An intriguing hormone released within a few minutes following stressor exposure is somatolactin. It belongs to the growth hormone/prolactin family and has been identified so far only in the pituitary pars intermedia of fishes. The function of somatolactin has been related to, for example, skin pigmentation, acid–base and calcium regulation, and lipid metabolism, but further studies are required to clearly define its role in the stress response.

Secondary Stress Response

Actions of adrenaline and cortisol

The most important physiological actions of CAs and cortisol are summarized in **Figure 1**. The main function of adrenaline is the rapid mobilization of energy to facilitate immediate action of the animal (fight or flight). Adrenaline further stimulates the breakdown of glycogen (glycogenolysis) in the liver, leading to a rapid rise of blood glucose. Furthermore, free fatty acid levels may become elevated. Given the prompt CA release following stressor perception, it is hardly possible to separate the effect of the stressor on plasma CA levels from the stress effects of capture and blood sampling on this parameter. Therefore, the rise in plasma glucose, which develops relatively slowly after stressor detection, is frequently used as an alternative parameter for the effect of a stressor on CA release. Glucose is the primary energy substrate for fish and is, in particular, needed by the brain and the muscles during stress. High-adrenaline levels further increase stroke volume and/or rate of the heart and promote blood flow, in particular, into the brain, muscles, and gills. Furthermore, adrenergic sympathetic innervation of the vascular system is involved. The increased perfusion of the gills stimulates the gas exchange and, together with a higher affinity of hemoglobin for O_2, induced by noradrenaline, facilitates the oxygenation of the tissues, in particular, brain and muscles.

In the first phase of the acute stress response, the actions of cortisol are less prominent than those of adrenaline. The most important functions of cortisol concern the reallocation of energy away from long-term investments in growth and offspring toward activities directed at immediate survival. A second important function concerns the repair of the hydromineral disturbance that accompanies stress in fishes (**see** section Tertiary Response). Thus, in fishes, cortisol functions both as glucocorticoid (GR) as well as mineralocorticoid (MR) hormone. In terrestrial vertebrates, the control of these functions is separated, with corticosterone or cortisol (depending on the species) as the GR, and aldosterone as the MR hormone. This functional separation at the hormone level apparently took place after the water-to-land transition of the vertebrates, around 380 million years ago. Other actions of cortisol are negative feedback control at all levels of the HPI axis. This does not imply that cortisol inhibits its own secretion under all conditions, for instance, during acute stress. In general, negative feedback processes are precisely regulated. For cortisol, the functional details are far from clear.

Receptors Involved in Cortisol Activity

Terrestrial vertebrates have GR and MR receptors for the GRs and aldosterone, respectively (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids and **The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis). In fishes, receptors with similar structure have been found. Two GR types have been reported, GR1 and GR2. Only one MR has been found so far. Furthermore, splicing isoforms have been described for GR and MR. GR1 have a much lower affinity for cortisol than GR2, indicating that GR1s are functioning at stress levels of cortisol. Expression of the mRNAs of the GRs and MR has been reported for many organs and tissues, including the telencephalon (in pallial areas homologous to the mammalian hippocampus), the CRF neurons in the pre-optic nucleus, and the ACTH cells in the pituitary. Thus, the three cortisol receptor types are all present in the three most important neural and endocrine areas related to the stress response. Their numbers can be manipulated by chronic stressor exposure.

Tertiary Response

If an animal can cope efficiently with a stressor through adaptation, or if the stressor is of short duration, homeostasis can be restored without serious consequences for the animal. The effects can be limited to improved performance in the future, such as increased predator avoidance capacity or disease resistance. In such cases, a tertiary response can hardly be observed. If the stressor has a chronic nature, and the adaptation to the stressor is laborious or even absent, the tertiary stress response is long-lasting and disabling. This was called distress by the pioneer of the field, Hans Selye, and distinguished from eustress, the stress that can be mastered successfully. In humans, eustress is challenging, rather than depressing. It makes humans and animals more resistant to the same or similar type of stressor, and this is the biological, adaptive function of the stress response.

During the tertiary response, the adrenaline and cortisol concentrations of the blood can vary from slightly to well above basal levels, much lower than the peak levels during acute stress. The chronically elevated adrenaline levels lead to depletion of the glycogen stores of liver and muscles, and to cardiovascular problems.

During chronic stress, the effects of cortisol are much more prominent than in the acute phase of the secondary response. The most important actions relate to energy balance, in particular, the replenishment of energy stores. This is performed by the diversion of energy substrates away from anabolic processes, in particular, growth and reproduction, and by the partial inhibition of the immune response. Whereas these actions occur in all vertebrates, one important action of cortisol is typical for fishes and concerns the hydromineral balance. All these actions of cortisol, which will be discussed briefly below, already start during the secondary stress response, but their effects become fully visible during the tertiary response.

Cortisol, Appetite, and Growth

Growth is inhibited rapidly during stress, via inhibition by cortisol of the pituitary growth hormone cells and the insulin-like growth factors (IGFs) produced by the liver. Food intake and food conversion efficiency are reduced. In trout, hypothalamic CRF mRNA levels are negatively correlated with food intake, as are the urotensin-1 mRNA levels in the urophysis. Leptin, the mediator produced by the liver that reduces appetite, may be involved. Growth can become negative by the catabolic activity of chronically high cortisol levels, which promote gluconeogenesis at the detriment of muscular proteins. This can lead to reduced scope for activity, emaciation, gastric and intestinal atrophy, and, finally, death (**see also Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism).

Cortisol and Reproduction

Stress, in particular when it is chronic, leads to inhibition of the reproductive hormones at all levels of the hypothalamo-pituitary-gonadal axis. In juvenile fishes, the onset of puberty is delayed. In sexually mature fishes, reproduction is decreased, or suppressed completely, as a result of delay or failure of egg development and ovulation, of suppressed spermatogenesis, and of disruption of courtship behavior. Reproductive success can be further affected by deleterious effects transmitted by elevated maternal cortisol levels on the survival changes of the offspring (see below). Inhibitory effects of cortisol have been demonstrated at all levels of the reproductive axis (although not studied in all species): the gonadotropin-releasing hormone (GnRH) producing neurons in the hypothalamus, the pituitary cells producing the gonadotropins I and II, and the gonadal cells producing the sex steroids (**see also Hormonal Responses to Stress**: Impact of Stress in Health and Reproduction).

Cortisol and Hydromineral Regulation

In fish, stressors have profound disturbing effects on hydromineral regulation, with substantial metabolic costs for its restoration. This is an important difference between fish and terrestrial vertebrates, connected with the aquatic lifestyle of fishes. Stressors affect hydromineral balance directly as well as indirectly (**see also Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish). Toxic stressors can act directly by damaging the osmoregulatory organs. In particular, toxic metal ions in the water can inhibit active ion transport mechanisms, for instance, NaKATPase, the biochemical equivalent of the sodium pump, and high-affinity CaATPase, the calcium pump, or can block ion channels. The gills (the main organs for ion exchange with the water), the gut (the location of water uptake in seawater fishes), and the kidneys are the main targets.

All stressors affect hydromineral regulation indirectly, via the adrenaline. The high-adrenaline levels, typical for stressed fish, do not only increase blood pressure, but also increase the permeability to water and ions of the respiratory surface, represented by the membranes lining the branchial lamellae. The blood spaces of the lamellae are separated from the water by thin endothelial and epidermal layers. This facilitates oxygen uptake and removal of carbon dioxide, but these delicate structures also make the gills a weak spot in the body surface. High-adrenaline levels increase the passive water and ion fluxes across the gill membranes, inducing hemodilution by increased ion losses and water inflow in freshwater fish, and, in marine fish, hemoconcentration by ion inflow and water outflow.

The mineralocorticoid functions of cortisol are focused on the stimulation of NaKATPase activity in gills, gut, and kidneys. The proliferation and differentiation of the branchial ionocytes (also called chloride cells, showing high NaKATPase activity) during stress are mediated by cortisol (**Figure 4**). Although originally

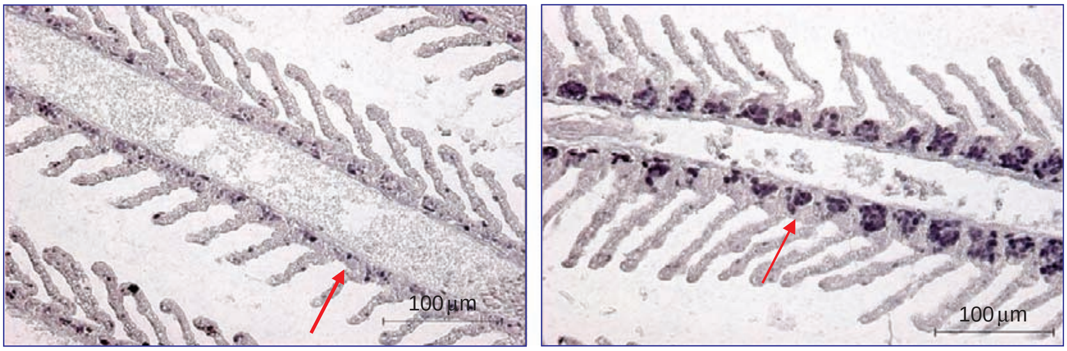


Figure 4 Gill filaments of Atlantic salmon (*Salmo salar*) immunologically stained with a monoclonal antibody raised against proliferating cell nuclear antigen (PCNA or cyclin), a molecule expressed in the nuclei of proliferating cells. Stained nuclei (arrows) are scarce in gills of control fish (left) and numerous in the gills of fish stressed by infestation with salmon lice (*Lepeophtheirus salmonis*; about 15 per fish). Almost all nuclei belong to cells differentiating into ionocytes (ion-transporting cells).

cortisol was considered to be primarily a hormone for hydromineral regulation in seawater, it is now generally accepted as equally important for marine and freshwater fishes, and for facilitating the hydromineral adaptations during migrations between freshwater and seawater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

The disturbing effects of stressors on passive water and ion movements in fish also stimulate the release of other hormones with hydromineral actions. In seawater fish, growth hormone promotes NaKATPase activity in the gills and gut. In freshwater fish, prolactin is the major hormone controlling the permeability to water and ions of several epithelia, including the branchial membranes. This function explains the stimulation of prolactin release that has often been reported in stressed freshwater fish. Both the osmotic uptake of water in freshwater fish and the osmotic loss of water in marine fish require compensatory actions by the kidneys, resulting in a urine flow that is high in freshwater and low in seawater. This means that the hormones controlling renal activity (e.g., natriuretic peptides), the neuropeptides vasotocin and isotocin, and the renin-angiotensin system are also indirectly implicated in the stress response. This also applies to stanniocalcin and calcitonin, hormones controlling the branchial calcium uptake, plasma calcium levels, and bone formation, and urotensin II, a hormone produced in the urophysis that has vasoconstrictor properties and stimulates the intestinal ion uptake in seawater (see also **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels).

Factors Modifying the Stress Response

Although the organismal stress response is essentially a uniform response to all kinds of stressors, within and between populations of animals, a great variety can be observed in the intensity and character of the responses to the same stressors. This variety is the result of the impact of several modifying factors acting at the individual or population level. Important factors are life stage, past experiences and acquired adaptations, genetic differences, maternal influences, and disturbance of the stress system by pollutants. Here, the focus is on the last three factors, since they have a direct relation with the stress hormones.

Innate Individual Variation: Proactive and Reactive Stress Coping Styles

Individuals within a population of animals show a great variety in the intensity and character of several behavioral and physiological aspects of the stress response. In many mammals, and in some bird and teleost species, a very similar bimodal distribution of this variety has been demonstrated, with individuals showing either a distinct proactive (bold) or reactive (shy) style, and others with a mix of intermediate behavior (**see also Social and Reproductive Behaviors**: Dominance Behaviors). Proactive animals show a shorter response interval to stressors, a higher locomotory activity and more aggression, and are more active in facing or avoiding stressors than reactive animals, which play a more waiting game. These individual differences in the style of coping with a stressor are normally consistent over lifetime, and connected with differences in brain activity and hormonal responses. Proactive animals show a relatively high-brain serotonin turnover and a high-BSC and modest HPI activity, resulting in high-CA and low-cortisol release during stress. Reactive animals show the reverse: lower brain serotonin turnover, lower BSC activity, and CA release, and a higher HPI-axis activation with a firm cortisol response (**Figure 5**). In animals living in a social group, proactivity is usually linked to both dominant behavior and

	Proactive (bold)	Reactive (shy)
Brain serotonin turnover	High	Low
Plasma adrenaline levels	High	Low
Cardiac output	High	Low
Reactivity	High	Low
Aggression level	High	Low
Locomotory activity	High	Low
Risk	Taking	Avoiding

Figure 5 Characteristics of the two coping strategies in fishes. Relative differences between pro-active and reactive individuals in physiological and behavioral responses to an inescapable stressor.

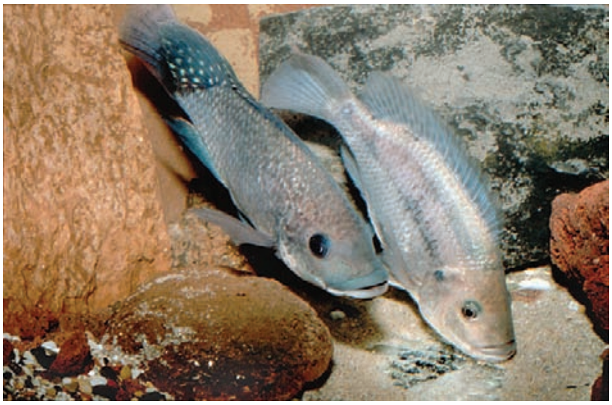


Figure 6 Two male tilapia (*Oreochromis mossambicus*). In confrontations the right fish, with the dark skin color and broad lips typical for dominant males (which have a higher testosterone level), showed a proactive, and the left fish, with the skin colour of lower ranks (and a lower testosterone level), showed a reactive type of behavior.

a high place in social hierarchy. In males, this is correlated with a high-testosterone level (**Figure 6**). A reactive coping style to stressors is typical for the lower subordinate ranks. These animals normally show less exploratory activity, have lower testosterone levels, are less aggressive, and have lower reproductive success. In mangrove killifish (*Kryptolebias marmoratus*) for instance, pre-fight cortisol levels relate negatively, and pre-fight testosterone positively, for initiating and winning a confrontation.

The coping style of a fish can be changed by cortisol administration from proactive to reactive, but it is clear that the stress-coping styles in fish have a firm genetic basis, as in mammals and birds. A selection program on trout has resulted in strains of low-cortisol responders with high-brain serotonin and CA-activity and a proactive stress-coping style, and high-cortisol responders with lower serotonin turnover and higher HPI activity and a reactive way of coping with stressors. Compared to mammals, in fishes, this type of research is still in its infancy. Nevertheless, it has become clear that these innate coping styles have an evolutionary history starting before the water-to-land transition of the vertebrates. This points to a high-survival value for both coping styles. Whereas, under some conditions, a proactive style with higher-testosterone levels may result in higher reproductive success, in other conditions, a reactive style may be advantageous. **Figure 7** shows the results of exposure of trout (*Oncorhynchus mykiss*) to water with a low-oxygen concentration. The fishes that recovered from a period of low oxygen had lower adrenaline and higher cortisol levels during hypoxia than the animals that did not recover. The swimming activity during that period was also significantly lower in the surviving fish. Moreover, the more risky behavior of proactive animals may reduce their lifetime, and this can imply that their total offspring is certainly not always higher than that of reactive animals. Research on mammals has shown that mixed populations of proactive and reactive animals are socially more stable than groups of only proactive or reactive animals.

Maternal Stress and Development of Offspring

Cortisol production probably starts already in the embryonic stage (see above). However, even unfertilized fish eggs contain cortisol, since this is transmitted from the mother to the yolk in the eggs of the developing gonads. When the mother shows elevated circulating cortisol levels, the eggs will also receive their share. If the mother is chronically and severely stressed, this will lead to absorption of the eggs and involution of the gonads. If the stress is not sufficiently high for the suppression of spawning, the eggs will receive elevated cortisol levels. Since elevated GRs can interfere with early development in many vertebrates, stress in the mother can affect the quality of the eggs and embryos, thereby reducing the chances of survival for the offspring, and lower the threshold of the stress response. Evidence from fishes, although limited, shows that developing eggs of stressed mothers,

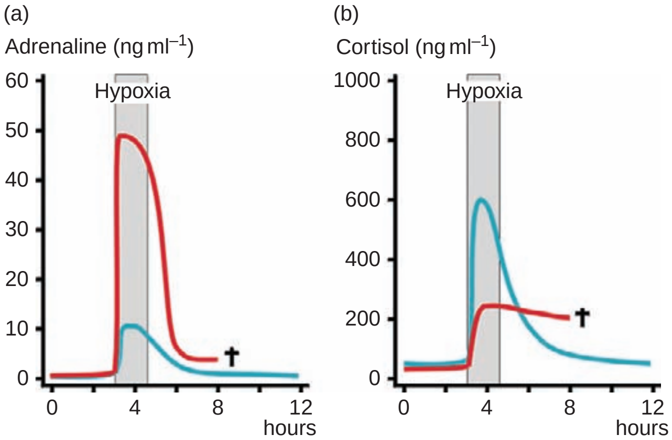


Figure 7 Concentrations of adrenaline (a) and cortisol (b) in blood plasma of recovering (r) and nonrecovering (nr) rainbow trout (*Oncorhynchus mykiss*) exposed to 90-min hypoxia at 4.8 kPa. This experiment shows that a high-adrenaline/low-cortisol response as in proactive fish is a disadvantage for surviving low-oxygen conditions (these fish did not recover), compared with a low-adrenaline/high-cortisol response as in reactive fish. Reproduced from van Raaij M, Pit D, Balm P, Steffens A, van den Thillart G (1996) Behavioral strategy and physiological stress response in rainbow trout exposed to hypoxia. *Hormones and Behavior* 30: 85–92, with permission from Elsevier.

or mothers experimentally exposed to high-cortisol levels, display faster depletion of energy resources in the eggs, lower growth rate, accelerated hatching, increased larval mortality, and abnormalities such as increased heart rates and asymmetrical otoliths (**see also Social and Reproductive Behaviors**: Dominance Behaviors, Sexual Behavior in Fish, and Socially Controlled Sex Change in Fishes).

Endocrine Disruption

Specific toxic effects of pollutants on the endocrine system may lead to disturbance of the endocrine-control mechanisms, a process called endocrine disruption. Cadmium inhibits cortisol synthesis via blocking of calcium channels in the membranes of the interrenal cells. Xenobiotics acting via the aryl hydrocarbon receptor can inhibit interrenal steroidogenic acute regulatory protein activity (the rate-limiting step in cortisol biosynthesis) as shown, for example, alpha- and beta-naphthoflavone. Other examples of endocrine disruption are GR downregulation by polychlorinated biphenyls and the effects of salicylate and other anti-inflammatory agents that disturb cortisol synthesis. The result is a diminished cortisol response to stressors and hydromineral challenges such as seawater adaptation, as has been demonstrated for fishes from many polluted surface waters. Furthermore, atrophied pituitary ACTH cells have been reported for such animals (**see also Hormonal Responses to Stress**: Impact of Stress in Health and Reproduction, **Toxicology**: The Toxicology of Organics in Fishes).

Concluding Remarks

In fishes, like in other vertebrates, stressors modify the activity of virtually all hormones and lead to adjustments of almost all aspects of physiology. Many hormones are produced at multiple sites and have multiple functions. Their interactions represent an intricate network firmly controlled by the brain. However, the focus of the primary stress hormones is on energy mobilization for activities directed at increasing chances of survival. When stress becomes chronic, the inhibition of other body functions may eventually lead to damage and atrophy in many organs, and reduced performance capacity. Although the response to a stressor is general rather than stressor specific, modifying factors give every stress response a unique character.

See also: **Brain and Nervous System**: Functional Morphology of the Brains of Agnathans; Functional Morphology of the Brains of Cartilaginous Fishes; Functional Morphology of the Brains of Ray-Finned Fishes; Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and Latimeria. **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids; The Hormonal Control of Osmoregulation in Teleost Fish. **Hormonal Responses to Stress**: Catecholamines; Impact of Stress in Health and Reproduction; Stress Effect on Growth and Metabolism. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction.

Social and Reproductive Behaviors: Dominance Behaviors; Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes. **The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis. **Toxicology**: The Toxicology of Organics in Fishes.

Further Reading

Balment RJ, Lu W, Weybourne E, and Warne JM (2006) Arginine vasotocin, a key hormone in fish physiology and behaviour: A review with insights from mammalian models. *General and Comparative Endocrinology* 147: 9–16.

Bernier NJ, Flik G, and Klaren PHM (2009) Regulation and contribution of the corticotropic, melanotropic and thyrotropic axes to the stress response in fishes. In: Bernier NJ, Van der kraak G, Farrell AP, and Colin J (eds.) *Fish Neuroendocrinology*, pp. 235–311. Amsterdam: Elsevier.

Evans DH, Piermarini PM, and Choe PC (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Review* 85: 97–177.

Hontela A (2005) Stress and the hypothalamo-pituitary-interrenal axis: Adrenal toxicology: Effects of environmental pollutants on the structure and function of the HPI axis. In: Moon TW and Mommsen TP (eds.) *Biochemical and Molecular Biology of Fishes, Environmental Toxicology,* vol. 6, pp. 331–336. Elsevier: Amsterdam.

Iwama GK, Afonso LOB, Todgham A, Ackerman P, and Nakano K (2004) Are hsps suitable for indicating stressed states in fish? *Journal of Experimental Biology* 207: 15–19.

Martínez-Porchas M, Martínez-Córdova LR, and Ramos-Enriquez R (2009) Cortisol and glucose: Reliable indicators of fish stress? *Pan-American Journal of Aquatic Sciences* 4: 158–178.

Oeverli O, Pulman KGT, Soerensen C, *et al.* (2007) Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral Reviews* 31: 396–412.

Pottinger TG (1999) The impact of stress on animal reproductive activities. In: Balm PHM (ed.) *Stress Physiology in Animals*, pp. 130–177. Boca Raton: CRC Press.

Reid SG, Bernier NJ, and Perry SF (1998) The adrenergic stress response in fish: Control of catecholamine storage and release. *Comparative Biochemistry and Physiology Part C* 120: 1–27.

Stolte EH, De Mazon AF, Leon-Kloosterziel KM, *et al.* (2008) Corticoid receptors involved in stress regulation in common carp, *Cyprinus carpio. Journal of Endocrinology* 198: 403–417.

Verburg-Van Kemenade BM, Stolte EH, Metz JR, and Chadzinska M (2009) Neuroendocrine–immune interactions in teleost fish. In: Bernier NJ, Van der Kraak G, Farrrell AP, and Colin J (eds.) *Fish Neuroendocrinology*, pp. 312–364. Amsterdam: Elsevier.

Wendelaar Bonga SE (1997) The stress response in fish. *Physiological Reviews* 77: 591–625.

Catecholamines

SG Reid, University of Toronto Scarborough, Toronto, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Biosynthesis of Catecholamines
Sites of Catecholamine Synthesis and Storage
Situations Eliciting the Release of Catecholamines into the Circulation
Mechanisms of Catecholamine Secretion
Effects of Plasma Catecholamines and Mechanisms of Action
Further Reading

Glossary

Adrenergic A general term pertaining to processes or structures related to catecholamines. For example, neurons that release noradrenaline as transmitter; catecholamines binding to adrenergic receptors (adrenoceptors), and adrenergically activated (catecholamine-activated) physiological processes.

Catecholamine-release threshold The point at which catecholamines are abruptly released into the blood (i.e., plasma catecholamine levels rise). The release threshold is usually defined in terms of the arterial oxygen partial pressure or the arterial oxygen content/concentration/oxygen-hemoglobin saturation level.

Cholinergic A general term pertaining to processes or structures related to acetylcholine. For example, neurons that release acetylcholine as transmitter. Nicotinic and muscarinic receptors are cholinergic receptors because they bind acetylcholine. Cholinergic synapses are found in both the central and peripheral nervous systems.

Chromaffin cells Cells from the sympathetic–adrenal lineage that synthesize and store catecholamines as well as release them into the blood (via the extracellular fluid).

Exocytosis The process by which neurotransmitters or hormones, which are stored in secretory vesicles or granules, are released from a nerve or endocrine cell when these storage granules fuse with the plasma membrane.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Hypoxemia A reduction in the oxygen content/concentration of the blood. Blood can be hypoxic without being hypoxemic.

***In vitro* perfused posterior cardinal vein (PCV) preparation** An experimental preparation used to measure the release of catecholamines from chromaffin cells within the walls of the posterior cardinal vein. The PCV is perfused with saline. The effects of various drugs or electrical nerve stimulation on catecholamine release can be assessed by measuring catecholamine levels in the outflowing perfusate.

Non cholinergic A term used to describe mechanisms of catecholamine release that occur independently of acetylcholine acting on nicotinic or muscarinic receptors.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Biosynthesis of Catecholamines

The catecholamine hormones, dopamine, noradrenaline (norepinephrine), and adrenaline (epinephrine), are synthesized and stored within adrenergic neurons and adrenal chromaffin cells where they function as neurotransmitters (dopamine and noradrenaline) and circulating hormones (noradrenaline and adrenaline), respectively. Catecholamine biosynthesis occurs via the Blaschko pathway beginning with the amino acid precursor, tyrosine (**Figure 1**). Tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to form dihydroxyphenylalanine (DOPA) in the rate-limiting step in catecholamine synthesis. TH activity is both under negative feedback control from dopamine and noradrenaline and under regulation by a variety of neuronal and hormonal factors.

DOPA is hydroxylated by amino acid decarboxylase (AADC or DOPA decarboxylase) to form dopamine. This enzyme is present in sufficient quantities that DOPA does not accumulate to any substantial extent. The first two steps of the pathway occur within the cytosol. Dopamine is then transported into secretory vesicles (granules) where it is hydroxylated by dopamine β-hydroxylase (DBH) to form noradrenaline. In some chromaffin cells, noradrenaline is transported from the secretory vesicle, back into the cytoplasm, where it is methylated to form adrenaline by the enzyme phenylethanolamine N-methyltransferase

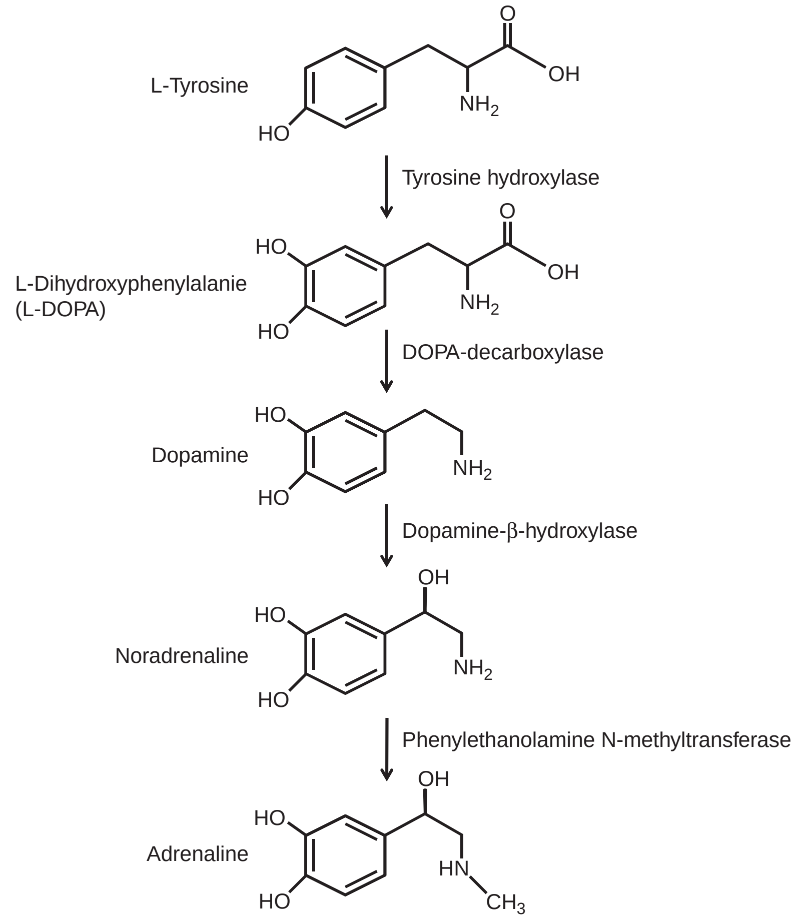


Figure 1 The catecholamine biosynthetic pathway.

(PNMT). Alternately, once synthesized, noradrenaline may remain within the secretory granules; and once synthesized within the cytosol, adrenaline is transported into secretory granules prior to release.

Sites of Catecholamine Synthesis and Storage

In fish, as in other vertebrates, catecholamines are synthesized within, and released from, chromaffin cells. The arrangement of chromaffin cells differs greatly among species. In mammals, chromaffin cells are found within the core of the adrenal gland, the adrenal medulla surrounded by the steroidogenic interrenal cells within the adrenal cortex. In birds and reptiles, chromaffin cells are closely intermingled with interrenal cells within an adrenal gland. In urodele amphibians, chromaffin cells are located within segmented bodies associated with sympathetic nerves on the ventral side of the kidney, while, in anuran amphibians, chromaffin cells form a distinct mass on the surface of the kidney.

Among different classes of fish, the arrangement of chromaffin cells also exhibits substantial diversity. In cyclostomes and agnathans (hagfish and lamprey; **see also Hagfishes and Lamprey**: Hagfishes, Lampreys: Energetics and Development, and Lampreys: Environmental Physiology), chromaffin cells are distributed within the systemic and portal hearts and in large veins and arteries. In elasmobranchs (sharks and rays; **see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays), chromaffin cells are associated with paravertebral ganglia. The axillary bodies, comprising chromaffin cells in association with gastric ganglia, are the primary source of circulating catecholamines in these fish. In teleost (bony) fish, chromaffin cells are located within the walls of the posterior cardinal vein (PCV) and its branches within the kidney tissue, primarily in the anterior regions of the kidney (head kidney; **Figure 2**; **see also Role of the Kidneys**: Histology of the Kidney).

Most fish release adrenaline and noradrenaline into the blood under the appropriate conditions. The relative amount of adrenaline versus noradrenaline varies,

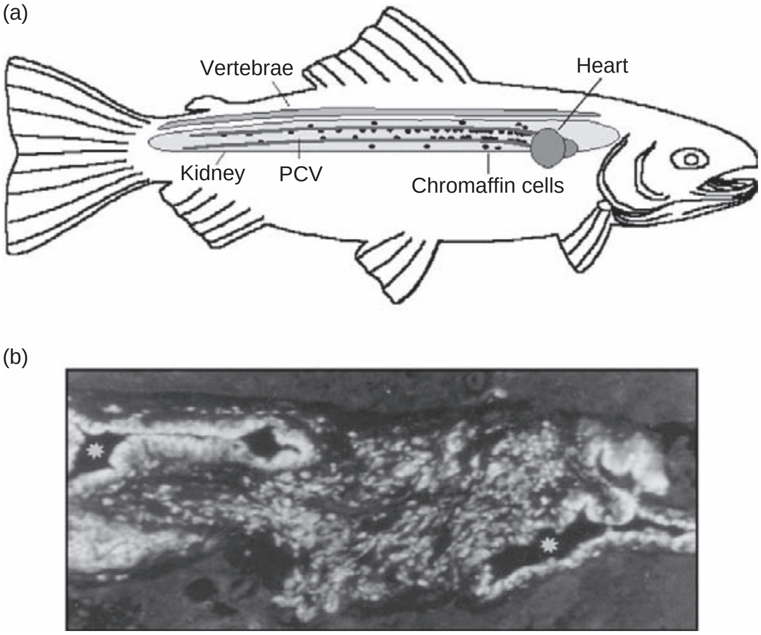


Figure 2 (a) The location of the catecholamine-containing chromaffin cells in a generic teleost fish. Chromaffin cells are found in the walls of the posterior cardinal vein (PCV) and scattered throughout the kidney tissue. (b) A cross section of the kidney and PCV from an Atlantic cod. (a) Modified from Reid SG, Bernier NJ, and Perry SF (1998) The adrenergic stress response in fish: Control of catecholamine storage and release. *Comparative Biochemistry and Physiology – Part C* 120: 1–27.

depending upon the species and the situation eliciting catecholamine release. As with other vertebrates, adrenaline and noradrenaline in fish are also stored in separate populations of chromaffin cells, although these populations are usually intermixed. Some chromaffin cells contain only adrenaline, whereas others contain only noradrenaline. Adrenaline-containing cells appear to contain the enzyme PNMT, whereas the noradrenergic cells do not.

Situations Eliciting the Release of Catecholamines into the Circulation

Catecholamine Release during Hypoxia

In teleosts, numerous stressful situations result in an elevation of plasma catecholamine levels. Conditions such as hypoxia (a decrease in the level of inspired oxygen in the water; **see also Hypoxia**: The Expanding Hypoxic Environment), hypercapnia (an increase in water CO_2 levels), exhaustive exercise, predation, ionoregulatory disruptions, and exposure to air usually lead to the release of catecholamines into the blood.

One of the most potent stimuli for catecholamine release in fish is exposure to environmental hypoxia. Hypoxic conditions are sensed by chemoreceptor cells (**see also Control of Respiration**: Oxygen Sensing in Fish) on the gills which then activate pathways ultimately leading to catecholamine release from the chromaffin cells. A number of studies, in a variety of teleost species, indicate that catecholamine release during hypoxia occurs when the oxygen content of the blood (or oxygen-hemoglobin saturation) decreases by approximately 50% (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). In other words, catecholamines are released when the partial pressure of oxygen in the arterial (Pa_{O_2}) blood reaches the P_{50} value (P_{50} is defined as the Pa_{O_2} level at which blood oxygen content is reduced to 50% of the maximal value; **Figure 3**(**b**)).

In experiments demonstrating this phenomenon, fish were exposed to varying levels of water P_{O_2} (Pw_{O_2}), usually between 30 and 150 mmHg. Blood samples were taken to measure arterial oxygen content, Pa_{O_2}, blood (hemoglobin) and plasma catecholamine levels. In all cases, the general profile of plasma catecholamine appearance in the plasma is very similar (**Figure 3**(**a**)). When water P_{O_2} is high or moderate, plasma catecholamine levels remain low (i.e., baseline levels). However, in all cases, a threshold is reached at which point plasma catecholamines increase. When plasma catecholamine levels are plotted as a function of blood oxygen content, the threshold at which catecholamines are elevated is approximately the same in all species of fish (i.e., at approximately a 50% reduction in blood oxygen content). When the same catecholamine data are plotted as a function of Pa_{O_2}, the release threshold is different among the

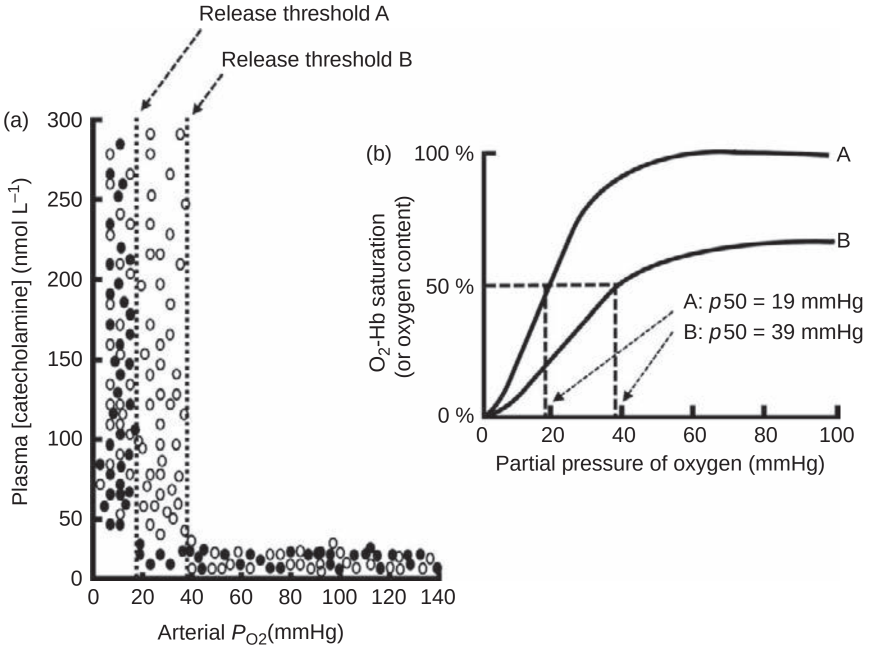


Figure 3 (a) The general relationship between plasma catecholamine levels and the partial pressure of oxygen in the arterial blood (P_{O_2}). (b) Generic oxygen equilibrium curves illustrating the relationship between the level of oxygen-hemoglobin saturation (or oxygen content) and the partial pressure of oxygen in the arterial blood in two groups of fish, group A and group B. The black symbols in (a) represent data points from group A, while the open symbols represent data points from group B. The dashed lines in (a) indicate the catecholamine release thresholds for both groups of fish (left line, group A; right line, group B). The higher affinity of hemoglobin for oxygen in group A results in a lower threshold for catecholamine release compared to group B.

species and correlates approximately to the P_{50} value for that species. The relationship between the Pa_{O_2} release threshold and the P_{50} value (from different fish) has a slope of approximately 1, indicating that catecholamines are being released when the oxygen content has fallen to approximately 50% (**Figure 4**).

Catecholamine Release during Hypercapnia and Exercise

Exposure to environmental hypercapnia frequently results in an elevation of plasma catecholamines. However, it is likely that it is due to an indirect effect of hypercapnia-

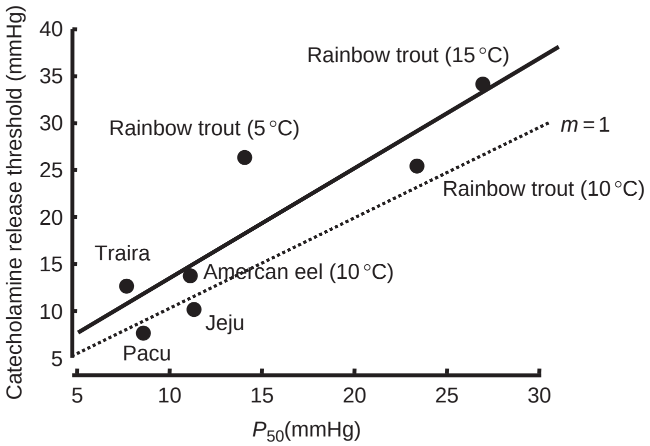


Figure 4 The relationship between the arterial P_{O_2} catecholamine release threshold (mmHg) and the P_{50} value (mmHg) using data from traira, pacu, jeju, American eel, and rainbow trout (at three different temperatures). The dashed line is a line of unity (slope = 1). The relationship between the catecholamine release threshold and the P_{50} value is very close to having a slope of 1, indicating that catecholamines are released into the blood when the arterial P_{O_2} falls to approximately the P_{50} level (i.e., at the point where blood oxygen content is reduced to 50%).

induced hypoxemia rather than a direct effect of hypercapnia *per se*. Hypercapnia is usually accompanied by a respiratory acidosis as CO_2 is hydrated to form an H^+ ion and a HCO_3^- ion (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion). Both CO_2 and H^+ ions compromise the ability of hemoglobin to bind oxygen and reduce the oxygen-carrying capacity of blood. As such, any elevation of plasma catecholamines during hypercapnia may simply result from CO_2 and H^+ ions reducing blood oxygen content – a known stimulus for catecholamine release (see above). However, nicotinic receptor-induced catecholamine release (see below) can be augmented by an acidic environment around the chromaffin cells or exposure of a fish to hypercapnia.

During exhaustive or strenuous exercise, catecholamine release serves to mobilize energy stores in an effort to increase plasma glucose levels. Within the liver, glycogenolysis and gluconeogenesis are stimulated, whereas glycolysis is inhibited. Catecholamines also assist in delivering oxygen to muscles by enhancing blood-oxygen-carrying capacity. Ionoregulatory disruptions, such as exposure to soft water, produce a thickening of the gills via proliferation of mitochondrial-rich (chloride) cells which, in turn, leads to a reduction in Pa_{O_2} and, possibly, blood oxygen content. Catecholamines may play a role in enhancing oxygen transport during initial exposure to soft water but chronic exposure may blunt any effects due to desensitization of the catecholamine release process and adrenergic receptors on effector cells such as the red blood cell.

Mechanisms of Catecholamine Secretion

Cholinergic Control of Catecholamine Release

Numerous mechanisms have been identified which can trigger, modulate, or inhibit the release of catecholamines from fish chromaffin cells. An extensive body of literature also exists on the mechanisms of catecholamine secretion in mammalian species. Near the end of the twentieth century, it was still, in general, dogmatic to state that there was little doubt that the primary mechanism leading to the release of catecholamines from chromaffin cells in fish (and other vertebrates) is stimulation of the chromaffin cells by acetylcholine (ACh) released from sympathetic nerves. In addition, in most species, it was thought that cholinergic-induced catecholamine release was mediated by ACh-activating nicotinic cholinergic receptors on the chromaffin cell (**Figure 5**). This leads to membrane depolarization, an

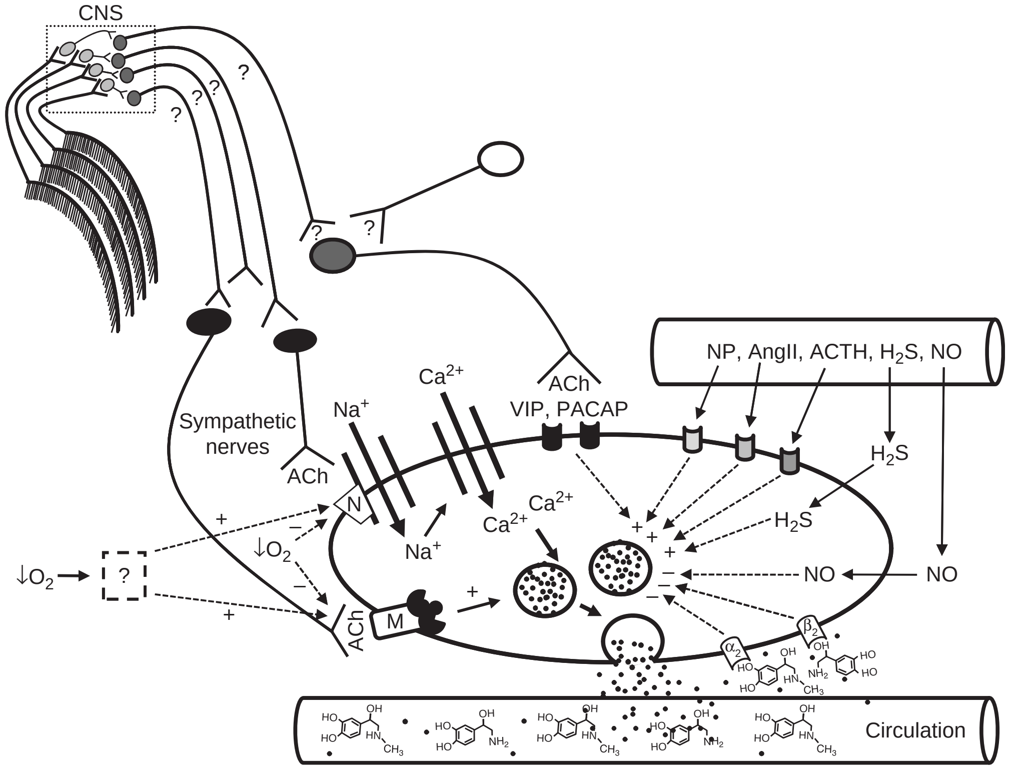


Figure 5 Cholinergic and non-cholinergic mechanisms of catecholamine secretion from a generic fish chromaffin cell. α_2, alpha-2-adrenoceptor; Ach, acetylcholine, ACTH, adrenocorticotropic releasing hormone; AngII, angiotensin II, β_2, beta-2-adrenoceptor; CNS, central nervous system; H_2S, hydrogen sulfate; M, muscarinic receptor; N, nicotinic receptor; NP, natriuretic peptide; NO, nitric oxide; PACAP, pituitary adenylyl cyclase-activating polypeptide; VIP, vasoactive intestinal polypeptide.

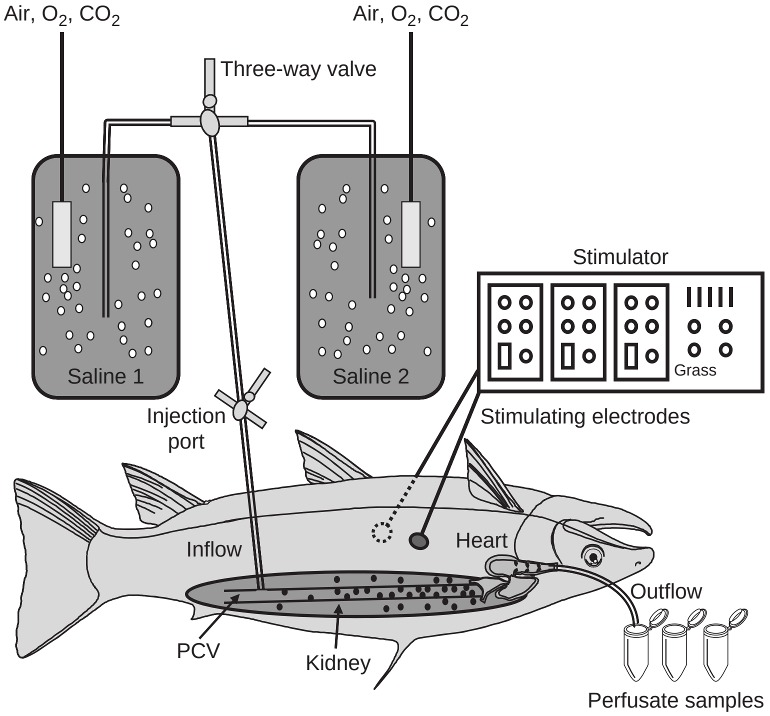


Figure 6 The *in vitro* (*in situ*) saline-perfused posterior cardinal vein (PCV) preparation used to study catecholamine release in fish.

influx of Ca^{2+} from the extracellular fluid, the fusion of catecholamine-containing secretory vesicles with the plasma membrane, and the release of their contents via exocytosis.

However, further studies have identified mechanisms other than nicotinic receptor stimulation that cause catecholamine secretion from fish chromaffin cells. Many of these studies have employed an *in vitro* saline-perfused PCV preparation (**Figure 6**). In this preparation, the PCV is cannulated near its caudal end to serve as an inflow, whereas the ventricle is cannulated to serve as an outflow. Saline is perfused through the inflow cannulae, flows through the PCV, across the chromaffin cells, and is collected in sample tubes via the outflow cannulae. Various pharmacological agents (e.g., receptor agonists and antagonists) can be added to the perfusion saline or given as bolus doses via an injection port. The effect of these agents on catecholamine secretion can be assessed by measuring catecholamine levels in the outflowing perfusate. A variation of this preparation uses an electrical stimulation technique to stimulate the sympathetic nerves that innervate the chromaffin cells.

Techniques such as these have revealed that direct activation of muscarinic receptors, by acetylcholine released from sympathetic nerves, can augment nicotinic receptor-induced catecholamine release from trout chromaffin cells and may also exert a direct secretory effect. On the other hand, studies on American eel chromaffin cells indicate that whereas nicotinic stimulation triggers catecholamine release, muscarinic receptor stimulation has a slightly inhibitory effect on noradrenaline release.

Cholinergic control of catecholamine secretion can be regulated or modified by a variety of factors. For example, perfusion of the *in vitro* PCV preparation with either adrenaline or noradrenaline prevented nicotinic or muscarinic receptor activation from triggering catecholamine release while reducing the ability of electrical stimulation to trigger release. These responses were mimicked by treatment with agonists of the adrenergic β_2 and α_2 receptors, respectively (**Figure 5**). Conversely, treatment with β_2 and α_2 receptor antagonists augmented catecholamine release triggered by nicotinic and muscarinic receptor stimulation. These studies illustrate that circulating plasma catecholamines can have feedback effects on the chromaffin cells, attenuating further release.

Exposure to environmental hypoxia can also regulate the release of catecholamines from fish chromaffin cells, albeit in a complicated manner. For example, perfusion of the *in vitro* PCV (chromaffin cell) preparation with hypoxic saline reduces catecholamine release in response to electrical field stimulation and activation of muscarinic and nicotinic receptors. On the other hand, if nicotine is injected into hypoxic fish *in vivo*, then the increase in plasma catecholamine levels is greater than that in normoxic fish following a nicotine injection. These opposite results indicate that regulatory processes at a site other than the chromaffin cells are modulating catecholamine release during hypoxia and overriding the inhibitory effects of hypoxia at the direct level of the chromaffin cell (**Figure 5**). Exposure to chronic hypoxia can also modify catecholamine release patterns by enhancing the sensitivity of the release process to cholinergic stimulation.

Noncholinergic Control of Catecholamine Release

Exposure to environmental hypoxia is one of the strongest triggers for catecholamine release in fish. However, nicotinic receptor stimulation is not absolutely required to trigger release under these conditions. If nicotinic receptors are experimentally desensitized by prolonged treatment with nicotine, catecholamine release during hypoxia still occurs. It is apparent that many mechanisms, noncholinergic in nature, can trigger catecholamine release from fish chromaffin cells. Indeed, in the most primitive group of extant fish, the cyclostomes, plasma catecholamine levels are elevated during stress despite the chromaffin cells receiving no innervation. In these animals, there must be humoral or paracrine processes that trigger the secretion of catecholamines. In 1998, Reid and colleagues published an extensive review on the mechanisms of catecholamine release in fish, describing the effects of humoral agents such as serotonin, angiotensins, natriuretic peptides (NPs), adrenocorticotropic hormone (ACTH), cortisol, opioids, assorted peptides, adenosine, histamine, and catecholamines. Since that time, a number of studies have continued to examine noncholinergic mechanisms of catecholamine secretion in fish (**Figure 5**), including regulation by angiotensin, vasoactive intestinal polypeptide (VIP), pituitary adenylyl cyclase-activating polypeptide (PACAP), and NPs and gasotransmitters such as nitric oxide (NO) and hydrogen sulfide (H_2S).

Angiotensin

In fish, as in other vertebrates, the renin–angiotensin system (RAS) is activated during periods of hypotension in order to restore blood pressure. When blood pressure drops, specialized cells in the kidney release an enzyme called renin, which converts a circulating peptide called angiotensinogen into angiotensin I. Angiotensin I is converted into angiotensin II by angiotensin-converting enzyme (ACE). Both angiotensins I and II can exert pressor effects although angiotensin II is the more potent form. Recent studies on trout have demonstrated that some of the pressor effects attributed to angiotensin II can actually be attributed to catecholamines that were mobilized by an angiotensin II-triggered mechanism. Histological studies have found the presence of angiotensin II receptors on rainbow trout chromaffin cells. Physiological studies have demonstrated that angiotensins I and II can trigger catecholamine release from trout and dogfish chromaffin cells. The RAS also appears to contribute to the maintenance of basal plasma catecholamine levels as inhibition of ACE causes a reduction in these levels. On the other hand, angiotensin II does not cause catecholamine release in the American eel nor do circulating catecholamines play a role in blood pressure restoration during hypotension in this species.

VIP and PACAP

In mammals, VIP and PACAP are often located in sympathetic nerve fibers where they function alongside acetylcholine as co-transmitters. Histological studies have identified the presence of VIP and PACAP in nerves innervating chromaffin cells in Atlantic cod, rainbow trout, European eel, and spiny dogfish. VIP-binding sites (that appear to be the VPAC receptor for VIP) are located on PNMT-positive and PNMT-negative chromaffin cells of rainbow trout. Both VIP and PACAP trigger adrenaline (but not noradrenaline) release from trout chromaffin cells with a similar degree of potency. This response can be abolished by antagonism of VIP receptors but not by antagonism of PACAP receptors. Electrical stimulation experiments demonstrated that VIP/PACAP-induced catecholamine release is triggered at low stimulation frequencies, whereas cholinergic mechanisms are activated at higher frequencies. If nicotinic receptors are desensitized by constant nicotine treatment, catecholamine release in response to low-frequency neural stimulation is not affected while release in response to high-frequency stimulation is reduced. The low-frequency-induced catecholamine release is not altered by treatment with muscarinic or nicotinic receptor antagonists but is reduced by treatment with a VIP receptor antagonist. VIP and PACAP, therefore, appear to function as co-transmitters in nerves innervating fish chromaffin cells and they trigger catecholamine release under mild-to-moderate stressful conditions when the nervous stimulation of the chromaffin cells is likely to be relatively low.

Natriuretic peptides

NPs are released into the blood in response to elevated blood pressure or plasma sodium levels. NPs are produced in the heart, brain, and chromaffin cells. Histological studies have found evidence for the presence of atrial natriuretic peptide (ANP) immunoreactivity in teleost chromaffin cells. Experiments on rainbow trout and American eels suggest that NPs cannot directly stimulate catecholamine secretion by direct actions on the chromaffin cells. However, in dogfish, NPs trigger noradrenaline release by mechanisms (on either the chromaffin cells or elsewhere) that depend, ultimately, on α-adrenoceptor stimulation.

Gasotransmitters

Gasotransmitters are small gaseous molecules that function as neurotransmitters. This family of neurotransmitters consists of nitric oxide (NO), carbon monoxide (CO), and H_2S. Gasotransmitters are not stored within secretory vesicles in nerve terminals but, rather, are produced when required. They diffuse from the nerve terminal into the target cell where they exert effects mediated by second-messenger systems. Both NO and H_2S have been shown to alter catecholamine release from fish chromaffin cells. For example, NO, derived from neuronal nitric oxide synthase (nNOS), inhibits catecholamine release in rainbow trout. This was demonstrated by introducing a NO donor into the *in vitro* PCV preparation and observing the abolition of electrically induced catecholamine release. Conversely, electrical-induced catecholamine release is augmented by the NOS inhibitors, L-NAME and 7-NI.

Repetitive hypoxia over a period of days decreases the capacity of rainbow trout to elevate plasma catecholamine levels in response to acute environmental hypoxia. This is due, at least in part, to hypoxia-induced increases in plasma NO, which in turn inhibit catecholamine release

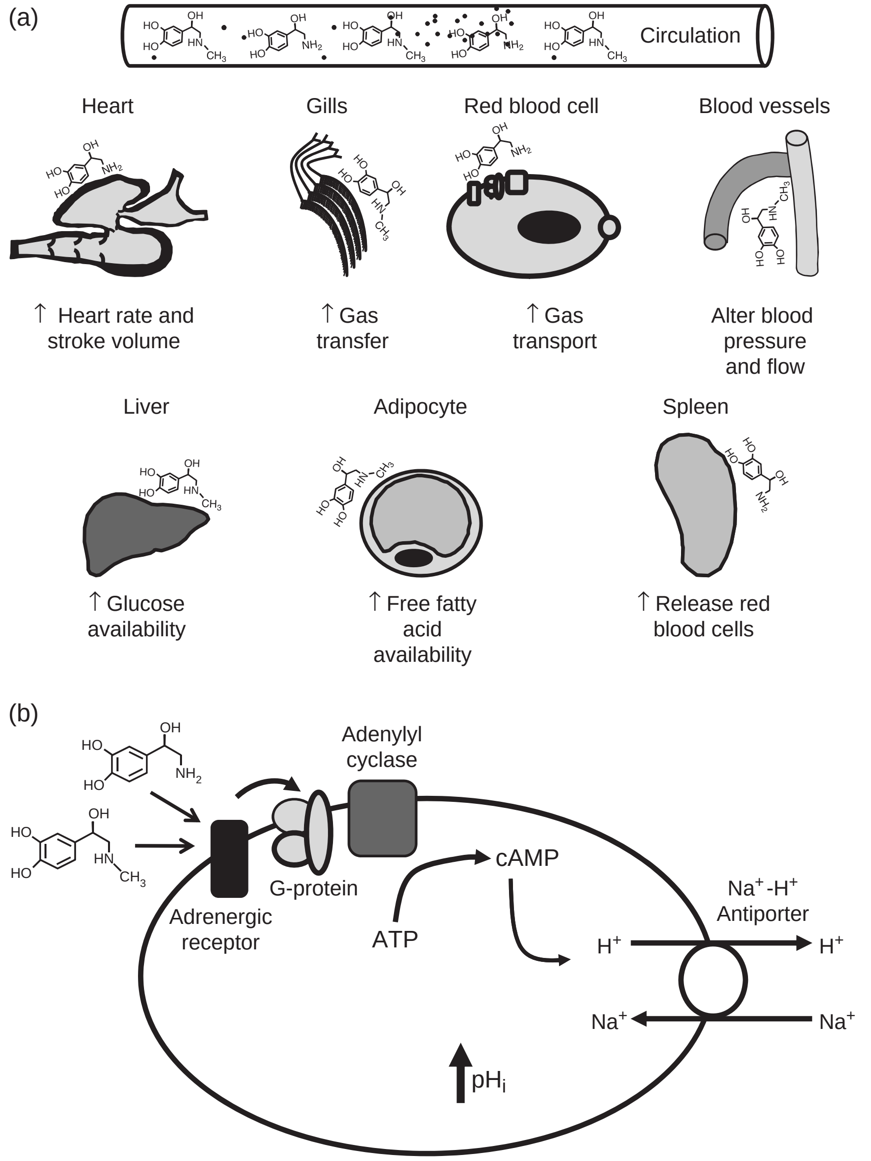


Figure 7 (a) Various target tissues affected by circulating catecholamines and the major effects elicited by the catecholamines on these tissues. (b) Adrenergically activated Na^+–H^+ exchange on the red blood cell leading to an elevation of intracellular pH which in turn increases the affinity of hemoglobin for oxygen (Bohr effect) as well as the carrying capacity of hemoglobin for oxygen (Root effect).

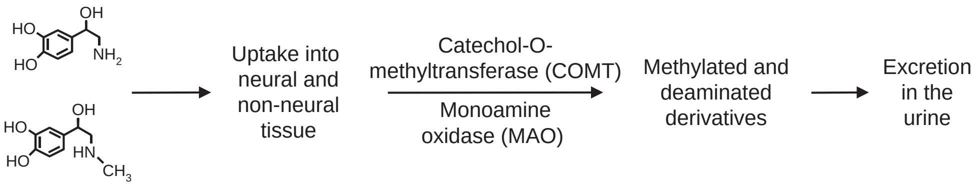

HO
HO
OH
NH2
HO
HO
OH
HN
CH3
Uptake into neural and non-neural tissue
Catechol-O-methyltransferase (COMT)
Monoamine oxidase (MAO)
Methylated and deaminated derivatives
Excretion in the urine

Figure 8 The general pathway of catecholamine degradation.

from chromaffin cells. Further evidence in support of NO-induced inhibition of catecholamine release during repetitive hypoxia comes from *in vitro* PCV preparations taken from fish exposed to repetitive hypoxia. Electrically stimulated catecholamine release in these preparations was reduced although this effect could be reversed by nNOS inhibitors.

H_2S can directly stimulate catecholamine secretion from trout chromaffin cells via membrane depolarization and calcium-evoked exocytosis. During exposure to environmental hypoxia, plasma H_2S levels increase as do plasma catecholamines. Furthermore, chromaffin cells will synthesize H_2S when incubated with L-cysteine, the precursor to H_2S, and H_2S is co-released from the chromaffin cells with catecholamines, suggesting an autocrine mechanism of augmenting catecholamine release.

Effects of Plasma Catecholamines and Mechanisms of Action

Although circulating catecholamines exert numerous effects on many different target tissues, the overall effect of these hormones is to reduce the detrimental effects of stress by optimizing cardiovascular and respiratory function while also mobilizing energy supplies. These effects are initiated by the binding of catecholamines to adrenergic receptors located on target tissue cell membranes. These receptors are generally classified as α_1, α_2, β_1, and β_2 adrenoceptors. Some of the key effects of plasma catecholamines are illustrated in **Figure** 7(**a**) and are described in detail in a 1992 review by Thomas and Perry and a 1998 review by Reid and colleagues.

Circulating catecholamines act on the heart to increase both heart rate and stroke volume and on blood vessels to alter blood pressure and flow (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels and Design and Physiology of the Heart: Physiology of Cardiac Pumping). At the level of the gills, catecholamines can enhance O_2 uptake from the water into the blood; at the red blood cell (see below), catecholamines increase both the affinity and capacity of the blood to carry oxygen while catecholamine-induced contraction of the spleen increases the amount of red cells in the circulation (**see also Blood**: Cellular Composition of the Blood). All of these effects serve to optimize cardiorespiratory function to ensure adequate delivery of oxygen to the tissues. At the liver, catecholamines stimulate gluconeogenesis and glycogenolysis, while at adipocytes they stimulate lipolysis. These actions increase the levels of circulating glucose and free fatty acids, respectively, to ensure adequate energy (fuel) supplies for metabolically active tissues (**see also Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism).

One of the most important physiological responses triggered by plasma catecholamines (at least in bony fish) is to increase the hemoglobin-oxygen-binding affinity as well as the carrying capacity of hemoglobin for oxygen (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange). The primary mechanism leading to these changes is adrenergically activated Na^+–H^+ exchange on the plasma membrane of red blood cells (**Figure** 7(**b**)). Both adrenaline and noradrenaline can activate β_2 adrenergic receptors on the red cell, which leads to the activation of adenylyl cyclase and cyclic adenosine monophosphate (cAMP) production. In turn, this leads to activation of Na^+–H^+ antiporters on the cell membrane that remove an H^+ ion from the red cell in exchange for a plasma Na^+ ion. This results in an acidification of the plasma and an alkalinization of the red cell. This increase in intracellular pH leads to increases in both the affinity of hemoglobin for oxygen (Bohr effect) and the maximal oxygen-carrying capacity of the blood (Root effect) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Catecholamines can also elevate blood-oxygen-carrying capacity by triggering splenic contraction and the release of additional erythrocytes into the blood (**see also Blood**: Cellular Composition of the Blood).

The adrenergic stress response is terminated (deactivated) by the uptake of circulating catecholamines into neuronal and non-neuronal tissue where they are converted to methylated and deaminated derivatives prior to excretion in the urine (**Figure 8**).

See also: **Blood**: Cellular Composition of the Blood. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Control of Respiration**: Oxygen Sensing in Fish. **Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Environmental Physiology. **Hypoxia**: The Expanding Hypoxic Environment. **Integrated Control and Response of the Circulatory System**: Hormone Metabolism in the Circulation. **Role of the Kidneys**: Histology of the Kidney. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Carbonic Anhydrase in Gas Transport and Exchange; Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; Hemoglobin.

Further Reading

Borges R, Carbone E, Nistri A, and Verkhratsky A (2008) Special issue on chromaffin cell physiology and pathology. *Acta Physiologica* 192(2): 143–335.

Fabbria E, Capuzzo A, and Moon TW (1998) The role of circulating catecholamines in the regulation of fish metabolism: An overview. *Comparative Biochemistry and Physiology – Part C* 120: 177–192.

Gallo VP and Civinini A (2003) Survey of the adrenal homolog in teleosts. *International Review of Cytology* 230: 89–187.

Kvetnansky R, Sabban EL, and Palkovits M (2009) Catecholaminergic systems in stress. Structural and molecular genetic approaches. *Physiological Reviews* 89: 535–606.

Perry SF and Capaldo A (2010) The autonomic nervous system and chromaffin tissue: Neuroendocrine regulation of catecholamine secretion in non-mammalian vertebrates. *Autonomic Neuroscience.*

Reid SG, Bernier NJ, and Perry SF (1998) The adrenergic stress response in fish: Control of catecholamine storage and release. *Comparative Biochemistry and Physiology – Part C* 120: 1–27.

Thomas S and Perry SF (1992) Control and consequences of adrenergic activation of red blood cell Na^+/H^+ exchange on blood oxygen and carbon dioxide transport in fish. *Journal of Experimental Zoology* 263: 160–175.

Stress Effect on Growth and Metabolism

TW Moon, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Catecholamines
Glucocorticoids
Other Related Hormones
Further Reading

Glossary

Gluconeogenesis The metabolic pathway responsible for glucose production from three-carbon precursor molecules, for example, amino acids.

Glycogenolysis The production of glucose from glycogen.

Hypothalamic–pituitary–interrenal (HPI) axis The HPI axis is the anatomical arrangement, whereby neuro-signals generated in the hypothalamus initiate the release of factors from the pituitary that move within the blood to the interrenal tissue to release glucocorticoids, subject to control by negative feedback loops at each level of this interactive system.

Signal transduction A process by which an extracellular signal (e.g., a hormone, the first messenger) initiates intracellular processes; generally involves a receptor, linking proteins, an enzyme that generates a second messenger (e.g., cyclic adenosine monophosphate), that activates downstream protein kinases which alters regulator-phosphorylation patterns.

Steroidogenesis The metabolic pathway by which steroids are enzymatically generated from cholesterol in cell types including the gonads and interrenals.

Introduction

This article examines metabolism and growth in fish as impacted by stress. Both factors are part of the organismal stress response with metabolism being important in terms of the secondary response, and growth being part of the tertiary response (**see also Hormonal Responses to Stress**: Hormone Response to Stress). When confronted by a stressor, the fish must respond and it does so by re-partitioning its energy between survival mechanisms and processes, such as growth and reproduction that are considered long-term commitments. A complex suite of nervous and endocrine functions are responsible for these re-partitioning activities and although we are now beginning to better understand these processes in mammals, our studies with fish remain primitive by comparison. To better understand the issues, the catecholamine (CA) and glucocorticoid pathways are briefly reviewed for their impacts on metabolism and growth.

Catecholamines

The CAs adrenaline (Adr) and noradrenaline (NAdr) are released from chromaffin cells (CCs) located to various body locations depending upon the fish group; within the advanced teleost fishes, these cells are primarily located within the walls of the posterior cardinal vein (PCV) in the anterior or head kidney generally intermixed with the steroidogenic interrenal cells (**see** section Glucocorticoids). The synthesis of Adr and NAdr within the CCs is discussed elsewhere (**see also Hormonal Responses to Stress**: Catecholamines), but suffice it to say that CAs are generally released from CCs to overcome acute cardiorespiratory and metabolic disruptions. Numerous stressors initiate such disruptions including hypoxia, anemia, acidosis, exhaustive exercise, and general physical disturbances.

Catecholamine Release and Content

Teleost fish CCs are innervated by sympathetic nerve fibers that release acetylcholine onto the cells ultimately releasing CAs from vesicles by Ca^{2+}-induced exocytosis into the PCV. Release can be induced using the mixed nicotinic/muscarinic agonist carbachol in rainbow trout (*Oncorhynchus mykiss*), but the specificity of cholinergic receptors on the CC shows significant species variability. A variety of noncholinergic agents are also known to elicit CA release in some fish species. Humoral agents, including serotonin (5-hydoxytryptamine (5-HT)-containing cells, are intermixed within the interrenal CCs), angiotensins, natriuretic peptides, adrenocorticotropic hormone (ACTH; **see** section Cortisol Release and Mechanisms of Action), opioid peptides, and CAs themselves have been shown to result in CA release. Probably, the most interesting and relevant nonhumoral factor is oxygen concentration (**see also Hormonal Responses to Stress**: Catecholamines).

Resting total Adr and NAdr levels in blood are generally $<20\,\mathrm{nmol{\cdot}l^{-1}}$, while stress-induced levels vary enormously across fish species being minimal in some Antarctic species to large in the salmonids ($>300\,\mathrm{nmol{\cdot}l^{-1}}$). These differences in levels between species result from factors including numbers of CCs and storage content, stress intensity, and turnover rates. Significant stress intensity is required to raise blood CA levels above the background in fish and, generally, this is correlated with a decrease in blood oxygen content.

Role of Oxygen

CA release is linked to a threshold PaO_2. Thus, the differences noted between fish species may reflect specific differences in hemoglobin-oxygen (Hb-O_2) binding affinities of blood (**see also Hormonal Responses to Stress**: Catecholamines). Examination of a variety of fish species has generally supported the link between hypoxemia, Hb-O_2 binding affinity (measured as P_{50}; **see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin), and CA release (**Figure 1**). Generally, teleost fish Hb has as low specific buffering capacity in part to

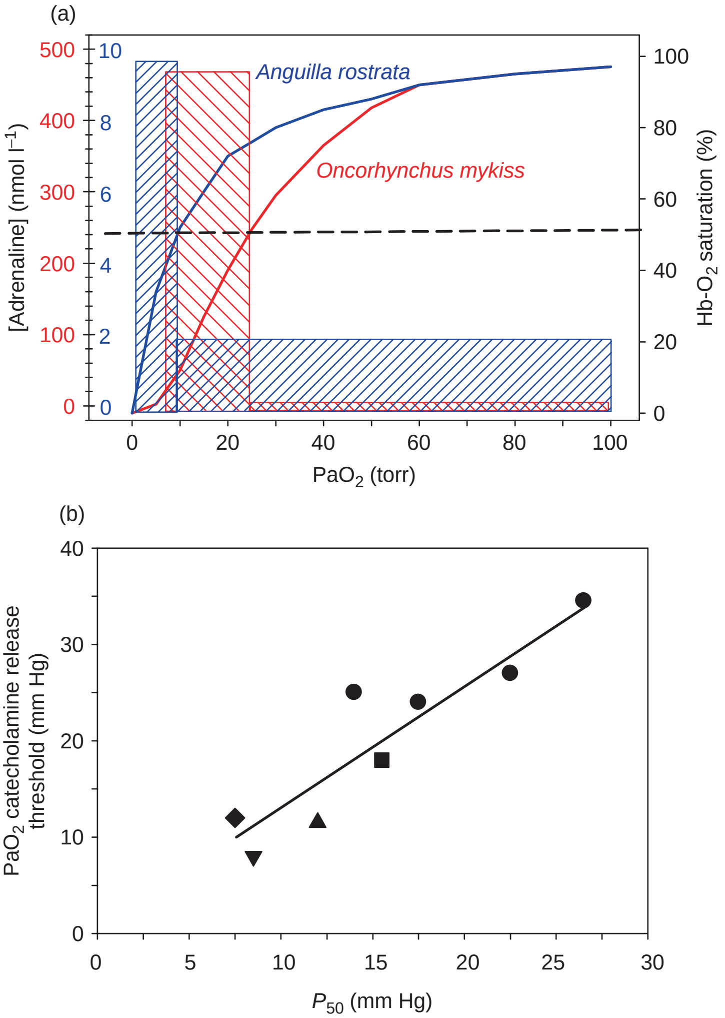


Figure 1 Plasma catecholamine levels are directly linked to arterial O_2 content. (a) O_2-saturation curves for the American eel (blue) and the rainbow trout (red) and the adrenaline concentration at various PaO_2 values. The dashed line represents approximately the 50% saturation value, which is the point where adrenaline levels rise. (b) Relationship between Hb-O_2 binding and the threshold for catecholamine secretion. Rainbow trout (circles); brown trout (*Salmo trutta fario*, square); American eel (up triangle); traira (*Hoplias malabaricus*, down triangle); and, jeju (*Hoplerythrinus unitaeniatus*, diamond). Reproduced from Perry SF, Reid SG, Gilmour KM, *et al.* (2004) A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 287: R188–R197, used with permission from American Physiological Society.

regulate the oxygen content of the swimbladder needed for neutral buoyancy. To ensure appropriate red blood cell (RBC), intracellular pH (pH_i) regulation and Hb-O_2 affinity, a novel CA-activated Na^+/H^+-exchanger (NHE) is located to the RBC membrane of many teleost fish and especially salmonids called the β-adrenergic NHE or simply the βNHE. Adr/NAdr binding to the βNHE initiates a signal transduction process that increases intracellular cyclic adenosine monophosphate (cAMP) content, activation of protein kinase A (PKA), phosphorylation of the βNHE, and activation of Na^+ influx and H^+ efflux. Physiologically, this process results in a rapid, but temporary RBC swelling and, importantly, increases pH_i and thus Hb-O_2 affinity and oxygen transport by Hb to metabolically active tissues. This β-adrenergic response specifically links changes in peripheral tissue metabolism to O_2 availability.

Metabolism and Catecholamines

Clearly, an acute stressor that reduces blood oxygen content results in the rapid release of CA. These circulating CAs affect tissue metabolism by binding to adrenergic receptors or adrenoceptors (ARs) located on membranes of responsive tissues to initiate signaling cascades (**Figure 2**). ARs are members of Family A (rhodopsin/β_2-adrenergic receptor-like) of the seven-transmembrane domain, G-protein-coupled receptor (GPCR) superfamily and evidence supports that the existing multiple AR subtypes (β_1-, β_2-, β_3-, α_1-, and α_2-ARs) arose during duplication events within the evolution of vertebrates. Fish ARs are similar to their homologs found across the vertebrates and especially within the well-characterized mammalian system.

CAs alter both carbohydrate and lipid metabolism in numerous fish species, both in the intact fish and in isolated hepatocytes. The most well-studied tissue is the liver and downstream of the hepatic ARs all components of the classic adenylate cyclase (ACase)/cAMP/PKA and inositol-phosphate/Ca^{2+}/protein kinase C pathways exist, leading to tissue metabolic changes (**Figure 2**). Changes may be modest, but this may be related to the timing of these changes, which generally are rapid and transient. CA concentrations giving 50% maximal hepatocyte glucose release can be as high as $\sim 1\ \mu\text{mol}\cdot\text{l}^{-1}$ and as low as $10\,\text{nmol}\cdot\text{l}^{-1}$. These values are generally above those reported for CA levels even in salmonids, but well above affinity values reported in fish AR binding studies ($1\text{–}20\,\text{nmol}\cdot\text{l}^{-1}$). Rainbow trout, bullhead catfish (*Ameiurus melas*; *A. nebulosus*), and American eel livers are classic β_2-AR-linked systems and incubations of primary hepatocytes from these species with β_2-agonists increase glycogen mobilization (glycogenolysis) through increases in cAMP and PKA activities activating glycogen phosphorylase a (GPase a); general β_2-antagonists (propranolol) block this response. Rainbow trout implanted with cortisol respond by increasing β-AR numbers on hepatocytes and production of cAMP. Although agonist-induced downregulation of ARs is common in mammalian studies, this is less so in fish possibly due to limited numbers of AR phosphorylation sites. Modest increases in gluconeogenesis from lactate and alanine are reported in some species, although this increase can be relatively large (common carp; *Cyprinus carpio*). The hyperglycemic effects of CA are enhanced by fasting and by chronic glucocorticoid application. Generally, these effects are more responsive to Adr than NAdr, with the possible exception of the copper rockfish (*Sebastes caurinus*) that is unusually responsive to NAdr.

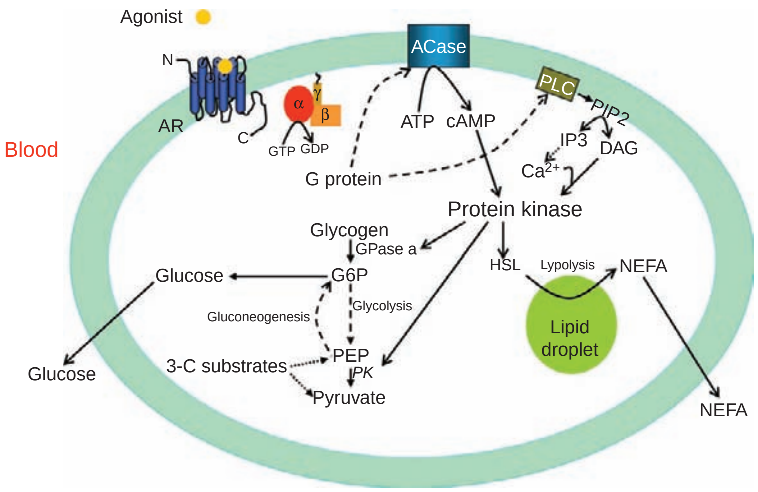


Figure 2 General cartoon for the action of a catecholamine agonist binding to a G-protein-coupled receptor (GPCR) in this case an adrenoceptor (AR) on a composite cell. The G protein is activated leading to the buildup of second messengers (cAMP, IP_3, DAG), which activate protein kinases that phosphorylate specific enzymes of glycolysis/gluconeogenesis and lipolysis. See text for description.

CAs stimulate plasma nonesterified fatty acid (NEFA) levels in mammals primarily by stimulating hormone-sensitive lipase (HSL) activities in adipose tissue (**Figure 2**). This effect is not always observed in teleost fishes, where both liver and adipose tissues can contribute to plasma lipid dynamics; Adr increases NEFA levels in some, but not all, fish while NAdr consistently lowers plasma NEFA in species where investigated. These differences may be related to stress intensity, predominant CA levels in the fish, AR-subtype composition and numbers on liver and adipose tissue, and the apparent high level of lipid mobilization and re-esterification found in some fish species. Certainly, all evidence points to a very different regulation of lipid mobilization in fish compared with mammals. The opposing adrenergic effects on NEFA metabolism in fish have been linked to hypoxia survival; fatty acid oxidation requires oxygen and by inhibiting NEFA mobilization at high CA concentrations, which in many fish represents NAdr content, NEFA and their oxidation intermediates will not compromise metabolic processes.

Muscle is the largest body tissue in fish. Swimming performance is reduced when circulating CA release is blocked by an antagonist. This antagonism can then be overcome by infusing Adr. Precisely how Adr is involved in contraction is unclear, although it may be related to shortening the twitch-relaxation cycle of skeletal muscle, or it may be indirect due to CA effects on other tissues important to swimming such as the heart. Exhaustive swimming generally results in muscle glycogen depletion and at least in some fish species increases in %GPase a, but there is no indication that this is linked directly to CA stimulation of β–ARs that do exist on fish muscle (**see** section Growth and CAs). Blood and muscle lactate pools are generally not in equilibrium in fish after exercise and evidence exists that the muscle lactate pool may contribute significantly to postexercise muscle glycogen recovery; the precise mechanisms for this glycogenesis and the role played by CA remain to be fully elucidated.

In summary, evidence supports a CA-induced increase in plasma glucose and NEFA content to provide tissues with energy needed during the stress response. This increase occurs primarily by Adr occupying β_2-ARs on tissue membranes. Under intense stress conditions as with deep hypoxia, NEFA mobilization is inhibited by NAdr preventing any negative affects of NEFA intermediates on metabolism. The precise metabolic role of CA in fish muscle remains to be elucidated.

Growth and CAs

Growth is generally compromised under both acute and chronic stress. However, there is little evidence for CA directly affecting growth at least in the short term. NAdr, along with numerous other humoral agents, directly inhibits growth hormone (GH) secretion from goldfish (*Carassius auratus*) pituitary cells *in vitro*, but NAdr is undetectable in the goldfish pituitary questioning the physiological importance of this observation.

Specific AR receptors (β_2-AR) exist on red and white skeletal muscle membranes of rainbow trout that, when occupied by β-agonists, increase cAMP intracellular concentrations, which suggests a classic cAMP/PKA signaling cascade. Feeding β-agonists to domestic animals increases muscle protein accretion, but studies in fish are equivocal and drug concentrations used far exceed levels found even under the most severe stress conditions. β_2-Antagonists are used as human drugs to treat cardiovascular problems and these drugs are now found in the aquatic environment; there is no indication to date that existing concentrations are adequate to affect the physiology of fish. There is some indication that CA may coordinate skeletal muscle recovery from exhaustive exercise at least in trout, but the precise mechanisms to account for this are not understood.

Glucocorticoids

The principal glucocorticoid in fish is cortisol (hydrocortisone). It is considered to be the primary indicator of the stress response, as unlike CA, basal and stress-induced levels can be easily measured. Cortisol is a multifaceted hormone expressing huge varieties of physiological and metabolic functions alone and in connection with other hormones (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids). Depending upon the fish species, the nature and intensity of the stressor, the affects of cortisol may differ. Certainly, stress-induced increases in plasma cortisol will directly affect glucose production, feeding responses, and, thus, growth, the immune response, hydromineral balance, and reproduction. Although cortisol is produced on demand at the level of the steroidogenic interrenal cells located to the head-kidney, the release process is initiated by a complex interaction within the HPI axis.

Cortisol Release and Mechanisms of Action

The principal interrenal secretagogue of cortisol is ACTH under the control of the hypothalamic factor corticotrophin-releasing factor/hormone (CRF/H). ACTH moves through the blood and binds to the melanocortin 2 receptor (MC2R), which belongs to the superfamily of GPCR linked to the ACase/cAMP/PKA signaling cascade ultimately increasing cortisol synthesis following stress. Levels of plasma ACTH rise prior to those of cortisol, but both are key indicators of responses to stressors including handling and confinement, crowding, thermal and osmotic shock, and chemical exposure in many species of fish. Cortisol

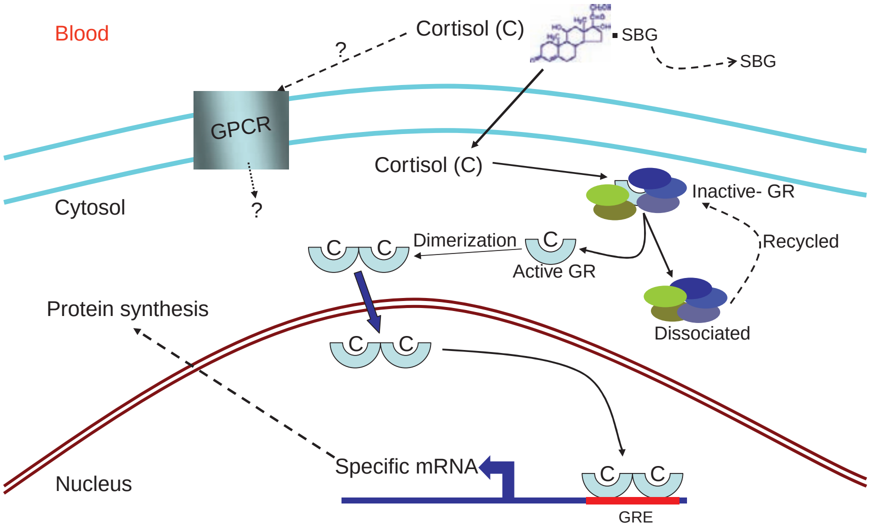


Figure 3 General cartoon of cortisol actions on a cell. Cortisol activates a cytosolic-localized nuclear receptor by displacing Hsps and other factors from it receptor (here a GR), that homodimerizes, moves to the nucleus where it binds to the GRE, located principally upstream of glucocorticoid responsive genes. The result is synthesis of gene-specific mRNA and ultimately proteins responsible for cortisol-induced metabolic changes. Some evidence exists for the rapid activities of cortisol by a membrane GPCR (G-protein-coupled receptor).

acts on cells by binding to corticosteroid receptors (CRs) that are ligand-activated transcription factors (TFs) located as complexes with other protein, including heat shock proteins (Hsps) in the cytosol (**Figure 3**). The phylogeny of CRs is complex but duplication events during evolution resulted in a glucocorticoid receptor (GR) and a mineralocorticoid receptor (MR). This phylogeny is discussed in detail elsewhere (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids).

As TFs, once cortisol binds to its CR, the complex moves to the nucleus where it is thought to homodimerize and bind to glucocorticoid response elements (GREs) generally found in the 5′ promoter sequence of glucocorticoid-sensitive genes (**Figure 3**). Putative GREs are reported on a number of fish genes (e.g., GH, myostatin, metallothionein, aromatase, and Na^+, K^+-ATPase subunit genes).

Cortisol and Metabolism

Generally, plasma cortisol levels rise with an acute stress, but the response is highly dependent upon the species and stress intensity. Handling or confinement, exhaustive exercise, and toxicant exposure will often elicit increases in plasma cortisol levels. Cortisol will be either free and thus physiologically available or bound to plasma steroid-binding globulins (SBGs) and thus not available (**Figure 3**); additionally, cortisol is metabolized within the liver and metabolic clearance rates (MtCRs) will additionally affect plasma levels. Free-cortisol content generally increases with acute stress and MtCR generally increases with chronic stress, leading to decreases or nonstressed levels of plasma cortisol.

Plasma glucose release is considered a secondary indicator of stress in vertebrates, but stress in fish can increase, decrease, or not change plasma glucose levels. In mammals, the rate-limiting gluconeogenic enzyme phospho*enol*pyruvate carboxykinase (PEPCK) contains a GRE, and cortisol or dexamethasone (DEX; a potent synthetic glucocorticoid) treatment stimulates PEPCK activity and stabilizes PEPCK messenger RNA (mRNA), and reduces hepatic glycogen content. The end result is an increase in gluconeogenesis and glycogenolysis (by activating GPase), ultimately increasing blood glucose (see **Figure 2**). In fish, there are significant interspecies differences in both glucose and hepatic glycogen and separating a stressor-specific effect by simply collecting the blood or tissue is extremely difficult. Using cortisol implants and isolated hepatocytes, the consensus is that cortisol or DEX treatment does increase hepatic gluconeogenic enzyme activities (including PEPCK), at least in some species. Some of the interspecific differences may arise from the apparent dissociation found between glucose content and the regulation of gluconeogenic enzymes (both activity and mRNA levels) observed in carnivorous fish species. However, tilapia (*Oreochromis mossambicus*) and rainbow trout hepatocytes do show a dose-dependent effect between cortisol and medium glucose in the absence of changes in glycogen, suggesting the primary cortisol affect is on gluconeogenesis. In rainbow trout with cortisol implants, GR downregulation does occur, which is thought to be associated with an increase in GR destruction by activation of proteosome activities.

GR mRNA is expressed in a variety of tissues including skeletal muscle. Reports exist that both cortisol and CAs may affect glycogenesis in fish skeletal muscle (**see** section Metabolism and CAs). Although controversial, glycogen production from lactate in fish white skeletal muscle is reported and, depending upon the glycogen content remaining following an exercise bout, both cortisol and Adr either increase glycogen production by increasing activities of glycogen synthase (at low glycogen) or increase glycogen breakdown by increasing activities of glycogen phosphorylase (at high glycogen). Both autoregulation of glycogen content by the prior glycogen status and the idea of a threshold value for this regulation are reported in mammals, but its precise role and its mechanistic basis remain to be explored in fish.

There is also general agreement that cortisol exerts a proteolytic function in fish white muscle and possibly liver; this action could fuel the gluconeogenic increases noted above. As mentioned earlier, direct evidence for this function is generally indirect or comes from studies of extreme or exaggerated behaviors (Pacific salmon migration, starvation, and exhaustive exercise) when cortisol levels rise. In general, plasma amino acid levels and hepatic amino acid transaminase activities rise in fish with cortisol implants. These amino acids are thought to be derived from white muscle proteolysis, which is extensive in migrating salmon; however, under normal conditions, where muscle represents >50% of total body mass, changes in muscle protein content are lost against very high background levels. To overcome this mass issue, other parameters are sometimes examined including ammonia output, which in theory, should rise with increased amino acid utilization. This does not always happen, although in resting Gulf toadfish (*Opsanus beta*) a highly significant correlation exists between increases in nitrogen excretion and plasma cortisol concentrations within normal resting levels (2–20 $\text{ng}\cdot\text{mL}^{-1}$). Mammalian tyrosine aminotransferase (TAT) contains a GRE that is considered the model for glucocorticoid action, but TAT activities do not increase with cortisol in most fish species. Alanine oxidation and gluconeogenesis do increase with cortisol in at least some fish hepatocyte preparations.

Lipids are a predominant fuel for normal fish metabolism and levels of all lipid classes are found in fish plasma and, as noted in section Metabolism and CAs, fish demonstrate very high resting lipolytic and re-esterification rates compared with mammals. Generally, increased cortisol increases plasma NEFA and peripheral lipolysis, and studies with salmon report cortisol activation of triacylglycerol lipase in mesenteric fat, red muscle, and liver. This latter effect was not seen in coho salmon (*O. kisutch*) smolts that already are naturally hypercortisolemic. Concurrent changes in some tissue lipid enzymes occur, but the precise role of these changes is not understood. In conditions of natural hypercortisolemia as during smoltification, these increases may relate to the intense restructuring of plasma membranes required during such events. Recent reports in mammals suggest that the increases in NEFA with cortisol are directly related to upregulation of lipase mRNA levels and activities, but also with cortisol acting permissively with other hormones. A detailed view of cortisol on lipids in fish is yet to be undertaken.

Cortisol and Growth

Cortisol is generally considered to decrease somatic growth in fish, but precisely how this comes about continues to be explored. Fish growth is a complex process modulated by the GH-insulin-like growth factor (IGF) axis and the neuroendocrine control of feeding. One or more GREs are reported within the promoter region of GH genes of numerous fish species, and using reporter gene assays, DEX stimulates transcriptional activities of the rainbow trout GH gene promoter, possibly through a cAMP response element. IGF is known to activate growth but plasma levels are kept low by a suite of hepatic-derived IGF-binding proteins (IGFBPs) and one of these (IGFBP-1) is very sensitive to stress and metabolic status. DEX was found to increase coho salmon hepatocyte IGFBP-1 mRNA and medium protein levels; increases in IGFBP-1 levels are known to decrease circulating IGF-I levels, the primary mitogen in fish. Confinement of gilthead sea bream (*Sparus aurata*) resulted in decreased IGF-I plasma levels consistent with decreased plasma GH levels and hepatic GH-receptor mRNA and suggested additional mechanisms to account for decreased growth during stress. In addition, chronic high-level increases in cortisol inhibit food intake in rainbow trout and goldfish while lower levels may actually increase food intake. The principal central and peripheral pathways responsible for such effects in fish are yet to be defined, but they may be related to cortisol effects on gut absorption or its affect on triiodothyronine (T3) levels.

In summary, cortisol affects many aspects of metabolism and growth, which in most cases are dependent upon the physiological status of the fish. In general, cortisol increases glycemia and aminoacidemia, and suppresses growth. These effects occur as a result of the peripheral action of cortisol binding to its receptor and activating transcription of key cellular proteins.

Other Related Hormones

Stressors and the stress response affect many fish tissues and multiple signaling systems, and not simply CAs and glucocorticoids. For example, CRF/H the principal secretagogue of ACTH by the pituitary can also activate secretion of melanocyte-stimulating hormone (αMSH) and even thyroid-stimulating hormone, both of which may act on tissues in quite different ways from ACTH. The best indication of this is the recent fish transcriptomic analysis done using low-density custom or high-density DNA microarrays for which a large number are presently available. Issues remain with the use of these arrays, but rainbow trout exposed to a moderate acute handling stress significantly increased a number of hepatic transcripts for hormone receptors and metabolic genes. These included receptors for testosterone, insulin, IGF and GH, glucose transporters, glucokinase, pyruvate kinase and, of course, PEPCK to note just a few. Using these new molecular techniques, it is clear that our initial view of the stress response in fish may need to be expanded to understand the full extent of the metabolic and growth interactions.

See also: **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids. **Hormonal Responses to Stress**: Catecholamines; Hormone Response to Stress. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin.

Further Reading

Aris-Brosou S, Chen X, Perry SF, and Moon TW (2009) Timing of the functional diversification of α- and β-adrenoceptors in fish and other vertebrates. *Annuals of the New York Academy of Sciences* 1163: 343–347.

Enes P, Panserat S, Kaushik S, and Oliva-Teles A (2009) Nutritional regulation of hepatic glucose metabolism in fish. *Fish Physiology and Biochemistry* 35: 519–539.

Fabbri E, Capuzzo A, and Moon TW (1998) The role of circulating catecholamines in the regulation of fish metabolism: An overview. *Comparative Biochemistry and Physiology* 120C: 177–192.

Kam JC and Milligan CL (2006) Fuel use during glycogenesis in rainbow trout (*Oncorhynchus mykiss* Walbaum) white muscle studied *in vitro*. *Journal of Experimental Biology* 209: 871–880.

Magnoni L and Weber J-M (2008) *In vivo* regulation of rainbow trout lipolysis by catecholamines. *Journal of Experimental Biology* 211: 2460–2466.

Mommsen TP, Vijayan MM, and Moon TW (1999) Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* 9: 211–268.

Nickerson JG, Dugan SG, Drouin G, Perry SF, and Moon TW (2003) Activity of the unique β-adrenergic Na^+/H^+ exchanger in trout erythrocytes is controlled by a novel β_3-AR subtype. *American Journal of Physiology Regulatory Integrative and Comparative Physiology* 285: R526–R535.

Owen SF, Huggett DB, Hutchinson TH, *et al.* (2009) Uptake of propranolol, a cardiovascular pharmaceutical, from water into fish plasma and its effects on growth and organ biometry. *Aquatic Toxicology* 93: 217–224.

Perry SF, Reid SG, Gilmour KM, *et al.* (2004) A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 287: R188–R197.

Thomas S and Perry SF (1992) Control and consequences of adrenergic activation of RBC Na^+/H^+ exchange on blood oxygen and carbon dioxide transport in fish. *Journal of Experimental Zoology* 263: 160–175.

Wiseman S, Osachoff H, Bassett E, *et al.*(2007) Gene expression pattern in the liver during recovery from an acute stressor in rainbow trout. *Comparative Biochemistry and Physiology* 2D: 234–244.

Impact of Stress in Health and Reproduction

L Tort, Universitat Autonoma de Barcelona, Cerdanyola, Spain

© 2011 Elsevier Inc. All rights reserved.

Impact of Stress on Reproduction
Impact of Stress on Health
Further Reading

Glossary

Atresia Any of a class of amines derived from a catechol that acts as chemical messengers (a hormone or neurotransmitter). The main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Catecholamine Any of a class of amines derived from a catechol that acts as chemical messengers (a hormone or neurotransmitter). The main catecholamines are dopamine, adrenaline, and noradrenaline and these are secreted by neurons of the autonomic nervous system or specific chromaffin cells of the head kidney.

Complement system Series of proteins of the innate immune system that are sequentially activated resulting in the release of cytokines and pathogen clearance through the membrane attack complex.

Cytokine Chemical messenger that activates or suppresses mechanisms of the immune system and may often influence other physiological regulatory systems.

Cytotoxicity Defense mechanism of the immune system producing cell death, mainly bacteria.

Glucocorticoid Molecule of lipidic nature secreted by the interrenal tissue in fish as a result of the activation of the hypothalamic–pituitary–interrenal axis after stress. The main glucocorticoid in fish is cortisol and it can mobilize glucidic reserves.

Gonadotropin Hormone produced in the pituitary gland that regulates the reproductive process in vertebrates. The main gonadotropins are the follicle-stimulating hormone and the luteinizing hormone.

Gonads Structures specialized in the production and feeding of gametes and synthesis of reproductive hormones.

Granulocyte A medium-sized white blood cell with variously shaped nuclei and a cytoplasm-containing granules that can perform phagocytosis.

Head kidney Cephalic portion of the kidney of fish in which several key functions during stress are performed, such as hematopoiesis, maturation of immune cells, and hormone secretion.

Hormone Signaling molecule released by a cell that either stimulates the cell of origin (autocrine), nearby cells (paracrine), or travels via the bloodstream to distant cells (endocrine).

Humoral immunity Array of defense mechanisms based on molecules, mainly proteins, that are found in the blood plasma.

Immune suppression Relevant reduction or disappearance of the capacity of response of one or several immune mechanisms.

Immunoglogulin An antibody playing a critical role in immunity.

Innate immunity A number of immune defense mechanisms that defend the host from infection by other organisms, in a nonspecific manner. These mechanisms do not depend on a previous recognition process and do not confer long-lasting or protective immunity to the host.

Interrenal cell Cell found in the head kidney of teleost fish that synthesizes and releases cortisol.

Lymphocyte A type of white blood cell involved in cellular defense and immunity. Granular lymphocytes are called natural killer cells and small lymphocytes produce immunoglobulins (B cells) or toxic granules to pathogens (T cells).

Lysozyme Protein that can be found in plasma, mucus, and other fluids of the animals, involved in the immune defense due to its lytic properties against bacterial cell walls.

Macrophage Specialized white blood cell matured from the monocyte that are able to perform phagocytosis.

Melanocyte-stimulating hormone (MSH) A peptide hormone synthesized in and secreted by the pars intermedia of the pituitary. It induces intracellular dispersion of melanosomes, resulting in skin darkening, and dispersion of xanthophore pigments, as well as longer-term morphological increases in melanophores and their melanogenesis.

Monocyte The largest white blood cell, produced in the head kidney that will be mobilized as a response to inflammatory signals and mature to macrophages.

Neuroendocrine axis Functional connection between structures of the brain, namely hypothalamus, and the pituitary gland that regulates physiological functions such as reproduction, growth, or stress response.

Neuropeptide Any member of a class of protein-like molecules released by the nervous system. They consist of short chains of amino acids, functioning as neurotransmitters (direct synaptic effects) or hormones (indirect modulatory effects).

Oocyte The female germ cell (gametocyte) from which an egg develops. After the onset of meiosis, oogonia become oocytes.

Pituitary An area of the brain involved with the release of regulatory hormones including adrenocorticotropic hormone.

Steroid Lipid compound consisting of a four-ring carbon skeleton (derived from cholesterol) and attached chemical groups. Some steroids act as hormones (17b-estradiol, 11-ketotestosterone) but others do not (cholesterol).

Stress State which alters the physiological equilibrium as a result of a challenge; the adaptive responses of an animal beyond the normal range or the normal functioning.

Stressor Any agent generating a situation of stress and inducing a coordinated response involving a transient modification of the physiological regulation.

Stressors cause a diverse array of consequences in animals depending on the type of stressor, its intensity, duration, and environmental circumstances. These consequences can be species specific. This article deals with these consequences in two areas, health and reproduction, as these two physiological systems can be significantly affected by stressors. Reproduction involves an increase in production of structures in the gonads and, sometimes, also elicits complex behaviors related to movement and aggression. Similarly, the production and maturation of immune cells and the increase of the synthesis of proteins, such as immunoglobulins, involve a delivery of energetic resources to support these processes. Therefore, stressful episodes may affect the correct or full function of these systems as resources are diverted to cope with the stress, thus compromising the reproductive ability and the immunocompetence.

Impact of Stress on Reproduction

Stressors in the natural fish populations may impair the reproductive process, therefore reducing the number of individuals (**Figure 1**). Many species that are cultured in captivity do not spontaneously ovulate or spermiate, although some of them may experience gonadal maturation. This indicates that the conditions must be optimal in order to fully reach the completion of the reproductive process (**see also Reproduction**: The Diversity of Fish Reproduction: An Introduction), otherwise, it is necessary to use artificial methods (exogenous hormones or abdominal stripping). From this observation, it is assumed that stress directly affects reproductive performance and that some stressors, either related to husbandry or simply because of captivity conditions, impair reproduction.

A number of studies have shown that stress induces a negative effect on reproductive fitness. However, not all stressors have been shown to alter reproduction (e.g., handling stressors), suggesting that the effects of stress on reproductive fitness strongly depends on the type of stressor, especially concerning the gamete quality. Even so recent studies indicate that, as with other performance activities, the deleterious nature of stress depends on the balance between input resources (food and reserves), the maintenance costs (standard metabolism, digestion, and exercise), and the reproduction-associated costs (see **Figure 2**). These reproductive costs will include the maturation processes (growth of gonads and

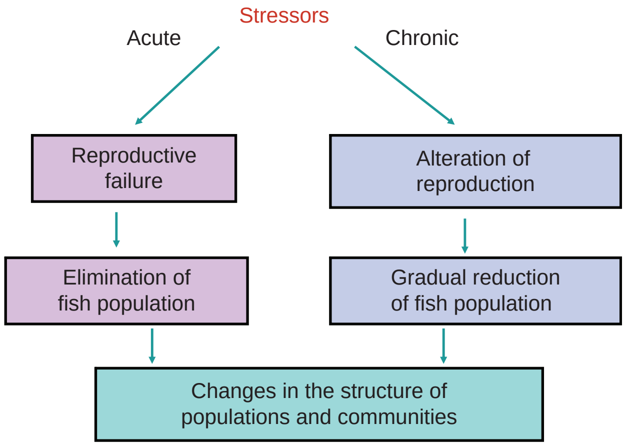

Stressors
Acute
Chronic
Reproductive failure
Alteration of reproduction
Elimination of fish population
Gradual reduction of fish population
Changes in the structure of populations and communities

Figure 1 Impact of stressors on the reproductive capacity of fish.

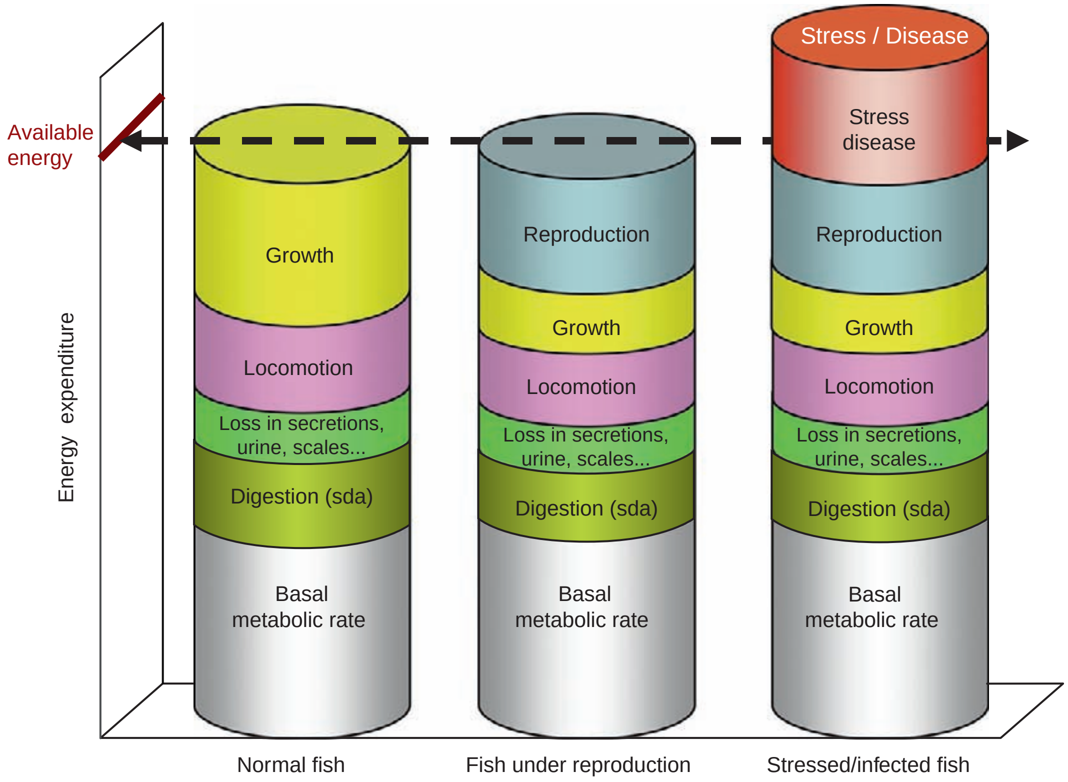


Figure 2 Energetic balance in fish under reproduction, stress, or infection compared to normal fish.

gametes) and reproductive activity (behavioral reproduction and spawning). Since stressors may impose an overload on resource expenditure to cope with the situation, this balance can be maintained if the energy available is sufficient. In this case, no deleterious effects may be observed; otherwise suppressive effects would take place impairing the reproductive performance. This would explain why, under stress, some studies do not observe changes, for instance, in the vitellogenin production, whereas others show oocyte atresia (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction).

Nevertheless, the reports on effects of stressors mostly show alterations in the reproductive performance. Thus, manipulations including confinement and capture may produce ovarian atresia. Handling results in lowered levels of reproductive hormones, such as 17β-estradiol (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction), fewer oocyte numbers (after stripping), and reduction of egg quality. Chronic or repeated handling stressors produce smaller eggs and reduce spermatic quality. Starvation decreases the levels of plasma and pituitary gonadotropins, and changes in the gonadosomatic index are observed in fish under low water oxygen conditions. Sudden alteration of the environmental temperature may induce reduced somatic condition and modifications in gonadal maturation.

Chemical stressors can greatly impair the reproductive process through various processes: by reducing egg numbers and size or inducing deformation of embryos; absence of spermatocytes; loss of organization in testes; and decrease or retardation in the spawning or increase of atresic eggs. In this manner, contaminants, such as heavy metals and pesticides, greatly impact reproduction, with severe population consequences. They can also interfere with the hormonal reproductive system of fish, causing effects such as sex change or arrest of reproduction.

Nutrition and food availability and quality are important in determining reproductive performance in fish. Insufficient nutrients may compromise reproductive success, as does the absence or the deficiency of certain diet components. For instance, lipid and fatty acid deficiency is associated with low fecundity and low-quality egg and sperm in fish under culture. Restriction of food resources and of specific components may either affect the energetic balance, preventing full reproduction or impairing hormonal equipment.

Pathogens (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular Fish Pathology) and parasites, by inducing stress depending on the degree of infection and duration of exposure, also affect reproduction since the endocrine and the immune systems interact during stress. Pathogens induce apoptosis in the ovary or changes in the maturation state, thus decreasing gamete quality and egg production. This effect may be mediated by pathogen-induced cytokines such as tumor necrosis factor alpha (TNFα). Similarly, parasites may prevent gonadal development and reproductive performance depending on their infestation degree.

Most fish depend on temperature and photoperiod to determine the main traits of their reproductive clock. Thus, sex determination, while genetically based, may suffer if alterations to temperature and photoperiod are severe.

Effects on the Reproductive Behavior

Stressors may affect reproductive behavior (**see also Reproduction**: The Diversity of Fish Reproduction: An Introduction), especially in species such as cichlids in which behavior is an important part of reproductive performance. Stressors can affect reproductive behavior in a number of different ways. First, handling stressors can prevent fish from performing reproductive activities, such as nest building, courtship, or migration. Second, the increase of circulating hormones after stress may interfere with the social status, thus reducing reproductive competence, and even affecting structures of the hypothalamus–pituitary–gonadal axis (HPG axis). Third, reproductive behavior may be a highly energy-demanding process that may not eventually be completed since the resources available are used to cope with the stressors.

Stressors causing social alterations may also be a reason for reduced reproductive performance, especially in hermaphroditic species. Some species, such as wrasse (fish from the family Labridae) or clown fishes (family Pomacentridae), change sex depending on factors, such as size and population density. Husbandry stressors affecting these factors or the related environmental conditions may alter reproductive abilities in these species (**see also Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish).

Effects on the Progeny

The effects of stressors may be deleterious not only for the progenitors but also for the progeny either in gamete quality and/or quantity. Fish with stress-induced low-levels of gonadal steroids have lower fecundity. The quality of the rearing phase of fish can affect the timing of successive reproductive events at later stages. The incidence of stress depends on the resources available, mainly on the nutritional input and quality; also, other factors related to the life quality may improve reproductive success. The effects of chemical stressors or contaminants on the progeny appear to be more severe, resulting in both egg reduction and malformations in embryos, and further reduction in the number of viable juveniles.

Overall, the stressor effects observed in the progeny of various fish species suggest that there are two general strategies for managing the stress. On the one hand, there is the strategy of preserving the health of the breeder and thus reducing the number of eggs, since fewer resources are available due to stressors. On the other hand, egg size and quality are maintained in the presence of the stress at a cost paid by somatic tissues. In the latter case, an increase in progeny cortisol levels is prevented by the progenitors (**Figure 3**). This could be the case of environmental challenges affecting reproduction in cultured fish. As an example, short-term, low-severity stressors associated with hatchery practices do not affect gamete quality of sturgeons.

Alterations of Reproductive Hormones

In all vertebrates, reproduction is a process basically controlled by hormones released at three different levels along the reproductive endocrine (HPG) axis – the hypothalamus, releasing the gonadotropin-releasing hormone (GnRH), the pituitary, releasing gonadotropins (GtH), and the gonads, producing gonadal steroids (progesterone, androgens, and estrogens). Given that the

Breeder self-protection.

- The breeder health is maintained after stress and cortisol treatment
- body weight is maintained and the production of eggs is readjusted
- The development of the ovary is compromised during vitellogenesis.

Progeny protection.

- gg size and quality are not affected when stress occurs during late vitellogenesis.
- Eggs are maintained and the cost is paid by somatic tissues.
- The rise/excess in cortisol levels in the progeny is prevented by the progenitors.

Protection mechanisms:

- Mature females show an atenuated response to cortisol.
- Cortisol-binding proteins prevent tissue receptor binding.
- Faster enzymatic action in follicles converting cortisol to cortisone.

Figure 3 Protection strategies for reproduction in fish under stress.

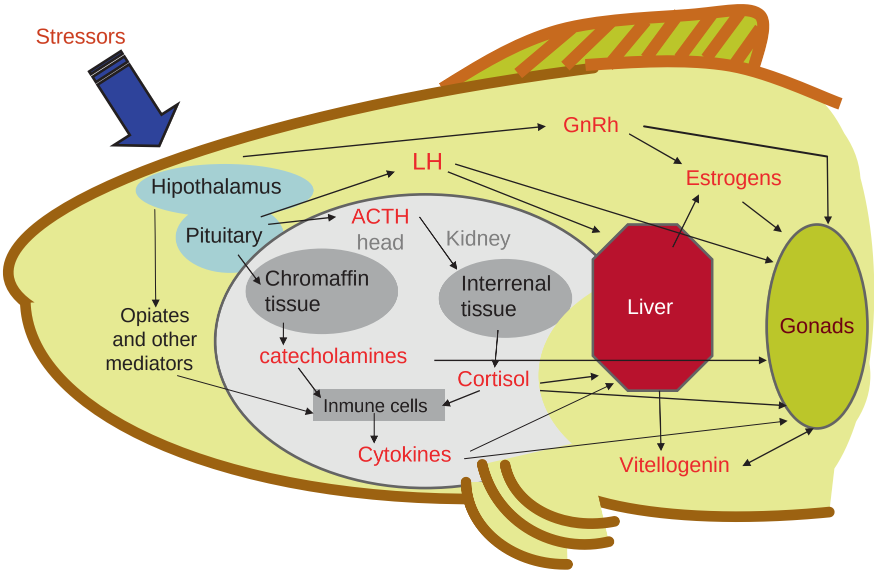


Figure 4 Scheme of the organs (hypothalamus, pituitary, head kidney, liver, and gonads) and messengers involved in stress, reproduction, and immunity.

stress response is also driven by another endocrine axis (hypothalamus–pituitary–interrenal (HPI); **see also The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis), involving the same neuroendocrine structures, and that the final hormone of the HPIaxis is cortisol (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids), a steroid hormone, it is apparent that the influence or interference of these messengers on both systems may exist during stress episodes (**Figure 4**).

Little is known about the direct effects of stressors on the release of GnRH and its effect on the gonadotrope cells of the pituitary. Acute confinement of male brown trout (*Salmo trutta)* induces an increase of plasma GtH, whereas in the white sucker (*Catostomus commersoni*) GtH is reduced 24 h after capture. Cortisol-mediated impairment of stress on reproduction does not appear to be mediated through inhibition of GtH secretion in rainbow trout (*Oncorhynchus mykiss*). Nevertheless, it is difficult to differentiate the effects of stress from that of cortisol on fish reproduction under stressful situations (**see also Hormonal Responses to Stress**: Stress Effect on Growth and Metabolism).

Regarding gonadal hormones, reduction in testosterone and 11-ketotestosterone (11KT) levels (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction) corresponds with an increase in cortisol, including after chronic confinement. Thus, higher population density reduces 11KT in males, as well as 17β-estradiol and testosterone concentrations in females. Brown trout subjected to crowding elevated plasma adrenocorticotropin hormone (ACTH) and cortisol levels and decreased testosterone and 11KT levels, despite elevated gonadotropin levels. In non salmonids, depression of most gonadal steroids with a concurrent increase of cortisol occurs after capture, transport, or hypoxia and also up to 5 days after confinement.

The Interaction between Reproductive Hormones and Stress Hormones

As mentioned before, the structure of hormones from both the gonadal axis and stress axis is similar, belonging to the family of cholesterol-derived steroids (**Figure 5**). Thus, variations in cortisol, or its precursors corticotropin-releasing factor (CRF) and ACTH, may influence the concentrations of gonadal steroids. Arginine vasotocin also appears to be part of the stress response of fish affecting reproduction. Urotensin-I is also corticotropic and it is involved in regulating cortisol during reproduction.

The diversity in hormone levels among species, particularly cortisol, makes it difficult to predict such interactions. For instance, plasma cortisol levels reported in immature female trout range from 0.5 to 20 ng mL^{-1} and up to 90 ng mL^{-1} in some species of salmon (*Oncorhynchus masou*), whereas levels in immature males range from 1 to 4 ng mL^{-1} in most fish species and up to 70 ng mL^{-1} in *O. masou*. In mature fish, the levels increase significantly. Thus, levels of mature females rise from 15 to 80 ng mL^{-1} in trout up to 180 ng mL^{-1} in *Oncorhynchus nerka* or 400 ng mL^{-1} in *O. masou*, and in males from 5 to 20 ng mL^{-1} in trout up to 80 ng mL^{-1} in other species or 350 ng mL^{-1} in *O. masou* or *O. tshawytscha*. Therefore, since the plasma cortisol levels of stressed fish may oscillate between 50 and 100–400 ng mL^{-1} or more,

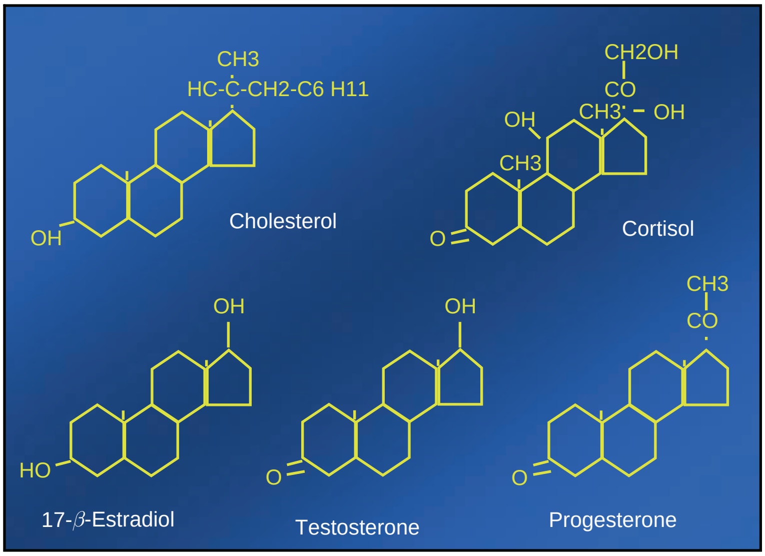


Figure 5 Chemical structure of the hormonal steroids.

cross-interactions between the sex and stress hormone axes will be seen in fish during the reproductive period, especially when a stressor is present. Thus, it is difficult to tease apart the effects of cortisol on reproduction versus the consequences due to the activation of stress-coping mechanisms in response to a stressor.

The effects of cortisol on reproductive hormones (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction) are diverse. A negative correlation between GtH and cortisol after stress exists. Cortisol administration decreases plasma estradiol and vitellogenin levels and reduces gonadal size. Inhibiting effects of exogenous cortisol administration on androgens exist, but are not seen if plasma cortisol concentration increases as a result of a natural increase due to the spawning period. Exogenously administered cortisol resulted in retarded oocyte growth, reduced condition factor, and lowered serum testosterone, and 17β-estradiol in tilapia (*Oreochromis* sp.). However, studies in goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), and one sparid (*Pagrus auratus*) reveal that cortisol does not affect testosterone and 17β-estradiol production at the level of ovarian steroidogenesis. Other *in vitro* studies show inconsistent results. In some studies, cortisol did not inhibit steroidogenesis or had deleterious effects on oocyte maturation in rainbow trout. In other studies, cortisol blocked the production and metabolism of hydroxyprogesterone in rainbow trout, thereby reducing the levels of estradiol and testosterone, but this was not seen in *Salvelinus* (**Figure 6**).

Inhibitory Mechanisms

Several hypotheses have been formulated to explain the alterations observed in the reproductive process in a stressed fish. They involve alterations in the endocrine axes and the mechanisms related to the processing of circulating steroids (**Figure 7**).

One interaction mechanism induces a lower intensity of response at the initial levels of the axes that would

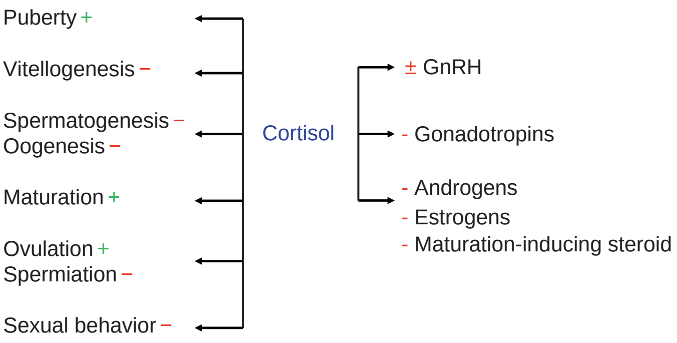


Figure 6 Stimulatory (+) or inhibitory (–) actions of cortisol on reproduction.

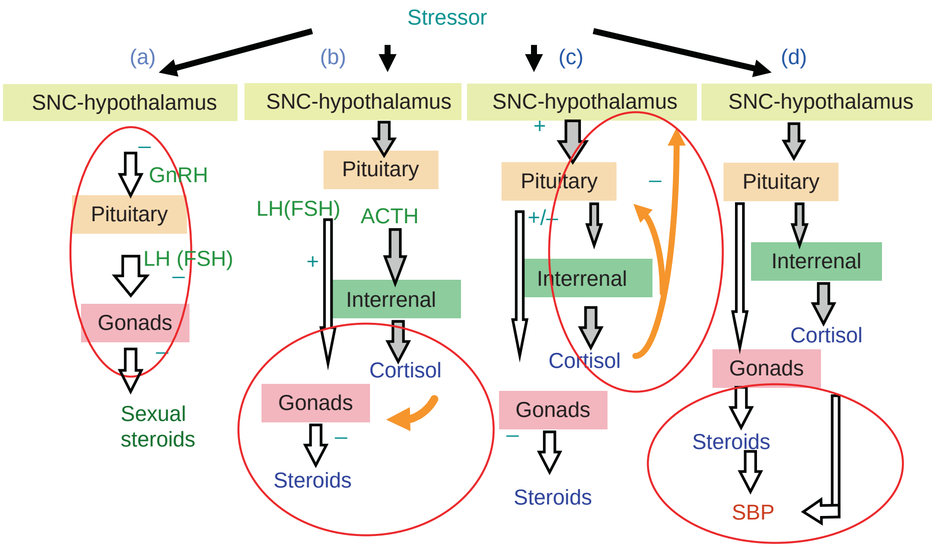


Figure 7 Possible mechanisms by which stress may affect the hormonal reproductive performance in fish. The red circle indicates the main point of action: (a) effects on SNC; (b) cortisol on gonads; (c) feedback; and (d) effects on circulating steroids.

reduce the full reproductive capacity. Under these circumstances, the hypothalamic and pituitary activation of gonads become less effective. The second mechanism involves cortisol altering gonadal efficiency by altering the production of the gonadal steroids and/or the production or quality of gametes. With this mechanism of steroidogenic inhibition, the effect of cortisol is exerted on the signal transduction of GtH, rather than at the pituitary level. A third mechanism consists of a feedback action of cortisol at the upper levels of the axis by blocking the synthesis or the secretion of gonadotropins or modulating the efficacy of such mechanisms. The fourth mechanism focuses on the steroid-binding protein (SBP). Estimations of the binding of the SBP indicate that the cortisol concentrations in plasma are over 100 times more than estradiol, which could displace the binding E2-SBP to cortisol-SBP. This would result in a higher rate of estradiol clearance, thereby affecting E2 efficacy in the activation and control of the reproductive activity.

Overall, the effects of stress on the reproductive ability of fish are mostly deleterious, but the effects will mostly depend on the duration and intensity of the stressor (see **Figure 8**). Regarding cortisol, its role may be as a modulator, rather than as a suppressor, since the deleterious effects are seen mostly in the early periods of reproduction and not at the later stages.

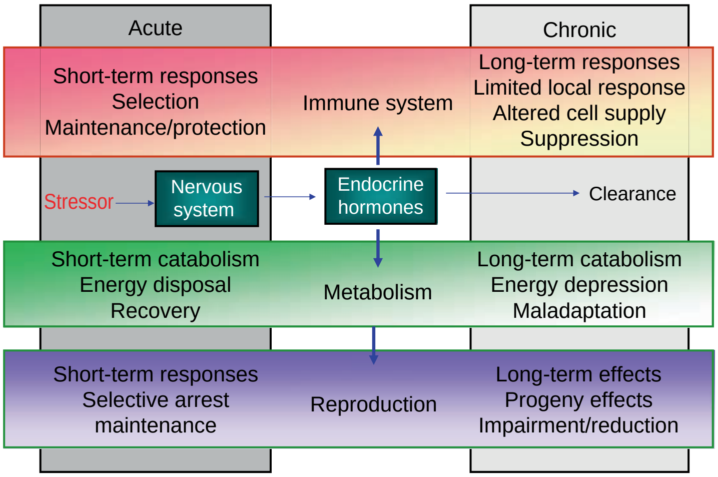


Figure 8 Comparison of short-term and long-term effects of stressors on the reproductive, metabolic, or immune performances.

Impact of Stress on Health

Fish, as in other vertebrates, maintain their integrity against infection by environmental pathogens (**see also** Cellular, **Molecular, Genomics, and Biomedical Approaches**: Molecular Fish Pathology) by activating the immune system, which is a complex network of specialized cells and molecules that are supported by other physiological systems (i.e., the other regulatory systems, neural and endocrine, and the energetic and metabolic system supplying resources for these functions). Stress, as a challenge to homeostatic equilibrium, may eventually modify such a balance and compromise the efficiency of the immune system.

The Neuro-Immuno-Endocrine Network

Similar to reproduction and growth (**see also Energy Utilization in Growth**: Energetics of Growth), the immune system and other defense processes are energetically demanding. In the event of an alarm situation, the stress response is by nature, an integrated response, thus affecting all physiological compartments. This means that the three main regulatory systems (i.e., neural, endocrine, and immune system) will be involved. Indeed, certain molecules of the neuroendocrine and immune networks belong to the same superfamilies and their functions are evolutionarily conserved.

Such molecules have been identified in higher vertebrates and some in zebrafish (*Danio rerio)*. Molecules such as heat shock proteins, acute phase proteins, or metallothioneins are present in most animals and have common generic functions involved in processes such as cell integrity, protection against oxidative processes, or cell regulation. Similarly, the influence of autonomic neurotransmitters is present in most fish. It has also been shown that the immune system can be regulated by specific peptides derived from the preopiomelanocortin (POMC) protein, the precursor of the pituitary hormones ACTH or alpha melanocyte-stimulating hormone (α-MSH), as some of these molecules are synthesized by immune cells.

Regarding the specific bidirectional connection between the immune system and the endocrine system, it has been shown that the recombinant Interleukin 1-β produce an increase of plasma cortisol or that cortisol inhibits the expression of TNFα. The receptor for interleukin (IL)1-β is present in both pars distalis and pars intermedia of the pituitary (**see also Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes) and that this cytokine stimulates the release of α-MSH and β-endorphin. At the same time, α-MSH modulates the production of cytokines by monocytes and macrophages. The corticosteroids affect phagocyte processes and modulate leukocyte responses. Some other hormones such as the GH and other interleukins may act directly on blood cells and stimulate their immune activity (see **Figure 9**).

In terms of the activation of responses at the endocrine and immune systems, such a connection is relevant since most often antigen recognition and stimulation share common stress response pathways that are also activated by physical or chemical stressors. For instance, warning mechanisms (acute phase proteins, heat shock proteins, cytokines, and neuropeptides) can be activated due to either pathogens or other stressors, thus modulating the hormone release and the involvement of metabolic–energetic responses, the cell protection systems, and the cellular and humoral immune responses (**Figure 10**).

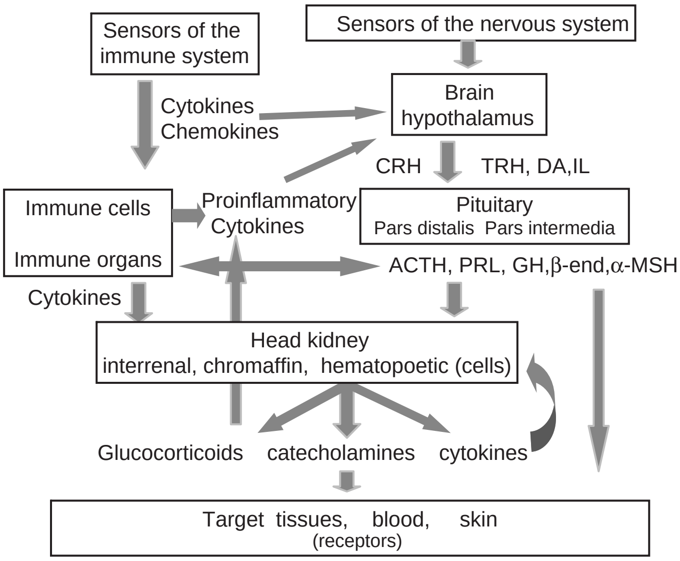

Sensors of the immune system
Sensors of the nervous system
Cytokines
Chemokines
Brain
hypothalamus
CRH
TRH, DA,IL
Immune cells
Immune organs
Proinflammatory
Cytokines
Pituitary
Pars distalis Pars intermedia
ACTH, PRL, GH,β-end,α-MSH
Cytokines
Head kidney
interrenal, chromaffin, hematopoetic (cells)
Glucocorticoids
catecholamines
cytokines
Target tissues, blood, skin
(receptors)

Figure 9 Flow diagram of the activation of the neuroendocrine and immune systems after stress.

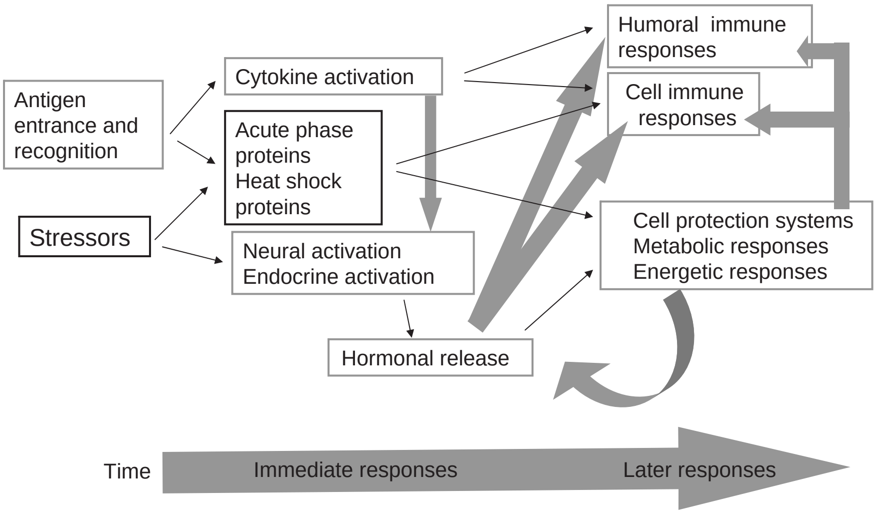


Figure 10 Time course activation of neuroimmunoendocrine responses and their interconnections.

The Role of the Head Kidney

In fish, another key organ in organizing the stress response is the head kidney (**see also Role of the Kidneys**: Histology of the Kidney), particularly concerning the activation of immune mechanisms driven by endocrine mediators. The head kidney is a key player in the endocrine response to stress (releasing cortisol (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids) and catecholamines (**see also Hormonal Responses to Stress**: Catecholamines) to the blood) and the hematopoietic response, producing different blood cell lines, including producing and maturing white blood cells for the immune defense and red blood cells for oxygen transport and delivery to the tissues (see **Figure 4**). Therefore, other than the neuroendocrine centers, this organ facilitates the stress response and the messenger molecules, such as neuropeptides, hormones, or cytokines, may play the role of the mediator between all the involved organs, not only through endocrine connections but also through direct paracrine action among the different cells present in the head kidney.

Stress and Immune Suppression

The overall effect of stressors often results in immune suppression. Yet, the effects on immune function depend on the type of stressor, its duration, and intensity; are varied; and even can be opposite; suppression may not always be observed. Thus, general rules on the effects of stressors on the immune system are lacking.

Stress normally compromises immunocompetence by diverting energy from immune function to the processes directly associated with coping with the stress (see **Figure 8**). Some of these changes are transient and without long-lasting immune suppression. Some initial or fast responses are stimulatory, at least at initial stages (see **Figure 10**). However, immune suppression is typically observed if the stressor persists, that is, it is chronic as opposed to acute.

Effects of Stressors on the Immune Function: Acute and Chronic Stressors

Overall, acute stressors may activate some immune responses and suppress others, whereas chronic stress normally results in immune suppression (see **Figure 8**).

An acute handling stress may induce a mitotic or proliferative response on the immune cells in the head kidney, spleen, or thymus. Handling stress can increase the number of glucocorticoid receptors in splenic and pronephric leukocytes, while handling and hypoxia produce an immediate increase of lysozyme and C3 complement proteins. All these responses should stimulate the immune system. By contrast, transport or high-density stress may suppress mitosis of head kidney leukocytes (**see also Blood**: Cellular Composition of the Blood).

Under chronic stress, fish experience immune suppression. Repeated chasing stress decreases the activity of the complement proteins. High fish density induces a decrease in the agglutinating capacity of blood and a reduction in the capacity of cytotoxicity and immune-related chemiluminescence. Dietary deficiencies depress nonspecific immune indicators. Pesticides, metals, or organic compounds may chronically induce immunosuppression, although phagocytic capacity can increase.

Genomic Assessment

Genomic analysis (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Global Gene Expression Profiling in Fish) confirms that the stress response is complex, involving most tissues, physiological compartments, and regulatory systems. Gene expression of the main metabolic and energetic pathways is upregulated. Genes involved in the cell protection mechanisms and energetic-demanding pathways, including metal-ion-binding proteins, glycolytic enzymes, and motor proteins, are transiently and rapidly induced. However, genes involved in immune response, cell proliferation and growth, signal transduction, apoptosis, protein biosynthesis, and folding increase more gradually and at later stages preparing the system for the phase of resistance. Therefore, the persistence of the stressor affects this second phase of gene expression, leading to immune suppression. Lipopolysaccharide (LPS)-stimulated fish macrophages show an expected increase of genes related to response to stress, humoral immunity, and apoptosis; administration of cortisol plus LPS suppresses this response. Nevertheless, the range of effects is more complex than simply the antagonism of LPS-induced transcriptional responses.

Immune Regulatory Hormones. The Role of Cortisol

Cortisol (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids) is a key mediator of immune suppression, as illustrated in numerous studies (**Figure 11**). For instance, macrophage respiration is suppressed after cortisol treatment, as is splenic nitric oxide and leukocyte production. Similarly, the mitogenic response to LPS is altered after incubation of leukocytes with cortisol. Head kidney monocyte-macrophage cell treatment with cortisol reduces the adherent capacity of such immune cells and increases the apoptotic index of lymphocytes and undifferentiated immune cells, suggesting cortisol-induced modulation of the hematopoietic potential of the fish (**see also Blood**: Cellular Composition of the Blood).

On the other hand, cortisol may activate an increase of leukocyte numbers in thymus and head kidney or myeloid and granulocyte numbers. In addition, a diversity of responses ranges from suppressive to activating, which is immune cell specific and dependent on cortisol concentration. Thus, cortisol may reduce B cells but increase the neutrophils, or may inhibit the lymphocyte proliferation and induce apoptosis, but not change their phagocytic competence.

Cortisol effects in peripheral tissues are equally varied. Cortisol does not consistently affect the phagocytic capacity or apoptosis of macrophages. Yet, cortisol clearly reduces pro-inflammatory cytokine expression in LPS-stimulated macrophages. The dual peripheral action of cortisol may be related to its two glucocorticoid receptors (GRs) found in most fish species. GR1 would be sensitive to high levels of cortisol (acute stress) and mediate the inhibition of LPS-induced cytokine production (TNFα, IL-12, or IFN). GR2 signaling-mediated modulation of humoral immunity would occur at lower cortisol levels. Current work on GRs should help to clarify their role in the physiological responses to stressors.

In summary, cortisol clearly modulates the immune response, rather than being a direct immunosuppressive agent. This modulatory role of cortisol fits with the role of other hormones of the HPI axis, which collectively ensure adequate supply of energetic and metabolic resources to cope with a stressor. This metabolic hypothesis allows some essential responses to be maintained, that is, phagocytosis in the tissues, while reducing other immune functions such as cell proliferation, which can be delayed until the recovery phase from the stress episode and refueling of resources.

Stress and Pathologies

Stress may result in pathologies. Stress is often the initial condition under which pathogens invade a host

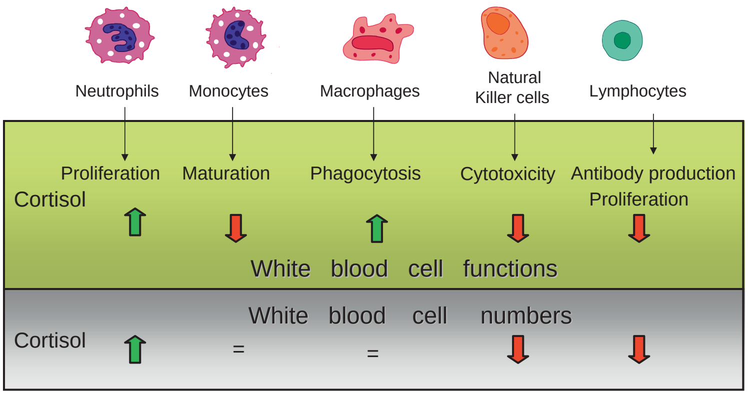


Figure 11 Reported effects of cortisol on the white blood cell numbers and function.

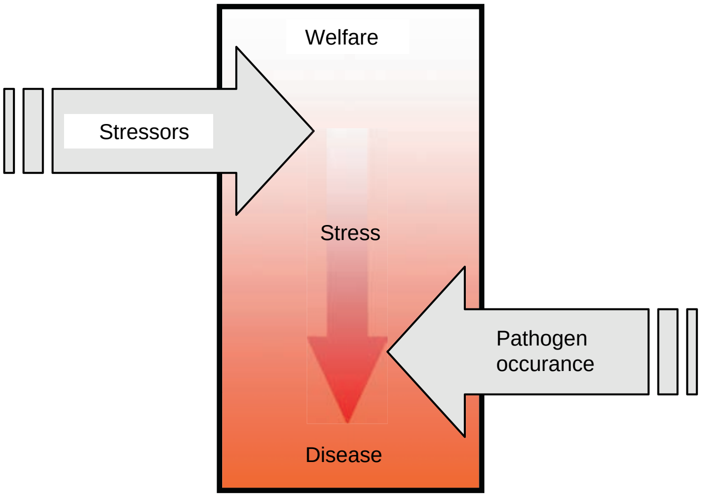


Figure 12 Stress affects facilitating pathogen infection and disease.

(**Figure 12**). Thus, pollution, contamination (**see also Toxicology**: The Toxicology of Metals in Fishes and The Toxicology of Organics in Fishes), and stress have been associated to both disease episodes or husbandry-related pathologies. For instance, cortisol increases cell death in fish previously contaminated with chemicals or high levels of cortisol increase the susceptibility of some fish to *Ichthyopthirius*. Thus, fish under compromised conditions (i.e., contaminated waters, presence of parasites (**see also Behavioral Responses to the Environment**: Manipulation of Fish Phenotype by Parasites), or sudden reductions of water temperature) show the classic hormone and metabolic response as in the chronic stress, as well as an increase in the susceptibility to pathogens, resulting in a heavy decrease of the immune competence and increased mortality.

The Integrated Immune Response to Stressors. Activation–Suppression–Modulation

The stress response may be modified by four factors: the species, the type of stressor, stress intensity, and stress duration. In addition, the response will depend on when response is monitored. Neural and HPI-axis responses occur in seconds to minutes, whereas metabolic and energetic responses take minutes to hours and days.

Stress duration and intensity may significantly change the immune status of the fish. Under acute stressors, the cost is short: high levels of circulating hormones are recorded but the energetic and metabolic consequences are limited. In these circumstances, an activating response is common but less so is suppression. Thus, an increase of cytokine production, an increase of other relevant proteins such as C3 or lysozyme, or the activation of enzymatic cascades such as the complement system may be observed.

However, the costs of chronic or repeated stressors are higher at medium term. Thus, the resources are diverted from other physiological processes, including the immune system, to cope with the stressor. The synthesis of new proteins, the production of immune cells in the head kidney, or the induction of inflammatory responses, which are costly processes, may be blocked until recovery. Therefore, responses based on these proteins or cells are compromised: a reduction of antibody synthesis, a reduction or arrest of monocyte maturation, and therefore loss of phagocytic potential.

In summary, indicators of immune suppression after stress are classified as innate, acquired, molecular, or cellular. However, none by themself is a reliable indicator, since each one changes depending on the conditions. A combination of indicators and/or a challenge test on disease resistance may be a better choice. A good immune indicator should ideally respond consistently to a wide range of stimuli, show a repetitive dynamics in terms of time and intensity for each stimulus, and reflect changes in both the innate and the adaptive response. Often, stress has two phases, initial activation and then suppression, each of which is reflected in several specific variables of the immune system changing (serum lysozyme, serum complement, number of lymphocytes, plasma immunoglobulins, white cell number and distribution, and nitric oxide production).

See also: **Behavioral Responses to the Environment**: Manipulation of Fish Phenotype by Parasites. **Blood**: Cellular Composition of the Blood. **Brain and Nervous System**: Functional Morphology of the Brains of Ray-Finned Fishes. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Global Gene Expression Profiling in Fish; Molecular Fish Pathology. **Energy Utilization in Growth**: Energetics of Growth. **Hormonal**

Control of Metabolism and Ionic Regulation: Corticosteroids. **Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish; Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress**: Catecholamines; Stress Effect on Growth and Metabolism. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Role of the Kidneys**: Histology of the Kidney. **The Pituitary**: Development of the Hypothalamus-Pituitary-Interrenal Axis. **Toxicology**: The Toxicology of Metals in Fishes; The Toxicology of Organics in Fishes.

Further Reading

Aluru N and Vijayan MM (2009) Stress transcriptomics in fish: A role for genomic cortisol signaling. *General and Comparative Endocrinology* 164: 142–150.

Bowden TJ (2008) Modulation of the immune system of fish by their environment. *Fish and Shellfish Immunology* 25: 373–383.

Campbell PM, Pottinger TG, and Sumpter JP (1992) Stress reduces the quality of gametes produced by rainbow trout. *Biology of Reproduction* 47: 1140–1150.

Castillo J, Teles M, Mackenzie S, and Tort L (2009) Stress-related hormones modulate cytokine expression in the head kidney of Gilthead seabream (Sparus aurata). *Fish and Shellfish Immunology* 27: 493–499.

Dhabhar FS (2009) A hassle a day may keep the pathogens away: The fight-or-flight stress response and the augmentation of immune function. *Integrative and Comparative Biology* 49: 215–236.

Engelsma MY, Huising MO, van Muiswinkel WB, *et al.* (2002) Neuroendocrine–immune interactions in fish: A role for interleukin-1. *Veterinary Immunology and Immunopathology* 87: 467–479.

Gornati R, Papis E, Rimoldi S, *et al.* (2004) Rearing density influences the expression of stress-related genes in sea bass (Dicentrarchus labrax L). *Gene* 341: 111–118.

Harris J and Bird DJ (2000) Modulation of the fish immune system by hormones. *Veterinary Immunology and Immunopathology* 77: 163–176.

Mackenzie S, Iliev D, Liarte C, *et al.* (2006) Transcriptional analysis of LPS-stimulated activation of trout (Oncorhynchus mykiss) monocyte/macrophage cells in primary culture treated with cortisol. *Molecular Immunology* 43: 1340–1348.

Milla S, Wang S, Mandiki SNM, and Kestemont P (2009) Corticosteroids: Friends or foes of teleost fish reproduction. *Comparative Biochemistry and Physiology* 153: 242–251.

Pankhurst NW and Van der Kraak G (2000) Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout in vivo through action of cortisol. *General and Comparative Endocrinology* 117: 225–237.

Reinecke M, Zaccone G, and Kapoor BG (2006) Stress response, reproduction and endocrine disruptors. In: *Fish Endocrinology*, vol. 2, pp. 571–912. New Hampshire: Science Publishers

Rice CD, Kergosien DH, and Adams SM (1997) Innate immune function as a bioindicator of pollution stress in fish. *Environmental Pollution* 96: 280–280.

Schreck CB (2010) Stress and fish reproduction: The roles of allostasis and hormesis. *General and Comparative Endocrinology* 165: 549–556.

Schreck CB, Contreras-Sanchez W, and Fitzpatrick MS (2001) Effects of stress on fish reproduction gamete quality and progeny. *Aquaculture* 197: 3–24.

Sunyer JO, Gómez E, Navarro V, Quesada J, and Tort L (1995) Depression of humoral components of the immune system and physiological responses in gilthead sea bream Sparus aurata after daily acute stress. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 2339–2346.

Varsamos S, Flik G, Pepin JF, Bonga SEW, and Breuil G (2006) Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass Dicentrarchus labrax. *Fish and Shellfish Immunology* 20: 83–96.

Verburg-Van Kemenade BML, Stolte EH, Metz JR, and Chadzinska M (2009) Neuroendocrine–immune interactions in teleost fish. In: *Fish Neuroendocrinology. Fish Physiology Series*, vol. 28, pp. 313–364.

HORMONAL CONTROLS

Hormones in Communication

Hormonal Pheromones

N Stacey, University of Alberta, Edmonton, AB, Canada

PW Sorensen, University of Minnesota, St. Paul, MN, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Goldfish and Related Carps
Female Preovulatory Steroid Pheromone
Female Postovulatory Prostaglandin Pheromone
Mechanisms of Pheromone Action
Male-Derived Hormonal Pheromones
A Goldfish Chemical Network?
Hormonal Pheromones and Species Specificity
Further Reading

Glossary

Androgen Any natural or synthetic steroid hormone that is principally involved in growth, development, and maintenance of the male reproductive system and secondary sexual characteristics. The major endogenous fish androgen is 11-ketotestosterone.

Electro-olfactogram (EOG) recording Measures olfactory sensitivity using extracellular recording of odor-induced changes in transepithelial voltage via an electrode placed near the surface of the olfactory epithelium and is thought to measure generator potentials of olfactory receptor neurons.

Estrogen Any natural or synthetic steroid hormone that controls female sexual development, secondary sexual characteristics, and stimulates egg production. The endogenous fish estrogen is 17-estradiol and together with progesterone (another steroid hormone), it regulates the development and function of sex organs.

Gonadotropin Hormone produced in the pituitary gland that regulates the reproductive process in vertebrates. The main gonadotropins are the follicle-stimulating hormone and the luteinizing hormone.

Hormonal pheromone A pheromone that contains at least one compound that is a hormone, a hormone precursor, or a hormone metabolite.

Luteinizing hormone (LH) A dimeric glycoprotein hormone released from the anterior pituitary gland that acts through gonadal membrane receptors to stimulate steroidogenesis and gametogenesis. LH is a gonadotropin.

Maturation-inducing steroid (MIS) A steroid produced by ovarian follicular cells to promote final oocyte maturation: 4-pregnen-17,20β-diol-3-one (17,20β-P) appears to be the MIS in the majority of fishes.

Odor A mixture of odorants that is both detectable and distinguishable by the olfactory system.

Odorant A chemical that binds to and stimulates an olfactory receptor.

Olfactory receptor A protein located in membranes of olfactory receptor neurons that binds odorants.

Olfactory receptor neuron (ORN) A neuron in the olfactory epithelium that contains olfactory receptors.

Pheromone A chemical, or mixture of chemicals, that is released to the environment by an individual and that evokes a specific and adaptive response in conspecifics: the term pheromone is used in many ways – our definition follows the original and is broad.

Pheromone active space The space (volume of water) in which a pheromone can be detected.

Prostaglandin Any member of a group of lipid compounds that are 20-carbon fatty acid derivatives containing a five-carbon ring. They mediate a wide range of physiological functions in fish, such as ovulation (follicular rupture), female sexual behavior, and also act as pheromones in some species.

Steroid Lipid compound consisting of a four-ring carbon skeleton and attached chemical groups. Some steroids act as hormones (e.g., 17β-estradiol and 11-ketotestosterone) but others do not (e.g., cholesterol).

Introduction

Imagine the benefits of being able to walk through a dark and crowded room and, with just a subtle sniff, determine not only the gender, but also the reproductive status of anyone you pass. Although beyond our chemosensory abilities, similar acts of chemically mediated social snooping are commonplace in fishes that have evolved the ability to smell and respond to extremely low concentrations of hormones and related chemicals released by conspecifics (other members of the same species). We refer to this group of odorants as hormonal pheromones, although only some are unmodified hormones, and the majority in fact might be hormonal precursors or metabolites. In this brief overview, we examine hormonal pheromone function primarily from the perspective of the common goldfish (*Carassius auratus*), because only in this species are we beginning to understand how hormonal pheromones are released and detected, how they exert their effects, and how their biological responses affect reproductive success. Nonetheless, there is growing evidence that hormonal pheromone systems are common, if not ubiquitous, among fishes.

Fish have evolved in a physical–perceptual world that differs from ours in several key characteristics. Particularly in large lakes and oceans, the three-dimensional, featureless, and often dimly lit living spaces of fish pose unique challenges to establishing social interactions and achieving reproductive synchrony. This is particularly obvious when we consider that the majority of fish engages in external fertilization using gametes that are short-lived in water. Along with the limitations that the aquatic medium places on several sensory modalities, it does confer one remarkable advantage: it enables the myriad of information-rich chemicals that fish inevitably release to the water to be readily transported to the olfactory organs of conspecifics, thereby facilitating the evolutionary origin of pheromone function.

Although it has been known for many years that fish have evolved sex pheromones to synchronize both gamete maturity (a primer effect) and behavioral interactions between individuals (releaser effects), the chemical nature of these pheromones has only recently become apparent. The idea that at least some fish pheromones might be endogenous hormones released into the external environment was first proposed by Kjell Døving (Oslo, Norway) in the 1970s, and received support from Lorenzo Colombo (Padua, Italy), who found that the male black goby (*Gobius niger*) releases a conjugated steroid, etiocholanolone glucuronide, to attract ovulated females to his nest for spawning. Since then, waterborne hormonal compounds (steroids, prostaglandins, and their precursors and metabolites) have been found to have biological effects in a variety of fishes. In addition, electro-olfactogram (EOG) recordings, which can quickly screen putative odorants for their olfactory activity (**see also Smell, Taste, and Chemical Sensing**: Neurophysiology of Olfaction and **Toxicology**: The Effects of Toxicants on Olfaction in Fishes), have shown that several hundred fish species from at least eight orders can detect hormonal compounds at the nanomolar-to-picomolar concentrations that would be expected of pheromones derived from hormones that already are at low concentrations in the bloodstream before they are released into the environment and further diluted.

These findings do not imply that all fish pheromones are exclusively derived from hormones. For example, sea lamprey (*Petromyzon marinus*) use bile steroids as a migratory pheromone (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and Lampreys: Environmental Physiology) and masu salmon (*Oncorhynchus masou*) use an amino acid as a female sex pheromone (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior), while other recent studies indicate that hormonal and nonhormonal odorants can function as a pheromonal mixture. Nonetheless, hormonal compounds are implicated in the great majority of teleost fishes where there is any indication of pheromone identity. Unfortunately, little, if any, attention has been paid to pheromones in the cartilaginous fishes (sharks, rays, and chimaeras), the lobe-finned fishes (lungfishes), or even the nonteleost ray-finned fishes (sturgeons, gars, and bichirs), with the result that the broader evolutionary significance of hormonal pheromones within the aquatic vertebrates is a mystery.

Goldfish and Related Carps

Carp is a common term for several dozen species in the family Cyprinidae that live in tropical and temperate freshwater lakes, streams, and rivers. Particularly well known are the common carp (*Cyprinus carpio*), the crucian carp (*Carassius carassius*), and the goldfish. Except for tubercles that form on the males' opercula and fins during the breeding season, these carps lack sexual dimorphism, spend their lives in apparently unstructured, mixed-sex aggregations, and employ a promiscuous mating system involving intense sperm competition among males. EOG studies show that goldfish, crucian carp, and common carp detect a remarkably similar suite of hormonal compounds, providing a possible explanation for the fact that they will occasionally hybridize under natural conditions. Carp hormonal pheromones are best understood in the goldfish, the only species for which a complete picture of hormonal pheromone function is beginning to emerge.

As with crucian and common carp, goldfish undergo yolk production (**see also The Reproductive Organs and Processes**: Vitellogenesis in Fishes) and oocyte growth in winter and early spring, and ovulate large numbers of oocytes several times over a protracted spring–summer spawning season. Laboratory studies and field observations indicate that local groups of females are triggered to begin ovulation by two key environmental cues: rising water temperature and the appearance of aquatic vegetation onto which the adhesive eggs are released. However, females that begin ovulation quickly release a steroidal pheromone that induces neighboring females to ovulate. These mechanisms, likely intended to promote reproductive synchrony, presumably have evolved because the release of large numbers of eggs swamps the ability of predators to eat the undefended eggs and young.

The environmental and social cues that trigger ovulation exert their effects very indirectly, by causing the pituitary gland to release a dramatic 12-h surge of luteinizing hormone (LH), which in turn acts on the ovarian follicles to stimulate synthesis of a maturation-inducing steroid (MIS) that prepares the oocytes to ovulate (**Figure 1**). During this preovulatory LH surge, males

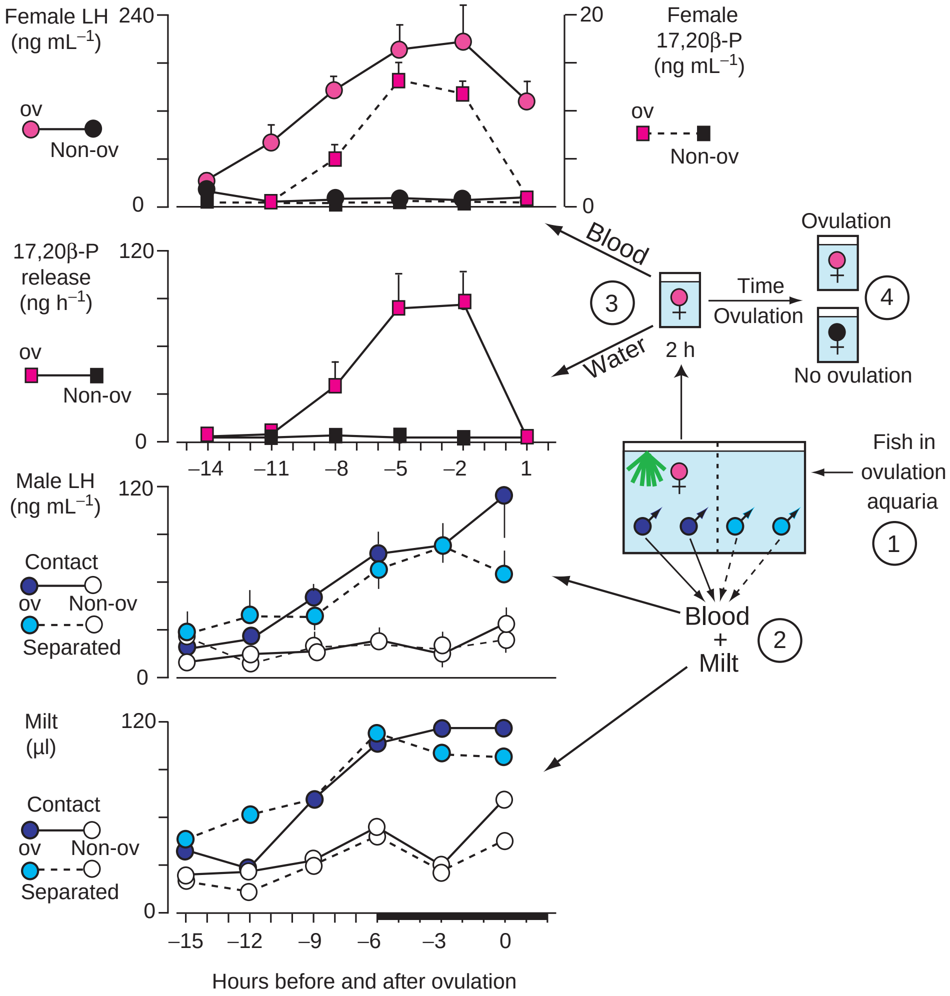


Figure 1 Male goldfish increase their blood LH concentrations and milt volumes if they are held with ovulating females, or separated from them by a perforated barrier. The right-hand side illustrates the experimental procedure. (1) Male and female goldfish were held overnight in warm water with aquatic vegetation, conditions that induced ovulation in half of the females. Half of the males were held behind a perforated barrier (separated) while the other half were in direct contact with females. (2) Males were removed from the experimental aquaria and immediately sampled for LH and milt. (3) Females were removed from the experimental aquaria, held for 2 h to obtain holding water for 17,20β-P determination, and then bled to measure blood concentrations of LH and 17,20β-P. (4) Females were checked frequently to determine if and when they ovulated. Graphs on the left-hand side of the figure illustrate the parameters measured. Adapted from Stacey NE and Sorensen PW (2006) Reproductive pheromones. In Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, vol. 24, pp. 359–412. San Diego, CA: Elsevier; Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior*, 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier; Stacey NE and Sorensen PW (1991) Function and evolution of fish hormonal pheromones. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes: Vol 1. Phylogenetic and Biochemical Perspectives*, pp. 109–135. New York, NY: Elsevier; and Stacey NE, Sorensen PW, Van Der Kraak GJ, and Dulka JG (1989) Direct evidence that 17α,20β-dihydroxy-4-pregnen-3-one functions as a goldfish primer pheromone: Preovulatory release is closely associated with male endocrine responses. *General and Comparative Endocrinology* 75: 62–70.

begin to follow and contact females, but the intensity of their behaviors increases dramatically when the females ovulate. At this point, and for the following hour or so, small and changing groups of males continuously chase a female, vigorously competing for position as she repeatedly enters vegetation, and then engaging in intense sperm competition as she quickly oviposits her eggs. In such a mating system, reproductive success of males will depend on the quantity and quality of sperm they are able to release, and on doing so, in proximity to released eggs. Although casual observation might suggest that these spawning behaviors are chaotic and rather unspecialized, they belie a surprisingly complex suite of pheromone-driven physiological and behavioral processes that begin soon after the surge in female LH and end when spawning ceases approximately 15 h later. During this brief time, hormonal pheromones function in three social contexts: between females, between males, and from female to male. The latter situation is best understood. Females first release steroid odors in the preovulatory period when the LH surge is stimulating final oocyte maturation, and then release prostaglandin odors during spawning.

Female Preovulatory Steroid Pheromone

If male goldfish or carp are held with females undergoing a preovulatory LH surge, or exposed to their odor, their blood LH concentration quickly increases, their social behaviors change, and within several hours they will have larger volumes of milt (sperm and seminal fluid) in their sperm ducts (**Figure 1**). In anosmic males (where the sense of smell is removed), these effects are completely blocked. The effects of preovulatory female odor are mediated by at least three specific and sensitive (picomolar threshold) olfactory receptors tuned to three key steroid odorants: the goldfish MIS (17,20β-dihydroxy-4-pregnen-3-one; 17,20β-P), a 20β-sulfated MIS metabolite (17,20β-P-su), and androstenedione (AD), an immediate precursor of testosterone. When added as single compounds to aquaria containing groups of male goldfish, both 17,20β-P and 17,20β-P-su rapidly increase male blood LH concentrations, whereas AD does not. However, all three odorants affect behavior, AD-inducing agonistic behavior between males, 17,20β-P-inducing weak chasing behavior, and 17,20β-P-su-inducing more intense but brief chasing bouts.

Although the female releases 17,20β-P, 17,20β-P-su, and AD simultaneously throughout the preovulatory period, there are two major reasons for believing that the male perceives these three steroids as a series of changing odors, rather than as a single odor mixture. First, each of the three key steroid odorants reaches a peak rate of release at a different time prior to ovulation – AD during the early surge, and 17,20β-P and 17,20β-P-su during the mid- and late surge, respectively. In both their endocrine and behavioral responses, males demonstrate the ability to discriminate mixtures differing in ratio, indicating they have the ability to discriminate among females at different stages in the preovulatory period. Second, and perhaps most importantly, the three key steroid odors are not released together: AD and 17,20β-P are released across the gills in an apparently continuous and passive manner, whereas 17,20β-P-su is released in pulses of urine. Based on release rates and EOG detection thresholds, it is estimated that males should detect the AD:17,20β-P mixture within only a few body lengths behind a moving female, whereas they should be able to detect 17,20β-P-su in urine patches at a much greater distance.

The primer and releaser effects of the preovulatory pheromone act in concert to increase male reproductive success (**Figure 2**). Microsatellite paternity studies show that if a male goldfish is exposed overnight to 17,20β-P (as would occur in the presence of an ovulatory female), and then allowed to spawn in competition with a control male, he sires the great majority of the offspring. This reproductive benefit of pheromone exposure is likely due to both behavioral and physiological factors. Pheromone-exposed males not only perform more spawning acts than control males, but also are more aggressive, a behavioral tactic strongly correlated with paternity. Furthermore, pheromone-exposed males not only ejaculate at least 10 times more sperm than control males, but their sperm are more motile as well, likely due to the action of LH-induced 17,20β-P synthesis in the testes. This increased motility apparently contributes to pheromone-enhanced paternity because, if sperm from 17,20β-P-exposed males are mixed with roughly equal numbers of control sperm and then allowed to compete for fertilization *in vitro*, they fertilize the majority of the eggs.

Female Postovulatory Prostaglandin Pheromone

Within minutes of ovulating, female carp cease releasing the preovulatory steroid pheromone, become sexually active, and begin to release an entirely different hormonal pheromone. Movement of ovulated eggs into the ovisac stimulates synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which in turn rapidly exerts two critical and almost simultaneous effects. First, $PGF_{2\alpha}$ enters the blood and then acts hormonally in the brain to stimulate female spawning behavior. This is easily demonstrated by injecting $PGF_{2\alpha}$ into nonovulated females, which soon perform spawning behaviors that are normal in all aspects except that no eggs are released. Second, $PGF_{2\alpha}$ and a key metabolite, 15-keto-prostaglandin $PGF_{2\alpha}$ (15K-$PGF_{2\alpha}$), are released as a postovulatory pheromone that not only triggers intense chasing and related courtship behaviors in males,

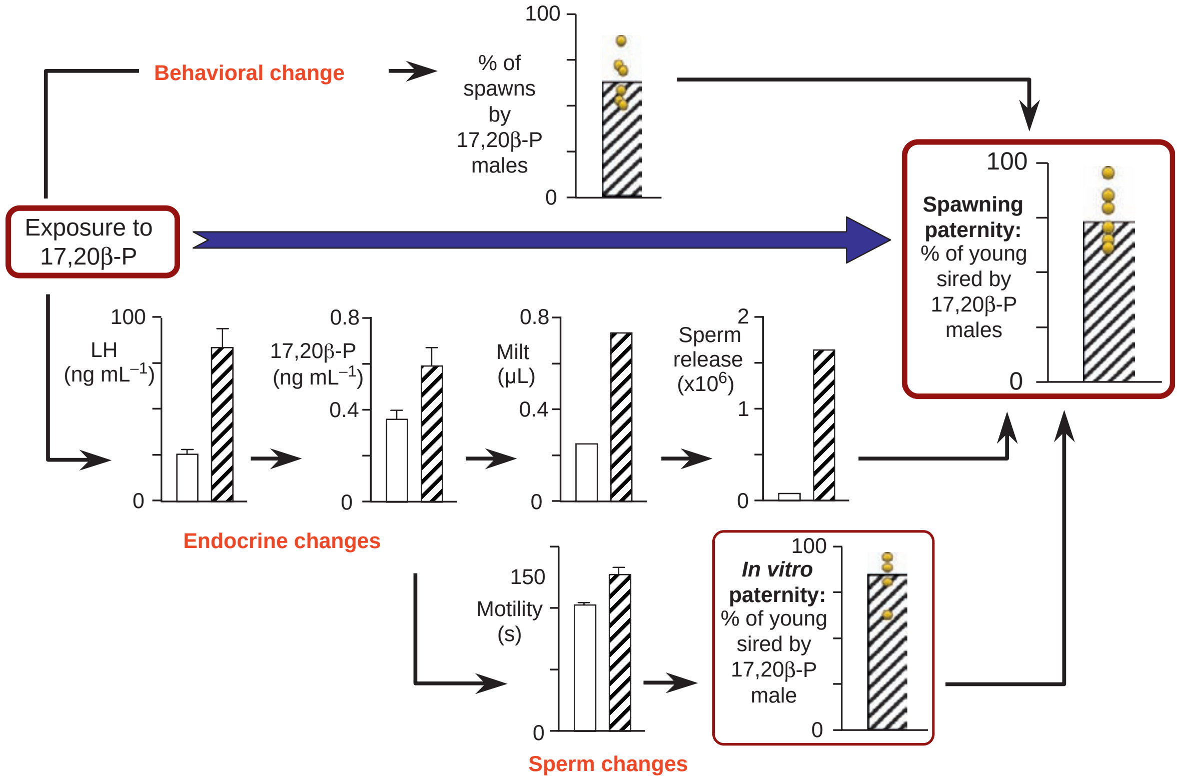


Figure 2 In each of six paired spawning competitions, DNA paternity studies showed that a male exposed overnight to pheromonal 17,20β-P sired a significantly greater percentage of the offspring than did his control male competitor (box at right). Each circle represents the percentage of paternity acquired by each 17,20β-P-exposed individual and the hatched bar represents the mean %. This effect on paternity likely is mediated by both behavioral and physiological changes. For example, in each of the six, paired competitions (top), 17,20β-P-exposed males performed a greater proportion of the total male spawning acts than did control males (again each circle represents one 17,20β-P-exposed individual). Moreover, these pheromone-exposed males (hatched bars) underwent rapid endocrine changes that enabled them to release dramatically more sperm than control males (open bars; middle and bottom graphs). Finally, it is likely that pheromone-induced 17,20β-P synthesis by the testis increases indices of sperm quality such as sperm motility. Such pheromone-induced sperm changes may account for the finding that in four, paired *in vitro* competitions, sperm from 17,20β-P-exposed males fertilized a greater percentage of the eggs than did sperm from control males. Adapted from Dulka JG, Stacey NE, Sorensen PW, and Van Der Kraak GJ (1987) A sex steroid pheromone synchronizes male–female spawning readiness in goldfish. *Nature* 325: 251–253; Hoysak DJ and Stacey NE (2008) Large and persistent effect of a female steroid pheromone on ejaculate size in goldfish. *Journal of Fish Biology* 73: 1573–1584; and Zheng W, Strobeck C, and Stacey NE (1997) The steroid pheromone 17α,20β-dihydroxy-4-pregnen-3-one increases fertility and paternity in goldfish. *Journal of Experimental Biology* 200: 2833–2840.

but also increases male LH concentration and milt volume, likely to maintain milt stores during spawning.

Although PGF$_{2\alpha}$ and 15K-PGF$_{2\alpha}$ appear to induce the same behavioral responses in males, EOG studies show that males have distinct olfactory receptors for these prostaglandins and are more sensitive to 15K-PGF$_{2\alpha}$ than to PGF$_{2\alpha}$. Whereas both PGF$_{2\alpha}$ and 15K-PGF$_{2\alpha}$ are released in urine (and therefore will be encountered as an odor mixture), only PGF$_{2\alpha}$ is released across the gills. From what is known of olfactory detection thresholds and the rates and routes of release, it appears that the urinary prostaglandin odor is able to inform males that an ovulated female is nearby, whereas PGF$_{2\alpha}$ released across the gills is detectable only in the female's immediate vicinity, and thus may identify her in a spawning group. Unlike the situation with the preovulatory steroid pheromone, which females appear to release in an unspecialized manner, female goldfish increase their urination rates just as they are selecting a spawning site in floating vegetation, suggesting that they release the postovulatory prostaglandin pheromone in controlled urinary pulses (**Figure 3**).

Mechanisms of Pheromone Action

The mechanisms mediating the actions of hormonal pheromones have been studied at several levels, from the olfactory sensory neurons, to the olfactory bulbs and tracts, and finally to brain centers through which olfactory input can affect behavior and pituitary function (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes).

Fish possess at least three morphologically distinct olfactory receptor neurons (ORNs) (**see also**

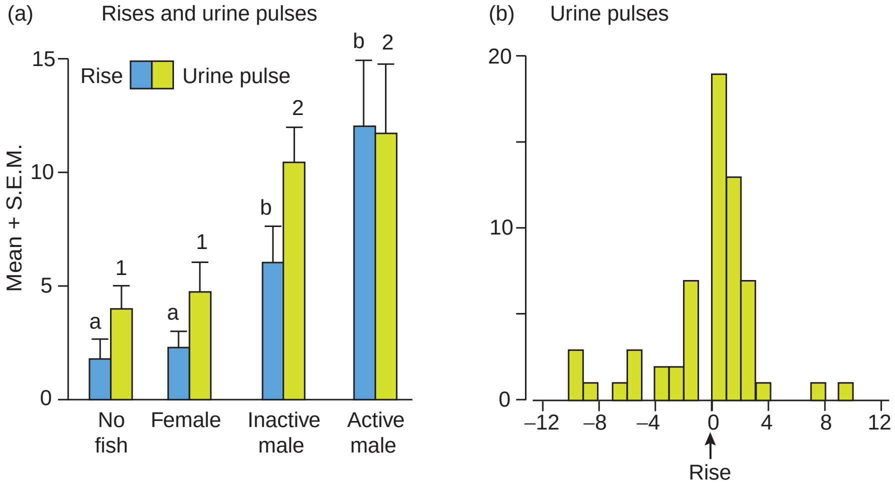


Figure 3 Female goldfish are specialized for signaling with the postovulatory prostaglandin pheromone by altering their urination frequency. (a) Immediately before each spawning act, females rise into floating spawning substrate. During a 60-min test, both rising and urine pulses increased in the presence of an inactive or active male, but not in the presence of a female. Letters (rise) and numbers (urine pulse) denote significant differences between treatments where bars sharing the same letter or number are not significantly different.
(b) Females urinate more frequently in the few seconds just preceding and following rising behavior. Bars indicate the total number of urine pulses released by eight females tested individually in the presence of sexually active males during a 60-min test. Adapted from Appelt CW and Sorensen PW (2007) Female goldfish signal spawning readiness by altering when and where they release a urinary pheromone. *Animal Behaviour* 74: 1329–1338; Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior*, 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier.

Smell, Taste, and Chemical Sensing: Morphology of the Olfactory (Smell) System in Fishes and Neurophysiology of Olfaction); current evidence suggests that it is the ciliated ORNs that detect pheromones. EOG recording studies reveal that, in addition to typically extreme sensitivity and specificity for their hormonal ligands, pheromone-sensitive ORNs in carps and other cyprinids display a consistent pattern of sexual dimorphism (**Figure 4**). For example, EOG responses to steroid odorants are not sexually dimorphic, but are equivalent in males, females, and prepubertal juveniles. In contrast, females and juveniles are much less sensitive to prostaglandins than mature males but, in response to androgen treatment, will develop male-typical EOG sensitivity within several weeks. This is one of the few clear vertebrate examples where sex steroids modulate the function of sensory neurons.

ORNs in the epithelium of the olfactory organs send their axons through the olfactory nerves to the olfactory bulbs, where ORNs with common receptor types converge in glomeruli, complex synaptic connections with the mitral cells, which in turn send their axons in the olfactory tracts to synapse in several brain areas (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes). Relatively little is known about how the brain transduces hormonal pheromone input into biological response (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). However, despite the fact that male goldfish respond to the female's preovulatory and postovulatory pheromones with an apparently similar effect (increased LH concentration and milt volume), it is clear that distinct mechanisms mediate this response to female preovulatory and postovulatory pheromones (**Figure 5**). For example, the preovulatory odorant 17,20β-P increases LH and milt by activating a simple neuroendocrine reflex, because it is effective both in males held in groups and in males isolated from all social stimuli. In contrast, the postovulatory prostaglandin odorants do not increase LH and milt in isolated males, but are effective only when males are grouped or spawning with PGF$_{2\alpha}$-injected females, suggesting that these postovulatory odorants induce their endocrine effects indirectly through the sociosexual behaviors they trigger among conspecifics.

Another key difference between the responses to preovulatory and postovulatory pheromones involves the brain mechanisms that link pheromone detection to LH release from the pituitary, and the role of LH itself (**Figure 5**). Even though both preovulatory and postovulatory female pheromones increase blood LH concentrations in males within 20 min, the milt increase induced by pheromonal 17,20β-P occurs in 3–6 h, whereas that induced by the postovulatory pheromone occurs in less than 1 h and, unlike the 17,20β-P-induced milt increase, is not delayed if the experiment is run in colder water. Pheromonal 17,20β-P increases milt only by stimulating LH secretion, whereas pheromonal prostaglandins trigger at least one additional mechanism. For example, if the goldfish pituitary gland is removed to eliminate the endogenous source of LH, male

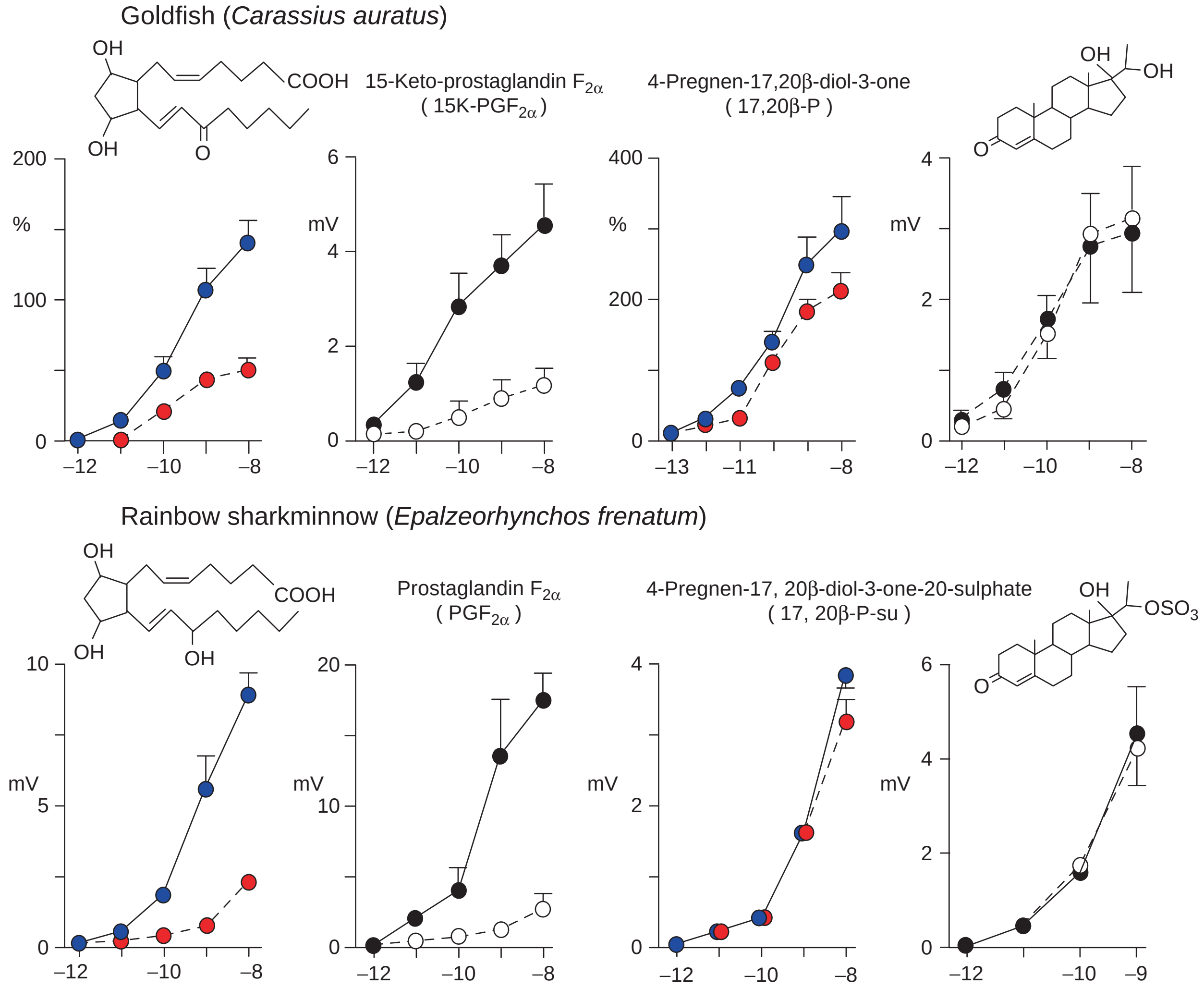


Figure 4 In cyprinid fishes, olfactory sensory responses to pheromonal prostaglandins are sexually dimorphic. EOG studies show that adult males (blue) are more sensitive to prostaglandins than are females (red), whereas males and females are equally responsive to pheromonal steroids. This adult difference likely is induced by male androgenic steroid hormones; because prepubertal juveniles treated with androgen (black circles) are more sensitive to prostaglandins than are untreated juveniles (white circles). Adapted from Belanger RM, Paszkowski MD, and Stacey NE (2009) Methyltestosterone-induced changes in electro-olfactogram responses and courtship behaviors of cyprinids. *Chemical Senses* 35: 65–74; Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior*, 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier; Stacey NE (2009) Pheromones and reproduction. In: Jamieson BGM (ed.) *Reproductive Biology and Phylogeny of Fish* (*Agnatha and Osteichthyes*), pp. 94–137. Enfield, NH: Science Publishers; Stacey NE and Sorensen PW (2006) Reproductive pheromones. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, vol. 24, pp. 359–412. San Diego, CA; and Stacey NE, Chojnacki A, Narayanan A, Cole TB, and Murphy CA (2003) Hormonally-derived sex pheromones in fish: Exogenous cues and signals from gonad to brain. *Canadian Journal of Phhsiology and Pharmacology* 81: 329–341.

goldfish still are able to increase milt volume when spawning with $PGF_{2\alpha}$-injected females but not when they are exposed to 17,20β-P, demonstrating that even though the postovulatory pheromone normally increases LH in intact males, it can also induce a milt response solely through an as-yet unknown extra-pituitary pathway.

The female goldfish's preovulatory and postovulatory pheromones also induce male LH release from the pituitary through distinct mechanisms (**Figure 5**). Under normal conditions, LH release from the goldfish pituitary is constantly inhibited by dopamine-containing neurons in the brain that make direct synaptic contact with pituitary LH cells (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). The preovulatory pheromone 17,20β-P appears to increase LH release by removing this dopamine inhibition because, if male goldfish are first injected with a dopamine receptor agonist to maintain dopamine inhibition, the effect of 17,20β-P on LH is blocked; in contrast, dopamine receptor agonists do not prevent LH increase in males spawning with $PGF_{2\alpha}$-injected females.

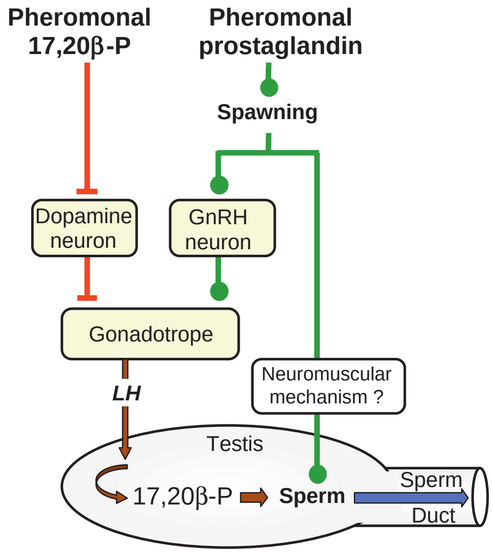


Figure 5 The preovulatory steroid pheromone and postovulatory prostaglandin pheromone of goldfish increase releasable sperm through different mechanisms. Pheromonal 17,20β-P appears to act only by inhibiting hypothalamic dopamine neurons that inhibit release of LH from pituitary gonadotropes. In contrast, pheromonal prostaglandin increases releasable sperm through at least two mechanisms. Pheromonal prostaglandin increases sperm by GnRH-induced LH increase, and also acts through a nonpituitary mechanism that has yet to be characterized. Adapted from Zheng W and Stacey NE (1997) A steroidal pheromone and spawning stimuli act via different neuroendocrine mechanisms to increase gonadotropin and milt volume in male goldfish (*Carassius auratus*). *General and Comparative Endocrinology* 105: 228–235; Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior*, 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier; and Zheng W and Stacey NE (1996) Two mechanisms for increasing milt volume in male goldfish. *Journal of Experimental Zoology* 276: 287–295.

Male-Derived Hormonal Pheromones

Given that their mating systems do not involve defense of a territory, mates, or offspring, it is perhaps not surprising that male carps have evolved complex mechanisms not only to anticipate ovulation and locate ovulated females, but also to increase their success in sperm competition. As discussed in the following section, interactions among male goldfish have dramatic and complex effects on testes and sperm that clearly appear to be adaptive tactics for sperm competition, although it is not yet clear if all these effects are mediated by hormonal pheromones. Nonetheless, two key findings suggest that interactions among males involve a pheromonal role for the steroid odorant AD. First, mature males release very large quantities of AD ($\sim$50 ng h^{-1}), and this release increases more than 10-fold when males begin to spawn. Based on release rates and EOG detection thresholds, spawning males have been estimated to produce an AD active space of $\sim$250 l h^{-1}; by comparison, it is estimated that the female's peak preovulatory release of AD ($\sim$100 ng h^{-1}) and 17,20β-P ($\sim$5 ng h^{-1}) generate active spaces of only 30 L h^{-1}. Second, aquarium studies demonstrate that AD released by dominant, sexually active males, mediates male–male interactions, stimulating agonistic interactions and inhibiting the milt increase that is normally induced by female pheromonal 17,20β-P. A key question, of course, is whether these effects might normally be exerted by AD released from males, or females, or both. Nevertheless, it is clear that AD serves as a hormonal sex pheromone in this species (and likely other carps) and that activity is linked with pheromone composition.

A Goldfish Chemical Network?

The preceding discussion is inevitably distorted by the fact that virtually all our understanding of goldfish hormonal pheromones pertains to female effects on males, and because it is convenient to discuss these effects in terms of an individual female sender and individual male receiver. However, hormonal pheromone effects are not only restricted to female effects on males, but also include intrasexual effects within each gender. Moreover, from the size of their pheromonal active spaces, and the proximity of goldfish in social groups, it is likely that these odorants operate to synchronize the final reproductive processes of many individuals within the local aggregations that form in early spring in typically turbid waters.

Male–male interactions affecting gonads and gametes are as dramatic as those between female goldfish, but evidently more complex, because males can either increase or decrease their milt production in response to the reproductive status of their male competitors. For example, if one male in a group is induced to increase milt stores by gonadotropin injection or exposure to 17,20β-P, his untreated tank mates will also increase their milt. On the other hand, males increase their milt volume when isolated from a group, and decrease their milt volume when returned. Finally, males that have been induced to increase milt volume (through either isolation or exposure to 17,20β-P) in turn stimulate milt production in additional males. These male–male effects might not be exclusively mediated by hormonal pheromones, and nothing is known about the underlying mechanisms, except that they do not involve a change in LH. Nonetheless, it is clear that males adaptively adjust milt production to the perceived level of sperm competition through cues provided by both males and females.

Putting all of this together, it appears that, prior to the occurrence of a female preovulatory LH surge, the male goldfish is both a source and a receiver of unknown cues that suppress sperm stores by maintaining basal concentrations of LH and testicular steroids, such as 17,20β-P

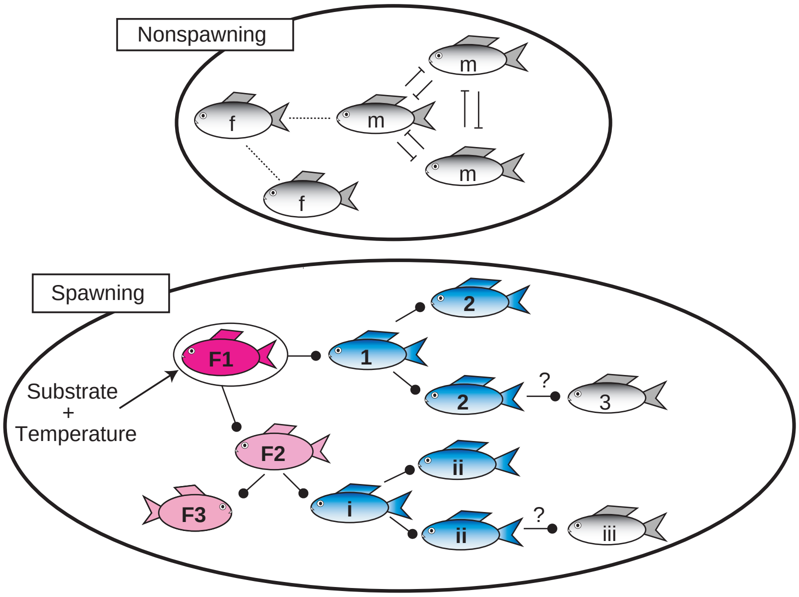


Figure 6 Proposed mechanism for pheromone-mediated synchronous ovulation and mass spawning in goldfish. In the nonspawning condition of early spring, when water temperature is low and aquatic vegetation (spawning substrate) has not begun to grow, males (m) are in a basal endocrine state and release unknown cues that suppress milt volume in other males. Laboratory experiments suggest that the transition to the spawning condition can occur when increasing water temperature and appearance of aquatic vegetation trigger an ovulatory LH surge in a single female (F1), releasing a preovulatory steroid pheromone that in turn triggers ovulatory LH surges in additional females (F2 and F3). The preovulatory pheromone also directly increases releasable sperm in males (1 and i) by increasing LH, and indirectly increases sperm in additional males (2 and ii) through an unknown mechanism; it is not known whether such indirectly stimulated males can increase releasable sperm in additional males (3 and iii). Adapted from Stacey NE (2003) Hormones, pheromones and reproductive behavior. *Fish Physiology and Biochemistry* 28: 229–235; Wisenden BD and Stacey NE (2005) Fish semiochemicals and the evolution of communication networks. In: McGregor PK (ed.) *Animal Communication Networks*, pp. 540–567. London: Cambridge University Press; Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior*, 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier.

(**Figure 6**). However, when exogenous stimuli such as warm temperature and aquatic vegetation trigger a preovulatory LH surge in at least one female, this stable, negative-feedback interaction among males is rapidly perturbed. Males and females encountering the preovulatory pheromone in turn increase their LH, thereby amplifying and disseminating the original stimulus and promoting synchronous final maturation (ovulation and increased sperm stores) of individuals within the local chemical network. Although it is difficult to exclude the possibility that these pheromone-mediated elements of goldfish reproductive physiology have been influenced by domestication, EOG and other studies both in the laboratory and in the field indicate that remarkably similar hormonal pheromone systems are used by the goldfish and the closely related crucian and common carps.

Hormonal Pheromones and Species Specificity

In general, the evolution of species recognition systems that enable reproductive interactions to be restricted to conspecifics is assumed to be a prerequisite for successful reproduction. Numerous studies have shown that many fish will behaviorally discriminate between the odor of conspecifics and the odor of another, even closely related, species. However, there are two key reasons why these observations might be difficult to reconcile with the fact that the use of hormonal compounds as pheromones appears to be widespread among the fishes: (1) sex steroids and prostaglandins are highly conserved throughout the vertebrates, making them poor candidates for species-specific odorants and (2) although EOG recordings have shown that distantly related taxa typically detect different steroid and prostaglandin odorants, they also show that the hormonal odorants detected by closely related species, such as the carps, can be remarkably similar. Our current understanding of hormonal pheromones does not allow us to reconcile these observations in the context of species specificity, because it is based almost entirely on simple laboratory experiments using single synthetic odorants, whereas insight into species specificity will require studies of the effects of whole body odors under natural conditions. Because pheromonal species specificity is a remarkably complex issue that is very poorly understood in fish, we end our brief review with several comments intended to stimulate further research in this area.

First, species specificity does not mean that all pheromones are unique, but simply distinct from those of historically encountered heterospecifics. Selection for specificity should be expected only where the fitness of a species is reduced by entering the pheromonal active space of heterospecifics. From this perspective, we predict that many fishes never experience such intimate reproductive sympatry, simply because they and neighboring species create only small pheromonal active spaces.

Second, although fish androgen (11-ketotestosterone), estrogen (17β-estradiol), and MISs (17,20β-P and 17,20β,21-P) have no ability to encode species specificity, this is not the case for their precursors and metabolites. EOG studies show that the MIS-related steroids in particular are highly diverse odorants in fishes because they can be synthesized and metabolized in many ways, making the number of MIS-related metabolites released by fish potentially enormous. Unfortunately, it is not known if fish have taken advantage of this potential for species-specific steroid odorants, because steroid metabolism and release is not an active area of research at this time.

Third, the olfactory receptors for hormonal pheromones are highly specific, such that a single enzymatic change to a potent steroid or prostaglandin odorant can make it undetectable at physiological concentrations. This characteristic of hormonal pheromone function is highly relevant to the issue of species specificity, because it means that even subtle changes in hormone metabolism can have drastic effects on the pattern of intimate reproductive sympatry.

Fourth, and perhaps most importantly, studies of hormonal pheromones have yet to test the possibility that hormonal pheromones might not be single compounds, but rather mixtures that could contain both hormonal and nonhormonal odorants. Indeed, the use of complex odor mixtures is perhaps to be expected, given that all insect and mammalian pheromones that have been fully characterized and then tested in field conditions are mixtures of two to seven compounds.

Finally, although responses to pheromones are generally assumed to be innate or unlearned, this need not be the case, particularly in long-lived species such as the carps that mate in large groups many times in their life and that would have numerous opportunities to associate hormonal and nonhormonal odorants with reproductive events.

In conclusion, two things are clear: many fishes detect hormonal odorants and many fishes discriminate between conspecific and heterospecific odors. A thoughtful consideration of the implications should lead to some very exciting research.

See also: **Hagfishes and Lamprey**: Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior; Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Olfaction. **The Reproductive Organs and Processes**: Vitellogenesis in Fishes. **Toxicology**: The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Appelt CW and Sorensen PW (2007) Female goldfish signal spawning readiness by altering when and where they release a urinary pheromone. *Animal Behaviour* 74: 1329–1338.

Belanger RM, Paszkowski MD, and Stacey NE (2010) Methyltestosterone-induced changes in electro-olfactogram responses and courtship behaviors of cyprinids. *Chemical Senses* 35: 65–74.

Cole TB and Stacey NE (2006) Olfactory responses to steroids in an African mouth-brooding cichlid, *Haplochromis burtoni* (Günther). *Journal of Fish Biology* 68: 661–680.

Derby CD and Sorensen PW (2008) Neural processing, perception and behavioral responses to natural chemical stimuli by fish and crustaceans. *Journal of Chemical Ecology* 34: 898–914.

Dulka JG, Stacey NE, Sorensen PW, and Van Der Kraak GJ (1987) A sex steroid pheromone synchronizes male–female spawning readiness in goldfish. *Nature* 325: 251–253.

Fraser EJ and Stacey N (2002) Isolation increases milt production in goldfish. *Journal of Experimental Zoology* 293: 511–524.

Hamdani EH and Doving KB (2007) The functional organization of the fish olfactory system. *Progress in Neurobiology* 82: 80–86.

Hoysak DJ and Stacey NE (2008) Large and persistent effect of a female steroid pheromone on ejaculate size in goldfish. *Journal of Fish Biology* 73: 1573–1584.

Kobayashi M, Sorensen PW, and Stacey NE (2002) Hormonal and pheromonal control of spawning behavior in the goldfish. *Fish Physiology and Biochemistry* 26: 71–84.

Olsén KH, Sawisky GR, and Stacey NE (2006) Endocrine and milt responses of male crucian carp (*Carassius carassius* L.) to periovulatory females under field conditions. *General and Comparative Endocrinology* 149: 294–302.

Scott AP and Sorensen PW (1994) Time course of release of pheromonally active steroids and their conjugates by ovulatory goldfish. *General and Comparative Endocrinology* 96: 309–323.

Sisler SP and Sorensen PW (2008) Common carp and goldfish discern conspecific identity using chemical cues. *Behaviour* 145: 1409–1425.

Sorensen PW, Pinillos M, and Scott AP (2005) Sexually mature male goldfish release large quantities of androstenedione into the water where it functions as a pheromone. *General and Comparative Endocrinology* 140: 164–175.

Stacey NE and Sorensen PW (2009) Fish hormonal pheromones. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds.) *Hormones, Brain, and Behavior,* 2nd edn., vol. 2, pp. 639–681. San Diego, CA: Elsevier.

Stacey NE, Sorensen PW, Van Der Kraak GJ, and Dulka JG (1989) Direct evidence that 17α,20β-dihydroxy-4-pregnen-3-one functions as a goldfish primer pheromone: Preovulatory release is closely associated with male endocrine responses. *General and Comparative Endocrinology* 75: 62–70.

Zheng W and Stacey NE (1997) A steroidal pheromone and spawning stimuli act via different neuroendocrine mechanisms to increase gonadotropin and milt volume in male goldfish (*Carassius auratus*). *General and Comparative Endocrinology* 105: 228–235.

Zheng W, Strobeck C, and Stacey NE (1997) The steroid pheromone 17α,20β-dihydroxy-4-pregnen-3-one increases fertility and paternity in goldfish. *Journal of Experimental Biology* 200: 2833–2840.

ENCYCLOPEDIA OF
FISH PHYSIOLOGY: FROM GENOME TO ENVIRONMENT

This page intentionally left blank

ENCYCLOPEDIA OF FISH PHYSIOLOGY: FROM GENOME TO ENVIRONMENT

Volume 3

ENERGETICS, INTERACTIONS WITH THE ENVIRONMENT, LIFESTYLES, AND APPLICATIONS

Editor-in-Chief
ANTHONY P. FARRELL
Department of Zoology,
University of British Columbia,
Vancouver, BC, Canada

AMSTERDAM BOSTON HEIDELBERG LONDON NEW YORK OXFORD
PARIS SAN DIEGO SAN FRANCISCO SINGAPORE SYDNEY TOKYO
Academic Press is an imprint of Elsevier
ELSEVIER
ACADEMIC PRESS

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NWI 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

Copyright © 2011 Elsevier Inc. All rights reserved

The following articles are US government works in the public domain and are not subject to copyright:
Energetic Models: Bioenergetics in Ecosystems
Hormonal Control of Metabolism and Ionic Regulation: The Hormonal Control of Osmoregulation in Teleost Fish
Swimming and Other Activities: Applied Aspects of Fish Swimming Performance
The Skin: Functional Morphology of the Integumentary System in Fishes; The Many Functions of Fish Integument

The following articles are Crown Copyright:
Fish Migrations: Eel Migrations
Social and Reproductive Behaviors: Sexual Behavior in Fish

No part of this publication may be reproduced, stored in a retrieval system
or transmitted in any form or by any means electronic, mechanical, photocopying,
recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at (http://elsevier.com/locate/permissions), and selecting *Obtaining permission to use Elsevier material*

Notice
No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein, Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Catalog Number: 2011923567

ISBN: 978-0-12-374545-3

For information on all Elsevier publications
visit our website at books.elsevier.com

Printed and bound in Italy

11 12 13 14 15 10 9 8 7 6 5 4 3 2 1

For supplementary material please visit the companion site:
http://www.elsevierdirect.com/companions/9780123745453

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER BOOK AID International Sabre Foundation

Editorial: Kate Mittell
Production: Nicky Carter

EDITORS

EDITOR-IN-CHIEF

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ASSOCIATE EDITORS

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island,
Charlottetown, PE, Canada

This page intentionally left blank

EDITORS' BIOGRAPHIES

Editor-in-Chief

Anthony P. Farrell, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Tony Farrell is a graduate of Bath University, where he was fortunate to study with Peter Lutz. His fortunes grew further when he moved in 1974 to Canada and the Zoology Department at the University of British Columbia (UBC) to complete his PhD degree under the excellent tutelage of Dave Randall. In 2004, Tony returned to UBC when he accepted an endowed research chair in sustainable aquaculture. In between these positions at UBC, Tony was employed at the University of Southern California (PDF), the University of New Brunswick (sessional lecturer), Mount Allison University (first real job), and Simon Fraser University (moving through the ranks to a full professor). In addition to highly controlled laboratory experiments on fish cardiorespiratory physiology, Tony is committed to working on animals in their own environment. Therefore, his research on fish physiology has taken him on an *Alpha Helix* expedition to the Amazon, the University of Gothenburg and the Kristineberg Marine Research Station in Sweden, the Portobello Marine Biological Station in New Zealand, the University of Christchurch and Massey University in New Zealand, the Bamfield Marine Science Station and the Huntsman Marine Station in Canada, the University of Aarhus in Denmark, the University of Adelaide Charles and Darwin University in Australia, and to the Danish Arctic Marine Station on Disco Island in Greenland. These travels have allowed him to work with many superb collaborators wordwide, as well as study the physiology of over 70 different species of fish. Tony has received a number of awards for his scientific contributions: an honorary degree from the University of Gothenburg in Sweden; Awards of Excellence from the American Fisheries Society for Fish Physiology, Conservation and Management; the Fry Medal from the Canadian Society of Zoologists; and the Beverton Medal from the Fisheries Society of the British Isles.

Associate Editor

Joseph J. Cech, Jr., Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

Joe Cech grew up in northern Illinois <1 km from the Salt Creek Preserve, where he spent many hours as a boy. Summer trips to northern Wisconsin, northern Minnesota, and southern Ontario increased his interest in aquatic habitats and their fishes. He graduated from the University of Wisconsin (UW, Madison), where he was fortunate to study oceanography under Robert Ragotzkie and fish biology under Art Hasler and Fran Henderson in the UW Laboratory of Limnology. Before completing his BS degree in zoology, he was fortunate to serve as the resident zoologist aboard the R/V *Dante Deo*, for a year, as it crossed the Caribbean Sea and the South Pacific Ocean. Completing his degree upon his return, he was hired as a research assistant at the University of Texas' Marine Science Institute (UTMSI), where he earned his MA and PhD degrees in zoology, under the supervision of Curly Wohschlag. During his graduate education in Austin (Texas), he studied fish biology under Clark Hubbs and physiology under Jack Myers and Jim Larimer. After a 2-year postdoctoral position in Maine, he was hired as a fish physiological ecologist faculty member at the University of California (UC), Davis, in the Department of Wildlife, Fish, and Conservation Biology.

During his 32 years as an active faculty member at UC Davis, his research on the physiological ecology of fishes took him to Canada (University of British Columbia, Bamfield Marine Station), Finland (University of Helsinki), and to other laboratories in the USA (University of Tennessee, Scripps Institution of Oceanography, R/V *David Starr Jordan* in the North Pacific Ocean, UTMSI, Oregon Institute of Marine Biology, and the UC Bodega Marine Laboratory) where he collaborated with many excellent scientists. During this period, he taught courses in physiology of fishes, physiological ecology, and field studies in fish biology; he supervised 25 graduate students (10 MS and

15 PhD) in the disciplinary areas of ecology, physiology, zoology, and pharmacology/toxicology at UC Davis. He is a fellow of the AIFRB and AAAS, and he received Awards of Excellence from the American Fisheries Society (Physiology Section, and California-Nevada Chapter) and the UC Davis Prize for Undergraduate Teaching and Scholarly Achievement. Finally, he co-authored a popular ichthyology textbook, with Peter Moyle, and he published more than 160 peer-reviewed publications on the physiological ecology of fishes. He is currently Professor Emeritus of Fish Biology at the University of California Davis.

Associate Editor

Jeffrey G. Richards, Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Jeffrey Richards is an associate professor in the Department of Zoology at the University of British Columbia, Canada. He started his academic career in the Department of Biology at Wilfrid Laurier University obtaining a BSc in 1995. He then went on to do an MSc at the University of Waterloo under the guidance of the late Prof. Richard Playle. With Prof. Playle, he studied environmental physiology and toxicology in fish. Following his MSc, he entered the laboratory of Prof. Chris Wood at McMaster University and focused his research on energy metabolism in swimming fish. He completed his PhD in 2002 and took up a postdoctoral fellowship at the University of British Columbia with Prof. Patricia Schulte. After a 2-year postdoctoral work, he started at the University of British Columbia as an assistant professor in 2004. His lab studies the relationship between plasticity and adaptive variation in the mechanisms that initiate, maintain, and coordinate metabolic energy balance in animals during exposure to environmental stress. He is particularly interested in the adaptations to low oxygen (hypoxia) in fish and other lower vertebrates. He prefers, whenever possible, to study animals in their natural habitat; thus, he travels extensively to unique and challenging environments, including the Amazon and the Tibetan plateau.

Associate Editor

E. Don Stevens, Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

Don Stevens did an MSc with the late Edgar Black on the effects of severe exercise on metabolites in trout in the Physiology Department at UBC after an undergraduate degree in mathematics and zoology at the University of Victoria. He then moved across the street to do a PhD on effects of moderate exercise on respiration and circulation in trout with Dave Randall, who just had been hired in the Zoology Department at UBC (summarized in a JEB classics article, *Journal of Experimental Biology* 211: 1521). His first position was at the Zoology Department at the University of Hawaii where he discovered that there was more to the fish world than trout, and that there was no such thing as the physiology of fish, rather there was a physiology of fishes. In Hawaii, he worked mostly on the biology of tropical tunas with a focus on their warm-bodiedness, in particular how they achieved higher than ambient body temperatures and the adaptive advantage of them. He then joined the Zoology Department at the University of Guelph working in the general area of comparative physiology with a focus on exercise effects until he retired in 2004. He now has an adjunct position at the Atlantic Veterinary College and continues to do research in fish physiology.

SECTION EDITORS

C Brauner
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

AB Butler
Department Molecular Neuroscience, George Mason University, Fairfax, VA, USA

BA Carlson
Department of Biology, Washington University, St. Louis, MO, USA

BM Casper
Department of Biology, University of Maryland, College Park, MD, USA

JJ Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA, USA

AP Farrell
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

G Goss
Biological Sciences, University of Alberta, Edmonton, AB, Canada

JB Graham
Department of Economics, University of California at San Diego, La Jolla, CA, USA

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

S Holmgren
Zoophysiology, University of Goteberg, Goteberg, Sweden

N Maclean
Biological Sciences, University of Southampton, Hampshire, UK

JA Nelson
Department of Biological Sciences, Towson University, Towson, MD, USA

KR Olson
Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA

C Olsson
Zoophysiology, University of Goteberg, Goteberg, Sweden

AN Popper
Department of Biology, University of Maryland, College Park, MD, USA

JG Richards
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

PM Schulte
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

RE Shadwick
Department of Zoology, University of British Columbia, Vancouver, BC, Canada

K Sloman
University of the West of Scotland, Paisley, Scotland, UK

ED Stevens
Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

AP Summers
Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, USA

M Vijayan
Department of Biology, University of Waterloo, Waterloo, ON, Canada

H-J Wagner
Universitaet Tuebingen, Tuebingen, Germany

This page intentionally left blank

CONTRIBUTORS

MV Abrahams
Memorial University of Newfoundland, St. John's, NL, Canada

RMcN Alexander
University of Leeds, Leeds, UK

D Alsop
McMaster University, Hamilton, ON, Canada

N Aluru
Woods Hole Oceanographic Institution, Woods Hole, MA, USA

D Álvarez
Universidad de Oviedo, Oviedo, Spain

B Austin
University of Stirling, Stirling, UK

M Axelsson
University of Gothenburg, Gothenburg, Sweden

R Baker
New York University Medical Center, New York, NY, USA

AM Bakke
Norwegian School of Veterinary Science, Oslo, Norway

JS Ballantyne
University of Guelph, Guelph, ON, Canada

S Balshine
McMaster University, Hamilton, ON, Canada

AH Bass
Cornell University, Ithaca, NY, USA

TL Beitinger
University of North Texas, Denton, TX, USA

TJ Benfey
University of New Brunswick, Fredericton, NB, Canada

M Berenbrink
University of Liverpool, Liverpool, UK

D Bernal
University of Massachusetts, Dartmouth, MA, USA

TR Binder
Hammond Bay Biological Station, Millersburg, MI, USA

BA Block
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

A Böhne
Université de Lyon, Lyon, France

NC Bols
University of Waterloo, Waterloo, ON, Canada

JK Bowmaker
University College London, London, UK

I Braasch
University of Oregon, Eugene, OR, USA

VA Braithwaite
Penn State University, State College, PA, USA; University of Bergen, Norway

MH Braun
University of Calgary, Calgary, AB, Canada

CJ Brauner
University of British Columbia, Vancouver, BC, Canada

DL Breitburg
Smithsonian Environmental Research Center, Edgewater, MD, USA

F Brunet
Université de Lyon, Lyon, France

J Buckley
University of Plymouth, Plymouth, UK

T Burt de Perera
University of Oxford, Oxford, UK

D Burton
Memorial University of Newfoundland, St. John's, NL, Canada

AB Butler
George Mason University, Fairfax, VA, USA

DC de Caprona
University of Iowa, Iowa City, IA, USA

AA Caputi
Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

BA Carlson
Washington University in St. Louis, St. Louis, MO, USA

MA Caruso
North Dakota State University, Fargo, ND, USA

MR de Carvalho
Universidade de São Paulo, São Paulo, Brazil

BM Casper
University of Maryland, College Park, MD, USA

T Castro-Santos
USGS Biological Resources Discipline, Turners Falls, MA, USA

JJ Cech Jr.
University of California, Davis, CA, USA

JN Cech
Howard Hughes Medical Institute, Boston, MA, USA

D Chabot
Institut Maurice-Lamontagne, Mont-Joli, QC, Canada

MJ Chacron
McGill University, Montreal, QC, Canada

K Cheney
The University of Queensland, Brisbane, QLD, Australia

KM Claeson
University of Texas at Austin, Austin, TX, USA

SJ Cooke
Carleton University, Ottawa, ON, Canada

SL Coombs
Bowling Green State University, Bowling Green, OH, USA

AJ Cotel
University of Michigan, Ann Arbor, MI, USA

LI Crawshaw
Portland State University, Portland, OR, USA

GT Crossin
Simon Fraser University, Burnaby, BC, Canada

S Currie
Mount Allison University, Sackville, NB, Canada

CP Cutler
Georgia Southern University, Statesboro, GA, USA

MN Dean
Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

J-P Denizot
CNRS, Gif sur Yvette, France

RH Devlin
Fisheries and Oceans Canada, West Vancouver, BC, Canada

RJ Díaz
Virginia Institute of Marine Science, Gloucester Point, VA, USA

KA Dickson
California State University Fullerton, Fullerton, CA, USA

P Domenici
CNR – IAMC, Torregrande (Or), Italy

SI Doroshov
University of California, Davis, CA, USA

RH Douglas
City University London, London, UK

WW Dowd
Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

K Dunlap
Trinity College, Hartford, CT, USA

S Egginton
University of Birmingham, Birmingham, UK

DJ Ellerby
Wellesley College, Wellesley, MA, USA

DG Elliott
Western Fisheries Research Center, US Geological Survey, Seattle, WA, USA

J Engelmann
University of Bielefeld, Bielefeld, Germany

DH Evans
University of Florida, Gainesville, FL, USA

DS Faber
Albert Einstein College of Medicine, Bronx, NY, USA

AP Farrell
University of British Columbia, Vancouver, BC, Canada

RR Fay
Loyola University Chicago, Chicago, IL, USA

LA Ferry
Arizona State University, Phoenix, AZ, USA

PA Fields
Franklin & Marshall College, Lancaster, PA, USA

IP Forster
Fisheries and Oceans Canada, West Vancouver, BC, Canada

ME Forster
University of Canterbury, Christchurch, New Zealand

ES Fortune
Johns Hopkins University, Baltimore, MD, USA

B Fritzsch
University of Iowa, Iowa City, IA, USA

DS Fudge
University of Guelph, Guelph, ON, Canada

D Galiana-Arnoux
Université de Lyon, Lyon, France

GLJ Galli
University of Manchester, Manchester, UK

AK Gamperl
Memorial University of Newfoundland, St. John's, NL, Canada

DP German
University of California, Irvine, CA, USA

TE Gillis
University of Guelph, Guelph, ON, Canada

KM Gilmour
University of Ottawa, Ottawa, ON, Canada

D Giordano
National Research Council (CNR), Naples, Italy

J Godwin
North Carolina State University, Raleigh, NC, USA

A González
University Complutense of Madrid, Madrid, Spain

RJ Gonzalez
University of San Diego, San Diego, CA, USA

A Gräns
University of Gothenburg, Gothenburg, Sweden

AY Gracey
University of Southern California, Los Angeles, CA, USA

JB Graham
University of California at San Diego, La Jolla, CA, USA

S Grillner
Karolinska Institutet, Stockholm, Sweden

H Guderley
Université Laval, Montreal, QC, Canada

TJ Hara
University of Manitoba, Winnipeg, MB, Canada

MC Hastings
Georgia Institute of Technology, Atlanta, GA, USA

AD Hawkins
Loughine Ltd., Aberdeen, Scotland, UK

LP Hernandez
George Washington University, Washington, DC, USA

JE Herr
University of Guelph, Guelph, ON, Canada

MJ Herrero-Turrión
Institute of Neurosciences of Castilla y León, Salamanca, Spain

TE Higham
Clemson University, Clemson, SC, USA

SD Hillyard
UNLV School of Dental Medicine, Las Vegas, NV, USA

EJ Hilton
Virginia Institute of Marine Science, Gloucester Point, VA, USA

SG Hinch
University of British Columbia, Vancouver, BC, Canada

MH Hofmann
University of Bonn, Bonn, Germany

S Holmgren
University of Gothenburg, Gothenburg, Sweden

DR Huber
University of Tampa, Tampa, FL, USA

P-P Hwang
Academia Sinica, Taipei, Taiwan, China

JM Jørgensen
University of Aarhus, Aarhus, Denmark

WR Jeffery
University of Maryland, College Park, MD, USA

E Jönsson
University of Gothenburg, Gothenburg, Sweden

M Jobling
University of Tromsø, Tromsø, Norway

DR Jones
University of British Columbia, Vancouver, BC, Canada

MG Jonz
University of Ottawa, Ottawa, ON, Canada

F Jutfelt
University of Gothenburg, Gothenburg, Sweden

IM Kaatz
Stamford, CT, USA

I Katsiadaki
Cefas Weymouth Laboratory, Weymouth, UK

A Kawano
University of Waterloo, Waterloo, ON, Canada

M Kawasaki
University of Virginia, Charlottesville, VA, USA

CJ Kennedy
Simon Fraser University, Burnaby, BC, Canada

P Kiilerich
Institut National de la Recherche Agronomique (INRA), Rennes, France

SS Killen
University of Glasgow, Glasgow, Scotland, UK

M Kinoshita
Kyoto University, Kyoto, Japan

F Kirschbaum
Humboldt-Universität zu Berlin, Berlin, Germany

CS Krahforst
East Carolina University, Greenville, NC, USA

Å Krogdahl
Norwegian School of Veterinary Science, Oslo, Norway

RHH Kröger
Lund University, Lund, Sweden

D Kültz
University of California, Davis, CA, USA

F Ladich
University of Vienna, Vienna, Austria

LEJ Lee
Wilfrid Laurier University, Waterloo, ON, Canada

B Levavi-Sivan
Hebrew University of Jerusalem, Rehovot, Israel

MW Levine
University of Illinois at Chicago, Chicago, IL, USA

D Liedtke
University of Würzburg, Würzburg, Germany

JH Long Jr.
Vassar College, Poughkeepsie, NY, USA

Z Lu
University of Miami, Coral Gables, FL, USA

JJ Luczkovich
East Carolina University, Greenville, NC, USA

WI Lutterschmidt
Sam Houston State University, Huntsville, TX, USA

P Machnik
University of Bayreuth, Bayreuth, Germany

N Maclean
University of Southampton, Southampton, UK

CP Madenjian
Great Lakes Science Center, Ann Arbor, MI, USA

H Malte
Aarhus University, Aarhus, Denmark

F Marino
University of Messina, Messina, Italy

NJ Marshall
The University of Queensland, Brisbane, QLD, Australia

GB McClelland
McMaster University, Hamilton, ON, Canada

CA McCormick
Oberlin College, Oberlin, OH, USA

SD McCormick
USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, USA

DJ McKenzie
Université Montpellier 2, Montpellier, France

DB McMillan
University of Western Ontario, London, ON, Canada

M Megías
University of Vigo, Vigo, Spain

AF Mensinger
University of Minnesota Duluth, Duluth, MN, USA

JD Metcalfe
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

WK Milsom
University of British Columbia, Vancouver, BC, Canada

CMR Le Moine
University of Ottawa, Ottawa, ON, Canada

JC Montgomery
University of Auckland, Auckland, New Zealand

TW Moon
University of Ottawa, Ottawa, ON, Canada

PC Morris
Heriot-Watt University, Edinburgh, UK

CD Moyes
Queen's University, Kingston, ON, Canada

CA Myrick
Colorado State University, Fort Collins, CO, USA

JA Nelson
Towson University, Towson, MD, USA

M Nikinmaa
University of Turku, Turku, Finland

GE Nilsson
University of Oslo, Oslo, Norway

S Nilsson
University of Gothenburg, Gothenburg, Sweden

RH Nóbrega
Utrecht University, Utrecht, The Netherlands

RG Northcutt
University of California, San Diego, La Jolla, CA, USA

D Northmore
University of Delaware, Newark, DE, USA

KR Olson
Indiana University School of Medicine – South Bend, South Bend, IN, USA

C Olsson
University of Gothenburg, Gothenburg, Sweden

K Pappas
Trinity College, Hartford, CT, USA

JC Partridge
University of Bristol, Bristol, UK

MA Peck
University of Hamburg, Hamburg, Germany

B Pelster
University of Innsbruck, Innsbruck, Austria

AE Pereda
Albert Einstein College of Medicine, Bronx, NY, USA

SF Perry
University of Ottawa, Ottawa, ON, Canada

S Pieperhoff
University of British Columbia, Vancouver, BC, Canada

F Piferrer
Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain

JE Podrabsky
Portland State University, Portland, OR, USA

MA Pombal
University of Vigo, Vigo, Spain

H-O Pörtner
Alfred-Wegener-Institute, Bremerhaven, Germany

AN Popper
University of Maryland, College Park, MD, USA

G di Prisco
National Research Council (CNR), Naples, Italy

P Prunet
Institut National de la Recherche Agronomique (INRA), Rennes, France

DJ Randall
City University of Hong Kong, Hong Kong, China

BJ Reading
North Carolina State University, Raleigh, NC, USA

SG Reebs
Université de Moncton, Moncton, NB, Canada

SG Reid
University of Toronto Scarborough, Toronto, ON, Canada

JG Richards
University of British Columbia, Vancouver, BC,
Canada

DA Righton
Centre for Environment, Fisheries and Aquaculture
Science, Lowestoft, UK

JW Robinson
University of Guelph, Guelph, ON, Canada

RE Rodríguez
Institute of Neurosciences of Castilla y León, Salamanca,
Spain

I Rodríguez-Moldes
Universidad de Santiago de Compostela, Santiago de
Compostela, Spain

PJ Rombough
Brandon University, Brandon, MB, Canada

I Rønnestad
University of Bergen, Bergen, Norway

GG Rosenthal
Texas A&M University, College Station, TX, USA

JL Rummer
University of British Columbia, Vancouver, BC, Canada

R Russo
National Research Council (CNR), Naples, Italy

FM Sánchez-Simón
Institute of Neurosciences of Castilla y León, Salamanca,
Spain

PM Schulte
University of British Columbia, Vancouver, BC, Canada

RW Schulz
Utrecht University, Utrecht, The Netherlands

W Schulze
University of Bayreuth, Bayreuth, Germany

S Schuster
University of Bayreuth, Bayreuth, Germany

CJW Scott
Heriot-Watt University, Edinburgh, UK

M Sebire
Cefas Weymouth Laboratory, Weymouth, UK

SM Secor
University of Alabama, Tuscaloosa, AL, USA

O Seppälä
EAWAG, Swiss Federal Institute of Aquatic Science and
Technology, and ETH-Zürich, Institute of Integrative
Biology (IBZ), Dübendorf, Switzerland

H Seth
University of Gothenburg, Gothenburg, Sweden

RS Seymour
University of Adelaide, Adelaide, SA, Australia

MA Sheridan
North Dakota State University, Fargo, ND, USA

HA Shiels
University of Manchester, Manchester, UK

KR Siegfried
Massachusetts General Hospital, Boston, MA, USA

JA Sisneros
University of Washington, Seattle, WA, USA

KA Sloman
University of the West of Scotland, Paisley,
Scotland, UK

LU Sneddon
University of Chester, Chester, UK

MC Soares
Universidade de São Paulo, São Paulo, Brazil

GN Somero
Stanford University, Pacific Grove, CA, USA

PW Sorensen
University of Minnesota, St. Paul, MN, USA

MW Sprague
East Carolina University, Greenville, NC, USA

N Stacey
University of Alberta, Edmonton, AB, Canada

EM Standen
McGill University, Montreal, QC, Canada

ED Stevens
University of Prince Edward Island, Charlottetown, PE,
Canada

H Straka
Ludwig-Maximilians-Universität, Martinsried-Planegg,
Germany

CV Sullivan
North Carolina State University, Raleigh, NC, USA

A Sundby
Norwegian School of Veterinary Science, Oslo, Norway

KS Sundell
University of Gothenburg, Gothenburg, Sweden

F Syeda
University of Birmingham, Birmingham, UK

DA Syme
University of Calgary, Calgary, AB, Canada

EW Taylor
University of Birmingham, Birmingham, UK

H Thorarensen
Holar University College, Saudarkrokur, Iceland

KB Tierney
University of Alberta, Edmonton, AB, Canada

L Tort
Universitat Autonoma de Barcelona, Cerdanyola, Spain

ED Tytell
Johns Hopkins University, Baltimore, MD, USA

C Verde
National Research Council (CNR), Naples, Italy

J-N Volff
Université de Lyon, Lyon, France

H Volkoff
Memorial University of Newfoundland, St. John's, NL, Canada

G von der Emde
University of Bonn, Bonn, Germany

M Vornanen
University of Eastern Finland, Joensuu, Finland

H-J Wagner
Universität Tübingen, Tübingen, Germany

MM Walker
University of Auckland, Auckland, New Zealand

KN Wallace
Clarkson University, Potsdam, NY, USA

T Wang
Aarhus University, Aarhus, Denmark

Y Wang
Queen's University, Kingston, ON, Canada

W Waser
University of Turku, Turku, Finland

JF Webb
University of Rhode Island, Kingston, RI, USA

PW Webb
University of Michigan, Ann Arbor, MI, USA

NC Wegner
Scripps Institution of Oceanography, La Jolla, CA, USA

SE Wendelaar Bonga
Radboud University Heiweg 8, EB Malden,
The Netherlands

CR White
The University of Queensland, St. Lucia, QLD, Australia

MP Wilkie
Wilfrid Laurier University, Waterloo, ON, Canada

TM Winegard
University of Guelph, Guelph, ON, Canada

JM Wilson
Centre for Interdisciplinary Marine and Environmental Research (CIIMAR), Porto, Portugal

RW Wilson
University of Exeter, Exeter, UK

RJ Wootton
Aberystwyth University, Aberystwyth, Wales, UK

PA Wright
University of Guelph, Guelph, ON, Canada

Y Yamamoto
University College London, London, UK

Z Yaron
Tel-Aviv University, Tel Aviv, Israel

D Zaccone
University of Messina, Messina, Italy

G Zaccone
University of Messina, Messina, Italy

DG Zeddies
Marine Acoustics, Inc., Arlington, VA, USA

IV Zhdanova
Boston University School of Medicine,
Boston, MA, USA

LI Zon
Howard Hughes Medical Institute, Boston, MA, USA

This page intentionally left blank

HOW TO USE THE ENCYCLOPEDIA

STRUCTURE OF THE ENCYCLOPEDIA

The material in the Encyclopedia is arranged as a series of articles within a certain topic.

There are three features to help you easily find the topic you're interested in: a contents list, cross-references to other relevant articles in the Encyclopedia, and a full subject index.

1. CONTENTS LIST

The contents list, which appears at the front of all volume, lists the entries in the order that they appear in the Encyclopedia. It includes both the volume number and the page number of each entry.

2. CROSS-REFERENCES

All of the entries in the Encyclopedia have been extensively cross-referenced. The cross-references which appear at the end of an entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere
ii. To draw the readers attention to parallel discussions in other entries
iii. To indicate material that broadens the discussion

Example

The following list of cross-references may appear in text and at the end of the entry
The Muscles|Cartilaginous Fishes Cranial Muscles

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **The Muscles**: Bony Fish Cranial Muscles. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

3. INDEX

The index includes page numbers for quick reference to the information you're looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4. CONTRIBUTORS

At the start of each volume there is list of the authors who contributed to the Encyclopedia.

This page intentionally left blank

CONTENTS

VOLUME 1 – THE SENSES, SUPPORTING TISSUES, REPRODUCTION, AND BEHAVIOR

SENSING THE ENVIRONMENT

BRAIN AND NERVOUS SYSTEM

Functional Morphology of the Nervous System: An Introduction *AB Butler* 1

Cranial and Spinal Nerves of Fishes: Evolution of the Craniate Pattern *B Fritzsch and DC de Caprona* 6

Functional Morphology of the Brains of Agnathans *MA Pombal and M Megías* 16

Functional Morphology of the Brains of Cartilaginous Fishes *I Rodríguez-Moldes* 26

Functional Morphology of the Brains of Ray-Finned Fishes *AB Butler* 37

Functional Morphology of the Brains of Sarcopterygian Fishes: Lungfishes and *Latimeria*
A González and RG Northcutt 46

Motor Control Systems of Fish *S Grillner* 56

Physiology of the Mauthner Cell: Discovery and Properties *AE Pereda and DS Faber* 66

Physiology of the Mauthner Cell: Function *DS Faber and AE Pereda* 73

Autonomic Nervous System of Fishes *S Nilsson* 80

Opioids and Opioid Receptors in Fishes *MJ Herrero-Turrión, FM Sánchez-Simón, and RE Rodríguez* 89

VISION

Vision in Fishes: An Introduction *H-J Wagner* 98

Physiological Optics in Fishes *RHH Kröger* 102

Photoreceptors and Visual Pigments *JK Bowmaker* 110

Adaptations of Photoreceptors and Visual Pigments *JK Bowmaker* 116

Inner Retina and Ganglion Cells *MW Levine* 123

Optic Tectum *D Northmore* 131

Behavioral Assessment of the Visual Capabilities of Fish *S Schuster, P Machnik, and W Schulze* 143

Color Vision and Color Communication in Reef Fish *J Marshall and K Cheney* 150

Extraretinal Photoreception *H-J Wagner* 159

Visual Adaptations to the Deep Sea *RH Douglas and JC Partridge* 166

see Communication Behavior: Visual Signals, Volume 1, p. 692
see Coloration and Chromatophores in Fishes, Volume 1, p. 489
see Bioluminescence in Fishes, Volume 1, p. 497

SMELL, TASTE, AND CHEMICAL SENSING

Chemoreception (Smell and Taste): An Introduction *TJ Hara* 183
Morphology of the Gustatory (Taste) System in Fishes *TJ Hara* 187
Morphology of the Olfactory (Smell) System in Fishes *TJ Hara* 194
Neurophysiology of Olfaction *TJ Hara* 208
Neurophysiology of Gustation *TJ Hara* 218
Chemosensory Behavior *TJ Hara* 227
see The Effects of Toxicants on Olfaction in Fishes, Volume 3, p. 2078
see Oxygen Sensing in Fish, Volume 2, p. 871

HEARING AND LATERAL LINE

Fish Bioacoustics: An Introduction *AN Popper and BM Casper* 236
Vestibular System Anatomy and Physiology *H Straka and R Baker* 244
Auditory System Morphology *AN Popper* 252
The Ear and Hearing in Sharks, Skates, and Rays *BM Casper* 262
Biomechanics of the Inner Ear in Fishes *MC Hastings* 270
Psychoacoustics: What Fish Hear *RR Fay* 276
Auditory/Lateral Line CNS: Anatomy *CA McCormick* 283
Physiology of the Ear and Brain: How Fish Hear *Z Lu* 292
Sound Source Localization and Directional Hearing in Fishes *DG Zeddies, RR Fay, and JA Sisneros* 298
Effects of Human-Generated Sound on Fish *AD Hawkins* 304
Acoustic Behavior *J Luczkovich, MW Sprague, and CS Krahforst* 311
Vocal Behavior of Fishes: Anatomy and Physiology *F Ladich and AH Bass* 321
Lateral Line Neuroethology *JC Montgomery and S Coombs* 329
Lateral Line Structure *J Webb* 336
see How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling, Volume 1, p. 684

DETECTION AND GENERATION OF ELECTRIC SIGNALS

Detection and Generation of Electric Signals in Fishes: An Introduction *BA Carlson* 347
Morphology of Electroreceptive Sensory Organs *JM Jørgensen* 350
Physiology of Ampullary Electrosensory Systems *MH Hofmann* 359
Physiology of Tuberous Electrosensory Systems *ES Fortune and MJ Chacron* 366
Active Electrolocation *G von der Emde and J Engelmann* 375
Electric Organs *AA Caputi* 387
Generation of Electric Signals *M Kawasaki* 398

Development of Electroreceptors and Electric Organs *F Kirschbaum and J-P Denizot* 409
see Shocking Comments: Electrocommunication in Teleost Fish, Volume 1, p. 699

MUSCLES, SKELETON, SKIN, AND MOVEMENT

Functional Anatomy of Bones, Muscle, and Skin: An Introduction *ED Stevens* 416

THE SKELETON

Cartilaginous Fish Skeletal Anatomy *KM Claeson and MN Dean* 419
Cartilaginous Fish Skeletal Tissues *MN Dean* 428
Bony Fish Skeleton *EJ Hilton* 434

THE MUSCLES

Cartilaginous Fishes Cranial Muscles *DR Huber, MC Soares, and MR de Carvalho* 449
Bony Fish Cranial Muscles *LA Ferry and L Hernandez* 463

THE SKIN

The Many Functions of Fish Integument *DG Elliott* 471
Functional Morphology of the Integumentary System in Fishes *DG Elliott* 476
Coloration and Chromatophores in Fishes *D Burton* 489
Bioluminescence in Fishes *AF Mensinger* 497
Hagfish Slime *DS Fudge, JE Herr, and TM Winegard* 504

BUOYANCY, LOCOMOTION, AND MOVEMENT IN FISHES

Buoyancy, Locomotion, and Movement in Fishes: An Introduction *ED Stevens* 516
Buoyancy in Fishes *RMcN Alexander* 520
Swimbladder Function and Buoyancy Control in Fishes *B Pelster* 526
Experimental Hydrodynamics *ED Tytell* 535
Undulatory Swimming *DJ Ellerby* 547
Functional Properties of Skeletal Muscle: Work Loops *DA Syme* 555
Paired Fin Swimming *EM Standen* 564
Maneuverability *PW Webb* 575
Stability and Turbulence *PW Webb and AJ Cotel* 581
Fast Start *P Domenici* 587
Feeding Mechanics *TE Higham* 597
Biomimetics: Robotics Based on Fish Swimming *JH Long Jr.* 603
see Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems, Volume 2, p. 935
see Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder, Volume 2, p. 929
see The Retia, Volume 2, p. 1119
see Deep-Sea Fishes, Volume 3, p. 1953

see Applied Aspects of Fish Swimming Performance, Volume 3, p. 1652
see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215
see The Effect of Exercise on Respiration, Volume 2, p. 812

REPRODUCTION

The Diversity of Fish Reproduction: An Introduction *KA Sloman* 613

THE REPRODUCTIVE ORGANS AND PROCESSES

Anatomy and Histology of Fish Testis *RW Schulz and RH Nóbrega* 616
Regulation of Spermatogenesis *RW Schulz and RH Nóbrega* 627
Vitellogenesis in Fishes *BJ Reading and CV Sullivan* 635
see Parental Care in Fishes, Volume 1, p. 670
see Endocrine Regulation of Fish Reproduction, Volume 2, p. 1500
see Socially Controlled Sex Change in Fishes, Volume 1, p. 662
see Nutritional Provision During Parental Care, Volume 1, p. 678
see Sexual Behavior in Fish, Volume 1, p. 656

BEHAVIOR AND PHYSIOLOGY

Linking Fish Behavior and Physiology: An Introduction *KA Sloman* 647

SOCIAL AND REPRODUCTIVE BEHAVIORS

Dominance Behaviors *KA Sloman* 649
Sexual Behavior in Fish *I Katsiadaki and M Sebire* 656
Socially Controlled Sex Change in Fishes *J Godwin* 662
Parental Care in Fishes *S Balshine and KA Sloman* 670
Nutritional Provision During Parental Care *KA Sloman and J Buckley* 678
see Hormonal Pheromones, Volume 2, p. 1553
see The Diversity of Fish Reproduction: An Introduction, Volume 1, p. 613

SENSORY SYSTEMS, PERCEPTION, AND LEARNING

How Fishes Use Sound: Quiet to Loud and Simple to Complex Signalling *IM Kaatz* 684
Communication Behavior: Visual Signals *GG Rosenthal and NJ Marshall* 692
Shocking Comments: Electrocommunication in Teleost Fish *K Pappas and K Dunlap* 699
Fish Learning and Memory *VA Braithwaite* 707
Nociception or Pain in Fish *LU Sneddon* 713
Spatial Orientation: How do Fish Find their Way Around? *T Burt de Perera* 720
Magnetic Sense in Fishes *MM Walker* 726
Circadian Rhythms in Fish *SG Reebs* 736
see Bioluminescence in Fishes, Volume 1, p. 497

see Acoustic Behavior, Volume 1, p. 311
see Behavioral Assessment of the Visual Capabilities of Fish, Volume 1, p. 143
see Chemosensory Behavior, Volume 1, p. 227

BEHAVIORAL RESPONSES TO THE ENVIRONMENT

A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food *MV Abrahams* 744
Effects of Compensatory Growth on Fish Behavior *D Álvarez* 752
Temperature Preference: Behavioral Responses to Temperature in Fishes *LI Crawshaw and JE Podrabsky* 758
Behavioral Responses to Hypoxia *KA Sloman* 765
Sleep in Fish *IV Zhdanova* 772
Manipulation of Fish Phenotype by Parasites *O Seppälä* 777
Anthropogenic Influences on Fish Behavior *KA Sloman* 783
see The Biology of Fish Migration, Volume 3, p. 1921

VOLUME 2 – GAS EXCHANGE, INTERNAL HOMEOSTATIS, AND FOOD UPTAKE

GAS EXCHANGE

Respiration: An Introduction *JJ Cech Jr. and CJ Brauner* 791

VENTILATION AND ANIMAL RESPIRATION

Plasticity in Gill Morphology *GE Nilsson* 796
Gill Respiratory Morphometrics *NC Wegner* 803
The Effect of Exercise on Respiration *H Thorarensen* 812
Efficiency of Gas Exchange Organs *H Malte* 820
Respiratory Gas Exchange During Development: Models and Mechanisms *PJ Rombough* 829
Respiratory Gas Exchange During Development: Respiratory Transitions *PJ Rombough* 838
Techniques in Whole Animal Respiratory Physiology *JJ Cech Jr. and CJ Brauner* 846
see Branchial Anatomy, Volume 2, p. 1095

CONTROL OF RESPIRATION

Generation of the Respiratory Rhythm in Fish *EW Taylor* 854
The Ventilatory Response to CO_2/H^+ *WK Milsom* 865
Oxygen Sensing in Fish *MG Jonz* 871

TRANSPORT AND EXCHANGE OF RESPIRATORY GASES IN THE BLOOD

Red Blood Cell Function *M Nikinmaa* 879
Hemoglobin *M Nikinmaa* 887
O_2 Uptake and Transport: The Optimal P_{50} *T Wang and H Malte* 893
Carbonic Anhydrase in Gas Transport and Exchange *KM Gilmour* 899

Carbon Dioxide Transport and Excretion *D Randall* 909
Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange *CJ Brauner and JL Rummer* 916
Evolution of the Bohr Effect *M Berenbrink* 921
Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder *W Waser* 929
Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems *M Berenbrink* 935
Hemoglobin Differentiation in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 944
see Cellular Composition of the Blood, Volume 2, p. 984

TISSUE RESPIRATION

Cellular Respiration *GB McClelland* 951
Mitochondrial Respiration *CD Moyes and CMR Le Moine* 959
Specializations in Mitochondrial Respiration of Fish *CD Moyes and CMR Le Moine* 966

CIRCULATION

The Circulatory System: An Introduction *AP Farrell* 973
Circulatory System Design: Roles and Principles *KR Olson* 977

BLOOD

Cellular Composition of the Blood *AP Farrell* 984
Erythropoiesis in Fishes *C Verde, D Giordano, R Russo, and G di Prisco* 992
see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Red Blood Cell Function, Volume 2, p. 879
see Hemoglobin, Volume 2, p. 887

DESIGN AND PHYSIOLOGY OF THE HEART

Cardiac Anatomy in Fishes *AP Farrell and S Pieperhoff* 998
Cellular Ultrastructure of Cardiac Cells in Fishes *GLJ Galli* 1006
The Outflow Tract from the Heart *DR Jones and MH Braun* 1015
Physiology of Cardiac Pumping *AP Farrell* 1030
Action Potential of the Fish Heart *M Vornanen* 1038
Cardiac Excitation–Contraction Coupling: Routes of Cellular Calcium Flux *HA Shiels* 1045
Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element *TE Gillis* 1054
Cardiac Cellular Length–Tension Relationship *HA Shiels* 1060
Intracardiac Neurons and Neurotransmitters in Fish *G Zaccone, A Mauceri, and D Zaccone* 1067
Accessory Hearts in Fishes *AP Farrell* 1073
The Coronary Circulation *AP Farrell* 1077

DESIGN AND PHYSIOLOGY OF ARTERIES AND VEINS

Anatomical Pathways and Patterns *KR Olson* 1085
Branchial Anatomy *KR Olson* 1095
Physiology of Resistance Vessels *KR Olson* 1104

Physiology of Capacitance Vessels *KR Olson* 1111

The Retia *ED Stevens* 1119

The Gastrointestinal Circulation *M Axelsson and H Seth* 1132

see Secondary Circulation and Lymphatic Anatomy, Volume 2, p. 1161
see The Coronary Circulation, Volume 2, p. 1077

DESIGN AND PHYSIOLOGY OF CAPILLARIES AND SECONDARY CIRCULATION

Capillaries, Capillarity, and Angiogenesis *S Egginton and F Syeda* 1142

Circulatory Fluid Balance and Transcapillary Exchange *KR Olson* 1154

Secondary Circulation and Lymphatic Anatomy *KR Olson and AP Farrell* 1161

INTEGRATED CONTROL AND RESPONSE OF THE CIRCULATORY SYSTEM

Integrated Control of the Circulatory System *KR Olson* 1169

Central Control of Cardiorespiratory Interactions in Fish *EW Taylor* 1178

Hormone Metabolism in the Circulation *KR Olson* 1190

Integrated Responses of the Circulatory System to Temperature *AK Gamperl* 1197

Integrated Responses of the Circulatory System to Digestion *H Seth and M Axelsson* 1206

Integrated Cardiovascular Responses of Fish to Swimming *AP Farrell* 1215

Integrated Responses of the Circulatory System to Hypoxia *AK Gamperl* 1221

see Hormone Metabolism in the Circulation, Volume 2, p. 1190
see Accessory Hearts in Fishes, Volume 2, p. 1073
see Respiratory Adaptations for Air-Breathing Fishes, Volume 3, p. 1861
see The Osmorespiratory Compromise, Volume 2, p. 1389

THE GUT

The Gastrointestinal Tract: An Introduction *C Olsson and S Holmgren* 1229
Appendix A 1232

GUT ANATOMY AND MORPHOLOGY

Gut Anatomy *C Olsson* 1268

Pancreas *MA Caruso and MA Sheridan* 1276

Development of Fish Gut *KN Wallace* 1284

see The Gastrointestinal Circulation, Volume 2, p. 1132

INTEGRATED FUNCTION AND CONTROL OF THE GUT

Gut Motility *A Gräns and C Olsson* 1292

Gut Secretion and Digestion *Å Krogdahl, A Sundby, and AM Bakke* 1301

Intestinal Absorption *KS Sundell I Rønnestad* 1311

Barrier Function of the Gut *F Jutfelt* 1322

Nervous System of the Gut *S Holmgren and C Olsson* 1332
Endocrine Systems of the Gut *E Jönsson and S Holmgren* 1341
see Integrated Responses of the Circulatory System to Digestion, Volume 2, p. 1206

OSMOTIC, IONIC AND NITROGENOUS-WASTE BALANCE

Osmoregulation in Fishes: An Introduction *DH Evans* 1348
Mechanisms of Gill Salt Secretion in Marine Teleosts *DH Evans* 1354
Mechanisms of Ion Transport in Freshwater Fishes *P-P Hwang* 1359
Water Balance and Aquaporin *CP Cutler* 1366
Osmosensing *D Kültz* 1373

ROLE OF THE GILLS

Morphology of Branchial Ionocytes *JM Wilson* 1381
The Osmorespiratory Compromise *RJ Gonzalez* 1389

ROLE OF THE KIDNEYS

Histology of the Kidney *DB McMillan* 1395
The Kidney *SF Perry* 1411

ROLE OF THE GUT

Gut Ion, Osmotic and Acid-Base Regulation *RW Wilson* 1419
Dogfish Rectal Gland *WW Dowd* 1429
see The Hormonal Control of Osmoregulation in Teleost Fish, Volume 2, p. 1466
see Barrier Function of the Gut, Volume 2, p. 1322
see Endocrine Systems of the Gut, Volume 2, p. 1341
see Intestinal Absorption, Volume 2, p. 1311

NITROGENOUS-WASTE BALANCE

Excretion of Ammonia *D Randall* 1437
Ureotelism *PA Wright* 1444

HORMONAL CONTROLS

THE PITUITARY

Development of the Hypothalamus-Pituitary-Interrenal Axis *D Alsop and N Aluru* 1450
Pituitary Gland or Hypophysis *DB McMillan* 1457

HORMONAL CONTROL OF METABOLISM AND IONIC REGULATION

The Hormonal Control of Osmoregulation in Teleost Fish *SD McCormick* 1466
Corticosteroids *P Kiilerich and P Prunet* 1474

HORMONAL CONTROL OF REPRODUCTION AND GROWTH

Endocrinology of Fish Growth *MA Sheridan* 1483

Endocrine Control of Sex Differentiation in Fish *F Piferrer* 1490

Endocrine Regulation of Fish Reproduction *Z Yaron and B Levavi-Sivan* 1500

Control of Appetite in Fish *H Volkoff* 1509

HORMONAL RESPONSES TO STRESS

Hormone Response to Stress *SE Wendelaar Bonga* 1515

Catecholamines *SG Reid* 1524

Stress Effect on Growth and Metabolism *TW Moon* 1534

Impact of Stress in Health and Reproduction *L Tort* 1541

HORMONES IN COMMUNICATION

Hormonal Pheromones *N Stacey and P Sorensen* 1553

see Hormone Metabolism in the Circulation, Volume 2, p. 1190

see Integrated Control of the Circulatory System, Volume 2, p. 1169

VOLUME 3 – ENERGETICS, INTERACTIONS WITH THE ENVIRONMENT, LIFESTYLES, AND APPLICATIONS

ENERGETICS

Energetics: An Introduction *JA Nelson* 1563

General Energy Metabolism *JA Nelson and D Chabot* 1566

Physiological Functions that Scale to Body Mass in Fish *CR White and RS Seymour* 1573

FOOD ACQUISITION AND DIGESTION

Energetics of Prey Capture: From Foraging Theory to Functional Morphology *LA Ferry* 1583

Energetics of Foraging Decisions and Prey Handling *S Killen* 1588

Digestive Efficiency *DP German* 1596

Cost of Digestion and Assimilation *SM Secor* 1608

Dietary Requirements of Fish Under Culture Conditions *IP Forster* 1617

see A Survival Guide for Fishes: How to Obtain Food While Avoiding Being Food, Volume 1, p. 744

ENERGY UTILIZATION IN GROWTH

Energetics of Growth *RJ Wootton* 1623

Growth: Environmental Effects *RJ Wootton* 1629

see Effects of Compensatory Growth on Fish Behavior, Volume 1, p. 752

SWIMMING AND OTHER ACTIVITIES

Energetics of Fish Swimming *DJ McKenzie* 1636

Cellular Energy Utilization: Environmental Influences on Metabolism *H-O Pörtner* 1645

Applied Aspects of Fish Swimming Performance *T Castro-Santos* 1652

see Dominance Behaviors, Volume 1, p. 649

see Integrated Cardiovascular Responses of Fish to Swimming, Volume 2, p. 1215

see The Effect of Exercise on Respiration, Volume 2, p. 812

see The Osmorespiratory Compromise, Volume 2, p. 1389

see Functional Properties of Skeletal Muscle: Work Loops, Volume 1, p. 555

ENERGETIC MODELS

Bioenergetics in Aquaculture Settings *M Jobling* 1664

Bioenergetics in Ecosystems *CP Madenjian* 1675

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

General Principles of Biochemical Adaptations *GN Somero* 1681

TEMPERATURE

Effects of Temperature: An Introduction *PM Schulte* 1688

Measures of Thermal Tolerance *TL Beitinger and WI Lutterschmidt* 1695

Proteins and Temperature *PA Fields* 1703

Mitochondria and Temperature *H Guderley* 1709

Temperature and Excitable Membranes *M Vornanen* 1717

Membranes and Temperature: Homeoviscous Adaptation *CD Moyes and JS Ballantyne* 1725

Heat Shock Proteins and Temperature *S Currie* 1732

Effects of Climate Change *H-O Pörtner and MA Peck* 1738

see Temperature Preference: Behavioral Responses to Temperature in Fishes, Volume 1, p. 758

see Integrated Responses of the Circulatory System to Temperature, Volume 2, p. 1197

HYPOXIA

The Expanding Hypoxic Environment *RJ Díaz and DL Breitburg* 1746

Respiratory Responses to Hypoxia in Fishes *SF Perry* 1751

Anaerobic Metabolism in Fish *Y Wang and JG Richards* 1757

Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia *JG Richards* 1764

see General Principles of Biochemical Adaptations, Volume 3, p. 1681

see Behavioral Responses to Hypoxia, Volume 1, p. 765

see Integrated Responses of the Circulatory System to Hypoxia, Volume 2, p. 1221

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

HAGFISHES AND LAMPREY

Hagfishes *ME Forster* 1771

Lampreys: Energetics and Development *MP Wilkie* 1779

Lampreys: Environmental Physiology *MP Wilkie* 1788

see Hagfish Slime, Volume 1, p. 504

see Functional Morphology of the Brains of Agnathans, Volume 1, p. 16

STURGEON

Physiology of Sturgeon *SI Doroshov and JJ Cech Jr.* 1800

CHONDRICHTHYES

Physiology of Sharks, Skates, and Rays *JS Ballantyne and JW Robinson* 1807

see Functional Morphology of the Brains of Cartilaginous Fishes, Volume 1, p. 26

see The Ear and Hearing in Sharks, Skates, and Rays, Volume 1, p. 262

BONY FISHES

Zebrafish *JG Richards* 1819

Crucian Carp *GE Nilsson* 1824

Life in Hot Water: The Desert Pupfish *SD Hillyard* 1831

Blind Cavefish *Y Yamamoto and WR Jeffery* 1843

see Eel Migrations, Volume 3, p. 1937

AIR-BREATHING FISHES

The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction *JB Graham* 1850

Respiratory Adaptations for Air-Breathing Fishes *JB Graham* 1861

Circulatory Adaptations for Air-Breathing Fishes *JB Graham* 1875

PELAGIC FISHES

An Introduction to the Biology of Pelagic Fishes *D Bernal* 1887

Physiology of Tuna *KA Dickson* 1903

Endothermy in Tunas, Billfishes, and Sharks *BA Block* 1914

see Tracking Oceanic Fish, Volume 3, p. 1928

FISH MIGRATIONS

The Biology of Fish Migration *TR Binder, SJ Cook, and SG Hinch* 1921

Tracking Oceanic Fish *BA Block* 1928

Eel Migrations *D Righton and J Metcalfe* 1937

Pacific Salmon Migration: Completing the Cycle *SJ Cooke, GT Crossin, and SG Hinch* 1945

see Spatial Orientation: How do Fish Find their Way Around?, Volume 1, p. 720

DEEP-SEA FISHES

Deep-Sea Fishes *AP Farrell* 1953

INTERTIDAL FISHES

Intertidal Habitats *PM Schulte* 1959

APPLIED ASPECTS OF FISH PHYSIOLOGY

CELLULAR, MOLECULAR, GENOMICS, AND BIOMEDICAL APPROACHES

Culture of Fish Cell Lines *NC Bols, A Kawano, and LEJ Lee* 1965

Pigment Genes and Cancer Genes *I Braasch and D Liedtke* 1971

Evolution of Fish Genomes *J-N Volff, F Brunet, A Böhne, and D Galiana-Arnoux* 1980

Fish as Model Organisms for Medical Research *JN Cech and LI Zon* 1986

Transgenesis and Chromosome Manipulation in Fish *N Maclean* 1998

Physiology of Triploid Fish *TJ Benfey* 2009

Growth Hormone Overexpression in Transgenic Fish *RH Devlin* 2016

Molecular and Chromosomal Aspects of Sex Determination *KR Siegfried* 2025

Molecular Fish Pathology *CJW Scott, PC Morris, and B Austin* 2032

Germ Cell Migration and Trans Sex *M Kinoshita* 2046

Global Gene Expression Profiling in Fish *AY Gracey* 2055

see Hemoglobin Differentiation in Fishes, Volume 2, p. 944
see Erythropoiesis in Fishes, Volume 2, p. 992
see Blind Cavefish, Volume 3, p. 1843
see Zebrafish, Volume 3, p. 1819

TOXICOLOGY

The Toxicology of Metals in Fishes *CJ Kennedy* 2061

The Toxicology of Organics in Fishes *CJ Kennedy* 2069

The Effects of Toxicants on Olfaction in Fishes *KB Tierney* 2078

see Anthropogenic Influences on Fish Behavior, Volume 1, p. 783

AQUACULTURE

Physiology of Fish in Culture Environments *CA Myrick* 2084

see Dietary Requirements of Fish Under Culture Conditions, Volume 3, p. 1617
see Bioenergetics in Aquaculture Settings, Volume 3, p. 1664
see Physiology of Triploid Fish, Volume 3, p. 2009
see Manipulation of Fish Phenotype by Parasites, Volume 1, p. 777

INDEX 2091

PREFACE

The *Encyclopedia of Fish Physiology: From Genome to Environment* is the first of its kind. It follows in the strong tradition built by the book series *Fish Physiology* that was pioneered in the late 1960s by Bill Hoar and Dave Randall and is still ongoing; however, the Encyclopedia differs markedly from this book series in three important respects.

Foremost, the Encyclopedia targets a much broader audience, not just the research community. My hope is that the Encyclopedia will serve as a general educational tool for biologists, as well as being of general use as an information resource for those interested in fishes. After all, the Encyclopedia is replete with magnificent examples of how fishes are marvelously adapted to almost every water body on the Earth, no matter how deep, how hot, how cold, how salty, or, in some cases, how dry!

Second, the articles span well beyond just the physiology of fishes. The intent here is to connect physiological functions, environmental factors, behavior, and the genome. This is the case even though the main focus here is on physiology.

Third, and as a result, the breadth of topics covered by the Encyclopedia far exceeds that of any focused volume of the *Fish Physiology* book series. Thus, the Encyclopedia is ambitious in both its content and its target audience.

The Encyclopedia contains a very impressive array of entries, each in the form of a discrete, self-standing article that is typically 3000–4000 words in length, not counting the ancillary material contained in figures, glossary, further reading, etc. In total, there are 252 articles written by over 200 international experts from over 20 countries.

The articles are arranged thematically. The larger thematic sections have an introductory article, written by a section editor, who explains contributions in that theme and places them in a broader and often historical context. Tributes are deservedly given to those who made key scientific advances.

The electronic format of the Encyclopedia was deliberate and a crucial requirement before this project even started. I have to thank Andy Richford for garnering Elsevier's support of this idea, as well as initiating this project when he was an acquisition editor. The electronic format, through 'hot keys', provides an easy and almost instant access to the glossary and related articles. Each article is generously linked to many others in this manner, offering the reader easy digressions or more detailed information and explanations. The 'Further Reading' section associated with each article provides additional resources.

This ambitious opus could not have been possible without the dedication and commitment of a great number of individuals. I had an idea that I have steered to completion, but others did most of the hard work. I am especially indebted to my associate editors, Don Stevens, Joe Cech, and Jeffrey Richards. They embraced my dream early on and stayed with me until completion. Without their wisdom, breadth of knowledge, and hard work, the Encyclopedia could not have come to fruition. I think we worked well together, and, on my part, it was a distinct pleasure to have such enthusiastic support.

I am equally indebted to the section editors: Colin Brauner, Ann Butler, Bruce Carlson, Brandon M Casper, Greg Goss, Jeff Graham, Toshiaki Hara, Sue Holmgren, Norman Maclean, Jay Nelson, Ken Olson, Catharina Olsson, Arthur Popper, Trish Schulte, Bob Shadwick, Kath Sloman, Adam Summers, Matt Vijayan, and Hans-Joachim Wagner. They shaped their section and carried the core of the editorial responsibilities for the contributed articles during the many rounds of review and revisions.

Finally, and importantly, there are over 200 authors and their collaborators, who contributed the articles. My hope is that your rewards go well beyond the recompense, which is a pittance for the time that I know you devoted to generating such marvelous articles. You are thanked for your dedication and trust in the project, and are congratulated for your respective skills and knowledge.

There is another side to the production of this Encyclopedia, and that is the Elsevier team, with whom the editors and authors interacted. This team was headed by Kristi Gomez, the acquisitions editor in San Diego. Milo Perkins, the

development editor in the Oxford office, was our go-to-man after Esmond Collins had set the ball rolling. Milo endured the rough and the smooth, and his rapid responses, as well as his flexible deadlines, were greatly appreciated. Kate Mittell worked quietly and efficiently in the background supporting Milo and was responsible for keeping me on track with the enormous glossary of terms. Nicky Carter, our project manager, superbly rounded out the team. We relied on her for the professional appearance and content of the final copy.

As Editor-in-Chief, I am ultimately responsible for any errors contained herein. If you find any, please let me know so that they can be corrected; likewise, if you feel there are important omissions, please let me know of these. They might be incorporated in future updates.

Bringing this Encyclopedia to its conclusion means one thing for me personally: Miriam, Dustin, Naomi, Nicole and Natalie will all be seeing a lot more of me over the next while! Miriam, your patience throughout was astounding, although it was pushed to the limit at times.

Tony Farrell
Vancouver

ENERGETICS

Contents

Energetics: An Introduction
General Energy Metabolism
Physiological Functions that Scale to Body Mass in Fish

Energetics: An Introduction

JA Nelson, Towson University, Towson, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Energy Use by Fish

Glossary

Entropy A thermodynamic property measuring the amount of disorder in the system. Greater disorder is energetically favorable; thus, entropy favors unfolding of proteins.

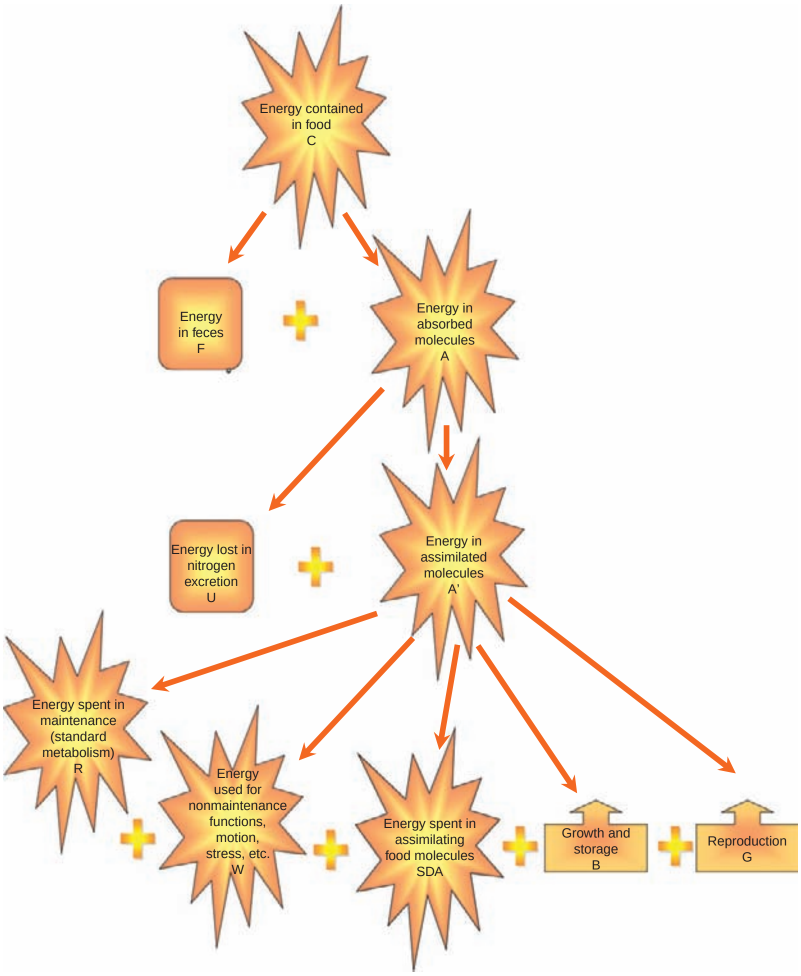
Specific dynamic action (SDA) The additional amount of oxygen or energy consumed above SMR as a result of feeding, measured during the period after feeding until metabolic rate returns to SMR; SDA represents the energetic cost of digestion, assimilation, and protein turnover.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Energy Use by Fish

Metabolism is the word used to describe the totality of energy-consuming, manipulative, and storage chemical reactions undertaken by organisms. The second law of thermodynamics demands that all processes increase the amount of entropy (disorder) in the universe. Thus, a highly ordered entity like a fish requires a constant input of energy to remain ordered, even in the absence of growing. In the process of obtaining and assimilating that energy, the fish randomizes the rest of the universe more than the ordering of the fish itself. This results in a net increase in the disorder of the universe and the possibility of fish life, but only with this constant energy input. Therefore, a primary goal of fish existence is to obtain sufficient energy to offset this universal randomization process. This energy needs to be of two types, chemical bond energy and high-energy electrons (reducing power) which, along with some minerals and macromolecules that fish are unable to synthesize themselves, constitutes the daily dietary requirement for that fish species (**see also Food Acquisition and Digestion**: Digestive Efficiency). A subsequent, but equally essential goal of fish life is to obtain these components in sufficient excess to fuel growth and reproduction, leading to eventual evolutionary success. This excess fuel can also support locomotion or other work done on the environment. A bioenergetics flow chart, such as **Figure 1**, summarizes the energy inputs and outputs of a fish.

Of the energy consumed in food (*C*), only a certain fraction is actually absorbed across the intestinal epithelium into the fish (*A*), with the remainder being lost as feces (along with a small, not depicted, fraction of energy lost from the sloughing of gastrointestinal cells into the eventual feces). Fish tend to be very efficient at retaining the energy contained in their food. Of the absorbed energy (*A*), nitrogen in excess of the animal's requirements for protein and nucleic acid growth or turnover is not available to the organism and is excreted. The energy contained in this lost nitrogen as well as any energy lost in processing it, for



Energy contained in food C
Energy in feces F
+
Energy in absorbed molecules A
Energy lost in nitrogen excretion U
+
Energy in assimilated molecules A'
Energy spent in maintenance (standard metabolism) R
+
Energy used for nonmaintenance functions, motion, stress, etc. W
+
Energy spent in assimilating food molecules SDA
+
Growth and storage B
+
Reproduction G

Figure 1 A bioenergetics flowchart for fish. Squares depict energy losses, stars potential or used energy, and rectangles energy storage. Details are contained in the text.

example, to urea, CN_2H_4O, is represented by the term U and is not available to the animal (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia and Ureotelism). The remaining energy (A') is available to the organism and can be used to support either maintenance functions (R), nonmaintenance activities (W), or the work of assimilating more food molecules (SDA) (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation). Excess energy is then available for storage or to support organismal growth (B) and gonad maturation (G) so that the animal can be successful from an evolutionary perspective and reproduce. This flow chart is often expressed in the form of an energy budget equation such as eqn (1) for modeling fish energy requirements:

$$C = (F + U) + (R + W + SDA) + (B + G) \qquad (1)$$

where, F is the fraction of ingested energy lost in the feces. This section of the encyclopedia focuses on this flow of energy through a fish. The section is divided into three main subsections: (1) energy acquisition, the energy cost to the fish to find, internalize, and extract the energy and other key molecules found in their food and how extrinsic and intrinsic factors influence that cost; (2) energy utilization, a detailed examination of the different ways that fish allocate the energy they procure from the environment and the effects of the variable aquatic environment on that allocation process; and (3) models and applications, where we explore how knowledge of the energy flow through fishes has led to advances in the management of exploited fish stocks and improvements in the aquaculture industry.

Energy acquisition encompasses the cost to the fish of finding food, successfully subduing it, manipulating it to the point that it can be swallowed, and then the cost of mechanically and chemically breaking down the macromolecules in food to a size that can be absorbed across the intestinal epithelium (digestion). Also included in this cost are the biochemical costs of manipulating the absorbed molecules into the proper forms to become self or storage compounds.

Lara Ferry-Graham opens this section with a consideration of how fish morphology matches up with the prey they consume, presumably minimizing energy acquisition costs, and how past evolutionary events may or may not have shaped these relationships. Shaun Killen continues with a discussion of the energetic costs of prey handling and ingestion, and how those costs may influence which prey items a fish actually attempts to consume. Donovan German then details what happens to the energy in food after it is ingested, that is, what factors determine the amount of energy extracted by a given fish on a given diet. Finally, Stephen Secor wraps up the 'energy acquisition' subsection with a detailed examination of the energetic costs associated with processing a meal.

The 'energy utilization' subsection deals with the main ways that fish can expend the energy they acquire with the exception of reproduction, which is dealt with in other areas of the encyclopedia (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). The chapter by Jay A. Nelson and Denis Chabot focuses on just the standard metabolism (SMR) term R of eqn (1). This is the energy required by the organism just to stave off the forces of entropy and includes both extrinsic and intrinsic determinants of SMR. Resolving the terms of eqn (1) is essential if we are to predict growth and thus production in any fish species, which is the subject of Bob Wooton's first chapter. He then goes on to explore how environmental factors can change energy allocation to growth in a second contribution. Environmental effects on fish energy allocation are also the subject of Hans-Otto Pörtner's chapter, but his focus is on how the environment influences energy consumption or metabolic rate. Finally, David McKenzie details the energy cost of exercise in fish and how that integrates with the other parts of the bioenergetic budget because, of course, most fish need to move to procure food in the first place, or to avoid becoming food themselves!

The last subsection of the bioenergetics section, 'models and applications' deals with how this bioenergetic information is put to work solving real-world problems of fisheries management and aquaculture. Equation (1) needs to be resolved on an ecosystem level if we are to understand how changes in a species' numbers will influence energy flow in their ecosystem. Chuck Madenjian details the history of bioenergetics use in fisheries management and presents several case studies where bioenergetics models have been essential in making good fisheries-management decisions. Finally, Malcolm Jobling presents a cogent argument for why all food production is basically an exercise in bioenergetics and how the aquaculture industry is steadily improving the bioenergetic basis of how it operates.

See also: **Food Acquisition and Digestion**: Cost of Digestion and Assimilation; Digestive Efficiency. **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Nitrogenous–Waste Balance**: Excretion of Ammonia; Ureotelism.

General Energy Metabolism

JA Nelson, Towson University, Towson, MD, USA

D Chabot, Institut Maurice-Lamontagne, Mont-Joli, QC, Canada

© 2011 Elsevier Inc. All rights reserved.

Metabolic Rates of Fish
Conclusions
Further Reading

Glossary

Adenosine triphosphate (ATP) An almost universal carrier of chemical bond potential energy; fish use ATP made from catabolism of foodstuff or body reserve molecules to fuel energy-dependent processes.

Direct calorimetry The measurement of waste heat produced by metabolic processes to assess the rate of these processes.

Entropy A thermodynamic property measuring the amount of disorder in the system. Greater disorder is energetically favorable; thus, entropy favors unfolding of proteins.

Indirect calorimetry The measurement of O_2 or foodstuff molecules consumed or CO_2 produced to assess the rate of metabolic processes.

Mass exponent, β (also called allometric exponent) The power function exponent for the relationship of metabolic rate as a function of body size.

Metabolic scaling How metabolism changes as body size changes; typically summarized by the mass exponent.

$\dot{M}_{O_2}$ Mass of oxygen consumed by an organism. The SI unit is micromoles or millimoles per unit time, but often expressed as milligrams of oxygen per unit time. It can also be divided by the mass of the fish (e.g., mg-O_2 h^{-1} kg^{-1}) in which case, it is called 'mass-specific oxygen consumption'.

Quantile A value that divides a data set into parts. Where the division is made depends upon the parameter *q*. If $q = 0.5$, half the data are below the quantile and half above, which gives the median. If *q* is given as a percent instead of a proportion, the quantile can be called a percentile.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Metabolic Rates of Fish

Measuring Metabolic Rate

Organismal energy use is thermodynamically inefficient, that is, about two-thirds of the potential energy of the reactants is lost as a less-useful form of energy known as heat during the execution of essential life processes. In addition, the catabolic reactions of the body that convert the energy in food to the adenosine triphosphate (ATP) required to fuel these process are themselves inefficient and generate heat. Thus, although the most useful indicator of total metabolic activity would be to measure the rate of total ATP turnover in an organism, in the absence of a convenient way to measure ATP turnover, accounting for the heat produced by these aggregate reactions is the best way to assess total metabolic activity, a process called 'direct calorimetry'. Unfortunately, the high heat capacity of water and the relatively low metabolic activity of fish result in a low 'signal:noise' ratio for direct calorimetry in aquatic studies. Therefore, we find limited measurements of metabolic rate by direct calorimetry available for the fishes; most studies instead rely upon a process called 'indirect calorimetry'.

Indirect calorimetry takes advantage of the fact that substances are consumed or produced during the catabolic conversion of foodstuffs to useful ATP energy. Eqn (2) depicts the complete aerobic respiration of an example foodstuff molecule, glucose:

$$\begin{aligned} &C_6H_{12}O_6 + 36\text{ADP} + 36\text{P}_i + 36\text{H}^+ + 6O_2 \\ &\quad \Rightarrow 6CO_2 + 36\text{ATP} + 42H_2O + \text{Heat} \end{aligned} \qquad (1)$$

Both oxygen (O_2) and carbon dioxide (CO_2) are gases at the range of temperatures and pressures encountered by fish; their stoichiometric consumption (O_2) or release (CO_2) can be monitored to gauge the rate of this reaction. Some studies also follow the decline in storage metabolites in starved fish as a way to monitor rates of respiration reactions. However, because starvation is a nonstandard physiological state, these types of experiments will not be discussed further here (**see also Gut Anatomy and Morphology**: Gut Anatomy). Because each foodstuff (protein, fat, or carbohydrate) produces different amounts of energy per amount of O_2 consumed or CO_2 emanated, accurate use of indirect calorimetry for bioenergetics requires a strict assessment of the substrate being respired. This is not a great challenge in laboratory situations, but may be impossible for most field studies because the exact composition of the diet is often unknown. Because measuring $[CO_2]$ in water is more challenging than measuring $[O_2]$, studies of fish metabolic rate over the past 30 years have relied upon measuring O_2 consumption almost exclusively, often without any attempt to relate the measurement back to energy usage. As such, oxygen consumption ($\dot{M}_{O_2}$) has actually become a measurement in its own right because without accounting for the substrate being oxidized or accounting for any anaerobic metabolism that occurs, it may be quite different from the actual metabolic rate. Recent careful comparisons of metabolic rate in endotherms using both direct and indirect calorimetry on the same animals suggest that the use of indirect calorimetry incurs routine errors of the order of 20%, with isolated cases as high as 35%.

Standard Metabolic Rate and its Measurement

$$C = (F + U) + (R + W + SDA) + (B + G) \qquad (2)$$

In eqn (2), a standard bioenergetics equation, (**see also Energetics**: Energetics: An Introduction) '*R*' represents the minimum rate of energy expenditure needed to keep a fish alive. It involves three broad classes of processes, the first of which is biosynthesis of macromolecules. Even when a fish is not growing, there is a constant, energy-requiring renewal of the macromolecules that make up the fish with molecules from the diet. The second class of processes concerns the chemical work of moving ions and molecules against concentration gradients or moving polar compounds across nonpolar membranes. These energy-requiring processes are essential for osmoregulation, the maintenance of internal cellular integrity, cellular communication, intra-organismal communication (e.g., action potentials or hormone release), and the transport of food molecules. The third class of processes concerns the internal mechanical work required to preserve organismal integrity (e.g., work done by the heart to accelerate blood).

R is often called basal metabolic rate (BMR) in endotherms and standard metabolic rate (SMR) in ectotherms. *R* allows for no activity, digestion, or reproduction, and care needs to be taken to exclude other sources of energy expenditure when measuring it. Measurement methodologies for BMR in endotherms are well established. In humans, for example, BMR is measured in subjects who are awake, supine, fasted for 12 h, motionless, after a 20–30-min period of rest, and isolated from external stimuli in a thermoneutral dark environment. Even so, BMR is not without criticism because metabolic rate can decrease further during sleep. Nevertheless, the stringent measurement conditions facilitate comparisons between studies and can also be applied to many nonhuman mammals.

By contrast, the conditions in which SMR is measured in aquatic ectotherms are more loosely defined. SMR is typically observed in postabsorptive (but not starving), resting organisms after acclimation to the experimental temperature and apparatus, isolated from outside stimuli (including sensory stimuli from potential predators and possibly conspecifics), during the part of the circadian activity cycle when $\dot{M}_{O_2}$ is lowest. Strictly speaking, SMR should be measured only in animals that are in a steady state (no growth or reproduction). However, the minimum metabolic rate of juvenile fish is often called SMR and the growth of many fish species is indeterminate, so the no-growth criterion is often not met. SMR of adult fish should be measured outside of the reproductive season, although in practice, the reproductive status of fish used in SMR determinations is not always known. Because there is usually some activity during measurements of metabolic rate, or because activity has not been monitored, many authors refrain from using the term SMR and instead use resting, fasting, or most commonly resting routine metabolic rate. However, this may be unduly restrictive and the term SMR should be allowed when activity level is measured and known to be at the minimum possible for the species.

Handling stress has undoubtedly inflated many published estimates of SMR in fishes. Human contact and air exposure need to be minimized before measuring SMR. Visual contact with humans or laboratory noises can elevate metabolic rate considerably even in the absence of movement. An acclimation period is required before $\dot{M}_{O_2}$ measurements can be used to estimate SMR. Handling stress and the novelty of the experimental setup may elevate $\dot{M}_{O_2}$ in fish for hours or even days. Sufficient acclimation can be verified by measuring $\dot{M}_{O_2}$ during the acclimation period to confirm that the fish has reached a state where repeatable measurements of minimum metabolic rate can be made. Striking a balance between ensuring that an animal is only postabsorptive and not starving with adequate acclimation may prove to be difficult. In social species, isolating fish to measure SMR may produce stress and increase $\dot{M}_{O_2}$. It may be necessary to determine SMR for groups of fish, after examination of the relationship between $\dot{M}_{O_2}$ and group size.

Spontaneous activity is extremely difficult to control or even measure in fish, yet activity can increase metabolic rate by an order of magnitude (**see also Ventilation and Animal Respiration**: The Effect of Exercise on Respiration). One approach has been to circumvent the problem by measuring $\dot{M}_{O_2}$ during forced activity at different speeds, and then by extrapolating the relationship between $\dot{M}_{O_2}$ and swimming speed back to zero swimming speed. However, fish can engage in spontaneous locomotor activity, have additional maneuvering costs at high speed, or be subject to stress, in addition to the sustained swimming at low speeds, thereby elevating $\dot{M}_{O_2}$ and the predicted $\dot{M}_{O_2}$ at zero speed. Because of this, $\dot{M}_{O_2}$measured at low swimming speed is generally less repeatable than at higher speeds, so if this method is employed, relatively high, but still aerobic, swimming speeds should be used. Another concern is that the routine metabolism of some organ systems (e.g., the gut) can be turned down with swimming activity, which, if left unaccounted, could lead to an underestimate of SMR.

An alternative method is to measure the level of spontaneous activity in a static respirometer, relate $\dot{M}_{O_2}$ to activity level (i.e., through regression analysis), and use the intercept (i.e., at zero activity) of this relationship as SMR. Yet another method is simply to estimate SMR only on measurements obtained when activity is zero. Some authors have employed anesthesia to eliminate activity from SMR measurements, but this method is not recommended because the anesthetic may interfere with other functions, including those responsible for SMR. However, for very active, dangerous or obligate ram-ventilating species, this may be the only tractable method available, in which case, the minimum dose to eliminate swimming activity should be used.

There is more uncertainty in estimating SMR when activity is not accounted for in static respirometry. Unless the period of minimum spontaneous activity is known for the species, $\dot{M}_{O_2}$ must be measured over at least 24 h; longer experiments increase confidence in estimated SMR by verifying that similar low levels of $\dot{M}_{O_2}$ are observed for the circadian minimum on multiple days. Long records of $\dot{M}_{O_2}$ can be obtained using two different respirometry techniques: open-flow respirometry and intermittent-flow respirometry. However, there is no standardized method to estimate SMR from these records. It may be nonadvisable to use the lowest value of $\dot{M}_{O_2}$ because it could be the result of measurement error. Often, an arbitrary number of the lowest values of $\dot{M}_{O_2}$ observed after the fish is deemed acclimated are averaged to obtain SMR, sometimes after removing 'outliers' that are arbitrarily also identified.

Another approach is to fit a mixture of normal distributions to the frequency distribution of $\dot{M}_{O_2}$ values. The mode with the lowest $\dot{M}_{O_2}$ can be interpreted as SMR; it constitutes the lowest value of $\dot{M}_{O_2}$ that is most frequently observed (**Figure 1**). This approach has two main advantages: (1) estimation of SMR is based on a large number of observations, and (2) it is unnecessary to manually select which measurements are used to estimate SMR. It turns out that this method does not work when there are many measurements with moderate activity or stress. It becomes impossible to discriminate the normal distribution corresponding to SMR from the distribution(s) corresponding to the moderately active or stressed fish and estimated SMR resembles routine metabolic rate (i.e., the metabolic rate of a moderately active fish, **Figure 2**).

Removing the 'noisy' part of the signal may improve the estimation of SMR by this approach, but the second advantage (see above) is negated (**Table 1**)

Yet another approach is to define SMR as a quantile: a proportion q (e.g., 0.05–0.25) of the measurements are assumed to be below SMR and the remaining $1 - q$ of the values above SMR. Models suggest that values of 0.10–0.15 result in small errors in estimating SMR across a broad range of activity and stress levels and could be used even in situations when fitting normal distributions (**Table 1**). However, the quantile approach has not yet been adopted widely to estimate SMR.

The postabsorptive state is also loosely defined in SMR studies on fish. Only rarely is gut-passage time measured for the same species under the same conditions; most studies rely on postabsorptive times from the literature that may not account for temperature or dietary differences (e.g., gut passage being significantly longer in herbivorous fishes (**see also Food Acquisition and Digestion**: Digestive Efficiency) or scaling effects (e.g., using published values based on fish of a different size; **see also Energetics**: Physiological Functions that Scale to Body Mass in Fish).

Body Size and SMR

Of the factors that contribute to differences in SMR, body size has to be considered the most pervasive. An animal's metabolic rate changes with body size in a nonproportional manner such that tissues of larger animals consume less energy per unit tissue than those of smaller animals (**see also Energetics**: Physiological Functions that Scale to Body Mass in Fish). The original mammal-centric literature on the subject dating to the nineteenth century considered this as a consequence of heat dissipation requirements by endothermic organisms and concluded that SMR scaled as the surface area to volume ratio or as body mass (M_b) to the two-thirds power:

$$\mathrm{MR} = CM_b{}^{0.67}$$

often expressed in the logarithmic form:

$$\log \mathrm{MR} = \log C + 0.67 \log M_b$$

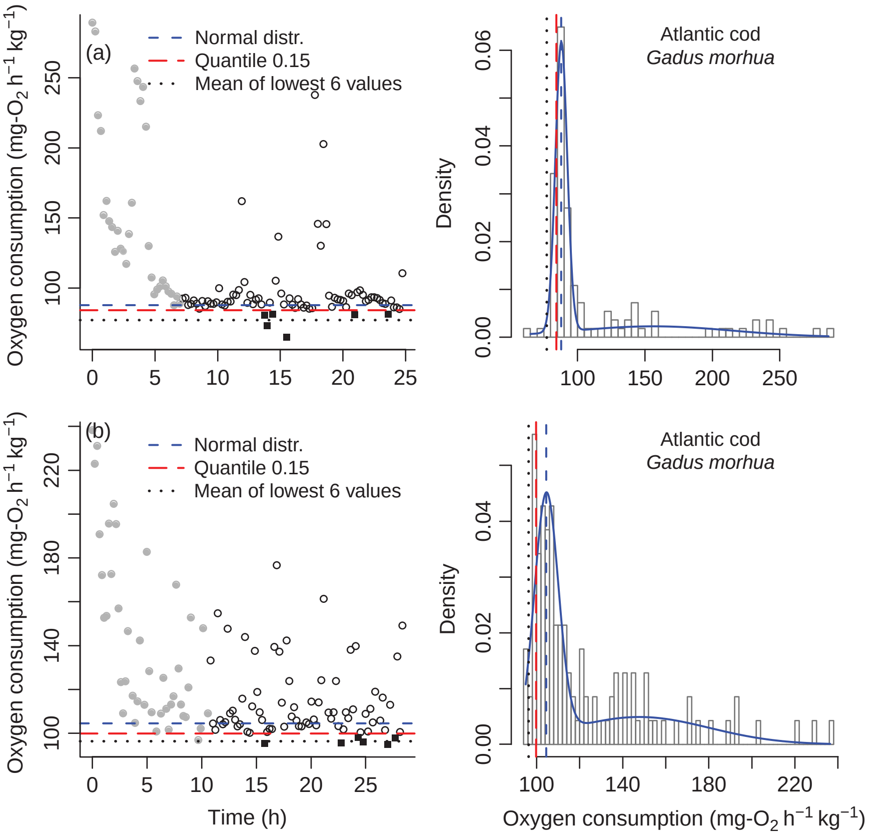


Figure 1 Oxygen consumption ($\dot{M}_{O_2}$) as a function of time in the respirometer (left), and frequency distribution of $\dot{M}_{O_2}$ values (right). (a) Juvenile Atlantic cod (*Gadus morhua*) with steady low levels of $\dot{M}_{O_2}$ and (b) juvenile cod with more variable $\dot{M}_{O_2}$ after acclimation. Gray circles and open circles represent values recorded during or after the acclimation period, respectively. The black squares are the six lowest values recorded after the acclimation period. Three estimates of SMR are shown: the average of the six lowest observations of $\dot{M}_{O_2}$, the lowest mode from fitting a mixture of normal distributions to the frequency distribution of $\dot{M}_{O_2}$ values shown in the right panels, and the quantile 0.15. The last two estimates were obtained using all available data, including the acclimation period.

where, MR is the metabolic rate, M_b the body mass, C the proportionality coefficient or MR at unit body mass, and 0.67 is β, the slope or power exponent describing the rate of change of metabolic rate with increasing body size. Subsequent empirical measurements throughout the twentieth century often arrived at a general interspecific 0.75 scaling coefficient for the metabolic rate of both ecto- and endotherms (i.e., $M_b^{0.75}$), but without a generally accepted physiological mechanism to account for it. Throughout the latter part of the twentieth century, measurements on fish generally returned a wide range of scaling coefficients. Knut Schmidt-Nielsen, in his seminal 1984 book on scaling, decried the huge variation in intraspecific β values for fish (0.37–1.1) and settled on a value of about 0.8 as the best. As the twentieth century was drawing to a close, several major new theories were advanced to either explain or discount the 0.75 scaling coefficient. These new theories created renewed interest in the field and stimulated many scientists to examine old data sets or undertake new measurements. To date, evidence has accumulated that metabolic scaling in fish may be higher than 0.75 (**see also Energetics**: Physiological Functions that Scale to Body Mass in Fish), especially if larval fishes are included. Scaling of metabolic rate under active conditions (**see also Swimming and Other Activities**: Energetics of Fish Swimming) appears to approach 1 for fish. Overall, SMR change with size is not proportional in juvenile through adult life stages in fish, and the exact scaling coefficients may be species specific. Certainly, the best way to correct for body-size differences, within a species, is to either find a carefully collected data set or construct one's own scaling factor, using specimens across a wide body-size range. Another way is to use an analysis of covariance (ANCOVA) to analyze data, using body mass as the covariate.

Sources of Variation in SMR

After correcting for body size, there still remains substantial inter-specific and intra-specific variability in SMR. Perusal of the literature can return values that vary by more than 70-fold for fish of similar lifestyles corrected

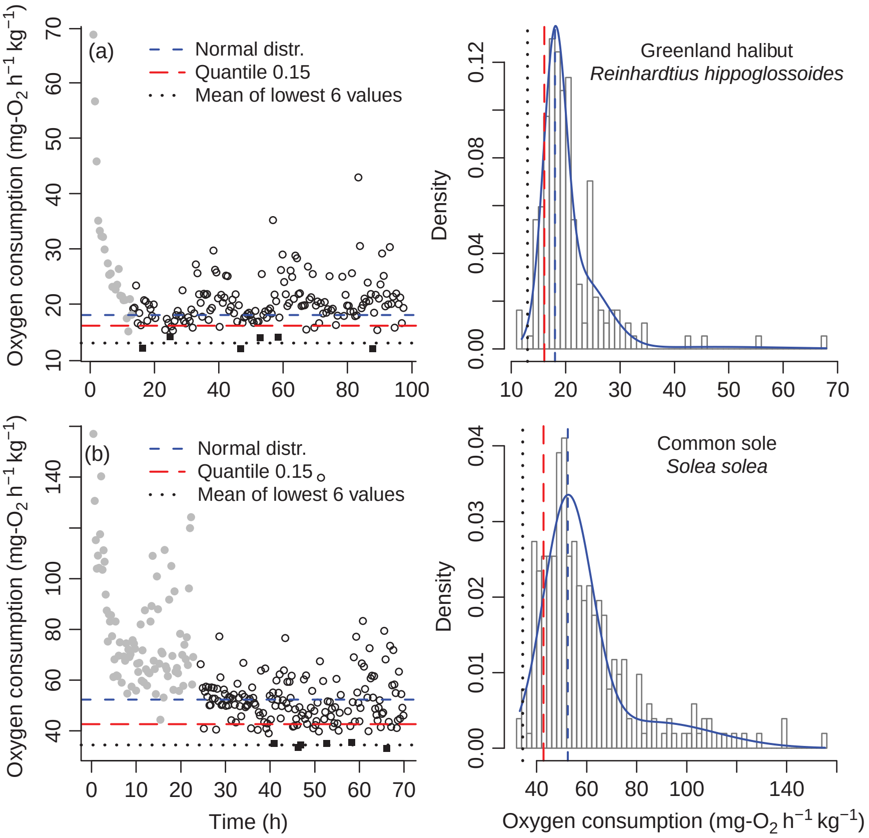


Figure 2 Oxygen consumption ($\dot{M}_{O_2}$) as a function of time in the respirometer (left), and frequency distribution of $\dot{M}_{O_2}$ values (right). (a) Mature female Greenland halibut (*Reinhardtius hippoglossoides*) and (b) juvenile common sole (*Solea solea*), both with high variability in $\dot{M}_{O_2}$ after the acclimation period. Symbols and lines are as in **Figure 1**.

Table 1 SMR estimated for the four fish shown in **Figures 2** and **3** using five different methods on four individuals from three species: averaging the six lowest values observed after the acclimation period, fitting a mixture of normal distributions to the frequency distribution of $\dot{M}_{O_2}$ measurements and selecting the leftmost mode, and taking the quantile 0.15, the latter two both with and without the acclimation period

			Normal distributions		*Quantile$_{0.15}$*	
Species	*Figure*	*Average of Six lowest values*	*All data*	*Excluding acclim. period*	*All data*	*Excluding acclim. period*
Atlantic cod	2A	77.1	87.6	87.2	84.2	83.1
Atlantic cod	2B	96.4	104.6	103.4	99.8	98.9
Greenland halibut	3A	13.0	18.1	18	16.1	16.1
Common sole	3B	34.4	52.7	47.9	42.7	41.1

for size and temperature. Much of this variation is due to experimental differences between laboratories, starting with how SMR was calculated. Many studies of standard or resting routine metabolic rate in fishes did not adequately control for human presence or elapsed time from human contact or transfer to the respirometer (see above). Thus, many of these values are inflated beyond standard or even resting routine levels. Another factor contributing to the large variance in standard metabolism estimates between species and studies are differences in water chemistry. Because of the constraints of Fick's first law of diffusion (eqn (3))

$$\mathcal{J}_{\mathrm{net}} = DA\Delta P_{\mathrm{gas}/\Delta d} \tag{3}$$

(where J_{net} is the net diffusional flux between two compartments, A the area over which the flux is occurring, ΔP the difference in partial pressure of the gas between the two compartments, Δd the distance separating the two compartments and D a constant), and the need for fish to extract O_2 and release CO_2, respiratory surfaces such as the skin or gills need to be large surface-area structures with a short diffusion distance to the external environment. These characteristics present an opportunity for the salt and water of the environment to also equilibrate across electrochemical gradients in water-breathing fish (**see also Role of the Gills**: The Osmorespiratory Compromise). Fish need to counteract these movements through active, energy-requiring processes. This fundamental conflict between the need to exchange respiratory gases and the relative cost of osmoregulation in water-breathing fish has been termed the 'osmorespiratory compromise'. Active osmoregulatory and acid–base processes will contribute to the animal's SMR, accounting for some of the variance between studies performed under different water chemistries. There is a fairly extensive literature attempting to define the cost of osmoregulation, that is theoretically at a minimum in environments iso-osmotic to body tissues, in fishes. Anadromous fishes and euryhaline fishes from primarily freshwater groups tend to conform to this theoretical prediction; however, euryhaline fish from primarily marine groups seem to generally have their lowest SMR's in full-strength seawater.

Even intraspecific, SMR measurements made within the same laboratory may vary as much as 10-fold between individuals of the same size at the same temperature. What accounts for this degree of variation in oxygen consumption? Studies of fish eggs have shown substantial genetic variation in SMR even before hatching. Studies of clonal fishes have also shown substantial environmentally induced variance in metabolic rate even in laboratory-reared fish, suggesting both genetic and environmental involvement. Respiratory rates of mitochondria corrected for temperature do not vary at the same magnitude as do whole animal metabolic rates among individuals of the same species or even across different species. Thus, differences in organismal metabolic rates corrected for body size and temperature differences come from different densities of mitochondria or differential patterns of either mitochondrial or whole tissue activation.

Some of the interspecific differences in SMR may arise from differential species-level activity patterns. The more aerobically active a species is, the more mitochondria they need to fuel those periods of activity. If the mitochondria can only be throttled down to a certain level, higher rates of standard metabolism are inevitable. It has been known for some time that active fish generally have a larger gill surface area and a higher muscle–mitochondrial density than less active fish. Thus, a more active species will have a relatively greater cost of osmo- and acid–base regulation and will have to maintain more metabolically expensive tissue like gills with high protein-turnover and ion-transport rates. A general linkage of SMR with activity metabolism has been found in many taxa and formed the basis of the activity metabolism hypothesis that was proposed to explain the evolution of endothermy in birds and mammals. Extrapolating this argument to the intraspecific level in fish, differential temporal selection regimes for either efficiency in standard metabolism or aerobic activity could account for the large variance in SMR that is apparent in extant populations. However, some studies have found that exercise training in fish can simultaneously increase muscle–mitochondrial density and decrease whole-animal SMR.

There is also some evidence that population-level differences account for some of the observed intraspecific variance in SMR. Regional differences in natural selection for SMR or traits linked to SMR could produce populations that differ significantly in SMR. There is some evidence that energetically expensive morphological traits that facilitate high rates of aerobic metabolism such as a high density of mitochondria (**see also Temperature**: Mitochondria and Temperature), large hearts (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes), and higher capillary densities (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis) may be outcomes of natural selection for fast recovery from exercise and not for exercise performance *per se*, because the highest rates of performance in fish are fueled anaerobically, and fish with higher resting metabolic rates recover faster from exhaustive exercise. This could result in a bimodal composition of some fish populations, one mode composed of cruising specialists (e.g., with higher SMR, higher aerobic performance, quick recovery from strenuous swimming, but low maximum-burst speeds) and a second mode composed of sprinting specialists (e.g., with higher maximum-burst speeds, but lower SMR, lower aerobic performance, and slow recovery from strenuous swimming), both maintained through disruptive (or diversifying) selection in the wild.

Temporal and geographic factors may also influence SMR, independent of the controlling factors of temperature and sexual maturation (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). Certainly, the evidence is better for the former. Several studies have shown seasonal cycles of SMR in sexually immature fish kept at constant temperature. Krogh suggested, as early as 1915, that polar ectotherms might compensate for the effects of temperature on reaction rate by elevating temperature corrected SMR ('idling the engine faster'). This was eventually termed the metabolic cold adaptation (MCA) hypothesis and found enough early support that it was generally accepted. However, recent studies have failed to find any difference in SMR of polar fish compared with SMR extrapolated to the same temperatures for temperate and tropical species. Therefore, early measurements of $\dot{M}_{O_2}$ in polar species may have overestimated SMR, because polar fish measured in cold water require longer acclimation periods (just to recover from handling stress) and longer periods to become post-absorptive (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation).

Conclusions

Despite a century of concerted effort, we have to conclude that the measurement of fish SMR is in its infancy. The lack of standardized methods and uncertainty regarding stress level or human contact in a given study leaves much of the published literature uninterpretable. The situation is far better for swimming metabolism (**see also Swimming and Other Activities**: Energetics of Fish Swimming), although repeat measurements on the same fish are rarely made to verify results. Similarly, published scaling coefficients for SMR in fish span an incredible range, with many near 0.85 for adults and juveniles and 1 for larvae. Considering the current uncertainty in fish SMR values and how they change with body size, parameterization of bioenergetic models for real-world applications can contain many uncertainties. SMR corrected for temperature and body size is quite variable both between species and within species. Much current research is focusing on the relationship between these intrinsic differences in SMR and factors that are directly related to fitness such as dominance hierarchies or aerobic scope.

See also: **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes. **Energetics**: Physiological Functions that Scale to Body Mass in Fish. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation; Digestive Efficiency. **Gut Anatomy and Morphology**: Gut Anatomy. **Role of the Gills**: The Osmorespiratory Compromise. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism; Energetics of Fish Swimming. **Temperature**: Mitochondria and Temperature. **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration.

Further Reading

Brett JR and Groves TDD (1979) Physiological energetics. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology,* vol. VIII, pp. 280–352. New York: Academic Press.

Cano JM and Nicieza A (2006) Temperature, metabolic rate, and constraints on locomotor performance in ectotherm vertebrates. *Functional Ecology* 20: 464–470.

Cech JJ, Jr. (1990) Respirometry. In: Shreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 335–362. Bethesda, MD: American Fisheries Society.

Chabot D and Claireaux G (2008) Quantification of SMR and SDA in aquatic animals using quantiles and non-linear quantile regression. *Comparative Biochemistry and Physiology* 150A: s99.

Clarke A and Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68: 893–905.

Darveau CA, Suarez RK, Andrews RD, and Hochachka PW (2002) Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* 417: 166–170.

Frappell PB and Butler P J (2004) Minimal metabolic rate, what it is, its usefulness, and its relationship to the evolution of endothermy: A brief synopsis. *Physiological andBiochemical Zoology* 77: 865–868.

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. VI, pp. 1–98. Academic Press: New York.

Jobling M (1994) *Fish Bioenergetics*. London: Chapman and Hall.

Killen SS, Costa S, Brown JA, and Gamperl KA (2009) Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society (London) B.* 274: 431–438.

Nelson JA (2002) Metabolism of three species of herbivorous Loricariid catfishes: influence of size and diet. *Journal of Fish Biology* 61: 1586–1599.

Reidy S, Kerr SR, and Nelson JA (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *Journal of Experimental Biology* 203: 347–357.

Schmidt-Nielsen K (1984) *Scaling: Why is animal size so important?* Cambridge: Cambridge University Press.

Steffensen JF, Bushnell PG, and Schurmann H (1994) Oxygen consumption in four species of teleosts from Greenland: No evidence of metabolic cold adaptation. *Polar Biology* 14: 49–54.

West GB and Brown JH (2005) The origin of allometric scaling laws in biology from genomes to ecosystems: Towards a quantitative unifying theory of biological structure and organization. *The Journal of Experimental Biology* 208: 1575–1592.

White CR and Seymour RS (2005) Allometric scaling of mammalian metabolism. *The Journal of Experimental Biology* 208: 1611–1619.

Physiological Functions that Scale to Body Mass in Fish

CR White, The University of Queensland, St. Lucia, QLD, Australia

RS Seymour, University of Adelaide, Adelaide, SA, Australia

© 2011 Elsevier Inc. All rights reserved.

Introduction
Scaling in Embryonic Fish
Ontogenetic Changes in Standard Metabolic Rate after Hatching
Interspecific Scaling
Further Reading

Glossary

Allometric Not proportional to body mass ($b <> 1$).

Critical partial pressure of oxygen The lowest partial pressure of oxygen at which a given physiological process can be maintained.

Interspecific Between different species.

Intraspecific Between individuals of the same species.

Isometric Proportional to body mass ($b = 1$).

Metabolic rate The rate at which an organism uses energy, often measured as rate of oxygen consumption.

Scaling exponent (*b*) A biological variable proportional to body mass^b.

Scaling Study of the relationship between biological variables and body mass.

Introduction

Body size affects practically every aspect of the biology of organisms. Among fish, the effects can be enormous, because the size range of adult fish spans 7.9 mm long and about 3 mg in a member of the carp family, *Paedocypris*, to 12.65 m and 21.5 tonnes in a whale shark, *Rhincodon*. The smallest fish is smaller than any other vertebrate animal, and the largest fossil fish, *Leedsichthys*, is said to equal the largest vertebrate, a blue whale. Thus, the size range in fish spans nearly 10 orders of magnitude, making the largest living fish about 10 billion times larger than the smallest. If one includes embryonic and larval stages, perhaps another order of magnitude can be added.

Changes in body size affect nearly all aspects of an animal's biology, including not only its morphology and physiology, but also the ecology, behavior, and life history. In most cases, measures of these aspects are not linearly related to body size, but are curved. The study of how they are related to body size is called 'scaling': variables that vary in direct proportion to body mass are said to scale isometrically, whereas those that do not are said to scale allometrically, meaning by another measure.

Curves of selected variables plotted on body mass are generally fit with a power equation of the form

$$Y = aM^b \tag{1}$$

where Y is the variable of interest, a the scaling factor (which defines the elevation or height of the curve), M the body mass (in defined units, usually grams as in this presentation), and b is the power or scaling exponent of the equation (which defines the shape and direction of the curve). In order to estimate the b value, it is generally required to obtain data spanning at least an order of magnitude range in M. The b value can be estimated over smaller ranges of M, but precision is reduced. Over very large ranges of M encompassing larval and adult fish, the b value often changes and fitting only a single exponent to the data may be inappropriate.

Sometimes data are expressed in mass-specific units, in a mistaken attempt to account for the effect of body mass, for example, metabolic rate (MR) per unit of body mass. In these cases, the a value is unchanged, but the mass-specific b value ($b_{m\text{-}s}$) is the negative complement of the whole-body one ($b_{m\text{-}s} = b - 1$). This shows that taking mass-specific values does not remove the allometric effect of body mass, except under the unique situation of isometric scaling ($b = 1$). Even if the mass range is small, say only twofold, the error would be 16% or 20% if b is 0.75 or 0.67, respectively.

The importance of allometry is the realization that most variables are far from being proportional to body size. This presentation can include only selected examples of scaling in fish. However, the most common one is the relationship between MR and body size, because MR affects many of the animal's other characteristics. Unlike some vertebrate animals that reach adulthood within a narrow range of body sizes, many fish species continue to grow throughout life. The study of scaling can be approached during ontogeny with intraspecific analyses; however, it is also interesting to compare fishes phylogenetically with interspecific analyses of adults. This presentation includes both approaches.

Scaling in Embryonic Fish

The largest fish egg is from the Coelacanth (*Latimeria chalumnae*) weighing as much as 325 g, although the egg capsules of sharks can exceed this. In these cases, the maternal delivery system supplies oxygen or the well-developed juvenile ventilates its capsule in later development. However, the free-living eggs of oviparous teleost fish are surrounded by a capsule that impedes diffusion of oxygen to the embryo. The eggs of these fish are limited to less than about 5 mm capsule size, for example, in the seawolf (*Anarhichas lupus*) or the Australian lungfish (*Neoceratodus forsteri*). It is apparent that the limitation in egg size is related to the difficulty for diffusion to supply adequate oxygen across the capsule. One line of evidence is that many fish parents tend their eggs and ventilate them to renew the oxygen around them (**see also Social and Reproductive Behaviors**: Parental Care in Fishes, Sexual Behavior in Fish, and **Reproduction**: The Diversity of Fish Reproduction: An Introduction). Diffusion through the capsule is slower than through pure water, which itself occurs some 250 000 times slower than through air. The gelatinous capsules of amphibian eggs are also known to limit oxygen diffusion, keeping them relatively small as well. By comparison, the eggs of reptiles, birds, and mammals are orders of magnitude larger than fish and amphibians, because the oxygen diffuses largely in the gas phase.

There are essentially three barriers in the way of oxygen uptake by fish embryos: the capsule, the perivitelline fluid around the embryo, and the embryo's tissue itself. Before the embryo's circulatory system is developed, it is possible to calculate the maximum size that an embryo can be and still be oxygenated by diffusion through its tissues. This depends on temperature and MR. At 20 °C, the carp (*Cyprinus carpio*) embryo is near the limit at about 0.5 mg. Embryos that are larger have higher respiration rates (**Figure 1**), and require circulatory systems and respiratory exchange organs. The capsule is the other major barrier to gas exchange that persists until hatching. Embryonic movements and ciliated epithelium circulate the

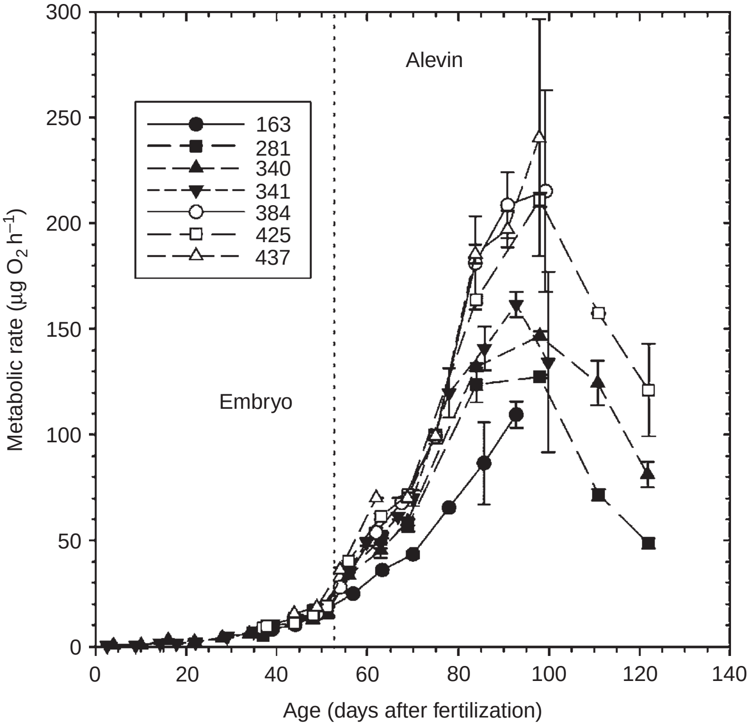


Figure 1 Metabolic rate of Chinook salmon (*Oncorhynchus tshawytscha*) embryos and alevins from eggs of seven selected sizes (163–437 mg). The vertical dashed line is the time of hatching. The effect of egg size is clear for the alevins – eggs with more resources produce larger larvae with higher rates of oxygen consumption. Reproduced from figure 1 of Rombough PJ (2007) Oxygen as a constraining factor in egg size evolution in salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 692–699.

perivitelline fluid sufficiently to deliver oxygen from inside the capsule to the respiratory surface.

Because fish eggs are generally round, it is relatively easy to measure the capsule surface areas, and relate them to the respiratory demand of the embryos. A study of Chinook salmon (*Oncorhynchus tshawytscha*) eggs of different sizes (163–437 mg) indicated that the surface area of the capsule, which scaled with egg size with $b = 0.67$, increased in larger eggs faster than respiratory demand, which scaled with $b = 0.30$. In a similar study on Atlantic salmon (*Salmo salar*), oxygen consumption was measured in eggs ranging in size of about 100–210 mg, yielding $b = 0.44$, which was again less than the exponent of egg surface area. A conclusion of the studies was that oxygen uptake is not limited by egg size. However, such scaling analyses can be misleading. The egg mass range was less than about 0.4 orders of magnitude, reducing the confidence in the value of *b*. The values are much less than scaling of early embryos and larvae of other species that are nearly isometric ($b = 1$), as discussed subsequently. In addition, the thickness of the capsule is assumed to be constant and independent of egg mass. It is not known whether capsule thickness is a constant in fish, or whether it increases with egg size. Because oxygen conductance is proportional to surface area divided by thickness, the gas conductance may scale with an exponent considerably less than that for surface area. In eggs of 11 species of amphibians, capsule thickness increases with egg size to the 0.84 power and effective surface area (the geometric mean surface area of the capsule wall) scales to the 0.52 power; conductance, therefore, scales with mass to the –0.35 power. It is evident that increasing metabolic demand in larger amphibian eggs is not matched by increasing conductance; in fact, conductance decreases. Therefore, the size of amphibian eggs is certainly limited by diffusion. It would be surprising if fish differed.

The role of the capsule in limiting oxygen uptake is apparent in the sensitivity of oxygen uptake to external partial pressure of oxygen (P_{O_2}). This is often measured by the critical P_{O_2}, above which oxygen uptake is not limited by diffusion, but below which it is. In Chinook salmon, the critical P_{O_2} increases with development until just before hatching, when critical P_{O_2} is nearly atmospheric (**Figure 2**). This illustrates that if the embryo did

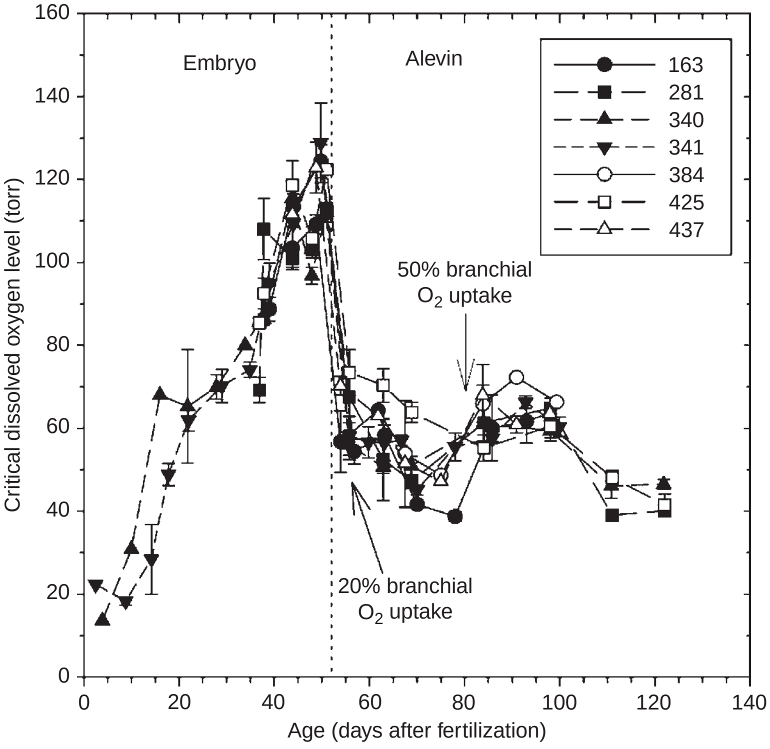


Figure 2 Critical partial pressures ($P_{O_{2c}}$) of Chinook salmon (*Oncorhynchus tshawytscha*) embryos and alevins from eggs of seven selected sizes (163–437 mg). The $P_{O_{2c}}$ is the value of external aquatic P_{O_2} below which the rate of oxygen uptake declines and above which uptake is independent of P_{O_2}. Because the capsule of embryos restricts diffusion, as the respiration rate of the embryo increases, it takes a greater external P_{O_2} to drive it into the capsule. Eventually, the $P_{O_{2c}}$ approaches atmospheric levels (~150 torr) just before hatching. After the capsule is removed at hatching, the diffusion barrier is removed, and the $P_{O_{2c}}$ drops. As the alevins grow, their increasing oxygen demand is met by increasing respiratory surface areas, so the values remain stable. Reproduced from figure 2 of Rombough PJ (2007) Oxygen as a constraining factor in egg size evolution in salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 692–699.

not hatch, increasing oxygen demand would become diffusion limited. After hatching, low critical P_{O_2} indicates that the barrier of the capsule has been removed and the gills and skin are capable of supplying sufficient oxygen (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms and Respiratory Gas Exchange During Development: Respiratory Transitions).

It is significant that the scaling of MR in hatching-stage fish is somewhat low. The same situation also occurs among amphibians ($b = 0.52$ at 30 °C and $b = 0.62$ at 15 °C) in relation to egg size. This is interpreted as an adaptation to diffusion limitation by the capsule in large eggs, because the value is low compared to respiration rates in other vertebrates and because the exponent increases at cooler temperatures that reduce oxygen demand. The price that the larger embryos pay for a relatively low respiration rate is a longer incubation time, because the rate of development depends closely on the rate of oxygen uptake. Consequently, larger amphibian eggs are incubated at lower temperatures and for a longer time. Such evolutionary trade-offs may well occur in embryonic fish. Therefore, rather than concluding that fish eggs are not diffusion limited because of a low exponent for metabolism, the exponent may in fact result from depression of MR due to the capsule, either proximately by a direct effect on respiration, or ultimately (through evolution) by an inherently low MR matched to the oxygen delivery capacity of the capsule.

Ontogenetic Changes in Standard Metabolic Rate after Hatching

There are many studies of MRs of individual species of fish over some mass range during their lives. Examples of mass-specific standard MR from 31 fish species are shown in **Figure 3**. Although the variability in the elevations of the data may be due to differences in temperature, activity levels, accuracy of measurement, and inherent differences between species, the exponents in each study should represent each species over the range of mass. Of course, the wider the mass range, the more confidence can be placed in the value of *b*. Most of the studies encompass mass ranges of about two orders of magnitude (a 100-fold difference), but some are less than one order. Even over similar mass ranges, the exponents are obviously quite variable, so there is little general information to be gained, other than to note that mass-specific respiration rate tends to decrease with increasing size, but not always.

The problems of narrow ranges of body mass have been recently overcome in several studies of standard MR in fish throughout their post-hatching life, ranging up to seven orders of magnitude in body size. Several species exhibit conspicuous breakpoints in allometric plots in which the exponents are high in the early larval stages but decrease abruptly in later stages. For example, carp with masses up to about 0.3 g show almost isometric scaling of MR ($b = 0.95$), but above this mass, the exponent is 0.82. Rainbow trout (*Oncorhynchus mykiss*) and sea bream (*Pagrus major*) also show distinct breaks during ontogeny, as do ocean pout (*Macrozoarces americanus*), lumpfish (*Cyclopterus lumpus*), and shorthorn sculpin (*Myoxocephalus scorpius*; **Figure 4**). The positions of the breakpoints and the values of *b* before and after the break vary greatly. There seems to be little correlation with metamorphosis or depletion of yolk, and the mass at the breakpoint can vary over two orders of magnitude between species. Whether a breakpoint actually exists or is a result of statistically forcing two allometric curves to fit the data is a moot point. To illustrate the problem, several analyses have been undertaken with the same data set for developing yellowtail kingfish (*Seriola lalandi*; **Figure 5**). Both a biphasic model and a quadratic model fit the data with high precision.

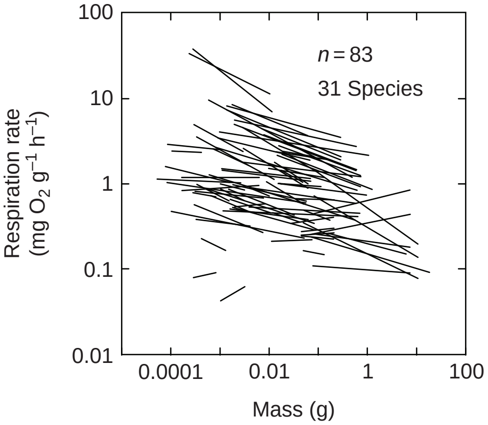


Figure 3 Meta-analysis of 81 selected studies of metabolic rate during growth in 31 species of fish. Each tick on the axes represents one order of magnitude. Reproduced from figure 2 of Post JR and Lee JA (1996) Metabolic ontogeny of teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 910–923.

Hypotheses to explain declining allometric exponents or breaks during ontogeny include changes in respiratory structures, differences in swimming ability, shifts in the proportions that different organs add to the total demand, and changes in water content. The first explanation implies that routine oxygen uptake is limited by diffusion in the respiratory gas exchangers, which is probably not true, at least because respiration rate can increase when the fish becomes active and gill perfusion increases (**see also Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish, **Integrated Response of the Circulatory System**:

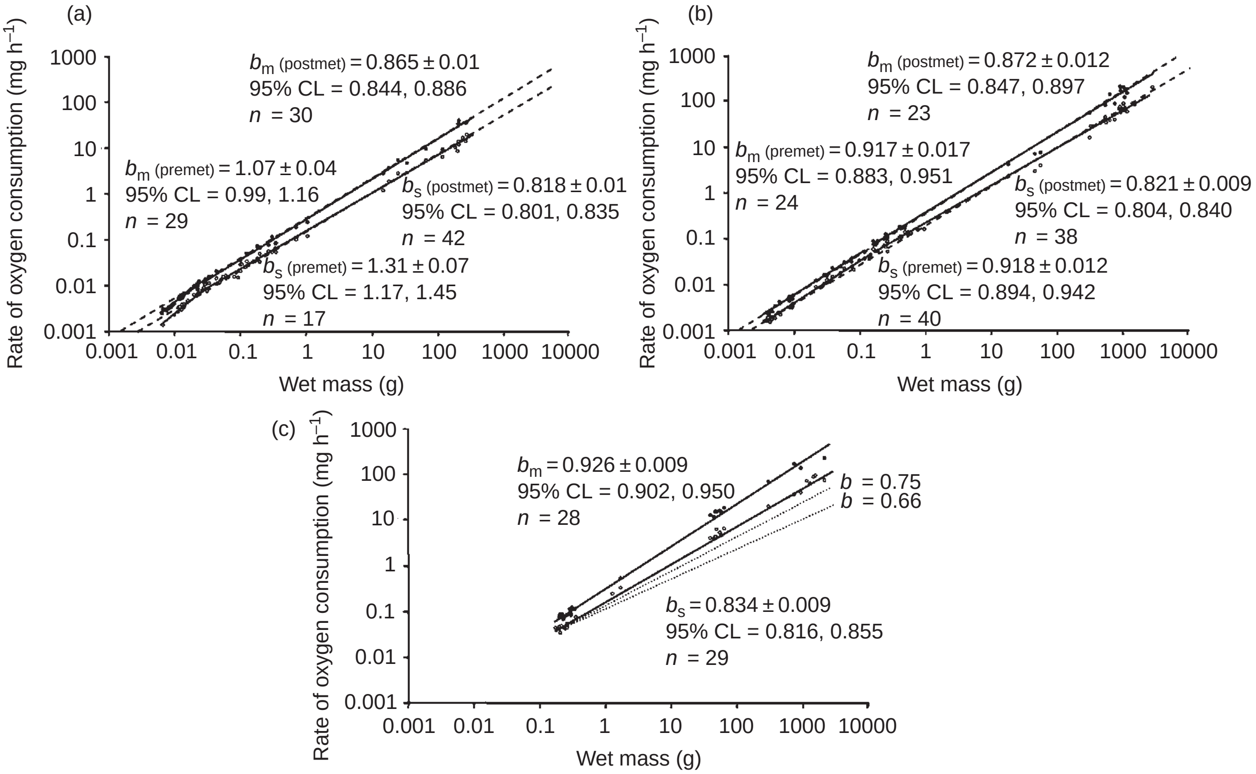


Figure 4 Rates of oxygen consumption during post-hatching development in (a) ocean pout (*Macrozoarces americanus*), (b) lumpfish (*Cyclopterus lumpus*), and (c) shorthorn sculpin (*Myoxocephalus scorpius*). Reproduced from figure 1 of Killen SS, Costa I, Brown JA, and Gamperl AK (2007) Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B: Biological Sciences* 274: 431–438.

Integrated Cardiovascular Responses of Fish to Swimming, Integrated Responses of the Circulatory System to Hypoxia, and **Control of Respiration**: The Ventilatory Response to CO_2/H^+). Even if there is a relationship between MR and gill area, it is likely that the demand for oxygen is the independent variable that ultimately influences gill development, not the reverse.

The second explanation concerns a shift in swimming ability, as fish pass through larval stages and finally reach adulthood. The argument is basically that swimming becomes more energetically efficient because there are shifts in swimming action and the fish becomes more hydrodynamically shaped. Greater efficiency would reduce MR at the same speed. This is consistent with the results from most fish, whether they are deemed to have a breakpoint in a biphasic allometry or a gradually decreasing *b*. On the other hand, the ability to increase aerobic MR above the standard metabolic rate (SMR) increases during ontogeny. This is measured as a factorial metabolic scope, which is the ratio of maximum metabolic rate (MMR) and SMR. Factorial scope increases from nearly 1 at hatching to about 6 at later stages in some species (**Figure 6**). A greater MMR, however, is not reflected in greater SMR, because the exponents differ, and the exponent for MMR is higher (**Figure 4**). Evidence from the literature suggests that athletic species, such as salmonids, have essentially isometric scaling of MMR ($b = 1$), while more sedentary benthic species show *b* values between 0.87 and 0.93. Thus, there is little basis for assuming that scaling of SMR is related to aerobic swimming performance.

The third explanation is that the decreasing value of *b* with ontogeny is possibly due to disproportionately high involvement of organs with high respiratory rate early in development. For example, the brains and kidneys are generally relatively larger in smaller fish, but decrease in relative size during ontogeny. This would also create a decreasing value of *b* with growth. Because it is difficult to assign MRs of specific organs to the whole animal, however, assessing the validity of this hypothesis may be difficult.

A fourth explanation relates to the water content of developing fish. Because the water content decreases during development in vertebrates in general, and most allometric studies measure wet mass, the energetic intensity of yolk-free younger fish may be figuratively diluted by higher water content. The result is that gradually decreasing water content causes allometric exponents to decrease. However, the extent of this effect has not been studied in fish.

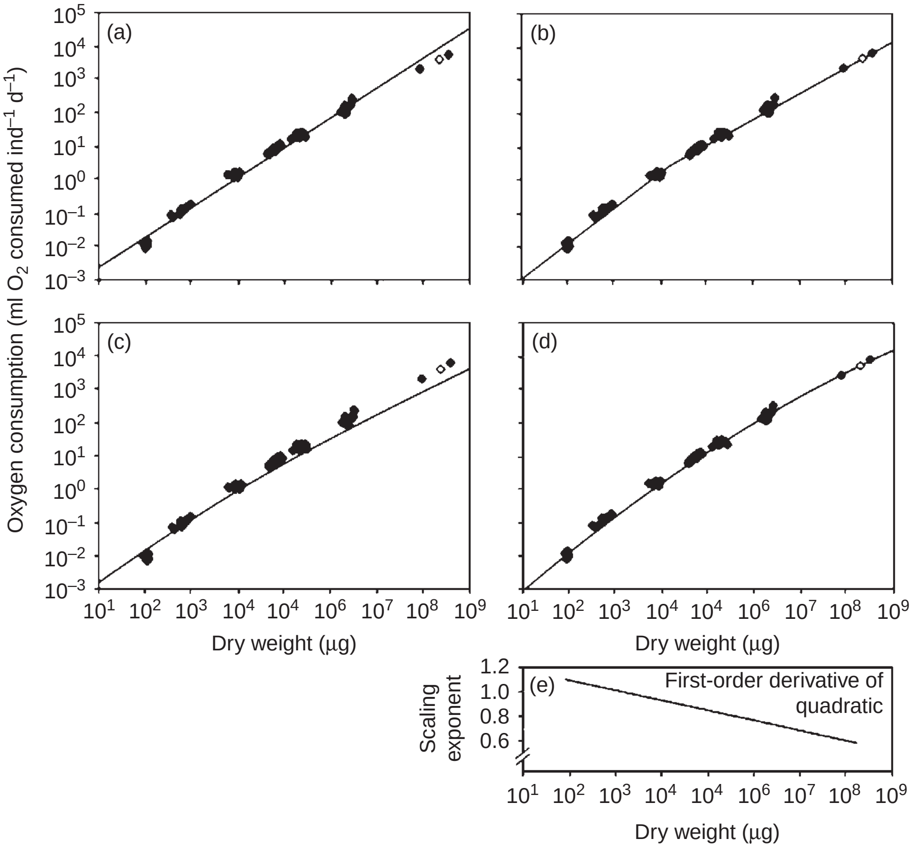


Figure 5 Oxygen consumption of yellowtail kingfish (*Seriola lalandi*) during ontogeny. Four functions were used to model the data: (a) LS linear regression; (b) LS segmented regression; (c) Bochdansky and Leggett model; and (d) LS quadratic regression. (e) Represents the first-order derivative of the quadratic regression ($y = 1.26 - 0.08x$) to illustrate the decreasing metabolic scaling exponent. From Moran D and Wells RMG (2007) Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. *Comparative Biochemistry and Physiology A* 148: 611–620.

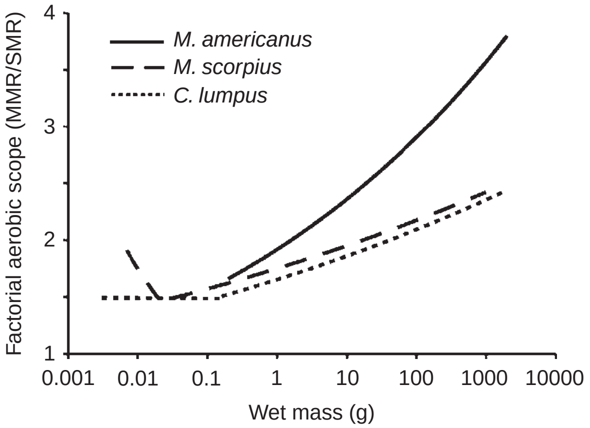


Figure 6 Ontogenetic changes in factorial aerobic metabolic scope (= maximum aerobic MR/standard MR) in three teleosts. Reproduced from figure 2 of Killen SS, Costa I, Brown JA, and Gamperl AK (2007) Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B: Biological Sciences* 274: 431–438.

There have been a few studies that examined the respiratory gas exchange structures in post-hatching fish and analyzed them allometrically. In post-hatching salmon, for example, scaling of the respiratory surface area appears to have a two-phase pattern (**Figure 7**). In the latter phase, the exponent (b = 0.86) is not greatly different from the scaling of MR at the time. Earlier, however, total surface area exceeds apparent requirements. Thus, respiration in early post-hatching larvae is not limited by surface area. It is clear that oxygen delivery to support SMR is not limited by respiratory structures in later development either, because the gills and skin can increase delivery for higher aerobic demands during activity.

Comparison of ontogenetic changes in SMR between species demonstrate that b is influenced by a range of factors, including temperature, aerobic scope, swimming mode, and lifestyle. The value of b decreases with temperature and is related to lifestyle and metabolic scope such that it is lowest in active pelagic species ($b = 0.70$), intermediate in benthopelagic species ($b = 0.78$), and highest in relatively sedentary benthic ($b = 0.80$) and abyssal species ($b = 0.86$). Swimming mode influences b in a similar manner, such that b is lower in athletic thunniform

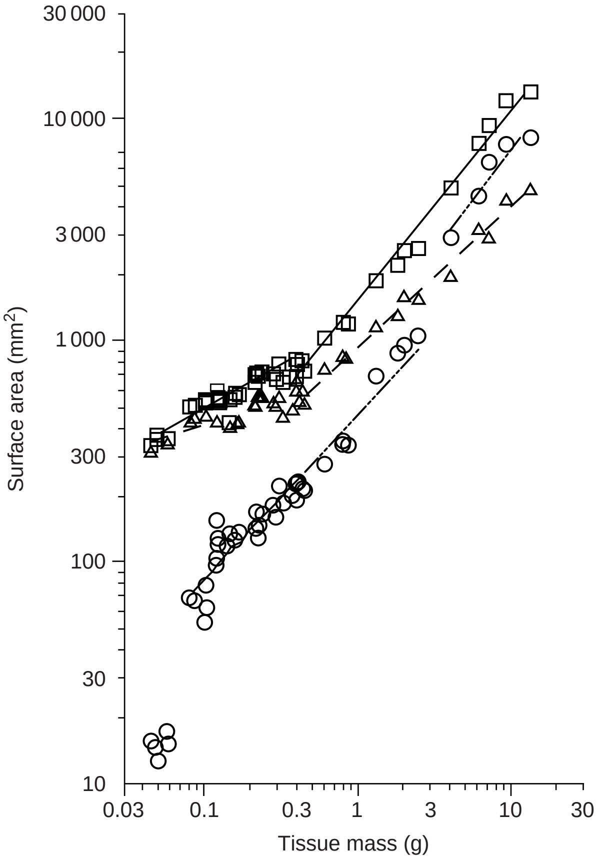


Figure 7 Relationship between the surface areas of the skin (△), the branchial structures (○), and the total (□) and body mass in chinook salmon (*Oncorhynchus tshawytscha*). Reproduced from Rombough PJ and Moroz BM (1990) The scaling and potentialimportance of cutaneous and branchial surfaces in respiratory gas exchange in young chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Experimental Biology* 154(1): 1–12.

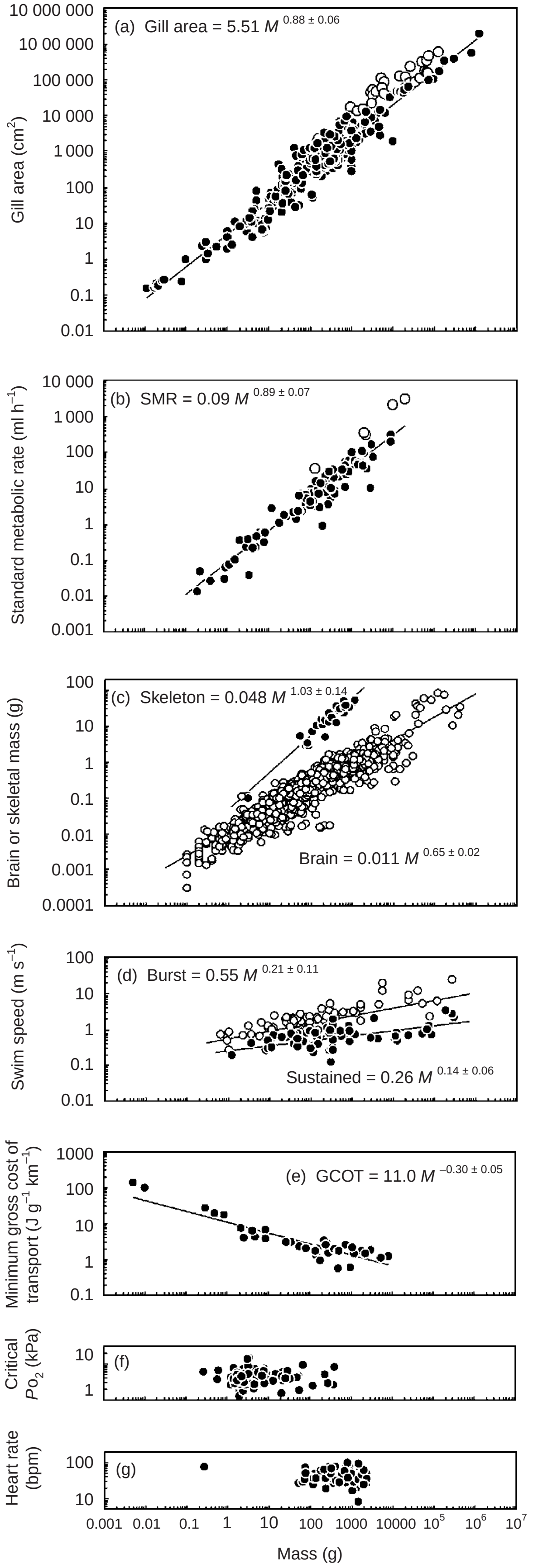
swimmers (b = 0.56) than in carangiform (b = 0.74), subcarangiform (b = 0.83), or anguilliform swimmers (b = 0.78). This effect of lifestyle and metabolic scope on b has been proposed to occur as a result of varying investment in energetically expensive tissues associated with different capacities for swimming during predator–prey interactions, but the hypothesis remains to be tested.

Interspecific Scaling

An advantage of comparative analyses of the relationships between physiological functions and body mass across a range of species is that it is possible to examine how evolutionary (rather than ontogenetic) changes in body mass influence physiology, as well as to examine data spanning very wide ranges of mass. For example, data for SMR and swim speeds are available for species spanning around 100 000-fold in mass, the relationship between brain mass and body mass can be examined for species spanning a 4.3-million-fold range of mass, and gill surface areas are available for species spanning 40-million-fold. It is notable, however, that while data for gill surface area, brain mass, sustained swim speed, and burst swim speed are available for large species (1200, 430, 290, and 270 kg, respectively), measurements of MR are currently limited to individuals no larger than 10–20 kg (lingcod (*Ophiodon elongatus*) and three species of tuna: albacore (*Thunnus alalunga*), Pacific bluefin (*Thunnus orientalis*), and Southern bluefin (*Thunnus maccoyii*)). Given the logistical difficulties involved in measuring the MRs of large fish, it seems unlikely that this will change in the foreseeable future and caution must be exercised with extrapolating from the available measurements to make estimates of SMR for larger species.

The interspecific scaling exponent of gill surface area (b = 0.88, **Figure 8**(**a**)) is similar to that of SMR (b = 0.89, **Figure 8**(**b**)). This similarity of scaling exponents, however, is not sufficient to demonstrate that gill area and SMR are related, although it seems likely that they are. At any given size, species with high SMR tend to have large gill surface areas. This is exemplified by large tunas, which have the highest gill surface areas, and SMRs of all fish (unfilled symbols in **Figures 8**(**a**) and **8**(**b**)). It is important to note that although the relationship between mass and SMR appears tight, it nevertheless conceals a great deal of variation: the SMR of species of similar mass at the same body temperature can differ by over an order of magnitude. Some of this variation is associated with climate, such that when measured at the same body temperature, species from high latitudes tend to have higher SMR than species from lower latitudes. This pattern is commonly referred to as 'metabolic cold adaptation', because species from cold environments have relatively high SMRs, but it remains a controversial hypothesis.

Skeletal mass of fish scales approximately isometrically ($b \approx 1$), although data are available for relatively few species (**Figure 8**(**c**)). Skeletal mass also scales approximately isometrically in cetaceans, but the skeletal mass of terrestrial mammals scales allometrically ($b > 1$). The difference between aquatic and terrestrial vertebrates might arise because most of the weight of aquatic animals is supported by water, whereas the skeletal elements of large terrestrial species need to be relatively robust compared to small species to support their mass against gravity. This is because the weight of a bone varies in proportion to its volume ($b = 1$), but its strength varies in proportion to its cross-sectional area, which scales with $b = 0.67$. It can therefore be predicted that the skeletal mass of animals that must support their weight against


(a) Gill area = 5.51 M 0.88 ± 0.06
Gill area (cm2)
10 000 000
10 00 000
100 000
10 000
1 000
100
10
1
0.1
0.01
(b) SMR = 0.09 M 0.89 ± 0.07
Standard metabolic rate (ml h−1)
10 000
1 000
100
10
1
0.1
0.01
0.001
(c) Skeleton = 0.048 M 1.03 ± 0.14
Brain = 0.011 M 0.65 ± 0.02
Brain or skeletal mass (g)
100
10
1
0.1
0.01
0.001
0.0001
(d) Burst = 0.55 M 0.21 ± 0.11
Sustained = 0.26 M 0.14 ± 0.06
Swim speed (m s−1)
100
10
1
0.1
0.01
(e) GCOT = 11.0 M −0.30 ± 0.05
Minimum gross cost of transport (J g−1 km−1)
1000
100
10
1
0.1
(f)
Critical Po2 (kPa)
10
1
(g)
Heart rate (bpm)
100
10
0.001
0.01
0.1
1
10
100
1000
10000
10^5
10^6
10^7
Mass (g)

gravity should increase allometrically ($b > 1$), because the cross-sectional area, not the mass, of bones must increase in proportion to animal mass.

Unfortunately, it is difficult to evaluate this hypothesis with data for fish, because all of the species for which skeletal masses are available weigh less than 1.2 kg. For these relatively small fish, skeletal masses are similar to those of similarly small terrestrial mammals. Large fully aquatic mammals (cetaceans), on the other hand, have skeletons that are a smaller fraction of body mass than large terrestrial mammals (elephants). It therefore seems that the large aquatic vertebrates have lighter skeletons than large terrestrial ones; however, measurements of skeletal weight of large bony fish (e.g., ocean sunfish (*Mola mola*) that weigh up to 2300 kg or blue marlin (*Makaira nigricans*) that weigh up to 900 kg) would be valuable in resolving the issue.

In contrast to skeletal mass, the scaling exponent of brain mass is clearly allometric ($b = 0.65$, **Figure 8**(**c**)). This value is close to that predicted for the scaling exponent of body surface area ($b = 0.67$), and the relationship between brain mass and body mass has been thought to arise as a consequence of the scaling of surface area, because the body surface is the interface between an animal and its environment. However, this hypothesis was rejected following the observation that the scaling exponent of brain mass for mammals, reptiles, and birds is significantly greater than that of surface area, and more closely approximates that of MR. A link between brain size and MR has also proved to be unsatisfactory, since in fish the scaling exponents of SMR and brain mass are different and, for any given size, there is no association between SMR and brain mass (this is also true for birds, and the existence of a link is contentious for mammals). However, mormyrid fish have exceptionally large brains, in similar proportions as humans and, undoubtedly, associated with electro-sensation (**see also Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes:An Introduction). The MR of the brains of these fish can account for 60% of their SMR.

Burst swimming speed scales with an exponent higher than sustained swimming speed ($b = 0.21$ and 0.14, respectively), though not significantly so (**Figure 8**(**d**)). The scaling exponent of burst speed is not significantly different from that of sprint speed in mammals ($b = 0.17$). However, the relationships between log-transformed sprint speed and log-transformed body mass in mammals are curved with a peak at a body mass of 119 kg. When plotted on log–log axes, the relationship for fish shows no such curvature and burst swimming speed increases throughout the mass range of species that are available (up to 290 kg, **Figure 8**(**d**)). However, the available data for many of the largest fish are not appropriate for inclusion in **Figure 8**(**d**), because body masses are generally unknown. Many of the largest fish are generally regarded as somewhat sluggish (e.g., ocean sunfish, basking sharks (*Cetorhinus maximus*), and whale sharks), and do not attain the high speeds of smaller species. For example, whale sharks and megamouth sharks (*Megachasma pelagios*) generally swim slowly (around $0.5\ \mathrm{m\,s^{-1}}$), but whale sharks can maintain speeds of $2\ \mathrm{m\,s^{-1}}$ for at least 10 min and can reach speeds of almost $4\ \mathrm{m\,s^{-1}}$ for short periods. These speeds fall toward the lower range of values recorded for other species. The fastest fish are probably either black marlin (*Makaira indica*) that are estimated to reach speeds of $36\ \mathrm{m\,s^{-1}}$, or Indo-Pacific sailfish (*Istiophorus platypterus*) that may reach speeds of $30\ \mathrm{m\,s^{-1}}$ (masses up to 750 and 100 kg, respectively). Swordfish (*Xiphias gladius*) that reach 650 kg have been measured swimming at $25\ \mathrm{m\,s^{-1}}$, while yellowfin tuna (*Thunnus albacares*; up to 200 kg) and wahoo (*Acanthocybium solanderi*; up to 83 kg) reach $21\ \mathrm{m\,s^{-1}}$. These are all smaller than the largest fish, yet they reach higher speeds. This suggests that the largest fish are not the fastest ones, just as the largest terrestrial animals are also not the fastest.

When MR is measured in flume respirometers at different swim speeds, the relationship generally increases in an upwardly bending curve, because the resistance of the water (drag) increases at higher speeds. Many fishes have been shown to swim voluntarily at an optimal speed that minimizes the energy cost of

Figure 8 Interspecific scaling of (a) gill surface area, (b) standard metabolic rate, (c) brain and skeletal mass, (d) burst and sustained swim speeds (unfilled and filled symbols, respectively), (e) minimum gross cost of transport at optimal swimming speed, (f) critical partial pressure of oxygen (P_{O_2}) for standard metabolic rate, and (g) resting heart rate. One data point for the largest body mass available for each species is shown for standard metabolic rate and brain mass; data for smaller body masses were excluded for clarity. All available data are shown for gill surface area, skeletal mass, swim speeds, cost of transport, heart rate, and critical P_{O_2}. Data for standard metabolic rate and heart rate are normalized to a body temperature of 20 °C using mean Q_{10} values calculated from available data (2.3 and 2.1, respectively). Allometric relationships (aM^b, with b shown ±95% confidence interval) were calculated by linear regression of log–log transformed data, using only the single measurement with largest M for each species; nonsignificant relationships for critical P_{O_2} and heart rate are not shown. Data for large scombrids are shown as unfilled symbols in (a) and (b) and were not included in regression calculations for gill surface area and standard metabolic rate. Data were compiled from FishBase website http://www.fishbase.org and a range of literature sources.

locomotion. The gross energy cost of locomotion at the optimum speed, compared allometrically over about six orders of magnitude body mass, scales with $b = 0.70$. However, the cost of transport is more often expressed as a mass-specific value, which represents the cost of transporting 1 g of fish a unit distance. Here, $b = -0.30$, showing that larger fish are more economical at transporting a unit of body mass than small ones (**Figure 8**(**e**)). Swimming in fish is also far more economical than any other form of transport, including running and flying, because of their hydrodynamic shape, lack of gravity effects, and generally continuous, undulatory swimming action.

Not all physiological functions vary with body mass. Both critical P_{O_2} for SMR (**Figure 8**(**f**)) and heart rate (**Figure 8**(**g**)) are not significantly related to body mass (i.e., b is not significantly different from zero). In the case of critical P_{O_2}, this probably arises because gill surface area and SMR scale with similar exponents (**Figures 8**(**a**) and **8**(**b**)). The partial pressure gradient required to sustain diffusion across the gills is therefore independent of body mass, all other things being equal. It is surprising that heart rate scales with $b \approx 0$ for fish, because large birds and mammals have lower heart rates than small ones, and the scaling exponent for heart rate is around −0.25 in these groups. However, most species of fish for which heart rate data are available weigh between 50 g and 2 kg, and high variability may obscure a trend (**Figure 8**(**g**)). The available data for zebrafish (*Danio rerio*) suggest that heart rates are possibly higher in small fish than large ones, but measurements are clearly required from fish of a greater range of body size.

See also: **Control of Respiration**: The Ventilatory Response to CO_2/H^+. **Detection and Generation of Electric Signals**: Detection and Generation of Electric Signals in Fishes: An Introduction. **Integrated Control and Response of the Circulatory System**: Central Control of Cardiorespiratory Interactions in Fish. **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Hypoxia. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Social and Reproductive Behaviors**: Parental Care in Fishes; Sexual Behavior in Fish. **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Models and Mechanisms; Respiratory Gas Exchange During Development: Respiratory Transitions.

Further Reading

Bokma F (2004) Evidence against universal metabolic allometry. *Functional Ecology* 18: 184–187.

Gibbs A and Somero GN (1990) Na^+ $-K^+$-adenosine triphosphatase activities in gills of marine teleost fishes: Changes with depth, size and locomotory activity level. *Marine Biology* 106: 315–321.

Glazier DS (2009) Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. *Journal of Comparative Physiology B* 179: 821–828.

Goolish EM (1991) Aerobic and anaerobic scaling in fish. *Biological Reviews* 66: 33–56.

Killen SS, Atkinson D, and Glazier DS (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters* 13: 184–193.

Killen SS, Costa I, Brown JA, and Gamperl AK (2007) Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B: Biological Sciences* 274: 431–438.

Moran D and Wells RMG (2007) Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. *Comparative Biochemistry and Physiology A* 148: 611–620.

Post JR and Lee JA (1996) Metabolic ontogeny of teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 910–923.

Rombough P (2006) Developmental costs and the partitioning of metabolic energy. In: Warburton SJ, Burggren WW, Pelster B, Reiber CL, and Spicer J (eds.) *Comparative Developmental Physiology. Contributions, Tools and Trends*,pp. 99–123. Oxford: Oxford University Press.

Rombough PJ (2007) Oxygen as a constraining factor in egg size evolution in salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 692–699.

Videler JJ (1993) *Fish Swimming*. London: Chapman and Hall.

White CR, Phillips NF, and Seymour RS (2006) The scaling and temperature dependence of vertebrate metabolism. *Biology Letters* 2: 125–127.

Relevant Websites

http://www.fishbase.org. – FishBase: A Global Information System on Fishes.

ENERGETICS

Food Acquisition and Digestion

Contents

Energetics of Prey Capture: From Foraging Theory to Functional Morphology
Energetics of Foraging Decisions and Prey Handling
Digestive Efficiency
Cost of Digestion and Assimilation
Dietary Requirements of Fish Under Culture Conditions

Energetics of Prey Capture: From Foraging Theory to Functional Morphology

LA Ferry, Arizona State University, Phoenix, AZ, USA

© 2011 Elsevier Inc. All rights reserved.

Overview of Competition Theory and Relevance
The Ecomorphological Paradigm
Foraging and Prey Capture Behavior
Performance Consequences
Further Reading

Glossary

Character displacement An idea linked fundamentally to the notion of competitive exclusion. Character displacement refers generally to changes in the morphology or behavior (any measurable character or trait) of a species in response to, or facilitating, the utilization of a new resource.

Competitive exclusion The process whereby two species diverge in their utilization of a limiting resource. Two species cannot utilize the same resource, if limiting, without competing and one ultimately excluding the other, to the extent that the resource is available.

Ecomorphological paradigm The structural or physical differences are a reflection of an organism's ecology.

Evolutionary stable strategy The strategy or behavior that offers an advantage compared with other strategies or behaviors. Such a strategy that, once fixed within a population, cannot be invaded by rare strategies.

Niche All the resources an organism utilizes in space and time.

Overview of Competition Theory and Relevance

In order to understand the role of competition in shaping diet and foraging patterns, it is necessary to first understand the concept of the niche. Many authors have attempted to define this, but perhaps the most popular definition is G. Evelyn Hutchinson's *n*-dimensional space that an organism occupies. This definition attempts to capture all the resources that an organism uses or interacts with in space and time. This utilization space defines the niche. Although this section focuses on diet, and therefore only a subset of the resources that an organism utilizes, this concept of niche is still useful. It is important to note that if resources are not limiting, competition will not occur, and other forces will be more important for shaping diet and foraging patterns.

Given that each organism, or set of organisms, has a niche, there exist several possible niche relationships. These are the included niche, unequal niches, equal niche breadth, and abutting or adjoining niches (**Figure 1**). In the case of the included niche, the niche

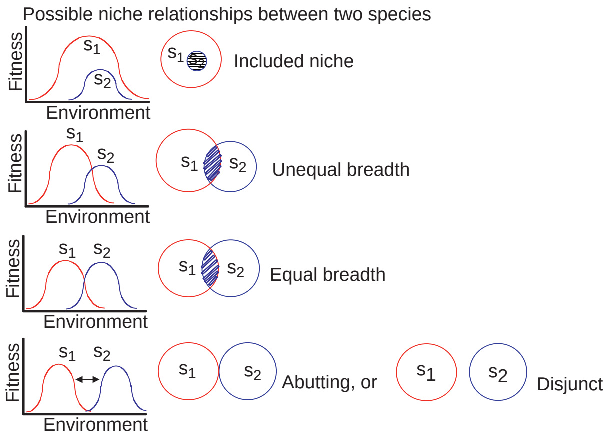


Figure 1 Graphical representation of various possible modes of niche interaction. Modified from Pianka ER (1983) *Evolutionary Ecology*, 3rd edn, 416pp. HarperCollins Publishers.

of species A is completely contained within species B. This scenario could arise when species B outcompetes A and limits it to a subset of the available resources. This scenario could also arise if resources were not limiting, and species A simply required a smaller quantity of the available resource. In the case of unequal niches, similar phenomena presumably lead to the area contained under the curve, but species A is also utilizing somewhat different resources than species B. Equal niches are produced when A and B are utilizing similar amounts of the available resources. Finally, in the case of abutting or adjoining niches, the resources utilized by species A and B do not overlap.

It is tempting, in the case of abutting or adjoining niches, to conclude that two species such as species A and B have gone through one or more of the possible relationships shown prior to exhibiting separate niches. We tend to assume that the two species progressed from a scenario wherein one suppressed the other to the point that their diets eventually diverged. Indeed, this sequence of events can take place and competition theory predicts that, when resources are limiting, they will be partitioned. Resource partitioning is achieved by what is known as limiting similarity, by making species A and B less similar. Dissimilarity is brought about by evolutionary character displacement and competitive exclusion. These interrelated ideas predict that as one species is excluded from a resource and forced to utilize another, selection will begin to favor different traits in the excluded species, and that the species will begin to adapt to the utilization of the new resource, showing traits more strongly favored for that resource or go extinct. Such selection requires that there be variation within the original source population, that these traits are heritable, and that suitable traits that confer differential fitness exist for selection to act upon. A word of caution is needed, however, when invoking this explanation for the observed distributions and patterns of resource distribution in the present-day populations. Indeed, Jim Connell treated this idea early on in the development of competition theory and its role in shaping ecological relationships with his famous paper 'Ghost of competition past'. However, demonstrating that competition occurred is possible, it is prevalent enough that many additional theories have been developed from the generalization that competition does occur and does shape resource utilization patterns, as well as the traits associated with the procurement of those resources.

The Ecomorphological Paradigm

Among these additional theories is the ecomorphological paradigm, popularized by noted functional morphologist Karel Liem. This paradigm states that structural or physical differences are a reflection of an organism's ecology (**Figure 2**). Therefore, the unstated underlying premise is

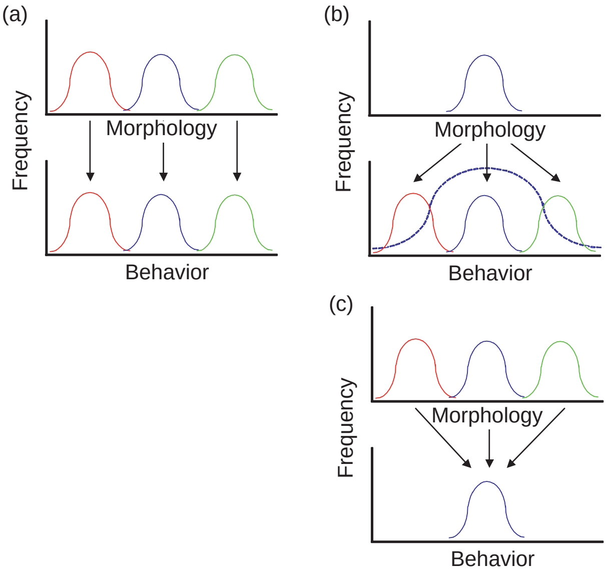


Figure 2 An expression of the ecomorphological paradigm, following from the style of niche representation in **Figure 1**. Panel (a) shows three different morphologies mapping onto three different behaviors, denoting one concept of specialization. Behavior in this sense is the action taken by an organism to utilize some aspect of the available resources. The behavior axis could just as easily be represented by a resource utilization axis. Panel (b) shows possible variations, where a species is more generalized and can perform all three behaviors with one morphology, perhaps through modulation. Modulation is the ability to choose a particular behavior from a repertoire of possible behaviors (the assumption is that the individual has the underlying ability to perform each). Another possible outcome is that the three behaviors are not distinct behaviors, but a continuum of behaviors, denoting another mechanism for generalization. Panel (c) describes a possible representation of Liem's paradox, where many different morphologies utilize one common behavior, in this case suction prey capture, and do not seem to map.

that the challenges associated with procuring resources are strong enough that they evoke change in the organism's phenotype. Competition is not necessarily required for such changes to evolve, but it is easy to see how such a force would drive change and subsequent diversification among organisms, all the more strongly. Indeed, this may serve to explain, at least in part, why we see the diversity of fish shapes that we do.

Mouth types have served as a prominent example in the literature of the ecomorphological paradigm. An example of a group of fishes that demonstrates the paradigm in a general sense is the wood-eating catfishes (**see also Food Acquisition and Digestion:** Digestive Efficiency). The wood-eating catfishes show differences in mouth shape, to some extent, as well as digestive morphology, associated with the amount of woody material in the diet. Similarly, suction-feeding fishes may show differences in the degree to which the jaws are protruded, or the heads expanded, for suction generation (**see also Buoyancy, Locomotion, and Movement in Fishes:** Feeding Mechanics). Jaw protrusion is thought to be one of the key evolutionary innovations in fishes and is evoked as one of the key reasons that fishes have diversified to the extent that they have. As it turns out, suction generation is actually quite energetically inexpensive, and therefore many fish use this as part of their prey-capture repertoire, not to mention gill ventilation. The trick, however, is in getting close enough to the prey for suction to be effective. Furthermore, differing contributions of swimming to the prey-capture event will change the energetic costs associated with feeding. This challenge for fish may be among the most important in shaping foraging behaviors.

An interesting challenge to the ecomorphological paradigm is Liem's paradox. The hallmark example of Liem's paradox is the cichlid fishes of the African rift lakes. The cichlid fishes exhibit a well-known diversity of mouth shapes often correlated, at least by authors describing the system, with incredibly novel diets and unique niches. It is often stated that these fishes underwent intense competition for resources within the rift-lake system, and therefore diverged sharply and partitioned the available resource space into many finely separated niches. It was curious, therefore, that studies of diet in these fishes sometimes showed that all of the species within the lakes were eating the same food source, zooplankton (**Figure 2(c)**). It was Beren Robinson and David Sloan Wilson, who put forth a logical explanation: when resources were seasonally abundant, all species ate the preferred food item, zooplankton, which was readily available and not limiting. When the lake levels dropped, and zooplankton became scarce, competition began and species began to utilize the food items that they appeared specialized for. That all fishes were able to perform a similar behavior (i.e., suction feed effectively on zooplankton) underscored the utility of suction feeding for aquatic prey capture and its prevalence, even with the odd changes to their morphology.

In other cases, the ecomorphological paradigm may fail entirely. While we still inherently believe that an organism's interaction with the environment shapes its morphology, there are many reasons the paradigm can fail. First, we may be studying a trait or morphological feature that we do not truly understand. Therefore, we, as scientists, fail to express the paradigm in a manner that is meaningful to the organism. For example, what is the role of the hammerhead cephalofoil on hammerhead sharks? Electrosensory? The cephalofoil is clearly covered with sensory ampullae, and the foil provides a larger surface than most other sharks would possess. Photosensory (i.e., visual)? The eyes are farther apart; therefore, the visual field may be enhanced. Hydrodynamic or locomotory? The foil may act like a wing, helping the shark to turn more easily. Furthermore, there are numerous casual observations of hammerheads using the cephalofoil to pin prey to the substrate. Are these advantages conferred by the foil, or merely ways to compensate for it? Which of these is the primary function? Which of these might merely be ways in which the foil is coopted? Why ultimately did it evolve? Similarly, the paradigm may fail because scientists attempt to ascribe function to a trait that actually has no functional value. Many morphological features also are not free to evolve in any manner and are subject to a variety of constraints. Traits may also be the product of developmental pathways, the convergence of growth zones, or inheritance of traits that long ago have lost any function.

Foraging and Prey Capture Behavior

The behaviors associated with the acquisition of food resources are shaped in part by the interactions among those organisms, including competition. This then begs the question: What is behavior? Behavior, in the most general sense, is the response of an organism to a stimulus. Behavior as a field within biology has a long and rich history. Prominent behavioral ecologist Niko Tinbergen suggested that there were four causes of behavior: (1) phylogenetic (historical) constraint; (2) mechanistic (physiological or morphological) constraint; (3) learned/experiential or ontogenetic constraint; and (4) adaptive significance. This suggests that, like morphological traits, behaviors are inherited, and can be shaped by evolution. However, also like morphological traits, they can be the mere product of constraints, particularly morphological constraints, and they may not always be functional or even beneficial.

This last point is important as it underlies the shift in the field from using optimal foraging theory (OFT) to predict or describe foraging behavior to the use of evolutionary stable strategies (ESSs) (**see also Food Acquisition and Digestion:** Energetics of Foraging Decisions and Prey Handling). It is not that OFT does not make accurate predictions. Indeed, it can be used quite effectively in many cases to model the parameters that shape individual foraging decisions or persistent population-level behaviors. However, the recognition that, in many cases, organisms represent a series of compromises, and that they often cannot make optimal decisions, has led to the use of ESS instead. ESS is that strategy or behavior that offers an advantage compared with other strategies or behaviors. They are those strategies that, just like genes or alleles, once fixed within a population, cannot be invaded by rare strategies. They are based on game theory, applied to biological concepts by John Maynard Smith, in which the outcome of an interaction depends upon the actions of the other players in the game.

There are broad categories of behavior associated with foraging that represent the steps between a hungry organism and a food item, which include (**Figure 3**): (1) encounter; (2) detection; (3) recognition; and (4) decision to ingest. So, in order for a particular food item to be found in the stomach of any given organism, that organism must encounter the organism, be able to detect it, recognize it as prey, and then make the decision to attack or otherwise ingest it. It must be noted that all of these categories have morphological bases, at least in part. For example, for an organism to encounter a particular mobile prey item, it must possess the appropriate locomotor ability to be in the general area of that prey item. However, for each category there is a strong behavioral aspect. Continuing from the same example, the moving organism above may tend to forage in particular parts of the water column, and this tendency may have been shaped by any of the four causes of behavior.

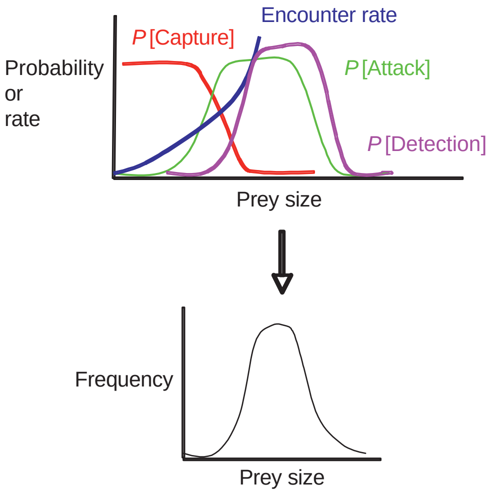


Figure 3 One possible graphical representation of the probabilities that affect what food items are ultimately taken by a foraging fish.

Of course, the tendency to be successful with that behavior is probably the ultimate cause for why it is observed. Each of the categories of behavior listed contains within it a probability distribution. There is a certain probability that our moving organism will encounter a prey item. This probability not only affects the act of encounter, but it interacts with the other steps in the chain of events as well. This means that the probability of encounter might easily then shape both the detection and recognition probabilities. This interaction can then move in both directions. The probability of successfully ingesting a prey item could easily influence the probability that an organism recognizes that item as a potential food item. If our organism has tremendous success with a particular type of zooplankter, it may begin to more readily recognize this as prey in the future.

Capture ability has both a behavioral component, such as applying the right trick at the right time, and a strong morphological component, such as having the right tools for the job. The line between behavior and morphology becomes quite blurred in terms of determining how and why certain items end up in the diet.

Performance Consequences

Changes to behavior or morphology will tend to have performance consequences. This means that a change will bring about an increase, or a decrease, in foraging success. This notion of performance consequences has been the driving force behind the study of the diversity of morphologies and behaviors exhibited within the polyphyletic group referred to as ‘fishes’. Fishes are the most taxonomically rich vertebrate group on the planet, and they inhabit nearly every realm including the terrestrial realm. The diversity in form and function associated with this richness is vast, and has spurred an entire field of research, often termed ecomorphology (or functional morphology) or ecophysiology.

It is easy to look at a typical filter-feeding fish, like a sardine, and see the long gill rakers, and immediately understand how they must function to sieve prey from the water, or, we look at a predatory fish, like a barracuda, and see the elongate jaws and sharp teeth and to understand that this is an active, highly piscivorous species. We look at the ilicium and esca on the head of a deep-sea angler fish and assume that this must be to lure prey closer to the mouth in the vast, deep sea (**see also Deep-Sea Fishes**). The ecomorphological paradigm, as detailed above, often holds true at these very general levels (though our assumptions also often go untested because we are willing to assume that what makes the most sense to us must be true). However, the interesting aspect to this research lies more in the exceptions to the rules. We know that most modern fishes rely on suction, at least in some part, to capture aquatic prey. So, it is interesting when we find a fish that feeds terrestrially, and cannot use suction because air is not dense or viscous enough, such as the four-eyed fish, *Anableps*, that leaps out of the water to capture prey. What are the performance consequences of feeding in air?

To answer this question, we need to undertake an analysis of the costs and benefits of a particular action or foraging mode. We must determine the important traits or behavioral elements associated with performing the action, and determine the cost or benefit of each of those elements compared with not performing said action. Usually, this requires a comparative approach, whereby individuals or species that perform the action to differing degrees are compared. A challenge for researchers lies in determining the metric to use for determining cost. In the case of avoiding predation, a simple metric is speed. Speed is quantifiable as velocity, for example, and has units that are both easily measured and understood and can be related back to energetics (**see also Swimming and Other Activities:** Energetics of Fish Swimming). What do we quantify with regard to foraging success? What is the value of an individual prey capture attempt? As newer and newer technology is applied to these sorts of research questions (**see also Buoyancy, Locomotion, and Movement in Fishes:** Feeding Mechanics), we begin to find ways to measure variables such as water velocity entering a fish mouth, and muscular work. These sorts of questions will continue to drive our understanding of fish foraging behavior and morphology, and the diversity that exists within these.

See also: **Buoyancy, Locomotion, and Movement in Fishes:** Feeding Mechanics. **Food Acquisition and Digestion:** Energetics of Foraging Decisions and Prey Handling. **Swimming and Other Activities:** Energetics of Fish Swimming.

Further Reading

Connell JH (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos* 35: 131–138.

Carroll A (2004) Muscle activation and strain during suction feeding in the largemouth bass *Micropterus salmoides*. *Journal of Experimental Biology* 207: 983–991.

Carroll A and Wainwright PC (2006) Muscle function and power output during suction feeding in largemouth bass, *Micropterus salmoides*. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* 143: 389–399.

Carroll AM and Wainwright PC (2009) Energetic limitations on suction feeding performance in Centrarchidae. *Journal of Experimental Biology* 212: 3241–3251.

Day SW, Higham TE, Cheer AY, and Wainwright PC (2005) Spatial and temporal patterns of water flow generated by suction feeding bluegill sunfish *Lepomis macrochirus* resolved by particle image velocimetry. *Journal of Experimental Biology* 208: 2661–2671.

Gross MR (1994) The evolution of behavioural ecology. *Trends in Ecology and Evolution* 9: 358–360.

Kajiura SM, Forni JB, and Summers AP (2003) Maneuvering in juvenile carcharhinid and sphyrnid sharks: The role of the hammerhead shark cephalofoil. *Zoology (Jena)* 106: 19–28.

Kajiura SM and Fitzgerald TP (2009) Response of juvenile scalloped hammerhead sharks to electric stimuli. *Zoology* 112: 241–250.

Liem KF (1993) Ecomorphology of the teleostean skull. In: Hanken J and Hall BK (eds.) *The Skull: Functional and Evolutionary Mechanisms*, vol. 3, pp. 422–451. Chicago, IL: University of Chicago Press.

McComb DM, Tricas TC, and Kajiura SM (2009) Enhanced visual fields in hammerhead sharks. *Journal of Experimental Biology* 212: 4010–4018.

Pianka ER (1983) *Evolutionary Ecology*, 3rd edn, 416pp. HarperCollins Publishers.

Robinson BW and Wilson DS (1998) Optimal foraging, specialization, and a solution to Liem's paradox. *American Naturalist* 151: 223–235.

Schaeffer B and Rosen DE (1961) Major adaptive levels in the evolution of the Actinopterygian feeding mechanism. *American Zoologist* 1: 187–204.

Energetics of Foraging Decisions and Prey Handling

SS Killen, University of Glasgow, Glasgow, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Motivation to Feed – When to Forage?
Habitat Selection – Where to Forage?
Searching for Food – How to Forage?
Prey Selection – What to Forage?
More on Prey Handling
Further Reading

Glossary

Aerobic scope The capacity for an animal to increase its level of aerobic metabolism beyond that which is required for maintenance alone; it sets the capacity for oxygen-consuming physiological processes that an animal may perform at a given time. Aerobic scope is the difference between minimal and maximal oxygen consumption rate.

Fitness (Darwinian) Describes the ability of a genotype to reproduce and propagate into the next generation. It is influenced by the survival and fecundity (reproductive output) of individuals.

Foraging Behaviors performed by animals when searching for, identifying, capturing, subduing, and consuming food.

Ideal free distribution A theoretical concept used to describe how animals distribute themselves across food patches; This distribution predicts that animals will disperse across patches in a manner that is proportional to the food available in each patch.

Maximal metabolic rate The maximum metabolic rate achieved by an animal. If measured as O_2 uptake, it would be termed V_{O2max}.

Optimal foraging theory A theoretical framework used to study the foraging decisions of animals. Incorporating several assumptions, it predicts that animals should forage in a manner that maximizes the net energetic intake.

Piscivore An animal that feeds on fish, either exclusively or as part of a wider dietary range.

Planktivore An animal that feeds on plankton, either exclusively or as part of a wider dietary range.

Prey handling Behaviors performed by a predator during the capture and consumption of a prey item, including the subduing, orientation, or sectioning of prey (biting, tearing) before consumption.

Standard metabolism In ectotherms, this term describes the minimal level of aerobic metabolism (per unit time) required for maintenance functions to sustain life. In this sense, it is analogous to basal metabolism in endotherms, though standard metabolic rate (SMR) is strongly influenced by environmental temperature and so it is not always constant within an individual.

Introduction

Fish must consume enough food to satisfy their energetic requirements for maintenance, growth, activity, and reproduction. From an evolutionary perspective, natural selection should favor the most efficient foragers. This concept forms the basis of optimal foraging theory (OFT), which posits that animals should behave in a manner that maximizes the rate of net energy intake. Energy input in the form of food is required to sustain biological function, and so it is assumed that net energetic intake is positively correlated with fitness. To achieve a maximum rate of energy accumulation, a fish must not only optimize energy intake through food ingestion, but also minimize the amount of energy spent on foraging. Another important consideration is time; time spent on capturing or handling prey may detract from further foraging (i.e., energy acquisition), or other important behaviors such as predator avoidance or reproduction.

While OFT provides a useful foundation for predicting how fish should behave while foraging, in reality, fish are subject to constraints that may lead to suboptimal energetic behavior. When observations do not agree with the theoretical predictions of OFT, however, it is often interesting and informative to study why this is so. There are numerous factors (e.g., predator avoidance) that affect fitness besides net energy accumulation, and it is impossible to simultaneously optimize all of these components in the performance of a behavior or physiological process. Furthermore, because all biological functions

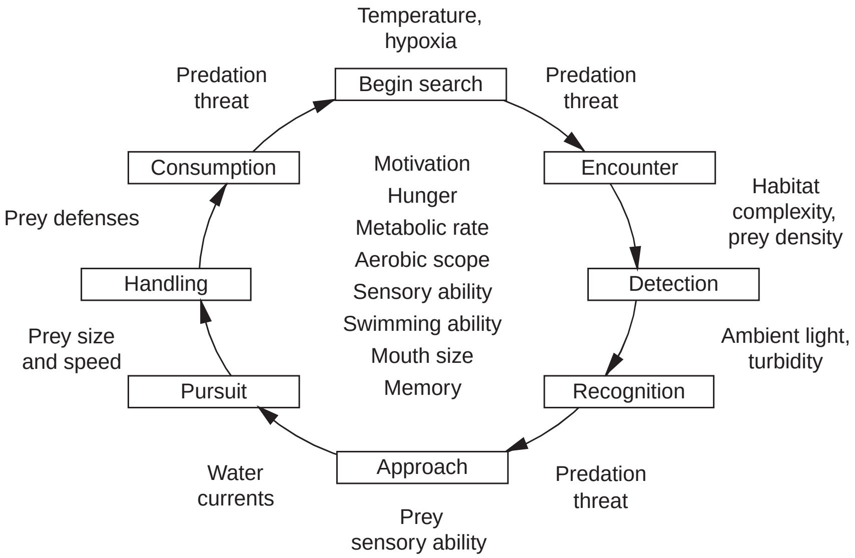


Figure 1 Components of a typical foraging cycle in fish, beginning with the onset of searching for prey and ending with prey consumption. Inside the circle are various internal factors (e.g., physiological and morphological characteristics) that may influence the outcome of the various phases of the foraging cycle. Outside the circle are various external factors (e.g., environmental variables and prey characteristics) that may influence the energy expenditure by the predator that occurs during each phase, the likelihood of each phase occurring, or the outcome. External factors most relevant to foraging energetics are listed alongside points in the cycle where they may have the greatest effect. Note that predation threat may have effects at numerous points along the foraging cycle. This is because a predatory threat could cause a foraging fish to abandon foraging behavior at all points throughout this cycle. Adapted from Hart PBJ (1997) Foraging tactics. In: Godin J-GJ (ed.) *Behavioural Ecology of Teleost Fishes*, pp. 104–133. New York: Oxford University Press, by permission of Oxford University Press.

require finite resources of time and energy, there are often inescapable constraints on their execution that restrict fish from maximizing their net-energy gain. While individual fish may not always forage in a manner that is energetically optimal, they still should adjust their behavior and physiological function within these trade-offs to maximize their overall fitness (**see also Food Acquisition and Digestion**: Dietary Requirements of Fish Under Culture Conditions).

After a fish is motivated to begin feeding, a typical foraging sequence proceeds with a search phase, followed by prey detection and recognition, an approach by the predator and a possible pursuit and attack, some handling of the prey, and then finally, consumption of the prey item by the predator (**Figure 1**).

There are numerous energetic trade-offs and constraints that affect the foraging decisions of fish at all points throughout this sequence.

The Motivation to Feed – When to Forage?

Internal Factors

Hunger

Individuals provided with an unlimited supply of food after fasting will initially display a high feeding rate, presumably due to increased hunger, but this rate will decrease as the animal satiates. Circulating concentrations of certain metabolic substrates and endocrine and paracrine substances are also important in regulating appetite (**see also Integrated Function and Control of the Gut**: Endocrine Systems of the Gut and **Gut Anatomy and Morphology**: Gut Anatomy). For example, injection of glucose, amino acids, or leptin tends to decrease feeding rates, whereas circulating ghrehlin will increase feeding. The change in appetite caused by fluctuations in metabolic substrates is mediated through hormonal and paracrine signaling between the gut and the brain and within the brain itself. Appetite can also be stimulated by certain olfactory and gustatory cues from the environment such as waterborne amino acids and carbohydrates.

Metabolic rate and aerobic scope

It is often hypothesized that the energetic requirements of maintenance may directly influence the motivation to forage in individual fish, possibly by increasing the frequency or intensity of hunger bouts. In ectotherms, the minimum amount of energy required to perform life-sustaining physiological functions can be quantified by measuring standard metabolic rate (SMR) (**see also Energetics**: General Energy Metabolism). While a relationship between resting metabolic demand and foraging motivation is often assumed to exist, there have been few attempts to confirm this link experimentally. Feeding rates tend to increase with temperature in fish, and this is at least partially due to an associated increase in metabolism and energetic demand. Still, the effect of metabolic rate on appetite in individual fish, independent of temperature, is not well understood. Indeed, territorial species may even show a negative correlation between SMR and foraging activity; aggressive individuals with correspondingly high SMRs may initially devote more time and energy to territorial defense as opposed to foraging. In the long term, however, this strategy may lead to increased foraging opportunities once a high-quality territory is established.

Another metabolic trait that may have an important influence on appetite is aerobic scope. Aerobic scope represents the capacity of an animal to increase its level of aerobic metabolism above that which is required for maintenance, and is bounded at its upper limit by an animal's maximal aerobic metabolic rate (MMR; **see also Swimming and Other Activities**: Energetics of Fish Swimming and **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration). This aerobic scope sets a limit on the simultaneous performance of oxygen-consuming physiological processes within an animal. In many fishes, feeding rate correlates well with aerobic scope, presumably because an elevated aerobic scope can accommodate the increase in metabolic rate associated with digestion and nutrient assimilation without compromising other physiological functions. Further research is needed to examine the relationship between appetite, maintenance metabolism, and aerobic scope in fish.

External Factors

Predation threat

Foraging makes animals more obvious to predators and also reduces the vigilance of individuals toward potential threats. For these reasons, fish generally decrease foraging in the presence of a predatory threat, and predator avoidance is a major constraint on the ability to maximize energy intake through foraging. In general, laboratory fish offered food in the absence of a predator eat more and grow faster than when a predator is present. The exact magnitude of such foraging reductions is influenced by the physiological state of an individual. Hungry or food-deprived fish display increased feeding in the presence of a predatory threat relative to that in well-fed fish, and they will resume feeding sooner after fleeing from an attack. It also has been shown that individual fish have distinct personalities, consistently behaving either more or less risky under the threat of predation (**see also Social and Reproductive Behaviors**: Dominance Behaviors). There is evidence to suggest that these behavioral differences among individuals are linked to genotype and intrinsic physiological factors such as metabolic rate, with individuals with increased SMR displaying increased risk-taking behavior during foraging. For example, in juvenile Atlantic salmon (*Salmo salar*), individuals with increased SMR tend to forage outside of covered areas more often and are thus able to attain higher rates of feeding. In contrast, larvae of many fish species decrease foraging under the threat of predation despite their high mass-specific metabolic rate, thereby suffering possible decreased growth, depletion of lipid stores, and increased mortality. However, growth-enhanced transgenic fish have such high appetites that they take more risks to feed, and as a result, are often eaten by predators in experimental situations (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish).

Temperature

Feeding in fish increases with increasing temperature until a peak at some intermediate species-specific temperature, and then shows a strong decline with further temperature increases, creating an optimum temperature for feeding and hence growth. The initial increase in feeding with temperature parallels the temperature-driven rise in metabolic rate. Interestingly, metabolic rate generally continues to increase with temperature beyond the point at which feeding rate begins to decline, and so changes in metabolic demand cannot solely be responsible for temperature-related changes in foraging motivation at least at these higher temperatures. Aerobic scope in fish follows a parallel pattern of response to temperature change as does feeding rate. In many species, the temperature at which aerobic scope is highest is also the peak temperature for feeding and growth rates (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation, **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism, **Temperature**: Membranes and Temperature: Homeoviscous Adaptation).

Hypoxia

Decreased environmental oxygen (hypoxia) strongly decreases appetite in water-breathing fish. Indeed, many fishes completely halt feeding when environmental oxygen becomes limiting. This is most likely due to the tremendous decrease in aerobic scope that occurs during hypoxia (both SMR and MMR decrease during hypoxia, but MMR shows a much greater decline), and likely serves to conserve some of the limited aerobic scope for other physiological processes besides digestion (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia and **Hypoxia**: Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia). Despite these constraints, some fish species that live in stratified waters (e.g., anchovy *Anchoa* spp.; mudminnow *Umbra limi*) have been observed to enter hypoxic-water layers briefly to capture prey, though they return to normoxic waters for digestion.

Habitat Selection – Where to Forage?

Food Availability and Predation Risk

As mentioned, fish that reduce foraging activity in the presence of an immediate predatory threat will incur an energetic cost, but such encounters can be avoided altogether if fish occupy a safer habitat. Often, this results in a behavioral trade-off between occupying a dangerous, resource-rich habitat, or a safe, resource-poor habitat. For instance, young lake fishes have increased foraging success while feeding on zooplankton and other prey in open environments. These same habitats, however, leave young fish exposed to being preyed upon, and so they instead may choose to forage in less-profitable but highly structured habitats that provide shelter. Such habitat shifts occur in environments with high predator populations and can cause reduced growth rates in young fish.

The relationship between predator-mediated habitat selection and energetic or nutritional demand in fish has not been investigated directly, though food-deprived fish tend to occupy habitats that are dangerous and more profitable as well. It is possible to estimate the energetic equivalence of predation risk in a dangerous foraging patch by observing predator-induced deviations from the ideal free distribution. Fish foraging on food items that are homogeneously distributed across two foraging patches show a nonequal distribution between the patches when one patch contains a predator. Fewer individuals use the risky patch, but those that do have greater access to food (per fish). The difference in food intake (per fish) between individuals in the risky and safe patches can be considered as the energetic equivalent of the imposed risk. This approach has been useful for gaining insight into the effects of factors such as gender and morphology on habitat selection, and could also be used to investigate the physiological thresholds that cause fish to select dangerous but profitable habitats (e.g., the effect of SMR, RMR, or aerobic scope on habitat selection).

Flow Velocity

For stream-dwelling fishes (e.g., juvenile salmonids), the choice of foraging location is influenced by localized differences in water flow. The encounter rate with drifting prey increases with flow velocity, but the actual number of prey captured declines at relatively high flows because prey are more difficult to catch. The energetic cost of foraging also increases with increasing flow velocity, because fish need to swim faster to keep up with the prevailing current. Furthermore, the energy spent on unsteady swimming during pursuits and prey capture increases with flow velocity due to increased drag. As a result of these opposing costs and benefits, there is an optimal velocity at which the net energy gain will be maximized. Observations in the laboratory and the field have shown that the flow velocity chosen by fish often matches with the predicted optimal velocity, though the exact foraging location selected also depends on factors such as prey size, prey density, the level of predation threat, and competition with other fish.

Territoriality and Aggression

Territoriality for foraging patches is well known in reef fish and stream-dwelling salmonids, though the energetic aspects of territoriality have been primarily studied in the latter (**see also Social and Reproductive Behaviors**: Dominance Behaviors). In juvenile salmonids, more aggressive individuals defend feeding territories by chasing away intruding conspecifics. The energy spent on these behaviors, per unit time, is at least twice the amount used for maintaining position in a flowing stream alone. Still, territorial fish have higher rates of feeding and growth compared to those that are not able to hold territories. Once a territory is established, the resident fish apparently spends less time and energy on aggressive behaviors, while nonterritorial fish continue to engage in aggressive encounters with other fish. Territorial fish also spend less energy on swimming because they spend more time maintaining station as opposed to maneuvering at an angle through currents.

Despite these apparent benefits of territoriality, the profitability of aggressiveness is context dependent. When aggressive and nonaggressive fish are given equal access to food, it is the nonaggressive individuals that grow faster. This may be due to the more-aggressive fish generally spending more energy on activity or having a higher SMR. Therefore, while aggressiveness may be beneficial when food resources are limiting, increased aggression apparently costs in situations where it is not possible to monopolize food resources.

Searching for Food – How to Forage?

Search Speed

Some fish species (e.g., tuna and salmonids) cruise through their environment actively searching for prey, whereas others remain nearly stationary, lying in wait until they ambush unsuspecting prey that happen to pass (sit-and-wait foragers; e.g., pike *Esox* spp.; sculpin *Myoxocephalus* spp.).

Each of these strategies carries distinct costs and benefits: cruise foragers incur greater locomotory costs, but increased prey-encounter rates; ambush foraging is less costly, but prey encounters are relatively rare. Between these two extremes, there is a continuum of search strategies, in which individuals swim through their environment but frequently pause to scan for prey (saltatory foragers; e.g., bluegill *Lepomis macrochirus;* arctic grayling *Thymallus arcticus;* and white crappie *Pomoxis annularis*). Species that use this strategy may be closer to either the cruise or sit-and-wait foraging strategies along the continuum depending on the relative durations of the swimming and pausing phases. This pattern of intermittent movement may allow for better detection of prey during pauses, and heighten the ability to detect predators while foraging. A potential drawback of saltatory foraging is that locomotory costs may be increased due to frequent acceleration and deceleration. On the other hand, saltatory searching may ensure that a given volume of water is searched more thoroughly, and so less swimming will be needed to search a given space.

For planktivorous fishes, including the larvae of many species, rates of prey intake increase with swimming speed, gradually level off, and possibly even decrease at high speeds (**Figure 2**).

At the same time, the costs of locomotion (energy per unit time) increase curvilinearly with swimming speed. Based on these considerations, fish should forage at the speed that provides the greatest difference between energy cost and energy return. Factors such as habitat and prey density also play a role in determining the optimal-foraging speed; fish should (and usually do) swim faster at low prey densities and slower at high prey densities. Fish also swim faster while foraging when they are hungry as compared to when they are well-fed.

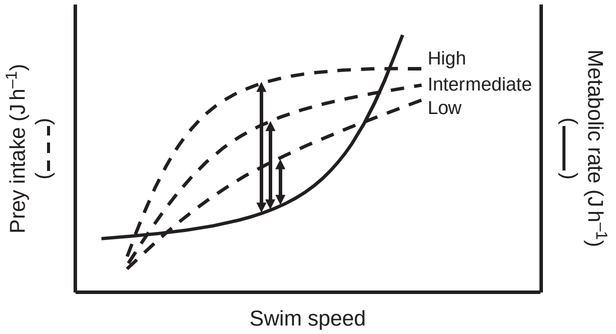


Figure 2 Schematic diagram illustrating changes in prey intake and metabolic rate (as estimated by measuring oxygen uptake) with swimming speed at three different prey densities (high, intermediate, and low). The predicted optimal swim speed at each density is that which allows for the greatest net energetic intake (largest difference between potential energy consumption and the costs of locomotion while foraging). Based on data from Ware DM (1975) Growth, metabolism and optimal swimming speed of pelagic fish. *Journal of the Fisheries Research Board of Canada* 32: 33–41 and Dabrowski K, Takashima F, and Law K (1988) Bioenergetic model of planktivorous fish feeding, growth and metabolism: Theoretical optimum swimming speed of fish larvae. *Journal of Fish Biology* 32: 443–458.

Laboratory and field observations generally match these theoretical predictions, though in some situations they do not. For example, fish foraging at high densities (e.g., fish shoals) tend to swim faster than their theoretically optimal speed. This could be due to the increased density of conspecifics and competition for available food, or differences in hydrodynamic efficiency when swimming in large groups. In other instances (e.g., some flatfish species), fish may not be able to achieve their optimal swim speed due to limited maximal aerobic capabilities. Furthermore, in some species (e.g., juvenile lumpfish *Cyclopterus lumpus*), foraging at the optimal swimming speed may comprise such a significant portion of the available aerobic scope that it would constrain the ability to perform additional physiological functions.

Search Path

Another consideration when actively foraging is the path that will be taken while scanning for prey. In habitats with low prey concentrations or prey that are clumped in small or spatially discrete patches, fish tend to search in a directed manner with low turning rates and low overlap of areas that have been searched already. In this type of directed foraging, the amount of spatial overlap with previously searched areas also decreases with experience and familiarity with a search range, which serves to decrease the energetic costs of searching. By contrast, fish feeding in habitats with high prey concentrations search in a more convoluted path with a higher turning rate and increased spatial overlap. A high turning rate while searching translates to increased foraging costs, but the fish are able to search a patch more thoroughly for prey.

The ability to search a given volume of water is not constant throughout ontogeny. Due to their limited visual range and swimming ability, larval fish have a very restricted search space as they move through the water column. The larvae of a few species have acquired interesting morphological adaptations to improve search efficiency, such as protruding eye stalks that increase visual range and therefore decrease the amount of swimming that is required to search a certain volume. As fish increase in size, their sensory systems and swimming ability improve, allowing them to search larger volumes of water more efficiently.

Foraging Modes

Many species display behavioral flexibility while searching for prey and are able to switch between alternate foraging modes in response to varying environmental conditions. Some species, for example, cruise forage when prey densities are low (e.g., lumpfish, juvenile salmonids, and nase *Chondrostoma nasus*), but become ambush foragers at high prey densities. Among ectothermic species that display search mode-switching behavior, the more energetically costly search mode is generally used when prey is scarce or in dispersed patches, and the less costly mode is used when prey is abundant or aggregated. This pattern is directly opposite to that observed in endotherms, for which the more costly search mode is used at high prey densities. This difference is most likely due to differences in metabolic demand; the relatively high metabolism of endotherms means that active foraging is only economical when prey is abundant. Meanwhile, less energy is required for activity in ectotherms, permitting them to actively seek out food when prey is scarce and conserve energy when prey is abundant. Reducing activity in this manner is also an effective way to conserve space within their aerobic scope, which is already relatively limited as compared to that of endotherms.

Prey Selection – What to Forage?

Prey Type and Size

The basic prey model (BPM), an offshoot of OFT, states that when selecting prey, energy gain should be maximized per unit of prey-handling time. Prey-handling time is often defined as the time between prey capture and ingestion, but many researchers also include the time used to pursue prey after detection. For a given prey type (e.g., mussels and minnows), large prey contain more energy than small prey but will take longer to capture, subdue, and consume. In fact, for a given prey type, there is usually an exponential increase in both the time and energetic costs of handling with size. Therefore, a trade-off exits between the energy provided by larger prey, and the time and energy required for their capture and handling. Studies with planktivorous fishes have found general agreement with the BPM, because these species tend to minimize time and energy spent foraging by selecting the largest prey items available. For piscivorous fishes, however, prey are often smaller than predicted. This is likely related to the inherent difficulty in capturing larger prey fishes, and variability regarding the energetic cost during pursuits. Piscivores may preferentially attack smaller fish because of the reduced chances of an escape or unexpectedly costly pursuit. Alternatively, it has been suggested that piscivores are indeed not size selective, but that estimates of prey-size preference based on gut contents simply reflect differential size-based capture success. There are also numerous anecdotal accounts of individual fish attempting to swallow prey items much larger than normally would be expected. For instance, it is not uncommon to observe a shorthorn sculpin *Myoxocephalus scorpius*, an ambush predator, with the tail of a much larger fish protruding from its mouth.

Numerous additional factors may affect moment-to-moment decisions when selecting prey. Habitat type may affect prey selection; fish tend to be less selective when foraging in complex environments. Learned responses by the fish may also alter the relative profitability of prey types, as prior experience with prey types reduces handling times. Prey selectivity may even change over the course of a single foraging bout. Fish tend to be less selective if they are hungry and become more selective as they feed. These factors are often overlooked, but could have important effects on tests of foraging models because they indicate that preferences for prey are highly dynamic. Possible physiological correlates of prey selectivity have not been investigated thoroughly, but there are numerous possible research avenues in this regard. For example, it could be hypothesized that fish with increased metabolic requirements (increased SMR) may be less selective when foraging.

Ontogenetic Diet Shifts

The diet of fish larvae is restricted to the smallest zooplankton, but as they grow, increased mouth gape size and swimming ability can allow for the capture and ingestion of a wider range of prey types. The ingestion of larger prey and increased feeding opportunities allow for increased growth, which in turn allows for the ingestion of even larger prey, and so on. Meanwhile, smaller prey that were once dietary staples may not be consumed at all once fish reach a larger size because of the relatively low energetic value that they provide. Such changes in diet with growth and development are widespread among fish species (**Figure 3**).

Many piscivorous fishes are planktivores as larvae, shift to feeding on benthic prey, and then reach a large enough size to begin feeding primarily on fish. In addition to gape size, an important driver for ontogenetic diet shifts may be changes in metabolic demand during ontogeny. If fish are unable to access larger prey during ontogeny, they can become growth stunted or show decreased body condition. Energetic demand can also change abruptly at certain points during development independent of size (e.g., during metamorphosis), and such changes in metabolism might necessitate changes in diet.

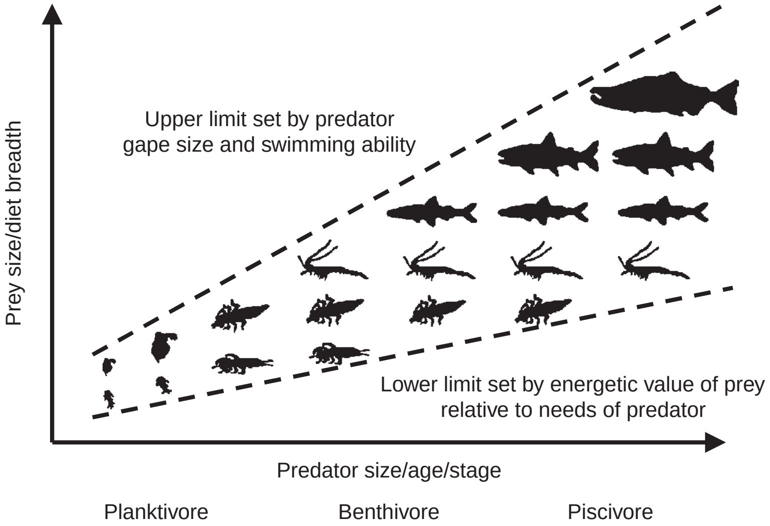

Upper limit set by predator gape size and swimming ability
Prey size/diet breadth
Lower limit set by energetic value of prey relative to needs of predator
Predator size/age/stage
Planktivore
Benthivore
Piscivore

Figure 3 Shifts in diet during ontogeny. As fish grow and develop, increased gape size and improved swimming ability allow for the capture and consumption of a greater range of prey types. Adapted with permission from Brett Johnson (http://welcome.warnercnr.colostate.edu/class_info/fw300/flashcrd/ontogeny.gif).

More on Prey Handling

Costs of Prey Pursuit and Capture

Maneuvering while chasing prey is more costly than is swimming at a fixed velocity. Juvenile Atlantic salmon in streams are less willing to travel across prevailing currents to intercept drifting prey at increased flow velocities. One reason why prey pursuits are costly is because they often require inefficient burst-type anaerobic swimming. Interestingly, the anaerobic capacity of fish may adapt to their diet. For example, fish that regularly feed on larger prey develop higher concentrations of white muscle lactate dehydrogenase (an important enzyme required for anaerobic metabolism) than those that feed only on smaller prey. This change in muscle biochemistry probably helps prepare these predators for the increased anaerobic performance required for pursuing larger fish. Though it usually does not require an anaerobic contribution, even filter feeding on planktonic prey is costly, because fish must swim constantly with increased drag caused by the flaring of the operculae and movement of water past the gill rakers. For larval fish, the costs of pursuing prey are substantial because the vast majority of larvae are active foragers and must overcome disproportionately large amounts of viscous drag due to their exceedingly small body size.

Prey Orientation and Ingestion

Piscivorous fishes most often attack prey by biting the head and swallowing the prey headfirst. There are a few species (e.g., pike, walleye *Sander vitreus*, and rainbow trout *Oncorhynchus mykiss*) that preferentially attack prey at the tail or middle of the body, but even these fish usually rotate the prey to be swallowed headfirst. This alignment reduces buccal and esophageal abrasion, because any spines protruding from the prey fish will be pushed down instead of outward. From an energetic perspective, headfirst attacks are advantageous for two reasons. First, they reduce overall handling time because the prey does not need to be re-oriented after capture (which often involves repeatedly ejecting and recapturing the prey). Second, rates of escape are increased when attacking the tail. This is especially true when attacking large prey, and piscivores feeding on larger species attack the head more often than when feeding on smaller species.

Some species switch between alternate foraging modes when capturing and ingesting prey. For instance, some species (e.g., American eel *Anguilla rostrata* and bluefish *Pomatomus saltatrix*) will either swallow their prey whole or, for larger items, tear the prey into pieces before ingesting the individual parts. In addition, several Chondrichthyan species (e.g., sawfish *Pristis* sp. and thresher shark *Alopias* spp.) use modified body parts as weapons to stun or injure prey, thus making them easier to subdue and consume. These modes of capture and consumption can allow a species to increase its dietary breadth, but are also energetically costly. Therefore, they are generally only used when prey that are small enough to be easily captured and swallowed whole are not available.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish. **Energetics**: General Energy Metabolism. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation; Dietary Requirements of Fish Under Culture Conditions. **Gut Anatomy and Morphology**: Gut Anatomy. **Hypoxia**: Metabolic Rate

Suppression as a Mechanism for Surviving Hypoxia. **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia. **Social and Reproductive Behaviors**: Dominance Behaviors. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism; Energetics of Fish Swimming. **Temperature**: Membranes and Temperature: Homeoviscous Adaptation. **Tissue Respiration**: Mitochondrial Respiration. **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration.

Further Reading

Abrahams MV and Dill LM (1989) A determination of the energetic equivalence of the risk of predation. *Ecology* 70: 99–1007.

Bone Q and Moore RH (2008) *Biology of Fishes*., 3rd edn. New York: Taylor and Francis.

Dabrowski K, Takashima F, and Law K (1988) Bioenergetic model of planktivorous fish feeding, growth and metabolism: Theoretical optimum swimming speed of fish larvae. *Journal of Fish Biology* 32: 443–458.

Gerking SD (1994) *Feeding Ecology of Fish*. San Diego, CA: Academic Press.

Gill AB (2003) The dynamics of prey choice in fish: The importance of prey size and satiation. *Journal of Fish Biology* 63 (supplement A): 105–116.

Hart PJB (1986) Foraging in teleost fishes. In: Pitcher TJ (ed.) *The Behavior of Teleost Fishes*, pp. 211–236. Baltimore: Johns Hopkins University Press.

Hart PBJ (1997) Foraging tactics. In: Godin J-GJ (ed.) *Behavioural Ecology of Teleost Fishes*, pp. 104–133. New York: Oxford University Press.

Helfman GS (1990) Mode selection and mode switching in foraging animals. *Advances in the Study of Behavior* 19: 249–298.

Hughes RN (1997) Diet selection. In: Godin J-GJ (ed.) *Behavioural Ecology of Teleost Fishes*, pp. 134–162. New York: Oxford University Press.

Jobling M (1981) *Fish Bioenergetics*. London: Chapman and Hall.

Killen SS, Brown JA, and Gamperl AK (2007) The effect of prey density on foraging mode selection in juvenile lumpfish: Balancing food intake with the metabolic cost of foraging. *Journal of Animal Ecology* 76: 814–825.

Lima SL and Dill LM (1990) Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology* 68: 619–640.

Milinski M (1986) Constraints placed by predators on feeding behaviour. In: Pitcher TJ (ed.) *The Behavior of Teleost Fishes*, pp. 236–253. Baltimore: Johns Hopkins University Press.

O'Brien WJ, Browman HI, and Evans BI (1990) Search strategies of foraging animals. *American Scientist* 78: 152–160.

O'Brien WJ, Evans BI, and Browman HI (1989) Flexible search tactics and efficient foraging in saltatory searching animals. *Oecologia* 80: 100–120.

Priede IG (1985) Metabolic scope in fishes. In: Tytler P and Calow P (eds.) *Fish Energetics: New Perspectives*, pp. 33–64. Baltimore: Johns Hopkins University Press.

Pyke GH (1984) Optimal foraging theory: A critical review. *Annual Review of Ecology and Systematics* 15: 523–575.

Reimchen TE (1991) Evolutionary attributes of headfirst prey manipulation and swallowing in piscivores. *Canadian Journal of Zoology* 69: 2912–2916.

Wang T, Lefevre S, Do TH, Nguyen VC, and Bayley M (2009) The effects of hypoxia on growth and digestion. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology,* Volume 27, pp. 362–397. London: Elsevier.

Ware DM (1978) Bioenergetics of pelagic fish theoretical change in swimming speed and ration with body size. *Journal of the Fisheries Research Board of Canada* 35: 220–228.

Digestive Efficiency

DP German, University of California, Irvine, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Concepts in Digestive Efficiency
Dietary Effects on Digestive Efficiency
Fish Digestive Strategies
Further Reading

Glossary

Apparent digestive efficiency Digestive efficiency not accounting for nondietary fecal material.

Detritus Degraded organic matter from nonfossil sources, including microbes.

Digesta Food in the GI tract that is in the process of being digested.

Digestive efficiency The ratio of energy or nutrient assimilated to energy or nutrient ingested.

Food intake The amount of food consumed.

Gastric Of or relating to the stomach.

Ingesta Food that has been ingested.

Intestinal bulb An outpocketing or enlargement of the proximal intestine that is not separated from the esophagus or intestine and lacks oxynticopeptic cells.

Nondietary fecal material Biochemical constituents in the feces that were not ingested with food; usually borne of the fish (endogenous input, such as sloughed intestinal cells and mucus) or endosymbiotic microorganisms residing in the fish's gastrointestinal tract.

Pyloric ceca Blind, finger-like projections stemming from the proximal intestine that contain pancreatic tissue and secrete digestive enzymes, thus facilitating digestion in some species.

Rectum The distal-most portion of the intestine, just preceding the anus, which acts to store fecal material before defecation.

Stomach An outpocketing of the proximal gastrointestinal tract that is separated from the esophagus and intestine by sphincter muscles, and features oxynticopeptic cells that produce hydrochloric acid and the proteolytic enzyme, pepsin.

True digestive efficiency Digestive efficiency after accounting for nondietary fecal material.

Concepts in Digestive Efficiency

To better understand what a fish is capable of absorbing from their food, one must determine the nutrients available in a given food type and account for losses that occur during digestion and absorption (**Figure 1**). Of all the losses, those to the feces are the greatest, and conveniently, the simplest to measure. Thus, digestibility is calculated as the ratio of food absorbed to the food ingested (**Figure 1**). Digestibility, or more appropriately, digestive efficiency, has important implications in the study of fish nutritional ecology, resource use, ecosystem-level studies, and aquaculture (**see also Energetic Models**: Bioenergetics in Aquaculture Settings).

However, before one can begin to study the digestive efficiency in fishes, the units of measurement must be clear. In many cases, energy may be the best currency against which to measure digestive efficiency, but in others, a different component of a fish's diet may be used (e.g., dry matter, organic matter, and protein; **see also Food Acquisition and Digestion**: Dietary Requirements of Fish Under Culture Conditions).

Essentially, efficiency concerns itself with the process of absorption, excluding pre-ingestive and post-absorptive processes, and thus, this article will focus on aspects of food consumption, digestion, and absorption as they relate to digestive efficiency.

When measuring digestive efficiency, the common practice is to allow a captive fish to eat a predetermined amount of food, or better, offer the fish unfettered access to food, allowing them to control the intake. Following defecation, feces are collected and the energy and/or nutrient content is measured in the food and in the feces (**Figure 1**). Included in the fecal material are not only undigested components of food origin, but also endogenous material from the fish itself (e.g., sloughed intestinal cells and mucus) or nondietary material from microorganisms residing in the fish's gastrointestinal (GI) tract. These nonfood inputs to the feces are commonly called 'metabolic fecal dry matter', or 'nondietary fecal nitrogen'. For this article, the more general term 'nondietary fecal material (NDFM) will be used.

If the NDFM of the feces is not accounted for, the digestive efficiency is 'apparent digestive efficiency', whereas if allowances are made for NDFM, it is called 'true digestive efficiency'. True digestive efficiency is the ratio of the quantity (i.e., energy and/or nutrients) absorbed to the quantity ingested and is greater than the apparent digestive efficiency by the amount of NDFM.

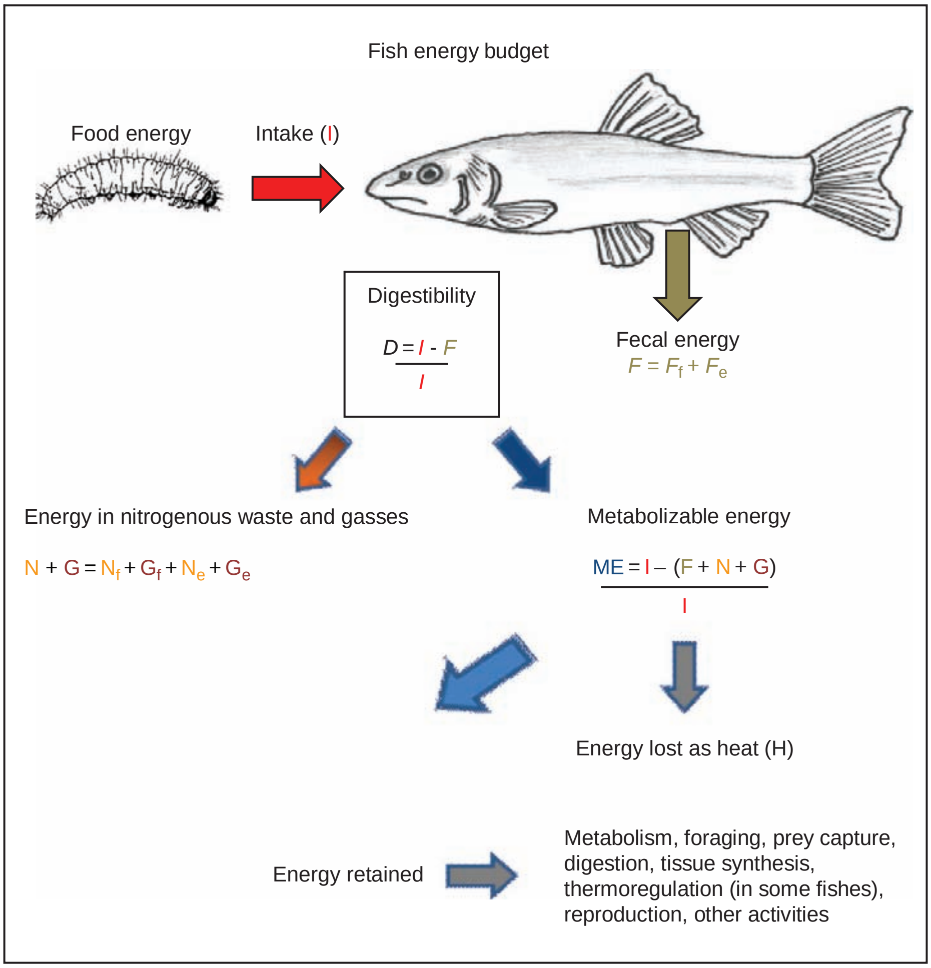

Fish energy budget
Food energy
Intake (I)
Digestibility
D = I - F / I
Fecal energy
F = Ff + Fe
Energy in nitrogenous waste and gasses
N + G = Nf + Gf + Ne + Ge
Metabolizable energy
ME = I – (F + N + G) / I
Energy lost as heat (H)
Energy retained
Metabolism, foraging, prey capture, digestion, tissue synthesis, thermoregulation (in some fishes), reproduction, other activities

Figure 1 Relationships among energy terms used in this chapter. F_e, N_e, and G_e correspond to endogenous energy (i.e., lost directly from the fish), whereas F_f, N_f, and G_f represent food energy lost directly in feces, nitrogenous waste, or gases, respectively. Total heat production is divided into several processes including thermoregulation, which is observed in some fish taxa (e.g., Tunas). River Chub (*Nocomis micropogon*) drawn by L.T. Crummett. Modified from Hume ID (2005) Concepts of digestive efficiency. In: Starck JM and Wang T (eds.) *Physiological and Ecological Adaptations to Feeding in Vertebrates* pp. 43–58. Enfield, NH: Science Publishers.

Because it is difficult to measure NDFM, true digestive efficiencies are not commonly reported for fishes. NDFM can be determined in fish by feeding isocaloric diets with a gradient (from low to high concentrations) of the nutrient of interest and plotting fecal loss as a function of nutrient intake (**Figure 2**). The point at which the regression line intercepts the *y*-axis can represent NDFM for that nutrient (or energy if a gradient of joules is used instead). However, this estimate of NDFM should be treated cautiously because it is an estimate. In a

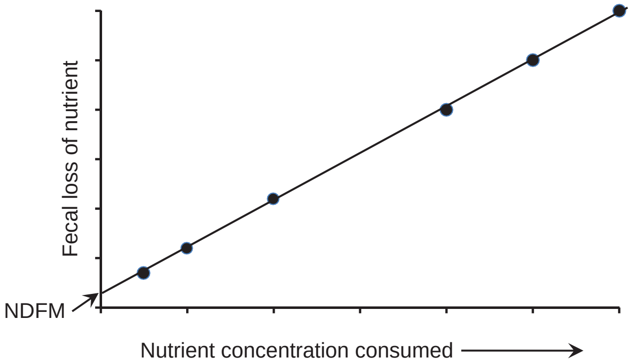


Figure 2 Determination of nutrient losses in feces as a function of nutrient ingested. Nondietary fecal material (NDFM) can be estimated by the *y*-intercept of the regression line.

nutritional physiology context, apparent digestive efficiency represents digestive efficiency in a natural setting because the losses of energy or nutrients to NDFM are real and must be replaced by the fish.

Attempts to measure digestive efficiency in wild-caught fishes have been made. These field measurements of digestive efficiency are performed by dissecting out a fish's GI tract immediately upon capture, obtaining digesta from the proximal and distal regions of the gut, and comparing nutrient or energy concentrations of the digesta from these two gut regions. This method assumes that digesta from the most proximal portion of the gut represent the nutrient or energy content of food and the digesta from the distal intestine represent the nutrient or energy content of the feces. Although these observations provide some insight into the digestive efficiency for fish in the wild, this methodology is problematical because food from the proximal GI tract is already being digested (and, thus, cannot be representative of food before it was consumed), and it is difficult to discern true fecal material from other digesta in the distal intestine. The sampling of digesta that are not completely digested, and thus cannot technically be considered fecal material, will lead to the underestimation of the digestive efficiency (**Table 1**). This is confounded by the fact that many fishes lack a discernable rectum. Therefore, digestive efficiency is best observed under controlled conditions using true fecal material.

An important point in the measurements of digestive efficiency is that a gram of food does not translate into a gram of fecal material. Because digestive efficiency is the comparison of concentrations, which are mass dependent, differences in mass between the amount of food consumed and feces produced must be accounted for. This is generally done in one of the two ways. First, and most accurately, is the total collection of food and feces. This is done with a specialized aquarium design, where feces and uneaten food fall through a grating at the bottom of a tank, where they can be collected and the fish cannot disturb them. The uneaten food or debris (commonly called 'orts') can be used to calculate the actual mass of food consumed (assuming that the amount of food offered to the fish is known). By knowing exactly how much food a fish ate and exactly how much feces they produced, a researcher can directly compare the energy or nutrient contents of one to the other, weighting the calculations by the mass of each:

$$1 - \frac{F}{I} \qquad [1]$$

where, I is the total amount of energy or nutrient consumed, and F is the total amount of energy or nutrient defecated (note that this equation is mathematically synonymous with the digestibility equation shown in **Figure 1**, $D = (I - F)/I$). Second, a researcher can use an inert marker that is indigestible by the fish, such as chromic oxide, titanium dioxide, or acid-insoluble ash. In these instances, the total amounts of food consumed and feces produced are unknown, and thus, the equation must be weighted for the amount of the indigestible marker in the feces and food:

$$1 - \left[\frac{a_i}{a_f} \times \frac{\mathrm{m}_f}{\mathrm{m}_i}\right] \qquad [2]$$

where a_i and a_f are the amount of indigestible marker in food and feces, respectively, and m_i and m_f are the amount of energy or nutrient in food and feces, respectively. Because acid-insoluble ash naturally occurs in food, it is commonly used in aquaculture and field digestive efficiency studies (**see also Food Acquisition and Digestion**: Dietary Requirements of Fish Under Culture Conditions).

However, ash is composed of minerals and some minerals are absorbed and excreted by fish during digestion, a process that can vary with the mineral balance of a particular fish and the fish's digestive tract anatomy (e.g., presence of an acidic stomach; see below). Thus, digestibility estimates based on ash can be biased based on the marker. Moreover, it is especially important to use acid-insoluble ash as opposed to total ash because marine fishes secrete carbonates in their feces (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation); not accounting for this will lead to overestimates of digestibility using total ash (by decreasing the ratio of a_i:a_f). Therefore, total collection is the most accurate way to measure digestive efficiency, but when this is not possible, an added marker (i.e., other than ash) may be a better option, as long as the marker is truly indigestible and does not interfere with digestion.

Table 1 Effects of fecal collection methods on estimation of digestive efficiency for different food components

	Digestive efficiency (%)		
Method	*Dry matter*	*Protein*	*Lipid*
Netting/siphoning from tank	84	95	97
Dissection (removal from true rectum)	80	90	97
Anal suction	79	90	97
Dissection (removal from distal intestine)	73	78	62

Reproduced from Jobling M (1994) *Fish Bioenergetics*. New York: Chapman and Hall, p. 107.

A consistent issue with digestibility studies is the potential leaching of nutrients or minerals from food or feces as they sit in water. Because fecal material for many fish species is encased in a mucus capsule, leaching occurs more readily from the food than from the feces. Leaching is a serious problem more so for soluble components (e.g., starches) in a diet or fecal material than for fibrous (e.g., cellulose) components, and it is recommended that feces be collected as frequently as possible to minimize contact with the water and potential leaching. Given the importance of an indigestible marker to a digestibility calculation, leaching could pose a serious problem for the accuracy of digestibility calculations reliant upon a leachable marker. For example, leaching of a marker from fecal material can cause an overestimation of digestive efficiency, whereas leaching of a marker from food can cause an underestimation. By not accounting for leaching, digestibility estimates based on a marker can become dubious.

Calculating losses of energy to nitrogen excretion (**Figure 1**) can be difficult in fishes in comparison to terrestrial animals, mainly because nitrogen excretion occurs in several locations in the fish, but primarily across the gills (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish; **Nitrogenous-Waste Balance**: Ureotelism and Excretion of Ammonia). Thus, metabolizable energy is not commonly used in studies of digestive efficiency in fish for nutritional-ecology purposes. However, nitrogen excretion and metabolic rates are commonly measured in aquaculture settings to gain insight on food conversion efficiency in fish (**see also Energetic Models**: Bioenergetics in Aquaculture Settings and **Food Acquisition and Digestion**: Dietary Requirements of Fish Under Culture Conditions).

Inherent in all digestive-efficiency measurements is the digestive strategy a fish takes to obtain nutrients from a meal. That is, does a fish maximize digestive efficiency for an overall meal, or the rate of intake of energy or nutrients? The theoretical frameworks available for fish digestion suggest that fish GI tracts are geared for maximal rate of net energy or nutrient gain. Moreover, compartment models of digestion suggest that there are several stages to the digestive process (**Figure 3**).

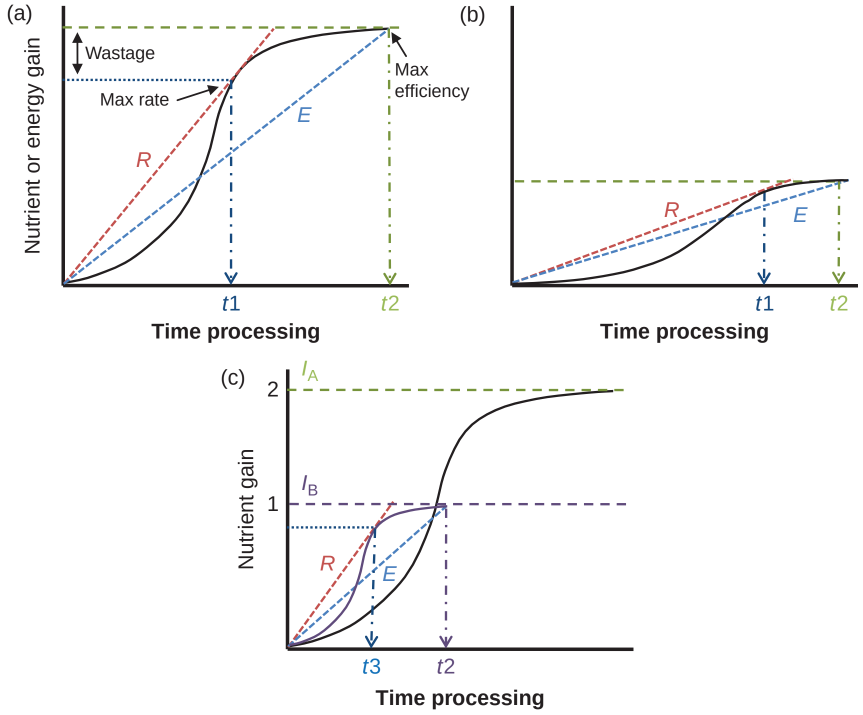


Figure 3 Cumulative nutrient or energy gained by a fish as a function of time spent processing a meal. (a): For high-quality food; (b) for low-quality food. The green horizontal dashed line represents the extractable nutrient ingested in the meal. The slope of the red line *R* represents the maximum rate at which the nutrient or energy can be absorbed from the meal. This is achieved by evacuating the contents at time 1 (*t*1) and taking a new meal, although a portion of the nutrient or energy consumed is lost in the feces (wastage). Maximum efficiency (blue line *E*) is attained by extending processing time to time 2 (*t*2); however, this is done at the cost of reduced digestive rate. For the low-quality food, long retention is unavoidable for maximum digestive rate. (c) For two ingested nutrients, I_A and I_B. Nutrients I_A and I_B are contained in the food in a 2:1 ratio, and thus, twice the amount of nutrient I_A is consumed as I_B. The fish is capable of assimilating the nutrients in a reciprocal 1:2 ratio, however, if they void the contents at time 3 (*t*3), since this is the point at which the rate of gain of nutrient I_B is twice that of I_A. Lines and times needed for maximum rate (*R*) and maximum efficiency (*E*) provided. Modified from Clements KD and Raubenheimer D (2006) Feeding and nutrition. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 47–82. Boca Raton, FL: CRC.

First, the fish must get past the structural defenses of the ingested food, which in many cases (e.g., for piscivorous fish), may be negligible. However, for fishes consuming lower-quality prey (e.g., plants or arthropods), there may be considerable energy expenditure gaining access to the more digestible components of a meal (i.e., getting past the structural polysaccharides in plant cell walls or arthropod exoskeletons to access the digestible inner tissues). Thus, the curves in (a) or (b) of **Figure 3** begin with shallow slopes (or may initially dip below the *x*-axis and be negative when considering that energy will be expended initially in prey handling and digestive processes), and rapidly rise once the digestible components of a meal are accessed. Following the period of rapid digestion, digestive rate progressively slows as less-tractable components of the meal remain. Line *R* in **Figure 3**, which runs from the origin tangential to the curve, depicts the optimal digestive rate, and suggests that digesta should be voided and a new meal taken at the point where *R* touches the main curve. Optimal digestive rates can be slower for lower-quality foods (**Figure 3(b)**), but optimal rates can also vary with the nutrient concentrations of the meal (**Figure 3(c)**).

A few predictions arise from this theoretical framework. First, optimal digestive rates will vary with diet. Second, because of lower nutrient or energy gain per amount of food consumed, fishes eating lower-quality foods should eat more, and, hence, have longer and larger digestive tracts than fishes consuming higher-quality foods. Finally, a fish should maximize retention of food for optimal absorption of the limiting component of their diet, whether it be energy (**Figures 3(a) and 3(b)**) or a specific nutrient, such as protein (**Figure 3(c)**). These predictions in relation to diet and fish GI tract structure and function will be explored throughout the rest of the article.

Dietary Effects on Digestive Efficiency

As the most species-rich group of vertebrates, fishes are found in waters throughout the planet and they consume nearly every type of food ranging from sediment, detritus, fruit, algae, and wood, to slime, scales, blood, arthropods, mammals, and other fish. Generally, animal material is higher in protein and more digestible than plant or detrital material (**Table 2**; **Figure 4**). There are differences in digestibility among animal and plant matter because fish generally lack the enzymes (e.g., cellulase and xylanase) that digest plant structural polysaccharides, such as cellulose and hemicellulose. Although some fish species (e.g., Silver drummer *Kyphosus sydneyanus*) have evolved symbioses with microbes that digest structural

Table 2 Digestive efficiencies of nutrients from natural foods consumed by fishes

Food type	*Family of representative species*	*Digestive efficiency (%)*	
		Protein	*Energy*
Detritus	Cichlids, loricariids	45–49	∼40
Macroalgae	Gobiids, pomacentrids, cichlids, stichaeids	45–80	30–70
Aquatic macrophytes	Cichlids, cyprinids	60–80	30–60
Oligochaetes	Salmonids, percids, cyprinids, pleuronectids	80–95	70–85
Crustaceans	Salmonids, percids	∼90	80–88
Fish	Percids, gadids	85–98	80–97

Reproduced from Jobling M (1994) *Fish Bioenergetics*. New York, NY: Chapman and Hall, p. 108.

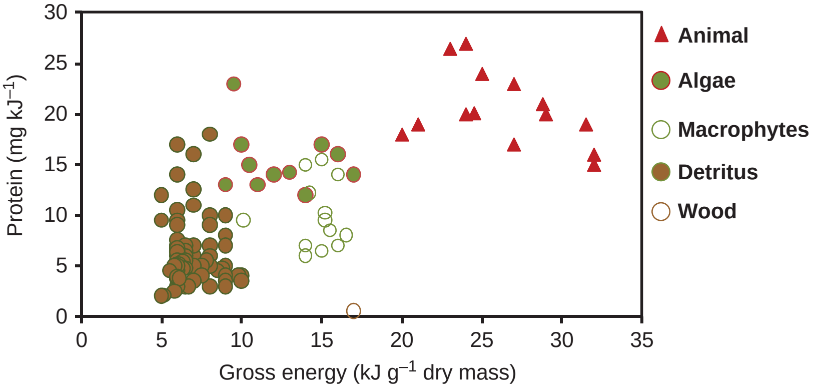


Figure 4 Protein and gross energy in food resources consumed in aquatic habitats. Redrawn from Bowen SH, Lutz EV, and Ahlgren MO (1995) Dietary protein and energy as determinants of food quality: trophic strategies compared. *Ecology* 76: 899–907.

polysaccharides in their GI tracts, many fish do not rely on microbial endosymbionts to do so; the fish largely ignore the recalcitrant polysaccharides in their diet and digest the soluble components of their food through the action of endogenous digestive enzymes (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion).

Nutrient Classes

Fishes consume a diversity of foods, but all require approximately the same 40 nutrients at the cellular level, and the general categories of macronutrients – proteins, lipids, and carbohydrates (**see also Food Acquisition and Digestion**: Dietary Requirements of Fish Under Culture Conditions). In terms of energy, lipids contain more than twice the energy (37.7 kJ g^{-1}) as protein or carbohydrate (each around 16.7 kJ g^{-1}). Clearly, fishes consuming different foods will encounter the macronutrients in different concentrations (e.g., carnivores will consume more protein and lipid and herbivores more carbohydrates) and, thus, will use different nutrients as fuel sources and will exhibit differences in digestive efficiencies of these nutrients (**Table 3**).

In general, fishes efficiently digest protein from most feedstuffs, regardless of their natural diet. Considering that 65–75% of fish carcass dry mass is protein, fishes need to efficiently absorb protein from food, if for nothing else, tissue maintenance and growth (although many carnivorous fishes metabolize amino acids for energy). Moreover, estimates of the fish dietary protein requirements from many taxa are 2–4 times greater than the amount needed by other vertebrates.

Many fishes consuming lipid-rich prey (e.g., carnivores and diatom-consuming fishes) primarily use lipids as an energy source, allowing protein to be utilized primarily for tissue maintenance and growth.

Carbohydrate utilization tends to be greater in herbivorous and omnivorous fishes than in carnivores for the obvious reason that carnivores naturally encounter less carbohydrate than herbivores or omnivores. In fact, some carnivorous fishes cannot tolerate high levels of carbohydrate in their diet. The differences between herbivores, omnivores, and carnivore are reflected in different digestive physiology (see below; **see also Integrated Function and Control of the Gut**: Intestinal Absorption and Gut Secretion and Digestion).

Dietary Quality

The quality of plant material (including algae) is gauged by the ratio of cell-wall components to digestible cell contents, which include soluble carbohydrates, proteins, and lipids. The cell-wall component to cell content ratio tends to be lower for algae and aquatic plants than for terrestrial plants because the former are buoyed by water, and hence, require less structural components than their terrestrial counterparts.

Comparatively, few species of fish (e.g., grass carp (*Ctenopharyngodon idella*)) feed on terrestrial plant material relative to the large number of algae-eating fish species (>400 species). Nevertheless, algae do possess structural polysaccharides (e.g., k-carrageenan and alginate) and some algal species synthesize toxic secondary compounds that can interfere with fish digestion. Although some algae can be relatively rich in protein (**Figure 4**), high-protein algal species are not always

Table 3 Summarized digestive efficiencies of protein, lipid, and carbohydrate in fishes with different natural diets consuming artificial feeds

		Digestive efficiency (%)		
Common name (species name)	*Natural diet*	*Protein*	*Lipid*	*Soluble carbohydrate (starch)*
Nile tilapia (*Oreochromis niloticus*)	Herbivorous/detritivorous	88–96	72–98	83–96
Common carp (*Cyprinus carpio*)	Omnivorous	80–93	92–96	77–96
Channel catfish (*Ictalurus punctatus*)	Omnivorous	66–87	76–97	59–78
Rainbow trout (*Oncorhynchus mykiss*)	Carnivorous	68–96	71–97	16–80
Red sea bream (*Pagrus major*)	Carnivorous	88–96	90–96	66–82
European sea bass (*Dicentrarchus labrax*)	Carnivorous	62–97	80–99	66–98

From Halver JE and Hardy RW (2002) *Fish Nutrition*, 3rd edn. San Diego, CA: Academic Press; Horn MH (1989) Biology of marine herbivorous fishes. *Oceanography and Marine Biology Annual Review* 27: 167–272; Jobling M (1994) *Fish Bioenergetics*, New York, NY: Chapman and Hall; Krogdahl Å, Hemre GI, and Mommsen T (2005) Carbohydrates in fish nutrition: Digestion and absorption in postlarval stages. *Aquaculture Nutrition* 11: 103–122; Stone DAJ (2003) Dietary carbohydrate utilization by fish. *Reviews in Fisheries Science* 11: 337–369; Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11: 107–184; Webster CD and Lim CE (eds.) (2002) *Nutrient Requirements and Feeding of Finfish for Aquaculture*. New York, NY: CABI Publishing.

preferred by herbivorous fishes. Detritus, which is defined as degraded organic matter, including microbes, is a food resource uniquely utilized by fishes (and some tadpoles) among vertebrate animals. Although detritus can be rich in many nutrients, including protein (**Figure 4**), it is often consumed with indigestible components, such as sediment, and is often dilute.

What a fish eats can vary as a function of habitat and/or season. For example, resources are relatively scarce in the deep sea (**see also Deep-Sea Fishes**), and, because of a lack of photosynthetic organisms, deep-sea fishes are almost exclusively carnivorous and eat intermittently when they encounter food. The deep-sea environment, because of the sparseness of resources, may therefore provide an example of circumstances where fish would maximize digestive efficiency over digestive rate (**Figure 3**).

Food availability varies by orders of magnitude among seasons in many aquatic systems (especially freshwater habitats), and algal biochemistry varies with season and nutrient input. Thus, what a fish consumes and how much they eat can change on different timescales. Many fish species consume an array of food items, counteracting deficiencies of particular nutrients in other consumed foods. For example, omnivory can be viewed as an effort to complement an abundant food source (e.g., algae) with one rich in protein (i.e., animal material).

Fish Digestive Strategies

Intake and Transit Time

Intake is perhaps the most important characteristic of fish feeding that is affected by dietary quality (**see also Food Acquisition and Digestion**: Energetics of Foraging Decisions and Prey Handling). In general, the lower the quality of the food (low-protein and/or low-energy content), the more the fish will have to eat to meet their demands. Higher intake in fish consuming lower-quality foods has been observed in aquaculture settings, in the laboratory, and in nature. However, when a fish is eating more food, they will pass digesta through the gut more quickly, which can compromise digestive efficiency (**Figure 3**).

Thus, there is a clear trade-off between intake, transit time, and digestive efficiency. A fish can deal with this in one of two ways. First, they can eat more at a given GI-tract size at the cost of evacuating their gut contents before they reach optimal digestive rate. However, this can be overcome by passing more material (and thus, energy and nutrients) through their gut per unit time to eventually meet their nutrient or energy demands. Conversely, fishes can increase the size and digestive capacity of their guts to accommodate increased intake and rapid gut transit without sacrificing digestive efficiency. Examples of both exist in the literature. One issue that arises in the second example above is that an increase in GI-tract size and function is energetically costly to maintain. Therefore, a fish has a further trade-off of increased energy expenditure on GI-tract maintenance to maintain digestive efficiency with higher intake (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation). Some fish can ingest 50% or more of body mass and digest the meal over at least a week, whereas for others 2% of body mass represents satiation and they can eat to satiation daily.

Intake is not affected solely by dietary quality, but also by temperature, body mass, and ecological factors (**Figure 5**; **see also Food Acquisition and Digestion**: Energetics of Foraging Decisions and Prey Handling; Energetics of Prey Capture: From Foraging Theory to Functional Morphology; **Energetics**: General Energy Metabolism; and **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). A fish will eat more as temperature increases, but only to a point where physiological disruption begins. Obviously, larger fish will eat more food on a gross-mass level than smaller fish. However, body mass directly affects intake in proportion to mass-specific metabolic rate (i.e., scales approximately as body mass$^{-0.25}$; **see also Energetics**: General Energy Metabolism), and thus, on a given diet, smaller fishes consume more food relative to their body mass than do larger fishes. Many fish species undergo ontogenetic dietary shifts (e.g., from carnivory to herbivory) and, thus, dietary differences can become a confounding variable in comparisons of intake among different-sized fishes of the same species.

Transit time through the gut is directly affected by ingesta particle size: the larger the particle, the slower the initial transit, as the surface area of a larger particle must be reduced by digestive processes before assimilation of nutrients can occur. Smaller particles tend to be easier to digest because of the shorter diffusive distance

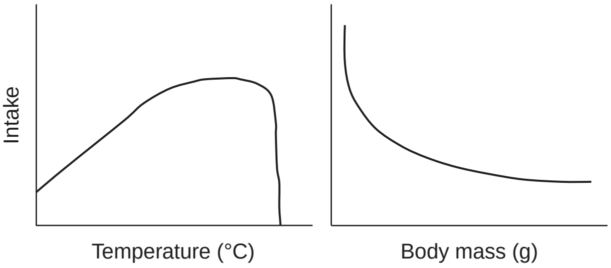


Figure 5 Influence of water temperature and body mass on intake in fish.

for digestive fluids to penetrate the digesta. Furthermore, different-sized particles can travel through the gut at different rates, usually with fluid and smaller particles traveling quicker. Because herbivorous and detritivorous fishes consume more food per day than carnivorous fishes, gut-transit time may be longer in carnivores than in herbivores/detritivores, especially in sit-and-wait predators that feed intermittently. However, herbivorous fishes that utilize hindgut fermentation to meet some portion of their daily energy needs can have longer retention of digesta in the hindgut than other species to allow sufficient time for microbial fermentation to occur.

Gut Morphology

One of the primary ways a fish can control transit time of digesta through the GI tract is through compartmentalization. In this regard, the transit of material from one compartment to another can be controlled, usually by particle size. Many fishes possess a gastric stomach that is separated from the intestine by a pyloric sphincter. The stomach is usually acidic (pH 2–4), and digestion begins in this region of the GI tract. In fishes that possess a stomach, up to 75% of transit time can be explained by residence time of digesta in the stomach alone, with digesta transit through the intestine happening relatively rapidly. However, not all fishes have an acidic stomach. Nearly 7600 species of fish (~25% of all described taxa) are considered 'agastric' (**see also Gut: Anatomy and Morphology**: Development of Fish Gut); some completely lack any outpocketing of the proximal region of the gut, whereas others maintain an alkaline muscular stomach that is more of a grinding apparatus, and others, an intestinal bulb (**Figure 6**). Some clades of fishes have co-opted

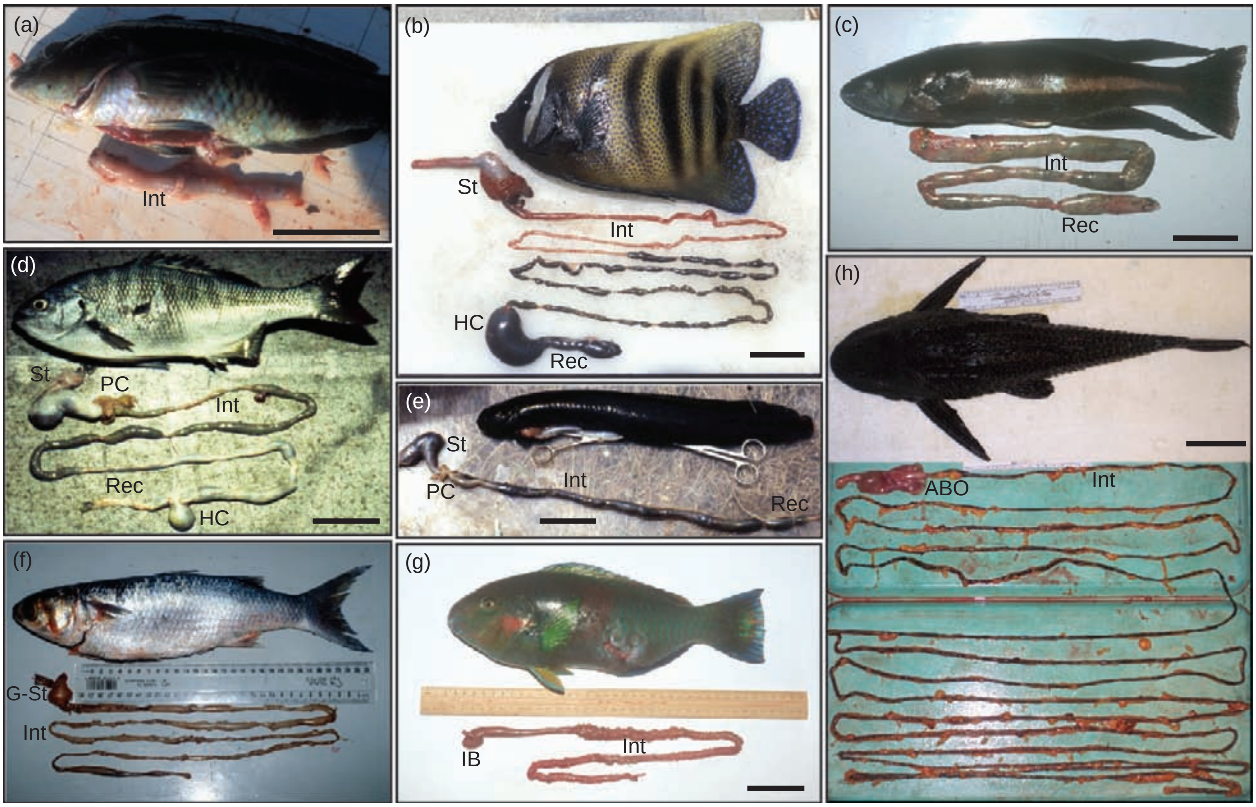


Figure 6 Fish GI tract morphology. (a) Yellow-saddled wrasse (*Notolabrus fuciola*, family Labridae), carnivore, with stomachless intestine; (b) Sixbar angelfish (*Pomacanthus sexstriatus*, family Pomacanthidae), omnivore, with stomach, long intestine, and hindgut chamber; (c) Butterfish (*Odax pullus*, family Labridae), herbivore, with stomachless intestine; (d) Zebra-perch sea chub (*Hermosilla azurea*, family Kyphosidae), herbivore, with stomach, pyloric ceca, intestine, and hindgut chamber; (e) Monkeyface prickleback (*Cebidichthys violaceus*, family Stichaeidae), herbivore, with stomach, pyloric ceca, and intestine; (f) Flathead grey mullet (*Mugil cephalus*, family Mugilidae), detritivore, with gizzard-like stomach, and long intestine; (g) Rivulated parrotfish (*Scarus rivulatus*, family Labridae), detritivore, with proximal intestinal bulb and sacculated intestine; (h) Vermiculated sailfin catfish (*Pterygoplichthys disjunctivus*, family Loricariidae), detritivore, with proximal stomach, which is an air-breathing organ (ABO) and not involved in digestion, followed by long intestine. St, stomach (acidic); G-St, gizzard-like stomach, which is more of a grinding apparatus (nonacidic); PC, pyloric ceca; Int, intestine; HC, hindgut chamber; Rec, rectum. Scale = 70 mm. Photo credits: AC, F and G, Kendall D. Clements; D and E, Michael H. Horn; H, Donovan P. German.

their stomachs for something else entirely: breathing air. Many loricariid catfishes have a thin-walled, alkaline proximal gut pouch that is not involved in digestion in any way (**Figure 6**(**h**),; **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction and Circulatory Adaptations for Air-Breathing Fishes). Less common than an acidic stomach is a hindgut chamber, which is also separated from the intestine by a sphincter, and can slow digesta flow (**Figures 6**(**b**) and **6**(**d**)). Many fish possess a sphincter separating the distal intestine from the rectum, also allowing for control of digesta-transit rate and particle passage in the distal intestine. Any type of 'colonic separating mechanism' and/or antiperistalsis allowing for selective retention of small particles, as observed in voles and rabbits, are unknown in fishes. However, scarines (parrotfishes) possess a sacculated intestine (**Figure 6**(**g**)) that may create vortices in the digesta flow, thereby allowing for selective retention of small particles and fluids within the sacculae, and allowing larger, less-digestible particles to pass through the lumen of the intestine. Selective retention of particles and fluid can allow a fish to have higher intake without the metabolic cost of increasing gut length.

Gut Length

One of the predictions of the models presented in **Figure 3** is that animals eating lower-quality foods will consume more food on a daily basis and, hence, will have larger digestive tracts than fishes consuming higher-quality foods. However, given that fishes exhibit a wide range of digestive tract morphologies (**Figure 6**), and these morphological features are also affected by phylogenetic history, these patterns are not always clear.

For example, fishes in the family Labridae (including Scarines and Odacines) tend to have short, stomachless GI tracts that lack chambers or ceca, regardless of the diet. However, within specific fish clades, in which all of the fish share a common evolutionary history, herbivores tend to have larger and longer guts than do carnivores, with omnivores sometimes showing intermediate gut lengths. Detritivorous (**Figures 6**(**f**)–**6**(**h**)) and coralivorous fishes tend to show the longest gut lengths because they generally have higher intake than fishes with other diets, largely due to the dilute nature of a detrital or coral-based diet. It is unclear whether an increase in gut length in fishes provides an increase in gut capacity, but in the few studies performed on atherinopsid fishes and loricariid catfishes, an increase in gut length translates into an increase in absorptive surface area (i.e., microvilli surface area) of the intestine. The folding patterns of the intestinal epithelium and microvilli on the surface of enterocytes act to increase the absorptive surface area in fish intestines (**Figure 7**).

An increased gut length does not always translate into longer retention of food in the gut, as this is largely determined by intake. For example, the detritivorous

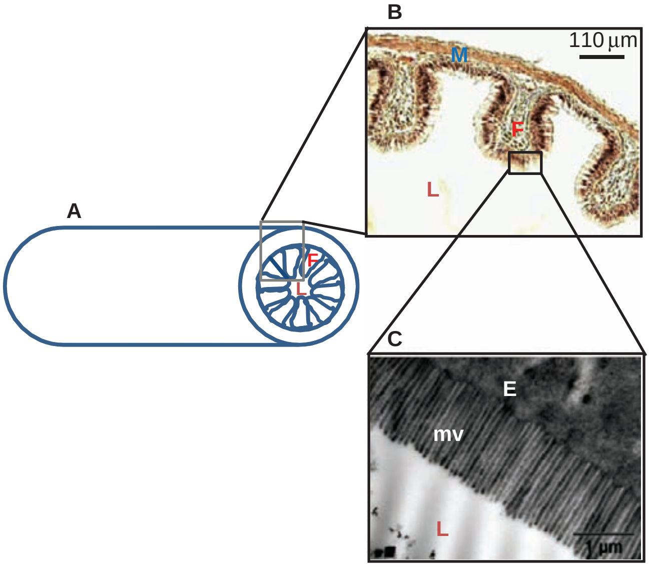


Figure 7 General structure of a fish intestine. (a) Illustration of a cross section of an intestine showing the intestinal folds along the mucosal lining of the intestine that increase the surface area. (b) Light micrograph of a histological preparation of the mid intestine of *Pterygoplichthys disjunctivus* showing the intestinal folds. (c) Transmission electron micrograph of the microvilli on the surface of epithelial cells of the mid intestine of *Pterygoplichthys disjunctivus.* The microvilli further increase the surface area of the intestine. E, enterocyte; F, intestinal fold; L, lumen of intestine; M, muscularis underlying the intestinal epithelium; mv, microvilli. Courtesy of DP German.

loricariid catfishes *Pterygoplichthys disjunctivus* and *Ancistrus triradiatus*, which each have gut lengths exceeding 18 times their body lengths, have digesta retention times of <4 h, whereas the herbivorous stichaeid fish *Cebidichthys violaceus* has a gut length averaging <2 times their body length and has retention times of digesta of >18 h. Thus, gut length offers little predictive power relating to digesta transit rate when comparing fish species with different evolutionary histories. Studies performed on a single species show that feeding a higher-quality diet (e.g., rich in protein) leads to a decrease in intake, an increase in gut-transit time, and in some cases, a decrease in gut length.

Mastication and Trituration

It is a general rule that fish do not chew food in their mouths as mammals do. However, fishes in many lineages possess well-developed pharyngeal teeth that act to grind and crush foods into smaller particles; these fishes are considered 'pharyngognathus'. Moreover, at least two families of fishes (Mugilidae and Acanthuridae) feature a gizzard-like stomach in which food is triturated against ingested sand or carbonates by muscular contractions of the stomach. Interestingly, pharyngognathus fishes and those with a gizzard-like stomach tend to be agastric, although some fish lineages (e.g., some cichlids) contain species that are pharyngognathus and possess an acidic stomach.

Digestive Enzyme Activities and Nutrient Transporters

Fishes possess the same suite of endogenous digestive enzymes as other vertebrates (**Figure 8**) and, based on the adaptive modulation hypothesis, one would predict that fish digestive-enzyme activities would be maintained at levels for optimal digestive rate of energy or nutrients from their diet. For carbohydrates, this largely appears to

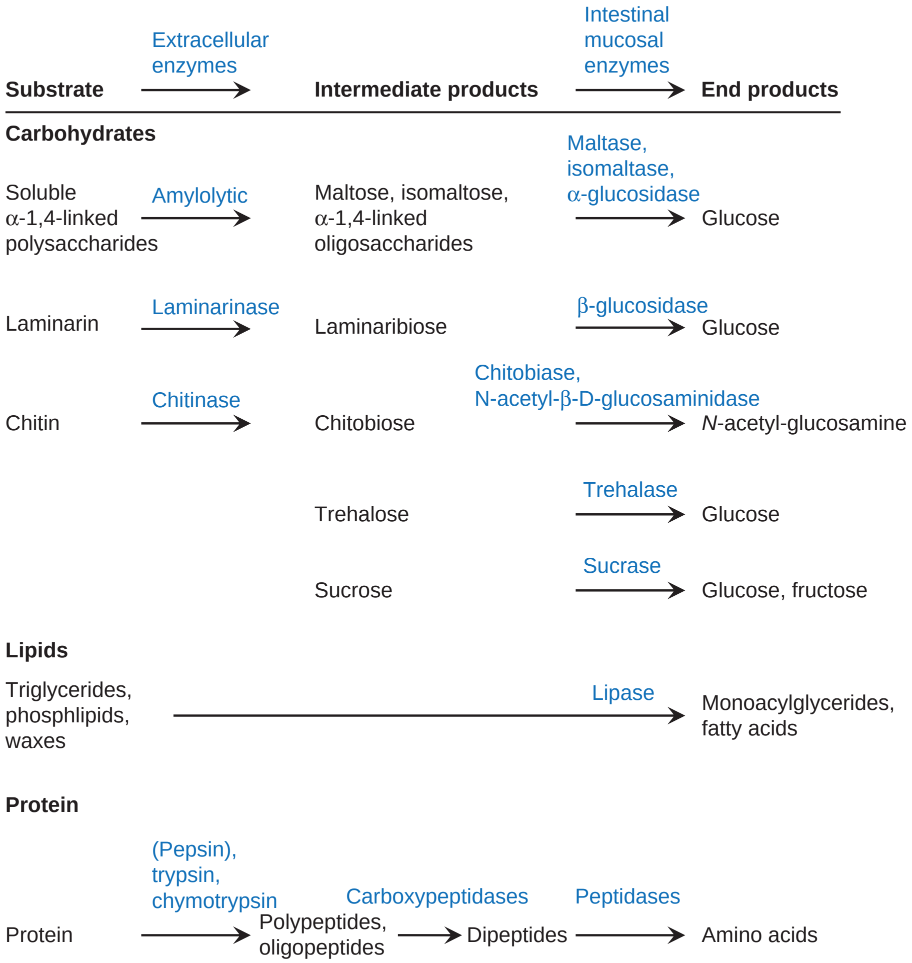


Figure 8 Principal nutrients in food and the endogenous digestive enzymes fishes produce to hydrolyze them. Note that the digestion of complex carbohydrates and proteins requires multiple steps, beginning with initial hydrolysis in the lumen by extracellular enzymes, and followed by membrane digestion by intestinal mucosal enzymes. Under protein, the enzyme pepsin is listed in parentheses because agastric species (i.e., those that lack an acidic stomach) generally lack this. Modified from Karasov WH and Martínez del Rio C (2007) *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton, NJ: Princeton University Press.

be true: herbivores and detritivores usually have higher carbohydrase activities (including polysaccharidase and disaccharidase activities) than carnivores, even when raised in the laboratory on a common low-starch diet.

However, as mentioned above, protein may be a limiting nutrient to many fish species, regardless of diet, so no consistent pattern relating to proteolytic enzyme activities has emerged. The same can be said for lipase activities.

Nevertheless, when digestive enzyme activities are put in the context of intake and digesta transit rate, herbivorous and detritivorous fishes, which eat more and pass food through their guts more quickly, may produce more digestive enzymes on a daily basis than carnivorous fishes, again illustrating the trade-off between intake, transit rate, and digestive efficiency. In those fish species that have endosymbiotic microorganisms involved in the digestive process (e.g., the herbivorous fish *Kyphosus sydneyanus*), digestive enzymes produced by microbes (e.g., carrageenase) may play a significant role in nutrient assimilation (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion).

As with digestive enzymes, fish possess similar intestinal-nutrient transporters as do other vertebrates, although fishes are less-well studied in this regard (**see also Integrated Function and Control of the Gut**: Intestinal Absorption). Generally, herbivorous fishes possess similar amino-acid-transport capabilities to those of carnivorous fishes (on the level of summed uptake capacity for the whole gut), again illustrating the importance of protein assimilation. However, herbivores and omnivores generally have higher glucose-transport rates than do carnivores. Moreover, this pattern appears to be fixed, as the feeding of a high-protein, low-starch diet to herbivorous and carnivorous fishes does not change the ratio of glucose transport to amino-acid transport.

Beyond nutrient-transport rates, the overall absorptive-surface area is important in comparisons of fishes with different diets. For example, herbivores have higher microvilli surface areas than carnivores among atherinopsid fishes, and loricariid catfishes consuming a low-quality detrital diet had higher microvilli surface areas than conspecifics consuming a high-quality algal diet. These studies suggest that fishes consuming lower-quality foods invest more energy in absorptive capacity as a mechanism to overcome their low-quality diet. The intestinal surface area of fishes (including intestinal folding patterns and microvilli surface area; **Figure 7**) decreases from the proximal to the distal intestine in all the fishes measured, suggesting that most absorption of nutrients from digestion occurs in the proximal and mid-intestine, although some protein absorption (and certainly ion and water absorption) may occur in the distal intestine. (**see also Integrated Function and Control of the Gut**: Intestinal Absorption).

Assimilation Markers

Beyond traditional methods of determining digestive efficiency, other chemical tracers can be used to quantify assimilation of different nutrients. For example, ^{14}C-labeled substrates (e.g., glucose and acetate) can be used to trace the direct assimilation of a nutrient into a fish. Because no ^{14}C is naturally excreted in the feces of the fish, digestive efficiencies using this technique represent true digestive efficiency, although losses to the water (e.g., urinary or incomplete ingestion) must be accounted for. The use of stable isotopes (e.g., δ^{13}C and δ^{15}N) in fish-feeding ecology has expanded in recent years. Although digestive efficiencies cannot directly be determined using stable isotopes, they do provide a mechanism to observe what portion of a diet is being assimilated into a fish's tissues based on its stable-isotopic signature.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish. **Deep-Sea Fishes**. **Energetic Models**: Bioenergetics in Aquaculture Settings. **Energetics**: General Energy Metabolism. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation; Dietary Requirements of Fish Under Culture Conditions; Energetics of Foraging Decisions and Prey Handling; Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **Gut Anatomy and Morphology**: Development of Fish Gut. **Integrated Function and Control of the Gut**: Gut Secretion and Digestion; Intestinal Absorption. **Nitrogenous-Waste Balance**: Excretion of Ammonia; Ureotelism. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism.

Further Reading

Bjorndal KA (1985) Use of ash as an indigestible dietary marker. *Bulletin of Marine Science* 36: 224–230.

Buddington RK, Chen JW, and Diamond J (1987) Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. *Journal of Physiology* 393: 261–281.

Choat JH and Clements KD (1998) Vertebrate herbivores in marine and terrestrial environments: A nutritional ecology perspective. *Annual Review of Ecology and Systematics* 29: 375–403.

Clements KD and Raubenheimer D (2006) *Feeding and Nutrition.* In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes,* 3rd edn., pp. 47–82. Boca Raton, FL: CRC.

Clements KD, Raubenheimer D, and Choat JH (2009) Nutritional ecology of marine herbivorous fishes: Ten years on. *Functional Ecology* 23: 79–92.

Collie NL and Ferraris RP (1995) *Nutrient fluxes and regulation in fish intestine*. In: Hochachka PW and Mommsen TP (eds.) *Metabolic Biochemistry,* vol. 4, pp 221–239. Amsterdam, The Netherlands: Elsevier Science.

Galetto MJ and Bellwood DR (1994) Digestion of algae by *Stegastes nigicans* and *Amphiprion akindynos* (Pisces: Pomacentridae) with an evaluation of methods used in digestibility studies. *Journal of Fish Biology* 44: 415–428.

German DP, Horn MH, and Gawlicka A (2004) Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary, and phylogenetic effects. *Physiological and Biochemical Zoology* 77: 789–804.

Halver JE and Hardy RW (2002) *Fish Nutrition*, 3rd edn. San Diego, CA: Academic Press.

Horn MH (1989) Biology of marine herbivorous fishes. *Oceanography and Marine Biology Annual Review* 27: 167–272.

Hume ID (2005) *Concepts of digestive efficiency*. In: Starck J Wang T (eds.) *Physiological ecological adaptations to feedingvertebrates,* pp. 43–58. Enfield, NH: Science Publishers.

Jobling M (1994) *Fish Bioenergetics*. New York, NY: Chapman and Hall.

Karasov WH and Hume ID (1997) *Vertebrate gastrointestinal system*. In: Dantzler WH (ed.) *Handbook of Comparative Physiology,* pp. 407–480. Bethseda, MD: American Physiological Society.

Karasov WH and Martínez del Rio C (2007) *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton, NJ: Princeton University Press.

Krogdahl Å., Hemre GI, and Mommsen T (2005) Carbohydrates in fish nutrition: Digestion and absorption in postlarval stages. *Aquaculture Nutrition* 11: 103–122.

Stevens CE and Hume ID (1995) *Comparative Physiology of the Vertebrate Digestive System*, 2nd edn. Melbourne: Press Syndicate of the University of Cambridge.

Stevens CE and Hume ID (1998) Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiological Reviews* 78: 393–427.

Stone DAJ (2003) Dietary carbohydrate utilization by fish. *Reviews in Fisheries Science* 11: 337–369.

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science.* 11: 107–184.

Webster CD and Lim CE (eds.) (2002) *Nutrient Requirements and Feeding of Finfish for Aquaculture*. New York, NY: CABI Publishing.

Cost of Digestion and Assimilation

SM Secor, University of Alabama, Tuscaloosa, AL, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Future Directions
Further Reading

Glossary

Postprandial Period after ingesting a meal.

Specific dynamic action The additional amount of oxygen or energy consumed above SMR as a result of feeding, measured during the period after feeding until metabolic rate returns to SMR; SDA represents the energetic cost of digestion, assimilation, and protein turnover.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Introduction

For all organisms, the repertoire of metabolic expenditures includes the energy that is spent on meal digestion and assimilation. The ingestion of a meal sets into motion cascades of cellular and enzymatic activities that result in the breakdown of the food, and the absorption, transport, and assimilation of its nutrients. The many steps involved in this nutritive journey each require an input of energy. The collective energy that is expended is commonly referred to as the specific dynamic action (SDA) of the meal. Also coined heat increment of feeding (HIF) and thermal effects of food (TEF), SDA is a mandatory component of an individual's energy budget, accounting for as much as 25% of its daily energy expenditure.

From the miniscule goby to the gigantic whale shark, all fish eat and hence experience an SDA response with each meal. Homer Smith's inaugural studies in the early 1930s on the air-breathing African lungfish, *Protopterus aethiopicus*, were the first to document fish SDA. Following a 30-year gap, the study of fish energetics was resurrected in the 1960s by the efforts of Canadian researchers William Beamish, Roly Brett, and Richard Saunders, who collectively laid the ground work for experimental studies in fish SDA. These studies demonstrated that the magnitude and duration of the SDA response are sensitive to meal size, type and composition, and body temperature and fish size. Many studies have been conducted on species important to commercial or recreational fisheries with an aim to manage better fish growth by minimizing the energy lost to SDA. For fish species not commercially important, studies have primarily explored ecological correlates of SDA.

First reviewed by Malcolm Jobling in 1981, fish SDA has continued to capture the interests of physiologists and aquaculturists, owing to the growing interest in postprandial metabolic responses and the contribution of digestive energetics to nutrition, growth, and the capacity for activity. Since Jobling's review, nearly 70 new studies (85 overall) have examined the postprandial metabolism of fishes. The following presents a brief description of the methods to quantify fish SDA, a taxonomic overview of fish SDA, the factors that influence SDA, and the energy-expending processes that generate the SDA response.

Measuring Fish SDA

The study of fish SDA is generally undertaken following a relatively simple experimental design. First, a baseline set of metabolic measurements, generally the standard metabolic rate (SMR) quantified as rates of oxygen consumption (V_{O_2} or M_{O_2} depending on whether the volume or mass of oxygen, respectively, is measured), are taken from individual fish at rest and postabsorptive (**see also Energetics**: General Energy Metabolism). If the fish maintains the same swimming speed after feeding, then the prefeeding cost of SMR and swimming can be subtracted from postprandial V_{O_2} to calculate SDA. An alternative approach is to measure V_{O_2} of postabsorptive fish over a range of swimming speeds (**see also Ventilation and Animal Respiration**: The Effect of Exercise on Respiration) and regress V_{O_2} against

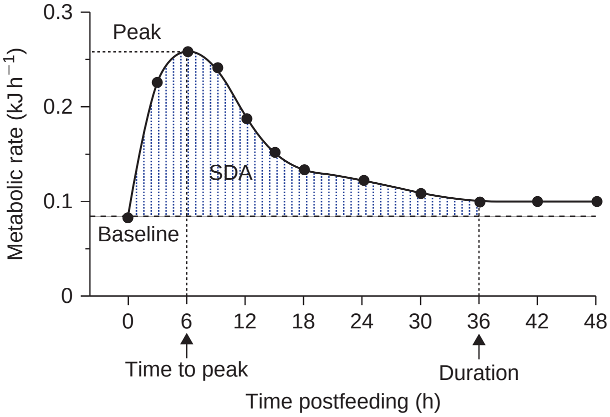


Figure 1 Hypothetical postprandial metabolic profile of a fish following ingesting a 20 kJ meal plotted against time postfeeding. Noted are variables commonly reported in studies of fish SDA.

swimming speed and assign the *y*-intercept (zero swimming speed) as SMR.

Once a baseline V_{O_2} is established, the fish are fed a natural or formulated diet to a targeted percentage of body mass or to satiety. Metabolic measurements are then resumed and thereafter recorded continuously or intermittently until metabolic rates return to baseline values. The postprandial metabolic response is characterized by plotting metabolic rate as a function of time postfeeding (**Figure 1**). SDA can then be quantified from the extra oxygen consumed above baseline values and assumptions regarding the substrate(s) being metabolized. Variables frequently quantified and analyzed (illustrated in **Figure 1**) include baseline (i.e., SMR, 0.088 kJ h^{-1}), postprandial peak in V_{O_2} (0.26 kJ h^{-1}), time to the peak V_{O_2} (6 h), factorial scope of peak V_{O_2} (peak V_{O_2}/SMR = 2.95), the duration of significantly elevated V_{O_2} (36 h), SDA (2.44 kJ), and the SDA coefficient (SDA as a percentage of meal energy = 12.2%).

Taxonomic Account of Fish SDA

Of the more than 30 000 known species of fish, the SDA response has been documented in at least 65 species. These species range in size from the 0.5 g zebrafish, *Brachydanio rerio*, to the 11 kg bluefin tuna, *Thunnus maccoyii* (**Table 1**). Experimental temperatures for SDA studies have ranged from −1 to 30 °C, with the majority of studies conducted between 20 and 28 °C. For many studies, especially those examining the effects of meal composition, fish were fed a pelleted diet with a formulated content of protein, carbohydrate, and lipid. Other studies used natural diets including mollusks, crustaceans, and other fish. Meal mass ranged from 0.25% to 24.2% of body mass, averaging 3.35 ± 0.23% (SE) of body mass. **Table 1** summarizes published fish SDA studies (a single study per species) for fish mass, relative meal size, meal type, the scope of peak postprandial metabolism, SDA, and the SDA coefficient.

Elasmobranchii

SDA has been examined in three species of elasmobranchs, the swellshark, *Cephaloscyllium ventriosum*, the spotted dogfish, *Scyliorhinus canicula*, and the spiny dogfish, *Squalus acanthias*. In these studies, body temperatures were maintained at 12–16 °C and sharks consumed either fish or squid meals that weighed 5.1–6.5% of the shark's body mass. Each species experienced a rapid postprandial increase in V_{O_2} that peaked 7–15 h after feeding at 1.9–3.0 times fasting levels (**Figure 2**(**a**)). Following the peak in V_{O_2}, metabolic rates declined, returning to fasting levels by 15 h and up to an estimated 120 h after feeding. For the three species, the SDA coefficient varied only between 12.0% and 19.8% (**Table 1**). Interestingly, juvenile *S. canicula* experienced a much lower SDA coefficient (6.0%) compared with adults (12.5%), partly, due to a shorter duration of their SDA response (46 h vs. 85 h).

Dipnoi

Of the six extant species of lungfishes, postprandial metabolism has been explored only for the African lungfishes, *Protopterus aethiopicus* and *P. annectens*, the former species being the first fish species to be examined for SDA. In that inaugural study by Homer Smith, lungfish forcefed pieces of beef heart (∼ 1% of body mass) responded with a rapid increase in metabolic rate that peaked within 24 h after feeding, followed by a subsequent decline in V_{O_2} that continued until the next meal. The study on *P. annectens* observed a sixfold increase in metabolic rate during the digestion of a chironomid-larval meal equaling 3.3% of lungfish body mass and determined that 60% of postprandial V_{O_2} is obtained from lung ventilation (**Figure 2**(**b**)).

Table 1 Tabulation of 67 species of fishes examined SDA response

Species	*Body mass (g)*	*T_b (C°)*	*Meal type*	*Meal mass (%)*	*Scope*	*SDA (kJ)*	*SDA coefficient (%)*
Chondrichthyes							
Carcharhiniformes							
Cephaloscyllium ventriosum	190	16	Fish	5.1	2.30	7.20	12.0
Scyliohinus canicula	740	15	Squid	6.5	2.99	27.7	12.5
Squaliformes							
_Squalus acanthias_*	4000	12	Fish	5.5	1.89	174	19.8
Sarcopterygii							
Lepidosireniformes							
Protopterus aethiopicus	510	25	Beef heart	0.98	2.30		
Protopterus annectens	151	27	Chironomid larva	3.3	6.49	1.96	10.6
Actinopterygii							
Anguilliformes							
Anguilla anguilla	72.8	25	Formulated diet	1.3	11.0	1.97	11.3
Anguilla rostrata	1.5	20	Formulated		6.6	0.15	
Cypriniformes							
_Acheilognathus lanceolate_*	3.5	25	Algae		3.43	0.084	
Brachydanio rerio	0.5	24	Formulated diet	5.0	1.80	0.019	4.63
_Carassius auratus_*	3.5	20	Algae		1.84	0.028	
Chalcalburnus chalcoides	0.1	20	Brine shrimp		1.98		
_Ctenopharyngodon idella_*	16.7		Algae			0.25	7.00
Cyprinus carpio	72.0	28	Formulated diet	1.0	2.66	2.24	15.5
Leuciscus cephalus	0.1	20	Brine shrimp		1.82		
_Phoxinus phoxinus_*	2.5	15	whiteworms	3.0		0.11	35.0
Pseudorasbora parva	1.18	25	Tubificid worms		7.03	0.44	58.6
Rhodeus rhombeus	10.5	30	Tubificid worms	8.2	1.73	0.18	7.69
Rhodeus lanceolatus	3.27	30	Tubificid worms	7.6	2.35	0.14	21.0
Scardinius erythrophthalmus	0.1	20	Brine shrimp		1.57		
Siluriformes							
Clarias gariepinus	0.0003	28	Brine shrimp		1.63		
Ictalurus punctatus	1025	22	Formulated diet	2.0	2.28		
Pelteobagrus vachelli	7.05	25	Fish	4.0	2.21		
Psedobagrus fulvidraco	1.18	25	Tubificid worms		5.39	0.38	50.8
Silurus asotus	37.4	25	Fish	9.0	3.19	2.69	16.3
Silurus meridionalis	46.5	27.5	Fish	8.3	2.92	2.52	12.7
Salmoniformes							
Oncorhynchus mykiss	12.5	15	Formulated diet	2.0		0.71	15.1
Oncorhynchus rhodurus	50.0	10	Fish	2.4		0.68	16.6
Oncorhynchus tshawytscha	520	10	Formulated diet		2.28		
Salmo salar	3.7		Formulated diet	2.7	1.71	0.041	2.48
Gardiformes							
_Gadus morhua_*	61.0	18	Fish	5.0	1.44	23.2	8.44
Melanogrammus aeglefinus	6	15	Formulated diet	1.7	1.44	0.057	
Scorpaeniformes							
_Anoplopoma fimbria_*	993	8.5	Fish	5.0	1.44	23.2	8.44
Myoxocephalus scorpius	74.5	15	Shrimp	12.7	2.86	7.64	16.0
Perciformes							
Anarhichas lupus	2000	9		6.0	2.40		
_Blennius pholis_*	15.6	10	Mussel	3.0	1.60	0.13	7.92
Channa argus	201	28	Fish	1.0		0.68	6.75
Coregonus lavaretus	0.051	10	Brine shrimp			0.021	13.0
Cirrhitichys bleekeri	21.1	25	Shrimp	5.3	1.97	0.81	14.9
Dicentrarchus labrax	42.0	25	Formulated diet	2.0	2.07	2.49	14.9
Eleotris oxycephala	40.0	30	Fish	3.6	2.15	0.75	13.7
_Esox lucius_*	18.6	12	Fish	7.8	2.35	0.92	12.6
_Etroplus suratensis_*	30.0	28	Formulated diet		2.33	0.48	
Harpagifer antarcticus	4.33	−0.5	Krill	14.6	2.38	0.41	10.1
_Harpagifer bispinis_*	3.0	10	Amphipod	15.0	3.60	0.13	
Kuhlia sandvicensis	44.0	23	Fish	2.4	2.21		17.5
Lepomis macrochirus	72.9	25	Formulated diet	1.8	1.80	3.11	16.1

(*Continued*)

Table 1 (Continued)

Species	*Body mass (g)*	*T_b (C°)*	*Meal type*	*Meal mass (%)*	*Scope*	*SDA (kJ)*	*SDA coefficient (%)*
*Lichia amia**	86.7	20	Fish	6.0	1.81	6.65	24.0
*Lithognathus lithognathus**	731	20	Clam	6.0	1.88	20.5	20.7
*Lithognathus mormyrus**	110	20	Clam	6.0	1.81	3.71	18.8
Macropodus chinensis	1.18	25	Tubificid worms		6.27	0.39	44.5
*Micropterus salmoides**	100	25	Fish	8.0		8.05	15.5
Notothenia neglecta	95.3	0.5	Shrimp	8.7	2.34	9.24	20.0
Odontobutis obscura	37.2	30	Fish	5.0	2.44	0.64	9.20
Oreochromis mossambicus	1.18	25	Tubificid worms		5.01	0.52	40.0
Oreochromis niloticus	95.0	28	Formulated diet	1.0	1.70	1.05	6.35
Orthodon microlepidotus	0.85	25.5	Brine shrimp	1.7	1.47		
Perca fluviatilis	2.45	20	Fish		4.19	0.65	13.5
*Pomadasys commersonni**	474	20	Clam	4.0	1.83	11.0	24.7
Sciaenops ocellatus	0.0001	24	Rotifer		2.52		
Seriola dumerili	308	19	Formulated diet		1.52		
Siniperca chuatsi	202	28	Fish	1.0		0.87	8.72
Sparus aurata	99.8	21	Formulated diet	1.5	3.18	0.95	19.8
Stizostedion vitreum	35.0	20	Formulated diet	1.6	1.80	0.70	5.58
*Tiliapia rendalli**	100	23.5	Algae	6.8		0.88	8.53
Thunnus maccoyii	11000	19.4	Fish	6.8	2.36	1628	38.9
Tridentiger obscurus	5.5	25	Algae		3.38	0.05	
Pleuronectiformes							
Pleuronectes platessa	32	10	Fish paste	3.8	1.97	1.12	16.5

An asterisk (*) next to species name indicates that SDA and/or SDA coefficient were calculated from published information. Meal mass (%) is the mass of the meal relative to fish body mass. Scope is quantified as maximum postprandial rate divided by basal metabolism. The SDA coefficient is quantified as SDA as a percentage of meal energy.

Actinopterygii

Anguilliformes

SDA has been examined in the European eel, *Anguilla anguilla*, and the American eel, *A. rostrata*, following the feeding on a formulated diet, which for *A. rostrata* varied in protein content from 30% to 40%. For both species, postprandial V_{O_2} peaked approximately 12 h after feeding and declined thereafter for the next 6–12 h (**Figure 2**(**c**)). For *A. anguilla*, the observed 11-fold increase in V_{O_2} is the largest known factorial increase in postprandial metabolism for a fish (**Table 1**).

Cypriniformes

The postprandial metabolic response has been quantified for a dozen species of cypriniforms, ranging in body mass from 0.1 to 318 g. Meals in these studies have included algae, tubificid worms, brine shrimp, and formulated pellets weighing 1–8% of fish body mass. The factorial scope of the peak in V_{O_2} averaged 2.51 ± 0.26%, while the SDA coefficient averaged 20.4 ± 2.5%. Studies on the common carp (*Cyprinus carpio*) have explored variation in SDA due to differences in meal type, meal composition, and meal size (**Figure 2**(**d**)).

Siluriformes

Although only five species of siluriforms have been studied for their SDA response, the southern catfish *Silurus meridionalis* has been examined meticulously for the impact of meal composition, meal size, feeding frequency, fasting duration, and body temperature on SDA (**Figure 2**(**e**)). Meal size has a profound impact on the postprandial metabolic response and SDA of *S. meridionalis*, as well as for the Chinese catfish, *S. asotus*. For both species, SDA increased stepwise with each increase in meal size. Across all experimental treatments (e.g., meal size, meal composition, and body temperature), *S. meridionalis* and *S. asotus* experienced a SDA coefficient that ranges between 7.7% and 24.7% (mean = 13.8 ± 0.6%).

Salmoniformes

Dominating the SDA studies on salmoniforms are those conducted on the rainbow trout, *Oncorhynchus mykiss* (**Figure 2**(**f**)). A goal for most of those studies was to determine the impact of meal composition, specifically protein and lipid concentrations, on SDA. Using diets formulated to different concentrations of protein and lipid, these studies aimed to determine the optimal combination of feed nutrients that would minimize SDA and maximize growth. Recent studies on rainbow trout have explored the relationships between postprandial metabolism and cardiovascular performance (**see also Design and Physiology of Arteries and Veins**: The Gastrointestinal Circulation).

Gadiformes

The commercial importance of the Atlantic cod, *Gadus morhua*, and haddock, *Melanogrammus aeglefinus*, has sparked studies examining the effects of body

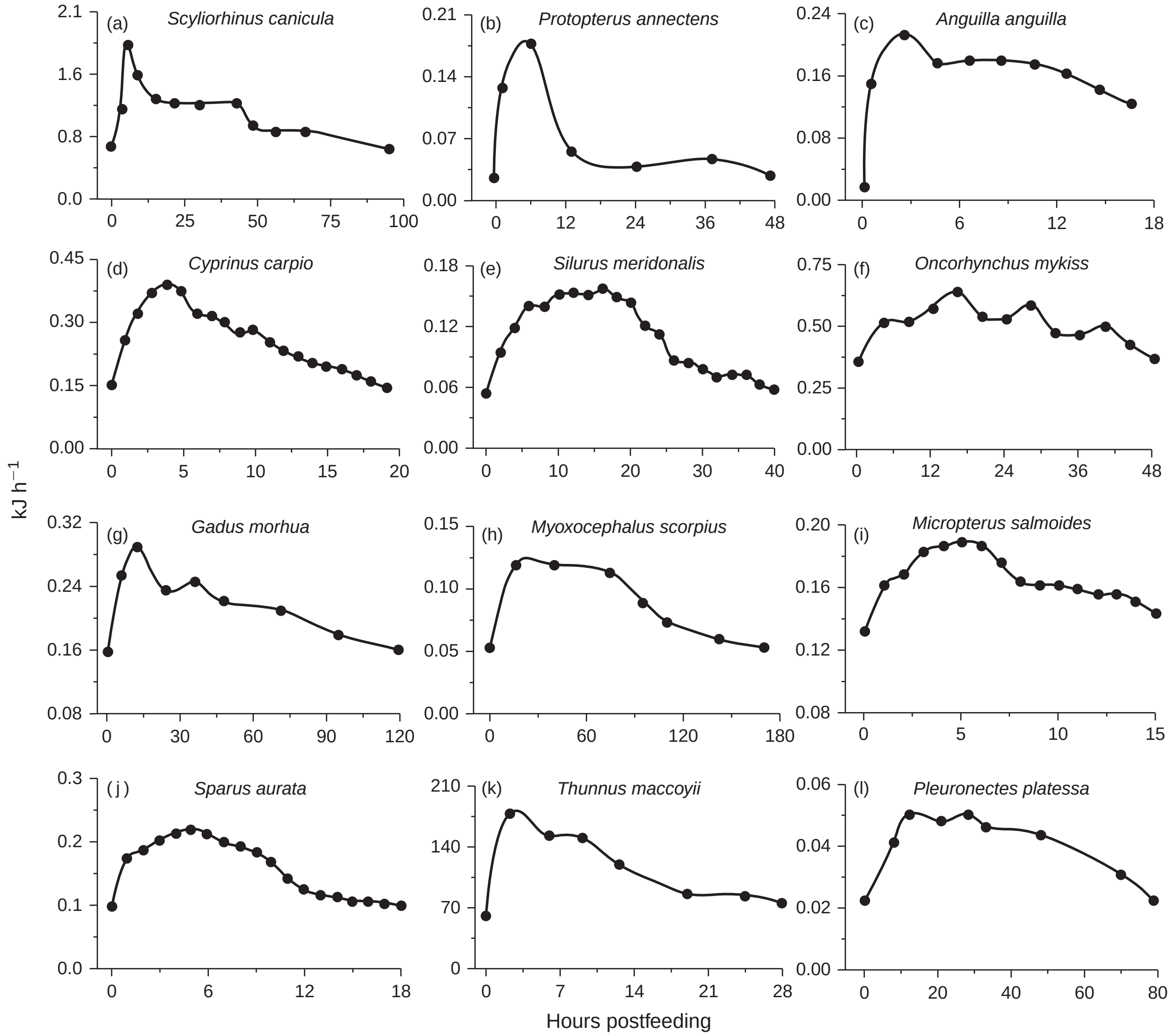


Figure 2 (a–j) Postprandial metabolic profiles of 12 species of fish representing three classes and 10 orders. Fish generally respond to feeding with a rapid increase in metabolic rate that generally peaks within 10 h after feeding. After the peak, metabolic rates return to prefeeding levels more slowly. Each figure was drawn from data presented in published articles. Among these studies, duration of postprandial measurements ranged from 15 to 180 h.

temperature, meal size, and body size on their SDA (**Figure 2**(**g**)). The SDA response has been quantified for cod larvae as small as 0.25–0.75 mg (dry mass) using microcalorimetry. Given that cod may be exposed to hypoxic events, research has determined that hypoxia decreases the postprandial peak in cod V_{O_2}, while increasing the duration of the metabolic response and SDA.

Scorpaeniformes

Two studies have separately quantified the SDA of the sablefish (*Anoplopoma fimbria*) and the shorthorn sculpin (*Myoxocephalus scorpius*) (**Table 1**). When exposed to two temperature regimes (5 and 15 °C) and digesting shrimp meals weighing 12.7–15.5% of fish body mass, the sculpins experienced a 160–250% increase in metabolic rate that generates an SDA equivalent to 16–17% of the meal's energy (**Figure 2**(**h**)).

Perciformes

Half of the fish species for which SDA studies have been conducted occur within the order Perciformes (**Figures 2**(**i**)–**2**(**k**)). Dominating this group are studies on the bluegill sunfish (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and Nile tilapia (*Oreochromis niloticus*). Studies range from a simple description of the postprandial metabolic response to a single meal at one temperature to studies examining interactions among several variables on SDA. Once again, the aim of studies on commercially important species (e.g., *Dicentrarchus*, *Lepomis*, *Micropterus*, and *Oreochromis*) was to identify the mixture of protein and lipid within the diet that minimized SDA and hence increased the energy that can be allocated to growth. For all perciform species, postprandial oxygen consumption peaked at 2.36 ± 0.07 times standard or resting metabolism, and the SDA coefficient averaged 16.8 ± 1.0%.

Pleuronectiformes

The European plaice (*Pleuronectes platessa*) is the only member of this fish order to be studied for postprandial metabolism (**Table 1** and **Figure 2**(**l**)). For young individuals (16–50 g), an increase in meal size increased SDA, variation in body temperature did not impact SDA, and varying protein content altered SDA. A separate study on larger individuals (30–2000 g) likewise observed an increase in SDA with meal size.

Determinants of Fish SDA

A goal of many fish SDA studies has been to identify the impact that features of the meal, characteristics of the fish, and/or environmental conditions have on the postprandial metabolic profile and the magnitude of SDA. Discussed below are the various determinants of fish SDA.

Meal type

Although a wide variety of natural food types (e.g., algae, shrimp, worms, clam, and various fishes) have been fed to fish to assess their SDA response, only a single study has measured SDA to more than one natural food item. For *L. macrochirus*, *M. salmoides*, and the donko, *Odontobutis obscura*, the SDA coefficient was less for the goldfish meal compared with that for the tubificid worm or earthworm meal. This difference may reflect partly in lower energy value of the worm meals (hence, SDA becomes a larger percentage of meal energy), rather than lower energy required to digest and assimilate the goldfish meals.

Meal composition

Whereas few studies have addressed how SDA differs among natural meals, considerable attention has been directed at how variation in nutrient composition, specifically levels of protein and fat, impact fish SDA response. The impetus for these studies is to identify the best balance of carbohydrates, lipids, and proteins that satisfies the fish's nutritional requirements, minimizes SDA, and maximizes growth. Meals have varied in relative carbohydrate, protein, and lipid content by as much as 100% protein, 100% carbohydrates, or 30% lipids, with various combinations of each. An earlier established prediction is that protein-based meals will generate a larger SDA response than either a high-fat or high-carbohydrate meal. This has been found to be true for studies on *M. salmoides, O. mykiss, P. platessa*, and the grass carp, *Ctenopharyngodon idella.*

A second prediction is that SDA increases with an increase in meal protein content. In support, 50%, 150%, and 700% increases in percent meal protein generated respective increases of 23%, 78%, and 300% in SDA for the fishes *O. mykiss, C. carpio, and O. niloticus* (**Figure 3**(**a**)). On the other hand, SDA did not increase with higher meal protein content in studies on *L. macrochirus* and the European seabass, *Dicentrarchus labrax.* Likewise, varying lipid content, while maintaining protein concentration constant, generated mixed results. An increase in lipid content reduced SDA for *D. labrax* and *O. niloticus*, whereas variation in lipid content had little effect on the SDA of *O. mykiss.* Whereas the emphasis in the past has been just on protein content, the combination of all three major nutrients may need to be taken into account when evaluating the impact of meal composition on SDA. Another difficulty in comparing diet composition is that the changes need to result in isocaloric diet treatments, which has not always been the case.

Meal size

Well documented among all vertebrate taxa is the impact of meal size on SDA. By controlling for meal type, body size, and body temperature, an increase in meal size, and thus in the work of digestion and assimilation, will result in a larger SDA response. Such has been the case for a number of fish studies that have measured SDA for 2–7 different meal sizes. In almost all studies, the postprandial peak in metabolism, duration of elevated metabolism, and SDA increase incrementally with an increase in meal size (**Figure 3**(**b**)). For the catfishes *S. meridionalis* and *S. asotus*, respective 37.5- and 32.2-fold increases in meal size resulted in respective 2.7- and 3.3-fold increases in peak V_{O_2}, 5.7- and 4.2-fold increases in duration, and 33.8- and 24.5-fold increases in SDA. Expectedly, a doubling of meal size, and hence effort, would double SDA. For those studies that varied in meal size while maintaining other variables constant, the response coefficient (Q_{2x}), which is the factorial increase in a parameter in response to a doubling of demand, can be calculated for postprandial peak V_{O_2}, duration, and SDA. For 13 fish studies, Q_{2x} averaged 1.29 ± 0.04 for peak postprandial V_{O_2}, 1.46 ± 0.06 for duration, and 2.00 ± 0.09 for SDA. As predicted, a doubling of meal size and hence the workload of digestion and assimilation generates a doubling in fish SDA.

Meal energy

While not mutually exclusive to meal composition, meal type, and meal size, meal energy is possibly the best predictor of SDA for a fish. The relationship between meal energy and SDA is generally linear with a slope between 0.1 and 0.2. Hence, regardless of meal composition, type, and size, for any fish under any condition, SDA is typically equivalent to 10–20% of ingested energy. **Figure 3**(**c**) illustrates data from studies with fishes <500 g wet body mass. The slope of the regression line is 0.131 ± 0.004 and meal energy can explain 81% of the variation in SDA.

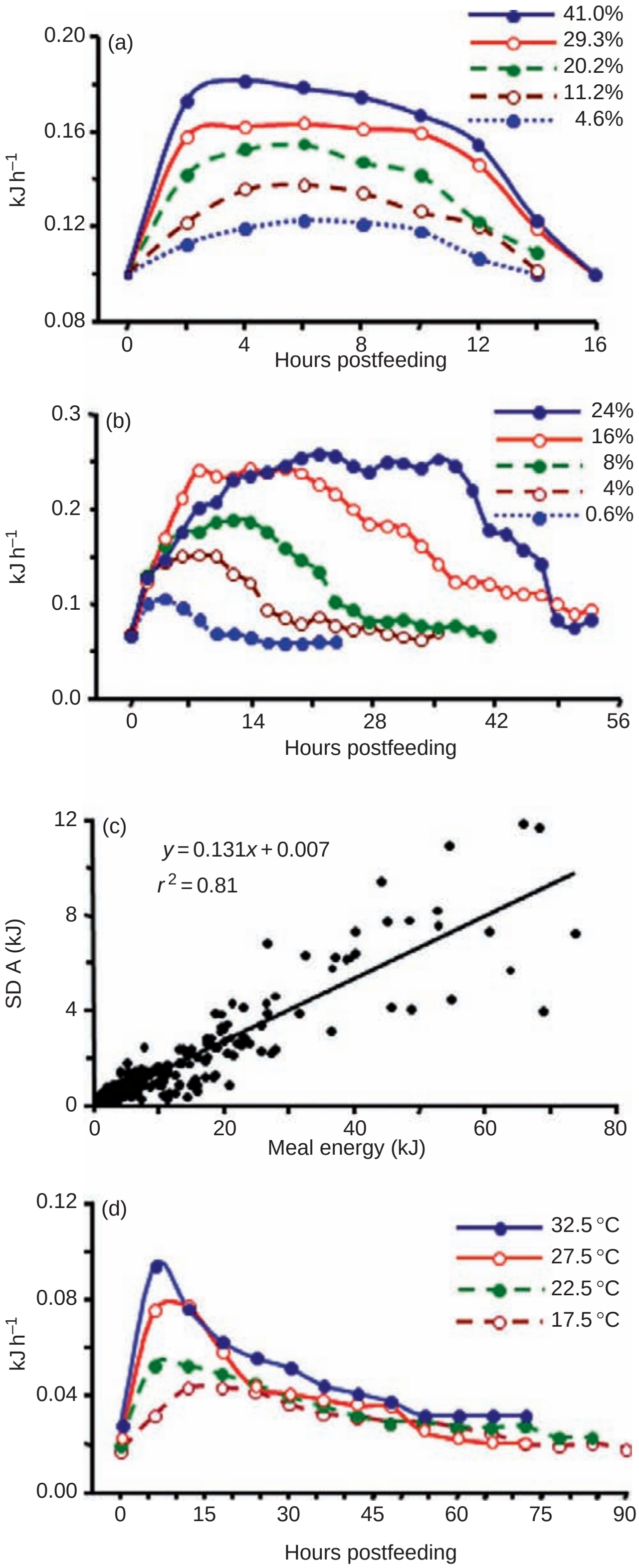


Figure 3 (a) Effects of protein content on the postprandial metabolic response of *Oreochromis niloticus*. With an increase in protein content, there is a corresponding increase in the magnitude of the SDA response. (b) Effects of meal size (% of body mass) on the postprandial metabolic response of *Silurus meridionalis*. As meal size increases, there is a matched increase in the magnitude and duration of the SDA response. (c) Linear relationship between meal energy (kJ) and SDA (kJ). This data set includes fishes that were less than 500 g at time of study. (d) Effects of body temperature on the postprandial metabolic profile of *Silurus meridionalis*. As body temperature increases, the postprandial profile becomes more elevated and shorter in duration.

Body size

With an increase in body size and a corresponding increase in meal size, absolute SDA should also increase. This is certainly true for those few fish studies that have examined body size effects on SDA. For young cod on the same diet, a 5.1-fold range in body mass was matched by a 5.3-fold range in SDA. For the yellowbelly rockcod, *Notothenia neglecta* digesting shrimp meals at −1 °C or 0.5 °C, a 90% and 140% increase in body mass for these respective temperatures generated 100% and 150% increases in SDA. Similarly, for the Antarctic plunderfish, *Harpagifer antarcticus*, digesting a krill diet and matched for relative meal size, a two- to threefold increase in body mass equaled the increase in SDA.

Body temperature

As ectotherms, fish are thermal conformers (with a few exceptions) whose metabolic rates vary as a function of ambient, and hence, body temperatures. A shift in body temperature alters not only basal and peak metabolism, but also the shape of the postprandial profile (3d). With an increase in body temperature, the postprandial profile becomes narrower and taller as the postprandial peak is reached sooner after feeding. With temperature influencing both basal and peak V_{O_2}, the factorial scope of peak V_{O_2} generally does not vary significantly with body temperature for fishes within a certain temperature range (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation and Effects of Climate Change). Assuming that a fixed amount of energy is expended to digest and assimilate a given meal regardless of body temperature, SDA should not vary with temperature. This has been found to be the case in studies on *D. labrax, P. platessa, O. obscura*, and the European minnow, *Phoxinus phoxinus*. Counter to this prediction, SDA was found to increase with body temperature for *G. morhua, S. meridionalis*, and the gilthead seabream, *Sparus aurata*.

Source of the SDA Response

What are the processes responsible for the SDA response? This question has intrigued scientists for more than a century. The list of possible actions include the sensory stimulation of metabolism by feeding, the actual work involved in meal breakdown and absorption, the assimilation and catabolism of proteins, fats, and carbohydrates, and protein cycling and synthesis (**see also Design and Physiology of the Heart**: Intracardiac Neurons and Neurotransmitters in Fish). A popular view for earlier fish studies was that SDA was partitioned between 'mechanical SDA', the cost of grasping, chewing, and gut peristalsis, and 'biochemical SDA', the cost of chemical breakdown, nutrient transport and circulation, catabolism of assimilated nutrients, and the synthesis of macromolecules (e.g., proteins and urea). Alternatively, as presented below, the processes contributing to SDA can be divided into pre-absorptive (prior to the entry of nutrients into circulation) and post-absorptive (after intestinal absorption) events.

Pre-absorptive contributions to fish SDA

Whether eating and swallowing constitutes a postprandial event and hence contributes to SDA is debatable. Fish undoubtedly vary in the effort expended to chew and swallow due to differences in meal size and type. Unfortunately, this cost is seldom measured since metabolic measurements typically begin after swallowing. Given their very short esophagus, there would be little contribution of esophageal peristalsis to SDA.

Fish vary tremendously in their ingested meal, from microscopic plankton to small fragments of a prey, to large intact prey. Regardless of meal type, the ingested item is broken down in the stomach (for those species that possess a stomach) to a soup-like chyme before it passes into the small intestine. Gastric breakdown of food is achieved by mechanical churning of the food by alternating contractions of circular and longitudinal smooth muscles and by the chemical actions of the proteolytic enzyme pepsin and hydrochloric acid (HCl) (**see also Integrated Function and Control of the Gut**: Gut Motility and Gut Secretion and Digestion). Muscle contraction, the production of pepsinogen (inactive precursor of pepsin), and the pumping of H^+ into the gastric-pit lumen (to form HCl) are energy-consuming processes. Given that one adenosine triphosphate (ATP) is utilized by the H^+/K^+ATPase for each H^+ pumped out of the gastric oxyntopeptic cell, the maintenance of an acidic gastric lumen during digestion contributes to the SDA response.

Intestinal activities that consume energy during digestion would include peristalsis, production, and actions of enzymes (e.g., disaccharidases and aminopeptidases), the production and secretion of regulatory peptides (e.g., cholecystokinin, gastric inhibitory polypeptide, and secretin), the transmembrane transport of nutrients (requiring ATP-driven maintenance of a sodium gradient), and any post-transport synthesis within intestinal cells (e.g., cellular hypertrophy and formation of chylomicrons). Even though these activities occur during digestion, it has been assumed that intestinal effort contributes modestly (if at all) to SDA. This view stems largely from studies for which fish were fed an inert compound (not absorbable) in their meal and their metabolic response was significantly reduced.

Post-absorptive contributions to fish SDA

Historically, investigators viewed fish SDA largely as a post-absorptive phenomenon, dominated by the costs of oxidative deamination of amino acids and protein synthesis. In support, a study on the channel catfish

(*Ictularus punctatus*) observed a significant 80% increase in V_{O_2} following the infusion into the caudal artery of an amino acid solution. The magnitude of this response was similar to that generated from the oral ingestion of the amino acids. Because absorbed amino acids are not stored, they must be rapidly incorporated into new proteins and/or catabolized. For the latter, fish expend energy to deaminate the absorbed amino acids, transaminate the amino group, oxidize the carbon residues or channel the carbon into gluconeogenesis or ketogenesis, and secrete the residual ammonia. Granted, the cost of ammonia production and secretion is generally assumed to be insignificant since it is readily diffused to most surrounding waters (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia and Ureotelism).

Amino acids, together with glucose and lipids that are not immediately catabolized, are instead directed into energy-consuming synthetic pathways. Fed fish experience a two- to fourfold increase in protein synthesis. The contribution of protein synthesis to SDA is realized when protein synthesis is experimentally blocked by the administration of cycloheximide, and the normal postprandial metabolic response is largely abolished. It has been estimated for the cod that protein synthesis accounts for 20–40% of SDA. Combined with the more modest contribution from the synthesis of glycogen and fat, the cost of biosynthesis (i.e., the cost of growth) is a dominant contributor to fish SDA.

Future Directions

Our understanding of fish bioenergetics and SDA would benefit from explorations directed at:

1. examining the SDA of more diverse species that possess unique feeding habits and ecologies;
2. identifying the pre-absorptive and post-absorptive events that generate the SDA response;
3. determining the impact that changes in salinity and oxygen tension have on SDA; and
4. investigating the interplay between the competing metabolic demands of activity (swimming) and digestion in dictating SDA.

See also: **Design and Physiology of the Heart**: Intracardiac Neurons and Neurotransmitters in Fish. **Energetics**: General Energy Metabolism. **Integrated Function and Control of the Gut**: Gut Motility; Gut Secretion and Digestion. **Nitrogenous-Waste Balance**: Excretion of Ammonia; Ureotelism. **Temperature**: Effects of Climate Change; Membranes and Temperature: Homeoviscous Adaptation. **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration.

Further Reading

Chakraborty SC, Ross LG, and Ross B (1992) Specific dynamic action and feeding metabolism in common carp, *Cyprinus carpio* L. *Comparative Biochemistry and Physiology A* 103: 809–815.

Eliason EJ, Higgs DA, and Farrell AP (2007) Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout. *Aquaculture* 272: 723–736.

Fu SJ, Xie XJ, and Cao ZD (2005) Effect of meal size on postprandial metabolic response in southern catfish (*Silurus meridionalis)*. *Comparative Biochemistry and Physiology A* 140: 445–451.

Guinea J and Fernandez F (1997) Effect of feeding frequency, feeding level and temperature on energy metabolism in *Sparus aurata*. *Aquaculture* 148: 125–142.

Hunt von Herbing I and White L (2002) The effects of body mass and feeding on metabolic rate in small juvenile Atlantic cod. *Journal of Fish Biology* 61: 945–958.

Jobling M (1981) The influences of feeding on the metabolic rates of fishes: A short review. *Journal of Fish Biology* 18: 385–400.

Jobling M and Davies PS (1980) Effects of feeding on metabolic rate, and the specific dynamic action in plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 16: 629–638.

Machida Y (1981) Study of the specific dynamic action of some freshwater fishes. *Reports of the USA Marine Biological Institute* 3: 1–50.

McCue MD (2006) Specific dynamic action: A century of investigation. *Comp Biochem Physiol A* 144: 381–394.

Ross LG, McKinney RW, Cardwell SK, *et al.* (1992) The effects of dietary protein content, lipid content, and ration level on oxygen consumption and specific dynamic action in *Oreochromis niloticus* L. *Comparative Biochemistry and Physiology A* 103: 573–578.

Secor SM (2009) Specific dynamic action: A review of the postprandial metabolic response. *Journal of Comparative Physiology B* 179: 1–56.

Soofiani NM and Hawkins AD (1982) Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua* L. *Journal of Fish Biology* 21: 577–592.

Tandler A and Beamish FWH (1981) Apparent specific dynamic action (SDA), fish weight and level of caloric intake in largemouth bass, *Micropterus salmoides* Lacepede. *Aquaculture* 23: 231–242.

Dietary Requirements of Fish Under Culture Conditions

IP Forster, Fisheries and Oceans Canada, West Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Nutrient Functions and Their Requirements
AAs/Protein
Fatty Acids
Minerals and Vitamins
Further Reading

Glossary

Dispensable amino acid (DAA) An amino acid that may be biochemically produced endogenously from precursors in sufficient quantity to meet the needs of the animal.

Indispensable amino acid (IDAA) An amino acid that must be supplied in the diet to meet the needs of the animal.

Introduction

About half of seafood consumed globally is farmed. Modern aquaculture includes a wide diversity of species and conditions – from carnivores to herbivores and from freshwater to marine species. Most of the global aquaculture production generally involves freshwater herbivores that are reared at low density in 'green water' ponds where nutrients are obtained within the pond, with little or no supplemental prepared feeds.

This paradigm is changing. Much of the recent growth in aquaculture has involved an increase in culture intensity, with fish being grown at higher densities. Many fish (especially carnivores like salmon) have required prepared supplemental feed from their inception as aquaculture species, and even those traditionally reared in green water environments are increasingly fed prepared feeds as their culture densities have increased.

An artificial fish feed for intensive aquaculture must meet the target species' nutrient requirements and this requires knowledge of what these nutritional requirements are. Matching dietary nutrients to the needs of the target species is important to permit maximum growth, greatest efficiency of feed utilization, and reduced environmental nutrient loading (pollution).

Nutritional scientists have developed a set of research approaches for determining nutrient requirements. Many aspects of animal nutrition are common among species enabling a transfer of knowledge from work with terrestrial animals to aquaculture. Consequently, great strides in a relatively short time (about 35 years) have been made in understanding the nutrient needs of fish species, resulting in considerable improvement in aquaculture feeds. However, considerable variability exists in specifics. In addition, the difficulties inherent in working in aquatic environments (nutrient leaching, uncertain feed consumption, etc.) have meant that the quantitative nutritional information obtained is generally less robust. Thus, for aquaculture to continue to grow in a sustainable fashion requires generation of feeds that efficiently meet the needs of the cultured species and this will require new basic understanding of nutritional needs at the species level and how these can be met by a variety of traditional and novel ingredients.

This article is concerned with nutrient requirements of cultured fish and the research approaches involved in their quantification. The Further Reading section contains reviews of nutrient requirements of cultured fish, including numeric values.

Nutrient Functions and Their Requirements

Animals are composed of a great number of substances, some of which are produced from precursors by operation of endogenous metabolic pathways and some of which are not. Substances that cannot be produced endogenously in sufficient quantities to meet the needs of the animal must be obtained exogenously, that is, from the diet (oxygen, water, and some minerals may be obtained through the gills or by drinking). Nutrients are substances derived from the diet that are necessary for life. Some nutrients supply energy, although energy is not itself a nutrient; it is a property of some substances that provides the capacity to do work (fuel).

Categories of nutrients include: amino acids (AAs; protein), fatty acids (lipids), vitamins, minerals, and carbohydrates. Water is a nutrient, but is not usually considered as such for aquatic animals. Some nutrients function only in the form in which they are ingested (e.g., vitamins and minerals). Others are subjected to catabolic and anabolic processes after being eaten and may have multiple roles. For example, AAs and fatty acids not only function as structural components of proteins and cell membranes, but are also sources of biochemical energy. Dietary carbohydrates, in contrast, predominantly function as energy sources. For example, starch is only a nutrient because it is a source of energy and is only useful to the extent that it is broken down into its constituents (glucose) and absorbed. Some carbohydrates when present in a diet have probiotic activity, that is, they act to promote the growth and/or activity of beneficial bacteria in the digestive tract, but this does not constitute a dietary requirement *per se*. Once a nutrient is used for energy it is functionally indistinguishable from other substances, whereas the specific structure of vitamins, minerals, some AAs, and fatty acids is necessary to retain their functionality.

Since nutrients are defined as substances needed for life, and since some nutrients are needed in the form they are in and cannot be produced endogenously to any great extent, it follows that there will be a dietary intake level of these nutrients below which the functioning of the animal will be impaired. These are essential nutrients. The lowest level of intake of an essential nutrient that permits maximum functioning, assuming that all other nutrients are present at requirement levels, is the requirement for that nutrient. The meaning of functioning is intentionally left vague, because there are different performance criteria that may be of interest. In aquaculture, growth is frequently the primary concern and the nutrient intake needed to maximize growth is used to define the requirement. Other important indices include: feed efficiency (weight gain per unit consumption), survival, resistance to stress or infection, and reproductive success. (Although feed conversion ratio (FCR) is more commonly seen, feed efficiency (FE) is preferable because it is in line with other responses. Weight gain and protein efficiency increase, so does FE, whereas FCR, which is an inverse index, decreases.)

Nutrient requirements are best described in terms of consumption (gram ingested per day as proportion of body mass), but are more frequently described in terms of proportion of diet (percent or milligram per kilogram diet) for convenience. The digestibility of a meal (what percentage is absorbed) is factored into such values. There is often not much difference between expressing requirements in terms of consumption or as proportion of diet, as there is a relationship between what an animal needs and its growth or metabolic rate, which is in turn affected by its feed intake. The requirement for some specific nutrients is described in terms of their relationship with other nutrients, when there is a close relationship between the two. For example, vitamin E (tocopherol) is an anti-oxidant protecting highly unsaturated fatty acids (which are also nutrients) from destructive oxidation and therefore the dietary requirement of vitamin E is positively related to the dietary level of these fatty acids.

Nutrient balance is important when considering nutritional requirements, as nutrients can interact with each other; one nutrient can improve the utilization of another (e.g., vitamin D improves calcium uptake and utilization), or nutrients can interfere with each other (some AAs compete with each other for intestinal absorption). Some nutrients, even when necessary for health, growth, and reproduction, may become toxic when present in excess (e.g., vitamins A and D, the mineral selenium). While toxic effects are rarely acute under practical conditions, they may become manifest if dietary excess continues for extended periods (**see also Toxicology**: The Toxicology of Metals in Fishes).

Although requirements can be determined with reasonable confidence, there is always some uncertainty and usually requirements are established based on results of several experiments. Given the uncertainty in requirement estimates, and the importance of ensuring that sufficient levels of all nutrients are present in a diet, allowances (requirement level plus an additional amount, a 'margin of safety') are sometimes used in practical situations.

AAs/Protein

Functions of AAs/Physiological Basis for Requirements

AAs are supplied in diets generally in the form of proteins. Although there are species differences, of the 20 or so AAs found in proteins, most aquatic animals studied require the same 10 of them – the indispensible AA (IDAA), sometimes referred to as the essential AA (EAA). The dispensable AAs (DAAs) are those that can be produced endogenously, either from simple compounds or from other AA. It is the IDAA requirement that is the basis for the protein requirement. In the body, AAs are found in the protein fraction; there is no storage depot for AA, as there is for lipids and carbohydrates, although the production of DAA can be accelerated as needed and IDAAs are obtained from catabolized proteins when physiological demand is greater than the supply from the diet. In addition to acting as the building blocks for protein, AAs are important source of biochemical energy. If an animal's energy needs exceed what is available from the diet, AA may be preferentially directed to catabolic processes with the liberation of energy. Conversely, adequate dietary energy reduces the catabolism of AA for energy, thereby reducing the overall dietary need for protein. This effect is known as protein sparing.

Beyond providing the material for protein anabolism and energy, AAs are precursors for many specialized biomolecules, including hormones, neurotransmitters, coenzymes, etc. For example, tyrosine is the precursor of the catecholamines dopamine, norepinephrine, and epinephrine and of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which are important in regulating the metamorphosis of flatfish and may play a role during adaptation of salmonids to seawater during smoltification. Serotonin and melatonin are derived from tryptophan and histamine is derived from histidine. While the generation of these and other biomolecules is dependent on the presence of the precursor AA, it is not likely that AA requirement is driven by the production of the biomolecules. In other words, the metabolic demand for the individual AA for protein synthesis is likely much greater than what is needed for generation of alternative biomolecules. Production of these hormones is highly regulated and not generally responsive to their dietary levels, although there is evidence for high dietary levels of some AAs affecting hormonal status under certain conditions. Serotonin in the diet has been shown to influence consumption of the amount and type of feed selected.

High circulating levels of specific AA can have considerable effects on the endocrine system of fish. For example, dietary level of the IDAA arginine influences plasma insulin levels. Most studies examining the requirements for specific AA focus on growth rate or other production values and do not consider the nonprotein aspects of AA nutrition, although this could provide valuable information.

Methods Used to Estimate AA Requirements

Most published studies on nutrition requirements for aquaculture examine protein and AA requirements because protein makes the greatest contribution to feed cost. Even so, IDAA requirements are known for only a handful of the myriad of aquatic species being cultured, and the values obtained are less refined than for most barnyard or laboratory animals as a result of technical difficulties (water leaching, uncertainty of consumption rate, etc.). By adapting techniques first used for terrestrial animals, most methods involve some form of dose–response, whereby the response is measured for a group of fish fed one of a series of diets containing graded levels of a specific AA. Usually, multiple responses are measured, including growth, protein accretion, feed efficiency, survival, and circulating levels of the AA. The lowest level giving the maximum response is declared the requirement.

There are significant practical difficulties with this approach, however. One is the development of a basal diet deficient in the AA being tested that fish will actually consume for the duration of the trials. Halver and co-workers, using an approach used in mammal research, developed a diet in the late 1950s that supplied all AAs as free AAs, thereby allowing independent manipulation of individual AA levels. This method permitted requirements to be worked out for a number of salmonid species, and this method was adopted for many other cultured fish species, including carp and eels, by other researchers. For animals that lack an acid stomach (e.g., carp; **see also Integrated Function and Control of the Gut**: Intestinal Absorption), neutralizing the diet with a strong base (sodium hydroxide) greatly improves the growth of the animals on the test diet (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). Alternatively, a protein source that is imbalanced for one or two IDAA may be used to determine requirements. An example of this is zein, a corn protein that is available in purified form that is extremely low in arginine and lysine.

Diets with AA in the free form are not well utilized relative to those contained in dietary protein, however. Yamada and co-workers force-fed rainbow trout (*Oncorhynchus mykiss*) diets containing either the milk protein casein or a mixture of free AA with the same profile. They found that the free AA peaked in the plasma of the fish at about 12 h postfeeding, while in the casein-fed fish, the peaks were about a third lower and were reached after about 24 h. The poorer utilization of dietary free AA relative to AA from protein is believed to be due to the rapid influx of AA flooding the body at levels beyond which the protein synthetic machinery can efficiently cope with. Since there is essentially no storage of AA or protein, any excess AA in the plasma will be directed toward catabolism, whereby they are broken down into other molecules with chemical energy released. This then could elevate the typical postprandial ammonia load to toxic levels (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). In AA requirement studies in which only one or a few AAs are supplied in free form, this effect would be magnified, as the balance of AA available for protein synthesis would be out of balance, with the free form arriving sooner after a meal and at higher proportions than the other IDAA derived from a meal. To counter this problem, free dietary AAs are often mixed with a binder that slows their rate of intestinal absorption. Examples of feed additives that have been used to slow the rate of absorption of free AA in requirement studies include gelatin, carboxymethyl cellulose, alginate, and carrageenan. Also, feeding smaller meals more frequently may reduce the disparity of timing and levels of free AA in the body. Interestingly, researchers have used the postprandial plasma levels of AA in fish as an indication of the maximum growth response. In this model, when a diet that contains a deficient level of a specific AA is fed, the AA is almost entirely used for protein synthesis leaving relatively low levels in the plasma, but when the level of the AA exceeds the requirement, more is available than can be used for protein synthesis and the plasma levels will be elevated.

Determining a dietary requirement, that is, the lowest level giving the maximum response, is not always as simple as it may seem. A number of analytical models are employed, each with its own benefits and concerns. Among the simplest of these is the broken-line model, under which the response (growth, feed efficiency, health, etc.) is expected to be linearly and positively related to dietary nutrient input up to the break point where there is no further increase, that is, an independence of input and response, which is the dietary requirement. Graphically, an upward-sloping straight line is followed by a horizontal line (**Figure 1**). Although this model gives a definite value to the requirement and has been successfully employed in many studies, it is sometimes criticized because it assumes that there is no variability in requirement among individuals. An alternative approach has used various smooth curved models, which in many cases provide a better fit than does the broken line and include quadratic, enzyme-kinetic (**Figure 2**), and exponential models. There can be considerable difference in the requirement value obtained from application of the different models. No matter which model is selected, the reliability of the findings will depend on the quality of the work. One of the most important considerations is that there must be sufficient data to establish the maximum. In the case of the broken-line model, this requires at least two, preferably three, levels producing responses below the maximum and at least three levels above it. Other considerations to improve reliability of AA requirements include ensuring that the growth, feed efficiency, and survival of the animals in the best-performing groups are not much less than what can be expected from animals fed a complete diet (a standard diet with an established history of producing good growth with the species under test).

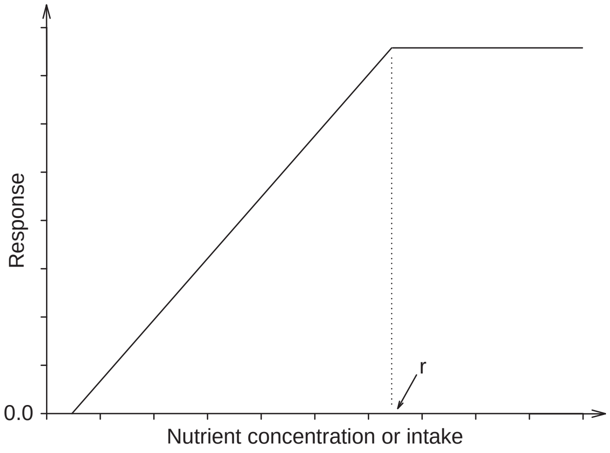


Figure 1 The relationship of physiological response to the level of a dietary nutrient, using the broken-line model. The sloped part of the line on the left represents the positive response to higher dietary nutrient levels under deficient conditions. The horizontal portion on the right indicates that there is no further change in response once the requirement has been reached. The concentration corresponding to the intersection of the two line segments is the requirement (r).

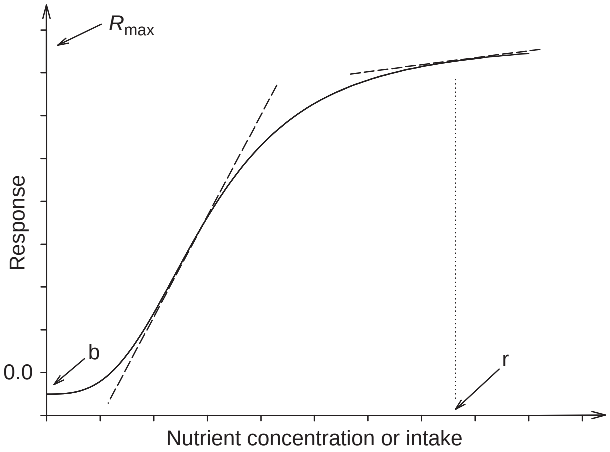


Figure 2 Relationship of physiological response to the level of a dietary nutrient, using enzyme-kinetics model. The dashed line on the left represents the maximum slope of the response curve. The dashed line on the right is the tangent of the response curve at 5% of the maximum slope and the requirement (r) is the corresponding concentration. R_{max} is the theoretical maximum response (asymptote of the curve) and b is the response at zero nutrient intake.

It is argued that it is necessary to establish the upper limit (tolerance) for requirements to ensure that the maximum has been achieved. This requires that dietary treatments be included to provide levels sufficiently high to induce a reduced response. This is not done very often. Mercer adapted an enzyme kinetics model to include a term describing a negative response to excessive dietary nutrient level – dietary inclusion levels above which the response is diminished. The double broken-line model can include a region in which the response is negatively related to dietary nutrient level.

An alternative method for estimating AA requirements is to relate the IDAA profile in muscle or whole body to that of the requirement for one AA. This method was first used in fish by Arai and co-workers and has been found to be effective at least as a first approximation, but should be followed up with more direct work (i.e., by dose–response).

Balance and Impact of Other Nutrients

In poultry some AAs, when present in a diet in high abundance, interfere with the utilization of other AA.

For example, high dietary levels of either lysine or arginine reduce growth rate unless the other AA is also increased. This effect between AA is known as antagonism. Lysine and arginine are known antagonists of each other, as are the branch-chained AA, leucine, isoleucine, and valine. The basis for this effect is at the level of intestinal absorption, where these AA groups utilize common routes of uptake which can be overwhelmed by excess levels of a specific AA.

In other instances, elevated levels of an AA will reduce the dietary need for a different AA. An example of this is the relationship of two sulfur-containing AAs: methionine and cysteine. Cysteine is produced from the IDAA methionine via a biochemical pathway that includes the DAA serine, and is thus not an IDAA. When cysteine is low in the diet, however, some of the methionine is converted to cysteine, increasing the dietary need for methionine for maximum protein synthesis (growth). If there is sufficient methionine in the diet, then the requirement for cysteine is low or nonexistent. Ensuring that there is sufficient cysteine in the diet reduces the need for dietary methionine, at least to the point where methionine is no longer converted to cysteine. Dietary cysteine is said to spare methionine and, generally, when reporting the methionine requirement for a species, the level of dietary cysteine is indicated. Frequently, the sum of methionine and cysteine requirements is reported. The IDAA phenylalanine may be converted to tyrosine and these two AAs have a similar relationship as methionine and cysteine.

Fatty Acids

Fatty acids are used both for biochemical energy and as structural components of cells. It is the structural functions of fatty acids that dictate their nutritional requirements. Not all functions are well understood, but most have to do with their role in the lipid bilayer of cell membranes and the production of certain signaling chemicals, such as prostaglandins.

Fatty acids are long chains of carbon atoms linearly strung together by single or double bonds, with one end terminating in a methyl group and the other in a carboxyl group. There are several kinds of fatty acids, defined by the chain length, the degree of unsaturation (number of double bonds), and the location of the double-bond closest to the methyl end. In standard nomenclature, the fatty acid designated as C18:4n-3 (stearidonic acid) has 18 carbon atoms in a row (C18), with four double bonds (:4), and with the last double-bond being 3 carbons from the methyl end (n-3). Saturated fatty acids (no double-bonded carbons) are readily produced from acetyl-CoA (CoA, coenzyme). Fatty acids can be elongated in pairs of carbons by enzymes called elongases, and so they exist in even chain lengths: 18, 20, and 22 carbons being the most important. The bonds between the carbons can be desaturated to form the various types of unsaturated fatty acids, provided that the appropriate desaturation enzyme (a desaturase) is expressed in the cells of the fish. Vertebrates lack the desaturases for desaturating carbons greater than position 9 from the carboxyl end (delta-9), so fatty acids of one kind (n-6 or n-3) can be elongated (e.g., C18:2n-6 to C20:4n-6), but not changed from one to the other (e.g., C18:2n-6 cannot be the basis of C18:3n-3 synthesis in vertebrates).

Determination of fatty acid requirements is similar to that for AA, but there are important differences. Obtaining bulk quantities of purified fatty acids for dose–response trials is much more expensive than it is for AA, so fatty acid requirement studies are much less common. The n-3 series of fatty acids are essential to maintain normal health and growth in aquatic species. It was thought that the sum of the 18, 20, and 22 carbon length n-3 fatty acids constituted the n-3 fatty acid requirement. It is now known, however, that the longer-chain unsaturated fatty acids, 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA), are the most important nutritionally and that the ability of fish to elongate and desaturate alpha-linolenic acid (18:3n-3) to generate these longer-chain fatty acids, while variable among species, is generally inefficient. Most commercial fish feeds are formulated with sufficient sources of EPA and DHA to meet the fatty acid requirement of fish. Cell membranes consist largely of a bilayer of phospholipids, each of which contains two fatty acids and a phosphate group. Cell membranes with high levels of EPA and DHA are more fluid and thereby functional at lower temperatures than those containing higher levels of fatty acids that are more saturated. Consistent with this is the loosely supported tendency for cold water fish to have a higher content of EPA and DHA relative to warmer water fish. The nutritional requirement for EPA and DHA is evidently higher for cold water species than for warm water ones. EPA and DHA are highly susceptible to auto-oxidation (degradation through oxidation) and diets that are especially high in these fatty acids generally are formulated with extra antioxidants. The level of vitamin E, which protects lipids from oxidation, needed by fish increases as the level of EPA and DHA increases.

Studies reported by Bell, Sargent, and others indicate that arachidonic acid 20:4n-6 plays an important role in some fish species, especially with regard to reproduction. Arachidonic acid regulates steroid production in at least some species, perhaps through conversion to a prostaglandin or thromboxane. As for DHA and EPA, arachidonic acid can be produced from shorter-chain fatty acids, notably linoleic acid 18:2n-6. It is proposed that the optimum ratio of DHA:EPA:arachidonic acid for marine fish is 10:5:1, which approximates the ratio of these fatty acids in fish eggs.

Minerals and Vitamins

Minerals have a myriad of functions in the body. Minerals cannot be manufactured from other constituents and so they must be acquired from sources external to the animal. Fish can obtain many minerals directly from their environment. Marine fish drink continuously to replace the water lost through their gills to the environment from osmotic action. To maintain a suitable osmotic internal environment, fish in saltwater actively excrete excess minerals into the intestinal lumen (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). In freshwater, the salinity is higher internally than externally and water floods into the fish through the gills. As a consequence, in freshwater, fish do not drink but excrete copious amounts of dilute urine. Clearly, the dietary intake of specific minerals that meets the nutritional needs of the fish in either seawater or freshwater will be highly modulated by the elemental concentration in the water.

Considerable levels of minerals are provided in most practical fish feeds from the principal protein sources; for example, meals made from terrestrial or aquatic animals. For this reason, and because supplemental minerals do not contribute greatly to the cost of feed, considerably less research has been done on mineral requirements than for AA. As terrestrial plant proteins are becoming more prominent in diets for fish in replacement of fishmeal, however, there is increased interest in mineral nutrition. This is especially true of phosphorus, which is an element that is required in greater amount than it can be acquired from the water. Most meat meals are good sources of phosphorous; however, plant protein products, while containing phosphorus, are poor sources, because the phosphorus is contained in phytate (the salt form of phytic acid) which is essentially unavailable to nonruminants. Phytate can also bind with divalent cations, for example, calcium, zinc, and magnesium, to render these unavailable. It is important to know the level of phytate present when considering the nutritional value of a feed because the minerals may be unavailable due to binding with phytate.

Like the other classes of nutrients, vitamins have several metabolic roles in animals, and need to be obtained through the diet. Under culture conditions, the cost of meeting the needs of the animals through supplements is generally sufficiently low so that there is relatively little research on quantifying the requirements for most species (ascorbic acid, vitamin C, is an exception to this). Requirements are determined using dose–response techniques, using growth, health, tissue levels, and enzyme activity (for those vitamins that act as coenzymes) as responses. The fat-soluble vitamins A and D have potential for accumulating in lipid stores to toxic levels. This is rare, but needs to be considered when developing feeds using alternative lipids, for example, lipids of marine origin from fish processing by-products, which may contain the fat-soluble vitamins A and D at above tolerable levels.

See also: **Integrated Function and Control of the Gut**: Gut Secretion and Digestion; Intestinal Absorption. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts. **Toxicology**: The Toxicology of Metals in Fishes.

Further Reading

Bureau DP and Cho CY (1999) *Introduction to Nutrition and Feeding of Fish.* Department of Animal and Poultry Science, University of Guelph. http://fishnutrition.uoguelph.ca/feedint.html (accessed June 2010).

Forster I (2000) Nutrient requirements. In: Stickney R (ed.) *Encyclopedia of Aquaculture*, pp. 592–600. New York, NY: Wiley.

Halver JE and Hardy RW (eds.) (2002) *Fish Nutrition*, 3rd edn. San Diego, CA: Elsevier.

Hernadez-Llamas A (2009) Conventional and alternative dose–response models to estimate nutrient requirements of aquaculture species. *Aquaculture* 292: 207–213.

Li P, Mai K, and Trushenski J (2009) New developments in fish amino acid nutrition: Towards functional and environmentally oriented aquafeeds. *Amino Acids* 37: 43–53.

National Research Council (2010) *Nutrient Requirements of Fish and Shrimp*. Washington, DC: National Academy Press.

Webster CD and Lim CE (eds.) (2002) *Nutrient Requirements and Feeding of Finfish for Aquaculture*. New York, NY: CABI Publishing.

ENERGETICS

Energy Utilization in Growth

Contents
Energetics of Growth
Growth: Environmental Effects

Energetics of Growth

RJ Wootton, Aberystwyth University, Aberystwyth, Wales, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Conclusion
Further Reading

Glossary

Anabolism Synthesis of materials via metabolic processes; a series of chemical reactions that builds larger molecules from smaller components, usually requiring energy in the process.

Bioenergetics Quantitative analysis of the energetic processes of living organisms.

Catabolism Metabolic processes that result in the release of energy from organic molecules.

Darwinian fitness Contribution of an individual to the gene pool of the next generation, often estimated as lifetime reproductive success (LRS).

Joule (J) Energy exerted by a force of 1 N acting to move an object through a distance of 1 m. It is the SI unit of energy.

Ontogeny Changes in the phenotype of an individual during its life span. Ontogeny is the outcome of a developmental program, encoded in an organism's genes, as well as interactions between the genotype of the individual and the environments it encounters, together with any maternal effects mediated through the egg itself.

Parameter A numerical constant in a quantitative relationship.

Somatic Body of an individual excluding the gonads.

Watt Rate of doing work, a measure of power as J s^{-1}.

Introduction

Growth, like all other physiological processes, is constrained by the laws of physics and chemistry. The laws of thermodynamics are of particular importance in the study of the bioenergetics of growth, forming the basis for the quantitative study of growth. The laws impose the constraints that during growth, energy can be neither created nor destroyed and that as useful work is done during the synthesis of materials required for growth, energy will be dissipated as heat. In studies of natural populations of fish or in aquaculture, growth is usually measured as changes in mass or length. However, growth can also be estimated as the change in energy content of fish, where energy content is measured by the heat generated when fish mass is burnt in an atmosphere of oxygen (bomb calorimetry), measured as joules (J), with the energy concentration $[E]$ expressed as J g^{-1}. The rate at which a fish uses energy can be expressed as power, measured in watts (J s^{-1}). Once growth is measured in energy units, energy conservation requires that growth = energy input − energy output, where energy input is the energy content of the food consumed and energy output is the energy lost in the form of fecal waste, excretory products (especially ammonia), maintenance metabolism, and as useful work is done. Growth is the energy retained in the body of the fish in the form of the energy of the chemical bonds in the organic molecules that have accumulated in the body. The relationship, growth = energy input − energy output, is a truism and can be applied, in principle, to any organism. The challenge is how to develop this principle in

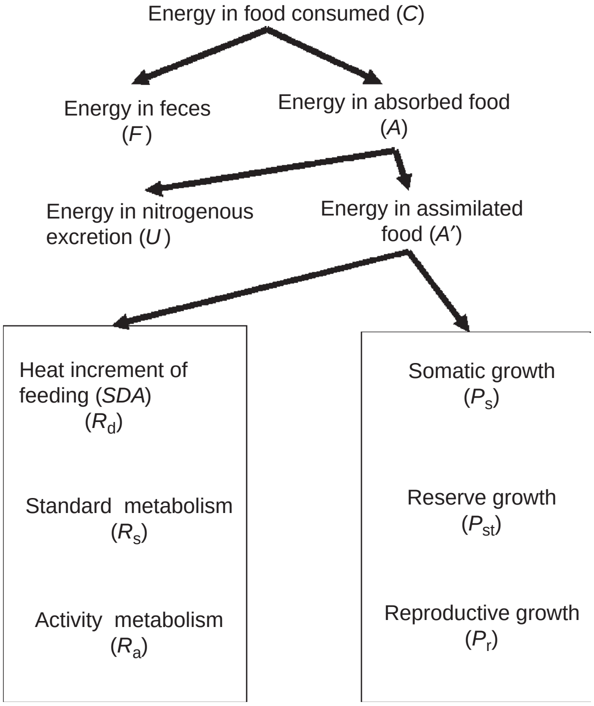

Energy in food consumed (*C*)
Energy in feces (*F*)
Energy in absorbed food (*A*)
Energy in nitrogenous excretion (*U*)
Energy in assimilated food (*A*′)
Heat increment of feeding (*SDA*) (R_d)
Standard metabolism (R_s)
Activity metabolism (R_a)
Somatic growth (P_s)
Reserve growth (P_{st})
Reproductive growth (P_r)

Figure 1 Pattern of energy allocation in individual fish described by Davis and Warren model.

a way that allows quantitative predictions to be made about the pattern of growth in specific circumstances for the identified species or groups of species of fish.

Growth has three components (**Figure 1**). The first is structural growth that builds the body of the fish, with its skeleton, muscles, nerve cells, and other tissues and organs, which allow the fish to function in its environment. In fish, the energy costs of this growth are predominantly the energy costs of the synthesis of proteins and structural lipids together with any costs of organizing the synthesized material into functioning components. The second component is reserve, or storage growth. This component provides a reservoir of energy that can be mobilized when the energy input in the form of food is insufficient to meet the individual's needs, but replenished when food availability is high. In fish, lipids generally form the main storage material, but some fish (e.g., the lungfish *Protopterus*) specialize in protein storage. Carbohydrates usually play only a minor role. The third component of growth is reproduction, represented by the germ cells, sperm, and eggs, which are eventually released during reproduction. For eggs, the main component is the yolk, made up of lipoprotein, while sperm are essentially packets of DNA wrapped in a lipid membrane.

Energy Budget

The three components of growth can be related to other aspects of the bioenergetics of the individual fish in the framework of an energy budget that itemizes the intake and output, the latter including growth over a defined time period. The budget must balance, because energy can neither be created nor destroyed (**see also Energetics**: General Energy Metabolism and **Energetic Models**: Bioenergetics in Aquaculture Settings). The basic equation is

$$C = F + U + R + P$$

where C is the food consumed, F the fecal waste, U the excretory losses produced by the metabolism of nitrogenous compounds, particularly proteins, R the heat lost during metabolism, and P is the total growth. All the items are measured in energy units. The heat-loss component, R, can be broken down into subcomponents:

$$R = R_S + R_D + R_A$$

where R_S is the standard metabolism, which is approximated as the heat expenditure of a resting, unfed individual; R_D the heat expenditure associated with the processing of the food consumed (sometimes called specific dynamic action, SDA); and R_A the additional heat expenditure of an active fish (**see also Energetics**: General Energy Metabolism). The growth component, P, can be subdivided as

$$P = P_S + P_{ST} + P_R$$

where P_S is the energy content of the structural growth, P_{ST} the energy content of the storage growth, and P_R the energy content of reproductive growth.

Conceptually, it is also useful to define absorbed energy (A) as $A = C - F$; and assimilated energy (A') as $A' = A - U$ or $A' = C - (F + U)$. A' is essentially the energy available to do useful work and to accumulate as growth.

Growth Efficiencies

The efficiency of a system is defined as output divided by input, where output and input are explicitly defined. Thus, in the context of the energy budget of an individual fish, the efficiency of growth can be defined. Three growth efficiencies are defined, depending on the input. For total growth, P, the efficiencies (expressed as percentages) are: $K_1 = 100PC^{-1}$ in which growth is related to the food consumed; $K_2 = 100PA'^{-1}$, where growth is related to assimilated energy; $K_3 = 100P(C - C_{\mathrm{MAIN}})^{-1}$, where growth is related to the energy of food consumed that is in excess of the maintenance ration (C_{MAIN}). C_{MAIN} is the level of food consumption when the individual fish is neither increasing nor decreasing in energy content, so growth and growth efficiency are zero.

Typically, for machines, including living organisms, it is not possible to maximize both the efficiency of a process and the maximum rate of that process. This suggests that a growing fish cannot maximize its growth rate and its growth efficiency simultaneously.

Energy Allocation and Its Fitness Consequences

An individual is faced with the problem of how the energy obtained by its feeding activities should be allocated among the components of the energy budget (**Figure 2**). From moment to moment, the fish must solve the problem of what pattern of energy allocation will maximize its Darwinian fitness, which here can be defined as lifetime reproductive success (LRS). The allocation must be made in the context of limitations of the time available for energy acquisition, the need to shelter from predators or adverse physical conditions, the intensity of competition for food, and eventually the need to devote time to achieve reproductive success (**see also Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology and Energetics of Foraging Decisions and Prey Handling). These limitations will mean that there are trade-offs. Energy allocated to maintenance, including expenditure on repair and defense against pathogens and parasites, will tend to increase the fitness component of survival. However, that energy is not available to be allocated to growth or reproduction. Energy allocated to somatic growth is not available for allocation to storage or reproductive growth. However, somatic growth means that the size of the fish increases, which also tends to increase the fitness component of survival. In addition, because fecundity in fish generally increases with fish size, allocation to somatic growth tends to increase future fecundity at the cost of allocation to present reproduction. Rapid somatic growth can also reduce the age at which the fish becomes sexually mature, thus decreasing the risk that it will die before reproducing. However, rapid growth can also diminish swimming performance. Allocation to storage growth has two possible advantages. It may allow the fish to survive adverse periods when feeding is poor, when the fish can mobilize its stored energy. Second, it may allow the fish to reproduce at times or in places that are advantageous to the progeny, but disadvantageous to the parents. Given these trade-offs, the optimal allocation of energy will change during the ontogeny of the individual fish and is dependent upon the abiotic and biotic environments. The pattern of energy allocation over the ontogeny of the fish defines the life-history pattern of that fish.

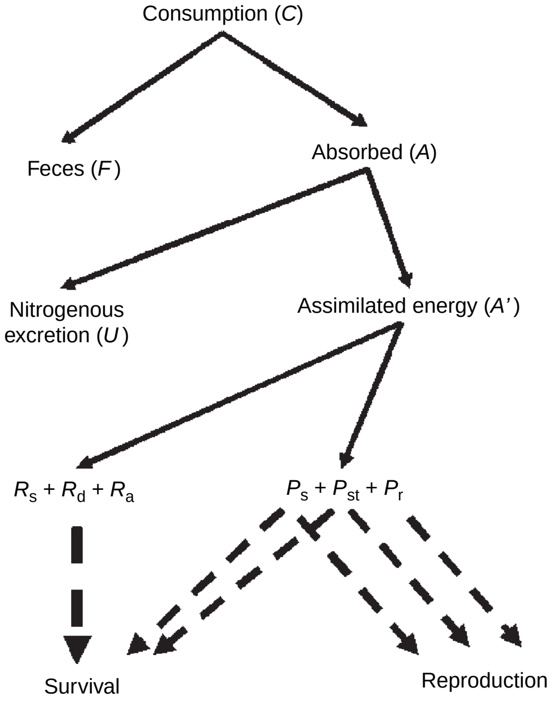


Figure 2 Effect of components of energy budget on components of fitness, that is, survival and reproduction, of an individual fish, indicated by broken lines.

Growth Models of Fish Based on Bioenergetics

von Bertalanffy model

In studies of fish biology and fisheries, the von Bertalanffy model has been probably the most influential growth model based on bioenergetic principles (**Figure 3**). Its development is associated with the names of Pütter and von Bertalanffy, and it was proposed as a general model of animal growth. It argues that growth rate is the difference between the rate of synthesis (anabolism) and the rate of breakdown (catabolism) of proteins, lipids, and

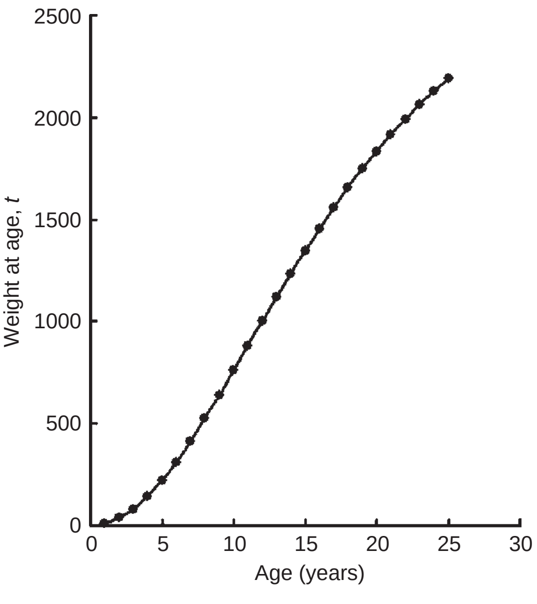


Figure 3 Example of von Bertalanffy growth model based on parameters describing growth of North Sea plaice (*Pleuronectes platessa*).

carbohydrates, with the anabolic and catabolic rates being related to body size. It then assumes that the rate of anabolism is determined by the surface area of the individual, but the rate of catabolism is directly proportional to body mass. Thus, growth rate (dW/dt) is expressed as $dW/dt = hW^{2/3} - kW$, where W is the body mass, and h and k the coefficients of anabolism and catabolism, respectively. A further assumption is that as the individual grows, it does not change in shape, that is, growth is isometric. That is, mass, W, and body length, l, have the relationship: $W = al^3$, where a and b are parameters. From these assumptions, von Bertalanffy growth equations are derived. For length, the model is $l_t = l_\infty\ [1 - e^{-k(t - to)}]$, and for mass, the model is $W_t = W_\infty\ [1 - e^{-k(t - to)}]^3$, where l_t is the length at age t, l_∞ the asymptotic length, W_∞ the asymptotic mass, e the base of natural logarithms, k a constant that defines the rate at which the growth curve approaches the asymptote, and t_o a constant that determines the hypothetical age at which the size of the fish is zero. The three constants, l_∞ (or W_∞), k, and t_o, determine the shape of the relationship between size and age, that is, the shape of the growth curve. Although based on physiological principles, the von Bertalanffy model has been used to fit curves to plots of size against age. In this role, it played a part in the derivation of the important Beverton and Holt model used in fisheries management.

A surprising feature of the von Bertalanffy model is that it does not include a term describing the rate of food intake. Growth cannot take place without an input of food that provides the required materials and energy; so, for a growth model to be predictive as well as descriptive, it has to include a term describing the rate of food consumption. This has led to a re-definition of the von Bertalanffy model in which the anabolic term is replaced by a term describing the rate of energy assimilation: ($j\ W_t^d$), where j and d are parameters. The rate of food consumption, C, is given by $C = (j\ W_t^d)a^{-1}$, where a is the assimilation efficiency ($A'C^{-1}$).

If $d = 2/3$, then the von Bertafanffy model is now expressed in terms of food assimilation and catabolism. Simulation studies indicate that the estimates of food consumption using this model are highly sensitive to the value of the parameter d.

Winberg model

Well before the modifications to the von Bertalanffy model that replaced the anabolic term by an assimilation term, Winberg introduced a balanced energy budget to describe the relationship among food consumption, growth, and metabolism. He identified input as the energy content of food consumed (C) and the outputs as growth (P) and metabolic expenditure (R). From a survey of the data then available, he suggested that assimilated energy represented 70% of the consumed energy, that is, $0.7C$. The energy budget was then expressed as $P = 0.7C - R$. Winberg argued that for fish in natural populations, R could be approximated as twice the standard metabolism measured under laboratory conditions; thus, $P = 0.7C - 2R_S$. In early studies of the bioenergetics of fish, this simple formulation provided a useful approximation of the magnitudes of growth and food consumption in natural populations. An early experimental study of the bioenergetics of the European perch, *Perca fluviatilis*, suggested that the Winberg equation could give a reasonable estimate of the maintenance metabolic rate, that is, when growth is zero, and of the metabolic rate of a fish on an unrestricted ration.

Davis and Warren model

A further step in the development of a bioenergetics model of growth was provided by the Davis and Warren model. This model is a flow diagram of the partitioning of the energy income among the energy outputs, the latter including any growth (**Figure 1**). The model thus provided a framework in which the pattern of energy allocation, in principle, could be described quantitatively. Each term in the energy budget equation for growth $P = C - (F + U) - R$ (see earlier) can be measured experimentally under defined conditions of individual body mass, water temperature, food availability, and other relevant environmental conditions. If four of the five terms in the energy budget equation are estimated empirically under defined conditions, the fifth can be obtained by calculation. For example, if C, F, U, and R are measured, P can be calculated, providing a prediction of growth under those conditions. That prediction can then be tested experimentally, by estimating the growth (in energy units) of individuals raised under the defined conditions.

The energy budget under defined conditions can be described in terms of the percentage allocation. The allocation by the grass carp, *Ctenopharyngodon idella*, when the fish were fed duckweed was summarized as $100C = 35F + 51R + 9P$, but, when fed tubificid worms, $100C = 9F + 8U + 61R + 22P$. This illustrates the effect of the better-quality animal diet on growth. A comparison of carnivorous with herbivorous species in terms of an energy budget suggested values of $100C = 27(F + U) + 44R + 27P$ for carnivores and $100C = 43\ (F + U) + 37R + 20P$ for herbivores.

Wisconsin growth model

The Davis and Warren model lies at the heart of the Wisconsin bioenergetics model that has found the most use as a predictive tool in fisheries biology (**see also Energetic Models**: Bioenergetics in Ecosystems). The model was made available as a computer package with a comprehensive manual, including data for a range of species on the parameters required to estimate the components of the energy budget. The model uses the energy budget equation to predict the growth of a fish of a given initial mass over a selected time period, typically 24 h. The mass of the fish at the end of the 24 h is then used as the starting point for the prediction of growth over the next 24 h and so on for as many days as required. Relevant factors such as temperature can also be changed on a day-by-day basis. The computer package allows the growth in body size of fish in different age classes (cohorts) to be simulated and so the bioenergetics of the individual fish can be extended to the bioenergetics of the population of fish.

In the Wisconsin model, growth energy, P, is converted to the increase in mass, ΔW, by dividing P by the energy density of the fish $[E]$, so $\Delta W = P\,[E]^{-1}$. A flavor of the model can be given by describing some of the relationships used to quantify components of the Davis and Warren energy budget. Food consumption, C, is estimated as $C = pC_{\mathrm{MAX}}\,f(T)$, where p is the proportion of C_{MAX} consumed, $f(T)$ is a function that describes the effect of temperature on food consumption, and the maximum rate of consumption $C_{\mathrm{MAX}} = a\,W_t^b$, where a and b are parameters. C is converted into energy units for use in the mass-balance equation by multiplying by the energy density of the food $[E_{\mathrm{FOOD}}]$. Metabolic expenditure, R, is estimated as $R = (\mathrm{ACT})R_{\mathrm{S}}\,g(T) + R_{\mathrm{D}}$, where ACT is a multiplier to define the level of activity and $g(T)$ is a function describing the effect of temperature on metabolism. If $\mathrm{ACT} = 2$, then active metabolic rate is twice the resting rate. Each of the relationships used has to be defined for the species of interest.

The Wisconsin model has been used to guide management strategies, especially for the Great Lakes in North America. A list of its uses includes the following: (1) the evaluation of the factors that constrain fish growth; (2) the evaluation of the impact of fish predators on prey populations; (3) quantification of the dynamics of bioaccumulation of contaminants such as polychlorinated biphenyls (PCBs); (4) prediction of the waste loads produced by an aquaculture system; and (5) enhancement of an understanding of the role that fish play in ecological processes in aquatic systems. The Wisconsin model is also incorporated as a subcomponent of individual-based models of fish life history and population dynamics.

The Wisconsin model also illustrates some of the problems in using bioenergetics models to predict growth in natural populations. The first problem is obtaining a realistic estimate of the level of activity of fish in a natural population so that the metabolic costs of activity can be estimated. A second problem is estimating the rate of food consumption in natural populations in the context of the availability of suitable food items (**see also Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology). This means that the Wisconsin model is often used to estimate food consumption on the basis of observed growth, rather than growth from observed consumption. A third problem is that the Warren and Davis and its successor, the Wisconsin model, do not include an explicit model of how energy should be allocated between the different outputs to maximize fitness. Although the structure of the Wisconsin model is general for any fish species, to apply it to a specific population of a species requires knowledge of a high number of parameters to define the relationships among the components of the energy budget and body mass, temperature, and other relevant factors. This means that care must be taken to examine the sensitivity of the predicted growth to the values of the parameters used in the model.

Dynamic energy budget model

A comprehensive bioenergetics model has been developed by the Dutch biologist, SALM Kooijman. Although this model is, in principle, applicable to all living organisms, it has been adapted to simulate the growth of fish. The crucial feature of this model is that it does explicitly include a rule for the allocation of energy among outputs. It assumes that assimilation rate is proportional to body surface area, that is, to $V^{2/3}$, where V is the structural volume of the individual, while maintenance rate is proportional to body volume, V (these proportionalities recall those used in the von Bertalanffy model). The assimilated products then enter a reserve pool, from where they are allocated to maintenance, somatic growth, and reproduction. A fixed fraction of the reserve, K, is allocated to maintenance and growth, while the remaining fraction, $1 - K$, is allocated to development and reproduction. If the rate of assimilation is low, priority is given to maintenance, and if the rate of energy utilization from the reserves cannot meet the costs of somatic maintenance, the individual eventually dies.

The dynamic energy budget (DEB) model (**Figure 4**) uses two state variables to describe an individual fish: the volume of the somatic body, V, and energy density of the reserves $[E]$, which describes the quantity of reserves, E, per unit of structural body volume. The DEB model clearly distinguishes between structural body and reserves. With these assumptions, the DEB generates a growth equation, which is mathematically, if not conceptually, equivalent to the von Bertalanffy model. Extensions of the basic model apply to embryos, which do not feed or reproduce, and juveniles, which feed, but do not reproduce. In some extensions of the model, a further allocation rule is proposed, which governs the allocation between somatic growth and stored reserves. A disadvantage of the DEB model is that the parameters used to develop the model are difficult to estimate.

In a simplified form, the DEB model has provided some insight into the nature of compensatory growth in salmonid fishes, and the simulation of the growth of Atlantic salmon, *Salmo salar*, in a Scottish stream. Although the model is

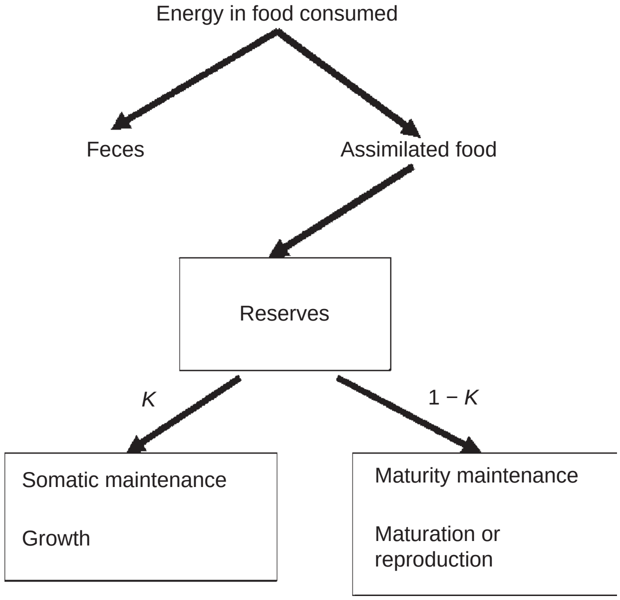


Figure 4 Dynamic energy budget (DEB) model of energy allocation in individual fish, showing *K*-rule.

conceptually more realistic than the Wisconsin model, because it does incorporate an allocation rule, it has not matched the use of the Wisconsin model in practical studies of fish growth.

Conclusion

As bioenergetic models of growth are based firmly on the laws of thermodynamics, they offer the prospect of providing realistic simulations of growth under defined environmental conditions. Even under conditions of relatively stable long-term environmental conditions, the ability to predict the growth trajectories of fish in natural populations or in aquaculture is valuable for conservation and resource management. With the predicted long-term changes in global temperatures and the consequences for the climate, the ability to predict the effects of the changes on the growth rate and related traits of fish will be essential to devise strategies that will reduce, as far as is possible, the impact of these changes. The models also provide a framework within which physiological studies on the neuroendrocrine control of energy allocation in the individual fish can be conducted. There continues to be the need to verify and validate the bioenergetic model used, by carefully designed laboratory and field studies that result in improvements to the structures of the models and the estimates of the parameters used in the models.

See also: **Energetic Models**: Bioenergetics in Aquaculture Settings; Bioenergetics in Ecosystems. **Energetics**: General Energy Metabolism. **Food Acquisition and Digestion**: Energetics of Foraging Decisions and Prey Handling; Energetics of Prey Capture: From Foraging Theory to Functional Morphology.

Further Reading

Brett JR and Groves TD (1979) Physiological energetics. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology*, 8, pp. 279–352. London: Academic Press.
Chipps SR and Wahl DH (2008) Bioenergetic modelling in the 21st century: Reviewing new insights and revisiting old constraints. *Transactions of the American Fisheries Society* 137: 298–313.
Hanson PC, Johnson TB, Schindler DE, and Kitchell JF (1997) *Fish Bioenergetics 3.0. WISCU-T-97-001*. Madison, WI: University of Wisconsin Sea Grant Institute.
Hartman KJ and Kitchell JF (2008) Bioenergetics modelling: Progress since 1992 symposium. *Transactions of the American Fisheries Society* 137: 216–223.
Jobling M (1994) *Fish Bioenergetics*. London: Chapman and Hall.
Kooijman SALM (2000) *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge: Cambridge University Press.
Tytler P and Calow P (1985) *Fish Energetics New Perspectives*. London: Croom Helm.
Van der Meer J (2006) An introduction to dynamic energy budget (DEB) models with special emphasis on parameter estimation. *Journal of Sea Research* 56: 85–102.
Wootton RJ (1998) *Ecology of Teleost Fishes*, 2nd edn. London: Chapman and Hall.

Relevant Websites

http://limnology.wisc.edu – Wisconsin Model.

Growth: Environmental Effects

RJ Wootton, Aberystwyth University, Aberystwyth, Wales, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Abiotic Factors
Biotic Factors
Conclusion
Further Reading

Glossary

Abiotic The chemical and physical (nonbiological, physical) variables of the environment (e.g., temperature).

Ad libitum Amount of food consumed when food is always present for individual fish.

Anabolism Synthesis of materials via metabolic processes; a series of chemical reactions that builds larger molecules from smaller components, usually requiring energy in the process.

Biotic Environmental factors related to living organisms. The biological variables of the environment, e.g., predation and competition.

Catabolism Metabolic processes that result in the release of energy from organic molecules.

Conspecifics Other individuals of the same species.

Dominance Behavioral ability of an individual to sequester resources in the presence of other individuals.

Ectothermic An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Heterospecifics Individuals from different species.

Ontogeny Changes in the phenotype of an individual during its life span. Ontogeny is the outcome of a developmental program, encoded in an organism's genes, as well as interactions between the genotype of the individual and the environments it encounters, together with any maternal effects mediated through the egg itself.

Photoperiod Durations of day and night during the 24-h diel cycle.

Ram ventilation A passive method of driving oxygen-containing water over the gills. The fish swims with its mouth open, so water flows in through the mouth and out past the gills.

Thermocline Zone of high rate of temperature change that separates a shallow, less-dense, warm water layer (epilimnion) from a deeper, more-dense colder-water layer (hypolimnion) in lakes, seas, and estuaries.

Introduction

Growth is central to the ecology of fishes because many factors relevant to fitness are size dependent, including prey size, risk of predation, survival during harsh conditions, and fecundity of females. In terms of the energy content of fish, growth is the difference between energy obtained in the food and the outputs of energy in the form of fecal and excretory losses and the heat generated by metabolic processes (**see also Energy Utilization in Growth**: Energetics of Growth).

The growth that an individual fish achieves depends on three constraints. The first is the genetic constitution of the individual. Growth responds to artificial selection indicating that there is underlying heritable, genetic variation for growth in fish populations. Second, the abiotic environment experienced by the fish will set constraints on growth. Third, the biotic environment will determine the extent of the growth potential that the fish can achieve as defined by its genotype and the abiotic environment experienced.

This article focuses on the effects of abiotic and biotic factors on growth. In analyzing the effect of abiotic and biotic factors on growth, a further factor has to be included. If the quality and quantity of food available does not change significantly, the growth rate, expressed as specific growth rate, g, decreases as the size of the fish increases. Specific growth rate is a measure of the size-specific growth rate. In mathematical terms, it is $g = \mathrm{d}W/W\ \mathrm{d}t$, where W is size (mass, length, or total energy content).

Abiotic Factors

Temperature

Metabolism, both anabolic and catabolic, is a set of chemical reactions catalyzed by enzymes; consequently, all physiological rates depend on temperature (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). Temperature controls the maximum rate at which growth can occur when food availability or other factors are not restricting growth rate. As the majority of fish are ectothermic (for exceptions, **see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks), their body temperature will essentially follow the temperature of the surrounding water. Experimental studies on the effect of temperature on growth rate show that three temperatures can be defined. There is a minimum (T_L) and a maximum temperature (T_U) at which the growth rate is zero. Between the minimum and maximum temperatures for growth, there is a temperature at which growth rate is maximized, identified as the optimum temperature for growth (T_{OPT}). This means that the relationship between growth rate and temperature is an asymmetric bell shape (**Figure 1**). This pattern largely reflects the relationship between *ad libitum* food consumption and temperature. For fish supplied with maximum rations, T_L, T_{OPT}, and T_U for growth in the European trout, *Salmo trutta*, a cold-water species, are approximately 5, 14, and 23 °C, respectively. Experimentally determined values will also depend on the population from which the experimental fish are taken and the details of the experimental protocols. If the ration size is restricted, the optimum temperature for growth decreases (**Figure 1**), reflecting the lower metabolic costs at low temperatures (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism).

Most laboratory studies of the relationship between growth and temperature use constant temperatures. However, fish in natural populations may experience cyclical changes in temperature, either because of day–night cycles in temperature or because voluntary movements of the fish take them into different thermal regimes. Experimental studies on the effect of cyclical temperature regimes on growth have not provided consistent results, probably because of differences in the experimental designs. The vertical migrations of juvenile sockeye salmon, *Oncorhynchus nerka,* through the thermocline, in some lakes in summer, may reflect the advantage of feeding in the warm surface waters, but metabolizing the results of feeding in the cooler, deeper waters.

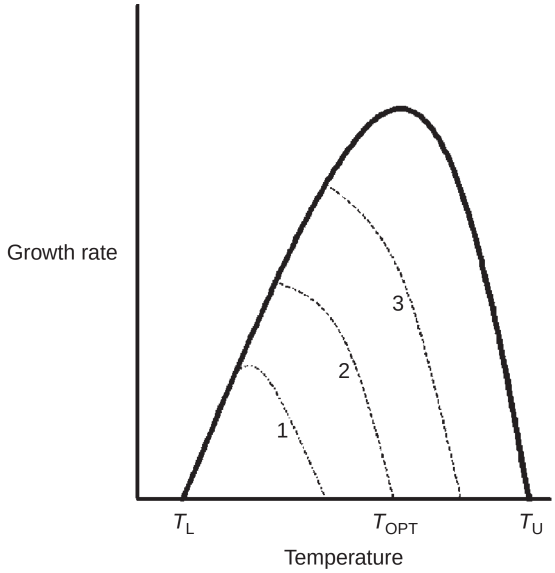


Figure 1 Schematic diagram of the relationship between growth rate and temperature, identifying the minimum, T_L; optimum, T_{OPT}; and maximum, T_U, temperatures for growth. Unbroken line shows relationship when fish fed *ad libitum*, broken lines show the relationship at restricted rations levels with level 1 < level 2 < level 3.

The relationship between temperature and growth rate for a given species of fish may vary with latitude. Experimental studies on the Atlantic silverside, *Menidia menidia*, which is found along the Atlantic seaboard of North America, demonstrated a pattern of counter-gradient selection. At a given temperature and with abundant food, silversides from northern populations grew faster than fish from southern populations. This was true even at high temperatures, even though the temperatures in which the northern population naturally live are lower throughout the year. This counter-gradient variation means that although silversides in the northern populations have a shorter growing season than that in the south, the higher growth potential of the northern fish means they can reach a size similar to that of the southern fish by the end of the growing season.

Oxygen

Oxygen is required for the aerobic metabolic processes that make energy available to do useful work (**see also Energetics**: General Energy Metabolism). However, oxygen has a low solubility in water, and this solubility decreases as water temperature increases. The oxygen content of water can be expressed either as the absolute concentration in mg L^{-1} or as a percent of saturation. Due to the effect of temperature on the solubility of oxygen, 100% saturation at a high temperature represents a relatively low absolute concentration (**see also Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P_{50}). Thus, at 10 °C and 100% air saturation (at sea level), dissolved oxygen is at a concentration of 11.3 mg L^{-1}, but at 25 °C, the oxygen concentration at 100% air saturation is at 8.2 mg L^{-1}. A further complication is that the oxygen concentration also decreases with an increase in water salinity.

There is the possibility that the rate of oxygen uptake by the fish is too low to support the rate of growth that would be permitted by the prevailing temperature. Thus, in some circumstances, oxygen can act as a limiting factor on growth and observed growth is lower than that predicted for the given temperature and available food (**Figure 2**). The

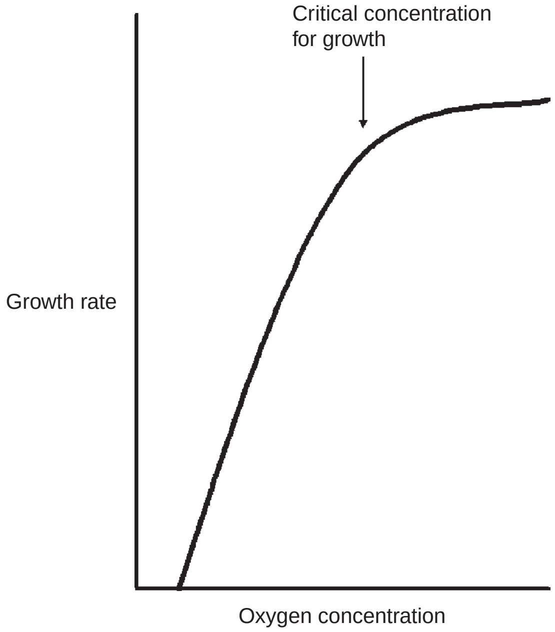


Figure 2 Schematic diagram of the relationship between growth rate and oxygen concentration, showing a representative critical oxygen concentration for growth.

environmental conditions in which oxygen may act as a limiting factor on growth are unusually high water temperatures, water in which there is a high demand for dissolved oxygen by decaying organic matter (biological oxygen demand, BOD), high densities of fish in a limited volume of water, and low rates of oxygen diffusion from air to water because of a barrier formed by ice or snow or stratification.

Experimental studies suggest that across a range of species, the critical threshold level of air saturation at which growth starts to be inhibited by hypoxia (i.e., low oxygen) is 50–70%. In the freshwater tropical species *Poecilia reticulata*, the critical concentration was about 3.0 mg L^{-1} and at 30 °C, 100% saturation would be about 7.6 mg L^{-1}. Most laboratory studies of the relationship between growth and oxygen level use a constant oxygen concentration. In natural environments, fish may experience regular or stochastic changes in oxygen concentrations such as those associated with day–night cycles in oxygen generated photosynthetically, or through water mixing induced by winds and storms.

Salinity

Fish have to do metabolic work to maintain a homeostasis of ionic concentrations in their body fluids. For those species that live the whole of their lives either in the sea water or in freshwater, this metabolic work will be consistent. However, some fish species move between waters of different salinities. Many salmonid species, for example, are anadromous, moving from freshwater, where they spend an early juvenile phase, into the seawater, where they undergo growth to sexual maturity. As a consequence, energy that could have appeared as growth (**see also Energy Utilization in Growth**: Energetics of Growth) is expended on ionic homeostasis (**Figure 3**). Salinity acts as a masking factor for growth. The costs of ionic homeostasis will change as the fish move between salinities. The juveniles of some species that spawn in the sea, such as the European sea bass, *Dicentrachus labrax,* move into estuaries, perhaps because of the growth advantage accrued by living at a reduced salinity. For a range of euryhaline species ($N = 23$), the best growth was achieved at salinities ranging from 0 to 35 ppt, but with a median value of 15 ppt. A study of growth in the early juvenile, glass-eel stage of the American eel, *Anguilla rostrata*, found that they grew faster in brackish water than freshwater, but that growth rates differed depending on the sites in eastern Canada from which the eels had been collected. This may be an example of an interaction between an abiotic factor and the genetic capacity for growth in eel populations.

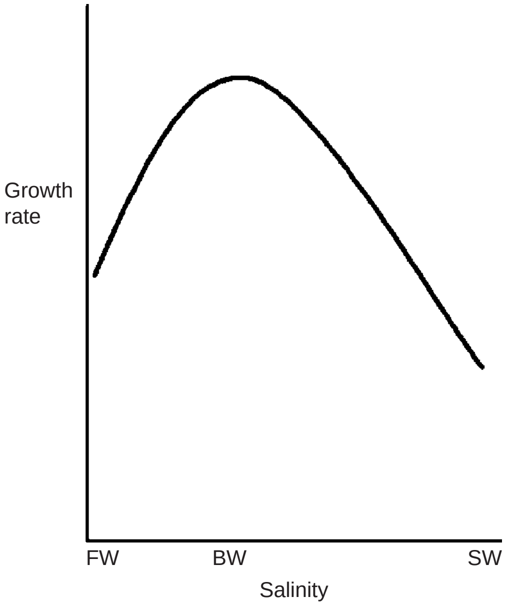


Figure 3 Schematic diagram of the relationship between growth rate and salinity for a euryhaline species. FW, freshwater; BW, brackish water; SW, seawater.

Current Speed

The speed of water flow potentially has opposing effects on growth. First, the energy the fish spends in swimming against a current will not be available to invest in growth. Second, the current flow may allow the fish to use, at least partly, ram ventilation for respiration, rather than the energetically more costly method of pumping the dense and viscous water over the gills. In some salmonids, including European trout and Arctic charr, *Salvelinus alpinus*, fish grew faster when forced to swim at speeds of about 1.5 body lengths s^{-1}. This is a relatively slow speed for salmonids. However, some species of fish showed no effect or even reduced growth if subjected to exercise, including the goldfish (*Carassius auratus*). A preliminary study detected no effect of speed of swimming over the range 0–1.0 body lengths s^{-1} in juvenile cod, *Gadus morhua*, a marine species.

Photoperiod

The effect of the diel cycle of light and dark on the growth of fish has, potentially, two components. For species that depend primarily on vision to detect their food, a longer period of light during the cycle will provide more time in which to feed. Other things being equal, this should result in faster growth. A second effect may be mediated through the control systems of the fish, due to the effect of photoperiod on the neuroendocrine system – as a consequence of light reception at the retina of the eye, the pineal gland, or perhaps other extra-retinal light receptors. In many species, these effects of photoperiod on the neuroendrocrine system control important processes in the life cycle, most notably sexual maturation. In salmonids, photoperiod is important in the control of smoltification, the physiological processes that prepare the juvenile salmonid for life in the sea. Experimental studies and studies using aquaculture systems demonstrate an increase in growth rates in some species, including Atlantic salmon, *S. salar*, subjected to long day lengths or even continuous light. Three possible mechanisms may lead to higher growth: the increased time for feeding; neuroendocrine changes that stimulate feeding; and neuroendocrine changes that direct resources into growth rather than other activities. Most of the research on the effect of photoperiodic regime on growth has focused on the light regimes that increase growth rates under aquaculture conditions and less has been directed at teasing apart the mechanisms by which higher growth rates are achieved.

Other Abiotic Factors

Although temperature, oxygen, salinity, and photoperiod are likely to be the most important factors influencing growth rates, in some contexts, other abiotic factors may become important. At high fish densities and low water-filtration/replacement rates, the ammonia/ammonium excreted by the fish can reach levels, in the water, that inhibit growth. Growth can also be inhibited at low pH levels or high [CO_2] such as those observed in areas affected by acid rain or decomposition of organic molecules.

Interactions of Abiotic Factors

Although experimental studies on the effects of abiotic factors on fish growth tend to study only one or two factors, under natural conditions, fish experience many factors simultaneously, with some of those factors showing short- and long-term variations. The factors also interact (e.g., the effects of temperature and salinity on oxygen concentration), while some are confounded (e.g., day length and temperature in temperate and boreal areas). Thus, while experimental studies are essential for an understanding of the effects of abiotic factors on growth, care needs to be taken in extrapolating experimental results to natural populations.

Biotic Factors

The abiotic factors provide the framework, which defines the maximum rate at which an individual fish can grow. Biotic factors determine what proportion of that maximum can be realized.

Food

Food is the biotic factor that controls the rate of growth (**see also Energy Utilization in Growth**: Energetics of Growth). If a fish cannot obtain food, it will lose mass and eventually starve, although fish can often survive long periods without food. Both the quantity and the quality of food available control the growth rate.

Food Quantity

Experimental studies show that the relationship between the growth rate and the quantity of food consumed is an asymptotic, curvilinear relationship (**Figure 4**). This relationship is defined by four levels of consumption. The growth rate of starved fish, that is, the rate at which the fish is losing mass, is defined by the value at which the curve intercepts the *y*-axis. The maintenance ration, C_{MAIN}, is defined by where the curve intercepts the *x*-axis. At this ration, there is no growth; the fish maintains a constant size. The optimum ration, C_{OPT}, is the ration at which the conversion of food into fish flesh is most efficient. Finally, C_{MAX} is the maximum ration the fish will consume under the prevailing abiotic conditions. With an increase in temperature, the loss of mass with starvation and C_{MAIN} increase, reflecting the effect of temperature on the rate of metabolism. Initially, C_{OPT} and C_{MAX} also increase, but at higher temperatures, beyond the optimum temperature for growth, they decline. The relationship also shows that a fish cannot maximize its growth rate and its growth efficiency simultaneously. Detailed, quantitative descriptions of

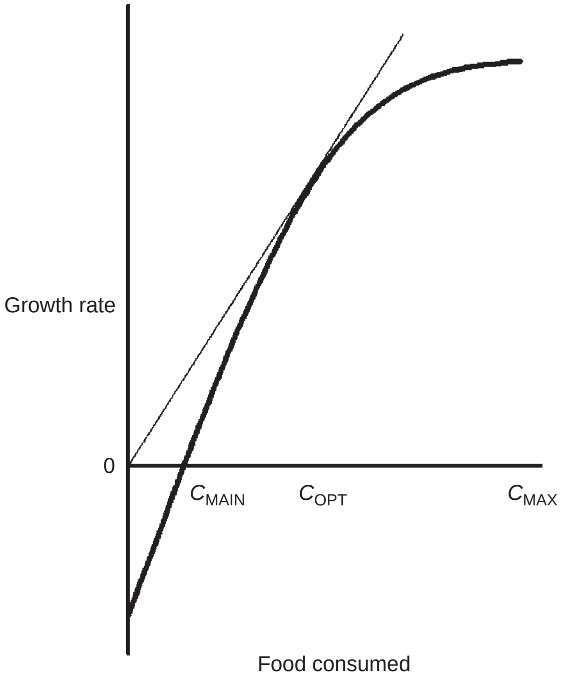


Figure 4 Schematic diagram of the relationship between growth rate and food consumption, identifying the maintenance ration, at which growth is zero, C_{MAIN}; ration at which growth efficiency is maximized, C_{OPT}; and growth rate with *ad libitum* food consumption, C_{MAX}.

the relationship between growth, rate of food consumption, and temperature have been determined for the European trout. A feature of these results was that the temperature and ration size at which growth efficiency was maximized were both lower than the temperature at which the growth rate was maximized. However, there are still relatively few studies on other fishes that define the relationships in detail as is available for the European trout.

Food Quality

Food quality in relation to fish growth has two aspects. The first is the nutritional quality of the food. Fish tend to have high requirements for protein and lipid in their diets. This is particularly true of carnivorous fish, such as the salmonids and gadoids. A consequence of this is that in the aquaculture of such species, to obtain good growth, the food provided contains good-quality protein, usually in the form of fish meal (**see also Energetic Models**: Bioenergetics in Aquaculture Settings). Omnivorous species such as the roach, *Rutilus rutilus*, one of the most common freshwater fishes of Western Europe, consume both plant and animal material. Roach fed on mealworms grew far faster than roach fed on grass, though the rates of consumption in both diets were close to the maximum. The growth efficiency for the roach fed on grass was 9%, whereas for those fed on mealworms it was 46%. These differences reflect the higher quality of animal foods.

A second aspect of food quality is the size of the prey eaten (**see also Food Acquisition and Digestion**: Energetics of Prey Capture: From Foraging Theory to Functional Morphology; Energetics of Foraging Decisions and Prey Handling). As fish grow, the size of the mouth (gape size) increases, which allows the fish to take larger prey, with the result that each prey taken provides more energy. However, the time taken to handle an individual prey tends to increase with prey size. Consequently, for a fish of a given size, there is a prey size that maximizes the rate of return per unit time. This is the optimum prey size. In species such as the European perch, *Perca fluviatilis*, or the European trout, if an individual can reach a size that allows it to switch from feeding exclusively on invertebrate prey to feeding on fish, the individual often shows an increase in growth rate after this switch. This is a reflection of the increased profitability of the prey. In aquaculture, the size of the food pellets fed to the fish reflects the changing size of the gape of the fish as they grow.

Social Interactions

The social environment of the individual fish can affect growth though several mechanisms, which influence the rate of feeding. The amount of food available can be affected by the presence of other animals. The propensity to feed may be affected by the presence of other, more dominant fish, even when food is available. Interactions with other animals can affect the time that can be spent on feeding or the energy expended on interactions. Social dominance is an excellent example of a social interaction (**see also Social and Reproductive Behaviors**: Dominance Behaviors) that results in differential growth.

Presence of conspecifics

The effect of conspecifics on feeding and hence growth depends on the nature of the behavioral interactions. One possibility is that there is competition by exploitation for food. This occurs simply because items of food consumed by one individual are then not available for being eaten by another individual. When food availability is limiting, individual fish have essentially the same chance of obtaining food. Differences in food consumption and, therefore, differences in growth are largely determined by the chance of encountering suitable food items.

However, if the behavioral interactions include dominance relationships, the dominant fish can sequester food at the expense of the subordinate fish. This leads to depensatory growth. The dominant fish grows at a faster rate than the subordinate fish and so the variance of fish size increases over time. Since dominance in fish is often related to fish size, as the dominant fish grow faster, their size advantage over the subordinate fish increases, which reinforces the ability of the dominants to sequester food. Observations on stream populations of salmonid fishes suggest that dominant fish can acquire the better localities for feeding. In the aquaculture of salmonids, fish are size-graded at regular intervals to reduce the differences in size among fish kept in a single tank, in an attempt to reduce the depensatory growth.

Even when food is readily available, the presence of dominant fish may inhibit the food intake of subordinate fish. The presence of the dominant fish stresses the subordinates and this leads to a loss of appetite in the subordinates. The importance of such a behavioral inhibition of feeding and hence growth in natural populations is uncertain (**see also Social and Reproductive Behaviors**: Dominance Behaviors).

In contrast, in fish species that typically live in shoals, the presence of conspecifics may stimulate feeding. This is because for such fish, isolation is a stressful condition, again leading to a suppression of appetite.

Presence of heterospecifics

If species that coexist in the same habitat have similar diets, there may be interspecific competition for food, with adverse consequences on the growth of individuals. In some North American lakes, juveniles of bluegill (*Lepomis macrochirus*) and pumpkinseed sunfish (*L. gibbosus*) both occur in the littoral zones and have similar diets and act as interspecific competitors. As they increase in size, the diets of the two species diverge such that interspecific competition relaxes.

The presence of predators can also affect growth rates because the predators may restrict their prey to habitats that are less favorable for feeding. Largemouth bass, *Micropterus salmoides*, feed on juvenile bluegill sunfish. In the presence of the largemouth bass, the bluegill juveniles tend to be restricted to the vegetated littoral and are not able to move in to more open water, where zooplankton is an abundant source of food, but the risk of predation by largemouth bass is much higher. The presence of a predator may also inhibit a fish from foraging for food.

Such interspecific interactions and their effect on fish growth can be complex. An example can be found among European perch and roach in lakes in northern Europe. In the early life-history stages, the two species can be interspecific competitors for zooplankton, with roach tending to be the superior competitor. Later in ontogeny, the perch may become predators of roach. The presence of roach may both inhibit and stimulate the growth of perch, depending on the ontogenetic stages of the individuals that are interacting.

Compensatory Growth

Compensatory growth is a phenomenon which has been demonstrated in the laboratory, but whose importance in natural populations is still uncertain. It occurs when fish, which have experienced a period of unusually slow growth (e.g., due to low rations or high levels of swimming activity), are returned to favorable conditions (e.g., in which food is unlimited and/or activity is low). Such fish then grow at an unusually high rate and may catch up in size with individuals that have never suffered the growth depression. It is of interest because it suggests that, even in good conditions, fish tend to grow at a rate slower than their maximum physiological capacity. This suggests, also, that there are costs, in terms of fitness, of high growth rates. Notably, some studies have described an inverse relationship between growth rate and swimming performance. Growth rates may be regulated by neuroendrocrine and endocrine mechanisms to reduce these costs.

Conclusion

Growth patterns are adaptive responses to the environmental conditions. The growth potential of an individual is defined by its genotype. However, the extent to which that potential can be realized will depend on the abiotic conditions experienced during the ontogeny of the individual, further modified by biotic factors, particularly the availability of food.

See also: **Energetics**: General Energy Metabolism. **Energy Utilization in Growth**: Energetics of Growth. **Food Acquisition and Digestion**: Energetics of Foraging Decisions and Prey Handling; Energetics of Prey Capture: From Foraging Theory to Functional Morphology. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Social and Reproductive Behaviors**: Dominance Behaviors. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism. **Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P_{50}.

Further Reading

Ali M, Nicieza A, and Wootton RJ (2003) Compensatory growth in fishes: A response to growth depression. *Fish and Fisheries* 4: 147–190.

Boeuf G and Le Bail P-Y (1999) Does light have an influence of fish growth? *Aquaculture* 177: 129–152.

Boeuf G and Payan P (2001) How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C* 130: 411–423.

Brett JR and Groves TD (1979) Environmental factors and growth. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology,* vol. 8, pp. 599–675. London: Academic Press.

Conover DO, Clarke LM, Munch SB, and Wagner GN (2006) Spatial and temporal scales of adaptive variation in marine fishes and their implications for conservation. *Journal of Fish Biology* 69 (supplement C): 21–47.

Davison W (1997) The effects of exercise on teleost fish, a review of recent literature. *Comparative Biochemistry and Physiology 117A:* 67–75.

Elliott JM (1994) *Quantitative Ecology and the Brown Trout*. Oxford: Oxford University Press.

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS and Randall DJ (eds.) *Fish Physiology,* vol. 6, pp. 1–98. London: Academic Press.

Halver JE and Hardy RW (2002) *Fish Nutrition*, 3rd edn. London: Academic Press.

Jobling M (1994) *Fish Bioenergetics*. London: Chapman and Hall.

McCullough DA, Bartholow JM, Jager HI, *et al.* (2009) Research in thermal biology: Burning questions for coldwater stream fishes. *Reviews in Fisheries Sciences* 17: 1–26.

Persson L and de Roos AM (2006) Food-dependent individual growth and population dynamics in fishes. *Journal of Fish Biology* 69 (supplement C): 1–20.

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology,* vol. 8, pp. 677–743. London: Academic Press.

Volkoff H, Canosa LF, Unniappan S, *et al.* (2005) Neuropeptides and the control of food intake in fish. *General and Comparative Endocrinology* 142: 3–19.

Wootton RJ (1998) *Ecology of Teleost Fishes*, 2nd edn. London: Chapman and Hall.

ENERGETICS

Swimming and Other Activities

Contents
Energetics of Fish Swimming
Cellular Energy Utilization: Environmental Influences on Metabolism
Applied Aspects of Fish Swimming Performance

Energetics of Fish Swimming

DJ McKenzie, Université Montpellier 2, Montpellier, France

© 2011 Elsevier Inc. All rights reserved.

Introduction
Why Swimming Requires Energy
Types of Fish Muscle and Swimming Activity
Environmental Influences and the Fry Paradigm
Estimating the Costs of Swimming in Wild Fish
Conclusions
Further Reading

Glossary

Aerobic metabolic scope The extent to which a fish can raise its aerobic metabolic rate above the minimal rate required to maintain life processes. The magnitude of the scope depends upon the ability of the respiratory and cardiovascular systems to provide oxygen and nutrients for aerobic activities.

Bio-logging and biotelemetry The measurement of physiological or physico-chemical information with sensors attached to (or inside) free-swimming fish. The information can be either stored (logged) or transmitted to a receiver (telemetered).

Cost of transport The unit cost of transporting a unit mass over a unit distance. This can be measured by respirometry of fish swimming at known speeds.

Drag power The inertial forces exerted by a viscous fluid such as water; the fish must exert an equal and opposite thrust power in order to move, which is generated by muscular work.

Excess post-exercise oxygen consumption (EPOC) The amount of oxygen that is consumed after exercise has stopped, which represents the oxygen cost of recovering from the exercise bout and includes the replenishment of oxygen stores and metabolites.

Red muscle Striated skeletal muscle that generates ATP aerobically and is used to perform sustained low-intensity swimming. Red muscle has many mitochondria and a good blood supply to provide oxygen and nutrients.

Swim-tunnel respirometer A piece of equipment that is designed to exercise fish at known speeds and measure their metabolic rate by respirometry.

Respirometry The measurement of metabolic rate as gas exchange, also known as indirect calorimetry.

White muscle Striated skeletal muscle that generates most ATP equivalents anaerobically and is used to perform bursts of high-intensity swimming. White muscle has few mitochondria and less blood supply than red muscle.

Introduction

Swimming is a defining feature of fish, almost all species swim at some point in their lifecycle. Fish swim routinely to find food, to escape predation, or to engage in social interactions such as territorial defense or reproduction. Some species inhabit lotic environments, which require them to swim just to maintain station. Many species swim to change their habitat, either as part of seasonal movements or for reproductive migration. The most famous long-distance migrants are salmonids of the genera *Salmo* and *Oncorhynchus* and eels of the genus *Anguilla* (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle and Eel Migrations).

Swimming requires energy because it is muscular work, primarily by the large blocks of axial muscle arrayed along each side of the body, known as myotomes, which are such a dominant anatomical feature of most fishes. Although active contraction occurs by the same adenosine triphosphate (ATP)-consuming molecular mechanisms as in all vertebrate skeletal muscle, fish have distinct muscle types. Each type uses specific metabolic pathways for the primary generation of ATP (aerobic vs. anaerobic) and power different types of swimming. Thus, swimming can range from gentle aerobic activity over long periods to brief bursts of intense anaerobic activity, depending on the context. In the laboratory, the energetic costs of such activities have been measured with swim flumes and techniques of respirometry. Respirometry estimates rates of ATP utilization indirectly as rates of oxygen uptake, because they are stoichiometrically related through oxidative phosphorylation (**see also Energetics**: General Energy Metabolism and **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming). One of the most difficult things to estimate is how much energy fish spend on swimming in their natural environment, because they are so difficult to study and follow underwater. This is becoming possible, however, through recent technological developments in biotelemetry and bio-logging (**see also Fish Migrations**: Tracking Oceanic Fish and Eel Migrations).

Why Swimming Requires Energy

Water is a dense, viscous medium that exerts inertial forces known as pressure drag. In order for a fish to move, it must replace the momentum lost to pressure drag by transferring kinetic energy from its body to the water. The amount of energy that is required depends on the shape of the fish and the texture of its body surface, as well as the nature of its propulsive movements (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). The overall rate of energy loss for a body relative to a water flow, often called the drag power (P_D), is described by the following equation:

$$P_D = 0.5\rho S C_b U^3 \qquad (1)$$

where ρ is the density of water, S is the wetted surface area of the body, U is the velocity, and C_b is a dimensionless drag coefficient. Body shape has a major influence on drag power, and many fishes have evolved a streamlined form. In order to move, the fish must generate thrust power that is at least equal to the P_D, but the propulsive movements themselves cause a large increase in C_b, by a factor of between three- and fivefold. Furthermore, because P_D is dependent on the cube of U, the energy required for swimming increases exponentially with increasing velocity.

The energy to overcome pressure drag comes from the hydrolysis of ATP in active muscle contraction. The largest proportion of work is performed by the swimming muscles themselves, which are composed of skeletal muscle fibers (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). There are also minor proportions of energy expended for gill ventilation, cardiac pumping (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping), and fin placement for maneuvering (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability). Some energy is also required for the nervous control of swimming activity.

Types of Fish Muscle and Swimming Activity

Fish swim with two types of striated skeletal muscle, aerobic red muscle and anaerobic white muscle (**Figure 1**). Aerobic red muscles, which typically maximally represent only about 10% of the total muscle mass, are used for slow to moderate, steady swimming. Anaerobic white muscles form the majority of the myotomal muscle blocks in most fishes, as well as the greatest proportion of body mass (sometimes over 50%), and are used for brief bursts of high-speed swimming. Laboratory research has investigated energetic costs of exercise in fish, often using protocols that take fish to extremes of their aerobic or anaerobic performance. This research has revealed much about the physiological mechanisms that support the costs of swimming, and what sets the energetic limits to each of these types of activity.

Aerobic Red Muscle and Sustained Aerobic Activity

This muscle is composed primarily of slow-twitch oxidative (SO; type I) fibers, which produce most power at low contraction (i.e., twitch) frequencies, and generate their ATP by mitochondrial oxidative phosphorylation, which produces 36 molecules of ATP for each glucose equivalent (**see also Energetics**: General Energy Metabolism). This requires substrates (lipids, proteins, and carbohydrates) to burn as fuel and a supply of oxygen as the terminal electron acceptor. The substrates and oxygen are generally

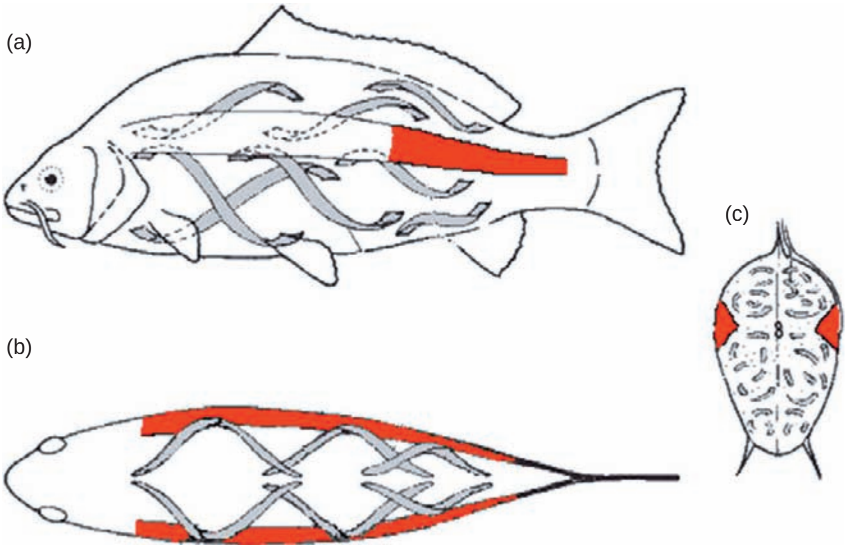


Figure 1 Longitudinal (a), dorsal (b) and transverse section (c) views of the position of different types of muscle in a representative teleost fish, the carp *Cyprinus carpio.* The red muscle is in red, a thin strip in the midline just below the skin. The white muscle is in gray and represents the majority of the myotomal muscle mass. Adapted from Rome LC, Funke RP, Alexander RMcN, *et al.* (1988) Why animals have different muscle fiber types *Nature* 355: 824–827.

provided to the muscle via the bloodstream, and red muscles have a rich blood supply and a high density of mitochondria (**see also Temperature**: Mitochondria and Temperature). They also have intracellular myoglobin, a transient oxygen store that, along with the many capillaries, gives them their characteristic red color. Thus, aerobic exercise performance depends upon factors that influence rates of substrate delivery for aerobic metabolism, such as gill morphology and surface area (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics), cardiac muscle function, and pumping capacity of the heart (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

The largest red muscle mass in most fishes is a strip that runs along each side of their body (**Figure 1**). This is used to beat the tail, in what is known as body-caudal fin (BCF) swimming (**see also Buoyancy, Locomotion, and Movement in Fishes**: Undulatory Swimming). This red muscle is used to swim at slow to moderate speeds, for activities that must be sustained for extended periods, such as foraging, holding station in rivers, or long-distance migration. Many fishes can also swim by moving their fins, in what is known as median-paired fin (MPF) locomotion (**see also Buoyancy, Locomotion, and Movement in Fishes**: Paired Fin Swimming and Maneuverability). The muscles powering the fins are generally skeletal red muscles and, in most species, MPF locomotion is for slow maneuvering. This ability is most pronounced in species that inhabit complex habitats, such as coral reefs. Some coral reef fishes actually can swim remarkably fast with particular sets of fins, notably the labriform and balistiform swimmers (from similarly named families, among others). Some species swim exclusively with fins, for example, the knifefishes of the Gymnotidae family, which have a modified anal fin that extends along much of the ventral side of their body, and which they undulate to propel themselves forward (or backward) through the water.

Most of what we know about aerobic swimming energetics comes from laboratory studies that utilize swim flumes or swim-tunnel respirometers. A schematic representation of one type of swim tunnel respirometer is shown in **Figure 2**. These are instruments that are designed to create a uniform (nonturbulent) water flow, and where the velocity of the current can be controlled. The fish swims spontaneously against the current, by a reflex known as rheotaxis, and different levels of swimming activity can be created by adjusting the speed of the water current. Their energetic costs can then be measured indirectly by respirometry (**see also Energetics**: General Energy Metabolism).

If a fish that uses BCF locomotion is exposed to progressive incremental increases in swimming speed, they exhibit a linear increase in tail-beat frequency with swimming speed. If oxygen uptake is measured at each step, there is an exponential increase in oxygen demand by the working muscles because P_D increases with the cube of U (eqn (1)). The shape of the curvilinear relationship between swimming speed and oxygen uptake is shown in **Figure 3(a)**; it typically is a power function:

$$\dot{M}O_2 = aU^b \qquad (2)$$

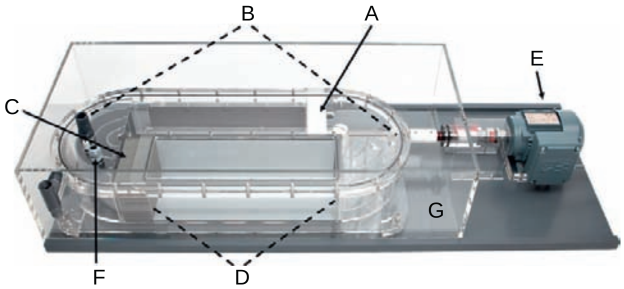


Figure 2 A photograph of a swim-tunnel respirometer. A propeller (not visible, (A)) generates a flow of water in an enclosed recirculating pathway (B), the flow is straightened with vanes and a honeycomb of tubing (C), so that it enters the swim chamber (D) with a uniform velocity profile. The fish swims spontaneously against the current in the swim chamber. The propeller is driven by a variable-speed electric motor (E), to obtain known current velocities and, therefore, swimming speeds. The whole recirculating pathway can be sealed, and oxygen uptake measured with an oxygen electrode (placed at F), from the decline in oxygen levels in the sealed chamber. The recirculating pathway is immersed in an outer chamber (G) and, at intervals, the system can be flushed, by a pump, with new aerated water from this outer reservoir, with water exiting through a chimney, to replace the oxygen consumed by the fish. A measurement of oxygen uptake can then be repeated. Photograph courtesy of Loligo Systems.

where $\dot{M}o_2$ is the rate of oxygen uptake, *a* and *b* are constants, and *U* is the swimming speed. The exponent *b* is always >1.

The capacity to swim aerobically depends on the ability of the fish to provide oxygen and substrates to the contracting muscle over and above their basal metabolic needs. Gill ventilation increases markedly with exercise, such that water flow over the gills is increased by up to tenfold. In some species, active ventilation of the gills ceases and they simply open the mouth and use their forward motion to create a current across the gills, (i.e., ram ventilation). The heart increases pumping activity, and cardiac output can go up by fivefold. There is also redistribution of blood, away from organs such as the liver and intestine, to preferentially perfuse the red muscle, brain, and heart. It has been estimated that blood flow to the red muscle can increase by up to 6 times when fish are at their maximum aerobic activity level (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

When fish swim aerobically, they can increase their $\dot{M}o_2$ by up to 10 times above their standard metabolic rate (SMR). This capacity to increase $\dot{M}o_2$ is known as aerobic metabolic scope, and the largest scopes are seen in larvae of coral reef species, which, for their size, are the champion swimmers of the fish world. The 10-fold increase in $\dot{M}o_2$ is, however, still less than some birds and athletic mammals, which can increase it by over 30 times when running or flying.

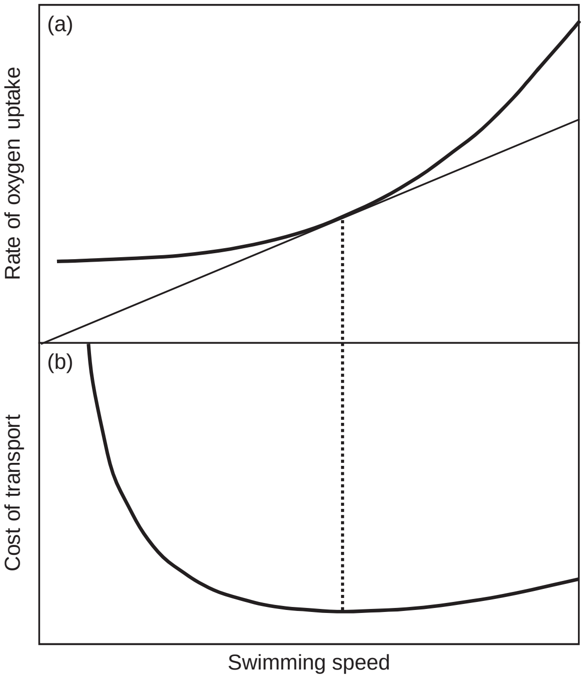


Figure 3 (a) The relationship between rate of oxygen uptake and swimming speed, where the tangent line intersects with this relationship, is the optimum swimming speed, at which the fish swims with the greatest energetic efficiency. (b) The cost of transport, as derived by dividing the rate of oxygen uptake by swimming speed. At the optimum swimming speed, the cost of transport (b) is minimal, as indicated by the vertical dotted line.

Most of the information about aerobic swimming energetics in fishes comes from studies on salmonids, especially of the genus *Oncorhynchus*, in particular the rainbow trout, *O. mykiss*. It is believed that salmonids reach their maximum possible rates of $\dot{M}o_2$ and, therefore, their maximum aerobic energetic flux, when they are exercising at their maximum sustainable aerobic swimming speed (as measured in a swim-tunnel respirometer), with their heart pumping at its maximum capacity. In other species, such as the European sea bass *Dicentrarchus labrax*, the maximum sustainable aerobic exercise does not evoke their entire aerobic metabolic scope, because they achieve higher maximum rates of $\dot{M}o_2$ if they are exercised when they are also digesting a meal (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation).

In **Figure 3(a)**, the point where the curve of $\dot{M}o_2$ versus *U* intersects with a tangent drawn from the origin indicates the speed at which there is the minimum cost of transport (COT). The COT is the energy required to transport a unit mass over a unit distance. If the COT is plotted against swimming speed, a U-shaped curve is created, with a distinct minimum (**Figure 3(b)**). The U-shape results from the high costs of SMR (relative to the net costs of swimming) at low speeds and the high costs due to the exponential rise in P_D at high speeds (eqn (1)). (Note that if the fish is not at its basal metabolism when inactive, switching off nonessential costs could contribute to this decrease at low-swimming speeds.) The minimum COT corresponds to the swimming speed at which the fish can travel the maximum distance for a given quantity of energy. It has been proposed that fish should, therefore, spontaneously swim at this speed in the wild, at least when performing long-term sustained aerobic swimming, such as during reproductive migrations (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance and **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). This idea is, however, still debated.

Relatively little is known about the energetic costs of labriform or balistiform locomotion, but these costs may be less than for BCF swimming, possibly because the body is held rigid while the fins beat, resulting in less average drag. These species will, however, transition to more expensive BCF locomotion to achieve very high swimming speeds.

In all studies using swim flumes, fish eventually reach a speed where they can swim no faster with aerobic red muscle alone. A gait transition occurs, and the fish supplement red muscle function with contraction of the powerful white muscles. This produces a burst of forward movement, a gait that is often called 'burst and glide' locomotion. The available evidence indicates a number of possibilities for the transition between gaits and each one acknowledges that it does not occur because the red muscle is exhausted or depleted of oxygen. One possibility is that the red muscle cannot contract at a faster rate to power faster tail beats (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). Another is that oxidative phoshorylation cannot generate ATP fast enough for the highest tail beat frequencies (**see also Hypoxia**: Anaerobic Metabolism In Fish). Another possibility is that the skeletal muscles strip the blood of oxygen, leaving the heart starved of oxygen (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). Fish have a single circulation; all blood leaves the heart and returns after it circulates through the gills and the body's systemic circulation. In many fishes, the heart gets most of its oxygen from this venous return. When fish exercise aerobically, their working red muscle extracts a great deal of oxygen from the blood so, as they swim faster and faster, there is less and less available for the heart. The fish may switch to white muscle with the red muscle extracting less oxygen from the blood, leaving just enough venous oxygen for the heart. This latter explanation appears to be particularly acute when fish swim at elevated temperatures (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature and **Temperature**: Effects of Climate Change).

The fact that red muscle is not fatigued when fish perform a gait transition in a swim-tunnel respirometer allows them to repeat maximum aerobic exercise many times, with virtually no recovery period between the tests.

Anaerobic White Muscle and High-Intensity Bursts of Activity

White muscle is composed primarily of 'fast glycolytic' (FG; type II) fibers, which produce the most power at high contraction frequencies and which rely almost exclusively upon endogenous intramuscular fuel stores to generate ATP anaerobically. This process is known as substrate-level phosphorylation, with the most dominant reaction being glycolysis, where glucose (stored as glycogen) is converted into pyruvate, and then lactate, to release only two ATP per glucosyl equivalent (**see also Hypoxia**: Anaerobic Metabolism In Fish). The fibers have little aerobic capacity and are much less-well vascularized than red muscle fibers with little to no myoglobin.

White muscle typically comprises most of the myotomal muscle mass (up to 90%; **Figure 1**) and is used for short bursts of high-speed swimming, such as predator–prey chases or to surmount velocity barriers during upriver migrations. The duration of burst swimming with white muscle is presumably limited by the availability of endogenous stores of high-energy phosphagens and glycogen. It may also be limited by accumulation of nonvolatile products of anaerobic metabolism (e.g., lactate or H^+). Anaerobic metabolism can reduce the pH of the muscle and the blood, raise the lactate and produce dramatic changes to cell organic phosphate pools and ion distributions. This may impair further muscle and, perhaps, nerve function.

When anaerobic exercise ceases, energetic costs must be repaid and metabolic equilibrium reestablished in the muscle and the whole animal. This is visible metabolically as excess post-exercise oxygen consumption, or EPOC. This used to be called the 'oxygen debt', a term that is still used today. The EPOC comprises the energetic costs of replenishing endogenous fuel stores, but also of reestablishing endocrine and other balances (e.g., the catecholamines, epinephrine, and norepinephrine, released during intense exercise), and of repairing any cell damage. Thus, attempts to directly relate the size of EPOC to the actual amount of ATP that was used to fuel anaerobic exercise may be futile. The energetic costs of EPOC have been measured most extensively using a protocol, whereby a fish is chased by the experimenter, who continually touches its tail until it can no longer make a rapid escape response. This might be equivalent to when a prey species is chased to exhaustion, for example, by a cormorant or a seal. At such exhaustion, white muscle stores of glycogen are profoundly depleted, and there is a severe drop in muscle and blood pH, associated with the production of lactate. The EPOC is then typically equivalent in magnitude to the maximum rate of $\dot{M}O_2$ observed during aerobic swimming, and persists for a number of hours. It can take up to 24 h to recover fully from such exhaustive exercise, depending on the water temperature. Thus, a major difference between maximal aerobic and anaerobic exercise is that fish can repeat maximal aerobic exercise immediately, whereas they cannot repeat maximal anaerobic exercise until they have replenished endogenous fuels and dealt with the end products.

Spontaneous Swimming Activity: What do Fish Actually do?

Laboratory studies of aerobic and anaerobic exercise have revealed much about the mechanisms that sustain swimming, and what sets the energetic limits for each type of activity. In the wild, it is unknown, however, to what extent fish might exploit their maximum aerobic or anaerobic performance. Recent research has allowed fish (smallmouth bass, *Micropterus dolomieu*) to swim spontaneously up a long channel in which they encountered different current speeds, and their swimming patterns were filmed to identify steady aerobic swimming versus bursts of anaerobic swimming. This revealed that the bass transitioned between muscle types, depending on the current speed, and often used bursts of anaerobic swimming to traverse areas of rapid flow even if, based on their swimming performance measured in a swim flume, they in fact could have negotiated that same current speed with aerobic swimming. Thus, they chose to cross the velocity barrier very quickly, rather than steadily, and they transitioned to anaerobic swimming before reaching their maximum capacity for aerobic exercise, even without knowledge of how long the section of rapid flow was. Furthermore, even when they used many bursts of anaerobic swimming in the channel, they showed virtually no EPOC or evidence of the white muscle metabolic imbalances that have been demonstrated in fish chased to exhaustion. Thus, fish transition dynamically between aerobic and anaerobic swimming. Also, they quite easily reestablish equilibrium in white muscle if it is not used too intensively and for a short duration, with little EPOC. Indeed, it has also been shown that steady aerobic exercise actually improves recovery from intense anaerobic exercise in some fishes. Thus, fish may use their maximum aerobic swimming performance only rarely (instead using bursts of anaerobic swimming interspersed with aerobic swimming), and they may never experience the large EPOC and extreme metabolic imbalances observed after prolonged chasing. This, of course, means that it may be very difficult to estimate actual energetic costs of swimming in wild fish at this level! In addition, the relative use of anaerobic versus aerobic swimming is an individual characteristic that varies substantially within a species. Costs of swimming have been estimated from energy store depletion in fish that are no longer eating, primarily migratory adult salmon and eels (**see also Fish Migrations**: Eel Migrations and Pacific Salmon Migration: Completing the Cycle).

Environmental Influences and the Fry Paradigm

Another reason why it is difficult to estimate energetic costs of swimming in wild fish is because their physiology is so profoundly influenced by physico-chemical (abiotic) conditions in their environment (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). The first person to model how environmental conditions might influence aerobic swimming energetics was a Canadian scientist named F.E.J. Fry, in the 1940s. Fry reasoned that, if environmental factors influence either the magnitude of SMR or the maximum capacity for oxygen uptake in fish, they would then influence aerobic metabolic scope. Environmental parameters will, therefore, set the boundaries within which all energetic demands and activities are constrained. Fry modeled how major factors would influence metabolism and physiology to define aerobic metabolic scope, as shown in **Figure 4(a)**.

Temperature: A Controlling Factor

Fish body temperature is generally near that of the surrounding water (except for some scombrids and lamnid sharks (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks). Fry termed temperature a controlling factor, because it sets rates for all metabolic processes, and so controls physiology and energetics (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation). The effects on aerobic metabolic scope are shown in **Figure 4(a)**. At low temperatures, all processes are depressed by the cold, so SMR is low but so is the capacity of the red muscles and heart to perform work. Thus, aerobic scope is small. As temperature increases, SMR rises but the warmth allows for greater red and cardiac muscle performance, so aerobic scope increases. As temperatures continue to rise, there is an inexorable rise in SMR, which eventually is so high that it is equivalent to the maximum capacity for $\dot{M}O_2$. Once again, therefore, aerobic scope is very small, and there is no oxygen available to allocate to swimming. The magnitude of aerobic metabolic scope therefore follows a bell-shaped curve with temperature (**Figure 4B**), with a clear optimum, and lower and upper limits (which may be lethal). Fish also show a bell-shaped curve for their aerobic swimming performance. Thus, overall energetics and

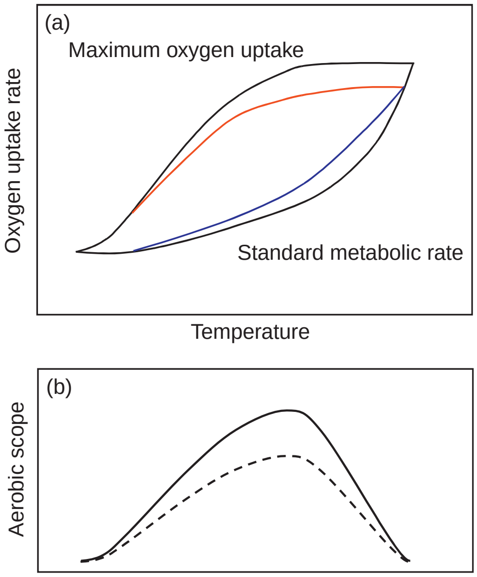


Figure 4 (a) The Fry paradigm, which models the effects of environmental factors on aerobic metabolic scope. Temperature is considered to be a controlling factor, which controls all metabolic processes. The solid black lines show how standard metabolic rate (SMR) and maximum oxygen uptake rate vary as a function of water temperature. The difference between these two rates is the aerobic metabolic scope, the capacity to provide oxygen for energetic fluxes such as swimming exercise. Note how the SMR shows an exponential increase with temperature. At low temperatures, maximum oxygen uptake is also low, because the cold inhibits all metabolic processes (including muscle performance). Therefore, scope is small. As temperatures rise, aerobic scope increases because the warming accelerates metabolic processes. Eventually, however, the effects of temperature on SMR make it rise to the point where it coincides with the fish's maximum oxygen uptake rate, so aerobic scope is again very small. The resultant aerobic metabolic scope is shown in (b), with a clear bell-shaped relationship and an optimum temperature range. In (a), the red line shows the effects of a limiting factor, such as oxygen, on maximum oxygen uptake rate. The blue line shows the effects of a loading factor, such as stress. These factors constrain aerobic scope, as shown by the dotted line in (b).

the capacity to allocate energy for swimming will depend on temperature, and it might be expected that fish will try to choose temperatures, if they can, which maximize their scope for aerobic activity.

Limiting Factors, in Particular, Availability of Dissolved Oxygen

Fry considered the availability of dissolved oxygen to be a limiting factor. Water has a low concentration of oxygen per unit volume and hypoxia, a decline in oxygen levels, can be a frequent occurrence in aquatic habitats (**see also Hypoxia**: The Expanding Hypoxic Environment). Of course, if there is less oxygen in the water, then, this will limit the maximum capacity for oxygen uptake, and therefore aerobic metabolic scope (**Figure 4(a)**). Other limiting factors may be pollutants or extreme turbidity, which may cause gill damage and therefore also limit $\dot{M}\mathrm{o}_2$ and aerobic scope.

Loading Factors

These are factors which cause a rise in SMR. Fry actually termed them 'masking factors', but loading factors is more explicit, because it makes clear that the effect is to place a load on the system by raising SMR and thereby constraining scope available for other activities such as swimming (**Figure 4(a)**). One major loading factor is an endocrine stress response, which can be biotic in nature (e.g., intraspecific aggression) or abiotic (e.g., exposure to salinity change or to pollutants). SDA is potentially another (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion and **Food Acquisition and Digestion**: Cost of Digestion and Assimilation)

Estimating the Costs of Swimming in Wild Fish

The energetic costs of swimming will, of course, depend on a species' lifestyle, but it has been estimated that swimming is a major component of energy expenditure for many fishes. Monitoring energetics of fish in their natural environment is a great technological challenge, because their physiology is so influenced by multiple environmental factors and they are so difficult to study and follow underwater. This challenge is now being addressed, with recent advances in techniques of biologging (measuring and storing biological information) and biotelemetry (measuring and transmitting information) that can provide relatively accurate information about swimming activity, and therefore energy expenditure, of fishes in the wild (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance). Biotelemetry is particularly useful because animals do not have to be recaptured to recover logged information, but the transmitter battery makes the sensors larger and heavier, therefore more likely to influence activity. In freshwater habitats, radio-telemetry is used whereas the high conductivity makes this impossible in seawater, where ultrasonic telemetry must be employed (**see also Fish Migrations**: Tracking Oceanic Fish).

Early radio-telemetry involved simply picking up signals about fish position in a water body, providing general information about distances swum in a given time interval, but no information about true activity levels or swimming speeds. Some studies have used externally attached speed-sensing devices, such as small paddle wheels, which trail behind the fish. These, however, can only be used on large animals, such as sharks, and tend to be relatively insensitive at low speeds and prone to damage or malfunction. More sophisticated techniques provide more precise data on metabolic costs of swimming by measuring and transmitting detailed information about biochemical or physiological correlates of oxygen uptake. These techniques all require that wild fish be captured, fitted with the transmitter, and then submitted to forced exercise in a respirometer, so that the relationships among the chosen physiological variable, swimming speed, and $\dot{M}O_2$ can be described. The animal is then released back into the wild.

It is possible, for example, to monitor heart rate as the electrocardiogram (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and Action Potential of the Fish Heart), the electrical activity that accompanies cardiac contraction, using electrodes and a transmitter placed inside the peritoneal cavity. Using heart rate to predict metabolic rate is, however, subject to error because this requires that other physiological traits, such as cardiac stroke volume (the amount of blood pumped per beat), oxygen extraction at the gills, and oxygen-carrying capacity of the blood, are either constant or vary in direct proportion with heart rate. Some transmitters use a cuff around the ventral aorta to monitor cardiac output and total blood flow, using a Doppler technique. This is more useful information than just heart rate, but the relationship with metabolic rate will still be confounded by any variations in tissue oxygen extraction, arterial oxygen saturation, and blood-oxygen-carrying capacity. Furthermore, cardiac activity can, of course, be affected by activities other than swimming in fish, such as digestion of food, and by external conditions such as hypoxia. Thus, telemetry of cardiac performance may be most useful for estimating the total energy expenditure in wild fish. This is, in itself, an important goal in fish ecophysiology.

There are techniques that can, however, measure swimming muscle activity directly. One method is the measurement and transmission of information about electromyograms (EMGs), the electrical activity that accompanies contraction of swimming muscles. Each axial myotome contracts once per tail beat, so an EMG can be used to derive tail beat frequency. If the relationships among tail beat frequency, swimming speed, and M_{O2} are measured on the captive fish, then, energetic expenditure for swimming can be estimated for the individuals after they have been returned to the wild. The sensors and transmitters can be placed inside the body cavity, so they do not contribute to drag and raise costs of swimming in themselves. This technique has permitted some quite detailed activity budgets to be constructed for free-swimming fish. For example, the cost of swimming activity for rainbow trout in a small lake was less than half their costs for SMR over a period of a year, and activity showed a daily pattern being lowest just before dawn. The rainbow trout also used less than 20% of their available aerobic metabolic scope.

The differential pressure tag (DPT) measures pressure changes at the tail as it beats during swimming. The DPT, therefore, measures changes in tail beat amplitude and force, and provides information about swimming effort. They are applied externally and require surgery to place the sensor through the caudal peduncle (the portion of the body just anterior to the caudal fin). Thus, they may influence natural behaviors in wild fish, and may be most useful for aquaculture applications.

The EMG or DPT transmitters can only, however, provide estimates of energy expenditure associated with red muscle activity, as the costs of white muscle activity are only visible as EPOC. The EPOC should, however, be visible as changes in cardiac activity (cardiac output). Furthermore, changes in water temperature will have a profound effect on the actual energetic costs of swimming. The best sensors, therefore, are those that can simultaneously measure a number of variables, such as body temperature, ECG, and EMG.

Conclusions

Laboratory studies have provided a significant amount of information about the function of fish swimming muscles, the mechanistic bases of swimming energetics, and the physiological factors which limit maximum aerobic and anaerobic performance, and which elicit a transition from aerobic to anaerobic swimming. Studies on fish swimming spontaneously indicate that they alternate dynamically between aerobic and anaerobic swimming, and may rarely exploit their maximum aerobic performance. The Fry paradigm provides a framework within which to explain how environmental factors will influence aerobic swimming energetics. It is to be expected that we will learn a great deal more about swimming energetics of fish in the wild, with the development of biologging or biotelemetry sensors that measure multiple factors simultaneously.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops; Maneuverability; Paired Fin Swimming; Undulatory Swimming. **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Physiology of Cardiac Pumping. **Energetics**: General Energy Metabolism. **Fish Migrations**: Eel Migrations; Pacific Salmon Migration: Completing the Cycle; Tracking Oceanic Fish. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Hypoxia**: Anaerobic Metabolism In Fish; The Expanding Hypoxic Environment. **Integrated Response of the Circulatory**

System: Integrated Cardiovascular Responses of Fish to Swimming; Integrated Responses of the Circulatory System to Digestion; Integrated Responses of the Circulatory System to Temperature. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance; Cellular Energy Utilization: Environmental Influences on Metabolism. **Temperature**: Effects of Climate Change; Membranes and Temperature: Homeoviscous Adaptation; Mitochondria and Temperature. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Beamish FWH (1978) Swimming capacity. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, vol. 7*, pp. 101–187. New York: Academic Press.

Blake RW (2004) Fish functional design and swimming performance. *Journal of Fish Biology* 65: 1193–1222.

Bone Q (1978). Locomotor muscle. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, vol. 7*, pp. 361–424. New York: Academic Press.

Briggs CT and Post JR (1997) Field metabolic rates of rainbow trout estimated using electromyogram telemetry. *Journal of Fish Biology* 51: 807–823.

Claireaux G, Couturier C, and Groison AL (2006) Effect of temperature on maximum swimming speed and cost of transport in juvenile European seabass, *Dicentrarchus labrax, Journal of Experimental Biology* 209: 3420–3428.

Cooke SJ, Thorstad EB, and Hinch SG (2004) Activity and energetics of free- swimming fish: Insights from electromyogram telemetry. *Fish and Fisheries* 5: 21–52.

Farrell AP (2007) Cardiorespiratory performance during prolonged swim tests with salmonids: A perspective on the temperature effects and potential analytical pitfalls. *Philosophical Transactions of the Royal Society B. Biological Sciences* 362: 2017–2030.

Fish FE (2009). Swimming strategies for energy economy. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion – an Etho-ecological Perspective*, pp. 90–122, Enfield, NH: Science Publishers.

Fry FEJ (1971). The effect of environmental factors on the physiology of fish. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, vol. 6*, pp. 1–98. New York: Academic Press.

Gollock MJ, Hunter KJ, Syme DA, *et al.* (2009) Potential methods for measuring the activity patterns and energy use of Atlantic cod *(Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences* 66: 1095–1106.

Jones DR and Randall DJ (1978) The respiratory and circulatory systems during exercise. In: Hoar WS and Randall DJ (eds.) *Fish Physiology, vol. 7*, pp. 425–501. New York: Academic Press.

Jourdan H, Dupont-Prinet A, Claireaux G, and McKenzie DJ (2010) An investigation of metabolic prioritization in the European sea bass, *Dicentrarchus labrax, Physiological and Biochemical Zoology* 83: 68–77.

Korsmeyer KE, Steffensen JF, and Herskin J (2002) Energetics of median and paired fin swimming, body and caudal fin swimming, and gait transition in parrotfish *(Scarus schlegeli)* and triggerfish. *(Rhinecanthus aculeatus). Journal of Experimental Biology* 205: 1253–1263.

Lee CG, Farrell AP, Lotto A, Hinch SG, and Healey MC (2003) Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. *Journal of Experimental Biology* 206: 3253–3260.

Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, and Healey MC (2003) The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho salmon (*O. kisutch*) salmon stocks. *Journal of Experimental Biology* 206: 3239–3251.

Marras S, Claireaux G, McKenzie DJ and Nelson JA (2010) Inter-individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass (*Dicentrarchus labrax*, Linnaeus 1758). *Journal of Experimental Biology* 213: 26–32.

McKenzie DJ and Claireaux G (2009). Effects of environmental factors on the physiology of sustained aerobic exercise. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion – an Etho-Ecological Perspective*, pp. 296–332. Enfield, NH: Science Publishers.

Peake SJ and Farrell AP (2004) Locomotory behavior and post-exercise physiology in relation to swimming speed, gait transition, and metabolism in free-swimming smallmouth bass *(Micropterus dolomieu). Journal of Experimental Biology* 207: 1563–1575.

Randall DJ (1982) The control of respiration and circulation in fish during exercise and hypoxia. *Journal of Experimental Biology* 100: 175–188.

Rome LC, Funke RP, Alexander RMcN, *et al.* (1988) Why animals have different muscle fiber types. *Nature* 355: 824–827.

Videler JJ (1993) *Fish Swimming*. London: Chapman and Hall.

Webb PW (1998) Swimming. In: Evans DH (ed.) *The Physiology of Fishes*, Marine Science Series, 2nd edn., pp. 3–24. Boca Raton, FL: CRC Press.

Cellular Energy Utilization: Environmental Influences on Metabolism

H-O Pörtner, Alfred-Wegener-Institute, Bremerhaven, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Aerobic Metabolic Pathways: Sustaining Functional Scope
Anaerobic Metabolic Pathways: Extending Limits to Function and Time
Further Reading

Glossary

Adenosine triphosphate (ATP) An almost universal carrier of chemical bond potential energy; fish use ATP made from catabolism of foodstuff or body reserve molecules to fuel energy-dependent processes.

Creatine phosphate An energy store alternative to ATP. Also known as phosphocreatine.

Critical swimming speed (U_{crit}) The U_{crit} is determined in a swim tunnel where the fish are forced to swim against a current. The current velocity is increased in steps and each step is maintained for a specified time (usually 10 min to 1 h) or until the fish fatigues.

Fatigue Inability of the exercising fish to sustain a set swimming speed. Cellular mechanisms eliciting fatigue are under debate.

Gibb's free-energy change Equals the energy available from ATP under cellular conditions, depends on concentration ratios of ATP over those of ADP and Pi as well as cellular pH and Mg^{2+} levels.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Introduction

Metabolic specialization and the level of energy turnover in teleost fish depend on the levels and variability of activity and on environmental factors. In the first place, fish are constrained to the vertebrate mode of metabolism, which, when compared to aquatic marine invertebrates, appears less flexible in terms of the pathways used, less reliant on the use of amino acids in metabolic pathways and osmoregulation and, possibly, less able to support survival under the most extreme environmental conditions. Nonetheless, teleost fish have conquered a wide range of freshwater and marine habitats, from streams to burrows in sediments to virtually all of the pelagic, deep, and shallow-water habitats. Examples of extreme adaptation do exist, exemplified by the extreme tolerance of crucian carp (*Carassius carassius*) to anoxia as well as by the specialization of Lake Magadi tilapia (*Alcolapia grahami*) to life in alkaline lakes, among others. These adaptive features not only require the expression of specialized processes providing tolerance (e.g., specialized mechanisms of energy production, of acid–base and ion regulation, or of antioxidant protection) but also require the expression of metabolic and regulatory features fueling those efforts and adjusting energy demand to the constraints frequently imposed by ambient extremes. For example, many previous efforts have investigated the capacities and regulation of metabolic pathways used in response to the environmental challenges of hypoxia and/or changing temperatures.

Global environmental change and its impacts on ecosystems have emphasized the need to interpret molecular, biochemical, and physiological findings in fish in an ecological context. Well-known metabolic features established earlier thus also need to be integrated into larger ecological frameworks. This re-interpretation with respect to ecological relevance should include an analysis of how metabolic features support or limit whole-organism functioning and survival at the ecosystem level, for example, under the influence of limited food availability or interactions with other species. Furthermore, integrative analyses of the interacting influences of abiotic and biotic stressors are required for a comprehensive understanding of species' response to environmental change. Individual metabolic pathways contribute to set the functional capacities of cells, organs, and whole organisms. Conversely, they also operate under constraints set by the capacity to supply the tissue with oxygen, which is challenged by environmental factors such as variable oxygen content, especially hypoxia, elevated CO_2 levels, fluctuating temperatures, or toxicants (e.g., hydrogen sulfide). These constraints thus are understood best from a whole-organism point of view.

The key role of all energy metabolism is to fuel energy-demanding processes in maintenance (e.g., protein turnover, anabolism, ion exchange and acid–base regulation, and cardiocirculatory activity) as well as whole-organism performances such as growth, reproduction, motor activity in foraging, migration, and escape (**see also Energetics**: General Energy Metabolism). Energy is gained from the hydrolysis of adenosine triphosphate (ATP), which is continuously re-phosphorylated from adenosine diphosphate (ADP) in oxidative or substrate-level phosphorylation in mitochondria, or anaerobic glycolysis. In a simplified view, the energy content of the adenylate system is adjusted high by maintaining free concentrations of ATP high over low ones of ADP and inorganic phosphate, that is, a high phosphorylation potential (wherein $[ATP]/[ADP]\ ^*\ [Pi]$ is generally on the order of 500). The energy content is quantified through evaluation of the Gibb's free-energy change of ATP hydrolysis ($\mathrm{d}G/\mathrm{d}\xi$, kJ mol^{-1}), a term influenced by intracellular pH, cellular magnesium levels, and the dissociation equilibria of inorganic phosphate as well as the kinetic constants of the enzymatic reactions involved (e.g., the creatine–kinase reaction):

$$\begin{aligned}\mathrm{d}G/\mathrm{d}\xi = {} & \Delta G'^{\circ} \\ & + RT\cdot\ln([\mathrm{Cr}]_{\mathrm{free,tot}}\cdot[P_{\mathrm{i}}]_{\mathrm{free/tot}}/[\mathrm{PCr}]_{\mathrm{free,tot}}\cdot[\mathrm{H}^{+}]\cdot K_{\mathrm{appCK}})\end{aligned}$$

where

$$\begin{aligned}K_{\mathrm{appCK}} &= [\mathrm{ATP}]_{\mathrm{tot}}\cdot[\mathrm{Cr}]_{\mathrm{tot}}/[\mathrm{ADP}]_{\mathrm{tot}}\cdot[\mathrm{PCr}]_{\mathrm{tot}}\cdot[\mathrm{H}^{+}] \\ &= K_{1,\mathrm{CK}}\cdot f_{\mathrm{ADP}}3\text{-}\ \cdot f_{\mathrm{PCr}}\text{-}/f_{\mathrm{ATP}}4\text{-}\end{aligned}$$

where the fractions *f* of ionized specimens of ADP, PCr, and ATP vary with pH and Mg^{2+} levels.

Aerobic Metabolic Pathways: Sustaining Functional Scope

Mitochondrial Functioning

The capacity and scope of aerobic energy metabolism has been identified as crucial in meeting long-term functional demands and in setting long-term tolerance to environmental parameters. It supports not only maintenance, but also crucial performances such as foraging, growth, and reproduction (**see also Energetics**: General Energy Metabolism). The capacity of aerobic metabolism supports the lifestyle of a species including its level of spontaneous or long-term activity. Such capacity is not only set by the capacity of the enzymes involved but is co-defined by the constraints imposed by the environmental abiotic regime. Constraints may be imposed, for example, by motor activity or by large temperature oscillations, when cool or warm temperature extremes cause the organism to exceed the limits of metabolic capacity. Constraints may also be imposed by ambient hypoxia, which reduces aerobic scope, or by elevated CO_2 levels, which may cause metabolic depression through disturbances in acid–base status. Shifted ion concentrations in ambient water (e.g., brackish vs. full-strength seawater) might enhance or reduce the costs for ion and acid–base regulation, thereby demanding a variable fraction of an organism's energy budget. Furthermore, acclimatization may occur and shift tolerance limits or windows, for example, when salmon or trout migrate between seawater and freshwater habitats or when temperate-zone fishes acclimatize, within limits, to a modified regime of ambient temperatures. The process of acclimatization may involve adjustments of metabolic capacities or shifts between metabolic pathways. For example, evolutionary adaptation to hypoxia involves improved mechanisms of oxygen uptake and a reduction in energy demand. In the cold, lipid metabolism tends to be emphasized more. In relation to climate zone and climate variability, temperature adaptation largely occurs by modulating metabolic capacities and thereby the level of standard metabolic rate and performance capacity.

Some generalizations are possible concerning how environmental and lifestyle constraints shape the level of aerobic scope available to an aquatic species. Oxygen limitations in hypoxic areas minimize aerobic scope for exercise. This reduction enables the organism to conserve available oxygen. As a trade-off, this reduction may still support successful competition by making room for the evolution of large-enough body sizes. Such trends may force all species of the hypoxic ecosystem into the slow lane. Such a phenomenon is also observed in polar oceans. Here it is hypothesized to be enforced by the obligatory exploitation of energy savings and stenothermy in the permanent cold. Conversely, aerobic scope might be maximized due to competition with other species at similar performance levels, up to the limits set to all competing species by environmental constraints, for example, hypoxia or permanently low temperature in cold oceans. As a trade-off between aerobic scope and body size, this may either limit aerobic scope or body size or both depending on environmental characteristics. Among a variety of possible patterns, body size is likely set to a maximum level in some species, associated with a reduction in aerobic scope (**see also Swimming and Other Activities**: Energetics of Fish Swimming).

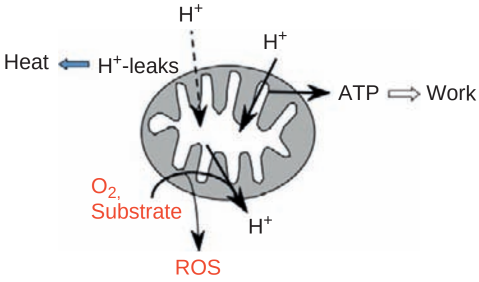


Figure 1 Scheme of main mitochondrial processes, which are associated with the capacities of ATP generation, including the oxidation of substrate through oxygen consumption, the buildup of mitochondrial membrane potential (proton motive force, $\Delta\psi$), and the rate of dissipative proton leakage as well as formation of oxygen radicals (ROS). All of these processes are subject to adaptive modification under the influence of environmental, functional, and lifestyle constraints.

Temperature adaptation involves the adjustment of aerobic scope of the whole organism (**see also Temperature**: Effects of Climate Change) and, as a precondition, of the scope of overall (per g tissue) mitochondrial capacities for substrate oxidation and ADP re-phosphorylation (**Figure 1**). First, mitochondrial capacities (per mg mitochondrial protein) fall upon cooling, following a simple Q_{10} relationship (the factorial drop in enzyme capacities with a 10° C drop in temperature) (**see also Temperature**: Effects of Temperature: An Introduction). Cold acclimatization or adaptation might involve an upregulation of mitochondrial capacity and/or of mitochondrial density to compensate for this Q_{10} effect. In liver, cold compensation is mainly accomplished by a rise in tissue mass, leaving mitochondrial protein per gram liver largely unchanged. Extremely high mitochondrial densities are found at low uncompensated functional capacities in red and cardiac muscle of Antarctic notothenioid fishes. Upregulation of enzyme capacities and mitochondrial respiratory capacity seems to be restricted to cold-exposed eurythermal fishes, for example, Northeast Arctic Atlantic cod (*Gadus morhua*). Some changes in the activities of membrane-bound enzymes are enabled by changes of membrane structure and enhanced membrane fluidity in cold-acclimatized fishes.

Conversely, warm acclimation should go hand in hand with reduced mitochondrial capacities as seen in temperate fish during summer, when citric synthase was reduced and cytochrome oxidase activities remained unchanged in white muscle of North Sea Atlantic cod. This response is not uniform, however, as seen in the Antarctic zoarcid (*Pachycara brachycephalum*) and the Antarctic notothenioid (*Pagothenia borchgrevinki*). Both species were shown to display some residual capacity to warm-acclimate, but their compensatory response differs from that in temperate fishes. In the polar species warm acclimation may reverse the strategy of energy savings in the cold resulting in elevated metabolic rates upon warm acclimation.

Cold-Induced Shifts to Lipid Metabolism

Development of enhanced mitochondrial density in the cold goes hand in hand with enhanced whole-body and intracellular storage of lipids and with the preferred use of lipids by mitochondria. Both trends are seen during cold acclimation in temperate fish (as in striped bass *Morone saxatilis* or rainbow trout *Oncorhynchus mykiss*) and, even more so, in cold-adapted polar fishes. It has been hypothesized that elevated mitochondrial contents provide tissues with a high capacity to maintain a high phosphorylation potential (see above). The low-energy turnover lifestyles in polar fishes would support a constantly high energy-status even further. Under these conditions, the glycolytic pathway is not cold-compensated, thereby favoring the use of noncarbohydrate substrates such as lipids. Interestingly, studies in plankton as well as in pelagic fishes show that elevated lipid levels are more typical of pelagic than benthic life forms. Their somewhat higher levels of motor activity would most likely be paralleled by somewhat higher rates of standard metabolism (SMR), as well as most likely higher mitochondrial densities and capacities. These trends would be stronger than in warm-water species as a consequence of mitochondrial proliferation at relatively low metabolic rates elicited by cold adaptation. High mitochondrial densities would lead to excess availability of mitochondrial intermediates such as citrate. Especially when costs are kept low during long periods of low to moderate activity levels in the cold and when phosphorylation potential is high, excess citrate is exported from the mitochondria into the cytosol where it fuels net fatty-acid synthesis. Energy savings are further supported by the gain of neutral buoyancy with elevated lipid levels.

Furthermore, elevated mitochondrial densities as seen in Antarctic fishes are paralleled by decreased myofibrillar volume fractions in the cell. The β-oxidation pathway located in the mitochondrial matrix is structurally favored in the cold over the glycogenolytic pathway, which is structurally associated with muscular fibrils. While both lipid synthesis and catabolism are favored in the cold in organisms at all levels of the food chain, the formation of high lipid stores will be supported even further at the upper end of the food chain, through the uptake of lipid-rich prey.

In summary, enhanced lipid accumulation and metabolism in the cold appear as by-products of increased mitochondrial densities and the associated shift to lipid metabolism. The enhanced cellular network of membranes associated with elevated mitochondrial densities has beneficial side effects by improving oxygen diffusion into the cell and to mitochondria. This helps in reducing the need for convective oxygen supply and thereby supports energy savings even further in the cold. Cost reductions are also involved, when muscle fibers are both fewer and larger, and capillary density is reduced as seen most clearly in Antarctic fish. This likely involves further energy savings due to reduced total surface area and ion-exchange activities of cellular membranes. These patterns of energy savings and reduced convective oxygen supply will, however, cause a narrowing of thermal windows in the cold, as a trade-off (**see also Temperature**: Effects of Climate Change).

Anaerobic Metabolic Pathways: Extending Limits to Function and Time

When ambient temperature approaches the upper or lower borders of the thermal window, oxygen deficiency generally may develop as a consequence of insufficient functional capacity. This deficit in functional capacity elicits transition to passive tolerance and associated systemic and cellular stress signals such as hormonal responses and oxidative stress. Importantly, passive tolerance also involves the use of anaerobic metabolic pathways and relies on sufficient anaerobic capacity. In fact, anaerobic metabolism may play an important role in setting the capacity of passive tolerance and accordingly, the length of this period. Here, the level of tolerance to temperature-induced hypoxemia benefits tolerance to ambient hypoxia and vice versa. Overall, mechanisms sustaining passive tolerance extend the capacity to survive under extreme conditions of temperature or hypoxia, but passive survival remains time-limited (**see also Temperature**: Effects of Climate Change).

While oxygen deficiency is reflected best by succinate accumulation in mitochondria, cytosolic anaerobic metabolism in fish muscle also responds to hypoxia. This anaerobic metabolism is always used when energy demand and metabolic rate exceed aerobic capacity, for example, during burst muscular activity for prey capture and predator avoidance (**see also Swimming and Other Activities**: Energetics of Fish Swimming). Reaching swimming velocities beyond a critical limit during short-term bursts depends on a large pool of high-energy phosphates, especially phosphocreatine, followed by the activation of anaerobic glycolysis and lactate formation, and finally adenylate degradation. Fully exploiting anaerobic capacity also depends on the capacity to first accumulate and then remove end products such as lactate and protons, including the ability to minimize and reverse associated pH disturbances. The increase in aerobic muscle design in the cold would explain why, as a trade-off, anaerobic capacity is reduced, especially in polar fish with their low-activity lifestyles. Accordingly, the contribution of anaerobic glycolysis during exercise is smaller in Antarctic notothenioids than in cold-acclimated temperate species. The contributions of the various pathways to supporting energy status (Gibb's free-energy change of ATP hydrolysis) during muscular exercise at various temperatures await further study. Recent comparative work in squid (*Lolliguncula brevis*) has shown that the various components of anaerobic metabolism are suitable to elevate the velocity reached during swimming, by buffering ATP levels and the Gibb's free energy of ATP hydrolysis and extending them above the limits required for ATPases to function. Such buffering occurs by the accumulation of free ADP and by glycolytic acidification. These principles would also apply to the time extension of passive tolerance during environmental extremes such as temperature or hypoxia, but on much longer timescales than during exercise (see below).

During cold acclimation, glycolytic enzyme activities increase in those temperate species that do not show large adjustments in aerobic metabolism (as in some salmonids). High mitochondrial densities constrain the space available for contractile proteins, glycogen stores, and glycolytic complexes. However, glycolytic capacity would decrease in species with a compensatory increase in oxidative capacity. Then, oxidative enzymes, creatine kinase, adenylate kinase, and AMP deaminase activities show cold compensation in Antarctic notothenioids, whereas glycolytic enzymes do not, at least in muscle. High mitochondrial densities constrain the space available for contractile proteins, glycogen stores, and glycolytic complexes.

Zoarcids are benthic sluggish fish with a low degree of spontaneous activity, such that the need for cold compensation in aerobic metabolism may be less than in more active species. This leaves some space for the compensation of anaerobic capacity. When cold-exposed (0° C), both cold-acclimated temperate (North Sea) and Antarctic zoarcids (*Zoarces viviparus* vs. *P. brachycephalum*) formed lactate in similar amounts during exercise, as seen in flounder (*Platichthys stellatus*), acclimated to 11° C. Accordingly, a similar degree of cold compensation of anaerobic pathways was possible in the temperate species as a result of cold acclimation and in the polar species, as a result of cold adaptation.

As a corollary, maximized aerobic cellular design in muscle of cold-acclimated or cold-adapted, slow-cruising pelagic fish is associated with minimized glycolytic capacity. By contrast, high-performance fish in tropical and temperate waters operate at lower mitochondrial densities but higher capacities than do polar fish. This allows them to maximize the use of both aerobic and anaerobic pathways and thereby swimming velocity. The pressure to enhance glycolytic capacity is likely alleviated in the

Antarctic and the deep sea where all ectothermic organisms, including both predators and prey, are tied to low-velocity largely aerobic lifestyles. Cellular space constraints and energy savings at cold temperatures are reflected in high mitochondrial densities, at the expense of myofibrillar density and, thus, contractile-force development.

These basic relationships are influenced by the change in body size during ontogeny of individual fishes. Baseline energy demand and, thus, SMR per unit fish and the need to maximize the aerobic machinery are reduced with increasing body size, thereby freeing cellular space for enhanced myofibril densities and, thus, force generation (**see also Energetics**: General Energy Metabolism). The reduction in mitochondrial density in larger fish opens cellular space for glycolytic components (glycogen granules, enzyme complexes, and buffer components), such that enhanced power generation during burst activity becomes possible.

In this context, an interesting observation is that maximum speed in Antarctic notothenioid fish seems more constrained by cold temperatures in larvae than in adults. Larval swimming velocity was 2–3 times below that of temperate and tropical larvae at their respective habitat temperatures. At first sight, this finding might either relate to the benthic origin of notothenioids or simply be due to the fact that high-performance predators do not exist for larval stages. However, both small body size and cold adaptation elicit similar adaptational traits in that mitochondrial densities are maximized. In adult (large) notothenioid fish, cold compensation led to mitochondrial densities close to cellular space limitations. The maximizing of aerobic capacity in small, including larval, fish therefore will likely reach an early limit in the cold. This may contribute to limited maximum swimming performance in larval notothenioid fish at cold temperatures (**Figure 2**). Energetic constraints on larval life are thus maximized in the cold, with the consequence that energy-saving strategies are maximized in larval, compared to adult, fish.

Extending Energetic Limits

There is a clear relationship between the use of carbohydrates and anaerobic pathways and the extension of exercise performance beyond aerobic limits. Similarly, this is true, also, for the capacity of passive perseverance under anoxia and thermal extremes. The onset of anaerobiosis beyond the critical swimming speed contributes to fatigue in muscle. A fall in pH affects the energy status of the muscle cells, quantified as the Gibb's free-energy change of ATP hydrolysis ($dG/d\xi$) and thereby the function of cellular ATPases (see above). At constant levels of the phosphates, a fall in pH initially causes a small

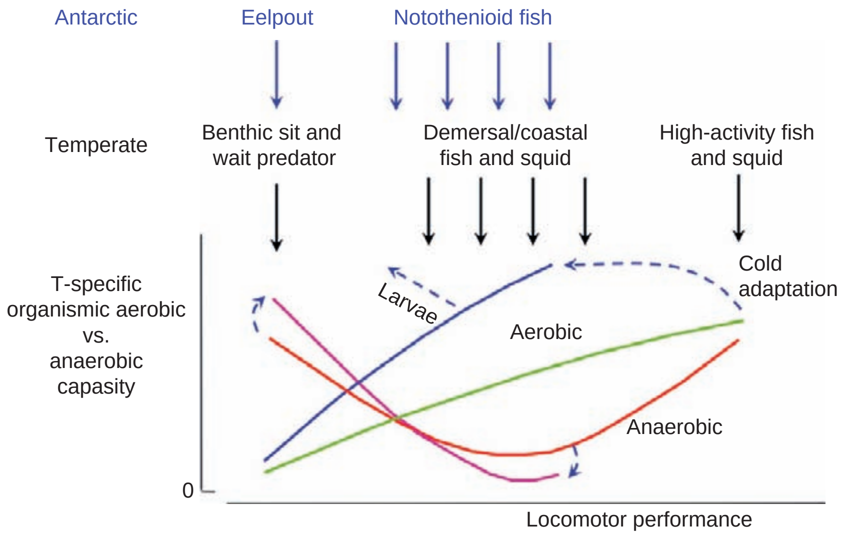

Antarctic
Eelpout
Notothenioid fish
Temperate
Benthic sit and wait predator
Demersal/coastal fish and squid
High-activity fish and squid
T-specific organismic aerobic vs. anaerobic capasity
Cold adaptation
Larvae
Aerobic
Anaerobic
0
Locomotor performance

Figure 2 Schematic depiction of whole-organism aerobic and anaerobic capacities in groups displaying different lifestyles and associated levels of locomotor performance in various temperature regimes. The graph shows the temperature-specific consequences of cold adaptation (broken arrows), i.e., consequences are compared at the same temperature. The figure illustrates how on a continuum between low and high levels of performance, the expression of anaerobic metabolic capacity follows a parabolic function (in temperate to warm-water species). Use of anaerobic metabolism is maximized in sluggish benthic species, minimized in moderately active aerobic cruisers and enhanced again in high-performance fish (and squid) at warmer temperatures. Cold adaptation (broken arrows) elicits reduced performance levels at maximized aerobic design like in pelagic Antarctic notothenioids (blue vs. green lines), especially for cold-adapted larvae (see text). In Antarctic fish, cold adaptation of anaerobic capacity (magenta vs. red lines) occurs in benthic sit-and-wait predators, but not in moderately active Antarctic fish. Here, anaerobic capacity appears reduced, as a trade-off in the maximization of aerobic design. Note that the figure does not differentiate between cold-adapted stenotherms and eurytherms.

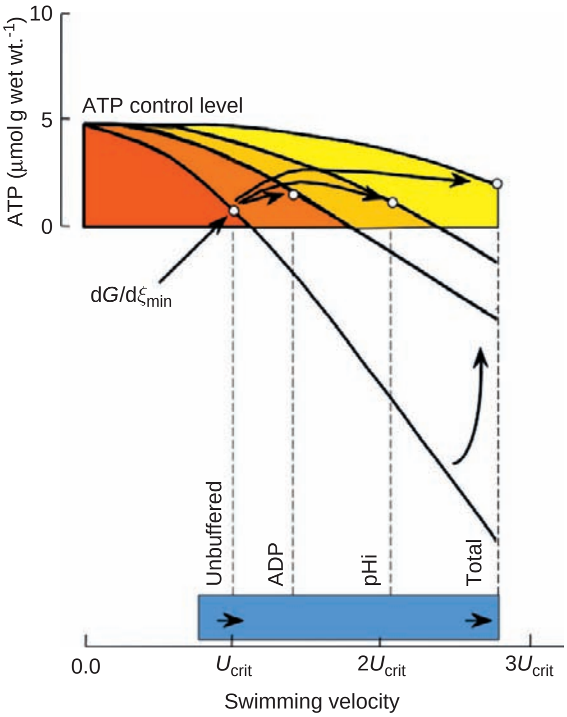


Figure 3 The role of the phosphagen system and anaerobic glycolysis in extending the period and velocity of swimming beyond the critical swimming speed U_{crit}. From a metabolic perspective this critical limit is best defined from the onset of supplementary anaerobic energy production. The figure adopts the respective patterns and model calculations as available for squid and extrapolates the principles into a general model to be tested in fishes. It illustrates how anaerobic metabolism provides power for time-limited swimming at supra-critical speeds. The buffering of ATP levels and Gibbs free-energy change of ATP hydrolysis is elicited by the combined effects of accumulation of free ADP levels and of intracellular acidification due to anaerobic glycolysis, both eliciting transphosphorylation from the phosphagen. Note that the present approach differs from the classical swimming protocols in fish physiology. The time protocol co-determines the swimming speed reached beyond U_{crit}. Maximum attainable velocity results higher as the swimming period beyond U_{crit} decreases.

reduction in standard and *in vivo* ATP free-energy change values by about 2 kJ mol^{-1} until a minimum is reached below pH 7.0. Further decrements follow the depletion of ATP and the phosphagen and the accumulation of ADP and inorganic phosphate (**Figure 3**).

The buffering of ATP levels leads to a buffering of ATP free-energy-change values, which are decreased initially by the accumulation of inorganic phosphate from net phosphagen depletion. Actually, this decrease can be considered advantageous, because both the acidosis and the accumulation of inorganic phosphate have some protective effect on ATP concentrations. As a consequence, the minimum level of ATP free energy is reached at higher ATP contents with the use of the phosphagen than without (**Figure 3**).

The accumulation of inorganic phosphate is very likely involved in limiting the degree of muscular activity or reducing the maximum swimming velocity. These processes are interpreted as early signs of fatigue. Inorganic phosphate may actually start to slow muscular performance at levels of ATP free energy higher than the minimum, limiting level. This may be beneficial when extending the time period during which anaerobic metabolism is to be used at low activity levels, for example, in hypoxic environments. At low performance levels, the rate of anaerobic glycolysis is low and the degree of free ADP accumulation small causing a delay in the depletion of the phosphagen and also less of a decrease in the Gibb's free-energy change of ATP hydrolysis over time. This reduced performance level keeps ATP free-energy change at higher levels. Reduced performance levels beyond the anaerobic threshold may be supported through the combined effects of low pH and elevated inorganic phosphate levels on muscular performance.

The anaerobic threshold is set low when oxygen availability is low, for example, due to ambient hypoxia. Reducing the energy requirements for locomotion in hypoxic environments delays the depletion of anaerobic resources including the phosphagen and also delays fatigue through a slower accumulation of inorganic phosphate. Development of an acidosis occurs unchanged and may support a reduction in performance prior to fatigue. Work in invertebrates shows in fact that intracellular pH falls more rapidly in hypoxia-tolerant species and may cause a protective decrease in performance before the energy status reaches a critically low level. A decrease in performance induced by acidosis is typically observed in many types of muscle tissue and may be protective during ischemia, long-term environmental stress, or extended periods of anaerobic exercise.

The acidosis also protects the levels of the adenylates and the Gibbs free-energy change of ATP hydrolysis. If metabolic expenditure is low, such as during hypoxia, effective lowering of pH$_i$ through ion exchange may contribute to a long-term use of anaerobic resources. The patterns of pH$_i$ regulation thus can strongly modulate the use of anaerobic resources. Progressive acidification first supports maximum levels of activity but then sets limits to the level of performance and, finally, the period of anaerobic exercise. These considerations can be extrapolated to strategies extending periods of passive tolerance to environmental extremes. Here the balanced use of anaerobic resources and energy stores also involves the downregulation of intra- and extracellular pH as seen in some animal models. In this case, the pH decrements elicit metabolic depression and thereby a time extension of passive tolerance.

As a corollary, aerobic scope sets and limits the capacity for long-term and long-distance cruising at high velocities. Similarly, the capacity for anaerobic performance sets the maximum energy turnover required for escape or attack on short timescales. In addition, it likely contributes to set the length of time when anaerobic metabolism is exploited under extreme environmental conditions, for example, during excursions into hypoxic environments linked to food uptake or during passive long-term sustenance under anoxia or thermal extremes.

See also: **Energetics**: General Energy Metabolism. **Swimming and Other Activities**: Energetics of Fish Swimming. **Temperature**: Effects of Climate Change.

Further Reading

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* 21: 1183–1226.

Chen Z, Cheng CH, Zhang J, *et al.* (2008) Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish. *The Proceedings of the National Academy of Sciences* 105: 12944–12949.

Egginton S, Skilbeck C, Hoofd L, Calvo J, and Johnston IA (2002) Peripheral oxygen transport in skeletal muscle of Antarctic and sub-Antarctic notothenioid fish. *Journal of Experimental Biology* 205: 769–779.

Finke E, Pörtner HO, Lee PG, and Webber DM (1996) Squid (*Lolliguncula brevis*) life in shallow waters: Oxygen limitation of metabolishm and swimming performance. *Journal of Experimental Biology* 199: 911–921.

Guderley H (2004) Metabolic responses to low temperature in fish muscle. *Biological Reviews* 79: 409–427.

Hardewig I, Peck LS, and Pörtner HO (1999) Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. *Journal of Comparative Physiology B* 169: 597–604.

Johnston IA, Calvo J, Guderley H, Fernandez D, and Palmer L (1998) Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *Journal of Experimental Biology* 201: 1–12.

Kassahn K, Crozier RH, Pörtner HO, and Caley MJ (2009) Animal performance and stress: Responses and tolerance limits at different levels of biological organisation. *Biological Reviews* 84: 277–292.

Kieffer JD (2000) Limits to exhaustive exercise in fish. *Comparative Biochemistry and Physiology A* 126: 161–179.

McClelland GB, Craig PM, Dhekney K, and Dipardo S (2006) Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish *(Danio rerio) Journal of Physiology* 577: 739–751.

Plaut I (2001) Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology A* 131: 41–50.

Pörtner HO (2002) Physiological basis of temperature dependent biogeography: Tradeoffs in muscle design and performance in polar ectotherms. *Journal of Experimental Biology* 205: 2217–2230.

Pörtner HO (2006) Climate dependent evolution of Antarctic ectotherms: An integrative analysis. *Deep Sea Research II* 53: 1071–1104.

Pörtner HO, Finke E, and Lee PG (1996) Metabolic and energy correlates of intracellular pH in progressive fatigue of squid (*Lolliguncula brevis*) mantle muscle. *Americal Journal of Physiology* 271: R1403–R1414.

Pörtner HO and Lannig G (2009) Oxygen and capacity limited thermal tolerance. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Hypoxia. Fish Physiology,* vol. 27, pp. 143–191. Burlington: Academic Press.

Pörtner HO, Lucassen M, and Storch D (2005) Metabolic biochemistry: Its role in thermal tolerance and in the capacities of physiological and ecological function. In: Farrell AP and Steffensen JF (eds.) *The Physiology of Polar Fishes: Fish Physiology,* vol. 22, pp. 79–154. Burlington: Academic Press.

Wieser W (1995) The energetics of fish larvae, the smallest vertebrates. *Acta Physiologica Scandinavica* 154: 279–290.

Applied Aspects of Fish Swimming Performance

T Castro-Santos, USGS Biological Resources Discipline, Turners Falls, MA, USA

Published by Elsevier Inc.

Background
Swimming Performance Data – Where Does It Come from?
Limitations of Available Data
Further Reading

Background

Any collection of articles on fish physiology would be incomplete without a thorough treatment of locomotion. Swimming, after all, is a unifying character of the vast majority of fishes. Locomotion determines a fish's ability to access habitats, defend territories, secure mates, capture prey, and escape from predators – it is clearly a fundamental aspect of the fitness of mobile organisms.

When we think of the physiology of locomotion, we are thinking about what fish are capable of doing: how biochemistry, muscle structure, innervation, circulation, and morphology all interact with the hydraulic environment to create opportunities for, and limits to, a fish's ability to move. On its own, physiology defines the limits to performance, and there are clear implications for fitness and adaptation. By association, this means there are also implications for management and conservation.

It is important to recognize, however, that physiological capacity is not tantamount to performance. It is what organisms do with their abilities – how they behave – that ultimately determines actual performance. Because of this, it is not possible to meaningfully review the conservation implications of physiology – or fitness implications generally – without integrating behavior into the discussion. This article considers performance to be the output of both physiology and behavior, and it is from this perspective that the ecological relevance of swimming performance can best be understood.

Threats to Fisheries Conservation at the Dawn of the Third Millennium

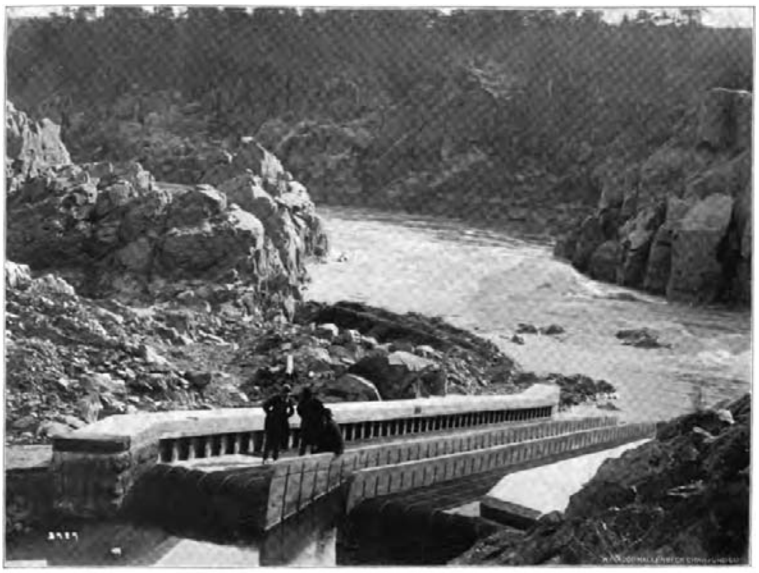
There has never been a time when environmental conservation and stewardship have been more important. When it comes to the conservation of fisheries, the consequences for humans are often dire. As human economies and populations grow, we turn increasingly to fisheries as a source of protein, and both commercial and artisanal fisheries are harvesting the oceans and waterways well beyond sustainable levels. In many commercial fisheries, most of what is captured is discarded as bycatch, creating a situation where fishing pressure affects not only target populations but nontarget ones as well.

Simultaneously, the by-products of industry and industrial agriculture put fisheries at unprecedented risk. Chemical pollution and eutrophication, sedimentation and turbidity, thermal pollution, and outright water withdrawals all act to reduce the resilience and productivity of the environments that we expect to support these fisheries.

In riverine environments, no single activity has posed a greater threat to fisheries than has the development of hydropower. This has been recognized for well over a century: The eighteenth and nineteenth centuries saw a proliferation of mill and logging dams built throughout Europe and eastern North America, leading to widespread losses of economically and ecologically important fish species (**Figure 1**). These losses were not limited to the riverine environment: many of the species affected by dams were diadromous migrants, species that routinely migrate between the marine and freshwater environments. These populations acted as vehicles conveying nutrients between ecosystems, and it long has been understood that, in many cases, losses of anadromous populations were also causing the depletion of coastal fisheries.

Dam development did not end with the Industrial Revolution. At the end of the nineteenth century, the invention of the electric dynamo provided the technological basis for the hydroturbine. This created an economic incentive for development of more and much larger dams, leading to further increases in the rate of dam development (**Figures 2** and **3**). Hydropower development slowed in North America and Europe by the late twentieth century. In recent years, however, developing economies in China, Brazil, and elsewhere have driven a global expansion in the number and scale of dam construction. Today, economic development coupled with concerns of global climate change mean that the value of hydropower, and hence, the scale of hydropower development, will continue to increase.

The effects of dams on riverine ecosystems are multifold. First, they alter habitat, converting lotic environments characterized by high velocities and turbulence (and thus high values of total kinetic energy) into a series of lentic pools with reduced kinetic energy and increased



ORIGINAL McDONALD FISHWAY WITH WATER SHUT OFF, IN GREAT FALLS OF THE POTOMAC.

Figure 1 McDonald fishway developed in 1850 to provide passage for migrating fish at milldams. This and several other fishways were developed in the nineteenth century without benefit of biological data – fish passage performance was almost universally poor. Reproduced from Cheney AN (1898) Fishways, their construction and use. In: *Third Annual Report to the Commissioners of Fisheries, Game, and Forests of the State of New York*, pp. 222–233. New York: Wynkoop Hallenbeck Crawford Co.

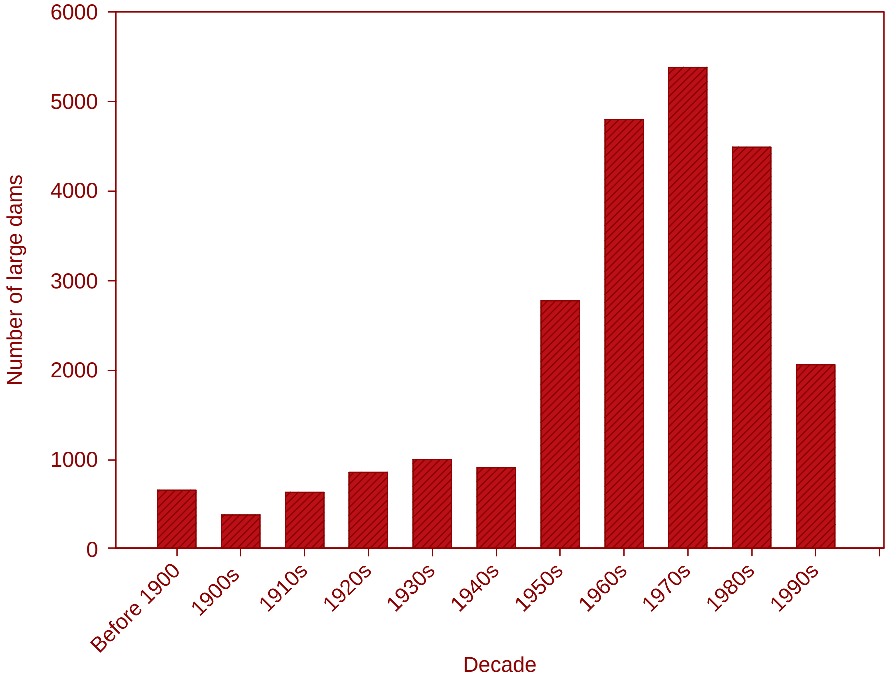


Figure 2 Worldwide large dam construction by decade; data do not include China. Note that this excludes millions of small (<10 m high) dams, many of which were built before 1900. From World Commission on Dams (2000) *Dams and Development: A New Framework for Decision-Making*. London: Earthscan Publications.

thermal stratification. This fundamentally alters habitat character, allowing for sediment deposition in riverbeds, raising or lowering temperatures, and otherwise creating environments that may be incompatible with native species, but that may promote non-native predators and other invasive species. In addition, behavioral cues used to navigate waterways may no longer be appropriate for traversing reservoirs, creating a situation in which traits such as swimming performance and navigation that evolved in lotic environments are no longer adaptive.

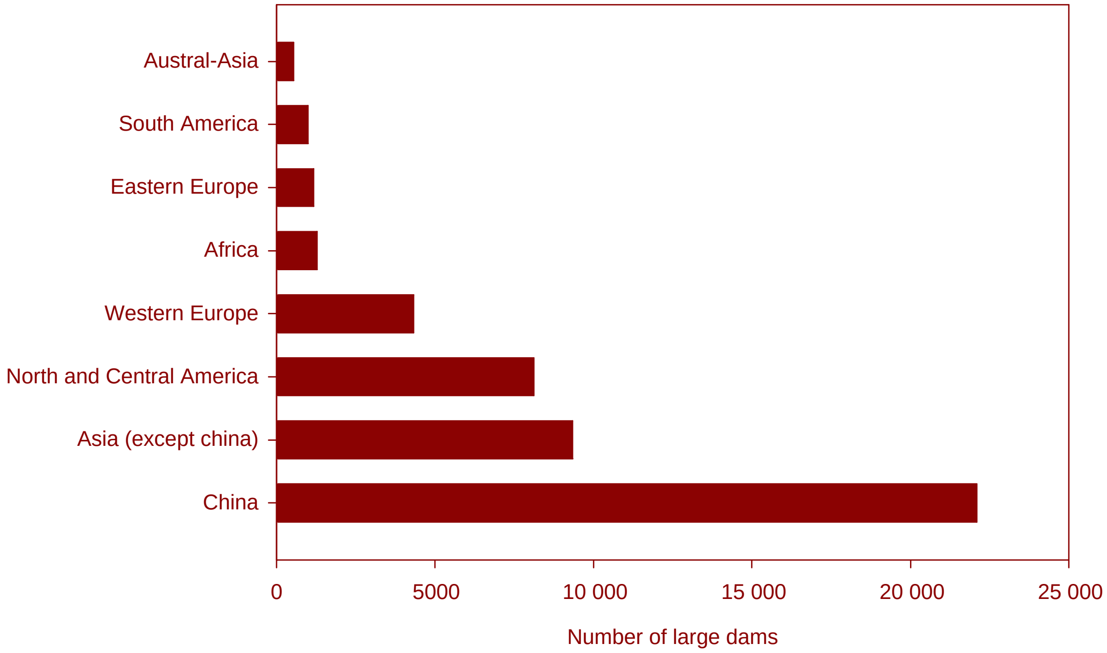


Figure 3 Large dam construction by geographical region. Note the massive construction in China, nearly all of which has occurred during the last 60 years. From World Commission on Dams (2000) *Dams and Development: A New Framework for Decision-Making*. London: Earthscan Publications.

Perhaps even more important than altering habitat, dams create barriers to movement. This is obviously important for philopatric (homing) anadromous species such as salmon – without access to their natal habitat, these populations can quickly go extinct. Downstream migrating juveniles, as well as adults of iteroparous and catadromous species, are also faced with the difficulty of finding passage routes around the dams and avoiding passage through turbines (**Figure 4**).

Barriers degrade habitat for nonmigratory species as well, reducing gene flow among resident populations and restricting access to important habitats. The development and application of telemetry technology in recent years has shown that movement along river and stream corridors is a much more ubiquitous and important trait than was thought previously, and even partial barriers to movement can cause subtle and chronic reductions to habitat quality. Importantly, the ability to traverse barriers is closely tied to swimming performance and behaviors. This means that development of mitigation measures to counter these dam effects will require a strong and holistic understanding of fish locomotion.

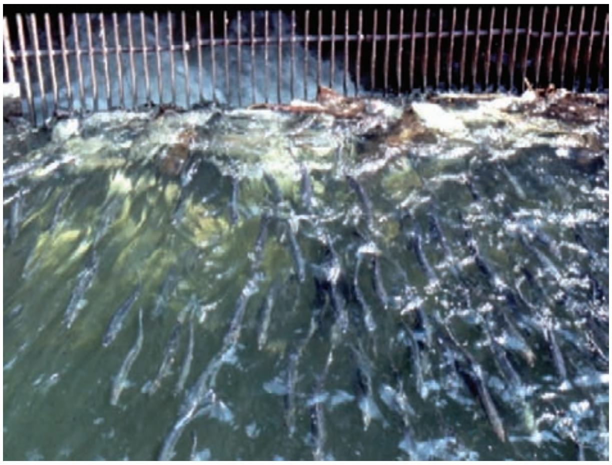


Figure 4 Adult American shad being impinged on powerplant intakes. Photo courtesy of Boyd Kynard.

In addition to dams, water withdrawals create situations in which large numbers of fish are killed. Withdrawals serve a number of functions, including cooling water for power plants, municipal water supplies, and agriculture. Rates of water withdrawal vary greatly around the world, with developed countries using far more per capita than do developing countries. As global economies develop, demand for water will continue to grow, with individual facilities often processing large proportions of local water bodies. Fish that are entrained at these facilities suffer very high mortality rates – designing intake structures to avoid entraining fish has become an important challenge.

Industrial and municipal withdrawals are often coupled with heated discharge. This is a major source of elevated temperatures in many water bodies, and compounds the ongoing effects of habitat destruction and climate change. As a result, temperatures of freshwater systems around the world are increasing at rates even greater than those that would be predicted from climate change alone.

Swimming performance has important implications for each of the conservation issues summarized above. Swimming ability can determine whether, and to what extent, a given population is susceptible to overfishing. Likewise, engineering criteria for providing fish passage at dams and exclusion from water withdrawals rely on swimming performance data. Finally, chemical and thermal alterations to habitat, whether from industrial effluent or from climate change are well known to affect swimming capacity, behavior, and energetics. Because of this, a good understanding of swimming ability, founded on appropriate and reliable data, is essential to the development of sound management practices, as well as solutions to mitigate for human activities.

Swimming Performance Data – Where Does It Come from?

Much of what we know about fish swimming performance reflects the historical context of social values and perceived needs. Some of this interest has stemmed from applications that have little to do with fish. For example, the salmonid body form influenced the development of hydrofoils in the eighteenth century, and the processes by and efficiency with which fish produce thrust continue to influence nautical engineering today.

Despite these interesting applications, conservation issues have consistently influenced the scope of scientific investigations. By the late nineteenth century, fisheries managers had become aware of the threats posed by milldams, and the resulting population declines of commercially important fishes in Europe and North America were viewed as one of the most important challenges confronting fisheries managers at the time. Several fishways – structures designed to provide routes of passage around dams for migrating fish – were developed during this period, but these structures were almost universally ineffective. Coincidentally, the field of fluid mechanics was undergoing rapid advances – contributions by Osborne Reynolds and others permitted the merger of the fields of biomechanics and hydraulic engineering. The first half of the twentieth century saw concerted efforts by hydraulic engineers to apply these new fields to fishway design in Europe, Great Britain, and North America. Although reliable methods for quantifying swimming performance were lacking, the importance of the topic was recognized, and engineers began testing their designs in laboratories using live fish (**Figure 5**).

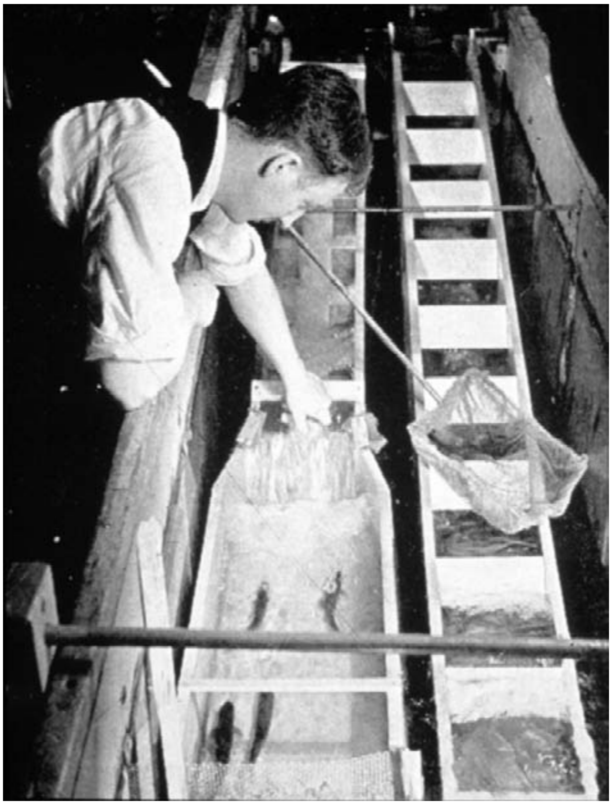


Figure 5 Fishway designs being tested at the University of Iowa hydraulics laboratory during the 1930s. Engineers at the time recognized the importance of empirical testing with live fish to verify performance. From McLeod AM and Nemenyi P (1940) *An Investigation of Fishways*. Iowa City: State University of Iowa.

During this period, biologists became interested in fish locomotion from the perspective of the nascent fields of ecology and evolutionary biology. Swimming performance and biomechanics developed into an area of basic research pursued independent of management and conservation concerns. Nevertheless, two areas of study arose from these efforts that have substantial conservation implications today. The first is the field of functional morphology – specifically the classification of different body forms and locomotor modes, which have substantial implications for swimming ability and behaviors as well as designs for protective devices (**see also Buoyancy, Locomotion, and Movement in Fishes**: Maneuverability). The second is the field of bioenergetics, whereby the energy consumed during locomotion and other activities can be related to ecology and trophic interactions (**see also Energetic Models**: Bioenergetics in Ecosystems).

Interest in bioenergetics led to the development of respirometers for quantifying swimming energetics. Several designs were developed, with the most popular being designs by Brett (**Figure 6**) and Blazka (**Figure 7**). These became important tools for a variety of applications and are still in widespread use today.

Although Brett was perhaps best known for his work on swimming energetics, he also developed two measures of swimming performance that have been widely used by

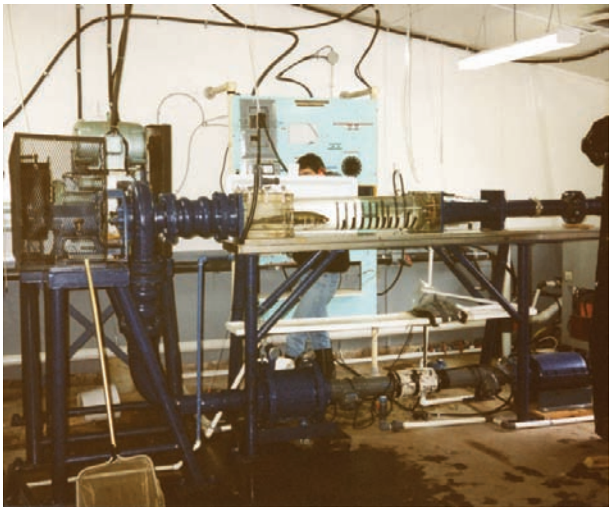


Figure 6 Photo showing the Brett respirometer. Fish are confined in an acrylic chamber and flow is regulated using a centrifugal pump. Note the long straight section on the upstream end: this helps establish uniform flow characteristics throughout the cross section of the swim chamber. Photo courtesy of Scott Hinch.

both researchers and managers. The first and most frequently replicated measure he called U_{crit}, or critical swimming speed. During the course of his early work, Brett noticed that salmon were able to sustain slow swimming speeds more or less indefinitely without fatiguing. This had an obvious application for salmon smolts that encountered hydroelectric dams as they migrated to the ocean. Brett assumed that if intake velocities were kept below the maximum sustainable swim speeds, then fish should never become entrained into turbine intakes, thus making it possible for them to seek and use safer alternate routes. Toward this end, Brett developed a standardized protocol whereby fish were placed in a swim chamber and subjected to incremental increases in flow velocity. Each increment was nominally set to one-half body length per second for a 60-min increment (subsequent researchers have used a range of velocity and time increments). U_{crit} is calculated as the final flow velocity, adjusted downward by the proportion of time remaining in the final time increment (**Figure 8**).

The velocity threshold below which swimming can be sustained indefinitely is ultimately governed by the physiology of the fish, that is, by its aerobic scope. This is largely a function of the quantity and distribution of mitochondria-rich musculature, and as such, varies with body size and species (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops). Most fish are ectotherms, meaning they are unable to regulate body temperature. Because of this, performance is typically reduced at temperatures both below and above what fish typically encounter, with optimum performance occurring over a relatively narrow range of temperatures. At low temperatures, their metabolism, and hence aerobic scope, is reduced. At high temperatures, the maintenance metabolic costs increase at a greater rate than the associated benefits, and eventually metabolism itself begins to fail. The range of thermal optima varies by species and represents a combination of physical limits and evolution; for example, cold-water-adapted species have lower thermal optima than warm-water-adapted species. For tropical

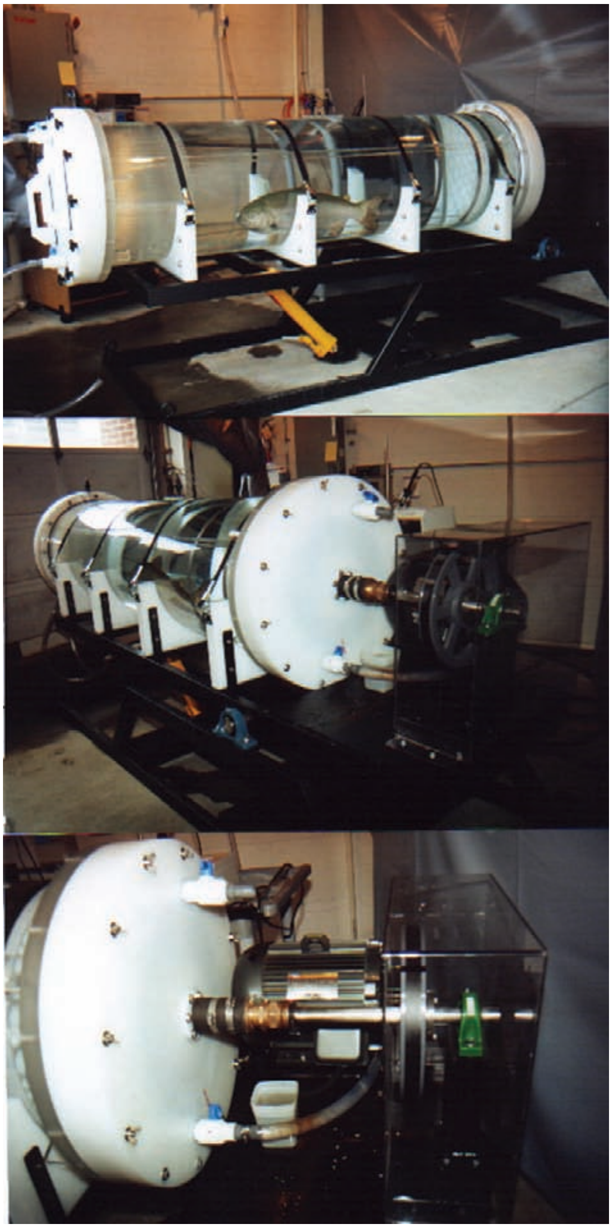


Figure 7 Images of a Blazka-type respirometer. The design incorporates two coaxial tubes. Fish are housed in a swim chamber in the internal tube, and an axial pump drives flow through the enveloping tube. These respirometers typically have a lower volume than Brett-type respirometers (**Figure 6**). Photo courtesy of Matthew Mesa.

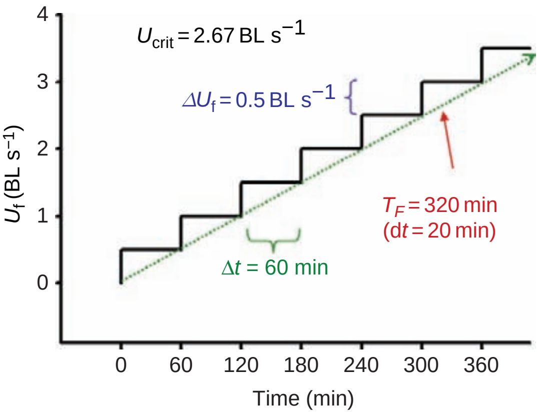


Figure 8 Schematic of U_{crit} protocol showing increments of velocity (ΔU_{f}) and time (Δt). Note that swim speed equals flow speed, and that U_{crit} interpolates the maximum speed that can be maintained for the interval duration, Δt.

migratory species (**Figure 9**), this may be advantageous. If these species' scopes for activity are greater, fish-passage solutions may be possible at the many new dams being built in the tropics that would not be possible in temperate regions.

The second important swimming performance metric that Brett helped develop was the relationship between swim speed and fatigue time (SSFT) for fish that are swimming at speeds greater than their maximum sustained swim speed (**Figure 10**). During the course of his studies, Brett noticed (and many subsequent authors have confirmed) the existence of two distinct modes of nonsustainable swimming. Once speeds exceed sustained levels, fish begin to recruit anaerobic musculature to power swimming. Often, this is noticeable by the onset of the so-called burst-and-coast swimming, a kinematic shift whereby fish use both aerobic and anaerobic

Figure 9 Freshwater Riches of the Amazon, by Ray Troll (www. trollart.com). The faunal diversity shown here is typical of the Amazon and other tropical rivers. Thousands of species inhabit these rivers, but little is known about swimming abilities or migratory habits. Extensive damming projects in development will likely impede movements of many of these species.

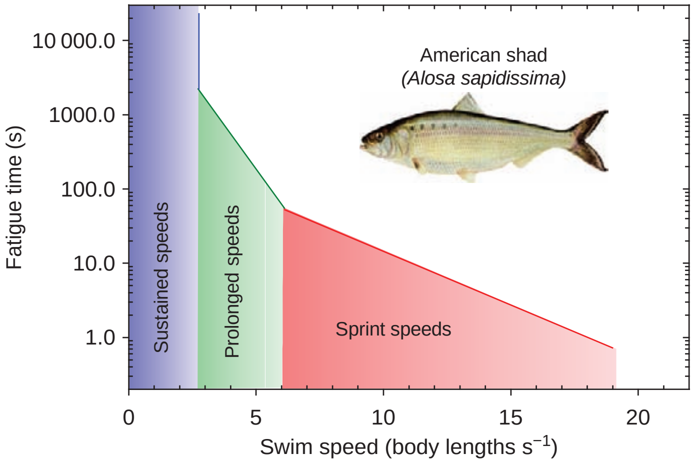


Figure 10 Swim speed–fatigue time relationship of American shad (mean body length = 45.5 cm). Similar relationships exist for many teleosts, although scope, scale, and slope can vary considerably by species and morphology.

physiology to power locomotion. This is typically called the ‘prolonged mode’, and Brett found that, for salmon, this mode characterized speeds that resulted in fatigue in more than 20 s. At some point, however, it is no longer possible to power locomotion with aerobic musculature, and fish enter a pure burst, or sprint mode. These two modes have distinct swim speed–fatigue time relationships, typically being much steeper in prolonged mode than in sprint mode.

Thus, sprinting comprises the broadest range of swim speeds, although endurance is necessarily shortened in its duration compared with prolonged mode. The relationship between swim speed and fatigue time has broad ecological relevance, (e.g., governing prey capture and predator avoidance). It also has important implications for fisheries and can influence capture of fish by mobile trawls and other common fishing gear (**Figures 11** and **12**). Because of this, researchers interested in bycatch reduction and gear efficiency have conducted many studies that have greatly contributed to our knowledge of swimming abilities of marine species.

As mentioned above, swimming performance is not fixed by species or even individual. An individual’s size can have dramatic effects on swimming ability, as can environmental conditions. The effect of size on swimming ability is largely a function of mechanics: larger fish typically have more propulsive area and a larger muscle mass, and so are able to move at greater absolute speeds. Because of this size effect on absolute swimming speed, swim speeds are often normalized by dividing by body length, and are reported in body lengths per second. Over a relatively small range of body sizes, this is a reasonable and effective approach. The danger of the approach, however, is that it assumes that

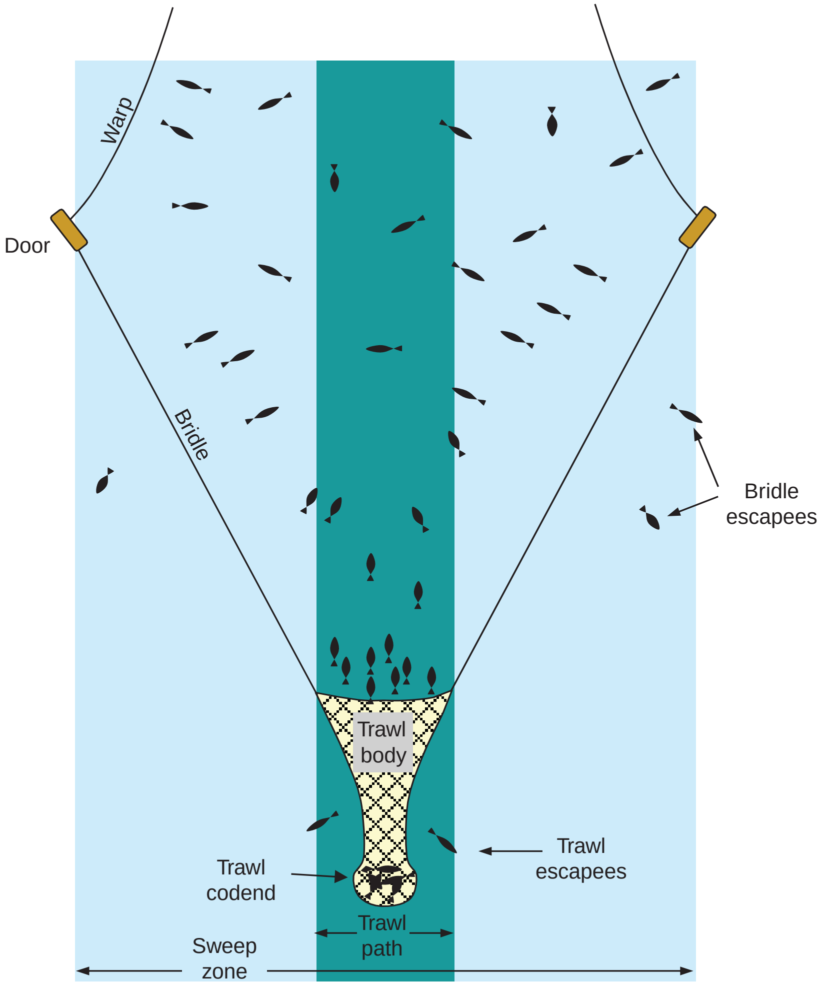


Figure 11 Schematic showing path of a typical otter trawl. Ability to escape depends on both swimming ability and behavior. Fish that are corralled by the bridle sweeps are more likely to be captured than those that swim across the sweeps. Capture likelihood is also affected by escape distance, speed of trawl, and mesh size. Redrawn from Castro-Santos T and Haro A (2006) Biomechanics and fisheries conservation. In: Shadwick RE and Lauder GV (eds.) *Fish Physiology Volume 23: Fish Biomechanics*, pp. 469–523 New York: Academic Press.

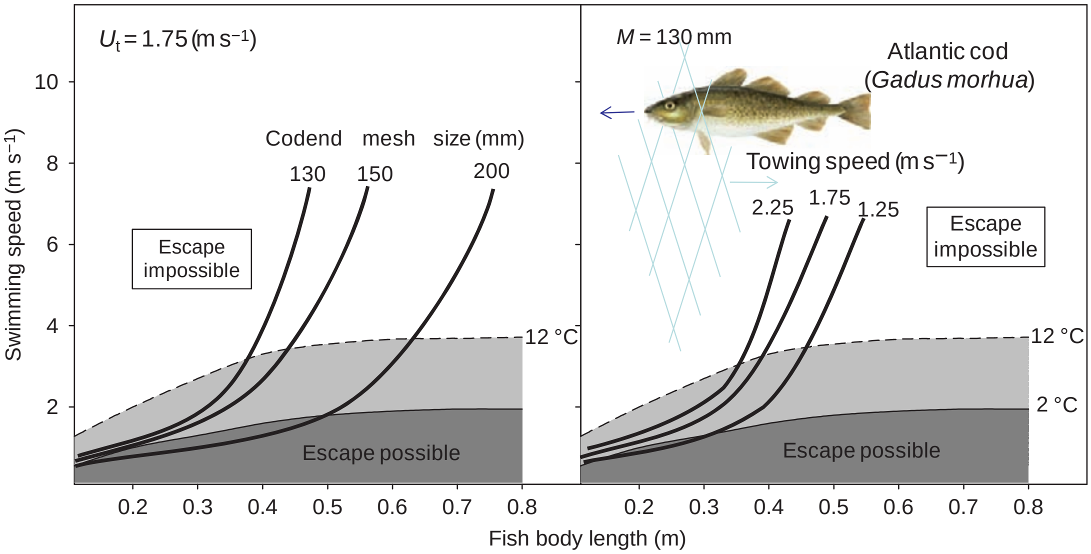


Figure 12 Effect of swimming ability, trawl speed, and mesh size on escape potential for Atlantic cod from the codend of an otter trawl. Shaded areas indicate zone in which escape is possible at 2 °C and 12 °C. Solid curves represent swimming speed required to escape through meshes (M) of different sizes (left panel) and through a fixed sized codend mesh at a range of tow speeds (U_t right panel). A vertical line drawn from the body size of the fish that intersects the solid curves within a shaded area indicates that it is possible for the fish to escape through the codend mesh at a given temperature. This type of analysis holds the potential for identifying gear design and fishing strategies that can target specific size classes and species. The information could be used to protect nontarget species and life stages as well as to improve fishing efficiency. He P (2010) Swimming capacity of marine fishes and it role in capture by fishing gears. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion, an Eco-Ethological Perspective*, pp. 484–512. Enfield, NH: Science Publishers.

swimming performance is isometric, that is, it changes uniformly with size. In fact, this is not the case: smaller individuals of a given species tend to have a greater relative performance than larger individuals, that is, swimming performance is allometric. This arises for several reasons, including allometry in morphology and physiology (**see also Swimming and Other Activities**: Energetics of Fish Swimming and **Energetics**: Physiological Functions that Scale to Body Mass in Fish). One consequence of this is that, in the absence of appropriate data, managers sometimes set velocity criteria for fishways and water withdrawals based on relative flow velocities, using data from very different-sized animals than those that they are trying to protect. Resolution of scaling effects on locomotion remains an area of active research and is of substantial interest to both managers and researchers.

Limitations of Available Data

The ease with which U_{crit} and SSFT can be measured (particularly the former) has made these valuable tools for laboratory studies. Because of this, there has been a proliferation of studies reporting U_{crit} for a large number of species under a range of conditions. The availability of this information and its apparent relevance to fish passage and protection have led to its ubiquitous application in fish-conservation measures.

However, just because data are available does not mean that they are applied appropriately or that they are even applicable to conservation efforts. Unfortunately, many examples exist of such inappropriate applications of swimming performance data. The reason so many studies have used the U_{crit} and related protocols is that these techniques are readily performed in a laboratory setting, where fish are confined to small chambers under very controlled conditions. These conditions may not exist in the field, however, and the apparatus used to collect the data prevents fish from exhibiting the full range of behaviors that are available to free-swimming fish. This means that performance in these chambers consistently underestimates natural capabilities. Moreover, no method has been developed using these structures that quantifies the willingness of fish to volitionally swim at a given speed, or against a particular current velocity. This is particularly important for upstream-migrating fish. Many of these migrants are strongly rheotactic, adapted to seek out zones of high-velocity flow used as cues for orientation and navigation.

This has important implications for design of fishways and other obstacles such as culverts at road crossings. Fish often show a reluctance to enter such structures; in the absence of a strong velocity jet, the resulting low entry rate leads to poor passage, regardless

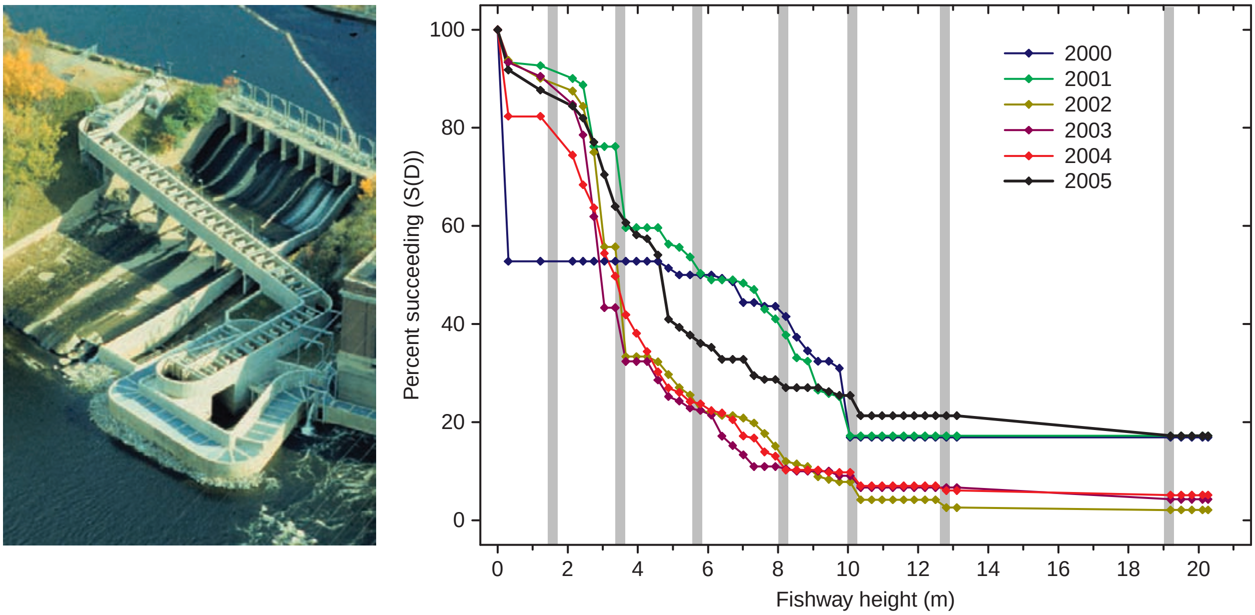


Figure 13 A fishway designed to pass Atlantic salmon and American shad. Although both species are sufficiently strong swimmers to pass this structure, their behaviors and tolerances are different: salmon pass the structure with ease, but only about 10% of American shad that enter pass successfully (right panel). Developments in telemetry technology have greatly improved the ability of researchers and managers to evaluate structures like this, but fewer than 1% of fishways have ever been rigorously evaluated.

of structure design. Because of the data limitations described above, management recommendations systematically underestimate the ability of fish to traverse these barriers, and, in their efforts to avoid creating velocity barriers, they err in the opposite direction and fail to provide sufficient stimulus for the fish to enter. In this way, management solutions intended to eliminate barriers can be ineffective (**Figure 13**), potentially causing more harm than benefit.

The limitations of existing swimming data – as well as potential solutions – have been made evident by a series of recent studies using open-channel flumes and volitionally swimming fish. Hydraulics in these flumes can be controlled to produce a broad range of velocities, and fish are provided a low-velocity zone or staging area downstream of the channel from which they are able to enter the channel at will (**Figures 14** and **15**). This method has been used by Stephen Peake and his

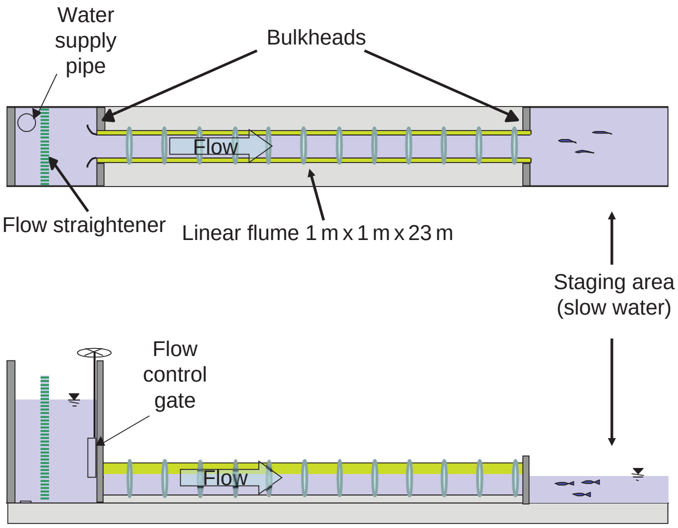


Figure 14 Open-channel flume used for volitional swimming studies. Velocities within these flumes are regulated by the control gate and flow source; the staging area downstream of the flume provides fish with a low-velocity zone from which they enter the flume volitionally. Progress up the flume can be monitored with video, or using telemetry arrays (indicated by ellipses).

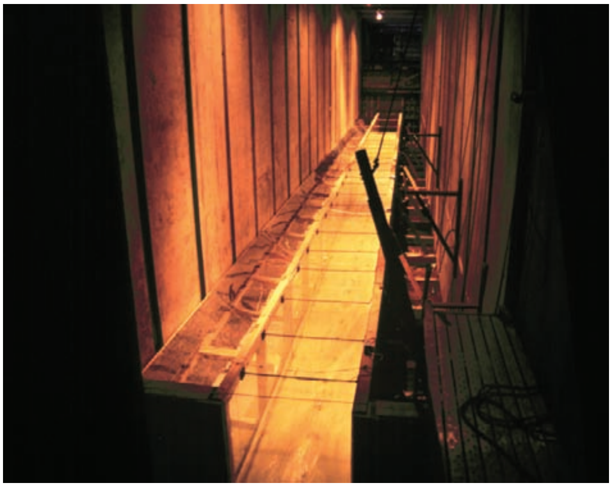


Figure 15 Open-channel flume prototype at the S.O. Conte Anadromous Fish Research Center, a large-scale facility designed to test full-scale prototypes of fishways and related structures. Fish have been tested in this flume against velocities of 0.5–5.0 $\mathrm{m\,s^{-1}}$.

colleagues to estimate sustained swimming speeds. The author and his co-workers have developed a similar approach that provides estimates of swim-speed versus fatigue-time relationships, as well as the extent to which a given velocity motivates fish to enter. Because of these structures' considerable length, it is also possible to measure directly the ability of fish to swim a given distance against a range of velocities, eliminating the need for (in fact, challenging) many of the assumptions about swimming performance that underlie current fishway designs.

Another advantage of studying volitional swimming performance is that it provides empirical evidence for not only what fish are capable of doing, but also what they are actually willing to do, and how well they do it. For example, for any flow velocity there exists an optimum swim speed that maximizes the distance a fish is able to traverse (**Figures 16–18**). Fish that swim too fast or too slow fatigue at shorter distances. The ability of fish to approximate their distance-maximizing optimum appears to be under selective control: anadromous species that must traverse high-velocity zones tend to do a better job

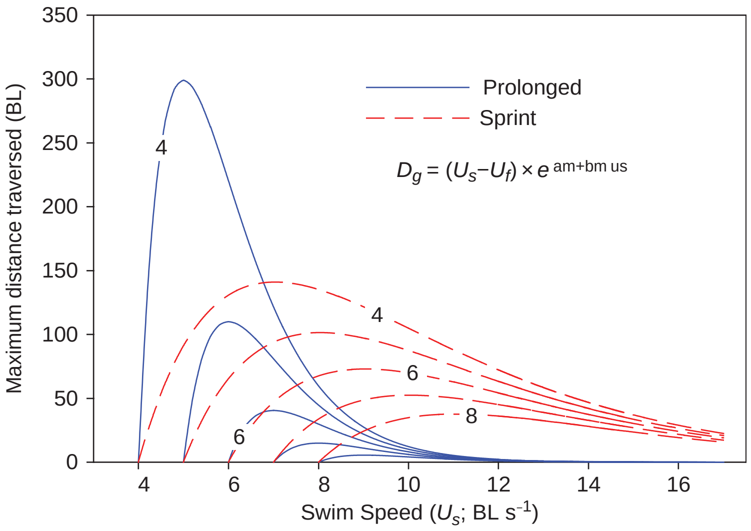


Figure 16 Maximum possible distance traversed (D_g) by American shad against a range of flow velocities (U_f), given the swim speed–fatigue time relationship for prolonged (blue) and sprint modes (red) shown in **Figure 10** (U_s = swim speed; a_m and b_m are intercept and slope terms for each mode). Curves represent flow velocities from 4–8 body lengths per second. Note that fish that swim at the speed of flow make no upstream progress, but swimming too fast also reduces range. Thus, distance maximization is a function of both the swim speed–fatigue time relationship and the ability of the fish to select the optimum swim speed (**Figure 17**). Modified from Castro-Santos T and Haro A (2010) Fish guidance and passage at barriers. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp. 62–89. Enfield, NH: Science Publishers.

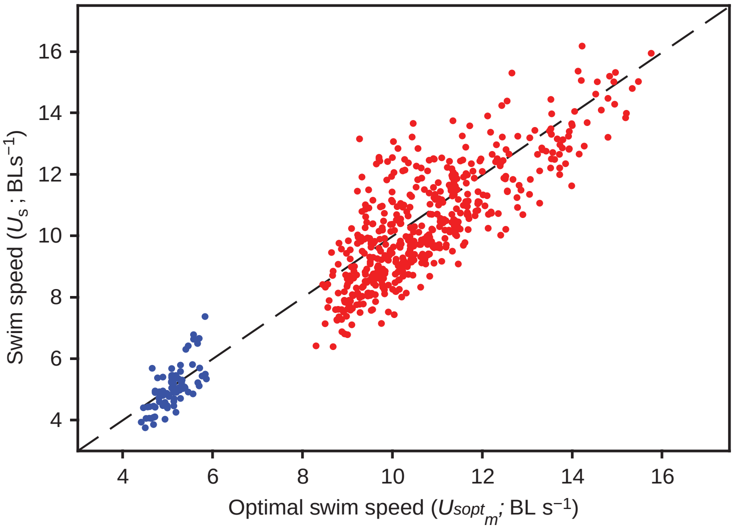


Figure 17 Swim speed optimization by American shad swimming in prolonged (blue) and sprint mode (red). The dashed line represents the optimal swim speed for maximizing distance, given the flow velocity (**Figure 16**), points above the line indicate fish that swam too fast and points below the line indicate fish that swam to slowly. Although on average American shad approximate the distance maximizing behavior, the magnitude of error limits actual distance traversed (**Figure 18**). Modified from Castro-Santos T and Haro A (2010) Fish guidance and passage at barriers. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp. 62–89. Enfield, NH: Science Publishers.

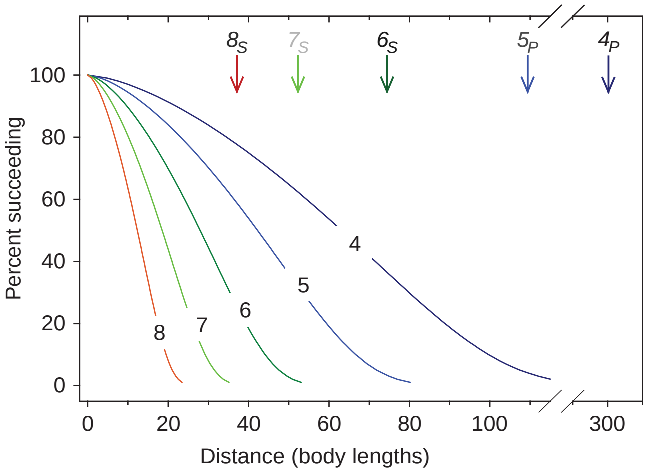


Figure 18 Consequences of the failure of American shad to select optimal swim speeds. Arrows indicate theoretical maximum distance of ascent against each flow velocity (4–8 body lengths per second). Curves represent models of distance of ascent based on empirical data. Note that individuals can never achieve their theoretical maximum potential – the extent to which they fall short is a result of behavior and varies by species. Estimates of performance that ignore either capacity or behavior are often inaccurate and intrinsically imprecise. Modified from Castro-Santos T and Haro A (2010) Fish guidance and passage at barriers. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp. 62–89. Enfield, NH: Science Publishers.

of picking the optimal swim speeds than resident species. Once again, this points to the importance of observing natural behaviors in field-like settings in order to gauge the ideal conditions for optimizing passage.

The lack of good information on free-swimming fish is not surprising when one considers the difficulty of observing them under controlled conditions. Behavioral data have been particularly difficult to acquire. Part of the reason for this is that behaviors are often quantitatively elusive: How should we identify and quantify relevant performance variables? Ideally, quantities should be derived from known or assumed fitness or applied relevance. When scientists are able to explicitly describe the assumed relevance of a given behavior, it becomes easier to determine appropriate metrics with which to quantify it.

The above caveats notwithstanding, laboratory studies have provided valuable information on swimming performance with important conservation implications. Swimming performance has repeatedly been shown to be affected by temperature and by exposure to pollutants, and to vary by life stage. In addition, the energetic costs of locomotion have been extensively studied. Migratory energetics can have important implications for spawning success and survival. This, in turn, drives the viability of populations as well as the size and resilience of fisheries. Barriers to movement, even if ultimately traversed, incur energetic costs that can counteract conservation efforts.

Ongoing efforts to study fish-swimming performance would do well to consider the important conservation challenges facing fisheries managers. Recognizing both the limitations and benefits of various approaches to studying swimming performance should help guide future research and resolve some pressing questions. In a world where humans and fish compete for ever-more limited resources, the importance of this knowledge will only increase.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops; Maneuverability. **Energetic Models**: Bioenergetics in Ecosystems. **Energetics**: Physiological Functions that Scale to Body Mass in Fish. **Swimming and Other Activities**: Energetics of Fish Swimming.

Further Reading

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* 21: 1183–1226.

Brett JR and Groves TDD (1979) Physiological energetics. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology Volume 8: Bioenergetics and Growth*, pp. 279–352. New York: Academic Press.

Castro-Santos T and Haro A (2006) Biomechanics and fisheries conservation. In: Shadwick RE and Lauder GV (eds.) *Fish Physiology Volume 23: Fish Biomechanics*, pp. 469–523. New York: Academic Press.

Castro-Santos T and Haro A (2010) Fish guidance and passage at barriers. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion: An Eco-Ethological Perspective*, pp. 62–89. Enfield, NH: Science Publishers.

Cheney AN (1898) Fishways, their construction and use. In: *Third Annual Report to the Commissioners of Fisheries, Game, and Forests of the State of New York*, pp. 222–233. New York: Wynkoop Hallenbeck Crawford Co.

Clay CH (1995) *Design of Fishways and Other Fish Facilities*. Boca Raton, FL: Lewis Publishers.

Domenici P and Kapoor BG (2010) *Fish Locomotion: An Eco-Ethological Perspective*. Enfield, NH: Science Publishers.

FAO (2002) *The State of the Worlds Fisheries and Aquaculture, 2002*. Rome: FAO.

Haro AJ, Smith KL, Rulifson RA, *et al.* (2009) Challenges for diadromous fishes in a dynamic global environment. Bethesda, MD, USA, American Fisheries Society Symposium 69.

Harrington JM, Myers RA, and Rosenberg AA (2005) Wasted fishery resources: discarded by-catch in the USA. *Fish and Fisheries* 6: 350–361.

He P (2010) Swimming capacity of marine fishes and it role in capture by fishing gears. In: Domenici P and Kapoor BG (eds.) *Fish Locomotion, an Eco-Ethological Perspective*, pp. 484–512. Enfield, NH: Science Publishers.

Lucas MC and Baras E (2001) *Migrations of Freshwater Fishes*. Oxford, UK: Blackwell Science.

McLeod AM and Nemenyi P (1940) *An Investigation of Fishways*. Iowa City: State University of Iowa.

Morita K and Yamamoto S (2002) Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. *Conservation Biology* 16: 1318–1323.

Peake S (2004) An evaluation of the use of critical swimming speed for determination of culvert water velocity criteria for smallmouth bass. *Transactions of the American Fisheries Society* 133: 1472–1479.

Rand PS, Hinch SG, MorrisonJ, *et al.* (2006) Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. *Transactions of the American Fisheries Society* 135: 655–667.

Videler JJ (1993) *Fish Swimming*. London: Chapman and Hall.

Wolter C and Arlinghaus R (2003) Navigation impacts on freshwater fish assemblages: The ecological relevance of swimming performance (review). *Reviews in Fish Biology and Fisheries* 13: 63–89.

World Commission on Dams (2000) *Dams and Development: A New Framework for Decision-Making*. London: Earthscan Publications.

ENERGETICS

Energetic Models

Contents

Bioenergetics in Aquaculture Settings
Bioenergetics in Ecosystems

Bioenergetics in Aquaculture Settings

M Jobling, University of Tromsø, Tromsø, Norway

© 2011 Elsevier Inc. All rights reserved.

Animal Bioenergetics
Defining Bioenergetics: Energy Flow and Partitioning
A Simple Model of Energy Partitioning
Units of Measurement
Chemical Analyses and Energy Determination
Energy Budgets: Growth Trials and the Biological Evaluation of Compound Feeds
Monitoring Feed Intake
Assessment of Nutrient Bioavailability
Metabolic Rates: Energy Expenditure and Rates of Nitrogenous Excretion
Growth and Nutrient Retention Efficiency
Past and Future
Further Reading

Glossary

Abiotic The chemical and physical (nonbiological, physical) variables of the environment (e.g., temperature).

Absorption Passage of nutrients across the gut and into the blood; uptake of fluid and solutes by cells and tissues.

Alkaloids A large group (*c.* 6000 are known) of toxic, basic nitrogenous compounds found in plants. The term alkaloid has not been precisely defined, but the group encompasses heterocyclic nitrogen bases derived from the amino acids – ornithine, lysine, phenylalanine, tyrosine, and tryptophan.

Amino acids Molecules with an amine group, a carboxylic group, and one of 20 R groups that are the building blocks of proteins.

Ammonotelic Excreting nitrogen mainly as ammonia (i.e., NH_3 or the ammonium ion, NH_4^+), as in most teleost fish and aquatic invertebrates. Total ammonia nitrogen (TAN) is the sum of the ammonia–nitrogen found in the unionized (NH_3) and ionized (NH_4^+) form.

Anti-nutritional factor (ANF) A substance that reduces the value of a feed or feedstuff, primarily via interference with the digestion, absorption, or metabolism of nutrients.

Ash The mineral residues remaining after combustion of feeds, feedstuffs, or plant and animal tissues at 450–500 °C.

Bioavailability (biological availability) Describes the proportion of a nutrient in a feed or feedstuff that may be utilized by an animal. Bioavailability of a nutrient can be subdivided into three constituent phases: availability in the intestine for absorption, absorption and/or retention in the body, and utilization. Bioavailability is studied to evaluate the quality of feeds and feedstuffs, and to provide data for establishing nutritional requirements.

Biotic factors Biological factors such as availability of food, numbers of conspecifics and competitors, and predators, etc., that affect the abundance, distribution, and life histories of individuals and species.

Carbohydrates Compounds containing carbon, oxygen, and hydrogen, and having the general formula $C_x(H_2O)_Y$ (although some compounds classifed as carbohydrates do not comply with this general definition). The group includes simple sugars (monosaccharides) and their derivatives, oligosaccharides and polysaccharides, such as starch and cellulose.

Carnivore Generally applied to animals that feed upon other animals, although there are also carnivorous (flesh-eating) plants that trap and digest insects and other small animals.

Crude fiber The insoluble carbohydrates remaining after boiling a feed or feedstuff in acid and alkali; this fraction represents the carbohydrates that are not readily available to, and are poorly utilized by most animals.

Crude protein The content of nitrogen in a feed, feedstuff, plant, or animal tissue multiplied by a factor (usually 6.25, since most proteins contain about 16% N) to provide an estimate of the protein content; this only gives a crude estimate of protein content because variable amounts of nonprotein nitrogen will be present in analyzed samples.

Digestion The process of solubilization and hydrolysis of ingested nutrients in the gastrointestinal canal into constituent molecules and elements suitable for transport across the intestinal wall.

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Endocrine Mode of secretion in which a chemical is released by a cell into extracellular fluid then enters the blood.

Endotherm An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Energy budget The balance of energy input and use in a biological system, expressed in terms of consumption (intake or ingestion), production (growth), respiration, fecal losses, and excretory losses.

Ether extract The portion of a feed, feedstuff, or tissue that is soluble in ethyl ether and is removed by extraction in this solvent; it represents the majority of the lipid fraction of a feed.

Excretion The elimination of waste materials from the body; specifically the elimination of waste materials produced by metabolism, for example, nitrogenous excretion refers to the elimination of nitrogenous compounds (ammonia, urea, uric acid, etc.) resulting, primarily, from the metabolism of proteins and nucleic acids.

Feces The undigested feed residues, plus some endogenous digestive secretions, sloughed cells of the intestinal lining, and bile metabolites that are expelled from the gut via the anus.

Feed efficiency Wet weight gain per unit feed consumed; it often calculated as gain divided by the amount of feed provided, thereby including an error relating to unconsumed feed waste; it may also be termed feed conversion efficiency or feed utilization (cf. feed:gain ratio).

Feed:gain ratio The term used in commercial aquaculture to express the quantity of feed required to produce a given weight gain; it is the reciprocal of feed efficiency.

Heat of combustion The heat produced when organic material is completely oxidized in an oxygen atmosphere to yield carbon dioxide, water, and oxides of nitrogen and sulfur; it is usually measured using a bomb calorimeter, and referred to as the gross energy of a feed or feedstuff.

Herbivore An animal that feeds exclusively on plants.

Homeotherm An animal that maintains a more or less constant body temperature regardless of external temperature variations; it is generally endothermic, being able to generate its own body heat.

Lipids Group of naturally occurring hydrophobic compounds including fats, oils, waxes, phospholipids, and others.

Macronutrients Substances required in relatively large amounts for normal growth and development; the macronutrients present in animal feeds are the proteins, lipids, and carbohydrates.

Maintenance requirement The amounts of food, or specific nutrients, needed to meet metabolic requirements, without any gain or loss of body weight; it is the level of intake that provides for the continuation of life processes but allows for no increase in biomass.

Metabolic rate A measure of the rate of metabolic activity within an organism; it is the rate at which an organism uses energy to sustain life processes. It is most usually measured as heat production or oxygen consumption (sometimes together with carbon dioxide production), but the latter only gives an estimate of the contribution of aerobic metabolism.

Metabolism An integrated network of biochemical reactions that occurs in living organisms; the biochemical processes by which the absorbed nutrients are transformed and stored (anabolism), broken down and energy made available for the performance of work (catabolism).

Micronutrient Nutrients needed in very small amounts.

Nitrogen-free extract (NFE): One of the six fractions in the system of proximate analysis; it is calculated by difference when the sum of the percentages of moisture, crude protein, ether extract, ash, and crude fiber is subtracted from 100. NFE is a complex mixture of compounds that may include cellulose, hemicelluloses, lignin, sugars, starch and pectins, organic acids, resins and tannins, pigments, and water-soluble vitamins.

Nutrient A substance used by an organism for maintenance, growth, and reproduction; it is designated as macronutrients (proteins, lipids, and carbohydrates) and micronutrients (vitamins and trace elements), depending upon the amounts required.

Nutrient requirement Minimum amount of a nutrient that is needed for the maintenance of growth, health, and reproduction, with no safety margin.

Omnivore An animal that feeds upon a mixed diet comprising both plants and animals.

Phytate Hexaphosphoinositol, the phosphate derivative of the sugar alcohol inositol, is found in plant seeds as the main storage form of phosphorus; fish are unable to digest phytate so the phosphorus is not bioavailable; phytate can inhibit the action of digestive enzymes, and can chelate with di- and trivalent metal ions, thereby reducing their biological availability; it is considered an important anti-nutritional factor (ANF) in plants used to manufacture fish feeds.

Poikilotherm An organism that cannot regulate body temperature except through behavioral means. An animal whose body temperature fluctuates with that of the environment.

Protease inhibitor A substance, often a protein (such as the trypsin inhibitors), that inhibits the actions of proteolytic enzymes, thereby reducing the efficacy with which an animal can digest protein.

Protein An organic molecule consisting of chains of amino acids, and containing chiefly C, H, O, N, and S. One or more polypeptide chain may comprise it, and such chains may also be associated with nonprotein components (prosthetic groups). It is essential in living organisms as enzymes and structural constituents of tissues.

Secondary metabolites (compounds) Compounds produced by microbes, plants, and some animals (e.g., antibiotics, alkaloids, and tannins) that are usually not essential to the growth of the organism, but may have other functions, for example, in chemical defense.

Spectroscopy Encompasses a range of techniques for acquiring information about atomic and molecular structure via the study of patterns of absorption or emission of electromagnetic radiation. Near-infrared spectroscopy is a technique that is used for the rapid analysis of the chemical composition of feeds and feedstuffs, and instruments that measure either reflectance (NIRS) or transmission (NIT) have been developed.

Tannins These are complex, soluble, phenolic secondary metabolites that play a prominent role in the general defense strategies of plants. They are believed to be by-products of the metabolism of the aromatic amino acid phenylalanine.

Vitamins These are organic compounds required by animals in small amounts for the maintenance of a variety of metabolic functions; these are classified as lipid soluble (vitamins A, D, E, and K) or water soluble (ascorbic acid, myo-inositol, choline, and the vitamin B complex). Vitamins are essential nutrients that must be obtained via the diet, and a lack of a vitamin will lead to the development of a deficiency disease.

Animal Bioenergetics

Experiments in bioenergetics were first carried out during the eighteenth century, and eventually the discipline matured to the stage where it was applied to solve problems within human and animal nutrition. This role for bioenergetics was initiated early in the twentieth century. Bioenergetics has been widely applied in animal science, where one of the major aims is to produce edible muscle. Interactions between farmed animals and their environments are often stressed, with research focusing on:

- the relationships between gas exchange and heat production;
- examination of the causes of energy expenditure, and the costs associated with the performance of various activities; and
- the establishment of a basis for the evaluation of feed ingredients and compound feeds in relation to energy requirements and expenditures.

The results of these various lines of research have been used to estimate the energy requirements of farmed animals, to predict the growth of animals on different feeding regimes and to assist in the development of compound feeds for terrestrial livestock and farmed aquatic animals. A major goal of aquaculture is the effective production of edible fillet (myotomal muscle), so it is important to know about the interactions of environmental and nutritional factors on muscle development and growth (**Figure 1**). Modern bioenergetics studies can increase this understanding.

Defining Bioenergetics: Energy Flow and Partitioning

Bioenergetics is the quantitative study of energy partitioning induced by the metabolic processes that occur in organisms for them to stay alive, grow, and reproduce; it includes events taking place at the

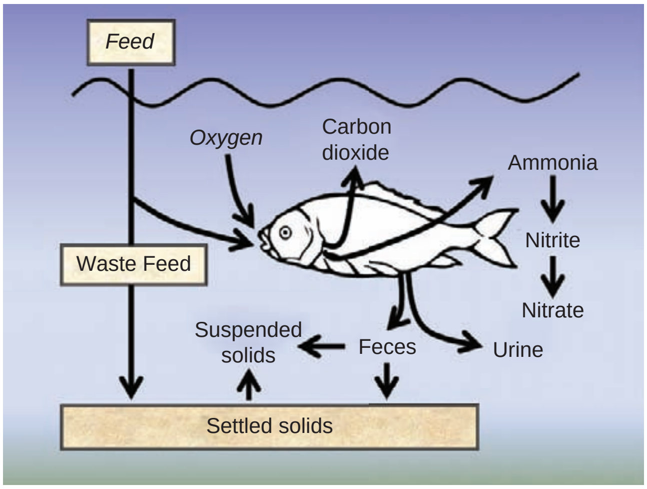


Figure 1 Two-way interactions between farmed fish and the environment may have marked influences upon the characteristics of the water (i.e., water quality) in which they are held. Adapted from Le Francois et al. (eds.) (2010) *Finfish Aquaculture Diversification.* Wallingford: CABI.

molecular and cellular level, as well as those that can be studied in whole organisms, populations, or ecosystems. In the broadest sense, a descriptive factorial scheme (**Figure 2**) can depict the fate of energy intake, and mathematical modeling or graphical representation can be used to investigate thef interrelationships among energy intake, metabolism, and energy retention.

Animals expend energy in the performance of physical activity, but even when resting animals may expend a considerable amount of energy to maintain the metabolic integrity of their body functions. For example, there is transport of substrates and metabolites in and out of cells, and chemical work is needed for tissue renewal and repair; the latter involves the turnover of proteins and the biosynthesis of lipids and carbohydrates (**see also Energetics**: General Energy Metabolism).

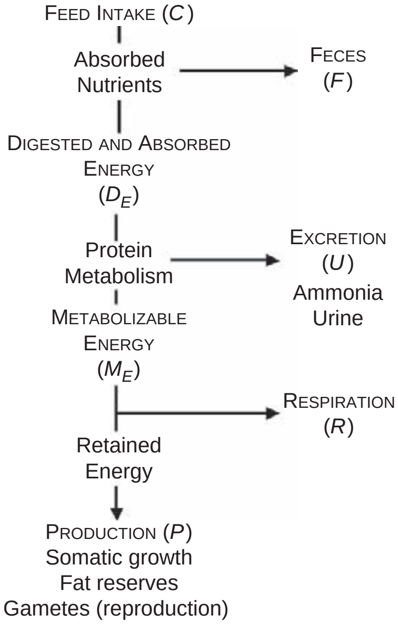

FEED INTAKE (C)
Absorbed Nutrients
FECES (F)
DIGESTED AND ABSORBED ENERGY (D_E)
Protein Metabolism
EXCRETION (U) Ammonia Urine
METABOLIZABLE ENERGY (M_E)
RESPIRATION (R)
Retained Energy
PRODUCTION (P) Somatic growth Fat reserves Gametes (reproduction)

Figure 2 Flow diagram illustrating the factorial scheme of energy partitioning and dissipation in the study of animal energetics.

How much energy animals are required to expend, and how much of their energy intake they retain as production is under the influence of a wide range of environmental factors (**Figure 3**; **see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). The environmental factors can be broadly divided into two main types, abiotic and biotic. Abiotic factors are those that relate to the nonliving, physical environment. For fish, and other aquatic animals, the abiotic factors include temperature, light intensity and day-length

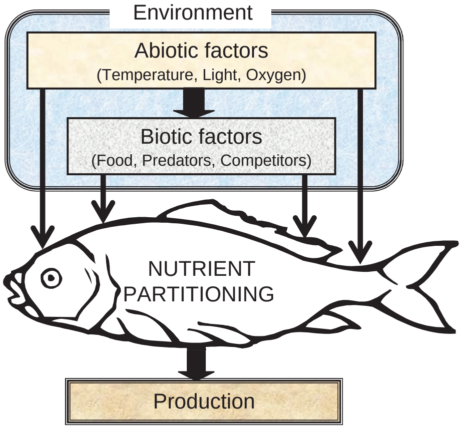


Figure 3 A wide variety of biotic and abiotic environmental factors influence energy partitioning and production of fish.

(photoperiod), oxygen concentration, water flow and currents, salinity, and other chemical properties of the water in which the animals live. The biotic factors, on the other hand, relate directly to the effects that organisms sharing habitats have on each other; biotic factors include the prey, predators, competitors, and parasites of the fish.

A Simple Model of Energy Partitioning

Food energy (C) is gross intake, usually expressed as a daily rate, but not all of this is available to the fish. Some is voided in the feces (F), and there are also losses as urinary excretion (U) and via diffusion from the body surface, including the gills of fish. From this, two quantities can be defined: digested and absorbed energy (D_E) and metabolizable energy (M_E) (**Figure 2**). D_E is the difference between food energy and fecal losses (i.e., $D_E = C - F$). M_E is the difference between food energy and the sum of losses in the feces, urine, and across the body surface, so $M_E = C - (F + U)$. In other words, M_E is the energy available for meeting the demands for staying alive, and for growing and reproducing.

The metabolic expenditure by an animal is often measured as the amount of heat produced (**see also Energetics**: General Energy Metabolism); this is usually called respiration (R). The difference between M_E and R is retained energy, usually termed production (P): $M_E - R = P$. When an animal is deprived of food ($C = 0$), body tissues are catabolized in order to support R, P is negative, and the animal loses body mass. On the other hand, if an animal eats some food but energy retention is zero over time (i.e., $P = 0$), there is an equilibrium and the animal is meeting its maintenance requirement (**Figure 4**).

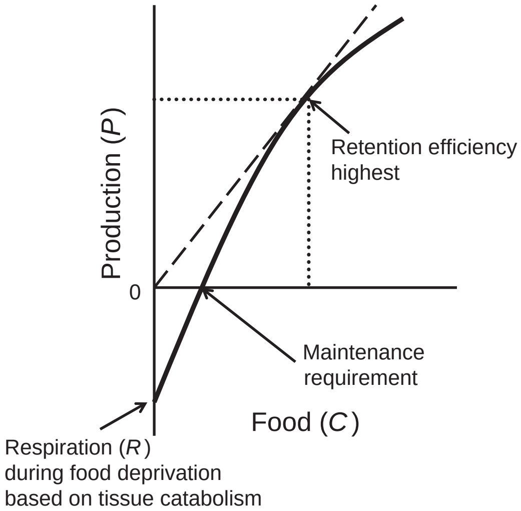


Figure 4 The relation between production and food intake, illustrating key points relating to energy retention.

As C increases so does R; R is greater at maintenance than it is in animals that are deprived of food, and as C increases above maintenance, R continues to increase. In a growing animal, an increase in C results in a proportion (r) of M_E being retained as P and a proportion $(1 - r)$ being lost in respiration (R). In other words, r is a measure of the efficiency with which energy is retained as production, or the energy utilization efficiency. Energy utilization efficiency is not constant but varies with the amount of food consumed. If the relationship between P and C is nonlinear, retention efficiency is highest at the point where a line drawn through the origin intersects with the plotted line that depicts the relationship between P and C (**Figure 4**). The efficiency with which C is retained as P is usually expressed as a percentage, the food conversion efficiency. In farming practice, the reciprocal of food conversion efficiency is sometimes used, that is, the amount of food required to produce a given unit of production or gain, the feed:gain ratio.

Units of Measurement

Bioenergetics is the study of biochemical transformations using units of energy as the common currency. In common parlance, for example, when we are undergoing weight reduction regimens we will often say that we are watching, or counting, our calories; the calorie is a unit used to measure energy, but the joule (J), an SI unit, has replaced the calorie in the scientific literature. The joule is named after James Prescott Joule, who was the first to demonstrate the equivalence of heat, work, and the various forms of chemical energy. The kilojoule ($1\ \text{kJ} = 10^3\ \text{J}$), rather than the joule, is most often the unit of choice in studies of animal energetics ($1\ \text{kJ} = 0.239\ \text{kcal}$; $1\ \text{kcal} = 4.186\ \text{kJ}$).

Bioenergetics involves the examination of biological rates, and power units (the watt in the SI system) are the appropriate ones to use when referring to such rates. The convention, however, is to retain the kilojoule and express rates in direct relation to periods of time. For example, food consumption is generally expressed as kilojoules per day (kJ d^{-1}) and energy expended on activity as kilojoules per hour (kJ h^{-1}).

Although bioenergetics relies upon energy units as common currency, it must be borne in mind that animals eat, digest, absorb, and metabolize food that is made up of chemicals. Animals have a requirement for, transform, and store macronutrients (proteins, lipids, and carbohydrates) and micronutrients (vitamins, minerals, and trace elements). As such, nutritional energetics revolves around the investigation of the absorption, biochemical transformation, and partitioning of nutrients. In the study of fish bioenergetics, the focus is most often on the fluxes and fates of energy, proteins, and lipids (**see also Energetics**: General Energy Metabolism).

Chemical Analyses and Energy Determination

Assessments of the chemical composition and energy value of feedstuffs, feeds, and animals are central to the study of the energetics and nutrition of farmed animals, including fish. The major energy sources in feeds are the organic macronutrients: lipids, proteins, and carbohydrates. These can be completely oxidized in a bomb calorimeter to yield carbon dioxide, water, oxides of nitrogen and sulfur, and heat. The three macronutrients yield different amounts of heat energy when completely oxidized: approximately 39 $\mathrm{kJ\,g^{-1}}$, 23.5 $\mathrm{kJ\,g^{-1}}$, and 17 $\mathrm{kJ\,g^{-1}}$ for lipids, proteins, and carbohydrates, respectively.

Chemical composition is measured using a variety of techniques. Some methods enable determinations to be made of individual amino acids, fatty acids, vitamins, and mineral elements, but the chemical analyses undertaken are not usually so comprehensive. Chemical composition is most frequently given as proximate composition in which six major fractions are recognized: moisture, crude protein, ether extract, ash, crude fiber, and nitrogen-free extract. Proximate analysis is time consuming and expensive, large samples are required, and the analyses are destructive. Alternatives can provide quick, nondestructive, quantitative assessments of the constituents of biological materials. These include X-ray computed tomography, total body electrical conductivity, near-infrared reflectance spectroscopy (NIRS), and near-infrared transmittance. NIRS has attained popularity because it is rapid and nondestructive, equipment costs are relatively low and the equipment is quite easy to use. NIR spectra can give information about physical and chemical properties, and NIRS is being used for assaying the chemical composition (protein, moisture and oil/fat) of feed ingredients, feeds and animal tissues, for species identification and for food authentication purposes.

Energy Budgets: Growth Trials and the Biological Evaluation of Compound Feeds

Chemical analyses provide a great deal of information about the nutritional properties of feeds, but biological trials are required to reveal the full value of feeds and their ingredients. For example, studies of digestion and absorption are required for the assessment of the bioavailabilities of nutrients to fish. Moreover, growth trials are fundamental for feed assessment, and for the determination of nutrient requirements. When conducting growth trials for feed assessment, it is seldom that data relating to all components of the energy balance equation are collected. Emphasis is usually placed upon the monitoring of food intake (C), fecal losses (F), and the energy and nutrients retained as production (P). Practical problems relating to the measurement of respiration (R) and excretion (U) over prolonged periods of time often preclude their inclusion in the assessment (**see also Energetics**: General Energy Metabolism).

Although neither R nor U is usually monitored in growth trials, their importance in determining the outcome of trials is recognized. For example, mammals and birds are homeothermic endotherms, whereas fish are poikilothermic ectotherms; these animals differ in metabolic characteristics and rates of energy expenditure. Homeothermic endotherms generate body heat and maintain a body temperature that differs from that of the environment. They have high metabolic rates and their maintenance requirements are high; if deprived of food, they lose body mass rapidly, and they have low starvation resistance (**Table 1**). Given that mammals and birds have relatively high maintenance requirements, the proportion of feed nutrients directed toward production may be low and so these animals are generally characterized as having relatively low retention efficiencies. Fish, being poikilothermic ectotherms, have a body temperature that changes as environmental temperature changes, and the main source of their body heat is the environment. Fish have lower metabolic rates than mammals and birds of similar size, and the maintenance requirements of fish are accordingly low. Fish are relatively efficient at converting food to body tissue, and have higher retention efficiencies than do mammals and birds of similar size.

Table 1 Comparison of metabolic characteristics and growth efficiencies of fish (poikilothermic ectotherms) and mammals (endothermic homeotherms)

	Fish	*Mammals*
Metabolic rate	Low	High
Starvation resistance	High	Low
Maintenance food requirement	Low	High
Food use for growth	High efficiency	Low efficiency

This comparison between mammals, birds, and fish can be extended to include generalizations about nutritional requirements and feed compositions. Most farmed mammals and birds are either herbivorous or omnivorous, and they are capable of digesting and metabolizing feeds that contain high concentrations of complex plant carbohydrates. Feeds formulated for these animals, such as poultry and pigs, usually have high carbohydrate content and relatively low protein and lipid contents (**Table 2**). Carbohydrates are an abundant and inexpensive energy source, so poultry and pig feeds are largely comprised of relatively cheap feed ingredients. In contrast to terrestrial farmed animals, many of the high-value, intensively farmed fish species are carnivorous. They are unable to digest and metabolize large amounts of dietary carbohydrate. These carnivorous fish rely upon feeds that contain relatively high proportions of proteins and lipids. Feed ingredients that contain these nutrients are more expensive than those that are rich in carbohydrate. Consequently, fish feeds are typically more expensive than those prepared for terrestrial livestock.

Monitoring Feed Intake

Effective feeding of farmed fish poses several problems not encountered when feeding terrestrial livestock. The feeds are exposed to water prior to being ingested, so they must be water stable with minimal loss of nutrients. Disintegration of feed will make it unavailable, and leads to undesirable water fouling. In addition, feeds should have physical and chemical characteristics that make them easy to detect and capture. As such, attempts are usually made to manufacture feeds that float at the water surface, or sink through the water column at prescribed rates that suit the fish's feeding preferences. A large proportion of the cost of aquaculture production is related to feeds and feeding, so feeds must be correctly formulated, and the quantities given to the fish should meet demand, without excessive wastage.

There are numerous factors that interact to influence feeding (**Figure 5**). Therefore, it is extremely difficult to predict when and how much a fish will eat. On intensive fish farms, feeding is usually highly automated, and several types of feeding systems have been developed. On-demand feeders are designed to circumvent problems arising from the unpredictability of feeding responses. The purpose of using on-demand feeders is to feed the fish effectively by presenting feed at the correct time and in sufficient quantity. On-demand feeders operate on the principle that both the timing and quantities of feed released are controlled by the fish: on-demand feeders are either self-feeders or interactive feeding systems.

When self-feeding, the fish control the delivery of feed by activating a trigger system. The trigger is activated either by the fish biting on, or displacing, the tip of a rod, or by pulling on a small bead or pellet-shaped object attached to a string. When the fish activates the triggering system, a portion of feed is released from a hopper.

Interactive feeding systems usually rely upon the detection of waste feed to control the amount delivered. The fish are provided with feed at regular intervals and registration is made as to whether or not the feed is consumed. This may be done by direct observation of the feeding responses of the fish, by following the fate of feed pellets using video recording, or by using special optical (infrared) or hydroacoustic (sonar or Doppler) sensors to monitor the distributions of the fish or detect uneaten pellets. Feed delivery is stopped or modified according to feedback signals from the sensor system, so the delivery of feed is controlled in relation to the willingness of the fish to eat.

When feed assessment studies are conducted, it is important to collect accurate information about food intake. Measurements are usually made by combining automatic feeding with the collection of waste feed to get information about the amount of food eaten by a

Table 2 Comparisons of feeding habits and feed compositions of selected terrestrial livestock species and farmed fish

	Terrestrial livestock (pigs and chickens)	*Carps and tilapias*	*Salmonids and sea basses*
Feeding habits	Omnivorous	Herbivorous Omnivorous	Carnivorous
Metabolic substrates	Carbohydrates Fats	Carbohydrates Fats	Fats Proteins
Feed components (nutrient composition)	High carbohydrate Low protein (*c*. 20%) Low fat (*c* 10%)	High carbohydrate Medium protein (*c*. 30%) Low fat (*c*. 10%)	Low carbohydrate (up to *c* 20%) High protein (38+%) Medium-to-high fat (up to *c* 35%)
Feed costs (per kg feed)	Low Cheap ingredients with high carbohydrate content	Low-to-medium	High Expensive protein-rich ingredients

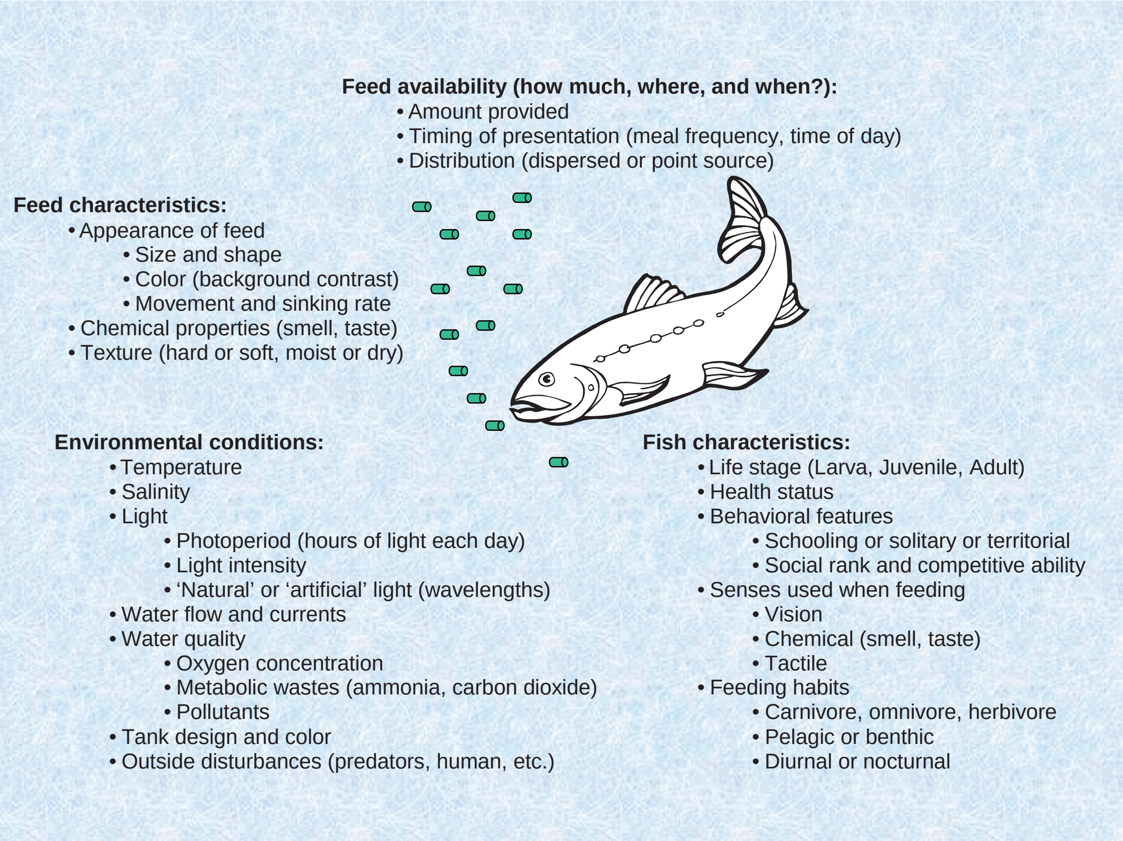

Feed availability (how much, where, and when?):
• Amount provided
• Timing of presentation (meal frequency, time of day)
• Distribution (dispersed or point source)
Feed characteristics:
• Appearance of feed
• Size and shape
• Color (background contrast)
• Movement and sinking rate
• Chemical properties (smell, taste)
• Texture (hard or soft, moist or dry)
Environmental conditions:
• Temperature
• Salinity
• Light
• Photoperiod (hours of light each day)
• Light intensity
• 'Natural' or 'artificial' light (wavelengths)
• Water flow and currents
• Water quality
• Oxygen concentration
• Metabolic wastes (ammonia, carbon dioxide)
• Pollutants
• Tank design and color
• Outside disturbances (predators, human, etc.)
Fish characteristics:
• Life stage (Larva, Juvenile, Adult)
• Health status
• Behavioral features
• Schooling or solitary or territorial
• Social rank and competitive ability
• Senses used when feeding
• Vision
• Chemical (smell, taste)
• Tactile
• Feeding habits
• Carnivore, omnivore, herbivore
• Pelagic or benthic
• Diurnal or nocturnal

Figure 5 The wide variety of factors that influence feeding in fish make prediction of food intake extremely difficult. From Le Francois et al. (eds.) (2010) *Finfish Aquaculture Diversification.* Wallingford: CABI.

group of fish. Techniques are also available that enable the food intake of individual fish to be monitored.

Assessment of Nutrient Bioavailability

Realistic assessments of feeds require that information is collected about the digestion and absorption of nutrients, that is, measurement of nutrient bioavilability is an important aspect of feed evaluation. In principle, $D_E = C - F$, so information about both C and F is required for the calculation of D_E. It is difficult to collect accurate information about food consumption and it is virtually impossible to collect all of the feces produced by fish during a growth trial. This means that D_E cannot be measured directly, and alternative indirect marker methods must be used for the estimation of nutrient bioavailability.

Many ingredients are used for preparing fish feeds. Some feed ingredients are now in short supply and are being replaced by alternatives. In particular, there is increased replacement of animal proteins, such as fish meal, with terrestrial plant proteins. The replacement of animal proteins with plant proteins presents several problems, particularly in feeds for carnivorous fish. Feeds given to carnivorous fish usually contain at least 38% protein, limiting the use of ingredients that contain moderate amounts of protein; plant protein sources usually have less protein than do animal products. Consequently, fractionation of plant materials is often carried out to increase the protein concentration in the feed ingredient.

In addition to their low protein concentration, many plants contain secondary compounds that reduce their value as feed ingredients. These compounds act as antinutritional factors (ANFs) by suppressing feeding or by interfering with the digestion, absorption, and utilization of nutrients. ANFs may interfere with protein digestion and absorption, bind to minerals and reduce their bioavailability, act as anti-vitamins, interfere with metabolic pathways, or disrupt endocrine signaling. The ANFs include protease inhibitors, alkaloids, tannins, phytate, and plant fibers. Some ANFs are heat labile, so thermal processing may be used to destroy them. ANFs present in the outer hulls or seed coats of plants may be removed and deactivated using de-hulling and heat treatment. Other ANFs are more resistant, and their removal or destruction may require treatment of plant materials with organic solvents or enzymes. Thus, a range of processing and fractionation technologies, including dehulling, heat treatment, enzyme treatments, aqueous or organic solvent extraction, and protein purification, may be applied to plant products to increase their value as feed ingredients.

Metabolic Rates: Energy Expenditure and Rates of Nitrogenous Excretion

There are many biotic and abiotic factors that influence energy expenditure and rates of excretion of poikilothermic ectotherms; temperature, oxygen availability, activity, and feeding are among the most important (**see also Energetics**: General Energy Metabolism and **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism). For example, an increase in environmental temperature results in an increase in energy expenditure that is reflected in an increase in oxygen consumption. Aquatic hypoxia is a state of reduced oxygen availability. In fish farming, attempts are made to minimize exposure of fish to hypoxic conditions to avoid the negative effects this has on feeding, energy expenditure, and growth.

Increases in swimming speed result in increases in rates of energy expenditure (**see also Swimming and Other Activities**: Energetics of Fish Swimming). Given the high energetic cost of activity, it is surprising that fish of some species grow faster and more efficiently when swimming against a modest water current than they do when reared in tanks with less pronounced water flows. This is most probably related to behavioral changes that result from exposure of fish to flowing water. The fish often form schools, and do not spend as much time and energy on exploratory activities and aggressive behaviors when engaged in directed swimming activity.

Energy expenditures of fed fish are higher than those of fish deprived of food; fed fish may be more active than unfed fish and this may contribute to their elevated metabolic rates. Food search and capture may lead to some increase in energy expenditure, but metabolic rates are elevated whenever there is food in the gut; metabolic rates are usually highest a few hours after feeding has ceased. As such, the increase in energy expenditure that follows feeding may largely result from the energy requirements for

- physical processing of food, digestion, and the absorption of nutrients;
- biosynthesis, turnover, and deposition of tissue macromolecules; and
- deamination of amino acids and synthesis of excretory products.

Following the ingestion of food there is an increase in motor activity of the gut, and digestion and absorption involve the synthesis and secretion of digestive enzymes and transport of nutrients across the gut wall; all result in increased energy expenditure. Tissue constituents are in a dynamic state; quite a large proportion of the energy expenditure associated with the ingestion of food is related to turnover, synthesis, and deposition of macromolecules (primarily protein, but also lipids and carbohydrates; **Figure 6**; **see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation. Rates of nitrogenous excretion also increase following feeding, so amino acid deamination and the synthesis of nitrogenous excretory products contribute to energy expenditure. Fish excrete most of their waste nitrogen as ammonia, that is, they are ammonotelic. Ammonia excretion increases following feeding and protein-rich feeds induce greater ammonia excretion than feeds of lower protein content. Although fish mostly excrete ammonia, they also excrete some urea, amino acids, uric acid, creatine, and creatinine; the amounts of the different nitrogenous compounds excreted vary with species and life-history stage, feeding conditions, feed composition, and environmental factors (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation).

Growth and Nutrient Retention Efficiency

Feeds are evaluated according to whether or not they are utilized effectively to sustain production. Information is collected on feed composition, amounts eaten, and animal production, and used to calculate how efficiently nutrients are retained. The calculation of an efficiency requires that there is both a numerator and a denominator, and it is imperative that the terms and units in which both are expressed are clear. The numerator is production and the denominator is feed consumption, but expressions of retention efficiency differ in sophistication and complexity. The crudest expression of retention efficiency sets the weight gain of the animal as the numerator and the weight of feed consumed as the denominator. This is an unsatisfactory estimate because it takes no account of possible differences in energy concentrations and chemical compositions between the feeds and the animals that consume them.

If the aim is to express retention efficiency in terms of energy or a specific nutrient, such as protein, it is essential to have information on the chemical compositions of each feed and of the animals at the beginning and end of a growth trial. Once information on chemical composition has been obtained, the numerator is either the energy or amount of nutrient accrued as production, and the denominator is feed consumption expressed in appropriate terms. The units expressing feed consumption can be the weight of a given nutrient that has been eaten, the total amount of gross feed energy consumed, consumption expressed in terms of digested and absorbed nutrients or energy, or the amount of metabolizable energy consumed by the animal.

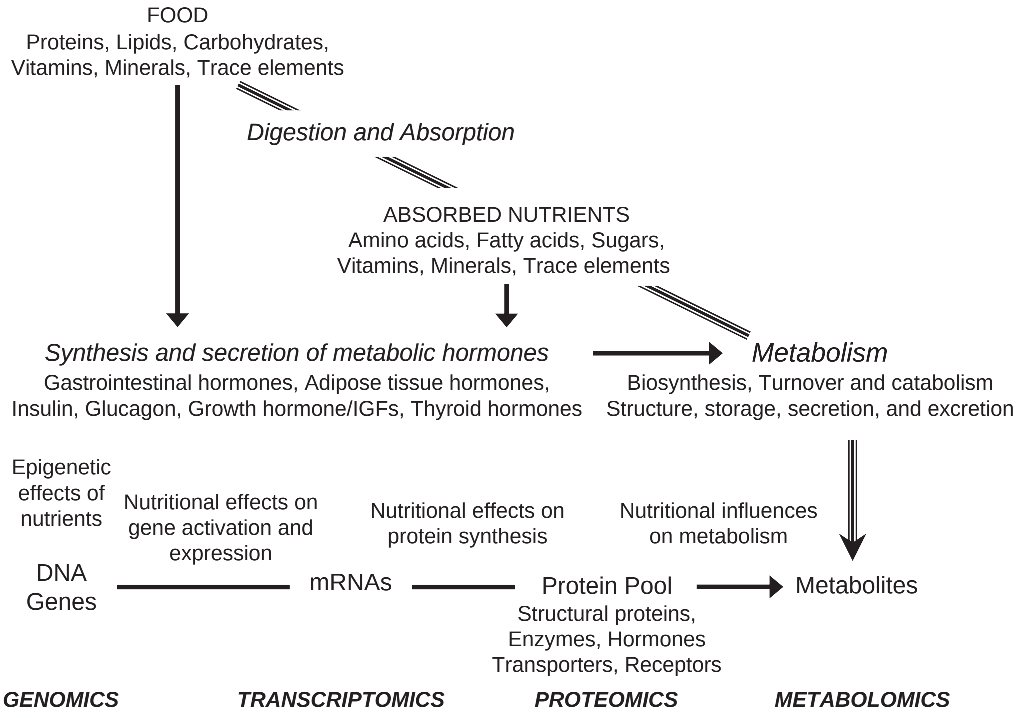

FOOD
Proteins, Lipids, Carbohydrates, Vitamins, Minerals, Trace elements
Digestion and Absorption
ABSORBED NUTRIENTS
Amino acids, Fatty acids, Sugars, Vitamins, Minerals, Trace elements
Synthesis and secretion of metabolic hormones
Gastrointestinal hormones, Adipose tissue hormones, Insulin, Glucagon, Growth hormone/IGFs, Thyroid hormones
Metabolism
Biosynthesis, Turnover and catabolism
Structure, storage, secretion, and excretion
Epigenetic effects of nutrients
Nutritional effects on gene activation and expression
Nutritional effects on protein synthesis
Nutritional influences on metabolism
DNA Genes
mRNAs
Protein Pool
Structural proteins, Enzymes, Hormones Transporters, Receptors
Metabolites
GENOMICS
TRANSCRIPTOMICS
PROTEOMICS
METABOLOMICS

Figure 6 Food nutrients induce changes in gene activation and transcription, protein synthesis and turnover and nutrient metabolism. Transcriptomic (gene expression profiling), proteomic (protein expression analysis), and metabolomic (metabolite composition analysis) monitoring provide holistic assessments of these changes.

Many expressions are used to describe the efficiency of animal production. This can cause confusion, and it also means that care must be exercised when comparing results among studies to ensure that the metrics and estimates are equivalent and comparable.

Past and Future

The biochemical transformations that sustain life and support production are a form of combustion, and this is the basis of bioenergetics. This discovery paved the way for experiments that combined the study of food digestion and absorption with measurements of heat loss, energy expenditure, production, and energy retention. These studies contributed to the development of schemes used to predict animal performance and food requirements, and provided guidelines for use in feed formulation. As such, bioenergetics is established as an important area of applied animal sciences.

On the negative side, the focus on energy as the common currency may overshadow the facts that nutrients have different fates within the animal body, and that energy partitioning is governed by how the different nutrients are transformed during their progress through metabolic pathways. In-depth understanding requires knowledge about the roles of specific nutrients and their metabolism, but such knowledge is fragmentary for the nutritional energetics of fish. This type of information must be obtained if dynamic response models are to be updated and improved in the face of new challenges. These challenges include concerns about the environmental impacts of farming systems, shortfalls in supplies of some traditional feed ingredients, an increased focus on product quality, and the pressure to develop production systems that are sustainable in the long term.

The applied animal sciences have now entered the '-omics' era: genomics, transcriptomics, proteomics, and metabolomics (**Figure 6**). The techniques developed in support of these disciplines provide powerful tools for the examination of the biochemical networks involved in metabolic regulation. These '-omics' disciplines integrate to provide a holostic overview of the information stored in the genes, the biochemical transformations resulting from enzymatic activity and the actions of metabolic hormones, and compositional changes that accrue from the retention and elimination of metabolites. This integration provides new insights into the factors that control the need for, and use of, nutrients and energy that are a prerequisite for the advance of nutritional energetics. For example, '-omics' techniques make it possible to study the effects of abiotic factors and nutrients on the activation or suppression of genes that code for metabolic enzymes, examine effects on protein translation, and discern rates of nutrient fluxes through metabolic pathways. Metabolomics allows mapping of the fates of nutrients via global monitoring of the metabolites present within cells and tissues. Such studies may lead to the identification of biomarkers that could have utility in growth trials, and the identification of specific nutrients that exert strong biological effects. Further, the large data sets generated by the '-omics' technologies, together with bioinformatics (analysis of metabolic pathways using a systems biology approach), are expected to instigate development of mathematical models to describe and predict the cellular responses to nutrients. This, in turn, should allow progress toward solving several of the problems facing the animal production industries.

See also: **Energetics**: General Energy Metabolism. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism; Energetics of Fish Swimming.

Further Reading

Blaxter K (1989) *Energy Metabolism in Animals and Man*. Cambridge: Cambridge University Press.

Cen H and He Y (2006) Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science and Technology* 18: 72–83.

Dumas A, Dijkstra J, and France J (2008) Mathematical modelling in animal nutrition: A centenary review. *Journal of Agricultural Science* 146: 123–142.

Fraser KPP and Rogers AD (2007) Protein metabolism in marine animals: The underlying mechanism of growth. *Advances in Marine Biology* 52: 267–362.

Gatlin DM, III, Barrows FT, Brown P, *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research* 38: 551–579.

Glencross BD, Booth M, and Allan GL (2007) A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition* 13: 17–34.

Halver JE and Hardy RW (eds.) (2002) *Fish Nutrition*, 3rd edn. San Diego: Academic Press.

Hoar WS, Randall DJ, and Brett JR (eds.) (1979) *Fish Physiology, Vol. VIII, Bioenergetics and Growth*. New York: Academic Press.

Houlihan D, Boujard T, and Jobling M (eds.) (2001) *Food Intake in Fish*. Oxford: Blackwell Science.

Ibanez E and Cifuentes A (2001) New analytical techniques in food science. *Critical Reviews in Food Science and Nutrition* 41: 413–450.

Jobling M (1994) *Fish Bioenergetics*. London: Chapman and Hall.

Johnson DE, Ferrell CL, and Jenkins TG (2003) The history of energetic efficiency research: Where have we been and where are we going? *Journal of Animal Science* 81(supplement 1): E27–E38.

Johnston IA, Macqueen DJ, and Watabe S (2008) Molecular biotechnology of development and growth in fish muscle. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard TJ, Jr. and Kaiser MJ (eds.) *Fisheries for Global Welfare and Environment*, pp. 241–262. Tokyo: Terrapub.

Kleiber M (1975) *The Fire of Life: An Introduction to Animal Energetics*, 2nd edn. New York: Robert E. Krieger Publishing.

Kussmann M, Rezzi S, and Daniel H (2008) Profiling techniques in nutrition and health research. *Current Opinion in Biotechnology* 19: 83–99.

McNab BK (2002) *The Physiological Ecology of Vertebrates: A View from Energetics*. Ithaca: Comstock Publishing.

Stickney RR (ed.) (2000) *Encyclopedia of Aquaculture*. New York: John Wiley.

Sundell K and Power D (eds.) (2008) *Special Issue: Functional Genomics in Sustainable Aquaculture. Reviews in Fisheries Science* 16(supplement 1): 1–166.

Wang J, Wu G, Zhou H, and Wang F (2009) Emerging technologies for amino acid nutrition research in the post-genome era. *Amino Acids* 37: 177–186.

Zdunczyk Z and Pareek CS (2008) Application of nutrigenomics tools in animal feeding and nutritional research. *Journal of Animal and Feed Sciences* 17: 3–16.

Bioenergetics in Ecosystems[1]

CP Madenjian, Great Lakes Science Center, Ann Arbor, MI, USA

Published by Elsevier Inc.

Fish Bioenergetics Modeling in Ecosystems: An Overview
A Simple Energy Budget for Fish
Wisconsin Bioenergetics Models for Fish
An Example of Fish Bioenergetics Modeling Guiding Fisheries Management
Estimating the Strength of the Predator–Prey Trophic Link: Other Examples
Use of Bioenergetics Modeling to Better Understand Fish Growth and Consumption
Role of Fish in Cycling Nutrients in Aquatic Ecosystems: Bioenergetics Model Applications
Contaminant Accumulation in Fish: Bioenergetics Model Applications
Limitations to Fish Bioenergetics Model Applications
Further Reading

Glossary

Amphipod Any of the small crustaceans belonging to the order Amphipoda.

Egestion Expulsion of feces.

Excretion Discharge of urine.

Haplochromine cichlid Any of the fishes belonging to the Haplochromini tribe within the family Cichlidae.

Polychlorinated biphenyls A class of organic compounds with 1–10 chlorine atoms attached to a biphenyl, which is a molecule composed of two benzene rings.

Respiration The metabolic process by which an organism assimilates oxygen and releases carbon dioxide and other products of oxidation.

Fish Bioenergetics Modeling in Ecosystems: An Overview

The laws of thermodynamics apply to the energy budgets of fishes (**see also Energetics**: General Energy Metabolism). Because energy is conserved, the amount of energy ingested by a fish should equal the sum of the total energy expenditures by the fish, the fish's growth, and waste heat production. The components of the fish's energy budget can be measured independently. Laboratory studies have played an important role in quantifying the various energy expenditures of fish (**see also Energetics**: General Energy Metabolism). Of all of the components in the fish's energy budget, fish growth is typically the component most easily measured. For fish populations in aquatic ecosystems, growth trajectories can be determined by sampling fish from the aquatic ecosystem and then weighing and aging these sampled fish.

Consumption of food by fish is not measured easily in the field (i.e., in lakes, rivers, and oceans). The traditional approach for estimating food consumption in the field is to examine and weigh stomach contents of fish caught in the field, and then to apply knowledge of gastric evacuation rates to estimate food consumption rate. The amount of food found in a fish's stomach can be highly variable within a given fish population, and therefore a substantial amount of sampling is usually required to obtain representative estimates of food consumption rates. In addition, food consumption rates may change over time. Moreover, use of gastric evacuation rates to estimate consumption rate also represents a modeling step, and this modeling step may involve several assumptions as well. For example, evacuation rates vary as a function of fish temperature, which is generally variable and unknown. An alternative approach toward estimation of food consumption in the field is to apply a fish bioenergetics model.

Use of bioenergetics models to estimate food consumption by fish in aquatic ecosystems has provided insights into fish ecology, food-web ecology, and contaminant biology of fishes, as well as facilitated sound management of fisheries. The ability to estimate food consumption via bioenergetics modeling has enabled fishery scientists to identify important factors regulating fish growth and consumption in aquatic ecosystems. Estimation of food consumption, based on bioenergetics modeling, has also allowed ecologists to assess the role of fish in cycling nutrients such as nitrogen and phosphorus in aquatic ecosystems. The availability of nutrients influences the rate of primary production, which serves as the base of the food web. In addition, estimation of food consumption via bioenergetics modeling has aided toxicologists in evaluating the importance of various factors affecting accumulation of contaminants, such as polychlorinated biphenyls (PCBs) and mercury (Hg), in fish (**see also Toxicology**: The Toxicology of Metals in Fishes, and The Toxicology of Organics in Fishes). Estimation of food consumption by fish populations via bioenergetics modeling has allowed scientists to quantify the trophic link between predator and prey populations within the aquatic ecosystem, and this type of bioenergetics model application has had important implications for managing valuable fisheries.

---

[1] This article is Contribution 1616 of the US Geological Survey Great Lakes Science Center.

Fish bioenergetics models may also be applied to aquatic ecosystems to predict fish growth for a given rate of food consumption. Although this type of bioenergetics model application is not as common as using the bioenergetics model to estimate food consumption by fish, predicting fish growth for a given rate of food consumption has been used to better understand factors affecting growth of fish in lakes, rivers, and oceans (**see also Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism).

A Simple Energy Budget for Fish

A simple energy budget for fish can be written as

$$G = C - R - F - U - S \tag{1}$$

where G is the growth, C the consumption, R the respiration, F the egestion, U the excretion, and S the release of gametes (typically at spawning; **see also Energetics**: Energetics: An Introduction) (**Figure 1**). A fish takes in energy through food consumption (C). The fish expends energy via respiration (R), which is defined as the metabolic process by which an organism assimilates oxygen and releases carbon dioxide and other products of oxidation. The fish also loses energy through egestion (F), which is the expulsion of feces. Energy is also lost through excretion (U), which is the discharge of urine. When a fish matures, energy is also lost through the release of milt or eggs at the time of spawning (S). Subtracting all of the energy losses (R, F, U, and S) from the energy taken in via consumption yields the energy incorporated into fish growth (G). Using algebra, eqn (1) can be rewritten as

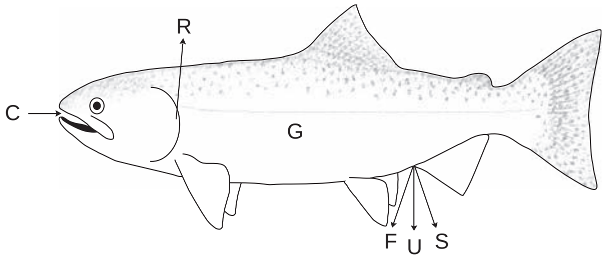


Figure 1 Diagram illustrating the energy budget for a fish. C, consumption; G, growth; R, respiration; F, egestion; U, excretion; and S, release of gametes at spawning.

$$C = G + R + F + U + S \tag{2}$$

where all of the terms are defined as in eqn (1). Summing the energy incorporated into fish growth with the various energy expenditure components yields consumption.

Wisconsin Bioenergetics Models for Fish

Beginning in the 1970s, researchers associated with the University of Wisconsin (UW) Center for Limnology (CFL) in Madison, Wisconsin (USA) have developed a suite of bioenergetics models for fish, and these models are generally called 'Wisconsin bioenergetics models'. A computer software package, prepared by a UW Center for Limnology research team, is available to the public. This software package includes bioenergetics models for a large variety of fishes: alewife (*Alosa pseudoharengus*), Atlantic herring (*Clupea harengus*), bloater (*Coregonus hoyi*), bluegill (*Lepomis macrochirus*), bluefish (*Pomatomus saltatrix*), burbot (*Lota lota*), coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*), dace (*Chrosomus* spp.), fishes of the family Coregonidae, lake trout (*Salvelinus namaycush*), largemouth bass (*Micropterus salmoides*), muskellunge (*Esox masquinogy*), Nile perch (*Lates niloticus*), northern pike (*Esox lucius*), pink salmon (*Oncorhynchus gorbuscha*), rainbow smelt (*Osmerus mordax*), sea lamprey (*Petromyzon marinus*), smallmouth bass (*Micropterus dolomieu*), sockeye salmon (*Oncorhynchus nerka*), steelhead (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*), tilapia (*Sarotheradon* spp.), walleye (*Sander vitreus*), walleye pollock (*Theragra chalcogramma*), weakfish (*Cynoscion regalis*), and yellow perch (*Perca flavescens*).

Typically, respiration is modeled as a function of fish weight and water temperature in these Wisconsin bioenergetics models. In some of these models, swimming speed is specifically included in the respiration component of the model, whereas an activity multiplier is included in the respiration component of other fish bioenergetics models. Egestion is modeled either as a function of consumption or as a function of both consumption and water temperature. Excretion is modeled as a function of consumption and egestion or as a function of consumption, egestion, and water temperature. Release of gametes is simulated by a fish losing a percentage of its body weight on the day of spawning. The time step for the Wisconsin bioenergetics models is typically 1 day.

To apply a Wisconsin fish bioenergetics model, four sets of information are required:

- water temperature regime experienced by the fish,
- diet composition of the fish,
- energy density of the fish's prey, and
- energy density of the fish.

These energy densities are used to determine the amount of energy contained in a given weight of the fish and its prey. The bioenergetics model is based on an energy balance (see eqns (1) and (2)), and therefore these energy densities are required for model application.

Each Wisconsin fish bioenergetics model can be applied in two different modes of operation:

- input fish growth to estimate consumption and
- input consumption rate to estimate fish growth.

In most cases, researchers are interested in estimating consumption, given a known amount of fish growth. However, in some instances, the researcher may be interested in predicting fish growth given a known consumption rate. Each Wisconsin fish bioenergetics model can be used to estimate consumption by a single fish or to estimate consumption by a population of fish. To apply the bioenergetics model to a population of fish, information on the population size and population mortality rate must be provided. Typically, when the bioenergetics model is applied to a single fish, the growth of the fish is chosen to be representative of an average fish within the fish population.

An Example of Fish Bioenergetics Modeling Guiding Fisheries Management

The classic example of fish bioenergetics modeling having an effect on fisheries management is the case of the stocking of salmonines (salmon and trout) into Lake Michigan (USA). The original top two native piscine predators in Lake Michigan were the lake trout and the burbot. Lake trout were extirpated from Lake Michigan during the 1950s, due to overfishing and sea lamprey predation. The sea lamprey, an invader from the Atlantic Ocean via the Welland Canal, also preyed on burbot, and this predation was responsible for the collapse of the burbot population during the 1950s. The alewife, a clupeid from the Atlantic Ocean, invaded Lake Michigan during the late 1940s. With a minimal amount of predator biomass in the lake, alewife abundance in Lake Michigan increased dramatically during the late 1950s and early 1960s, and peaked in 1966. Through lampricide treatments in tributary streams, sea lamprey abundance in Lake Michigan was greatly reduced during the early 1960s. A major salmonine stocking program for Lake Michigan was launched in 1965, with millions of Chinook salmon, lake trout, coho salmon, steelhead, and brown trout (*Salmo trutta*) stocked each year. Stocking of salmon and trout continues through today (2010) (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development, Lampreys: Environmental Physiology).

During the 1970s and early 1980s, researchers at the UW CFL developed bioenergetics models for salmon and trout, and then applied these models to populations in Lake Michigan. The results from these bioenergetics model applications revealed that estimates of annual consumption of alewives by salmon and trout populations in Lake Michigan increased from about 5000 metric tons in 1967 to over 50 000 metric tons by 1981. According to the modeling results, Chinook salmon were responsible for most of the annual consumption of alewives by Lake Michigan salmonines. The UW researchers then compared their estimates of annual consumption of alewives by salmon and trout in Lake Michigan with estimates of annual production of alewife biomass in Lake Michigan. From this comparison, these researchers estimated that Lake Michigan salmon and trout consumed as much as 33% of the annual alewife production in Lake Michigan in some years.

Based on the bioenergetics model results, the Center for Limnology researchers concluded that eventually the salmon and trout consumption would be sufficiently great to cause a substantial reduction in alewife abundance in Lake Michigan. These researchers warned managers of the recreational fisheries for Lake Michigan salmonines that the alewife population was headed for a collapse due to intense predation by the salmonines on alewives. Fishery managers from three of the four states surrounding Lake Michigan (Wisconsin, Illinois, and Indiana) responded to these warnings by reducing their stocking rates of Chinook salmon into Lake Michigan during the 1980s. Several additional Chinook salmon-stocking-rate reductions, with compliance from all four states (including Michigan), have been transacted during the 1990s and 2000s. Although alewife abundance in Lake Michigan has remained at relatively low levels during 1982–2008, when compared with abundances during the 1960s and early 1970s, the alewife population did not suffer a complete collapse during the 1990s or 2000s. The recreational fishery for Chinook salmon in Lake Michigan has remained an economically important fishery through the 2000s. Thus, from the standpoint of perpetuating the Chinook salmon fishery in Lake Michigan, these decisions to reduce Chinook salmon-stocking rates were effective decisions and were based on the results of bioenergetics modeling. The case of salmonine stocking in Lake Michigan clearly illustrates the utility of bioenergetics modeling in guiding fishery management decisions. Modeling studies on the dynamics of the

Lake Michigan alewife population and on Chinook salmon–alewife predator–prey interactions in Lake Michigan conducted during the 1990s and 2000s have corroborated the contention that Lake Michigan salmonines were exerting a strong predatory effect. Hence, the conclusion reached by UW Center for Limnology researchers in the 1980s concerning the strength of the trophic link between alewives and salmonines in Lake Michigan has been validated by subsequent studies.

Estimating the Strength of the Predator–Prey Trophic Link: Other Examples

Bioenergetics models have been used to estimate the strength of the predator–prey trophic link to quantify the predatory effect of introduced or invading fish populations on native fish populations. For example, bioenergetics modeling has been applied to the exotic Nile perch (*L. niloticus*) population in Africa's Lake Victoria to estimate annual consumption of endemic fishes by Nile perch. The Nile perch was introduced into Lake Victoria, where this piscivore fed on a variety of fishes, including the endemic haplochromine cichlids. Using bioenergetics modeling, researchers were able to estimate the degree of predation on haplochromines under various amounts of fishing mortality imposed on Nile perch. Consequently, this modeling exercise was useful in exploring various management strategies to reduce the predatory effect of Nile perch on the endemic haplochromines. In another example, bioenergetics modeling was used to estimate the annual consumption of native cutthroat trout (*Oncorhynchus clarki*) by the introduced lake trout in Yellowstone Lake (Wyoming, USA) and to evaluate the effectiveness of a lake trout-control program.

Bioenergetics models have also been used to quantify the predatory effect of native fish on invader populations. For example, dreissenid mussels invaded Lake Michigan (USA) and Lake Huron (Canada and USA) during the 1990s. A native benthivorous fish, the lake whitefish (*Coregonus clupeaformis*), has fed upon the dreissenid mussels in these two lakes. Bioenergetics modeling was applied to the lake whitefish populations in both lakes to determine the extent to which the lake whitefish populations were capable of reducing the population growth rate of dreissenid mussels. In another example, bioenergetics modeling has been applied to the burbot population in eastern Lake Erie (Canada and USA) to estimate its predatory effect on the round goby (*Neogobius melanostomus*), a benthic fish which invaded Lake Erie during the 1990s. The native burbot began to feed heavily on round gobies in eastern Lake Erie during the 2000s. Bioenergetics model results indicated that the burbot was the major contributor toward the predatory control of round gobies in the offshore waters of eastern Lake Erie.

Use of Bioenergetics Modeling to Better Understand Fish Growth and Consumption

Bioenergetics modeling can be applied to the average fish from a fish population to assess the effects of environmental factors on fish growth and consumption in aquatic ecosystems. For example, bioenergetics modeling has revealed that the faster growth of yellow perch in the central basin of Lake Erie compared with the western basin of Lake Erie was not due to a difference in water temperature between the two basins. Rather, the faster growth was attributable to greater availability of larger benthic invertebrate prey in central Lake Erie. In another study, bioenergetics modeling was used to determine if the relatively cold water temperature regime experienced by lake trout on Sheboygan Reef, a deep offshore reef in Lake Michigan (USA), was the primary factor for the slower growth of lake trout on Sheboygan Reef compared with lake trout from nearshore regions of Lake Michigan. Bioenergetics modeling results indicated that lake trout growth was not constrained by cold water temperatures on Sheboygan Reef. Rather, the low availability of small prey fish limited the growth of juvenile lake trout on Sheboygan Reef. A similar type of bioenergetics model application has shown that in most areas of the North Atlantic, growth of Atlantic cod (*Gadus morhua*) is not limited by cold water temperatures.

Bioenergetics modeling is well suited to address the effects of climate change on fish growth, because water temperature regime is an input to the bioenergetics model. Climate-change models can be used to predict future changes in water temperature. These predictions of water-temperature regimes can be used as an input to fish-bioenergetics models to generate predictions of changes in fish growth under various climate-change scenarios. For example, results from a modeling study indicated that growth rate of yearling largemouth bass in Lake Erie would increase by nearly of factor of 6 under a climate-warming scenario corresponding to the doubling of carbon dioxide in the Earth's atmosphere, provided that prey availability increased in accord with the increase in water temperature.

Bioenergetics modeling can also be used to estimate changes in fish-consumption rates due to changes in food supply. For example, the dreissenid-mussel invasions of Lake Michigan and Lake Huron are associated with declines in the abundance of a native amphipod *Diporeia*. Growth and condition of both alewife and lake whitefish have decreased in response to lower abundance of *Diporeia*, because this amphipod was an important component of the diet of both fishes. Bioenergetics modeling revealed that the overall feeding rate of alewives decreased during the dreissenid mussel invasion, whereas lake whitefish-feeding rate actually increased during the dreissenid mussel invasion. In addition, the bioenergetics modeling indicated that alewives did not compensate for lower *Diporeia* abundance by increasing their feeding rate on zooplankton. In contrast, lake whitefish fed on dreissenid mussels during the invasion at a rate higher than the rate at which lake whitefish fed on *Diporeia* before the mussel invasion.

Role of Fish in Cycling Nutrients in Aquatic Ecosystems: Bioenergetics Model Applications

Fish excrete nitrogen and phosphorus, two nutrients essential for the growth of phytoplankton and other aquatic plants. Phosphorus has been identified as the limiting nutrient for primary production in most freshwater ecosystems, whereas nitrogen is the limiting nutrient in many marine ecosystems. The phosphorus budget for a fish can be written as

$$P_U = P_C - P_G - P_F - P_S \quad (3)$$

where P_U is the phosphorus excreted, P_C the phosphorus consumed, P_G the phosphorus allocated to growth, P_F the phosphorus lost through egestion, and P_S the phosphorus lost through release of gametes at spawning. Knowing the phosphorus content in the urine of the fish, in the feces of the fish, in the somatic tissue of the fish, in the gonadal tissue of fish, and in the food of the fish, fish bioenergetics modeling can then be used to estimate the amount of phosphorus excreted by a population of fish within an aquatic ecosystem. In an analogous manner, fish bioenergetics modeling can also be used to estimate the amount of nitrogen excreted by a population of fish within an aquatic ecosystem. In addition, bioenergetics modeling can be used to estimate the degree to which a fish population can serve as a sink for phosphorus or nitrogen within an aquatic ecosystem.

Fish-bioenergetics-model applications have shown that certain fish populations play an important role in cycling nutrients within some aquatic ecosystems, while other fish populations have little effect on nutrient cycling within the ecosystem. For example, via bioenergetics modeling, excretion and egestion of phosphorus by the alewife population in Lake Michigan during the mid-1970s was estimated to be equivalent to the phosphorus regeneration by Lake Michigan zooplankton. Thus, alewives played an important role in phosphorus recycling within the Lake Michigan ecosystem during the mid-1970s. In another example, bioenergetics modeling revealed that populations of trout introduced into mountain lakes of the Sierra Nevada of California (USA) were regenerating phosphorus within the lakes at a rate approximately equal to the rate of atmospheric deposition of phosphorus into these lakes. Consequently, trout introductions into these mountain lakes had a substantial effect on the cycling of phosphorus within these lake ecosystems. In contrast, the round goby population in central Lake Erie was estimated to excrete less than 1% of the phosphorus needed by the benthic community for primary production. Thus, the bioenergetics modeling results indicated that the round goby invasion of central Lake Erie had very little influence on phosphorus cycling within the central Lake Erie ecosystem.

Contaminant Accumulation in Fish: Bioenergetics Model Applications

Bioenergetics modeling has been invaluable in identifying the major factors regulating contaminant accumulation in fish in aquatic ecosystems. For example, direct uptake of PCBs from the water into fish via the gills was once believed to be the major pathway of PCBs into fish living in lakes, rivers, and oceans. However, application of a bioenergetics model to Lake Michigan lake trout revealed that nearly all of the PCBs in adults entered the lake trout via food consumption. In addition, bioenergetics modeling has been used to quantify the effects of fish growth on rates of contaminant accumulation in fish. For example, female walleyes grow considerably faster than male walleyes, with mean weight at age for mature females ranging from 20% to 50% higher than that for mature males. Despite the substantially higher growth rate for females, bioenergetics modeling has shown that this higher growth rate for females will only account for roughly a 15% higher PCB concentration in males compared with same-aged females. This growth dilution effect is the phenomenon of faster-growing fish having lower contaminant concentrations than slower-growing fish, under equivalent conditions. Thus, bioenergetics modeling has shown that growth dilution typically accounts for only a modest proportion of the observed differences in fish contaminant concentrations.

Limitations to Fish Bioenergetics Model Applications

All mathematical models have limitations and caveats associated with their use, and fish bioenergetics models are no exception to this rule. Several problems with fish-bioenergetics-model applications have been identified. For example, values of several of the model parameters for a particular fish species may have been borrowed from the bioenergetics model for another fish species, and these borrowed values may not be appropriate for model application. In addition, fish activity may vary from one aquatic ecosystem to another, and therefore the preset fish activity level included in the bioenergetics model for a particular fish species may not be appropriate for every model application. Also, if fish are not properly acclimated to the respiration chamber in the laboratory, then their respiration rates may be elevated compared with normal conditions in the aquatic ecosystem (**see also Energetics**: General Energy Metabolism). If these elevated respiration rates are then incorporated into the fish bioenergetics model, then the bioenergetics model will overestimate consumption when applied to fish in aquatic ecosystems. Fish bioenergetics models should be evaluated in both the laboratory and the field to determine their reliability in estimating consumption and growth.

See also: **Energetics**: General Energy Metabolism. **Hagfishes and Lamprey**: Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Toxicology**: The Toxicology of Metals in Fishes; The Toxicology of Organics in Fishes.

Further Reading

Bunnell DB, Johnson TB, and Knight CT (2005) The impact of introduced round gobies (*Neogobius melanostomus)* on phosphorus cycling in central Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 15–29.

Hansen MJ, Boisclair D, Brandt SB, *et al.* (1993) Applications of bioenergetics models to fish ecology and management: Where do we go from here? *Transactions of the American Fisheries Society* 122: 1019–1030.

Hanson PC, Johnson TB, Schindler DE, and Kitchell JF (1997) *Fish Bioenergetics 3.0*. University of Wisconsin, Sea Grant Institute, WISCU-T-97-001, Madison, Wisconsin.

Hayward RS and Margraf FJ (1987) Eutrophication effects on prey size and food available to yellow perch in Lake Erie. *Transactions of the American Fisheries Society* 116: 210–223.

Hewett SW and Johnson BL (1987) *A Generalized Bioenergetics Model of Fish Growth for Microcomputers*. University of Wisconsin, Sea Grant Institute, WIS-SG-87-245, Madison, Wisconsin.

Hoar WS, Randall DJ, and Brett JR (eds.) (1979) *Fish Physiology, volume VIII, Bioenergetics and Growth*. New York: Academic Press.

Jobling M (1994) *Fish Bioenergetics*. London: Chapman and Hall.

Kitchell JF, Schindler DE, Ogutu-Ohwayo R, and Reinthal PN (1997) The Nile perch in Lake Victoria: Interactions between predation and fisheries. *Ecological Applications* 7: 653–664.

Kleiber M (1975) *The Fire of Life: An Introduction to Animal Energetics*, 2nd edn. New York: Robert E. Krieger Publishing.

Kraft CE (1993) Phosphorus regeneration by Lake Michigan alewives in the mid-1970s. *Transactions of the American Fisheries Society* 122: 749–755.

Madenjian CP, DeSorcie TJ, and Stedman RM (1998) Ontogenic and spatial patterns in diet and growth of lake trout in Lake Michigan. *Transactions of the American Fisheries Society* 127: 236–252.

Madenjian CP and O'Connor DV (1999) Laboratory evaluation of a lake trout bioenergetics model. *Transactions of the American Fisheries Society* 128: 802–814.

Madenjian CP, O'Connor DV, Pothoven SA, *et al.* (2006) Evaluation of a lake whitefish bioenergetics model. *Transactions of the American Fisheries Society* 135: 61–75.

Ney JJ (1993) Bioenergetics modeling today: Growing pains on the cutting edge. *Transactions of the American Fisheries Society* 127: 736–748.

Rice JA and Cochran PA (1984) Independent evaluation of a bioenergetics model for largemouth bass. *Ecology* 65: 732–739.

Ruzycki JR, Beauchamp DA, and Yule DL (2003) Effects of introduced lake trout on native cutthroat trout in Yellowstone Lake. *Ecological Applications* 13: 23–37.

Stewart DJ, Kitchell JF, and Crowder LB (1981) Forage fishes and their salmonid predators in Lake Michigan. *Transactions of the American Fisheries Society* 110: 751–763.

Stewart DJ, Weininger D, Rottiers DV, and Edsall TA (1983) An energetics model for lake trout, *Salvelinus namaycush*: Application to the Lake Michigan population. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 681–698.

Weininger D (1978) Accumulation of PCBs by Lake Trout in Lake Michigan. PhD Thesis, University of Wisconsin.

Whitledge GA and Hayward RS (1997) Laboratory evaluation of a bioenergetics model for largemouth bass at two temperatures and feeding levels. *Transactions of the American Fisheries Society* 126: 1030–1035.

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

General Principles of Biochemical Adaptations

GN Somero, Stanford University, Pacific Grove, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Noncovalent Bonding as a Basis for Environmental Stress
Optimal Values for Biochemical Systems: The Conservative Side of Adaptation
Conservation of Protein Structure and Function
Conservation of an Appropriate Internal Milieu
Conservation of Membrane Function: Homeoviscous and Homeophasic Adaptation
Conservation of Gene Regulatory Ability
Summary
Further Reading

Glossary

Alphastat regulation Regulating the pH of blood and intracellular fluids in a manner that conserves the fractional dissociation state ($\text{alpha}_{\text{imid}}$) of the imidazole side-chains of histidyl residues of proteins.

Homeophasic adaptation Modification of the lipid composition of cellular membranes, notably in the head groups of phospholipids, to achieve conservation of the ability to form nonlamellar phases, which may be critical for endocytosis and exocytosis.

Homeoviscous adaptation Modification of the lipid composition of cellular membranes that achieves stability in the static order (fluidity) of the bilayer. Counteracts temperature-dependent changes in membrane fluidity (viscosity) mainly by changes in saturation level of fatty acids.

Noncovalent chemical bonds A diverse set of bonding interactions that do not involve sharing of electrons, but instead are based on polar effects, ionic interactions, hydrogen bonds, van der Waals interactions, and the hydrophobic effect. Noncovalent (weak) chemical bonds are primarily responsible for the higher orders of structure of large molecules – proteins, nucleic acids, and lipids. Noncovalent bonds have bond energies similar to the thermal energy of cells; so, they break and reform continually, giving proteins, lipids, and nucleic acids their intrinsic capacities to undergo conformational changes needed for function. The marginally stable structures established by reliance on weak bonds also make proteins, lipids, and nucleic acids highly sensitive to perturbation by temperature, hydrostatic pressure, pH, and the osmotic composition and concentration of biological fluids.

Orthologs Homologous genes (i.e., sharing a common evolutionary ancestor) that are found in different species.

Osmolyte A low-molecular-weight constituent of cells, including small organic molecules (amino acids, polyhydric alcohols, urea, and methylammonium compounds) whose concentrations are adjusted during osmoregulation to maintain cellular volume and establish an intracellular milieu compatible with optimal protein function.

Introduction

The diverse physiological capacities of fishes – including energy metabolism, locomotion, differentiation, growth, active transport, nerve-impulse conduction, and reproduction – are based on a set of biochemical processes and structures that are highly sensitive to perturbation by the environment, notably to conditions of temperature, hydrostatic pressure, osmotic composition and concentration, and pH. The success with which fishes have managed to sustain these biochemical capacities, while colonizing a vast range of habitats that differ in their physical and chemical properties, thus, is a reflection of pervasive adaptation at the biochemical level.

The purpose of this article is to present some general principles of adaptation that explain how these diverse biochemical systems succeed in functioning under widely different environmental conditions, thereby helping to support the remarkable diversification we find among the 25 000 or so species of fishes. The analysis is constructed around two distinct and complementary issues. First, the discussion is on how alterations in the abiotic (physical and chemical) environment perturb biochemical systems, thereby shifting key structural and functional properties of the systems into suboptimal ranges, where physiological function is compromised. Second, some general mechanisms of adaptation that account for the abilities of organisms to restore these perturbed systems to optimal states of structure and function are sketched out.

In developing this analysis, it will be necessary to define as rigorously as possible what is meant by ‘optimal’ values of structural and functional properties of diverse biochemical systems. Although this analysis might seem destined to head us into choppy philosophical waters, in fact, comparative biochemical studies during the past few decades have provided a basis for a sound, quantitative understanding of what ‘optimal’ means for a variety of biochemical systems. Studies of a taxonomically diverse suite of fishes and other animals from widely different habitat conditions have revealed striking patterns of conservation in the values of environmentally sensitive biochemical traits. These conserved values, which are examined in several systems and in a variety of stress-related contexts, serve as quantitative indices of how closely a biochemical system is poised to its optimal range of values, those at which physiological systems work best. These optimal values can be viewed, then, as the targets of the processes that underlie adaptation to environmental stresses.

Noncovalent Bonding as a Basis for Environmental Stress

All biochemical systems have higher-order structures stabilized by noncovalent bonds. These bonds are sometimes referred to as weak chemical bonds to indicate that their bond energies are of the same order of magnitude as the thermal energy of the system. Thus, these weak bonds continually form and break at biological temperatures. Despite their labile nature, noncovalent bonds are responsible for the formation and stabilization of the membrane bilayer, the conformations and assembly states of proteins, the binding of ligands to proteins and nucleic acids, and the capacities of nucleic acids to support closely regulated transcription of genes and translation of the resulting messenger RNA (mRNA) into proteins.

To one not familiar with the linkages between structure and function in these diverse molecular systems, it might seem puzzling as to why evolution has led to such a profound dependence on biochemical structures and reactions that are so intrinsically sensitive to perturbation by the physical (temperature and hydrostatic pressure) and chemical (ionic composition and pH) features of the environment and the cell. The most general explanation of this reliance on noncovalent bonds is based on the critical role that changes in molecular shape and organization have in most aspects of biochemical activity. The precision and sensitivity of all biochemical processes are based in large measure on reversible changes in the three-dimensional shape (conformation) and organizational state (e.g., assembly of different proteins linked to a common function) of lipids, proteins, and nucleic acids. Thus, all organisms face a common problem in coping with changes in the abiotic features of their environments: the properties of biochemical systems that give these systems their remarkable abilities to form the structures and drive the processes essential to life also render them highly sensitive to environmental change.

Optimal Values for Biochemical Systems: The Conservative Side of Adaptation

It follows from this analysis that the core, weak-bond-dependent biochemical systems responsible for cellular structure and function must adapt their properties to offset perturbation from the environment, so as to maintain these diverse systems in an optimal range for function. **Figure 1** presents a general model of what this conservative type of adaptive response achieves. Shown are the responses of homologous systems of a variety of species evolutionarily adapted to different values (low, medium, and high) of the environmental variable in question, to an acute change in this environmental factor. The responses could also be viewed in the context of acclimatization to environmental changes occurring during an individual organism’s lifetime. Even though each system exhibits a sharp response to an acute change in the environmental factor, at the normal range of environmental conditions

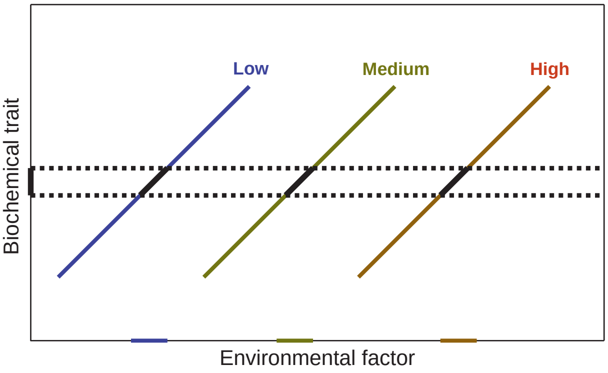


Figure 1 A model showing how an environmentally sensitive trait, such as one based on noncovalent bonds, in species adapted to different values of an environmental factor such as temperature is influenced by acute changes in this factor and by evolutionary adaptation or acclimatization. The three species (or three acclimatization states) illustrated, low, medium, and high, have highly conserved values for the trait in question at their respective adaptation or acclimatization conditions (thick line segments in response functions and on the abscissa). The conserved range is shown by dashed lines. These conserved ranges for traits are defined as optimal values for the trait.

experienced by the organism, either during its evolutionary history (genetic adaptation) or at the season in question (acclimatization), the value of the system is strongly conserved. Empirically, these conserved values are defined as optimal. What makes them optimal in terms of function and mechanism is discussed next in the context of each major class of large molecular systems.

Conservation of Protein Structure and Function

Proteins provide excellent illustrations of the conservative nature of biochemical adaptation portrayed abstractly in **Figure 1**. They likewise present fascinating examples of the complementary roles played by adaptive changes in a large molecule – here, in amino acid sequence – and in the milieu in which large molecules carry out their activities. As proteins are so strongly affected by changes in temperature, enzyme–temperature interactions, notably those involving binding reactions, provide a good starting point for this analysis (**see also Temperature**: Proteins and Temperature).

The initial step in an enzymatic reaction is the binding of ligands (substrates and cofactors) to the enzyme's catalytic site(s). Binding ability is usually quantified by determining the Michaelis–Menten constant (K_m) of the ligand, which is measured in units of substrate concentration (e.g., mmol L^{-1}) and equals the concentration of substrate or cofactor that is sufficient to cause the reaction to proceed at half-maximal velocity (V_{max}). Strong binding is indicated by a low value of K_m. For most enzymes, acute increases in temperature cause a loss of binding ability (increase in K_m) as shown in **Figure 2** for a glycolytic pathway enzyme, lactate dehydrogenase-A (LDH-A). Loss of binding ability with rising temperature is thought to be largely due to temperature-dependent changes in the three-dimensional configuration of the regions of the protein involved in ligand recognition. However, despite the acute effects of temperature change on binding, when comparisons of orthologs from differently temperature-adapted species are compared, a high degree of conservation of K_m is observed at the normal body temperatures of the species. These values of K_m can be regarded as optimal values for binding ability. (**see also Temperature**: Proteins and Temperature).

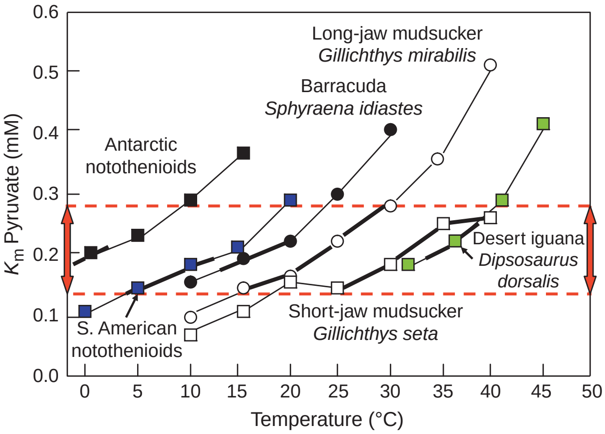


Figure 2 The effects of adaptation temperature and acute shifts in measurement temperature on binding of the substrate pyruvate (indexed by K_m^{pyruvate}) by orthologs of lactate dehydrogenase-A. Dark line segments illustrate K_m^{pyruvate} values at the physiological temperature ranges of the different species. The dashed lines and vertical thick arrows on the ordinates show the conserved range of K_m^{pyruvate} values for the species. Modified from Hochachka PW and Somero GN (2002) *Biochemical Adaptation*. New York: Oxford University Press, Figure 7.7.

Conservation of an Appropriate Internal Milieu

Organic Osmolytes

The osmolyte composition and concentration and the proton activity (pH) of intra- and extracellular fluids have strong effects on proteins. To a large degree, the changes in osmolyte composition and concentration that are observed during osmotic regulation and the fine-scale adjustments of pH made during acid–base regulation, and in response to shifts in body temperature, reflect the importance of conserving critical properties of proteins such as K_m. Discovery of the key roles of organic osmolytes in establishing an appropriate milieu for protein function and structure was an important achievement of comparative biochemistry over the past three decades. Although most of these studies have involved osmotically conforming marine and estuarine invertebrates, species that display wide variation in osmolyte composition and concentration in response to changes in external osmolality, the conclusions drawn from these studies apply to fishes as well, notably to osmoconforming marine elasmobranchs and hagfishes whose body fluids have osmolalities close to that of the surrounding medium.

Cells of osmoconforming marine invertebrates and hagfishes contain a suite of small organic molecules that individually have little or no effect on protein structure or function at physiologically relevant concentrations. These osmolytes thus are termed compatible osmolytes. In marine elasmobranchs and the coelacanth, a different strategy is found, the counteracting-solute strategy. Here, a mixture of urea, a protein denaturant, and methylammonium solutes such as trimethylammonium N-oxide (TMAO) and glycine betaine, which are potent stabilizers of proteins, is employed. When the appropriate ratio of urea to methylammonium solutes is present, there is no net effect on protein stability or function: the destabilization caused by urea is fully offset by the stabilization achieved by methylammonium solutes such as TMAO. Thus, the conservation of critical protein characteristics, such as ligand-binding ability and an optimal balance between protein stability and flexibility, can be achieved through accumulating either a suite of organic osmolytes that are inherently nonperturbing at physiologically relevant concentrations or a set of two or more types of osmolytes that have offsetting effects on protein stability and function.

The use of protein-stabilizing osmolytes, such as TMAO, is not restricted to the context of osmotic adaptation. Recently, Paul Yancey, the discoverer of the counteracting-solute mechanism, and colleagues showed that depth-related changes in osmolyte composition and concentration offset the effects of elevated hydrostatic pressure on proteins. TMAO not only can offset perturbation caused by a chemical, urea, it also offsets disruption of protein structure and function by a physical factor, hydrostatic pressure. Comparisons of the tissue-fluid compositions of bony fish collected at different depths revealed that the concentration of TMAO increased in a regular manner with increasing depth of occurrence (**Figure 3**). The effect of depth of occurrence on [TMAO] was seen among conspecifics collected from different depths as well as between species with different depths of occurrence, suggesting active depth-related regulation of TMAO. Other studies by Yancey and colleagues showed a similar trend in a variety of marine invertebrates collected at different depths. Elasmobranch fishes, too, showed an interesting trend: shallow-living species contained the canonical 2:1 ratio of urea to methylammonium compounds long noted in comparative studies of elasmobranches living in shallow habitats. However, with increasing depth of capture, the ratio shifted toward a more stabilizing mixture, that is, a lower [urea]:[TMAO] ratio. In species from the greatest depths sampled, the concentration of TMAO exceeded that of urea.

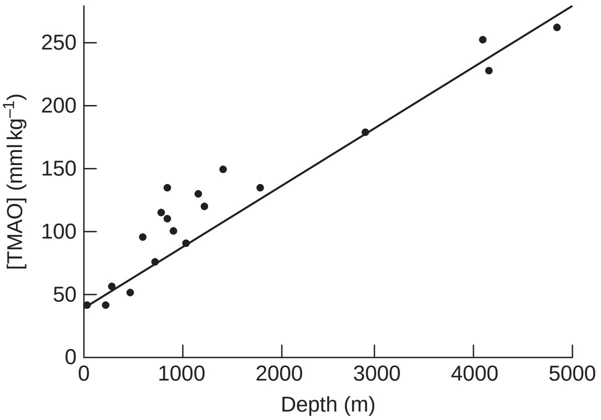


Figure 3 The relationship between depth of capture (m) and concentration (mmol kg^{-1}) of trimethylamine-N-oxide (TMAO) in a variety of marine bony fishes. The depth-related trend in concentration of TMAO is found among conspecifics collected at different depths as well as among different species of fish. Modified from Samerotte AL, Drazen JC, Brand GL, Seibel BA, and Yancey PH (2007) Correlation of trimethylamine oxide and habitat depth within and among species of teleost fish: An analysis of causation. *Physiological and Biochemical Zoology* 80: 197–208, Figure 2.

pH

The hydrogen ion also plays an important role in conserving protein function and structure. At physiological pH values, only a single amino-acid side-chain, the imidazole ring of the amino acid histidine, can be titrated by changes in pH (**Figure 4**). For a wide range of physiological processes, it is important to retain this titration capacity, to ensure that biochemical reactions can respond appropriately to the acid–base status of the cell and blood. Largely for this reason, regulatory processes function to conserve the charge states of imidazole residues. As the dissociation of the imidazole side-chain is temperature-dependent – the pK of the reaction exhibits a shift of approximately –0.018 pH unit per degree Celsius increase in temperature (however, the response is not quite linear with temperature over the biological temperature range (**Figure 4**, left panel)) – increases in body temperature drive dissociation of the proton from the imidazole ring. Thus, unless counterbalanced through acid–base regulation, changes in cell temperature can disrupt processes dependent on an appropriate charge state of imidazole side-chains. To avoid this type of thermal perturbation, a pH regulatory strategy termed 'alphastat regulation' is observed in almost all cells, under almost all circumstances. 'Alpha$_{\text{imid}}$' refers to the fractional charge state of imidazole side-chains (**Figure 4**), and alphastat regulation denotes regulatory processes that function to maintain this stable charge state. Alphastat regulation maintains the stability of protein function, including ligand-binding ability and subunit aggregation state, two common pH-sensitive characteristics of proteins. Thus, the adaptive changes in proteins that result from evolutionary shifts in amino-acid composition are defended in the context of acute fluctuations in pH through alphastat regulation. For enzymes that employ an imidazole residue in the active site, for instance, lactate dehydrogenase, for which binding of pyruvate requires a protonated imidazole ring (**Figure 4**, right panel), alphastat regulation is a vital component of the overall capacity of the system to retain stable ligand binding in the face of temperature change. When pH is maintained constant across all temperatures (pHstat conditions), K_m^{pyruvate} values for LDH-A orthologs vary over a much wider range than those under the alphastat conditions that pertain in the cell (**Figure 4**, right panel). Conservation of optimal

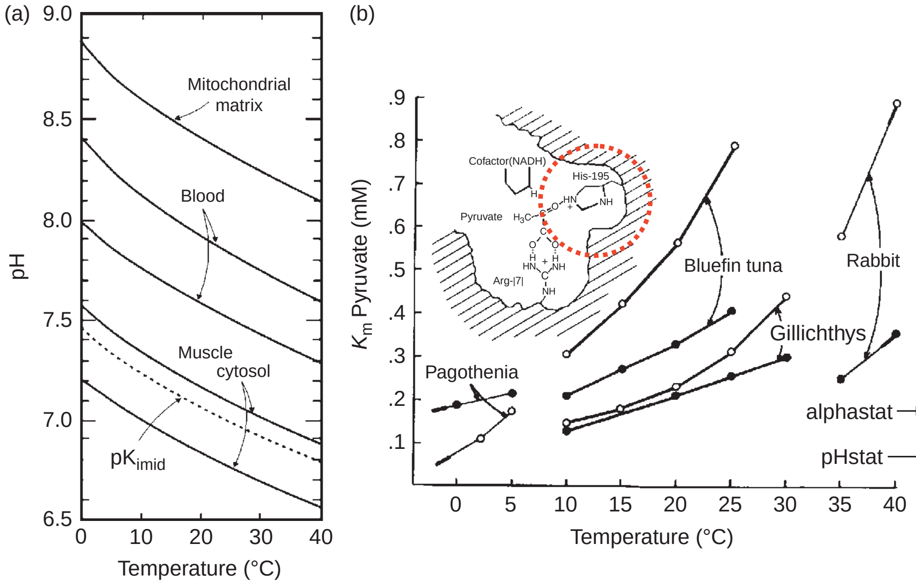


Figure 4 The effects of measurement pH on orthologous LDH-As of different species of vertebrates. Left panel: the observed variation in extracellular (blood)-, intracellular (muscle cytosol)-, and mitochondrial matrix pH as a function of temperature. Note that the temperature-dependence of pH follows that of the dissociation constant of the imidazole side-chain of histidine, pK$_{imid}$. Right panel: the effect of a biologically realistic assay pH (alphastat regulation) and temperature-independent pH (pHstat regulation) on the $K_m{}^{pyruvate}$ of LDH-A orthologs. Alpha$_{imid}$ = [imid–H$^+$]/{[imid] + [imid–H$^+$]}. Modified from Hochachka PW and Somero GN (2002) *Biochemical Adaptation*. New York: Oxford University Press, Figure 7.16.

conditions for proteins thus involves close regulation of the pH of the milieu. Optimal pH values in the cytoplasm are essentially those at which the charge states of imidazole side-chains are conserved, as shown in **Figure 4**.

Conservation of Membrane Function: Homeoviscous and Homeophasic Adaptation

The principles of conservation of optimal values of key structural and functional traits outlined for proteins apply for cellular membranes as well, which are composed of approximately equal masses of proteins and lipids. Changes in temperature exert complex effects on membranes due, as in the case of protein-based structures, to influences on noncovalent bonds. Temperature-sensitive noncovalent bonds, such as those mediated by van der Waals interactions among the aliphatic chains of membrane phospholipids, largely establish the physical state of the bilayer. The expression 'physical state' encompasses a number of membrane properties, including the static order (fluidity) of the bilayer and the propensity of the membrane to form nonlamellar structures. To maintain the optimal physical state of the membrane, that is, to support the membrane's critical functions as a barrier, a site of transport (including exo- and endocytosis) and, in the mitochondrion, a locus of ATP production, the lipid composition of the bilayer must be regulated to compensate for the effects of temperature and, in deep water habitats, those of pressure.

Two different aspects of conservation of physical state have been examined in detail in fishes. Homeoviscous adaptation, the conservation of the static order of the membrane bilayer, is noted in comparisons of species evolutionarily adapted to different temperatures and in conspecifics acclimated (or acclimatized) to different temperatures. **Figure 5** illustrates these relationships for brain synaptosomal membranes from vertebrates adapted to temperatures between −1.9 °C (Antarctic notothenioid fish) and approximately 39 °C (birds and small mammals).

The regulatory responses that lead to homeoviscous adaptation involve modification in the acyl-chain composition (chain length and degree of saturation (double-bond content)) and head-group composition of phospholipids. Increases in double-bond content of acyl chains typically increase membrane fluidity. Thus, decreases in saturation with falling adaptation or acclimation temperature are normally observed in fishes and other ectotherms. Shifts between classes of head group can also modulate the static order of the bilayer.

Changes in head group, for example, shifts between choline and ethanolamine, are also instrumental in establishing homeophasic adaptation, the retention of the appropriate propensity for generating the nonlamellar, hexagonal-phase membrane states required for endocytosis and exocytosis.

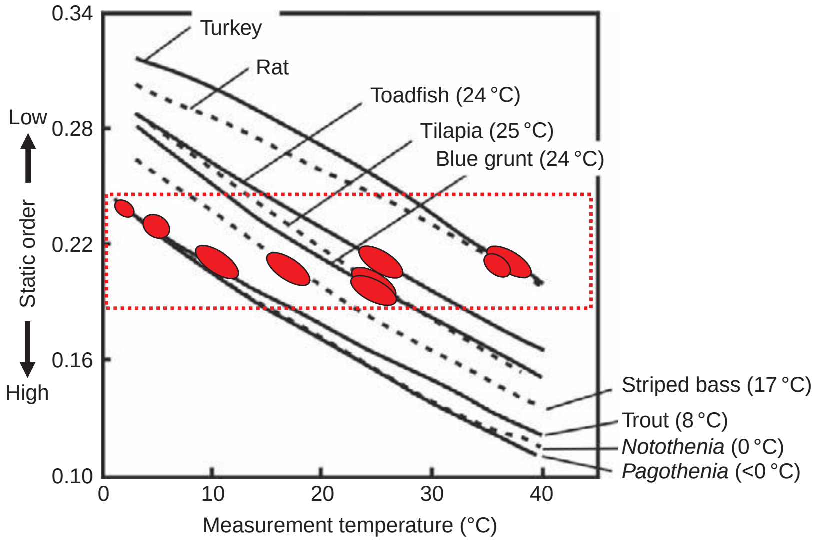


Figure 5 Homeoviscous adaptation of brain synaptosomal membranes. The effect of measurement temperature on membrane static order (measured as fluorescence anisotropy of the probe 1,6-diphenyl-1,3,5-hexatrience (DPH)). Filled ovals indicate the static order at the different species' temperatures of adaptation. Modified from Logue JA, DeVries AL, Fodor E, and Cossins AR (2000) Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *Journal of Experimental Biology* 203: 2105–2115, Figure 1.

Optimal properties of membranes can be regulated quite rapidly by substituting different acyl chains on phospholipids or modifying the phospholipid's head groups. Changes in cholesterol content have also been observed in thermal acclimation, with rising temperatures promoting higher cholesterol levels. By conserving optimal physical states for membrane lipids, optimal functional states for membrane-localized proteins are conserved.

Conservation of Gene Regulatory Ability

Adapting to an acute environmental stress frequently involves modulating gene expression in appropriate manners. Thus, sustaining optimal capacities for tightly regulated gene expression is a vital component of an organism's repertoire of responses for dealing with environmental perturbations of the biochemical machinery of the cell.

Due to the roles of noncovalent bonding in DNA and RNA secondary structures and in the interactions among nucleic acids and gene-regulatory proteins, such as transcription factors, abiotic stress would be predicted to have major impacts on the regulation of gene expression. Currently, much less comparative information is available on the effects of abiotic factors, such as temperature, hydrostatic pressure, and osmolality, on DNA and RNA structure and function than in the case of proteins and lipids. Nonetheless, even at this relatively early stage of comparative investigation of adaptation to stress in transcription and translation, adaptive patterns are emerging that resemble in many ways those discussed above for proteins and membranes. Here, we consider but one example of the types of adaptive responses that cells might use in maintaining an optimal state of DNA secondary structure for supporting transcription. While still somewhat conjectural, this illustration of temperature–DNA interactions may reflect a broad suite of adaptive processes that involve effectors of optimal nucleic-acid secondary structure.

DNA secondary structure is highly temperature dependent because of its reliance on hydrogen bonding between the two DNA strands. Yet, to be active in transcription, DNA must have a secondary structure that is sufficiently open to allow the complex transcriptional machinery, which comprises transcription factors and RNA polymerases, to function correctly. There is evidence from several studies, including one of fish acclimated to different steady-state temperatures as well as to an environmentally realistic regimen of diurnal variation in temperature, that DNA structure is closely regulated in a manner that conserves transcriptional ability. The extremely eurythermal killifish, *Austrofundulus limnaeus*, which experiences daily temperature fluctuations between approximately 24 °C and 38 °C, showed a strong temperature dependence in expression of a gene encoding a protein named high-mobility-group-B1 (HMGB1). This gene-regulatory protein is important in establishing the openness – and, thereby, the transcriptional potential – of DNA in a global manner. HMGB1 proteins are not specific transcription factors; rather, they modulate the openness of DNA throughout much of the chromosome, thus allowing specific transcriptional regulators to exert their effects on their target genes. Upregulation of the gene encoding HMGB1 occurred in steady-state cold-acclimated *A. limnaeus* and during the cold phase of a diurnally cycling temperature regimen. If the changes in mRNA for HMHG1 protein are reflective of changes in this rapidly turning-over protein, then an important form of temperature compensation of transcriptional activity could result. Thus, because DNA's double-helical structure is stabilized by reduced temperature, introducing higher concentrations of a protein that opens up DNA structure could offset the effect of cold on transcriptional capacity. Likewise, at high temperatures when DNA's structure may fluctuate into too open a configuration, reduction in concentrations of HMGB1 protein could offset this effect of heat on regulatory competence. Although the importance of this putative temperature-compensatory shift in DNA structural stability remains to be documented, it would appear that the concept of conserving an optimal structural state, as discussed for proteins and membranes, applies for DNA as well in the context of transcriptional potential.

Summary

The thrust of the above analysis has been to illustrate the conservative nature of biochemical adaptations that function in the face of abiotic stress to sustain the structural and functional characteristics of protein-, lipid- and nucleic-acid-based biochemical systems. Through adaptive changes in structures of the large molecules themselves and through compensatory shifts in osmolyte systems, pH, and other large molecules (e.g., HMGB1 proteins) that affect these biochemical systems, critical physiological processes such as enzymatic function, membrane transport, and signal transduction and transcription of DNA are sustained in the face of shifts in the abiotic environment. In all cases, we find that these conservative adaptations lead to the retention of optimal values for critical properties of the biochemical systems that underlie the physiological activities that are required for life, piscine or otherwise, across a wide range of environments.

See also: **Temperature**: Proteins and Temperature.

Further Reading

Buckley BA, Gracey AY, and Somero GN (2006) The cellular response to heat stress in the goby *Gillichthys mirabilis*: A cDNA microarray and protein-level analysis. *Journal of Experimental Biology* 209: 2660–2677.

Fields PA (2001) Protein function at thermal extremes: Balancing stability and flexibility. *Comparative Biochemistry and Physiology* 129A: 417–431.

Hazel JR (1995) Thermal adaptation in biological membranes – Is homeoviscous adaptation the explanation? *Annual Review of Physiology* 57: 19–42.

Hochachka PW and Somero GN (2002) *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford University Press.

Logue JA, DeVries AL, Fodor E, and Cossins AR (2000) Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *Journal of Experimental Biology* 203: 2105–2115.

Podrabsky JE and Somero GN (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *Journal of Experimental Biology* 207: 2237–2254.

Samerotte AL, Drazen JC, Brand GL, Seibel BA, and Yancey PH (2007) Correlation of trimethylamine oxide and habitat depth within and among species of teleost fish: An analysis of causation. *Physiological and Biochemical Zoology* 80: 197–208.

Somero GN (2004) Temperature adaptation of proteins: Searching for basic 'strategies'. *Comparative Biochemistry and Physiology* 139B: 321–333.

Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *Journal of Experimental Biology* 208: 2819–2830.

Yancey PH, Clark ME, Hand SC, Bowlus RD, and Somero GN (1982) Living with water stress. *Science* 217: 1214–1222.

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

Temperature

Contents

Effects of Temperature: An Introduction
Measures of Thermal Tolerance
Proteins and Temperature
Mitochondria and Temperature
Temperature and Excitable Membranes
Membranes and Temperature: Homeoviscous Adaptation
Heat Shock Proteins and Temperature
Effects of Climate Change

Effects of Temperature: An Introduction

PM Schulte, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Temperature and Its Measurement
Temperature, Thermal Energy, and Heat
Effects of Temperature on Rate Processes
Q_{10}
Arrhenius Breakpoint Temperature
Effects of Temperature on Weak Bonds
Strategies of Thermal Adaptation and Acclimation
Further Reading

Glossary

Abiotic factors Nonliving chemical and physical factors in the environment (e.g., temperature and salinity).

Activation energy The minimum amount of energy required to initiate a chemical reaction.

Arrhenius breakpoint temperature (ABT) The temperature at which the activation energy of a biological reaction changes abruptly. ABT is correlated with maximum habitat temperature for some species.

Arrhenius equation An equation describing the effects of temperature on reaction rates due to the increase in kinetic energy of molecules, which causes more molecules to exceed the activation energy of the reaction.

Arrhenius plot A graph showing the relationship between the natural logarithm (ln) of a measured reaction rate on the ordinate (*x*-axis) plotted against the inverse of temperature (1/*T*) in kelvin on the abscissa (*y*-axis) that is used to show the effects of temperature on the rate of a reaction.

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Endotherm An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Eurytherm An ectotherm able to survive over a broad range of environmental temperature.

Heat Form of energy resulting from random molecular movements that flows between objects at different temperatures.

Heterotherm Organisms that have both homeothermic and poikilothermic characteristic (see regional heterotherm and temporal heterotherm).

Homeotherm Organisms that maintain their body temperature within a relatively narrow range despite changes in environmental temperature.

Kinetic energy Energy due to motion.

Maxwell–Boltzmann distribution A probability function describing the distribution of kinetic energy of particles in a sample of an ideal gas. The shape of this distribution changes with temperature, with a larger proportion of particles with higher energies as temperature increases.

Poikilotherm An organism that cannot regulate body temperature except through behavioral means. An animal whose body temperature fluctuates with that of the environment.

Q_{10} Factoral change in the rate of a biochemical reaction or process with a 10 °C increase in temperature.

Regional heterotherm An organism that maintains a relatively constant temperature in some parts of the body, while allowing temperature in other parts of the body to vary with environmental temperature (e.g., tunas).

Temporal heterotherm An organism that maintains a relatively constant body temperature through generation of metabolic heat at some times of day or during some seasons, while allowing body temperature to vary with external temperature at other times of day or during some seasons (e.g., hibernators and organisms that undergo daily torpor).

Thermal energy Total energy of a system as a result of kinetic energy of the translational, rotational, and vibrational movement of molecules and potential energy of atomic interactions such as hydrogen bonds.

Temperature Measurement of the average kinetic energy or heat content within a substance or system.

Stenotherm An ectotherm able to survive only within a narrow range of environmental temperatures.

Introduction

Temperature has profound effects on biological systems at all levels of organization from the molecule to ecosystems through its effects on the rates of biological reactions, and on the formation and stabilization of weak intermolecular interactions. Consequently, understanding the fundamental effects of temperature is central to a core understanding of how all organisms, including fish, interact with their environment.

Temperature's effects are so profound that organisms have evolved a variety of strategies for either avoiding or compensating for the effects of temperature. These thermal strategies can be described using two sets of terms. The terms 'poikilotherm' and 'homeotherm' distinguish organisms based on the constancy of their body temperature. Poikilotherms have a body temperature that varies with the temperature of the environment, whereas homeotherms have a relatively constant body temperature. The terms 'endotherm' and 'ectotherm' distinguish animals based on the source of heat that affects body temperature. For endotherms, the majority of body heat is generated internally as a consequence of metabolic processes, and many endotherms have specialized physiological mechanisms for heat generation. For ecotherms, the majority of body heat is derived from the external environment. Most mammals and birds are homeothermic endotherms that maintain a relatively constant body temperature as a result of metabolic heat generation. Most fishes are poikilothermic ecotherms whose body temperature varies as a result of changes in environmental temperature. However, some fishes can be considered to be homeothermic ecotherms because they live in a very thermally stable environment, or use behavioral mechanisms to seek out microhabitats with a specific temperature.

Although all ecotherms generate heat as a result of standard metabolic processes (see also **Tissue Respiration**: Cellular Respiration and Mitochondrial Respiration, the low metabolic rate of most ectotherms does not generate sufficient internal heat to balance heat loss to the environment. For fishes, an additional challenge is the rapid loss of internally generated heat to the aquatic environment as the blood passes through the gills. Some fishes, such as tuna and marlin, have evolved a variety of strategies for reducing the loss of this heat to the environment, as well as for generating additional body heat (see also **Design and Physiology of Arteries and Veins**: The Retia, **Pelagic Fishes**: Physiology of Tuna, and Endothermy in Tunas, Billfishes, and Sharks). However, even these fishes are unable to maintain their entire body at a constant temperature in the face of environmental temperature variation. Instead, only certain parts of their body are maintained at a relatively constant temperature, while temperature in other regions of the body varies with the environment. Animals that employ the strategy of selectively defending the temperature of only some parts of the body are termed regional heterotherms. Temporal heterotherms allow their body temperature to track environmental temperatures at some times, while maintaining a relatively constant body temperature at other times.

Because most fishes are ectotherms, temperature plays a particularly critical role in determining their distribution and abundance. Temperature sets both the absolute limits at which fishes can live and influences their ability to function within those limits. For example, fishes can be found living actively only within a temperature range from a few degrees below 0 °C up to about 45 °C, and only a very few species of fish can even survive at all of temperatures across this range, with most species specializing to live at a much narrower range of temperatures. Fishes that can live across a wide range of temperatures are termed 'eurytherms', whereas fishes that specialize to live only at a narrow temperature range (and who cannot survive outside this range) are termed 'stenotherms'. The thermal sensitivity of an animal such as a fish is often summarized using a tolerance or performance curve, which relates the value of some parameter of tolerance (such as lethality, ability to maintain equilibrium, or sensitivity to touch) or performance (such as fecundity, growth, metabolic rate, or swimming performance) to temperature (**Figure 1**). These curves provide information on several parameters, including: (1) the optimal temperature for performance (T_{opt}); (2) the thermal breadth (or window), which defines the range of temperatures over which performance meets some specified threshold; and (3) the maximum and minimum tolerated temperatures for short-term survival (see also **Temperature**: Measures of Thermal Tolerance).

In order to understand the factors shaping performance curves and why temperature is so important for the lives of fishes, it is necessary to have a strong understanding of the effects of temperature on living systems. To do so, we must first define what is meant by the word temperature and distinguish this concept from the related words thermal energy and heat.

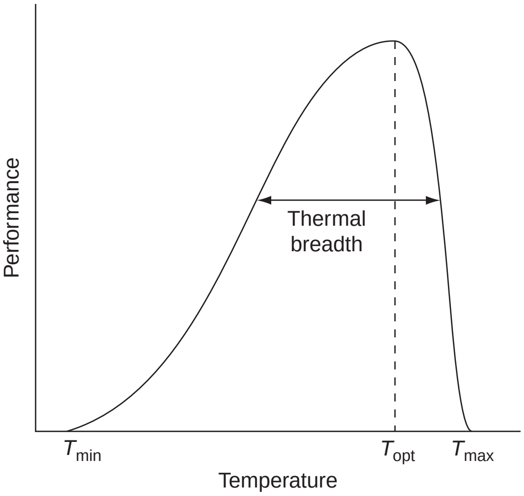


Figure 1 Thermal performance curve. This curve shows a hypothetical relationship between a performance parameter such as growth, swimming speed, or enzyme reaction rate and temperature. T_{opt} is the optimal temperature for performance. T_{max} is the maximum temperature at which performance is greater than 0. T_{min} is the minimum temperature at which performance is greater than 0. The thermal breadth (or thermal window): the temperature range over which performance exceeds some predetermined level. It is also possible to define an upper and lower T_{p} (pejus temperature) at which performance is statistically significantly lower than the performance at T_{opt}.

Temperature and Its Measurement

Temperature can be defined as a measure of the average translational kinetic energy (E_{k}) associated with the movement of a group of molecules. Translational kinetic energy (E_{k}) can be calculated as $E_{\mathrm{k}} = (1/2)mv^2$ (where m is the mass of the molecules and v their velocity). Thus, a group of fast-moving molecules will have a higher average kinetic energy and thus a higher temperature than a group of molecules that are moving more slowly, provided the molecules are of the same mass. But because molecules of greater mass have more kinetic energy at the same speed as molecules with lower mass, the molecules in a lead ball move more slowly than the molecules in an aluminum ball when the two balls are at the same temperature.

Temperature is measured on a variety of arbitrary scales, the most scientifically relevant of which are the Kelvin and Celsius scales. The kelvin (K) is the SI base unit of temperature. Note that temperatures reported in kelvin do not have a degree symbol, and the unit kelvin is not capitalized (although the symbol for the kelvin is a capital K, and the K is capitalized when we refer to the Kelvin scale). The Kelvin scale is designed so that 0 K is at absolute zero, a hypothetical temperature at which all molecular movement stops. The other major reference point on the Kelvin scale is the triple point of water (the temperature at which the solid, liquid, and gaseous phases of water are in equilibrium). The triple point of water is set as exactly 273.16 K. This number seems as if it is entirely arbitrary, but in fact it is a historical artifact of the development of the Kelvin scale from the existing Celsius scale. The Celsius scale was originally defined such that 0 °C was at the freezing point of water, and that 100 °C was at the boiling point of water. Each unit on the Kelvin scale (K) was set as the same as the degree Celsius (°C). In 1954 the Celsius scale was redefined such that absolute zero is at – 273.15 °C, and 0.01 °C is at the triple point of water. This definition sets both the degree Celsius and the kelvin as 1 part in 273.16 parts, which is the difference between absolute zero and the triple point of water. The Kelvin scale and the Celsius scale are thus readily interconvertible: 0 °C is exactly 273.15 K. To convert degrees Celsius into kelvin, one can simply add 273.15 to the temperature in °C.

Temperature, Thermal Energy, and Heat

Temperature, thermal energy, and heat are related concepts, but they are not the same. As discussed above, temperature can be operationally defined as a measure of the average kinetic energy of the molecules in a system. Thermal energy, in contrast, is the total energy contained in a system and depends not just on the kinetic energy from the translational movement of the molecules in the system, but also on the energy from the rotational and vibrational movement of the molecules, on their electrical interactions, and on the total number of molecules in the system. A milliliter of water contains much less thermal energy than does a liter of water at the same temperature. Likewise, a liter of air contains less thermal energy than does a liter of water at the same temperature, because water contains more molecules per unit volume than does air.

Heat is a word used to describe the transfer of energy from a system at higher temperature to a system at lower temperature. The word heat is also sometimes (incorrectly) used to describe the total thermal energy possessed by a system, but the word heat is more properly confined to describing the process of transfer of thermal energy from one system to another. This confusion arises, in part, because the amount of heat necessary to raise a given mass of a substance by a specific unit of temperature is termed the specific heat (or specific heat capacity). Given that heat is readily lost from blood to either water or air across a respiratory structure (gills or lungs, respectively), and that water can store more thermal energy than air, it becomes obvious why fishes have more difficulty retaining metabolic heat than do terrestrial animals.

There is a strong relationship between temperature and thermal energy. When we add thermal energy to a system, the temperature of the system generally increases. When we remove thermal energy from a system, the temperature of the system generally decreases. Note, however, that adding or removing energy does not always cause a change in temperature. It can also cause a change in state. For example, when thermal energy is removed from a liter of water, the water may freeze without changing temperature. Similarly, ice can melt when thermal energy is added, again without changing temperature. This change in state means that the average kinetic energy of the ice molecules in the moment before the ice melts is the same as the average kinetic energy of the water molecules in the moment after melting. Thermal energy is absorbed by the process of melting, but it does not result in a change in kinetic energy. Instead, the addition of thermal energy results in a change in weak bond formation between the molecules, which affects their potential (not their kinetic) energy. From this brief discussion, we can see that thermal energy has important effects both on the formation of weak bonds among molecules and on the movement of these molecules. As we discuss below, both of these processes are critically important factors when assessing the effects of temperature (or more correctly, thermal energy) on fish.

Effects of Temperature on Rate Processes

Increasing the temperature of a system increases the thermal energy of the system, which then causes an increase in the rates of most chemical reactions. The increase in the rate of chemical reactions is, in part, due to an increase in the number collisions between molecules per unit time. In order for two molecules to undergo a chemical reaction, they must first come into contact. Thus, increasing the number of collisions between molecules increases the number of contacts that can lead to a chemical reaction. However, this factor can only account for a very small proportion of the observed increase in reaction rate with temperature. For example, at biologically relevant temperatures, reaction rates roughly double (i.e., increase by 100%) with a 10 °C increase in temperature. We can approximate the frequency of collisions between particles as proportional to the square root of the temperature in kelvin. If the temperature of a system were increased from 10 to 20 °C (from 283.15 to 293.15 K), the frequency of the collisions would increase by a factor of 1.018-fold (or 1.8%), which is far lower than the observed ~100% increase in reaction rate. Thus, other factors must be involved in the increase in reaction rates with temperature.

Far more important for increasing the rates of reactions with temperature is an increase in the energy with which molecular collisions occur. Chemical reactions occur only when the energy of the molecular collision exceeds the activation energy (E_a) of the reaction (**Figure 2**). The Maxwell–Boltzmann distribution (**Figure 3**) can be used to describe the fraction of molecules with different kinetic energies in a population of molecules at a specified temperature, and allows

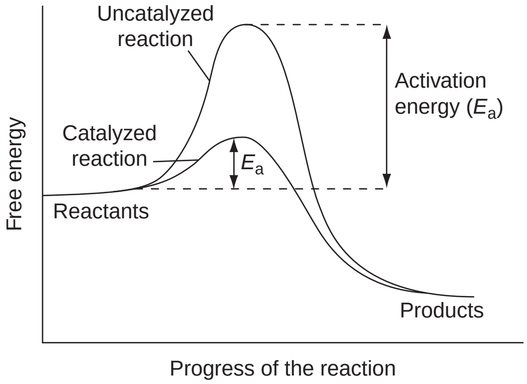


Figure 2 Free energy diagram for a spontaneous (exergonic) reaction. Chemical reactions occur through the formation of a high-energy transition state (the activated complex). The energy needed to form this transition state is called the activation energy (E_a) and is the difference between the energy of the reactants and the transition state. Catalysts such as enzymes reduce the activation energy of a reaction relative to that of the uncatalyzed reaction.

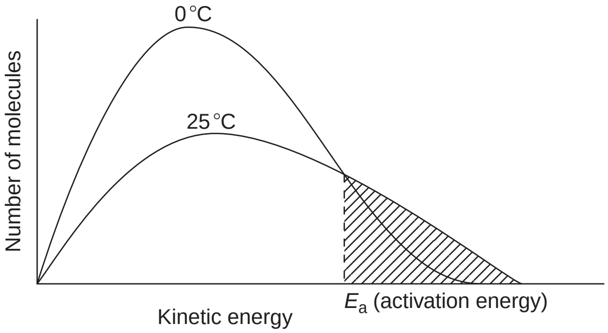


Figure 3 Effects of temperature on the Maxwell–Boltzmann distribution. The Maxwell–Boltzmann distribution is a probability distribution that shows the number of molecules with a given kinetic energy as a function of kinetic energy. In order for a reaction to proceed, the molecules involved must have kinetic energy equal to or greater than the activation energy (E_a). The number of molecules that exceed the activation energy is shown by the area under the curve in the shaded area of the diagram. As temperature increases, the Maxwell–Boltzmann distribution shifts down and to the right, and more molecules exceed E_a. This change in the shape of the distribution accounts for much of the increase in the rate of chemical reactions with temperature.

the estimation of the fraction of the molecules in a population that exceed the activation energy of a reaction. When temperature increases the thermal energy of the system, the fraction of molecules with high energy also increases. This increase in energy can be observed as a change in the shape of the Maxwell–Boltzmann distribution, which shifts down and to the right. Overall, this typically results in an increase in the number of molecules that exceed the activation energy of a reaction. Thus, more collisions with sufficient energy to cause a reaction occur per unit time at a higher temperature, and this effect causes the reaction to proceed more rapidly.

The effect of temperature on the rates of reactions can be quantified using the Arrhenius equation (named after the Swedish physical chemist Svante Arrhenius, who derived it based on earlier work by Van't Hoff). The Arrhenius equation is usually written as

$$k = A\ \mathrm{e}_{\mathrm{a}}^{-E}/RT \quad \text{or} \quad \text{In}\ k = -E_{\mathrm{a}}/RT + \text{In}\ A$$

where k is the rate of a reaction, A a term called the frequency factor or the pre-exponential factor (which is generally constant across biologically relevant temperature ranges), E_a the activation energy of a reaction, R the gas constant, and T the temperature in kelvin. Using the Arrhenius equation, we can calculate the effect of temperature on reaction rate. The Arrhenius equation clearly shows that the effects of temperature on reaction rates are exponential. Assuming biologically relevant values for E_a and A, the Arrhenius equation predicts that the rate of a reaction should roughly double when the temperature is increased from 10 to 20°C (i.e., increase by ~100%). Thus, unlike the effects of increasing the rate of molecular collisions, the effects of increasing the number of molecules with energy greater than the activation energy can account for most of the observed effects of increasing temperature on reaction rates.

One important point to remember when applying the Arrhenius equation to biological reactions (which are often enzyme catalyzed or involve other types of changes in protein structure in order to occur) is that E_a (the activation energy of the reaction) is an evolved property of the protein involved in the reaction. For example, enzymes act as catalysts because they reduce the E_a of a reaction, and the degree to which they reduce E_a may differ between species, and may vary with temperature. Differences in E_a between species and with temperature could alter the way that temperature affects a biological reaction. In addition, other factors (such as substrate concentrations or other cellular conditions) may vary with temperature *in vivo*, further complicating the analysis of the effects of temperature on biochemical and physiological processes in living organisms.

Q_{10}

Biologists often quantify the effects of temperature on the rates of physiological processes using the parameter Q_{10}, which is the unitless ratio of reaction rates at temperatures that differ by 10 °C (or 10 K). Q_{10} can be calculated as

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$$

where T_2 and T_1 are the temperatures in degrees Celsius or kelvin, and R_2 and R_1 are the rates of a biological process or reaction. If the rate of a reaction is independent of temperature, then Q_{10} will be equal to 1. If the rate of a reaction doubles with temperature, then Q_{10} will be equal to 2. Typically, the Q_{10}'s of enzyme-catalyzed reactions and other biological processes at biologically relevant temperatures are between 1 and 3.

Arrhenius Breakpoint Temperature

The effects of temperature on a biological reaction are often displayed using an Arrhenius plot, which relates the natural logarithm of a measured reaction rate to the inverse of temperature ($1/T$) in kelvin. This plotting method linearizes the otherwise exponential relationship between temperature and reactions rates (**Figure 4**). The y-intercept of the Arrhenius plot allows the calculation of the pre-exponential factor (A) and the slope of the line is $-E_a/R$, and is thus related to the apparent activation energy of the reaction. For biological reactions and metabolic processes, Arrhenius plots may have a breakpoint at which the slope of the

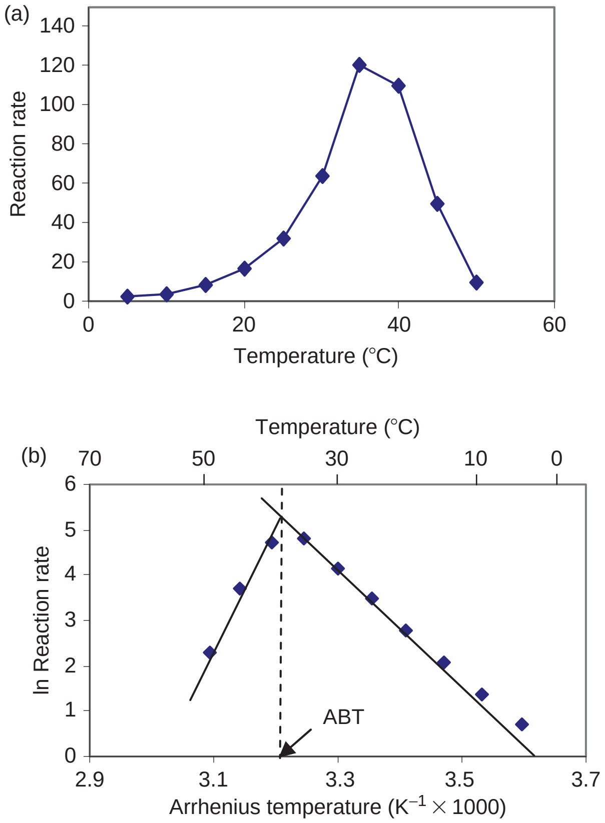


Figure 4 Effect of temperature on the rates biological reactions. Panel (a) shows how temperature affects the rate of a hypothetical reaction. Panel (b) shows the same data as an Arrhenius plot in which the natural logarithm (ln) of the reaction rate is plotted against inverse of the temperature in kelvin (K^{-1}). The slope of a line on an Arrhenius plot is an index of the activation energy of the reaction. Arrhenius plots for biological reactions often have discontinuities (or breakpoints) at which the slope of the line changes abruptly. The temperature at which these breakpoints occur is called the Arrhenius breakpoint temperature (ABT).

line changes abruptly. The temperature at which this breakpoint occurs (the Arrhenius breakpoint temperature, ABT) is often considered to be the first point at which loss of biochemical or physiological function may be occurring. At least in some species, ABTs are correlated with habitat temperature, with species from warmer habitats having higher ABTs for a given biochemical reaction or physiological function than do species from cooler habitats.

Care must be taken in applying Arrhenius plots to any multistep process such as mitochondrial respiration or whole-organism metabolic rate. The Arrhenius equation assumes that a reaction is governed by a single rate-limiting step. Processes such as mitochondrial respiration are composed of many biochemical reactions, each with its own activation energy, and thus its own slope on an Arrhenius plot. As a simplifying assumption, the rate of multistep process is often assumed to be governed by one of its steps (e.g., the rate-limiting enzyme in the pathway). However, it is possible that the step that is rate limiting within a multistep pathway could change with temperature, resulting in an Arrhenius plot with a complex shape.

Effects of Temperature on Weak Bonds

In addition to effects on rate processes, changes in temperature (and thus thermal energy) have important effects on the formation of noncovalent (weak) bonds between molecules. Weak bonds, which include bonds such as hydrogen bonds, ionic bonds, van der Waals interactions, and hydrophobic interactions, play a critical role in determining the secondary, tertiary, and quaternary structure of proteins and nucleic acids, and the behavior of biological membranes (see also **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations). An increase in thermal energy destabilizes hydrogen bonds, ionic bonds, and van der Waals interactions, whereas an increase in thermal energy stabilizes hydrophobic interactions (see also **Temperature**: Proteins and Temperature). In general, increases in temperature will tend to destabilize proteins to the point that they eventually denature and become nonfunctional. At very low temperatures, proteins become too rigid to perform their functions effectively. Cells have a variety of mechanisms to help stabilize proteins and reduce or mitigate the damage caused by protein-denaturing stressors such as high temperatures. The best known of these mechanisms are a family of proteins known as the heat shock proteins (hsp's) which help stabilize and refold proteins (see also **Temperature**: Heat Shock Proteins and Temperature).

The effects of temperature on weak bonds are also critical for the functioning of biological membranes, which are stabilized by a combination of hydrogen bonds and hydrophobic interactions. Changes in these interactions result in alterations in the fluidity of the membrane, which can alter the function of membrane-embedded proteins (see also **Temperature**: Membranes and Temperature: Homeoviscous Adaptation). For example, changes in membrane function can affect the function of mitochondrial proteins and affect the ability of the cell to generate energy using aerobic metabolism (see also **Temperature**: Mitochondria and Temperature). Temperature effects on cell membranes may be particularly critical for excitable cells such as neurons and muscle cells that rely on electrical signals for communication (see also **Temperature**: Temperature and Excitable Membranes).

Strategies of Thermal Adaptation and Acclimation

Physiological processes and biological reactions are carried out by proteins and membrane-bound compartments whose structure and composition have been shaped by evolution. Thus, changes in macromolecular structure as a result of adaptation have the potential to compensate for the effects of temperature on biological reactions and the formation and stabilization of weak intermolecular interactions. Sir Joseph Barcroft (1934) eloquently expressed this idea saying: "…nature has learned so to exploit the biochemical situation as to escape from the tyranny of a single application of the Arrhenius equation."

The extent to which fish, and ectotherms in general, are able to escape the tyranny of thermodynamics is still a matter of vigorous debate in the literature. For example, knowledge of thermodynamics suggests that temperature effects are likely to impose trade-offs such that it is difficult to evolve to function well at both high and low temperatures. However, this and other related questions remain unresolved and are likely to occupy thermal biologists for many years to come. These questions may, in fact, become increasingly important as anthropogenic effects on the global climate result in shifts in the temperature of natural environments (see also **Temperature**: Effects of Climate Change).

See also: **Design and Physiology of Arteries and Veins**: The Retia. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks; Physiology of Tuna. **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations. **Temperature**: Effects of Climate Change; Heat Shock Proteins and Temperature; Measures of Thermal Tolerance; Membranes and Temperature: Homeoviscous Adaptation; Mitochondria and Temperature; Proteins and Temperature; Temperature and Excitable Membranes. **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration.

Further Reading

Angilletta MJ, Huey RB, and Frazier MR (2010) Thermodynamic effects on organismal performance: Is hotter better? *Physiological and Biochemical Zoology* 83(2): 197–206.

Barcroft J (1934) *Features in the Architecture of Physiological Function*. Cambridge: Cambridge University Press.

Bullock TH (1955) Compensation for temperature in metabolism and activity of poikilotherms. *Biological Review* 30: 311–342.

Chang H (2007) *Inventing Temperature: Measurement and Scientific Progress*. Oxford: Oxford University Press.

Cossins AR and Bowler K (1987) *Temperature Biology of Animals*. New York: Chapman and Hall.

Hazel JR (1995) Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology* 57: 19–42.

Hochachka PW and Somero GN (2002) *Biochemical Adaptation: Mechanism and Process in Physiological Evolution,* 466 pp. Oxford: Oxford University Press.

Hofmann GE and Todgham AE (2010) Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. *Annual Review of Physiology* 72: 127–145.

Johnston IA and Bennet AF (eds.) *Animals and Temperature: Phenotypic and Evolutionary Adaptation.* Cambridge: Cambridge University Press.

Moyes CD and Schulte PM (2008) *Principles of Animal Physiology*. San Francisco, CA: Pearson Education Inc.

Prosser CL (1973) *Comparative Animal Physiology*. Philadelphia, PA: Saunders.

Somero GN (1995) Proteins and temperature. *Annual Review of Physiology* 57: 43–68.

Somero GN (2004) Adaptation of enzymes to temperature: Searching for basic strategies. *Comparative Biochemistry and Physiology B* 139(3): 321–333.

Measures of Thermal Tolerance

TL Beitinger, University of North Texas, Denton, TX, USA

WI Lutterschmidt, Sam Houston State University, Huntsville, TX, USA

© 2011 Elsevier Inc. All rights reserved.

Temperature-Dependent Physiology: The Basis of Thermal Tolerance
Environmental Temperatures (T_e's) and Fishes
Thermal Tolerance of Fishes: A Brief History
Laboratory Measures of Thermal Tolerance
Influences on Thermal Tolerance
Ecophysiological Theory of Thermal Tolerance
Biological Applications of Thermal Tolerance
Concluding Remarks
Further Reading

Glossary

Abiotic The chemical and physical (nonbiological) variables of the environment, (e.g., temperature)

Biotic Environmental factors related to living organisms. The biological variables of the environment, e.g., predation and competition.

Ectotherm Organism whose body temperature is obtained and governed by heat exchange with its environment.

Endotherm An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Fitness Contribution of an individual to the next generation relative to other individuals, i.e., the proportion of the individual's genes in all the genes of the next generation. Traits affecting an organism's survival and reproductive success are tightly connected to fitness, i.e., fitness = survivorship' fecundity.

Heat Form of energy resulting from random molecular movements that flows between objects at different temperatures.

Lethal dose Concentration which kills 50% of the population (LD-50) in a specified time.

Loss of righting response Inability for an organism to right itself due to loss of sensory and motor control.

Poikilotherm An organism that cannot regulate body temperature except through behavioral means. An animal whose body temperature fluctuates with that of the environment.

Spasm Onset of muscular contractions caused by a physiological reaction to high temperature.

Temperature Measurement of the average kinetic energy or heat content within a substance or system.

Temperature quotient or Q_{10} A measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C. The Q_{10} is calculated as: $Q_{10} = (R1/R2)^{10\ (T2-T1)}$, where R is the rate at a particular temperature and T the temperature in degrees celsius.

Temperature tolerance polygon Derived from a plot of temperature tolerance on acclimation temperature in $^{\circ}C^2$.

Thermoregulation Ability of an organism to behaviorally or physiologically regulate its body temperature.

Van't Hoff rule A rule of thumb that the Q_{10} for a biological or biochemical rate process equals or approaches 2.0, that is, approximately doubles for a 10 °C increase.

Temperature-Dependent Physiology: The Basis of Thermal Tolerance

Temperature is by far the most pervasive abiotic factor governing an organism's biology. Some fishes have more physiological control over temperature than others, for example, tunas and billfishes (**see also Pelagic Fishes**: Physiology of Tuna and **Fish Migrations**: Tracking Oceanic Fish). However, most fishes have low metabolic rates that cannot produce significant metabolic heat, which in combination with little or no insulation makes it difficult to maintain a temperature much different than that of the surrounding water. Thus, physiological and metabolic rate processes in fishes are governed mainly by environmental temperature (T_e).

How significantly T_e influences a fish's physiological rate processes may directly indicate its sensitivity or tolerance to temperature. Not only is exposure to low or high temperatures important, but also the magnitude and speed of T_e change (ΔT_e). We are all familiar with thermal shocking observed when fish are too quickly transferred from one thermal environment to another.

The basis of an organism's tolerance for temperature is governed by its temperature-dependent rate processes, which can be quantified using indices such as the Q_{10} (**see also Temperature**: Effects of Temperature: An Introduction). Measures of upper and lower thermal tolerance describe the functional range of temperatures for an organism, while measures such as Q_{10} and the critical thermal increment (E_a) indicate how physiological rate processes change with increasing or decreasing temperature.

Environmental Temperatures (T_e's) and Fishes

Both the presence and the success of a fish within a habitat depend upon a complex combination of environmental factors. Of these factors, T_e has been defended as the single most important and pervasive factor. Thus, a fish's fitness (survivorship × fecundity) depends on its species-specific physiological tolerance for changes in environmental factors. Following F.E.J. Fry's classification of environmental factors by their influences on animals, a classic abiotic factor such as temperature can control the rates of physiological processes, limit locomotion and hence distribution, mask the influence of other environmental factors, direct an orientation when present as a gradient, and be lethal.

These multiple effects may be surprising since temperature, that is, heat *stricto sensu*, has only two main effects on living systems. In addition to influencing the rates of chemical reactions and physiological processes, heat influences the three-dimensional shapes of biomolecules, including enzymes and DNA (**see also Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations and **Temperature**: Proteins and Temperature). Although the vast majority of fishes are obligate poilikothermic ectotherms, they are not at the mercy of T_e. The cells, tissues, and organs of fishes have evolved the capacity to acclimate, which allows them to function over a wider range of body temperatures. Fishes have also evolved behavioral measures to avoid lethal levels of heat by seeking out and occupying thermally favorable habitats. The bottom line to this brief discussion is that T_e plays a major role in the lives of fishes.

Thermal Tolerance of Fishes: A Brief History

Simply, too much or too little heat can kill organisms. As concluded by F.E.J. Fry in the late 1940s "possibly the most dramatic effect of temperature is to act as a lethal agent." Although several hypotheses have been proposed, the mechanisms resulting in thermal death are unknown. It is likely that there are several different causes of thermal death. Lethality certainly precedes coagulation of proteins at high temperature and the formation of ice crystals at low temperatures. The unknown and specific causes of thermal death have not curtailed studies of lethal temperature. The subset of environmental temperatures that a species can tolerate is an important component of its *n*-dimensional niche and the basis for understanding thermal environments and distributional patterns of fishes.

The two major contemporary methods to quantify temperature tolerance were independently developed in the 1940s. In the early 1970s, aquatic temperature studies in the US, including thermal tolerances of aquatic organisms, were stimulated by concerns of thermal pollution from cooling systems of electric generating stations, particularly those associated with nuclear reactors. More recently, thermal tolerance research has become increasingly important for understanding the impacts of global climate change (**see also Temperature**: Effects of Climate Change). Quantifying a species thermal tolerance, in particular its upper lethal temperatures, is critical to assessing the potential biological/ecological impacts of anticipated increases in temperature predicted for specific ecosystems (e.g., the Great Lakes) and the entire biosphere.

Laboratory Measures of Thermal Tolerance

Incipient Lethal Temperatures

In 1942, F.E.J. Fry and co-workers at the University of Toronto introduced the temperature static, plunge, incipient lethal temperature (ILT), or simply the Fry method for temperature tolerance assessment of fishes. A temperature that is lethal to exactly 50% of a sample of fish in a specified time is empirically determined. Endpoints are designated as the incipient lower lethal temperature (ILLT) and the incipient upper lethal temperature (IULT). ILTs are determined in groups of fish (previously acclimated to the same temperature) that are instantaneously transferred from their acclimation temperature and plunged into one of a series of constant temperatures thought to contain the lethal temperature. ILLT and IULT are dependent, to varying degrees among fish species, on the acclimation temperature. Therefore, if ILLT and IULT are repeatedly determined over a range of acclimation temperatures, a

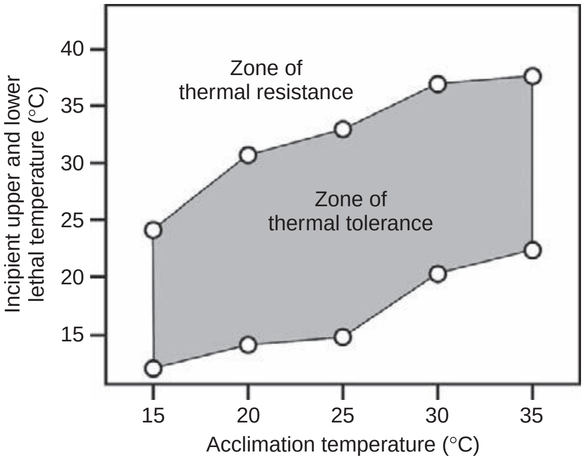


Figure 1 Thermal tolerance polygon showing the incipient lethal temperatures as the zones of thermal tolerance and thermal resistance.

temperature tolerance polygon can be constructed (**Figure 1**), separating temperature data into zones of resistance (outside the polygon) and tolerance (within the polygon). The slopes of the data lines then provide information on the role and degree of temperature acclimation.

Polygons are generated from the resistance times where 50% mortality is observed for a set of test temperatures (**Figure 2**(**a**)). These test temperatures are then regressed against resistances times for 50% mortality (**Figure 2**(**b**)) and the estimated 24 h IULT for 20 °C is determined (i.e., approximately 35 °C). Usually, this relationship is not linear with respect to resistance time; hence, data are transformed as probits and plotted against the logarithm of resistance time. The mainstays of this approach include (1) constant test temperatures, (2) mortality as the test endpoint, and (3) estimated ILLT and/or IULT at specified time intervals (e.g., 12, 24, or 96 h). If a temperature tolerance polygon is generated (**Figure 2**(**c**)), the most extreme temperature tolerances (i.e., ILLT and IULT) can be estimated. Here, **Figures 2**(**a**) and **2**(**b**) show how the single point of 35 °C is generated for the polygon shown in **Figure 2**(**c**). By finally calculating the area of the polygon in °C^2, the degree of species eurythermicity is determined. Stenothermal fish have a small area, and eurythermal fish a large area. The first ILT polygon was determined for goldfish (*Carassius auratus*), and to date, temperature tolerance polygons for <50 fish species have been published.

Temperature tolerance polygons can be further divided into intrinsic (genetic) and acquired (acclimation) temperature tolerances (**Figure 3**). In the presentation, acclimation-dependent high and low thermal tolerances equal 170 and 253 °C^2, respectively. The results ($n = 21$ species) indicated that (1) acclimation temperature was responsible for about 50% of total thermal tolerance polygon, and (2) lower acclimation-dependent areas were nearly 3 times greater than upper acclimation-dependent areas. This suggests that acclimation plays a larger role in low rather than in high thermal tolerance.

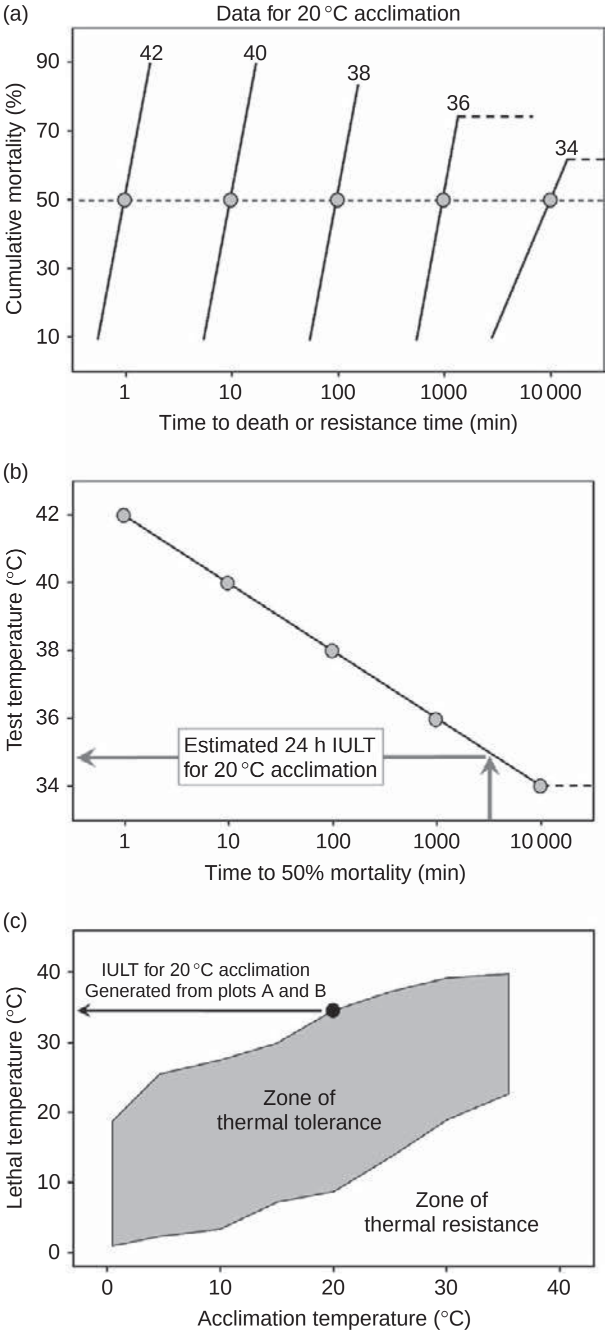


Figure 2 Determination of 24-h IULT for fish acclimated to 20 °C. (a) Estimation of times to 50% mortality in samples of fish exposed to constant test temperatures of 34–42 °C. Ordinate is in probits (inverse cumulative distribution function) and abscissa logarithmic (log 10). (b) Estimation of 24-h IULT. (c) Placement of 24-h IULT of 35 °C on the temperature tolerance polygon.

Critical Thermal Methodology (CTM)

Prior to the 1940s, a few researchers reported temperature tolerances of fishes exposed to changes in temperature

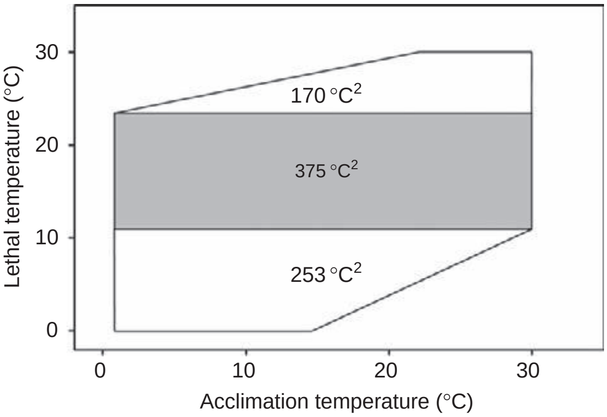


Figure 3 Generalized temperature tolerance polygon separated into acclimation temperature-independent tolerance (gray) and the lower and upper acclimation temperature-dependent areas.

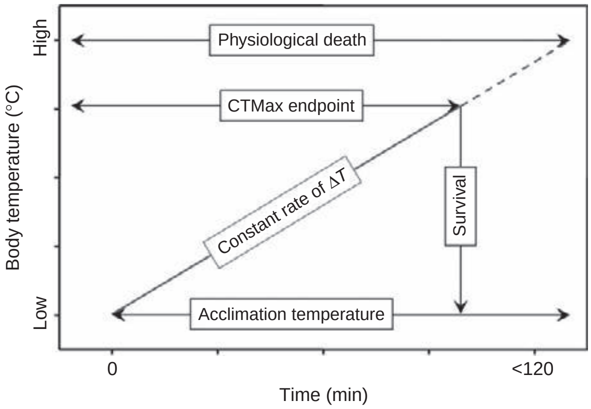


Figure 4 Generalized CTM methodology showing constant increase in T_b over time until CTMax occurs prior to physiological death.

with either lethal or sublethal endpoints. However, Cowles and Bogert's classic study published in 1944 of desert reptiles introduced and defined the critical thermal maximum (CTMax) as the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death. Recovery from this incapacitation is the criterion for determining whether or not the animal has been exposed to high temperatures. From the ecological viewpoint, CTMax is the lethal temperature. Although Cowles and Bogert also defined the critical thermal minimum (CTMin), they did not quantify exposures of reptiles to decreasing temperatures. In temperature tolerance research on bluegill (*Lepomis macrochirus*), Cox further defined the CTMax as the:

> Arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses the ability to escape from conditions that will promptly lead to its death when heated from a previous acclimation temperature at a constant rate just fast enough to follow T_e without a significant time lag.

When this experimental approach included the exposure of fishes to decreasing temperatures (i.e., CTMin), a clarification of the abbreviations became necessary. CTM now refers to the general methodology – critical thermal methodology – and CTMax and CTMin as the measured endpoints.

The keys to CTM include (1) consistent ΔT during trials, (2) ΔT should be fast enough to prevent reacclimation during a trial and slow enough to allow deep body temperature (T_b) to track T_e without a time lag, usually 0.3–1.0 °C min^{-1}, (3) choice of an obvious, repeatable near-lethal but sublethal endpoint such as the loss of righting response (LRR) or the onset of spasms (OSs), and (4) the caveat, that once its CTM endpoint is observed, the animal will survive if immediately captured and quickly returned to its pretest acclimation temperature (**Figure 4**).

Just as ILTs are used to generate a thermal tolerance polygon (**Figure 3**), both CTMax and CTMin measured over a range of acclimation temperatures can be used to construct a dynamic thermal tolerance polygon.

Comparison of ILT and CTM

These two approaches attempt to quantify thermal tolerance from different perspectives: static versus dynamic temperature exposures and lethal versus sublethal endpoints. Neither method is superior to the other. The ILT method may offer a more complete reflection of a species temperature tolerance. With additional data, it is possible to elucidate smaller polygons of growth and reproduction within the temperature tolerance polygon. In contrast, the CTM requires far less fish, is nonlethal (hence, threatened species can be tested), can be conducted over a relatively short time (usually <2 h), and is an excellent tool to bioassay biotic and abiotic factors that may influence thermal tolerance. Finally, it is also theoretically possible to predict CTM endpoints from resistance data generated by ILT trials.

CTMax Endpoints

Much discussion has been given to using the OS versus the LRR as the most appropriate CTMax endpoint. Although OS may be more biologically meaningful due its increased precision and greater physiological relevance, it has been recommended that both endpoints be collected and reported for data completeness and for comparisons with past studies. Because the OS occurs only a few degrees above the LRR, it is easy to continue heating trials until both endpoints are obtained. Second, studies invoking the production of heat shock proteins may require the neurological and possibly neuroendocrine responses of the more severe thermal stress associated with OS.

CTMin Endpoints

Most fishes experiencing decreasing temperatures during CTMin determinations exhibit neither OS nor consistent LRR. As T_e continues to decrease, swimming activity becomes sporadic, then ceases with fish often lying on the bottom of the test chamber still respiring. Since an obvious, overt endpoint is not apparent, the most often reported endpoint is the fish's inability to respond to prodding with a glass rod. This endpoint has been used extensively in one of the author's laboratories (TLB). If fishes at this endpoint are returned to their pretest acclimation temperature, they will generally survive.

Other Laboratory Methods

Another dynamic temperature method used to estimate lethal temperatures of fishes is the chronic lethal method (CLM). The CLM is similar to the CTM with two exceptions. First, the rate of ΔT during trials is 1 °C per day or slower. Second, death is employed as the CLM endpoint. The low rates of temperature change allow two competing processes to occur. A slower ΔT potentially allows fish to acclimate to the changing thermal conditions during an experimental trial, potentially extending their lower or upper temperature limits. Simultaneously, a slower ΔT allows more time for heat (or the lack of heat) to exert its lethal effects. The same fish species under the same acclimation temperature will demonstrate the following upper thermal tolerance relationship where CTMax > CLMax > IULT.

Laboratory Temperature Tolerance: Two Major Variables

Whether attempting to quantify thermal tolerance with either static or dynamic temperature methods, two variables need to be considered: temperature and exposure time or rate. When exposed to a series of static, high temperatures, fish acclimated at the same temperature will withstand 40 °C longer than either 42 or 44 °C. Tolerance time will be inversely related to test temperature. As a consequence, it is necessary to report both exposure temperature and time. Simply reporting a temperature is incomplete and analogous to reporting a lethal toxicant concentration as 4.2 mg L^{-1} without reporting the time of exposure. In temperature dynamic methods, these two variables are combined into a rate of ΔT. As opposed to the static method, both key variables are changing during a trial.

Influences on Thermal Tolerance

A fish's thermal tolerance is typically greater under a single environmental factor than its tolerance under a combination of environmental factors. Reproducing the holocoenotic environment and its variability in the laboratory is not feasible. However, experimenters must recognize that a combination of environmental, chemical, physical, and biological factors such as thermal history, photoperiod, diel and seasonal cycles, size, reproductive status, stress, concentration of respiratory gases, pH, salinity, and diet are just some factors that must be considered. Thus, measures of thermal tolerance must consider possible multifactor interactions and standardize methods as much as possible to allow for comparisons of data among studies. In this section, we introduce and illustrate how thermal tolerance may be influenced by some commonly and not so commonly considered factors.

Acclimation Temperature

Stress and ultimately death at extreme temperatures can be thought of as an effect of temperature. In contrast, altering thermal limits via acclimation is a response to temperature. In this framework, effects are considered negative, disruptive to homeostasis, and possibly lethal. Responses are adaptive attempts to restore homeostasis or at least minimize the probability of lethality. Adjustment of thermal tolerance limits via temperature acclimation is an obvious response to heterothermal environments.

Temperature tolerances of fishes are influenced by a cornucopia of physical, chemical, and biological factors. Among these, temperature acclimation is probably the most important, yet least understood. As T_e's change, most ectotherms make internal responses involving their biochemistry and physiology to maintain homeostasis. It is important to note that changes in set temperature and resulting physiological rate processes of a fish fully acclimated to 10 °C are quite different from those of fish fully acclimated to 30 °C. Further, these same physiological rates are quite different between full acclimation versus habituation (e.g., short exposure time of a few hours) to 10 °C.

Both upper and lower lethal temperatures of fishes increase as T_e increases during the transition from winter to summer. Conversely, they both decrease with transition from summer to winter. Thus, fishes subjected to heterothermal environments consistently adjust their lethal limits to bracket T_e providing a safety zone between T_e and lethal temperatures.

Many researchers erroneously assume that fishes are acclimated to a particular temperature because they have been held at a constant temperature in the laboratory. Acclimation involves much more than just holding temperature. Exposure of fishes to higher T_e invokes accruement of heat tolerance and, simultaneously, attrition of cold tolerance. Conversely, during exposure to lower T_e, heat tolerance is lost and cold tolerance is gained. Research indicates that rates of heat and cold accruement and attrition are a function of (1) direction of T_e change, (2) the amount of T_e change, and (3) species-specific thermal tolerance. For example, channel catfish (*Ictalurus punctatus*) transferred from 10 to 20 °C required 21 days to reacclimate, whereas only 3 days were required when fish were transferred from 30 to 35 °C. Similarly, loss of heat tolerance (i.e., decrease in CTMax) occurs more slowly. Readjustment of high-temperature tolerance of catfish transferred from 20 to 10 °C and 35 to 30 °C required 54 and 9 days, respectively (**Figure 5**). Thus, heat tolerance is gained 2.5- to 3-times more rapidly than heat tolerance is lost. Fishes therefore require different amounts of time to adjust temperature tolerance limits to different magnitudes and directional changes in T_e. In addition, the rates of reactions required for the acclimation processes are likely to proceed at slower rates at cold temperatures compared with warmer ones.

In addition to acclimating fish at constant temperatures, diel oscillating sine waves of temperature create thermocycles or thermoperiods which may influence measures of thermal tolerance. Some researchers hypothesize that thermocycles better simulate natural exposures to temperature. In other cases, fishes are exposed to thermocycles in an attempt to extend the upper limits of thermal tolerance. Finally, other investigators have attempted to determine if thermocycles result in temperature tolerances consistent with fishes being acclimated to the maximum, average, or minimum temperature of the thermocycle.

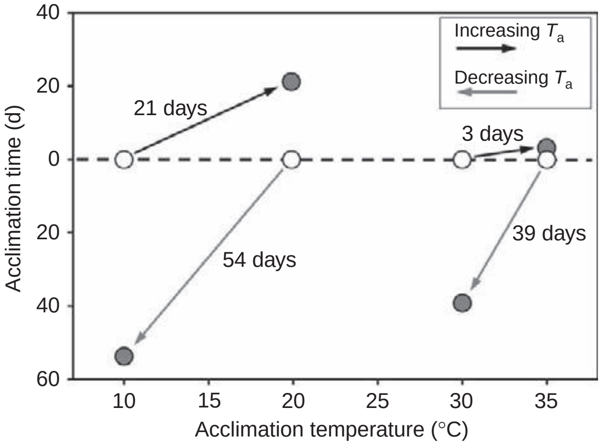


Figure 5 Relative acclimation times for increasing and decreasing acclimation temperature.

Seasonality and Photoperiod

There are numerous examples of seasonal effects on thermal tolerance. Both LRR and OS for two centrarchid species were shown to be higher during the summer than winter (**Figure 6**). Most likely, these seasonal differences in thermal tolerance are due to acclimation affects of temperature related to seasonal change. However, we must still consider the holocoenotic environment and, therefore, we cannot rule out other seasonal influences such as photoperiod. The shorter photophase (light) and longer scotophase (dark) associated with winter months influence thermal tolerance. Shorter photophase in winter causes an increase in the hormone melatonin, which functions to suppress some physiological processes. Exogenous melatonin treatments have been shown to lower thermal tolerance in an amphibian and mammal, further illustrating how even neuroendocrine responses to environmental cues add to the complexity of multifactor interactions that confound studies of thermal tolerance. The production of antifreeze proteins in some fishes off Newfoundland in response to the fall equinox is an excellent example of how a critical aspect of temperature acclimation is triggered by seasonal photoperiod.

Environmental Stressors

Although the field of stress biology is far too vast for even a modest summary, it is worth mentioning that measures of thermal tolerance are used in research as bioassays for environmental stressors. Physiological condition and the ability to tolerate extreme temperatures are compromised

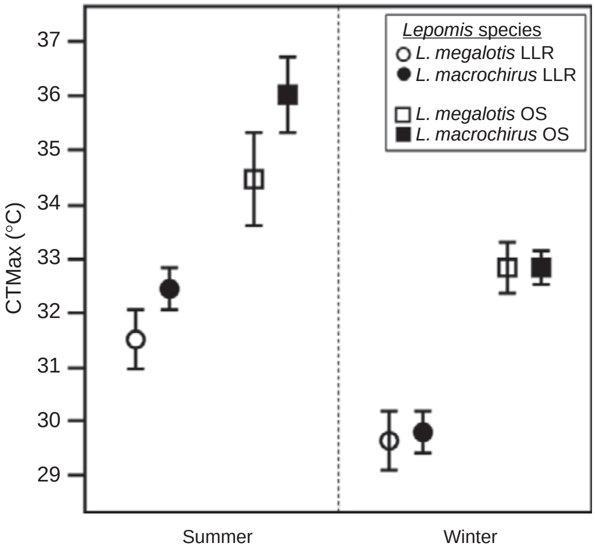


Figure 6 Seasonality of thermal tolerance. Modified from Schaefer JF, Lutterschmidt WI, and Hill LG (1999) Physiological performance and stream microhabitat use by the centrarchids Lepomis megalotis and *Lepomis macrochirus. Environmental Biology of Fishes* 54: 303–312.

under stressful conditions. Poor habitat quality and pollutants directly influence physiological health, reducing thermal tolerance (**Table 1**). A majority of chemicals tested produced significant decreases in temperature tolerance at mg L^{-1} (ppm) concentrations and lower. Poor environmental quality and reduction in physiological health also make fish more susceptible to infection, disease, and increased parasitism. Interactions between parasites and their fish hosts are often deleterious and thus lower physiological performance. In nature, parasites may have negligible effects on a host's overall physiological performance if environmental conditions are optimal. However, under suboptimal environmental conditions, parasites can lower physiological performance and fitness. Lutterschmidt and co-workers showed a clear inverse relationship between total helminth parasite load and thermal tolerance, demonstrating that thermal tolerance can be influenced by parasites and that parasite load should be a further consideration in studies of thermal tolerance (**see also Toxicology**: The Toxicology of Metals in Fishes and The Toxicology of Organics in Fishes).

Reproductive Status and Ontogeny

Reproduction can place a significant demand upon a fish's physiological condition. Reproduction in salmon is so demanding that it results in complete exhaustion and death. For many fish, reproduction is less detrimental to physiological condition but can influence thermal tolerance. In addition to reproductive condition or status, the developmental stage or ontogeny of fish may have associated limits of thermal tolerance. Although an adult may survive particular T_e regimes, earlier developmental stages may be unable to cope with such thermal conditions.

Population and Genetic Influences

Ward and Seely's landmark study in 1996 demonstrated that harsh T_e and environments select for genotypes and play an evolutionary role in conferring increased plasticity of physiological traits. Since this publication, considerable research has addressed both intra- and interspecific differences in thermal tolerance and the relationship between genetic variation and thermal tolerance. Such studies have increased our understanding of ecophysiological theory and the role that genetically based variation in thermal tolerance plays in the evolutionary ecology of fishes. In addition, many studies investigating environmental stress have assessed genetically influenced development of morphometric traits through measures of fluctuating asymmetry rather than thermal tolerance. Increased fluctuating asymmetry in morphological traits occurs under environmental and genomic stress, leading to a reduction in developmental homeostasis. Thus, measures of fluctuating asymmetry can help in determining if populations of fishes are under environmental or thermal stress.

Ecophysiological Theory of Thermal Tolerance

Studies of physiological tolerance, including thermal tolerance, are central to understanding the distributions of fishes and how they may have adapted to these geographic regions. Both biogeography and macroecology attempt to explain such distributional patterns. Liebig's law of the minimum suggests that distributions are governed by those factors for which species have the narrowest tolerance and least adaptability. Species have different abilities in coping, acclimating, and adapting to T_e and its variation. Temperature adaptation, unlike acclimation, requires genetic change over evolutionary time. Investigations of how harsh thermal environments select for genotypes and allow for plasticity in physiological traits are central to ecophysiological theory. Physiological plasticity leads to increased performance breadths, niche diversification, increased geographic distribution, changes in genetic population structure, and ultimately changes in

Table 1 CTM of fishes as a bioassay of chemical exposure

Chemical	*Species*	*Test*	*Effect*
Cadmium	*Cyprinella lutrensis*	CTMaximum	Yes
	Pimephales promelas	CTMaximum	Yes
	Lepomis cyanellus	CTMaximum	Yes
Copper	*Pimephales promelas*	CTMaximum	Yes
Nitrite	*Ictalurus punctatus*	CTMaximum	Yes
Cyfluthrin	*Pimephales promelas*	CTMaximum	Yes
		CTMaximum	Yes
Endothal	*Cyprinella lutrensis*	CTMaximum	No
Phenol	*Pimephales promelas*	CTMaximum	No
		CTMaximum	No
Selenite	*Pimephales promelas*	CTMaximum	Yes

communities and ecosystems. These invoke theoretical discussions in biology, central to the study of physiological ecology and evolutionary biology.

Biological Applications of Thermal Tolerance

Measures of thermal tolerance may be important in the future assessment of global climate change. The importance of thermal tolerance to the biogeography of fishes is realized when estimating the potential impacts of environmental perturbations on fish populations. Global climate change is a significant environmental threat to numerous species and several studies have documented the effects that climate change will have on the ecology of fishes (**see also Temperature**: Effects of Climate Change). Despite the high specific heat of water and, hence, its resistance to rapid temperature change, global climate change will have a great effect on fishes. This is particularly true for fishes living in shallow, isolated streams, ponds, and lakes. Numerous researchers have specifically addressed how climate change will cause local extinctions, reduce species richness, and affect the distributional status of fishes, including commercial fisheries. However, little is known about the physiological mechanisms that mediate the impacts of climate change. Rates of T_e change, predicted by some models, will exceed the adaptive capacities of most fishes. There is little doubt that temperature exerts selection pressures on the microevolutionary processes of communities and community structure by selecting against individuals with narrow ranges of thermal tolerance. Future investigations of thermal tolerance will be invaluable to fish ecologists, especially in light of the potential impacts of global climate change on the normal thermal regimes of environments.

Concluding Remarks

The history and vast literature clearly demonstrate the importance of thermal tolerance to the field of fish biology. We briefly introduced and illustrated how thermal tolerance is measured and how it has played an important role in our understanding of the ecology and physiology of fishes. Our hope is that this information gives adequate direction for those students of ichthyology and physiological ecology to further explore this truly interesting area of research.

See also: **Fish Migrations**: Tracking Oceanic Fish. **Pelagic Fishes**: Physiology of Tuna. **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations. **Temperature**: Effects of Climate Change; Effects of Temperature: An Introduction; Proteins and Temperature. **Toxicology**: The Toxicology of Metals in Fishes; The Toxicology of Organics in Fishes.

Further Reading

Beitinger TL and Bennett WA (2000) Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes* 58: 277–288.

Bennett WA, McCauley RA, and Beitinger TL (1998) Rates of gain and loss of heat tolerance in channel catfish. *Transactions of the American Fisheries Society* 127: 1051–1058.

Cowles RB and Bogert CM (1944) A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History* 83: 265–296.

Cox DK (1974) Effects of three heating rates on the critical thermal maximum of bluegill. In: Gibbons JW and Sharitz RR (eds.) *Thermal Ecology*, pp. 158–163. Alexandria, VA: National Technical Information Services.

Erskine DJ and Hutchison VH (1982) Reduced thermal tolerance in an amphibian treated with melatonin. *Journal of Thermal Biology* 7: 121–123.

Fletcher GL, Idler DR, Vaisius A, and Hew CL (1989) Hormonal regulation of antifreeze protein gene expression in winter flounder. *Fish Physiology and Biochemistry* 7: 387–393.

Fry FEJ (1947) *Effects of the Environment on Animal Activity,* University Toronto Studies in Biology, Series number 55, Publication of the Ontario Fish Research Laboratory, vol. 68, 62pp. Toronto: University of Toronto Press.

Fry FEJ, Brett JR, and Clawson GH (1942) Lethal limits of temperature for young goldfish. *Review of Canadian Biology* 1: 50–56.

Hutchinson VH (1976) Factors influencing thermal tolerance of individual organisms. In: Esch GW and McFarlane RW (eds.) *Thermal Ecology II*, pp. 10–26. ERDA (Energy Research and Development Administration) Symposium Series CONF750425, U.S. National Technical Information Center, Oak Ridge, TN., 404p.

Klerks PL and Blaha MA (2009) Heat tolerance as an indicator of climate change impacts: General considerations and a case study in poeciliid fish. *Environmental Bioindicators* 4: 46–66.

Lutterschmidt WI and Hutchison VH (1997) The critical thermal maximum: Data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology* 75: 1553–1560.

Lutterschmidt WI and Hutchison VH (1997) The critical thermal maximum: History and critique. *Canadian Journal of Zoology* 75: 1561–1574.

Lutterschmidt WI, Schaefer JF, and Fiorillo RA (2007) The ecological significance of helminth endoparasites on the physiological performance of two sympatric fishes. *Comparative Parasitology* 74: 194–203.

Parsons PA (1992) Fluctuating asymmetry a biological monitor of environmental and genomic stress. *Heredity* 68: 361–364.

Pyron M and Beitinger TL (1993) Temperature tolerance after spawning in female and male fathead minnows, *Pimephales promelas*. *Texas Journal of Science* 45: 319–323.

Schaefer JF, Lutterschmidt WI, and Hill LG (1999) Physiological performance and stream microhabitat use by the centrarchids *Lepomis megalotis* and *Lepomis macrochirus*. *Environmental Biology of Fishes* 54: 303–312.

Shimada Y, Shikano T, and Seikai T (2006) Ontogenetic changes in tolerance for environmental stress in Japanese flounder *Paralichthys olivaceus* during early development. *Nippon Suisan Gakkaishi* 72: 169–173.

Somero GN (2005) Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* 2: 1–9.

Ward D and Seely MK (1996) Adaptation and constraint in the evolution of the physiology and behavior of the Namib Desert tenebrionid beetle genus *Onymacris*. *Evolution* 50: 1231–1240.

Proteins and Temperature

PA Fields, Franklin & Marshall College, Lancaster, PA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Effects of Temperature on Enzyme Function
Structural Modifications in Proteins Important for Temperature Adaptation
Location of Amino Acid Substitutions Is Important in Enzymic Temperature Adaptation
Further Reading

Glossary

Catalytic rate The rate at which an enzyme converts substrate into product.

Conformation The three-dimensional shape a folded protein occupies in aqueous solution. One protein molecule can occupy a number of different conformations.

Denaturation The process by which a protein unfolds from the native state to a random coil that is no longer functional. Denaturation can be caused by high or low temperature, pressure, or chemical denaturants.

Enthalpy A thermodynamic parameter measuring the total heat energy of a system. In a folded protein, enthalpy is roughly correlated with the number of bonds maintaining a particular conformation.

Entropy A thermodynamic property measuring the amount of disorder in the system. Greater disorder is energetically favorable; thus, entropy favors unfolding of proteins.

Enzyme homologs Enzymes that share the same catalytic function in different species, and that arose from the same molecular ancestor.

Ligand A molecule that binds to another molecule, such as an enzyme substrate cofactor, or signaling molecule (Latin ligare = to bind).

Native state The three-dimensional conformation or set of conformations in which an enzyme maintains catalytic activity.

Poikilotherm An organism that cannot regulate body temperature except through behavioral means. An animal whose body temperature fluctuates with that of the environment.

Substrate affinity A measure of the likelihood or strength of binding between substrate and enzyme.

Temperature compensation A change in the rate of a biological process, such as enzymatic rate, to adjust for the accelerating effect of high temperature or the decelerating effect of low temperature. Temperature compensation can occur through acclimation or adaptation.

Introduction

As poikilotherms, fish can experience a wide range of body temperatures, from ~45 °C in pupfish (*Cyprinodon* spp.; **see also Bony Fishes**: Life in Hot Water: The Desert Pupfish) in the southwestern desert of North America or intertidal fish of the tropics and subtropics, to the freezing point of seawater (–1.9 °C) for polar fish such as Antarctic notothenioids. Further, many species of fish occur in environments where temperature can fluctuate greatly on a seasonal or even daily basis. Maintaining adequate physiological performance across this broad range of temperatures requires a variety of adaptations, especially on the biochemical level, many of which involve changes in the composition of macromolecules. For example, temperature-induced changes in the physical properties of membranes in response to thermal stresses are well described, especially in the context of optimization of membrane viscosity to maintain membrane fluidity and the function of embedded membrane proteins (homeoviscous adaptation; **see also Temperature**: Temperature and Excitable Membranes and Membranes and Temperature: Homeoviscous Adaptation). In addition, there is growing evidence that nucleic acids involved in both functional and information-storage roles may be under selective pressure to alter stability in response to environmental temperature. This article focuses on the acute effects of temperature on proteins, especially catalytically functional proteins (enzymes), examines the structural modifications that allow proteins to function optimally in stressful temperature regimes, and describes the locations within enzymes where such structural modifications are most likely to occur.

Effects of Temperature on Enzyme Function

Most enzymes are remarkably specific in the reactions they catalyze, efficiently binding substrate and forming product, while excluding from the binding site molecules that often are structurally very similar to these ligands. Such enzyme specificity is based largely on the detailed interaction between the active site and substrate, dependent partly on geometry – the three-dimensional complementarity between ligand and active site structures – and partly on thermodynamic interactions, for example, between oppositely charged groups or hydrophobic regions.

In addition to substrate affinity, however, appropriate enzyme function also depends on adequate catalytic rate. A highly specific enzyme with negligible turnover of substrate to product would be useless! Thus, in addition to three-dimensional complementarity between substrate and enzyme, adequate flexibility in the enzyme molecule is also necessary. Most enzymes must undergo conformational changes to proceed through the catalytic cycle. Specifically, the enzyme must occupy one conformation appropriate for binding ligands, but be able to modify that conformation in order to produce the transition state and protect that intermediate from substances in the medium (including, for instance, water molecules) that might interfere with the catalytic chemistry. Finally, a further conformational shift is necessary to release product and return the enzyme to its original state, ready to bind another substrate molecule. An overly stable or rigid enzyme molecule, then, would inhibit catalysis by increasing the activation energy necessary to move from one conformation to another. These two attributes of enzymes, structural specificity and flexibility, are both affected by temperature, but in ways that make optimization of enzyme function to a broad range of temperatures difficult.

Substrate Affinity and the Apparent Michaelis–Menten Constant, K_m

The affinity of an enzyme for its substrate is most commonly measured using the apparent Michaelis–Menten constant, K_m, which can be defined most simply as the amount of substrate necessary to allow an enzyme to function at half its maximal velocity. (A more rigorous definition of K_m, employing on- and off-constants of substrate and product, is not necessary for this discussion of the effects of temperature on enzyme function.) Thus, K_m has units of concentration (molar) and a higher K_m value indicates an enzyme with lower substrate affinity (i.e., a higher substrate concentration is necessary to allow the enzyme to reach a certain reaction rate). If an enzyme is assayed across a range of temperatures, the most common pattern encountered is that the K_m of the enzyme rises with temperature (**Figure 1**, heavy black line). To understand this process, it is informative to imagine not one static enzyme molecule encountering substrate, but many enzyme molecules, each of which can occupy a range of conformations. At low temperatures there are relatively few such conformations, and most are able to bind substrate, leading to a relatively low K_m. As temperature increases, however, enzyme molecules are able to occupy a broader range of conformations, a greater proportion of which cannot adequately bind substrate because of modifications to the geometry of the active site. Any one enzyme molecule may shift between binding-competent and binding-incompetent conformations, but in aggregate, the population of enzymes has a lower binding affinity. In essence, the increased heat energy present in the medium (i.e., the increased kinetic energy of solute molecules and the protein itself) induces the breakage of some of the weak interactions that are responsible for holding the active site of the protein in a given conformation, allowing other conformational states to be entered (**see** the section ‘Structural modifications in proteins important for temperature adaptation’). Experimentally, we observe this as an increase in K_m.

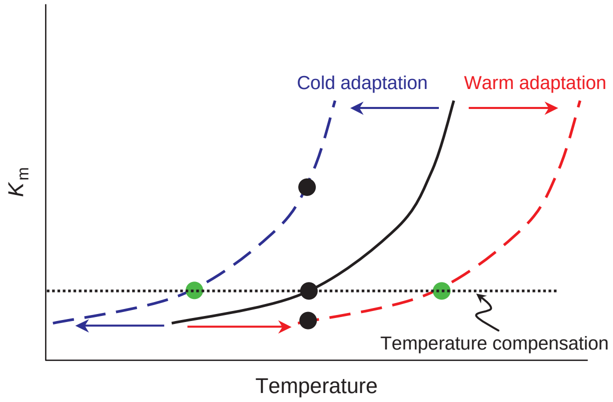


Figure 1 An idealized representation of the effect of temperature on the apparent Michaelis–Menten constant, K_m. Higher temperature increases K_m (thick black line), representing a loss of substrate affinity. Over evolutionary time, adaptation to warm (red arrows) or cold (blue arrows) temperature can lead to temperature compensation – the cold-adapted homolog has a much higher K_m than the warm-adapted homolog at a single measurement temperature (black dots), but the enzymes have similar K_m values when measured at each species’ physiological temperature (green dots).

Given the sensitivity of enzyme substrate affinity to temperature, and the importance of adequate substrate binding to enzyme activity, it is not surprising that enzymes of species adapting to new environmental temperatures demonstrate modified K_m’s. Specifically, as enzymes adapt to colder conditions, K_m values increase at a given measurement temperature, while adaptation to warmer temperature leads to decreased K_m values (**Figure 1**, black dots). The resulting temperature compensation in K_m is such that substrate affinity of the enzyme remains similar at the new environmental temperature (**Figure 1**, green dots). In fact, compensation in substrate affinity has been observed repeatedly in studies of enzyme homologs of closely related fish species living in different thermal environments. Generally, enzymes from more warm-adapted species have lower K_m values at a given measurement temperature, but have similar K_m values within the range of temperatures to which they are adapted. The pattern for cold-adapted species is reversed.

Catalytic Rate and the Turnover Number, k_{cat}

If substrate affinity were the only attribute important in enzyme adaptation to temperature, it would seem plausible that enzyme molecules would adapt to become as rigid as possible, in order to maintain active site structure and affinity regardless of the temperature experienced. However, as described above, excessive rigidity in the enzyme molecule impairs enzyme function by limiting catalytic rate. Catalytic rate in enzymes is measured by the turnover number, k_{cat}, defined as the number of substrate molecules converted to product per active site per unit time (k_{cat} is reported in units of reciprocal time, s^{-1}). As with K_m, k_{cat} for most enzymes is temperature sensitive and increases with increasing temperature. This is because increased thermal energy induces the breakage of weak bonds maintaining enzyme three-dimensional structure, allowing the enzyme to undergo necessary conformational changes more quickly. The result is that enzyme activity tends to increase exponentially with temperature until enough thermal energy is present to cause the relatively sudden denaturation of the enzyme molecule and loss of enzyme activity (**see also Temperature**: Effects of Temperature: An Introduction).

As with substrate affinity, studies of enzyme homologs from different thermal environments show that the flexibility of catalytically important regions of the molecule is increased in enzymes adapting to colder temperatures through the loss of stabilizing interactions, leading to greater mobility with less available thermal energy. Conversely, in those enzymes adapting to warmer conditions, enzyme structure is stabilized through the formation of greater numbers of weak noncovalent interactions.

Based on the above description of the effect of temperature on K_m and k_{cat}, it appears that there are competing and incompatible selective pressures on enzymes adapting to temperature change – increases in rigidity will enhance substrate affinity at the expense of catalytic rate; increased flexibility will increase catalytic rate, but negatively affect substrate affinity (**Figure 2**). The former would be expected in enzymes adapted to warmer temperatures (**Figure 2**, red circle), and the latter in enzymes adapted to cold (**Figure 2**, blue circle). Importantly, then, it appears that while an enzyme may become adapted for optimal function (balancing substrate affinity and catalytic rate) across a narrow temperature range, it may be impossible for an enzyme to maintain optimal function across a broader range of temperatures (**Figure 2**, green circle).

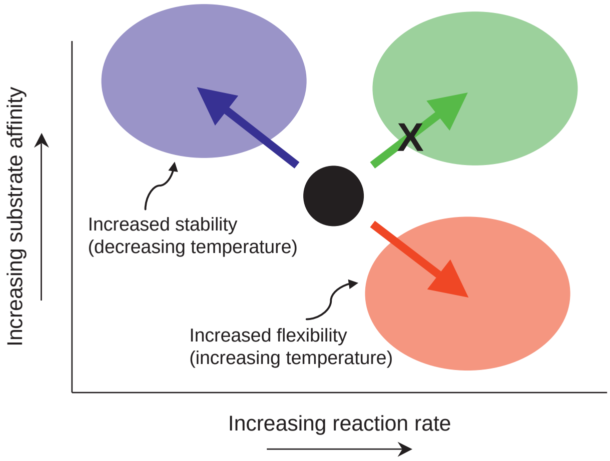


Figure 2 An idealized view of the trade-off between substrate affinity and reaction rate in enzymes. An enzyme with a given substrate affinity and catalytic rate at one measurement temperature (represented by the black circle) will be affected by changes in temperature as shown by the blue-and red-shaded areas – cold increases substrate affinity while decreasing catalytic rate, and higher temperature has the opposite effect. Optimization of enzyme function to maintain or increase both affinity and rate (green shaded area) as temperature changes may not be possible.

Structural Modifications in Proteins Important for Temperature Adaptation

Over short periods of time, that is, within the lifetime of an individual, poikilotherms can use a number of strategies to compensate for changes in enzyme activity due to temperature fluctuations. For instance, organisms can increase enzyme activity at colder temperatures by increasing the copy number of enzymes in the cell, although this may be energetically costly. In addition, for those organisms with the luxury of multiple isoforms of enzymes (and tetraploid fish taxa such as carp (Cyprinidae) and salmon (Salmonidae) are notable here), it is possible that different isoforms will become adapted to, and expressed under, different conditions.

Over evolutionary time periods, it is more likely that selection will optimize enzyme performance through structural modifications. In other words, genetic mutations (leading to amino acid substitutions) will accrue, tending to increase molecular flexibility during cold adaptation, and increase stability during warm adaptation. After considering the balance between stabilizing and destabilizing forces that occurs within proteins in solution, this section describes the types of amino acid substitutions that have been found in enzymes adapting to different temperatures.

Factors Leading to Protein Stabilization or Destabilization

Given the above description regarding the importance of both stability and conformational flexibility in enzyme function, it is not surprising that enzymes in aqueous solution are only minimally stable, with denaturation and renaturation of local regions of the molecule occurring as the enzyme moves from one conformation to another. In fact, although there may be thousands of stabilizing interactions among residues within an average enzyme molecule, the net stabilization of the molecule often is energetically equivalent to only a few hydrogen bonds. This is because there are powerful destabilizing forces acting on the enzyme as well. Unfavorable interactions between amino acids within the polypeptide play an important role in destabilizing the molecule, as do favorable interactions between buried residues and the solute (mainly water) upon unfolding. However, the dominant destabilizing factor affecting proteins is the increase in entropy that occurs during unfolding. Because a denatured protein can occupy many more conformations than the same protein in native form, unfolding leads to an increase in entropy, an energetically favorable condition that is continually pushing the folded form toward denaturation. Thus, the balance between stability and flexibility described above in the context of optimal catalytic function must also be understood as a balance between destabilizing forces (mainly entropic) and stabilizing forces (mainly enthalpic, i.e., based on the formation of weak attractive interactions among atoms) within the protein molecule. Adaptation of an enzyme molecule to function optimally at a new temperature thus involves amino acid substitutions that can affect the magnitude of entropy and enthalpy changes between the native and denatured states.

The types of interactions that stabilize an enzyme molecule, including changes in amino acid composition, hydrophobic interactions, van der Waals interactions, salt bridges between oppositely charged residues, and covalent bonds such as disulfide bridges, are relatively well understood. It is important to emphasize, however, that because of the complexity of proteins made up of many hundreds of amino acids, and the multitude of possible interactions among these residues, it still remains challenging to determine *a priori* how a particular amino acid substitution will affect the stability of a protein in aqueous solution.

Modification of Amino Acid Composition

A hallmark of temperature adaptation in enzymes is modification of the mobility of local regions of the molecule that move during catalysis. Thus, amino acid substitutions that affect structural flexibility have an important role in temperature adaptation. For example, the amino acid glycine is unusual in having no side chain, and thus the peptide backbone on either side of a glycyl residue is relatively free to rotate without physical interference (i.e., there is a minimum of steric hindrance) from neighboring residues. Therefore, mutations increasing the proportion of glycyl residues increase the flexibility of protein structures and allow shifts in conformational states with an input of less thermal energy from the medium.

Conversely, amino acids with bulky side chains, such as the aromatic amino acids tryptophan, tyrosine, and phenylalanine, tend to inhibit rotation of the peptide backbone, simply because the large side chains of these amino acids are difficult to move past neighboring residues without large-scale unfolding of the protein. As a result, these types of residues tend to make protein structures more rigid. A special example of an amino acid that stabilizes proteins through increasing the rigidity of the backbone is proline, which is the only amino acid whose side chain binds to the backbone in two places. The ring structure of proline severely limits the rotation of the backbone near a prolyl residue, and greatly increases the rigidity of the protein in that location. Indeed, researchers examining amino acid composition of organisms adapted to relatively warm or relatively cold environments have discovered a tendency to increase the proportion of glycyl residues in cold-adapted forms, while increasing prolyl residues and aromatic side chains in more warm-adapted species. Note, however, that studies describing changes in proportions of particular amino acids during temperature adaptation do not necessarily address the location of these substitutions within the protein molecule. The location of temperature-adaptive substitutions with respect to the active site, then, is as important as the type of substitution (**see** section 'Location of amino acid substitutions important in enzymic temperature adaptation').

Hydrophobic Interactions

Hydrophobic interactions arise among amino acid residues that are relatively nonpolar (e.g., valine, leucine, and alanine). Because these residues cannot form hydrogen bonds, they interact less favorably with water molecules in the surrounding medium than the water molecules do with one another. It is important to note that stabilization of proteins through hydrophobic interactions does not depend on special interactions between nonpolar residues *per se*, but instead involves the energetic favorability of water–water interactions in comparison to water–residue interactions. The presence of a nonpolar amino acid in water leads to the formation of ordered water molecules that can only hydrogen-bond to a limited subset of neighbors, that is, a cavity is formed in the water structure in which surrounding water molecules lose some rotational and translational freedom. If the nonpolar structure is buried in the interior of a polypeptide – that is, the protein remains folded – the water molecules in solution are free to hydrogen-bond with a greater number of neighbors, increasing the entropy (and energetic favorability) of the system. Thus, a core of hydrophobic residues tends to stabilize the native state of the protein.

The nature of the interactions between amino acid residues and water, and among water molecules themselves, makes the hydrophobic effect temperature dependent. Simply put, within the temperature range fish experience, the hydrophobic effect is weak at lower temperatures and becomes stronger at high temperatures. At low temperatures, close to the freezing point of water, water molecules in the surrounding medium have relatively little kinetic energy, and so will form relatively long-lived hydrogen bonds with neighbors. Under these conditions, the introduction of a nonpolar amino acid residue to the aqueous solution will not force many extra water molecules into more ordered interactions – there is a relatively small entropic penalty, and thus proteins stabilized by hydrophobic interactions will be less stabilized and more likely to unfold in the cold (this is the basis of cold denaturation of proteins). Conversely, at higher temperatures, when water molecules form and break hydrogen bonds with neighbors much more rapidly, introduction of nonpolar side chains becomes more disruptive – the ordered water structures that must form are energetically more costly. Thus, at higher temperatures hydrophobic interactions become more stabilizing. Again, empirical evidence supports these observations – generally, more warm-adapted enzymes tend to have a greater proportion of nonpolar amino acids.

Hydrogen Bonding, Ionic Interactions (Salt Bridges), and van der Waals Interactions

In addition to hydrophobic interactions, proteins can be stabilized by a number of other weak (i.e., noncovalent) interactions dependent on the types of contacts among amino acids in the three-dimensional structure of the native state. For example, in the average-sized protein there can be many hundreds or thousands of hydrogen bonds among polar amino acid residues, or between polar side chains and the amide or carbonyl groups of the peptide backbone. Not surprisingly, among proteins adapted to cold, the necessity for greater flexibility generally leads to a reduction in intramolecular hydrogen bonds. Similarly, salt bridges, which are attractive electrostatic interactions among oppositely charged ionizable side chains, can also provide stability to protein structures. Salt bridges form between the acidic (i.e., negatively charged) residues of aspartate and glutamate and the basic (positively charged) residues of lysine, arginine, and, sometimes, histidine. Comparative analyses of enzymes from species adapted to different temperatures reveal that warm-adapted homologs tend to have relatively more charged residues, presumably because salt bridges between pairs of these residues add to protein stability.

A further type of interaction significant in stabilizing the three-dimensional structure of a protein involves efficient packing of residues and concomitant van der Waals interactions. These interactions occur when uncharged atoms are brought into close proximity: fluctuations in the positions of electrons around the nucleus of one atom can produce a temporary dipole that induces a complementary dipole in the neighboring atom. The weak dipoles attract each other, drawing the two atoms together until they reach an optimal distance in which attractive and repulsive forces balance. Because stabilization via van der Waals interactions depends on close association of interacting atoms, efficient packing of residues in the interior of a folded protein may lead to greater stabilization by encouraging the formation of more, stronger van der Waals pairs. Efficient packing seems to depend in part on residue volume (i.e., the sum volume of atoms in the residue side chain – tryptophan for example – having a greater volume than alanine (227.8 vs. 88.6 Å^3)), and thus increased van der Waals stabilization correlates with a greater number of residues of relatively large-volume amino acids. Again, given our expectations of temperature adaptation in enzymes, it is not surprising that warm-adapted enzymes generally have increased average residue volumes relative to their cold-adapted homologs, thereby enhancing van der Waals interactions.

Disulfide Bonds

Disulfide bonds are highly stabilizing covalent interactions between the sulfhydryl groups of neighboring cysteinyl residues. Due to the strength of the –S—S– bond, these interactions provide extreme stabilization against denaturation. However, because of the highly reducing environment of the cytoplasm disulfide bonds do not appear to be employed in the stabilization of intracellular enzymes, regardless of the temperature the species experiences. Instead, disulfide bonds are most prevalent in proteins targeted for extracellular function.

Location of Amino Acid Substitutions Is Important in Enzymic Temperature Adaptation

The structural modifications described above have important effects on the stability of enzyme molecules, and evidence indicates that each (except for disulfide bonds) can be used to modify enzyme activity for optimal function at different temperatures. It is important to note, however, that temperature-adaptive amino acid substitutions do not appear haphazardly throughout the structure of an enzyme. Instead, such stabilizing or destabilizing substitutions tend to occur in discrete locations relative to mobile regions of the enzyme.

Remarkably, active site residues – those amino acids that bind substrate and cofactor and are directly involved in the catalytic chemistry mediated by the enzyme – rarely are modified during adaptation to different temperatures. In other words, it appears that the three-dimensional relationship of these residues with ligands is so important to catalysis that the residues cannot be substituted without severely affecting substrate affinity and catalytic rate. Thus, as a nearly universal rule, enzyme adaptation to temperature does not involve changes to the active site.

Instead, studies of homologs of a number of enzymes from species adapted to both cold and warm temperatures indicate that temperature-adaptive substitutions tend to occur in regions bordering the active site, and especially those regions that must move during catalysis. An idealized enzyme molecule can be imagined to have a relatively static core, which does not move during catalysis and is highly stable. The static core acts as a scaffold on which is built the active site and mobile structures nearer the periphery of the molecule. These mobile structures, which may comprise loops, α-helices, or larger components based on multiple secondary structures, control access to the active site and create the catalytic vacuole through their movements. While the mobile structures themselves may retain appreciable rigidity, small areas of the protein act as hinges allowing the structures to move. Analysis of differentially adapted homologs indicates that these hinge regions are the predominant locations of functionally relevant amino acid substitution during enzymic temperature adaptation.

See also: **Bony Fishes**: Life in Hot Water: The Desert Pupfish. **Temperature**: Effects of Temperature: An Introduction; Membranes and Temperature: Homeoviscous Adaptation; Temperature and Excitable Membranes.

Further Reading

Bjelic S, Brandsdal BO, and Aqvist J (2008) Cold adaptation of enzyme reaction rates. *Biochemistry* 47: 10049–10057.

Chiuri R, Maiorano G, Rizzello A, *et al.* (2009) Exploring local flexibility/rigidity in psychrophilic and mesophilic carbonic anhydrases. *Biophysical Journal* 96: 1586–1596.

Fields PA (2001) Review: Protein function at thermal extremes: Balancing stability and flexibility. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* 129: 417–431.

Georlette D, Damien B, Blaise V, *et al.* (2003) Structural and functional adaptations to extreme temperatures in psychrophilic, mesophilic, and thermophilic DNA ligases. *Journal of Biological Chemistry* 278: 37015–37023.

Hochachka PW and Somero GN (2002) *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford University Press.

Schrank T, Bolen D, and Hilser V (2009) Rational modulation of conformational fluctuations in adenylate kinase: Implications for allostery and functional adaptation in proteins. *Proceedings of the National Academy of Sciences of the United States of America* 106(40): 16984–16989.

Siddiqui KS and Cavicchioli R (2006) Cold-adapted enzymes. *Annual Review of Biochemistry* 75: 403–433.

Somero GN (2004) Adaptation of enzymes to temperature: Searching for basic "strategies." *Comparative Biochemistry and Physiology – Part B: Biochemistry and Molecular Biology* 139: 321–333.

Zavodsky P, Kardos J, Svingor A, *et al.* (1998) Adjustment of conformational flexibility in a key event in the thermal adaptation of proteins. *Proceedings of the National Academy of Sciences of the United States of America* 95: 7406–7411.

Zhou XX, Wang YB, Pan YJ, *et al.* (2008) Differences in amino acids composition and coupling patterns between mesophilic and thermophilic proteins. *Amino Acids* 34: 25–33.

Mitochondria and Temperature

H Guderley, Université Laval, Montreal, QC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Fish Mitochondria and Thermal Change
Further Reading

Glossary

Acyl chain The nonpolar hydrocarbon chains composing the central core of biological membranes. Phospholipids typically contain two acyl chains, whereas triglycerides contain three. Acyl chain length ranges between 10 and 26 carbons and the number of double bonds influences their conformation. In this article, the term fatty acid is used interchangeably with acyl chain.

Apoptosis Programmed cell death that can be initiated by mitochondrial liberation of cytochrome C.

Arrhenius break temperature Temperature at which the relationship between maximal rates and assay temperature shows an abrupt transition.

Bilayer The basic phospholipid structure of biological membranes. Two layers of phospholipids are placed such that their polar head groups are at opposite sides, whereas the nonpolar acyl chains join each other in the center. The structure is like a sandwich with the head groups being the bread and the acyl chains the filling.

Cardiolipin A phospholipid only found in mitochondria that contains four acyl chains conjugated to a diglycerol backbone.

Electron transport system (ETS) A series of multisubunit protein complexes and two mobile electron carriers, embedded in or associated with the inner mitochondrial membrane. The ETS transfers electrons from reducing equivalents to oxygen to form water and in so doing forms an electrochemical gradient.

Mitochondria Organelles that synthesize most of the adenosine triphosphate (ATP) needed by the cell, using oxygen as the final electron acceptor.

Oxidative phosphorylation The production of ATP by the F_1F_0ATPase using the electrochemical gradient produced by the ETS. Oxidation by the ETS is coupled with phosphorylation by the F_1F_0ATPase.

Phospholipids Molecules composed of a polar head group and two nonpolar acyl chains.

Reducing equivalents Molecules that transfer electrons in oxidation–reduction reactions.

Saturation (unsaturation) Acyl chains that do not contain double bonds are said to be saturated, containing all the hydrogen possible. Unsaturated acyl chains contain at least one double bond and polyunsaturated acyl chains contain more than one (up to six in the case of DHA) double bond.

Introduction

Mitochondria are often referred to as the powerhouse of the cell, as they are the site of oxidative phosphorylation in eukaryotic cells. Oxidative phosphorylation is the most efficient biological means by which fuels, such as carbohydrates and proteins and lipids, are converted into the cellular energy currency, adenosine triphosphate (ATP). First, the Krebs cycle, located primarily in the mitochondrial matrix, generates reducing equivalents from these fuels. The electron transport system (ETS) uses these reducing equivalents to produce a proton gradient across the inner mitochondrial membrane. Oxygen is the final electron acceptor of the ETS. This electrochemical gradient provides the thermodynamic impetus for a multisubunit protein complex in the inner mitochondrial membrane, the F_1F_0ATPase, to add a phosphate group to adenosine diphosphate (ADP) and produce ATP. When ATP is produced by anaerobic glycolysis, the yield of ATP per glucose is approximately 10-fold lower than that as a result of oxidative phosphorylation. As acidification of the cytoplasm accompanies glycolytic ATP production, its duration is restrained by the limited tolerance of pH fluctuations typical of living systems.

Although mitochondria provide the cell with most of its ATP and, in a sense, potential energy for work for the remainder of the cell, living with mitochondria has its difficulties. To begin with, the cell needs to keep conditions comfortable for mitochondria. If the mitochondrial membrane potential drops below a critical value, apoptosis can be triggered, resulting in controlled, but nonetheless irreversible, cell death. Thus, under extreme conditions when the mitochondrial membrane potential is drastically diminished due to a lack of oxygen, glycolytically generated ATP may be used by the F_1F_0ATPase to pump protons in the reverse direction, to re-establish the mitochondrial membrane potential.

Another negative aspect of mitochondrial activity is the production of reactive oxygen species (ROS) by the ETS. ROS can damage proteins, lipids, and deoxyribose nucleic acid (DNA). Particularly when mitochondria are idling, poised for ATP synthesis, ROS production can be considerable due to side reactions in which oxygen combines with electrons and becomes more reactive. Proton leak across the inner mitochondrial membrane is activated by ROS. The ensuing decrease in the membrane potential slows down ROS formation, but reduces the overall ratio of ATP produced to the amount of oxygen consumed.

Thus, having mitochondria has benefits and costs (**Figure 1**): the efficiency of ATP production is vastly increased by oxidative phosphorylation, but if the polarization of the inner membrane is not maintained, apoptosis may result. Maintaining the inner membrane potential when ATP synthesis is slow favors the production of ROS that damage cellular components. While proton leak minimizes ROS formation, it liberates energy as heat, and not as useful ATP. Regulation of mitochondrial activity must strike a balance between these conflicting requirements.

Mitochondrial membranes have interesting structural particularities. The inner membranes have the highest protein density of all intracellular membranes. As only a small number of lipid molecules is thought to separate the proteins, localized changes in membrane phospholipids may be critical in establishing the activities of membrane proteins. In all vertebrates examined to date, mitochondrial membranes do not contain cholesterol, in contrast to the plasma membrane, which generally contains cholesterol. The mitochondrial inner membrane is the only cellular structure containing cardiolipin, a phospholipid composed of four acyl chains attached to a di-glycerol backbone. Cardiolipin molecules are tightly associated with several protein complexes of the ETS, in particular, cytochrome C oxidase (CCO) and the F_1F_0ATPase, as well as with adenine nucleotide translocase (ANT). An unusual acyl chain, vaccenate, is a central element of the oxygen-transport system in CCO from the eukaryotic cows to the prokaryotic soil bacteria, *Paracoccus denitrificans*. The conservation of vaccenate in this critical position across such a huge phylogenetic distance argues for an important functional role besides an ancient origin. These specific structural attributes and phospholipid requirements of mitochondrial membrane proteins suggest not only that these lipids play critical functional roles but also that thermal change could perturb mitochondrial performance. But what would these consequences of thermal change be?

As fish are water breathers, changes in habitat temperature translate into changes in body temperature for most species. Temperature affects the kinetic energy of molecules and modifies rates of diffusion and molecular interactions. As thermal change has differential impacts upon the weak bonds (hydrophobic and hydrophilic) responsible for tertiary protein structure, it can affect the stability and the activity of critical mitochondrial proteins (**Figure 2**) (**see also Temperature**: Effects of Temperature: An Introduction).

Thermal change can also profoundly alter membrane properties, by causing phase shifts in phospholipid bilayers. Those for whom this concept is foreign need only to think of how butter melts when heated. These transitions between gel and liquid phases occur over a range of temperatures for lipids. The more saturated a fat (e.g., butter), the lower the temperature at which the gel state is favored. Saturated lipids have linear acyl chains that become ordered more easily than the kinky acyl chains of unsaturated fats. Lipids with polyunsaturated acyl chains have extreme acyl-chain curvature and high transition temperatures. One of the most highly unsaturated acyl chains, docohexanoic acid (DHA; 2:6*n*-3),

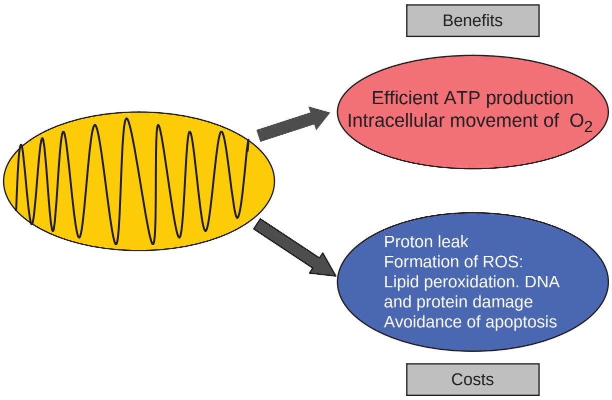


Figure 1 Costs and benefits of mitochondria.

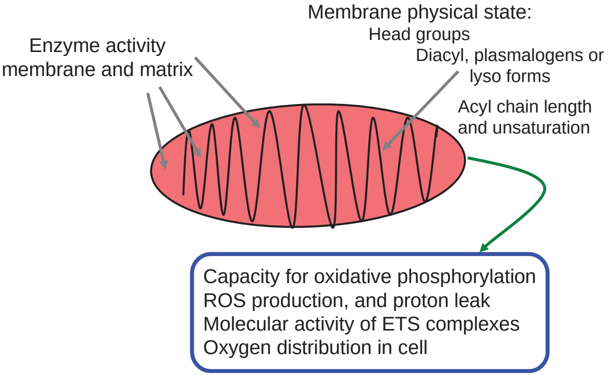


Figure 2 What mitochondrial properties are sensitive to thermal change?

assumes a pyramidal shape in phospholipid bilayers. This conformation facilitates dynamic modifications of membrane structure. Cholesterol tends to prevent phase changes; so, its absence in mitochondrial membranes suggests that they would be more likely to be perturbed by thermal change than other intracellular membranes. Phospholipid head groups also influence membrane order. Phosphatidylethanolamine (PE) destabilizes membranes whereas phosphatidylcholine (PC) increases membrane stability. Thus, both the phospholipid and acyl-chain composition of a membrane will influence its physical state and how it is affected by thermal change.

The physical state of cellular membranes has been proposed to act as a metabolic pacemaker by setting the rates at which membrane-bound proteins carry out their activities. Therefore, the impact of temperature on the physical state of mitochondrial membranes could have far-reaching functional consequences. The fact that, of cellular membranes, mitochondrial membranes show the most complete thermal compensation of fluidity and dynamic physical state may reflect the importance of maintaining the balance between the positive and negative roles of mitochondria under a range of environmental conditions.

Particularly for fish from temperate zones, both diurnal and seasonal fluctuations in environmental conditions can be pronounced. Strong seasonal cycles in temperature, photoperiod, and food availability are typical, especially for anadromous or catadromous species. Daily thermal fluctuations in shallow ponds or intertidal pools can be more pronounced than seasonal changes in marine habitats. Just as aquatic habitats differ in their seasonal and diurnal temperature fluctuations, the fish exploiting these habitats vary in the temperatures and thermal fluctuations they can tolerate. These tolerances change during development with larval and juvenile stages often having narrower limits than adults. Thermal tolerances also change with an individual's thermal history and can differ between latitudinally separated populations. Such tolerances are a major determinant of biogeography and are of increasing interest in the context of global climate change.

It is likely that fish that better tolerate thermal fluctuations will have proteins that are damaged little by typical thermal excursions, or they possess better rescue systems to resurrect damaged proteins. Fish that tolerate thermal instability should either have a mitochondrial membrane composition that minimizes the impact of thermal change upon its functional properties, or be able to rapidly adjust composition in response to thermal change. In the following an overview of how fish mitochondria respond to acute thermal change and how changes in macromolecular design have allowed adaptation of mitochondria to vastly different thermal regimes over evolutionary time is given. This is followed by an examination of how fish can modify their mitochondria in response to thermal acclimation. While knowledge in this area is grounded in decades of study, it is rapidly expanding, with the application of genomic and proteomic techniques that allow concomitant examination of the expression of thousands of genes. Such molecular studies are confirming previous findings and are extending our understanding of the plethora of changes occurring during transitions between thermal regimes in eurythermal fish.

Fish Mitochondria and Thermal Change

Most experimental studies of thermal effects vastly simplify thermal dynamics in comparison with the patterns observed in nature. Acute thermal effects are evaluated using isolated preparations, thereby minimizing *in vivo* adjustments that could mitigate the impact of thermal change. These acute thermal shifts are vastly larger and often exceed the range of temperatures encountered by the species. Studies of thermal acclimation expose fish to constant temperatures, often at constant, and therefore unrealistic, photoperiods. Sampling fish at different seasons in their habitat is more realistic, but interpretation of results is complicated by concomitant changes in biotic and abiotic factors. Few studies have examined the time course of the response to temperature. Thus, our knowledge of the effects on temperature upon fish mitochondria can be compared to an impressionist painting, with broad strokes providing a reasonable overview, but details lacking for most species. The impressive diversity of fish, not only in their phylogeny and evolutionary history, but also in their habitats and lifestyles, should prevent biologists from assuming that there is just a single response of fish mitochondria to temperature.

Responses to Acute Thermal Change

For enzymes, covariation of pH and temperature minimizes the impact of thermal change on substrate affinities, but maximal rates remain subject to the tyranny of Arrhenius (**see also Temperature**: Effects of Temperature: An Introduction). Insofar as maximal rates of substrate oxidation are concerned, a similar picture applies to fish mitochondria: maximal rates rise with temperature, even though maximal capacities vary seasonally and rates at habitat temperature fall during winter months (**Figure 3**). The thermal sensitivity of mitochondrial affinities for carbon and adenylate substrates is less well documented, but affinities for carbon substrates tend to be maintained, when pH covaries with temperature. The ADP affinity of rainbow trout (*Oncorhynchus mykiss*) muscle mitochondria and its thermal sensitivity vary seasonally, with ADP Km_{app} increasing with assay temperature only during warm periods (**Figure 4**). Acute thermal change does not perturb the pH gradient across the inner membrane of mitochondria from carp

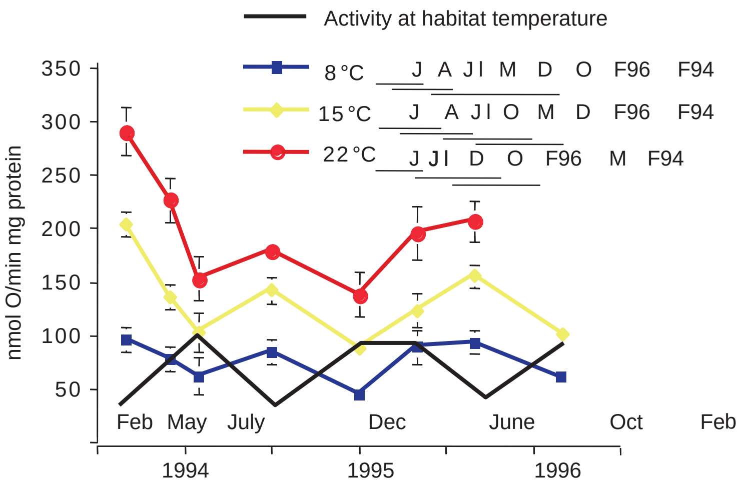


Figure 3 Seasonal cycles of maximal oxidative capacity of muscle mitochondria (at 8, 15, and 22 °C) from naturally acclimatized trout.

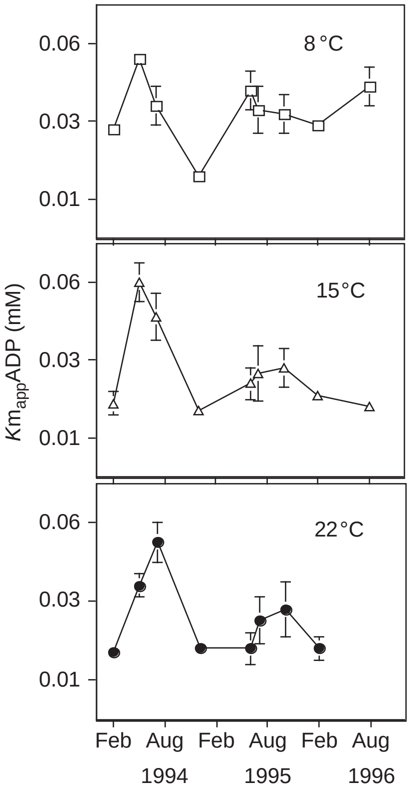


Figure 4 Seasonal cycles of ADP affinity in trout muscle mitochondria.

muscle. Q_{10} values for maximal rates of mitochondrial substrate oxidation vary seasonally, but maximal rates always fall as temperature drops (**Figure 3**). This consistent, acute thermal sensitivity of mitochondrial oxidative capacities may underlie the frequently observed compensation of oxidative capacity during thermal acclimation of cold-active species (see below).

During acute thermal change, mitochondrial membranes undergo changes in physical state that could be mitigated *in vivo*. Thus, when desert cyprinids (*Agosia chrysogaster* and *Notropis lutrensis*) are sampled at different times of the day at a wide range of temperatures, the membrane phospholipid head-group composition differs. Indeed, head-group composition can change quite rapidly, with marked shifts completed within 2 days of thermal transfer. Such mechanisms may alleviate the functional impact of acute thermal change *in vivo*. Protective effects of heat-shock proteins could also minimize acute thermal effects during exposure to extreme temperatures *in vivo*.

Evolutionary Thermal Adaptation

The specialization of fish mitochondria for different habitat temperatures is shown by interspecific comparisons of mitochondrial respiration rates. On the one hand, one relationship describes the thermal sensitivity of oxidation rates of mitochondria isolated from muscle of a variety of percid fish from Antarctic to subtropical habitats. For each species, assay temperatures closely bracketed habitat temperatures. On the other hand, the Arrhenius break temperatures (ABTs), obtained from curves relating respiration rates to assay temperature, vary markedly between tropical and Antarctic fish. In these assays, mitochondrial respiration rates are measured at temperatures up to 25 °C above habitat values. Mitochondria from tropical fish show considerably higher ABTs for the different respiratory states than mitochondria from Antarctic fishes. When measured at a common temperature, membrane fluidity is lower in the species from warm waters than in those living at cooler temperatures. Thus, membrane properties of mitochondria have adjusted to typical habitat temperatures over evolutionary time, but protein-specific mitochondrial respiration rates for fish from polar waters have not increased to compensate for the cold temperatures. Over short-term evolutionary timescales, mitochondrial function seems quite conserved. Thus, despite specialization of Arctic and Brook chars (*Salvelinus alpinus* and *Salvelinus fontinalis*) for different thermal habitats and divergence of their protein structures, ETS complex activities and thermal sensitivities were maintained in naturally occurring hybrids.

When fish species with similar lifestyles are compared, fish inhabiting permanent cold have higher mitochondrial volume densities in their muscles than those living in warmer or more variable climes. These high mitochondrial contents by far surpass those in mammalian heart and presumably curtail the space that can be devoted to other organelles, including the myofibrils that are responsible for contractile activity. Various interpretations have been given for these high volume densities, including thermal compensation of capacity and facilitation of oxygen distribution. This mitochondrial abundance may enhance rates of ROS production, explaining the high levels of superoxide dismutase, the major enzymatic protector against ROS, in Antarctic species. Abundant mitochondria also require maintenance of the membrane potential despite proton leak. The associated metabolic cost would increase standard metabolic rate and become problematic when ventilatory costs reduce the availability of oxygen.

Membrane phospholipid composition differs markedly with thermal habitat, such that the membrane physical state is similar at different body temperatures. To this end, modifications in the unsaturation and in the proportion of long-chain polyunsaturated fatty acids are accompanied by shifts in the relative levels of the major phospholipids (choline, serine, ethanolamine, etc.) (**Figure 2**). Membrane phospholipid composition follows tissue temperature more than central temperature. These compositional modifications conserve the dynamic nature of the membrane, such that organismal temperature is approximately centered between the temperatures of the phase transitions (fluid–gel transition and formation of inverted vesicles). The concept of homeoviscous adaptation (**see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation), which refers to the maintenance of membrane fluidity or viscosity, is often used to justify the compositional changes. The greater polyunsaturation of membrane phospholipids at low body temperatures also facilitates the movement of oxygen within the more fluid hydrophobic core of the membrane. However, greater polyunsaturation of phospholipids at low temperatures may increase proton leak and enhance susceptibility to lipid peroxidation. Presumably to mitigate these difficulties, polar fish maintain exceptionally high levels of lipid antioxidants, such as vitamin E.

Response of Fish Mitochondria to Thermal Acclimation

The metabolic strategy used by a fish species to respond to thermal acclimation should depend upon whether it remains active at all seasons. Thermal acclimation is typically studied by holding fish at different temperatures for several weeks while leaving other parameters as constant as possible. When thermal acclimation modifies rate functions so that the rate at the new temperature is not simply dictated by their Q_{10}, thermal compensation is said to have occurred.

Herbert Precht separated responses to thermal acclimation (or acclimatization) into overcompensation, perfect compensation, partial compensation, no compensation, and inverse compensation. These categories illustrate the strategies available for coping with thermal change. For example, when fish are faced with an upcoming winter, they can:

1. slow their processes by following Q_{10} effects (no compensation),
2. depress metabolism by enhancing thermal effects (torpor and negative compensation), or
3. use partial or perfect compensation to offset Q_{10} effects.

Isolated mitochondria from cyprinids, salmonids, and sculpins fall in the last category. Mitochondrial enzyme levels in muscle increase with cold acclimation of numerous cold-active species, suggesting that oxidative phosphorylation is particularly sensitive to cold. Intriguingly, mitochondrial proliferation in the anoxia-tolerant crucian carp (*Carassius carassius*; **see also Bony Fishes**: Crucian Carp) is similar to that in cold-active species. Crucian carp release ethanol to maintain their energy status during prolonged winter anoxia, and markedly depress metabolic demands during these periods. Tissue-specific responses may occur; for example, cold acclimation of carp decreases uncoupling protein-1 (UCP-1) levels in the liver but increases them in the brain.

Many studies have shown that cold acclimation can increase muscle oxidative capacities in temperate-zone fish (**Figure 5**). Muscle oxidative capacities rise (as demonstrated by rises in the activity of mitochondrial enzymes), through increases either in mitochondrial content or in the oxidative capacities of mitochondria. An increased proportion of oxidative fibers in the musculature may contribute. Mitochondrial proliferation can occur within glycolytic or oxidative fibers. The response of striped bass, *Morone saxatilis*, has been particularly well characterized: cold acclimation increases muscle oxidative capacity, mitochondrial volume density, lipid concentration, and lipid oxidation capacity. The increase in muscle lipid contents (both triglycerides and mitochondrial membranes) partially offsets the impact of cold on the intracellular diffusion of oxygen. Increases in the capacity for lipid oxidation and storage are also seen during cold acclimation and seasonal cold acclimatization of rainbow trout. Antioxidant systems respond to thermal acclimation, but as ROS production rises with temperature, while susceptibility to ROS damage increases with cold-induced lipid polyunsaturation, the patterns are difficult to interpret. In rainbow trout, optimal swimming performance occurs after acclimatization (in outdoor ponds) to 11 °C and despite increases in mitochondrial capacities, rates at habitat temperature fall in winter (**Figure 3**). While mitochondria are a primary target of thermal compensation in oxidative and glycolytic muscle, compensation is rarely perfect and performance may be optimal at intermediate temperatures.

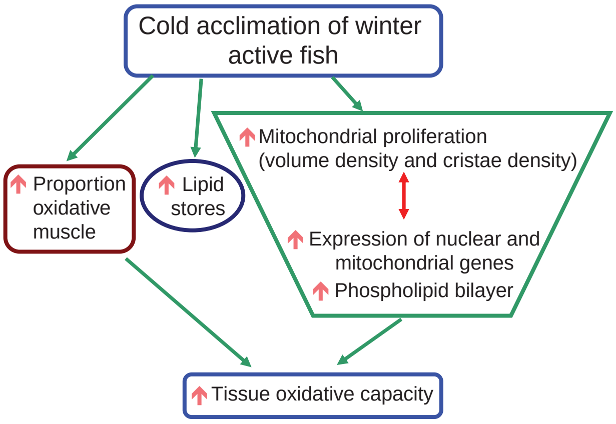

Cold acclimation of winter active fish
↑ Proportion oxidative muscle
↑ Lipid stores
↑ Mitochondrial proliferation (volume density and cristae density)
↑ Expression of nuclear and mitochondrial genes
↑ Phospholipid bilayer
↑ Tissue oxidative capacity

Figure 5 Cold acclimation of winter active fish.

Mitochondrial proliferation requires coordinated expression of nuclear and mitochondrial encoded genes, and relies on the integrated activity of DNA-binding proteins and their coactivators. In contrast to mammals, the nuclear respiratory factor-1 is the primary activator of mitochondrial proliferation in fish, while the peroxisome proliferator-activated receptor is linked with fatty-acid metabolism. All the ETS complexes contain many subunits, with most encoded by the nucleus but some in mitochondria. Understanding of the regulation of their synthesis is far from complete. Sufficient quantities of membrane must be produced to lodge the mitochondrial proteins. Oxidative capacities of existing mitochondria may be enhanced by either increasing the number of ETS complexes per inner membrane area or changing the phospholipid composition.

Thermal acclimation causes extensive remodeling of the phospholipid and fatty-acid composition of mitochondrial membranes, leading to changes in the activity of mitochondrial enzymes per milligram mitochondrial protein. This may be the basic response to thermal change. Within hours after transfer to cold temperatures, changes in phospholipid structure are initiated. Changes in phospholipid head groups occur after 8 h, the ratio of saturated to monenoic acyl chains shifts within 2–4 days of thermal transfer, whereas changes in the proportions of long-chain polyunsaturated fatty acids require weeks of thermal acclimation. Cold acclimation of cyprinids (**Figure 6**) markedly changes mitochondrial phospholipids, increasing the proportion of PE to the detriment of PC and raising the proportion of unsaturated and polyunsaturated fatty acids in PC, but not in CL. The changed lipids account for the increased activity of succinate dehydrogenase, CCO and the F_1F_0ATPase with cold acclimation. Activation of the Δ9-desaturase by changes in membrane order with falling temperature increases the unsaturation of the central core of the phospholipid bilayer. Production of Δ9-desaturase mRNA during cold acclimation further increases enzyme number and activity. Shortly (2–4 days) after transfer to their new temperature, the oxidative capacities of rainbow trout muscle mitochondria change actually accentuating the impact of thermal change, but longer acclimation leads to thermal compensation (**Figure 7**). During warm acclimation of rainbow trout, the phospholipid composition of isolated mitochondria changes more than contents of cytochromes or ANT. Marked changes of acyl-chain composition in minor phospholipids, such as the plasmalogen forms of PE and PC as well as cardiolipin, suggest that specific compositional changes in these

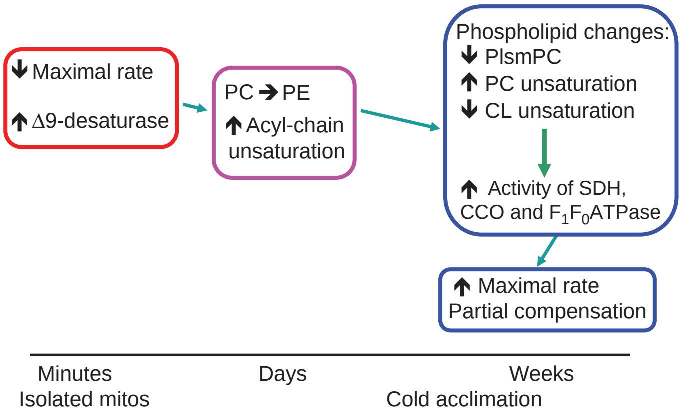


Figure 6 Responses of isolated mitochondria to cold acclimation in cyprinids.

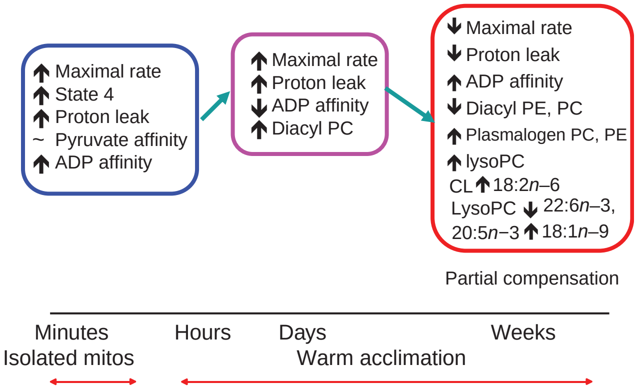


Figure 7 Time course of responses of muscle mitochondria during warm exposure: rainbow trout.

phospholipid classes modulate the activity of the ETS complexes. Cold-acclimated rainbow trout have more PE in the inner half of the inner mitochondrial membrane than warm-acclimated rainbow trout. The enrichment of membranes from cold-acclimated rainbow trout (5 °C) with these membrane-destabilizing lipids helps maintain the dynamic behavior of the membrane at low temperatures. The response to thermal acclimation can vary among latitudinally separated populations, with northern populations of killifish (*Fundulus heteroclitus*) compensating more for low temperatures than the southern populations.

Responses to laboratory acclimation may differ from those occurring when fish are acclimatized to natural changes in photoperiod and temperature, or are sampled in their habitat at these temperatures. These changes were noticed when comparing the oxidative capacities of mitochondria from rainbow trout sampled at various periods during their annual cycle. The compensatory increase in maximal rate was particularly marked during the autumnal decrease in temperature. In other words, although habitat temperatures had only begun to fall, the increase in mitochondrial oxidative capacity was greater than needed to compensate for the actual thermal change, suggesting that the fish were anticipating the expected seasonal change. Effectively, rainbow trout acclimated to short day lengths at warm temperatures increase the oxidative capacity of isolated muscle mitochondria, but to a lesser extent than trout acclimated to short day lengths and cold temperatures. Thus, photoperiod control allows fish to prepare their mitochondria for the upcoming season. Furthermore, if thermal acclimation is carried out at seasonally inappropriate photoperiods, fish performance differs radically from that obtained under appropriate photoperiods. The signals involved are yet to be identified.

Temperature and Fish Mitochondria in the Genomic Era

Whereas most of the foregoing article is based on experimental tests of hypotheses concerning specific parameters, such as mitochondrial oxidative capacities, activities of mitochondrial enzymes, or phospholipid composition, the study of thermal acclimation has plunged into the genomic age with the development of DNA chips allowing the examination of changes in expression of thousands of genes. Together with

Transcriptomics:
Cold acclimation of carp enhances expression of approximately 3400 genes involved in:

- RNA processing
- Translation initiation
- Modification of higher membrane (Δ9 desaturase)
- Mitochondrial metabolism
- Muscle atrophy
- Proteasome components
- Unknown functions….

Figure 8 Transcriptomics: cold acclimation of carp enhances expression of approximately 3400 genes involved in various processes.

proteomic and metabolomic analyses, these powerful approaches show when and how functional groups of genes respond to environmental change and reveal responses of gene sequences of unknown function. This exploratory approach has the potential to move understanding to an entirely new level. A pioneering study followed the tissue-specific responses of carp during cold acclimation using a gene chip with approximately 13 000 sequences. While induction of the Δ9-desaturase in all tissues and increases in expression of mitochondrial proteins confirmed previous findings, other increases in expression were more surprising (**Figure 8**). For example, the ubiquitin-linked proteasomic pathway and genes stimulating muscle atrophy were markedly activated during cold exposure. When links between the changes in expression and the levels of the product are examined, the correlations between changes in message and in the protein products are positive, but vary in time as well as in strength. As the message and the protein are at different stages of a highly regulated sequence, such confirmation is needed for sound interpretation of expression patterns.

These discovery-based investigations will identify new processes involved in thermal change in fish as well as clarify the integration between previously identified processes. With the increasing availability of sequence data and complementary DNA (cDNA) micro-arrays for many fish species, our understanding of the responses to temperature should increase by leaps and bounds in the coming years. Given the impressive phylogenetic diversity of fish and the variety of habitats they exploit, study of mitochondrial responses to temperature among these species will reveal central processes in the coevolution between mitochondria and the organisms they serve.

See also: **Bony Fishes**: Crucian Carp. **Energetics**: General Energy Metabolism. **Temperature**: Effects of Temperature: An Introduction; Membranes and Temperature: Homeoviscous Adaptation. **Tissue Respiration**: Cellular Respiration; Mitochondrial Respiration.

Further Reading

Abele D and Puntarulo S (2004) Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comparative Biochemistry and Physiology – Part A* 138: 405–415.

Gracey AY, Fraser J, Weizhong L, *et al.* (2004) Coping with cold: An integrative multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences of the United States of America* 101: 16970–16975.

Guderley H (2004) Metabolic responses to low temperature in fish muscle. *Biological Reviews* 79: 409–427.

Hazel JR (1995) Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology* 57: 19–42.

Hulbert AJ and Else PL (1999) Membranes as possible pacemakers of metabolism *Journal of Theoretical Biology* 199: 257–274.

Kraffe E, Marty Y, and Guderley H (2007) Changes in mitochondrial oxidative capacities during thermal acclimation of rainbow trout, *Oncorhynchus mykiss*: Roles of membrane proteins, phospholipids and their fatty acid compositions. *Journal of Experimental Biology* 210: 149–165.

Pörtner HO, Lucassen M, and Storch D (2005) Metabolic biochemistry: Its role in thermal tolerance and in the capacities of physiological and ecological function. In: Steffensen JF and Farrel AP (eds.) *Fish Physiology, Volume 22, The Physiology of Polar Fishes*, pp. 79–154. San Diego, CA: Academic Press.

Precht H (1958) Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: Prosser CL (ed.) *Physiological Adaptations,* Symposium General Physiology, pp. 50–78. Washington, DC: American Physiology Society.

Sidell BD (1998) Intracellular oxygen diffusion: The roles of myoglobin and lipid at cold body temperature. *Journal of Experimental Biology* 201: 1119–1128.

Somero GN (2004) Adaptation of enzymes to temperature: Searching for basic strategies. *Comparative Biochemistry and Physiology – Part B* 139: 321–333.

Temperature and Excitable Membranes

M Vornanen, University of Eastern Finland, Joensuu, Finland

© 2011 Elsevier Inc. All rights reserved.

What Is an Excitable Membrane or Cell?
Ion Channels Are Necessary for Excitability
How Does Temperature Affect Excitability?
Significance of Membrane Lipids in Thermal Responses of Excitable Cells
Evolutionary Thermal Adaptation of Excitability
Further Reading

Glossary

Action potential A small and transient change in voltage across the cell membrane. The action potential is possible because of the activity of ion channels embedded in the cell membrane, selectively allowing certain inorganic ions to pass through their pore.

Activation Transformation of the ion channel from the resting closed state (i.e., inactivation gate open and activation gate closed) to the open state by the opening of the activation gate.

Conduction velocity The rate at which an action potential proceeds along the cell membrane.

Deactivation Transformation of the activation gate of an ion channel from its open position to the closed position.

Depolarization The reduction in electrical potential difference (voltage) measured across a cell membrane. This means the inside of the cell becomes less negative with respect to the outside of the cell. Depolarization occurs during the upsweep of the action potential (opposite of repolarization).

Excitable membrane A cell membrane of neurons and muscle cells (and some glandular cells) which generates self-propagating action potentials. Nonexcitable cells can produce local voltage changes that dampen quickly.

Homeoviscous adaptation Adaptation of the lipid membrane to changing temperature via alterations in its chemical composition, which counteracts temperature-dependent changes in membrane fluidity (viscosity) mainly by changes in saturation level of fatty acids.

Inactivation Transformation of the ion channel from an open state to a closed state (i.e., activation gate open, inactivation gate closed) by the closure of the inactivation gate.

Inward current A flow of positively charged ions across the membrane into the cell.

Ion channel A protein or a group of proteins that form water-filled pores in the cell membrane. It allows a rapid flow of ions through the cell membrane. Each channel type is specific for one inorganic ion (sodium, calcium, potassium, or chloride).

Ion channel gating Conformational changes of ion channel proteins responsible for open and closed states of the ion channel.

Ligand-gated channel A transmembrane ion channel whose opening is activated by the binding of a specific physiologically active compound (ligand) to channel protein.

Lipid rafts The microdomains of cell membrane mainly consisting of cholesterol, sphingolipids, and proteins. These are centers of cellular signaling that float freely within the lipid bilayer.

Outward current A flow of positively charged ions across the membrane out of the cell.

Refractory period A finite period of time after the action potential during which a new action potential cannot be generated, due to time- and voltage-dependent recovery of ion channel from inactivation.

Repolarization A change of membrane voltage back to the polarized state that prevailed before the action potential was generated, that is, increase in the negativity of membrane potential.

Resting membrane potential The stable negative membrane potential (–70 to –90 mV) of the resting cell.

Threshold potential The value of membrane voltage needed to excite a full-blown action potential. It is the membrane voltage (about –60 mV) at which sodium influx exceeds potassium efflux across the cell membrane and results in depolarization of the cell membrane.

Voltage-gated channel An ion channel whose opening and closing are governed by changes in membrane voltage. Opening and closing of the channel take certain time, that is, the operation is voltage and time dependent.

Voltage sensor A special domain of the ion channel protein that senses the voltage across the cell membrane. Movement of the voltage sensor causes opening and closing of channel gates.

What Is an Excitable Membrane or Cell?

All animal cells maintain a voltage difference across the cell membrane, which is termed the 'resting membrane potential' (RMP). The RMP is used to power transport of substances across the membrane against their concentration gradients. In addition, changes in the RMP can act as signals within the cell. Most changes in the RMP dampen within a short distance of their origin, and cannot be used as long-distance signals. However, some cells (including neurons, skeletal, cardiac, and smooth muscle cells and some endocrine glands) can produce transient changes in their RMP that are called action potentials (APs). (In fish skeletal muscles, only the fast focally innervated fibers produce APs, while multiply innervated slow fibers are activated by local potentials. Thus, in a strict sense, all slow fibers in fish are not excitable.) APs can propagate along the cell membrane without change in amplitude, and are thus a means of fast transmission of information across long cellular distances, which can be the order of meters for nerve axons. Cells that are able to produce these self-propagating APs are referred to as electrically excitable.

In the resting state, electrically excitable cells have a negative RMP of −60 to −100 mV relative to the extracellular medium. A change in membrane potential from the RMP in the positive direction is termed as 'depolarization', while a change from the RMP in the negative direction is termed as 'hyperpolarization'. The AP begins with a small (50–100 mV) and transient change of voltage across the cell membrane from the RMP in the positive direction (**Figure 1**). This depolarization is then followed by a repolarization of the membrane potential toward the RMP, which then may (or may not) be followed by a brief hyperpolarization, depending upon the cell type. The ability to produce APs is critical for the functioning of the nervous and muscular systems. Thus, ectothermic organisms such as fish must maintain electrical excitability as environmental temperature changes in order to maintain physiological functions.

Ion Channels Are Necessary for Excitability

APs are produced by the opening and closing of ion channels. Ion channels are pore-forming membrane proteins that allow passage of certain inorganic ions through otherwise impermeable lipid membrane. These channels are triggered to open and close by changes in membrane potential (voltage-gated channels) or binding of a physiologically active compound, for example, a neurotransmitter, to the channel (ligand-gated channels). The presence of voltage-gated channels is required for the generation of APs.

Opening and closing of voltage-gated ion channels are produced by complex changes in three-dimensional conformation of the channel protein, which can be described as changes in the position of molecular gates of the channel pore (**Figure 2**). Voltage-gated ion channels often have two gates: an activation gate and an inactivation gate. In the resting, nonconducting channel, the inactivation gate is open and the activation

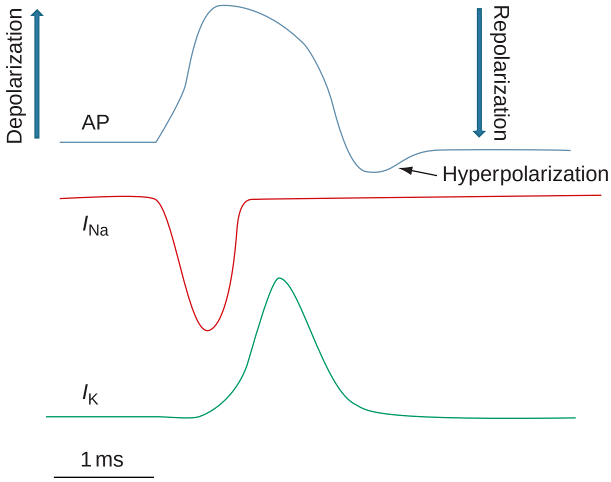


Figure 1 Schematic presentation of the neuronal action potential. An action potential is produced by the sequential opening of sodium and potassium channels. Opening of sodium channels causes inward flow of sodium ions, which results in rapid depolarization of the membrane potential slightly above the zero membrane potential. Closure of sodium channels and the delayed opening of potassium channels cause repolarization of membrane potential by the net outward potassium current. Differences in the rate of opening, that is, in the rate of the voltage sensor displacement, between sodium and potassium channels produce the balance of sodium and potassium currents needed for action potential generation.

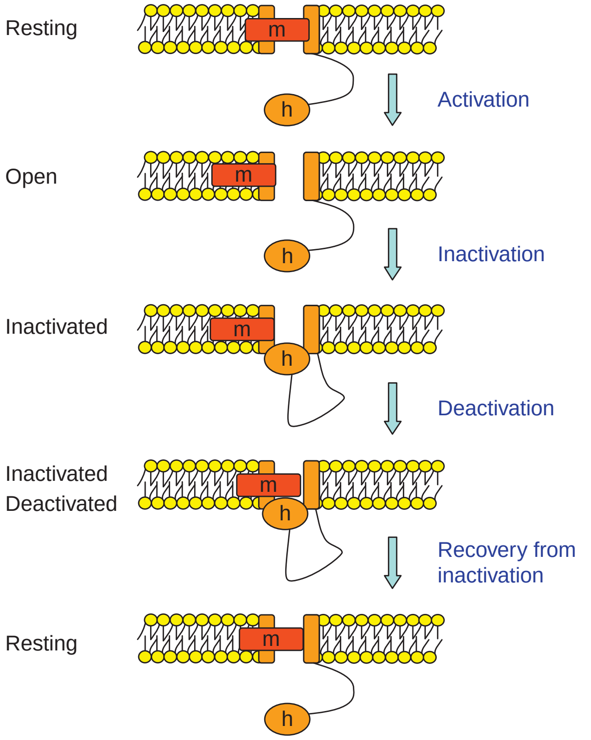


Figure 2 Opening and closing of the ion channel by the movements of the voltage-dependent gates. An ion channel cannot reopen unless it has resumed the resting state. This is a time- and voltage-dependent process and makes the cell membrane refractory to stimulation immediately after the action potential. The order of deactivation and recovery from inactivation varies between voltage-gated channels.

gate is closed. When the activation gate opens, the channel changes to open state (activation), that is, it allows passive flow of ions through its open pore. Next, the inactivation gate closes (inactivation) and makes the channel nonconducting again (inactivated state). The channel cannot reopen, unless it achieves the resting state again, that is, the inactivation gate must open (recovery from inactivation) and the activation gate must close (deactivation). This gating process is determined by changes in membrane voltage, which the channel senses with a positively charged domain of the channel protein called the 'voltage sensor'. In the resting cell, the voltage sensor is close to the inner surface of the membrane (attracted by the negative RMP) and moves in the outward direction when cell membrane starts to depolarize. Movements of the voltage sensor cause opening and closing of the gates.

The depolarization phase of an AP is triggered by an initial graded depolarization of the excitable membrane. This is normally caused by a spread of AP from the neighboring cell, as in cardiac and smooth muscles, or by an opening of a ligand-gated ion channel by a neurotransmitter in the postsynaptic membrane of neuron or skeletal muscle cell. When the depolarization reaches a level known as the threshold potential, sufficient voltage-gated channels are opened that a self-reinforcing positive-feedback loop occurs, resulting in an AP.

How Does Temperature Affect Excitability?

Excitability of cell membrane can be altered by temperature as a result of temperature-dependent changes in (1) RMP, (2) the generation of APs, (3) the velocity of AP propagation, and (4) the duration of the AP. These alterations in membrane excitability are reflected in alterations in neuromotor functions such as sensory transduction, motor reflexes, locomotion, heart function, learning, and memory of fish.

Temperature dependence of excitability stems from the function of ion channels. Passive flow of ions through an open ion channel has a low-temperature dependence ($Q_{10} = 1.0–1.4$; **see also Temperature**: Effects of Temperature: An Introduction), similar to that of diffusion. Therefore, ion permeation through the open channel has only relatively a small effect on temperature dependence of ion currents. In contrast, the opening and closing of ion channels have activation energies similar to enzymatic reactions and therefore function of the channel is temperature dependent. Usually, the Q_{10} of ion channel gating is around 3, but can be as high as 8. At the single-channel level, the frequency of ion channel openings and closings and the duration of open and closed times are affected by temperature. At a macroscopic level, these appear in temperature sensitivity of channel activation, inactivation, deactivation, and recovery from inactivation (**Figure 3**).

Effects of Temperature on RMP

The value of the membrane potential is determined by those ion channels that are open, and reflects a balance of the equilibrium potentials for each ion and the relative permeabilities of the membrane for each ion. In most excitable cells, the RMP is maintained by a small potassium efflux across cell membrane via the inward rectifier potassium (Kir2) channels that mediate an inwardly rectifying potassium current, the I_{K1}. Other ion conductances of the resting cell are only a fraction of the potassium conductance and therefore RMP is close to the equilibrium potential of potassium ions (–80 mV). Acute decreases in temperature have been observed to cause a depolarization of the membrane. For example, in the extraocular muscle of the Antarctic teleost, *Pagothenia borchgrevinki*, RMP changes 22 mV/10 °C. Depolarization is often due to the reduced density of the I_{K1}, as in trout cardiac myocytes (**Figure 4**). The decreased potassium conductance appears as increased membrane resistance.

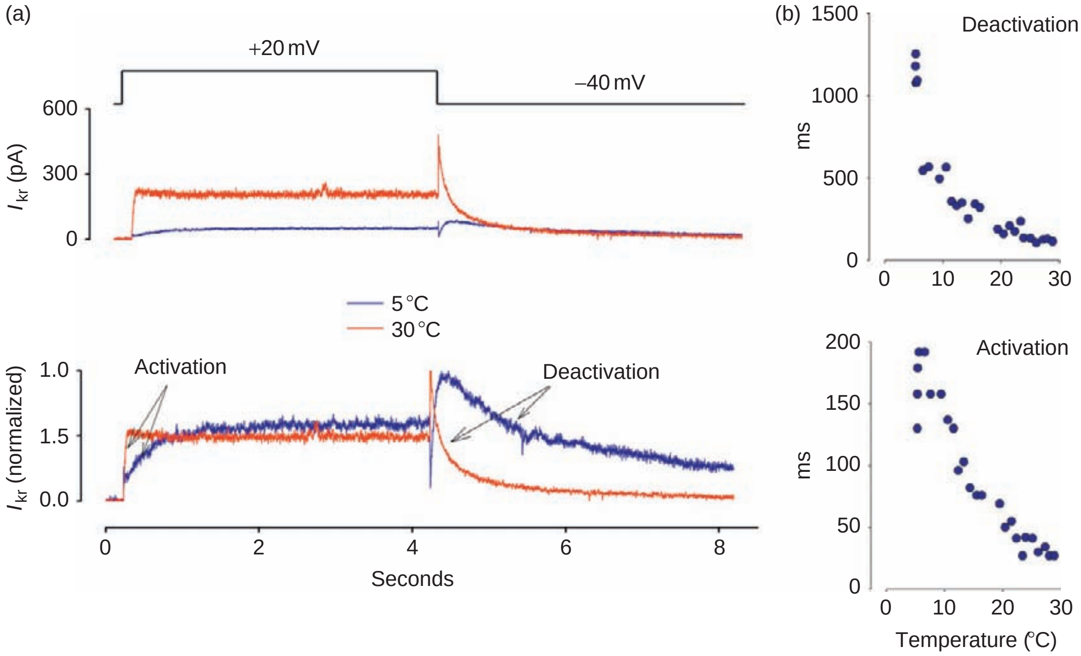


Figure 3 Temperature changes density and kinetics of ion currents. (a) The rapid component of the delayed rectifier potassium current (I_{Kr}) at 5 and 30 °C generated by the voltage-gated potassium channel Kv11.1. The upper panel shows temperature-dependent difference in current density. Effect of temperature on activation (depolarizing pulse to +20 mV) and deactivation (repolarizing pulse to –40 mV) kinetics is clearly evident from the normalized tracings of the lower panel. (b) Temperature dependence of activation and deactivation kinetics of the I_{Kr} between 5 and 30 °C. Deactivation kinetics is composed of two kinetically different components, but for clarity only the time constant of the rapid component is indicated. Activation kinetics is given as a time to half-maximal activation. All recordings and data are from the same atrial myocyte from a rainbow trout heart.

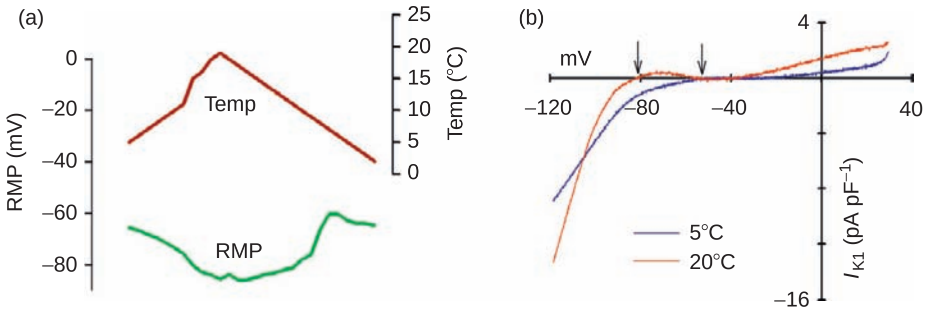


Figure 4 Effect of temperature on resting membrane potential. (a) Reversible changes of resting membrane potential (green line) of trout ventricular myocyte. (b) Inward rectifier current, I_{K1}, through Kir2 channels of the same myocyte. Resting membrane potential (arrows) is set by the reversal potential of the I_{K1}.

This depolarization of the membrane at the RMP improves excitability of the membrane, because less depolarizing current is needed to bring membrane potential to the threshold of AP generation. If everything else remains constant, decreased potassium conductance would be expected to increase the excitability of neurons and muscle cells in the cold.

On the other hand, acclimation of animals to the cold often results in compensatory increase in the density of the I_{K1}, which counteracts the direct effect of temperature on I_{K1} and tends to stabilize RMP. In the majority of the fish species examined, cardiac I_{K1} is upregulated in the cold. However, in rainbow trout (*Oncorhynchus mykiss*), I_{K1} is depressed in cold acclimation, which enforces the effects of acute temperature change, that is, increases excitability of myocytes.

Species-specific responses to temperature in the density of I_{K1} are associated with altered expression of Kir2 channels. The Kir2 subfamily has five members (Kir2.1–Kir2.5), and three of them (Kir2.1, Kir2.2, and Kir2.5) are expressed in the fish heart. In the rainbow trout heart, enhancement of the I_{K1} with warm acclimation is associated with increased expression of the Kir2.2 subunits, although Kir2.1 is the main Kir2 transcript in both warm-acclimated and cold-acclimated hearts. In contrast, in the crucian carp (*Carassius carassius*), the cold-induced increase in the density of I_{K1} is based on strong upregulation of the Kir2.5 transcripts at the expense of Kir2.2. Arguably, the Kir2.5 is the cold-adapted isoform and the Kir2.2 is the warm-adapted isoform of the fish heart. Rainbow trout heart does not express Kir2.5, which may explain the absence of thermal compensation of the I_{K1} with cold acclimation. Transcripts of the Kir2 channels are also abundantly present in brain and skeletal muscle of the fish, but effects of temperature on skeletal muscle and brain Kir2 channels have not been examined.

Effects of Temperature on the Velocity of AP Conduction

Increases and decreases in temperature accelerate and decelerate, respectively, conduction velocity of APs. For example, in Mauthner cells of the goldfish (*Carassius auratus*) brainstem, conduction velocity of AP is reduced by the cold, affecting the fast escape behavior (startle response) of the fish. Similarly in the vagus nerve of the carp (*Cyprinus carpio*), conduction velocity is strongly dependent on temperature in both unmyelinated and myelinated nerve fibers.

The rate of impulse conduction is dependent on the upstroke rate of AP, which is determined by the sodium current (I_{Na}), the largest inward current in most excitable cells. When the threshold potential is exceeded, a large number of sodium channels open almost simultaneously causing a rapid change of membrane voltage. Changes in conduction velocity of AP in an excitable cell will therefore largely result from changes in the magnitude and kinetics of I_{Na} and their temperature dependence.

An acute decrease in temperature decreases the density of the I_{Na}, which is expected to decrease upstroke velocity and amplitude of AP with a subsequent decrease in the speed of impulse conduction. Thus, direct effects of cold temperature on I_{Na} tend to decrease excitability by reductions in impulse conduction velocity.

The acute effects of low temperature on I_{Na} could be alleviated by compensatory increases in the density and/or kinetics of I_{Na} with cold acclimation. Indeed, the density of I_{Na} in rainbow trout cardiac myocytes increases with cold acclimation and, as well, activation of sodium channels occurs at more negative voltages. While increased current density and reduced threshold of I_{Na} activation will increase excitability of fish cardiac myocytes, the thermal compensation is only partial (density of I_{Na} at 4 °C is 59% of the density at 18 °C). There is also partial thermal compensation in the rate of I_{Na} inactivation.

I_{Na} is produced by the activity of voltage-gated sodium channels, which consist of a pore-forming α-subunit and one or two ancillary β-subunits. The family of vertebrate sodium channels has nine members ($Na_v1.1$–$Na_v1.9$) which differ with regard to electrophysiological properties and expression profiles. The compensatory changes of the rainbow trout cardiac I_{Na} are associated with a small change in the expression of sodium channel α-subunits (ventricular muscle expresses three sodium channel α-subunits: $Na_v1.4$, $Na_v1.5$, and $Na_v1.6$). Relative transcript (messenger RNA (mRNA)) levels of these channels in the warm-acclimated ventricle are 61.5%, 38.4%, and 0.1% for $Na_v1.4$, $Na_v1.5$, and $Na_v1.6$, respectively. With cold acclimation, the portion of $Na_v1.4$ increases to 81.8% at the expense of $Na_v1.5$ (18.2%).

In contrast to rainbow trout, crucian carp shows inverse thermal compensation: I_{Na} density is strongly depressed in the winter-acclimatized fish so that the density of I_{Na} at 4 °C is only 21% of that at 18 °C in summer-acclimatized fish. There is also no thermal compensation of I_{Na} kinetics. Thus, in crucian carp, thermal acclimation of I_{Na} decreases excitability of cardiac myocytes. It should be noted that this occurs simultaneously with increase in I_{K1}, which also decreases excitability. Thus, I_{Na} and I_{K1} act synergistically to reduce excitability of the crucian carp heart. This inverse thermal compensation of I_{Na} probably reflects the inactive lifestyle of the crucian carp in its habitat, which becomes severely hypoxic and even anoxic during winter.

Effects of Temperature on AP Duration

AP duration affects the frequency response of excitable cells. When an AP has swept along a nerve or muscle cell, a second AP cannot be initiated immediately after the previous AP. A finite time, known as the refractory period, must elapse before another AP can be elicited. This stems from the time and voltage dependence of ion channel gating. The return of activation and inactivation gates to their initial positions in the resting channel requires certain time at the RMP and only after which the channel can open again. The refractory period sets a limit to the frequency at which APs can be conducted along a nerve or muscle fiber.

In view of this, AP duration is important for all neuromuscular and cardiac function (**see also Design and Physiology of the Heart**: Action Potential of the Fish Heart), since information transmission is often dependent on the frequency of impulses, a factor that is limited by temporal width of an AP. Sensory information is frequency-modulated in that the strength of response is directly related to the frequency of APs elicited in the sensory nerve. The rate of locomotion is dependent on contraction frequency of skeletal muscle fibers. In addition, cardiac pumping is partially frequency-modulated because cardiac output is dependent on the heart rate (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping).

Acute decreases in temperature broaden APs. For example, the AP of the goldfish brainstem Mauthner cell is 31.3% broader at 5 °C and 26.9% narrower at 25 °C compared with fish acclimated at 15 °C. AP broadening in the Mauthner cell is due to prolongation of both depolarization and repolarization phases of the AP, suggesting that density/kinetics of both sodium and potassium currents are depressed in the cold. Unlike neuronal and skeletal APs, cardiac myocytes have a long AP duration (0.3–3 s) with a distinct plateau phase near the zero membrane potential. In six species of fish, temperature between 4 and 18 °C affected cardiac AP duration with a mean Q_{10} of 2.4 and 1.9, for the atrial and ventricular muscle, respectively.

Temperature compensation is evident in AP duration of both nerve and muscle cells of fish: acclimation to cold counteracts the acute effects of low temperature and prevents excessive lengthening of AP by the cold. Ion channels responsible for nervous and skeletal APs in fish are poorly understood, and at present molecular data on ion channels exist only for cardiac muscle. Duration of cardiac AP is regulated by a delicate balance between inward calcium current (I_{Ca}) and outward potassium currents (I_{Kr}, I_{Ks}, and I_{K1}). All these currents are sensitive to temperature and contribute to temperature-dependent changes in AP duration. In rainbow trout heart, density and kinetics of I_{Ca} do not change much under thermal acclimation. In contrast, the repolarizing potassium currents I_{Kr} and I_{K1} are strongly upregulated by cold acclimation in several fish species. Increased density of the I_{Kr} seems to be a ubiquitous response in teleost myocardium and this current is probably of prime importance in the regulation of AP duration. Increase in I_{Kr} is mainly due to cold-induced increase in the number of channels. I_{K1} is also upregulated in majority of species in the cold.

Significance of Membrane Lipids in Thermal Responses of Excitable Cells

Membrane lipids provide the physical environment where ion channel proteins go through complex conformational changes necessary for opening and closing of channels. Not surprisingly, ion channel function is dependent on chemical composition and physical properties of the lipid membrane. These requirements may involve interactions of the channel with special lipid components of the membrane, for example, cholesterol, or certain physical qualifications, such as fluidity of the lipid bilayer.

As discussed in articles **Temperature**: Proteins and Temperature and Mitochondria and Temperature, acute cooling decreases the fluidity (increases the viscosity) of the membrane which could impede conformational changes of the channel. On the other hand, temperature acclimation induces changes in the composition of lipid membrane that tend to maintain physical properties of the membrane independent of temperature changes. Although membrane lipids are known to modulate ion channel gating, there is currently no direct evidence indicating that membrane fluidity or homeoviscous adaptation affects ion channel function in excitable membranes of fish or any other ectotherm.

Lipid membranes are heterogeneous structures consisting of areas that differ in physical properties and molecular composition. Specific areas enriched in cholesterol and sphingolipids form microdomains called 'lipid rafts' that float in the more fluid lipid phase, like icebergs on the ocean. A number of vertebrate ion channels, including members of sodium, calcium, and potassium channels, reside in lipid rafts. Cholesterol of the lipid rafts exerts inhibitory action on several ion channels and therefore cholesterol removal (e.g., using β-methylcyclodextrin) from the membrane increases current densities in mammalian systems. For example, Kir2.1 channels are partially silenced, that is, stabilized in the closed state, by cholesterol.

In fish, temperature acclimation affects the cholesterol content of cell membranes, including the cells of the central nervous system. Cholesterol's inhibitory effect on Kir2.1 channels is largely dependent on leucine in the position 222 of the channel that mediates the interaction to cholesterol. Similar to mammals, the corresponding residue in the fish Kir2.1 is leucine, while Kir2.2 and Kir2.5 channels have isoleucine (which cannot mediate the interaction) in this position. In rainbow trout heart, Kir2.1 is abundantly expressed and, therefore, in this species and tissue cholesterol content of the membrane could affect I_{K1}. In contrast, in crucian carp heart, I_{K1} is produced mainly by Kir2.2 and Kir2.5 subunits and, therefore, the effect of cholesterol content on cardiac I_{K1} is expected to be minor.

Evolutionary Thermal Adaptation of Excitability

A fish's body temperature is variable and usually lower than in endotherms. An interesting question is: Are ion channels of the ectothermic fish somehow adapted to low temperatures in comparison to those of the endotherms, or is the ion channel performance simply scaled down with temperature in fish?

Comparison of Fish and Mammals

Most of the data regarding the effects of temperature on the functions of excitable cells in fish and mammals are available for cardiac muscle. The peak density of I_{Ca} in rat cardiac myocytes is about 16 $\mathrm{pA\,pF^{-1}}$ at 36 °C and

7 pA pF^{-1} at 23 °C. In rainbow trout cardiac myocytes, the peak I_{Ca} is 5.5 pA pF^{-1} and 2 pA pF^{-1} at 21 and 7 °C, respectively. Therefore, at the equivalent temperature, the density of I_{Ca} is similar in mammalian and fish hearts, that is, there seems to be no evolutionary temperature compensation in the density of I_{Ca}, a very important current for cardiac contraction (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element).

The I_{Ca} in rainbow trout atrial myocytes has a Q_{10} of 1.8–2.0 between 4 and 21 °C, while in mammals the Q_{10} varies from 2.3 to 5.8 at >22 °C. The lower Q_{10} for fish I_{Ca} may relate to their natural temperature variability. The rate of I_{Ca} inactivation shows no evolutionary temperature compensation: at 25 °C, the fast and slow time constants of I_{Ca} inactivation in rabbit ventricular myocytes are 12.6 and 51 ms, respectively, while the values are 27 and 73 ms in rainbow trout ventricular myocytes at 20 °C. Although less data are available for I_{Na} than I_{Ca}, similar conclusions as for I_{Ca} seem to apply to sodium channel function. There is little evolutionary thermal compensation in the function of inward currents, neither in current density nor in gating kinetics. As a consequence, the size of I_{Ca} and I_{Na} is scaled down in a temperature-dependent manner.

The situation for repolarizing potassium currents seems to differ from the inward currents. The peak density of the rapid component of the delayed rectifier, I_{Kr}, is generally substantially larger in fish cardiac myocytes than in mammalian cardiac myocytes. Among six teleost species, the ventricular I_{Kr} varies between 0.6 and 5 pA pF^{-1} at 11 °C, while in mammalian ventricle the I_{Kr} density is about 1 pA pF^{-1} or less at >22 °C. There seems to be also thermal compensation in activation, inactivation, and deactivation kinetics of the fish I_{Kr} because the rate of channel gating in fish at 11 °C is similar to that of the mammalian channels at room temperature (22–24 °C).

In addition, the density of the I_{K1} is larger in fish than in mammalian cardiac myocytes. In mammals, ventricular I_{K1} is generally between –4 and –12 pA pF^{-1} at temperatures above 22 °C, while the density of the I_{K1} varies between –7 and –25 pA pF^{-1} at 18 °C in fish ventricular myocytes. On the other hand, the density of the adenosine triphosphate (ATP)-sensitive potassium current (produced by Kir6 channels) is much higher in mammals than in fish.

Current data suggest that there is some evolutionary thermal adaptation in repolarizing potassium currents in fish. However, practically all data are from the cardiac muscle, a tissue in which large species-related differences exist in the composition of repolarizing currents. Therefore, the true adaptive value of those compensations remains an open question.

Comparison of Antarctic and Temperate Fishes

The nerves and muscles of Antarctic fish operate in extreme and stable cold. Therefore, appropriate thermal adaptations in excitability of nerve and muscle cells might be expected when compared with temperate or tropical fish.

Findings from neuromuscular systems suggest that there is relatively little evolutionary temperature compensation in muscle function, while the nervous system shows more extensive adaptation. Indeed, partial temperature compensation has been documented for conduction of peripheral sensory nerves and in the burst frequency of motoneuronal firing associated with the saccade eye movement. These findings suggest that the excitability, and therefore ion channel function, of central and peripheral neurons is thermally adapted. Compensation in conduction velocity and firing frequency suggests that adaptation involves changes in the function of depolarizing sodium current (conduction) and repolarizing potassium currents (AP duration). However, the molecular arrangements behind these adaptations are unknown, but could involve changes in channel isoforms analogous to those of thermal acclimation. Changes in the lipid membrane and/or altered interaction of channel proteins with membrane lipids could also be involved.

See also: **Design and Physiology of the Heart**: Action Potential of the Fish Heart; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping. **Temperature**: Effects of Temperature: An Introduction; Mitochondria and Temperature; Proteins and Temperature.

Further Reading

Bezanilla F (2008) How membrane proteins sense voltage. *Nature Reviews. Molecular Cell Biology* 9: 323–332.

Cossins AR and Prosser CL (1978) Evolutionary adaptation of membranes to temperature. *Proceedings of the National Academy of Sciences United States of America* 75: 2040–2043.

Harper AA, Watt PW, Hancock NA, and MacDonald AG (1990). Temperature acclimation effects on carp nerve: A comparison of nerve conduction, membrane fluidity and lipid composition. *Journal of Experimental Biology* 154: 305–320.

Hassinen M, Haverinen J, and Vornanen M (2008) Electrophysiological properties and expression of the delayed rectifier potassium (ERG) channels in the heart of thermally acclimated rainbow trout. *American Journal of Physiology* 295: R297–R308.

Hassinen M, Paajanen V, and Vornanen M (2008) A novel inwardly rectifying K^+ channel, Kir2.5, is upregulated under chronic cold stress in fish cardiac myocytes. *Journal of Experimental Biology* 211: 2162–2171.

Haverinen J and Vornanen M (2004) Temperature acclimation modifies Na^+ current in fish cardiac myocytes. *Journal of Experimental Biology* 207: 2823–2833.

Haverinen J and Vornanen M (2009) Responses of action potential and K^+ currents to temperature acclimation in fish hearts: Phylogeny or thermal preferences? *Physiological and Biochemical Zoology* 82: 468–482.

Käkelä R, Mattila M, Hermansson M, *et al.* (2008). Seasonal acclimatization of brain in a eurythermal fish is *Carassius carassius* mainly determined by temperature. *American Journal of Physiology* 294: 1716–1728.

MacDonald JA (1981) Temperature compensation in the peripheral nervous system: Antarctic vs. temperate poikilotherms. *Journal of Comparative Physiology A* 142: 411–418.

Montgomery JC and MacDonald JA (1984) Effects of temperature on nervous system: Implications for behavioral performance. *American Journal of Physiology* 259: R191–R196.

Prosser CL and Nelson DO (1981) The role nervous systems in temperature adaptation of poikilotherms. *Annual Review of Physiology* 43: 281–300.

Szabo TM, Brookings T, Preuss T, and Faber DS (2008) Effects of temperature acclimation on a central neural circuit and its behavioral output. *Journal of Neurophysiology* 100: 2997–3008.

Membranes and Temperature: Homeoviscous Adaptation

CD Moyes, Queen's University, Kingston, ON, Canada

JS Ballantyne, University of Guelph, Guelph, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Structure of Cellular Membranes
Environmental Effects on Fluidity
Conclusions
Further Reading

Glossary

Acyl group A fatty acid esterified to another moiety, glycerol in the case of phosphoglycerides.

Amphipathic A molecule with one hydrophilic and one hydrophobic end.

Ceramide Sphingosine esterified to a fatty acid.

Cholesterol A steroid that exerts complex effects on membrane fluidity.

Diacylglycerol A molecule with two fatty acids esterified to a glycerol backbone, typical in the first and second position.

Glycolipid Distinct from phosphoglycerides, glycolipids are diacylglyceride plus a carbohydrate.

Homeoviscous adaptation Modification of the lipid composition of cellular membranes that achieves stability in the static order (fluidity) of the bilayer. Counteracts temperature-dependent changes in membrane fluidity (viscosity) mainly by changes in saturation level of fatty acids.

Hydrophobic This term, which literally means water-hating, describes chemical tendency of molecules to interact through mutual avoidance of water.

Lysophospholipid A phospholipid with the organic component of its polar headgroup removed, or diacylglycerol plus phosphate.

Membrane order The degree to which membrane constituents are able to undergo free rotation and lateral movement.

Phosphoglyceride A molecule composed of a glycerol backbone with fatty acids esterified to positions 1 and 2, and a phosphate group linking glycerol to a polar headgroup.

Phospholipids Molecules composed of a polar head group and two nonpolar acyl chains.

Phosphotide Diacyl glycerol esterified to an inorganic phosphate.

Polar headgroup A hydrophilic molecule that is attached to a lipid backbone, to create an amphipathic lipid.

Sphingolipid A lipid consisting of a sphingosine backbone, esterified to a fatty acid and a polar headgroup.

Introduction

Biological membranes permit cells to organize their inner workings in useful ways. Cell membranes allow animal cells to create differences in internal and external ions, enabling them to generate membrane potentials (**see also Temperature**: Temperature and Excitable Membranes). Organelle membranes allow cells to isolate metabolic pathways and other cellular functions in discrete regions. Membranes play vital roles in structural organization, acting as anchor points for the cytoskeleton. Although membranes confer benefits to organization of cellular functions, they also represent barriers to free movement of ions and molecules.

Membrane proteins mediate electrical and chemical communication and catalyze transfers of molecules from one side to the other. The ability to carry out these vital functions depends upon the membrane proteins forming appropriate interactions with membrane lipids. The role of membrane lipids in both structure and function depends upon maintaining the appropriate degree of intermolecular movement. Membrane constituents must be able to move within the membrane. If a membrane is too fluid, then its ability to act as a barrier is compromised. If a membrane is too rigid, then it compromises the function of membrane proteins that require room to move.

Physicochemical challenges, such as temperature, can change the fluidity of membranes. Cells and organisms can remodel their membrane lipids to compensate for deleterious changes in fluidity, a response known as homeoviscous adaptation (HVA). In doing so, cell membrane fluidity at the new temperature is returned to a desired state. HVA has been shown in virtually all taxa as a response to temperature changes. The same patterns of membrane differences are also seen when comparing species of fish that have evolved in different thermal environments, from the Antarctic to the Amazon. The term is now applied to any adaptive response to conditions that perturb membrane fluidity, including pressure, pH, ionic strength, specific ions, and organic solutes.

Structure of Cellular Membranes

As outlined in the fluid mosaic model of membrane structure, phospholipids (PLs) are arranged laterally into a sheet, with polar headgroups displayed on one surface and hydrophobic tails aligned along the other surface, forming a monolayer. The membrane consists of two of these monolayers arranged tail to tail, with polar headgroups forming the inner and outer surface. Central to the fluid mosaic model is the ability of the molecular constituents to move. Proteins and lipids rotate within the membrane and each component moves laterally along the monolayer (PL, proteins) or bilayer (proteins).

The physical properties of the membrane are due to the cumulative influences of the membrane constituents and the way they are brought together to form the membrane. Each polar headgroup occupies a volume of space on the surface of the membrane. The hydrophobic core of the bilayer is essential to maintain a barrier to hydrophilic molecules, such as ions and most metabolites. Small hydrophobic molecules such as oxygen can pass between the polar head groups, enter the bilayer and exit on the other side of the membrane.

Lipid-Dependent Proteins

Integral proteins are firmly attached to or embedded in membranes, so much so that they cannot be released by the action of mild detergents. They may be embedded in one monolayer, penetrate across the bilayer, or be anchored covalently to another membrane constituent. Integral proteins include membrane transporters and plasma membrane receptors. In contrast, peripheral proteins are loosely associated with membrane components (PL, integral proteins, and carbohydrates) and can be released with a mild detergent. Examples of peripheral proteins include cytochrome *c*, the cytoskeletal protein spectrin, and many soluble protein kinases. The degree of interaction between membrane protein and lipid is important because integral proteins experience the effects of changes in membrane fluidity, whereas peripheral proteins may not.

Proteins are influenced in complex ways by the surrounding lipids. Some proteins have lipid moieties incorporated directly into their structures. For example, the mitochondrial enzyme cytochrome oxidase has an unusual requirement for a specialized lipid molecule, cardiolipin. When cardiolipin is removed from the enzyme, its catalytic properties suffer dramatically. Other membrane proteins, such as the transporter Na^+/K^+ adenosine triphosphatase (ATPase), have more complex membrane requirements. In the absence of lipid, activity is lost.

Many other proteins are influenced in nonspecific ways by fluidity. When membrane proteins undergo a catalytic cycle, they change the volume of space they occupy. In an environment where such volume changes are restricted, the catalytic properties of the protein suffer. Thus, maintenance of fluidity allows catalytic proteins (enzymes and transporters) to change shape within the lipid environment.

Lipid Components

The lipid component of membranes includes an astonishing array of molecules, including phosphoglycerides (PGs), sphingolipids (SLs), glycolipids, cholesterol, and membrane antioxidants. The complexity in structures makes it difficult to come up with a terminology that avoids ambiguity. With a focus on physical properties of membranes, we discuss the two main lipid elements of membranes: amphipathic and hydrophobic molecules.

Cellular membranes are often said to be composed primarily of PLs, which, in the strictest sense, refer to lipid molecules with phosphate attached (see **Figure 1**). This includes all PGs but excludes other amphipathic membrane lipids, such as glycolipids and many SLs. Collectively, these amphipathic lipids share an overall shape and specific chemical features. The hydrophobic component typically has a two-tailed structure that extends deep into the bilayer, whereas the hydrophilic component – the polar headgroup – is more globular and sits on the surface of the bilayer. In all of these molecules, a fatty acid contributes one (SL) or two (PG) of the tails. The fatty acids can vary in the length (typically 16–22 carbon atoms long) and the degree of saturation (0–6 double bonds). The polar headgroup can be one of many organic compounds (amino acids, amino sugars, polyols, monosaccharides, oligosaccharides, and modified sugars).

In an effort to make this article as accessible as possible, we continue with the convention of referring to the various amphipathic molecules as PL, acknowledging that this is not, in the strictest sense, accurate. However, it is worthwhile to consider the chemical distinctions between the various amphipathic membrane lipids:

1. PGs are composed of diacylglycerol + phosphate + polar headgroup. The most common PGs are phosphotidylcholine (PC), phosphotidylethanolamine (PE), phosphotidylserine (PS), and phosphotidylinositol (PI).

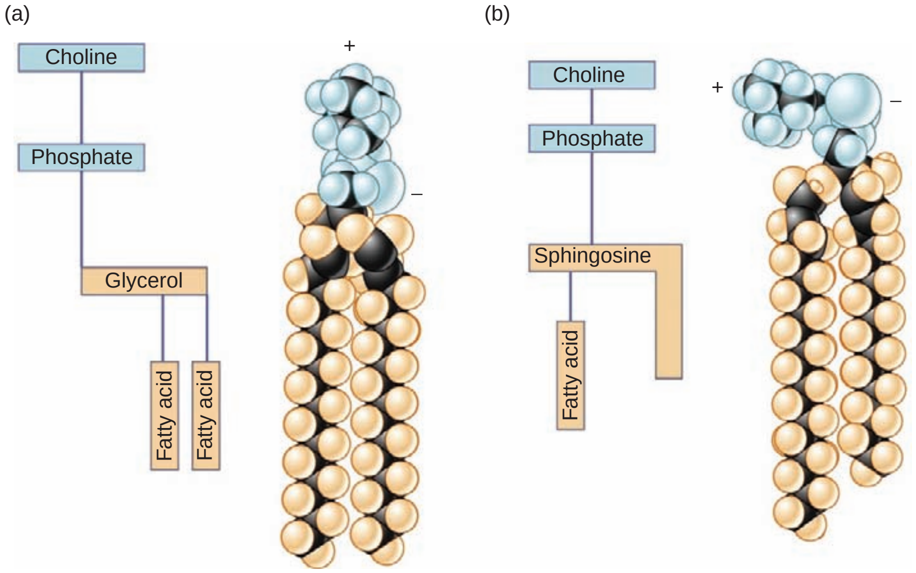


Figure 1 Structures of the amphipathic components of cellular membranes. (a) Phopshoglycerides; (b) sphingolipids. From Moyes CD and Schulte PM (2006) *Principles of Animal Physiology*, 2e, figure 2.36 (p.56).

2. SLs are composed of sphingosine + fatty acid (collectively ceramide) + a polar headgroup. Some are true PLs (e.g., ceramide + phosphocholine is sphingomyelin) but others lack phosphate. Sphingoglycolipids (ceramide + carbohydrate) include cerebrosides (ceramide + monosaccharide), sulfatides (ceramide + a sulfated sugar), globosides (ceramide + glycosaminoglycan), or gangliosides (ceramide + oligosaccharide decorated with sialic acid, a seven-carbon sugar).
3. Glycolipids are in the strictest sense carbohydrate plus lipid (without phosphate). They include diacylglycerol + a monosaccharide, as well as many of the SLs.

In addition to the amphipathic molecules, there is a collection of hydrophobic molecules within membranes, the most important of which is cholesterol (**Figure 2**). This steroid has a four-ring structure that creates a wedge-like shape. This allows it to insert within the hydrophobic tail zone, fitting best alongside of SL molecules. However, it can intersperse among other amphipathic membrane lipids as well. Cellular membranes also contain other hydrophobic compounds, including vitamin A (retinoic acid) and vitamin E (α-tocopherol). These vitamins are important antioxidants, conferring protection from oxidative damage, which influences membrane properties.

Regional Membrane Specializations

Cells are able to control which types of lipids are where within the plasma membrane. Many cells exhibit polarity in cell membranes, where the lipids in one region are distinct from those elsewhere in the cell. Perhaps the best example of this is distinction between the plasma membranes in the apical versus basolateral regions of transport epithelial cells (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes). For an epithelial cell, such as a gill epithelium, the tip of the cell exposed to the external world is called the apical membrane. The sides of epithelial cells interact with each other through tight junctions, which are belts of protein–protein connections that allow cells to adhere to each other to limit paracellular exchange and also prevent membrane constituents from moving from one end to the other. Below the tight junction zone, on the sides and the bottom of the cell, is the

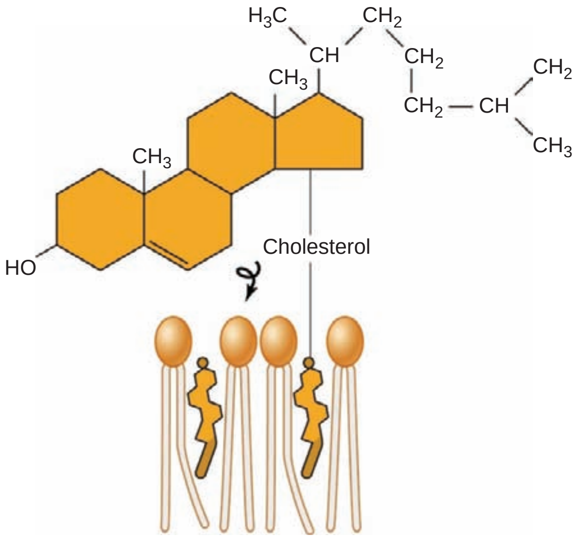


Figure 2 Structures of hydrophobic components of cellular membranes. From Moyes CD and Schulte PM (2006) *Principles of Animal Physiology*, 2e, figure 2.44 (p.65).

basolateral membrane. Many cells show distinct differences between the lipid composition of the apical and basolateral membranes.

Within any particular region of the membrane, lipids can coalesce into physically and chemically distinct islands. There are regions where SL and cholesterol accumulate, leading to a general thickening of the membrane, which in turn allows the accumulation proteins with longer transmembrane domains. These regions, called lipid rafts, are important sites for signal transduction. They have a distinct lipid profile and, thus, when changes in membrane composition occur, they can follow different trajectories in the main region of the membrane and the isolated lipid rafts. In some cases, regions of lipid rafts form invaginations into the cell, structures called caveolae. Like other lipid rafts, they play important roles in signal transduction, increasing the surface area of the membrane and providing deeper access into the cell interior.

There are also differences in the nature of the lipids in the inner and outer monolayers of the bilayer. Some amphipathic lipids are distributed roughly equally in the two layers. Within most plasma membranes, much of the PE and PS are in the inner monolayer, whereas much of the PC and sphingomyelin are in the outer leaflet. When cells initiate programmed cell death, the enzyme flippase takes internal PS and flips it into the external monolayer, sending a signal (extracellular access to PS) to the immune system to encourage removal of the apoptotic cell.

Mechanisms for Controlling Membrane Composition

All cells exert control over the composition of their membranes via diverse pathways, modifying them as needed. The main mechanism by which membrane lipid composition is altered is through the intracellular sorting pathways. Much of the synthesis and modification of membrane lipids occurs in the endoplasmic reticulum (ER) and Golgi apparatus. Endocytosis removes regions of membranes and carries the resulting vesicle into the cell for modification. Vesicle membranes fuse to the ER and Golgi, where the constituents can be modified and returned to the cell membrane by exocytosis. This cycle of intracellular traffic allows cells to remodel the plasma membrane, as well as membranes of the lysosome, ER, and Golgi.

Cells also have a capacity to remodel some membrane lipids *in situ*. One mechanism is deacylation and reacylation. Phospholipase A_2 removes a fatty acid from a membrane PL, then lysophospholipid acyltransferase attaches a replacement fatty acid that is more suitable. Cells possess ATP-dependent enzymes that transfer PL between monolayers. Flippases bring PL inward, from outer to inner monolayers, and floppases send PL from the inner to the outer monolayer. These enzymes are central to establishing the membrane asymmetries that are critical for cellular function. As noted above, translocation of PS from the inner to the outer monolayer is an early step in programmed cell death. However, it is also likely that flippases can be used in membrane remodeling in response to changing environmental conditions.

Membrane Fluidity

PLs play important roles in membranes because of their overall shape, their amphipathic nature, and the ability to form weak bonds laterally within the monolayer. The physical nature of the membrane is governed by the magnitude of the weak bonds between membrane constituents. Throughout most of the membrane, the PLs are not so well connected as to prevent rotation and lateral movements. This condition has been called a fluid, liquid, or disordered state. At any point in time, there will be small regions within the membrane that are much more rigid, where rotation and lateral movements are very restricted. These regions are considered viscous (less fluid), gel like (less liquid), or ordered.

It is now clear that there is a third state within membranes: the liquid-ordered state. Lipid rafts are small, isolated patches of the cell membrane where fluidity remains but much more order exists. The molecule that is central to the maintenance of this liquid-ordered state is cholesterol, which we discuss in the next section. Cells build membranes to ensure that much of the membrane remains in the dynamic liquid state, and, as discussed later, when environmental conditions impose a solid-ordered state, cells compensate to regain fluidity.

The Unusual Role of Cholesterol

Cholesterol plays a complex role within membranes and many questions remain about its function. It is a critical component in the lateral organization of monolayer. Working in conjunction with SL, cholesterol acts as a focal point for the formation of lipid rafts, helping to maintain the liquid-ordered phase. Apart from cholesterol, lipid rafts are dominated by SL and PG that have long, saturated fatty acids. Regions rich in these lipids would tend to become more solid and ordered. Cholesterol is able to disrupt these interactions, ensuring that the lipids in these islands remain sufficiently fluid. Thus, cholesterol ensures that fluidity is maintained in membrane regions that would otherwise become ordered.

Cholesterol is also important in limiting the permeability of membranes. The membrane core is hydrophobic, so membranes generally have a low permeability to small hydrophilic solutes. Even in the simplest of membranes, the lipid tails are not sufficiently linear to eliminate spaces between PL molecules. Low-molecular-weight solutes can penetrate the membrane, jumping from gap to gap within the membrane core. Cholesterol reduces this minor permeability essentially by filling in the gaps between PL molecules, driving surrounding fatty acids closer together. Thus, cholesterol induces order in fluid regions, thereby reducing permeability. The complex effects of cholesterol on membrane fluidity mean that changes in cholesterol content and distribution are central to HVA.

Environmental Effects on Fluidity

Although HVA is known to occur in response to many environmental factors, temperature is the best studied. Whether changing rapidly, as fish swim through a thermocline, or daily, or seasonally, changes in temperature exert effects on membranes. Under normal conditions, membrane lipids ensure that the membrane maintains an appropriate fluidity, a balance between order and disorder. When membranes become too ordered, restricting lateral movement of membrane constituents, they also experience a detrimental thickening. When membranes become too disordered, the membrane lipids move too much and the membrane loses its integrity as a barrier. In many situations, changes in temperature result in relatively minor effects on fluidity until a critical temperature is reached, triggering a dramatic structural transition that is catastrophic for membrane function. This change in membrane structure at the transition temperature can be abrupt or gradual depending on its lipid composition. Cholesterol plays a major role in determining how abrupt the transition is. Cellular responses, including adaptational changes, to the effects of temperature change need to include responses that modify the transition temperature (e.g., lower it in cold environments), maintain appropriate fluidity, and maintain membrane thickness.

The time course of responses varies with the most rapid responses involving thermal modulation of existing desaturases and demethylases to alter saturation and headgroup (PE vs. PC) content. Processes requiring induction of protein synthesis take longer (e.g., Δ5 and Δ9 desaturases). Responses such as would be required for diurnal adaptation in intertidal areas or desert ponds may be limited to the more rapid modifications. The time course is also influenced by the direction of temperature change with changes from cold to warm temperatures occurring more rapidly than those from warm to cold.

Though HVA is a very common response to temperature in fish, and, in fact in all organisms, there are many examples where HVA is not seen. Some fish species do not remain active at cold temperatures (e.g., overwintering) and may not undergo HVA to the same extent as fish that remain active. Although the membrane lipids have been examined in some dormant overwintering crustacean species, it has not yet been examined in fish. Membrane fluid properties may be altered as part of metabolic depression to reduce membrane leakiness and downregulate membrane ion pumps. This could be viewed as a reverse HVA. This has been observed in estivating African lungfish (*Protopterus dolloi*).

Although temperature is the best studied, other physiochemical factors can alter fluidity, including hydrostatic pressure and diverse chemical effectors such as solute profile, ionic strength, pH, and toxicants. While these factors can certainly influence fluidity experimentally, whether they do so under natural conditions and whether they trigger HVA are not always clear. Some may interact with temperature effects.

Adaptive Remodeling of Membranes in HVA

One of the outcomes of HVA is to help maintain function of membrane-bound proteins such as enzymes and transporters. A plot of the log of enzyme activity versus inverse temperature in kelvin (Arrhenius plot) demonstrates how changing temperature affects a membrane-bound enzyme (**Figure 3**; **see also Temperature**: Effects of Temperature: An Introduction). There is a characteristic temperature at which the change in activity abruptly changes. Below this temperature, the activity rapidly decreases. Modification of membrane lipids (HVA) changes the temperature at which the enzyme activity abruptly declines with the result that the activity at the new colder acclimation temperature is restored to values similar to that at the warmer acclimation temperature.

The fluidity of a membrane depends on many qualitative and quantitative features of the lipid components, and, not surprisingly, adaptive changes in fluidity can target different molecules. Each of the following strategies has been shown to occur in some example of HVA, although not every strategy occurs in each cellular membrane. Mitochondria, for example, lack cholesterol and thus it

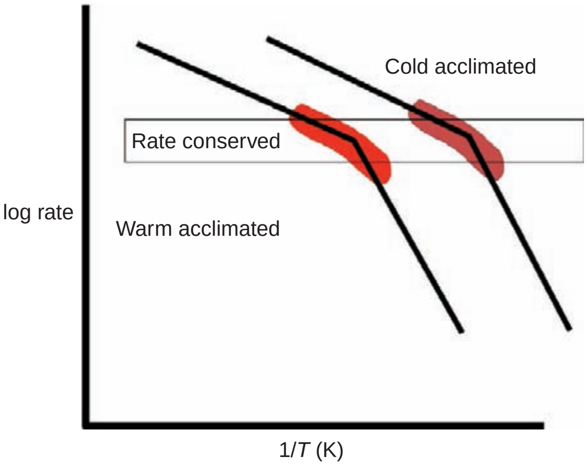


Figure 3 Effects of temperature acclimation on membrane-bound enzyme activity.

Table 1 Mechanisms of homeoviscous adaptation of membranes due to cold acclimation

Lipid component	*Change observed*	*Type of membrane or tissue involved*
Head group	PE more PC less PE:PC higher	Plasma membranes, mitochondrial membrane
	Sphingomyelin higher	Plasma membranes
	PI increase	Mitochondria (inner membrane)
	PS decrease	Mitochondria (inner membrane)
	Cardiolipin no change or decrease	Mitochondria
Phospholipid acyl chain saturation	Less saturated fatty acids More Monoenoic fatty acids More Polyunsaturated fatty acids	Plasma membranes, mitochondria
Phospholipid acyl chain double bond position	Increased n-3, n-6, n-9	Plasma membrane
Phospholipid acyl chain position	Position 1 – more unsaturated fatty acids	Plasma membrane
Phospholipid acyl chain length	Increased when unsaturation increases	Plasma membrane
Gangliosides	Increased sialic acid content	Brain membranes
Phospholipid acyl linkages	Reduced PE plasmalogen content	Brain
Cholesterol	Reduced	Plasma membranes, smooth and rough endoplasmic reticulum
Raft domains	Cholesterol content decreased Phospholipid content increased	Plasma membranes

does not contribute to HVA in this organelle. **Table 1** summarizes the known responses during cold adaptation.

Headgroup modification

The conical shape of PE tends to disorder membranes and thus can be used to offset the ordering effect of low temperature. PC, on the other hand, assumes a more cylindrical shape in the membrane conferring a more ordered state. Thus, the PE/PC ratio increases in situations where there is a need to fluidize the membrane. This can be accomplished *in situ* by converting the PE into PC by adding three methyl groups via N-methylation. This is one of the most rapid responses of HVA and is involved in diurnal changes in membranes such as those seen in desert pupfish (**see also Bony Fishes**: Life in Hot Water: The Desert Pupfish).

Deacylation–reacylation

Deacylation–reacylation in cold acclimation replaces a less unsaturated acyl chain (e.g., 18:1) with an unsaturated fatty acid (e.g., 22:6n-3). The reassigning of acyl chain positions within a PL may be an important role for this process, for example, changing a membrane with PCs 18:0 18:0 and 18:1 18:1 to PCs 18:0 18:1 18:0 18:1 may alter membrane properties with no net change in membrane composition. This mechanism may be important in rapid responses and during cold acclimation since existing acyl chains can be used.

Fatty acid saturation

The acyl chains attached to various PL headgroup have an important effect on membrane fluidity and are modified to maintain membrane fluidity. These can be modified in several ways. Changing the fatty acids available for the normal turnover of membrane acyl chains can be accomplished by modifying the relative rates of production of saturated and unsaturated fatty acids. The higher Q_{10} of fatty acid synthetase than that of desaturases results in a greater proportional production of saturated fatty acids at higher temperatures simply due to the effects of temperature on enzyme activities.

Desaturation and elongation

Animals are able to add a double bond in the $\Delta 9$ position and thus can synthesize 16:1 and 18:1. They cannot add double bonds beyond the $\Delta 9$ position and thus n-6 and n-3 series fatty acids are essential in animals. The n-6 fatty acids such as linoleic acid (18:2n-6) can be further desaturated and elongated by animals to produce 20:3n-6 and 20:4n-6. Similarly, n-3 fatty acids such as linolenic acid (18:3n-3) can be elongated and desaturated to produce 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid). Adding double bonds has the effect of introducing kinks into the acyl chain, which effectively shortens the chain length. Elongation by adding two carbons is an important part of HVA since it helps maintain membrane thickness. Thus, although lengthening acyl chains would have the effect of increasing the transition temperature, it is necessary to maintain membrane thickness and this is offset by the decrease in transition temperature due to the addition of double bonds.

Sialization

As important components of nervous tissue, ganglioside properties must be regulated to maintain nerve conduction. Gangliosides can be modified by sialization. At low temperatures, the increased sialization that is observed increases the polarity of the molecules and helps fluidize membranes. Antarctic fish have the highest levels of sialization of gangliosides of any vertebrate and represents a clear adaptation to frigid water temperatures.

Plasmalogen content

Ether-linked lipids occupy a smaller profile in the membrane and hence confer order and reduce permeability. Plasmalogen content especially in PE tends to be higher at high temperatures.

Cholesterol content

Cholesterol disrupts the packing of gel-phase lipids and has an ordering effect on fluid-phase lipids. The membrane content of cholesterol, as expressed by the cholesterol:PL ratio, can be altered to change the fluid properties. In general, the effects on fluid-phase lipids seem to be more important in HVA. Raft domain cholesterol content declines with cold acclimation. Cholesterol also has the effect of broadening the transition temperature as its proportion in the membrane increases. The mechanisms whereby cholesterol dynamics are mediated have not been established for HVA.

Sensing and Responding to Changes in Fluidity

Anticipation of seasonal changes can be cued by photoperiod for whole organism anticipatory responses, while more rapid temperature changes may be signaled by temperature change itself with individual cells being responsible for their own adaptation. A variety of hormones have been implicated in temperature adaptation of fishes, including prolactin, glucagon, insulin, thyroxine, and cortisol, but the extent to which these mediate HVA is not clear. There are temperature-sensitive cells in the preoptic region of the central nervous system of fish that determine preferred body temperature. Because of the thermosensitivity of electrical sensors such as the ampullae of Lorenzini of elasmobranchs and similar ampullae of teleost fish, they may function as thermoreceptors in fish. As membranes undergo conformational changes in response to temperature, they can be used as sensors to initiate cellular changes needed in response to temperature stress. Since isolated cells from fish are capable of adapting to temperature change, much of the adaptive response may not require elaborate central sensing or processing. Several aspects of the biochemical machinery responsible for membrane remodeling are sensitive to temperature. The higher Q_{10} (**see also Temperature**: Effects of Temperature: An Introduction) of fatty acid synthetase than that of desaturases results in a greater proportional production of saturated fatty acids at higher temperatures simply due to the effects of temperature on enzyme activities. Membrane-bound desaturases are sensitive to temperature in such a way that they are activated at low temperatures as membranes stiffen, and the resultant increase in unsaturated fatty acids when incorporated into membrane lipids fluidizes the membrane, thereby inactivating the desaturase in a feedback regulatory phenomenon. The Q_{10} for cholesterol synthesis is high ($\sim$3), enhancing the availability of cholesterol for membranes at higher temperatures. Similarly, Q_{10} effects result in preferential synthesis of PC compared to PE at higher temperatures.

Conclusions

Biological membranes are complex and dynamic, making it challenging to study their structure and function. The HVA response is a common phenomenon, exhibited by fish, as well as other animals, and other eukaryotes. Despite the rich history in this field, and the ubiquity of the response in eukaryotes, many questions remain about how the changes in membrane constituents seen in HVA alter the biophysics of membranes. The mechanisms by which cells sense and respond to changes in membrane fluidity also remain an active area of research.

See also: **Bony Fishes**: Life in Hot Water: The Desert Pupfish. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes. **Temperature**: Effects of Temperature: An Introduction; Temperature and Excitable Membranes.

Further Reading

Gracey AY, Fraser EJ, Li W, *et al.* (2004) Coping with cold: An integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences of the United States of America* 101: 16970–16975.

Hazel JR (1984) Effects of temperature on the structure and metabolism of cell membranes in fish. *American Journal of Physiology* 246: R460–R470.

Hazel JR (1995) Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology* 57: 19–42.

Mouritsen OG (2010) The liquid-ordered state comes of age. *Biochimica et Biophysica Acta* 1798: 1286–1288.

Mouritsen OG and Zuckermann MJ (2004) What's so special about cholesterol? *Lipids* 39: 1101–1113.

Quinn PJ (1981) The fluidity of cell membranes and its regulation. *Progress in Biophysics and Molecular Biology* 38: 1–104.

Sinensky M (1974) Homeoviscous adaptation – a homeostatic process that regulates viscosity of membrane lipids in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* 71: 522–525.

Singer SJ and Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. *Science* 175: 720–731.

Heat Shock Proteins and Temperature

S Currie, Mount Allison University, Sackville, NB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Heat Shock Proteins
HSPs and Fish
HSPs in the Cold
Plasticity of the Heat Shock Response
Thermal/Cross Tolerance
Triggers of the Heat Shock Response
Molecular Regulation of the Heat Shock Response
Physiological Regulation of the Heat Shock Response
Conclusion
Further Reading

Glossary

Critical thermal maximum (CTM) The maximum temperature that can be tolerated by an organism. In fish, this variable is most often measured by acutely increasing the temperature (e.g., by 2 °C h^{-1}) until the fish loses equilibrium.

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Endotherm (adj. endothermic) An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Eurytherm An ectotherm able to survive over a broad range of environmental temperature.

Heat shock protein A protein that interacts with other proteins, promoting productive folding pathways to ultimately stabilize cellular proteins and membranes.

Heat shock response The induction of heat shock proteins in response to a thermal stress.

Molecular chaperones Proteins that interact or chaperone other proteins to guide folding into the correct three-dimensional structure; heat shock proteins are molecular chaperones.

***Pejus* temperature** Latin for turning worse; it represents the upper thermal limit of regular animal function.

Protein denaturation A change in the three-dimensional structure of a protein that renders the protein nonfunctional.

Stenotherm An ectotherm able to survive only within a narrow range of environmental temperatures.

Introduction

The capacity of fish to cope with temperature change has fascinated physiologists for decades. Issues of climate change and the warming trends observed in our oceans and freshwater systems have certainly accelerated interest in this area. Because the body temperatures of most fish species are within 0.1–1.0 °C of water temperatures, understanding the biological effects of temperature change is particularly compelling. Fish, like all organisms, live within a limited range of body temperatures where growth, reproduction, foraging, and behavior are optimum. During cooling and warming, performance may be compromised and at temperatures outside thermal limits, mortality is likely (**see also Temperature**: Measures of Thermal Tolerance.

At or beyond these thermal limits or critical temperatures, a fish's aerobic capacity is diminished, proteins begin to aggregate or misfold, and cellular membranes are impaired. In order to cope with temperatures near the thermal limit, fish rapidly turn on the inducible heat shock proteins (HSPs; the number (e.g., HSP70 and HSP90) indicates the apparent molecular weight, in kilodaltons, of the HSP family) in their cells to protect proteins and membranes from temperature damage. This phenotypic response is generally considered adaptive, where the induction of HSPs may extend the life of the cellular constituents and thus the organism. It is important to note that the induction of HSPs is but one of several molecular responses used by fish to cope with temperature stress (e.g., changes in enzymes, cell signaling intermediates, and membrane dynamics).

Heat Shock Proteins

The HSPs are one of the most highly conserved groups of proteins in nature, found in nearly every species examined to date – from bacteria to humans. These proteins are molecular chaperones in that they interact with other proteins, promoting productive folding pathways and ultimately stabilizing cellular proteins and membranes. HSPs are not only important during cellular stress; they are present in cells constitutively, such that under normal growth conditions they help to guide protein folding and movement throughout the life of the cell, performing important housekeeping functions associated with protein synthesis and maturation. When the cell is subjected to stress, HSPs facilitate recovery of protein structure by helping to prevent protein aggregation, encouraging appropriate refolding and/or targeting proteins damaged beyond repair for destruction. Temperature stress is certainly the most well-known and consistent inducer of the HSPs but because their induction is not limited to heat stress (see below), these proteins are also known as stress proteins. It is now well established that HSPs are critical for proper cell functioning in all organisms including fish – both under normal conditions and following stress.

The heat shock response was discovered by Ritossa in 1962 when he observed puffing of the polytene chromosomes in fruit flies that had been accidentally exposed to heat shock after one of his lab mates accidentally turned up the temperature on his incubator! This puffing Ritossa observed represented rapid (i.e., in minutes) synthesis of new genes. These same genes, which we now know were hsp genes, were also synthesized with uncouplers of ATPases (ATP, adenosine phosphate) and with recovery from anoxia. It took several years before the impact of this important and serendipitous finding was felt within the scientific community.

HSPs are traditionally grouped into families according to their molecular weight, each with several members: high-molecular-weight HSPs (~100–110 kDa), HSP90 (85–90 kDa), HSP70 (68–73 kDa), and low-molecular-weight HSPs (16–47 kDa). All family members are expressed in fish cells (**Table 1**), although the most well-known family is the HSP70 family, with both constitutive (heat shock cognate (HSC)70; the constitutive isoform of the HSP) and inducible (HSP70) forms. Fish possess high levels of HSC70 within their cells and have at least two isoforms of the inducible HSP70. There is a high level of genetic similarity or conservation between HSP70 and HSC70 in fish.

Because body temperatures of ectotherms fluctuate with the environment, their cells and proteins have a greater likelihood of experiencing thermal injury. Thus, fish may have an enhanced need for HSPs compared to organisms with a more stable body temperature such as mammals. So, despite their ubiquity, one could argue that HSPs are more important in the survival of ectotherms, like fish, than in endotherms.

HSPs and Fish

With one exception (see below), the heat shock response is present in all freshwater and marine fish species in which it has been studied (**Table 1**). Much of the research on the heat shock response with high temperature in fish has been performed in teleosts, although there is now evidence that this response is also present in elasmobranchs and agnathans. Two members of the

Table 1 HSPs and examples of fish (whole animal, tissues, and/or cells) exhibiting induction with thermal stress

HSP (gene and/or protein)	*Example fish species*
HSP70 (inducible)	*Danio rerio, Fundulus heteroclitus, Gadus morhua, Gillichthys mirabilis, Oligocottus maculosus, Oligocottus snyderi, Oncorhynchus mykiss, Oreochromis mossambicus, Oryzias laptipes, Petromyzon marinus, Pimephales promelas, Pleuronectes americanus, Poeciliopsis* sp.*, Salmo salar, Salvelinus fontinalis, Sparus sarba, Xiphophorus maculates, Umbra limi*
HSC70 (constitutive)	*Austrofundulus limnaeus, Danio rerio, Fundulus heteroclitus, Gillichrhys mirabilis, Poeciliopsis* sp.*, Oligocottus maculosus, Oncorhynchus mykiss, Sparus sarba*
HSP70/HSC70 (not distinguished)	*Acipenser medirostris, Ambloplites rupestris, Cyprinus carpio, Ictalurus natalis, Oligocottus maculosus, Pimephales promelas, Salmo trutta, Squalus acanthias*
HSP90	*Acipenser medirostris, Austrofundulus limnaeus,Danio rereio, Fundulus heteroclitus, Gillichthys mirabilis, Oligocottus maculosus, Oncorhynchus mykiss, Onchorhymcus tshawytscha, Petromyzon marinus, Pimephales promelas, Pleuronectes americanus, Poeciliopsis* sp.*, Salmo salar, Umbra limi*
HSP60	*Pimephales promelas, Poeciliopsis lucida*
HSP30	*Austrofundulus limnaeus, Gillichthys mirabilis, Oncorhyncus mykiss, Poeciliopsis* sp.
HSP27-28	*Pleuronectes americanus, Poeciliopsis lucida*
HSP22	*Austrofundulus limnaeus*
HSP40	*Gillichthys mirabilis*
HSP47	*Danio rerio*
HSP100 (100-108 kDa)	*Gillichthys mirabilis, Pimephales promelas, Poeciliopsis lucida, Salmo salar*
HSP10	*Cyprinus carpio*

cold-adapted, Antarctic Nototheniidae family of icefishes (*Trematomus bernacchii* and *Harpagifer antarcticus*) are examples of only a very few organisms that appear to lack the heat shock response. These stenothermal fish never encounter temperatures above 0 °C and die of heat death above 4 °C. These notothenioids have the ability to synthesize HSPs, but do not induce HSPs upon temperature stress. Their strategy is to maintain high basal levels of the constitutive and inducible HSP70 to aid with increased protein damage and problematic protein folding at low temperatures. This lack of a heat shock response may mean that their range of thermal tolerance is attenuated. In light of current and predicted increases in seawater temperature, these cold-water fish could be in serious jeopardy from thermal stress.

HSPs in the Cold

Many of the physiological effects of heat stress, such as increased protein denaturation, are also apparent in exposure to the cold. There is some evidence in bacteria, plants, insects, and mammalian cells, indicating that HSPs are induced with cold stress. In fish, although there is limited information on the induction of HSP genes or proteins with cold, members of the small HSP family (e.g., HSP10) are turned on with cold stress in fish as are HSP70 and HSP90. Notably, HSP90 and HSP70 may have an important role in stabilizing other transcription factors that are turned on in the cold, such as the hypoxia inducible factor (HIF)-1α).

Plasticity of the Heat Shock Response

As noted above, HSPs are turned on with stressors other than temperature. In some fish, HSPs are induced with low oxygen or hypoxic stress exposure to contaminants such as heavy metals and during disease. However, in many cases these stressors result in species- and HSP-specific responses. To illustrate, hypoxia-sensitive fish such as rainbow trout (*Oncorhynchus mykiss*) exhibit a robust induction of HSP70 with chronic and acute heat stress, but hypoxia does not induce HSP70, although there is some indication that lower-molecular-weight HSPs are turned on. On the other hand, hypoxia-tolerant fish (e.g., sculpins, *Oligocottus maculosis* and mudminnow, *Umbra limi*) appear to induce HSP70 with exposure to low oxygen. The heat shock response to high temperature in fish also exhibits considerable tissue specificity for reasons that are not completely clear, but may be related to differences in the degree of protein damage incurred by each tissue. The suite of specific HSPs induced during a temperature stress is also highly variable. In thinking about temperature stress in fish specifically, the heat shock response is further affected by thermal history, the intensity and magnitude of the temperature stress, the geographic population, and the developmental stage of the fish.

Thermal History

Although the fish heat shock response occurs at temperatures within the physiological limits for the species, the stress needed to induce HSPs is strongly related to the thermal niche of the animal. In general, cold-water species will induce HSPs at a lower temperature than warm-water species. However, both the minimal temperature at which HSPs are induced (the threshold induction-temperature) and the temperature of maximal synthesis are quite variable and this variation is due to several factors.

First, the recent thermal history of the fish is an important consideration in the nature of the heat shock response. Depending on the species' thermal niche, fish will often experience fluctuations in temperature on a daily and seasonal basis and this variation will influence the induction and magnitude of the heat shock response. Second, there is evidence in both eurythermal and stenothermal fish that exposures to chronically high temperatures lead to higher levels of HSPs, possibly because these conditions are more stressful and result in more protein damage. On the other hand, daily, cyclical fluctuations in environmental temperatures (i.e., >10 °C) such that would be experienced by eurythermal fish such as killifish (*Fundulus heteroclitus*) or sculpins are not necessarily coordinated with increases in HSPs. The rapid induction of HSPs observed during daily temperature fluctuations in intertidal invertebrates such as snails does not necessarily occur in fish and this may reflect different fish life-history strategies and species variations in cellular stress tolerance for fish.

Intensity of Thermal Stress

Keeping the thermal history of the fish in mind, another factor influencing the nature of the heat shock response is the intensity of the heat stress. In fish, the earliest temperature where aerobic function begins to decline is known as the *pejus* (Latin for turning worse) temperature, the upper thermal limit for regular animal function. Interestingly, HSPs are not induced at these temperatures in fish, even though physiological function is compromised. HSPs appear to be induced when fish go beyond this *pejus* temperature to a critical thermal temperature, likely when circulatory and ventilatory activity begins to fail, protein structure is threatened, and protein damage has accrued. For example, the induction of HSP70 in rainbow trout acclimated to 13 °C occurs precisely at 24 °C, a temperature where salmonid fish typically have little aerobic scope for physiological activity. Although it is tempting to approximate an overall temperature change at which

HSPs will be induced in fish (e.g., 10–12 °C above acclimation temperature), considering the influences of thermal history, such an estimate would be imprudent.

The intensity of the heat stress is more than just the temperature of induction; it is also determined by the amount of time the fish or cell is experiencing the stress as well as the rate of temperature change. These factors exacerbate the extent of protein damage and result in a more robust heat shock response (i.e., higher levels of HSPs), but it is unclear if a higher heat shock response is adaptive or deleterious. Indeed, overexpression of HSPs is thought to have negative consequences for growth and development. On the other hand, lack of HSP induction may have deleterious effects on thermal/stress tolerance (see below). Thus, in evaluating the nature of the heat shock response, one could speculate that the HSP induction temperature is a more appropriate indicator of an adaptive response than the actual magnitude of the response. Regardless, this point underscores the importance of understanding the overall fitness implications of the induction of HSPs with temperature stress.

Temporal Variation

There is a temporal component to the heat shock response that is also dependent upon the intensity of the heat stress. In fish, the heat shock response reaches its highest levels only after the stress has been removed and the animal or cell is returned to optimal physiological temperatures. It has been suggested that because the synthesis and function of HSPs demand considerable energy, the recovery process offers a higher potential for the aerobic generation of ATP. The heat shock response in fish is transcriptionally regulated. The kinetics of gene regulation and transcription are such that maximal HSP gene expression usually occurs within an hour after the stress with protein expression peaking from 8 to 24 h during recovery from the stress. Again, it is difficult to generalize the time course of the heat shock response to all fish and fish tissues as it is species and tissue specific, as well as dependent on the particular HSP being studied.

Geographical Population Variation

The magnitude of the heat shock response and its threshold induction temperature also varies with different populations of the same fish species. This has been effectively illustrated by Schulte and colleagues in the estuarine killifish (*Fundulus heteroclitus*) who have shown that although the coding sequences of HSP70 genes do not differ between northern and southern populations, there are distinct population differences in HSP induction temperatures and in the magnitude of response. These differences may be indicative of one population experiencing more protein-damaging stress than the other and/or one population being better equipped to cope with temperature stress.

Development and Aging

Fish, like most other organisms, exhibit characteristic patterns of HSP expression during embryonic development, and enhanced heat resistance is correlated with the induction of several HSPs. As a fish ages, the magnitude of the heat-induced expression of HSPs declines and the subcellular localization of specific HSPs changes. Furthermore, younger cells (e.g., nucleated red blood cells) have a greater capacity to mount a heat shock response compared to older cells. This more robust heat shock response in younger fish/cells may partly explain the general phenomenon of why juvenile fish are more tolerant to high temperature than their older counterparts.

Thermal/Cross Tolerance

There is considerable evidence correlating HSP induction patterns and resistance to damaging effects of temperature stress (i.e., thermotolerance) in several species of fish. This suggests that the heat shock response, at least in part, allows fish to survive temperatures that would otherwise be lethal. However, without mechanistic information linking the expression of specific HSPs to the thermotolerant state, we still do not understand how HSPs may confer temperature tolerance in fish. Experiments on genetically tractable fish such as zebrafish (*Danio rerio*) will soon fill this knowledge gap. Regardless, the heat shock response in fish is important for thermal flexibility and has a protective role in a fish's natural tolerance to environmental change.

HSPs are also thought to have a role in cross-protection or cross-tolerance, whereby the exposure to one stressor affords protection against another distinct stressor. This phenomenon has been observed several times in fish and fish cells. For example, a prior heat shock, sufficient to induce HSP70, is associated with increased tolerance to a subsequent osmotic shock. As was the case with thermotolerance, there is strong correlative evidence linking HSP induction to the mechanism of cross-tolerance in fish, but we have yet to directly establish HSPs as mediators of this phenomenon.

Triggers of the Heat Shock Response

Although the signal triggering the heat shock response has not been thoroughly elucidated, there is considerable evidence, including in fish, that the presence of damaged or denatured proteins is the proximate trigger for the induction of HSPs. Wherever protein synthesis occurs, there is the danger of improper folding or aggregation. During stresses such as high temperature, the probability of protein misfolding, damage, and/or aggregation increases and if this damage becomes too high, cell death is likely. HSPs prevent inappropriate protein interactions and effectively reduce the degree of protein damage in the cell (**Figure 1**). In fish, the heat shock response is triggered at temperatures that lie within the upper reaches of their normal body temperatures, providing indirect evidence that thermal damage to proteins occurs under natural environmental conditions.

If, however, proteins are denatured or damaged beyond repair, they must be removed from the cell. Molecular chaperones are an important part of this process. A small chaperone called ubiquitin covalently and irreversibly binds to damaged proteins so that they will be directed to the proteasome for destruction. This protein is ubiquitous among organisms and we know that fish cells make use of this ubiquitin–proteolytic system to ensure protein quality control so that malfunctioning proteins do not accumulate in the cell.

Molecular Regulation of the Heat Shock Response

Given the variation that exists in the heat shock response, one model will not explain the regulation of all families of HSPs in all species. However, a few generalizations on the mechanisms of HSP regulation can be made. The genes encoding HSPs can be grouped into three regulatory classes: (1) constitutively expressed during growth and development, (2) constitutive and inducible, and (3) strictly inducible. The primary form of regulation of HSPs in fish and other organisms is through transcription, but there is also a degree of posttranscriptional regulation (e.g., messenger RNA (mRNA) stability, translational regulation, and subcellular localization) in the stress-induced increase of HSPs. Most HSP genes do not contain introns, so the mRNA is rapidly translated into new proteins within minutes to hours of exposure to a stressor.

In all organisms, the heat shock response is mediated by a family of heat shock transcription factors (HSFs). There are at least four known HSF genes (HSF1, HSF2, HSF3, and HSF4) where HSF1 is the master regulator of the heat shock response in eukaryotes. To date, HSF1 and

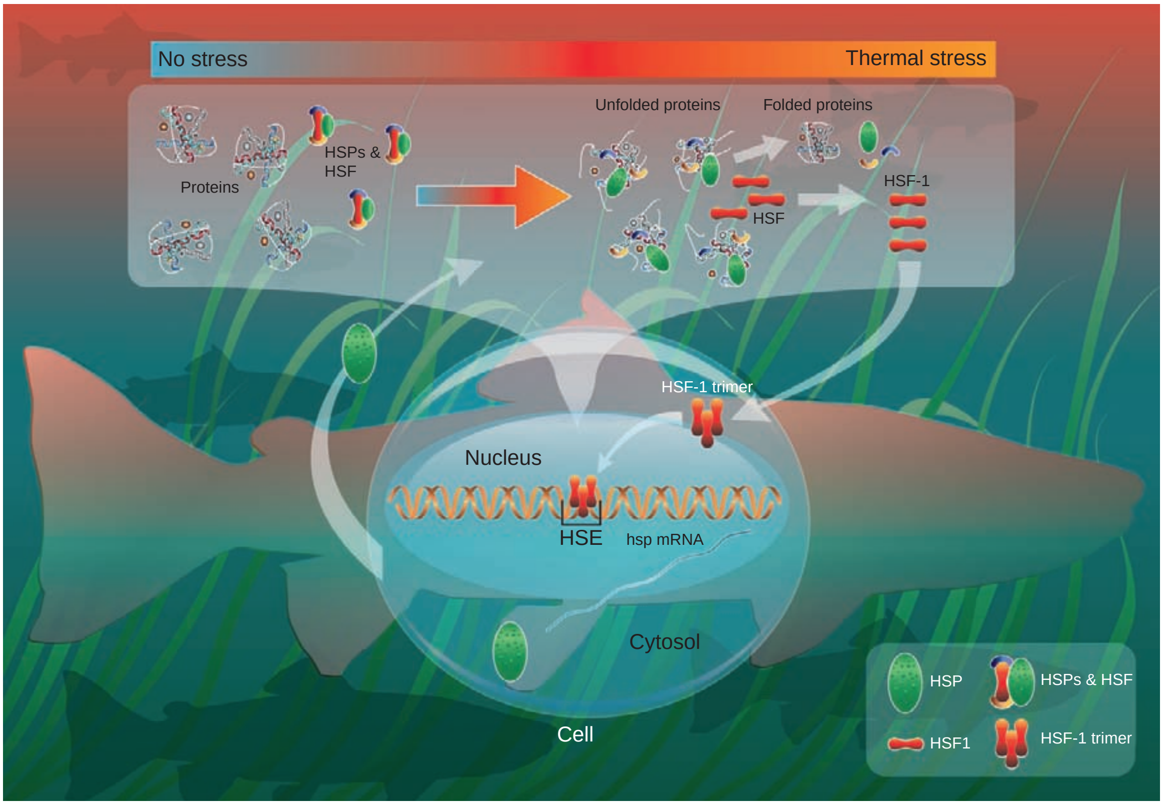


Figure 1 A model depicting the molecular regulation of HSP induction in fish cells. Under optimum temperature conditions, HSPs are transiently associated with HSF-1 monomers in the cell. During thermal stress, proteins may unfold and/or aggregate and this process triggers the release of HSPs from the HSF. The free HSF monomer moves into the cell nucleus where it trimerizes and binds to specific HSE regions of the DNA, resulting in transcription of HSP genes. These genes are translated to HSPs within the cytosol that can chaperone accumulating denatured proteins.

HSF2 have been identified in fish and two HSF1 genes (HSF1a and HSF1b) have been identified in rainbow trout. Furthermore, the regulation of both HSF1 and 2 in fish is very similar to that of other vertebrates. Under nonstress conditions, these transcription factors are maintained in a non-DNA-binding form as a monomer through transient interactions with HSPs (e.g., HSP70, HSP90, and HSP40; **Figure 1**). Induction of the heat shock response, likely through an accumulation of damaged, unfolded proteins, recruits HSPs from the HSF, removing the repressive effect and allowing the unbound HSF to move into the nucleus. Once inside the nucleus, HSF monomers trimerize and bind to specialized sequences on the DNA where they are activated through phosphorylation, resulting in transcription and subsequent translation of heat shock genes and proteins, respectively. The main DNA targets for HSF binding are heat shock elements (HSEs) within promoter regions of heat shock genes (**Figure 1**). High rates of transcription are maintained only when HSF1 trimers remain bound to HSEs. When either the stress signal is removed or damaged, proteins are no longer generated, the heat shock response is rapidly diminished. This quick downregulation of the heat shock response in the absence of stress is important to reduce energetic costs of HSP synthesis and to prevent any possible deleterious effects of overexpression. The variation we observe in the heat shock response in fish may be explained by differences in the regulatory regions of the different fish HSFs (post-translational modification), the HSF DNA-binding kinetics as a function of thermal history, and/or the interaction of HSFs with HSPs and other chaperones.

Physiological Regulation of the Heat Shock Response

It is now clear that aspects of the physiological responses to temperature stress in fish, such as changes in stress hormones and fatty acids, play a role in the regulation of the heat shock response. Acute heat stress in fish results in transient increases in the catecholamines, adrenaline and noradrenaline, and in the glucocorticoid stress hormone, cortisol, as well as changes in cell membrane dynamics leading to the release of polyunsaturated fatty acids (PUFAs). In fish, these humoral factors all directly but differentially impact the heat-induced expression of HSPs. Overall, catecholamines and PUFAs enhance, whereas cortisol attenuates the heat shock response. Clearly, the physiological regulation of the heat shock response in fish is complex but is likely optimally coordinated to ultimately delay or prevent thermal injury during heat stress (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids, **Hormonal Responses to Stress**: Catecholamines, Hormone Response to Stress, and Stress Effect on Growth and Metabolism).

Conclusion

Climate change is emerging as a significant threat to the world's dwindling fish stocks. It is therefore critical that we have a solid understanding of the physiological and molecular mechanisms underlying the capacity of fish to maintain key biological functions to help us predict the consequences of thermal stress on populations and ecosystems. The induction of HSPs is an integral component of a fish's response to temperature change. To appreciate the impact of HSP expression on animal fitness, future research must establish a direct role for HSPs in stress tolerance and integrate HSP induction with whole animal physiological function.

See also: **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids. **Hormonal Responses to Stress**: Catecholamines; Hormone Response to Stress; Stress Effect on Growth and Metabolism. **Temperature**: Measures of Thermal Tolerance; Proteins and Temperature.

Further Reading

Currie S, Reddin K, McGinn P, McConnell T, and Perry S (2008) β-Adrenergic stimulation enhances the heat shock response in fish. *Physiological and Biochemical Zoology* 81: 414–425.

Dietz TJ and Somero GN (1992) The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). *Proceedings of the National Academy of Sciences of the United States of America* 89: 3389–3393.

Fangue NA, Hofmeister M, and Schulte PM (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *Journal of Experimental Biology* 209: 2859–2872.

Feder ME and Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243–282.

Hochachka PW and Somero GN (2002) *Biochemical Adaptation*. New York: Oxford University Press.

Hofmann GE, Lund SG, Place SP, and Whitmer AC (2005) Some like it hot, some like it cold: The heat shock response is found in New Zealand but not Antarctic notothenioid fishes. *Journal of Experimental Marine Biology and Ecology* 316: 79–89.

Morimoto RI (1998) Regulation of the heat shock transcriptional response: Cross talk between a family of heat shock factors, molecular chaperones and negative regulators. *Genes and Development* 12: 3788–3796.

Pörtner HO and Farrell AP (2008) Physiology and climate change. *Science* 322: 690–692.

Räbergh CMI, Airaksinen S, Soitamo A, *et al.* (2000) Tissue-specific expression of zebrafish (*Danio rerio*) heat shock factor 1 mRNAs in response to heat stress. *Journal of Experimental Biology* 203: 1817–1824.

Ritossa F (1996) Discovery of the heat shock response. *Cell Stress and Chaperones* 1: 97–98.

Effects of Climate Change

H-O Pörtner, Alfred-Wegener-Institute, Bremerhaven, Germany

MA Peck, University of Hamburg, Hamburg, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Ecosystem Level Phenomena: Populations and Communities
Physiological Background of Climate-Change Impacts on Ecosystems
Performance Patterns and Energy Budget
Physiology-Based Projections of Impacts
Further Reading

Glossary

Aerobic scope The capacity of an animal to increase its level of aerobic metabolism beyond that which is required for maintenance alone and thereby support activities such as muscular exercise, growth or reproduction; it sets the capacity for oxygen-consuming physiological processes that an animal may perform at a given time. Aerobic scope is the difference between minimal and maximal oxygen consumption rate.

Climate change The global and local trends of changes in atmospheric climate conditions, which are, on average, associated with trends of warming and acidification, as well as expanding hypoxic zones in the world's oceans.

Life-history stages Successive stages of shape, morphology, functioning, and lifestyle during development from the egg into adulthood.

Ocean acidification A trend of reduction in the pH of ocean waters which is elicited by the dissolution of accumulating atmospheric CO_2.

Oxygen- and capacity-limited thermal tolerance (OCLT) A physiological concept explaining the specialization of animals on limited ranges of ambient temperatures (thermal windows) and their intolerance to temperature extremes. It builds on the finding that the functional capacity of tissues, including those involved in oxygen supply, is restricted to a limited range of temperatures and supports aerobic scope within that range.

Introduction

Ongoing climate change is projected to affect individual organisms during all life stages, thereby impacting populations, communities, and the functioning of ecosystems. These impacts of climate change on organisms can be direct, for example, through changing water temperatures, hypoxia events, ocean acidification, or climate-induced shifts in hydrodynamics and sea level, or indirect and mediated through changes in the composition of the food chain.

All effects occur via impacts on individual specimens in a population, involving phenomena at four interlinked levels of biological organization:

1. Whole-organism physiological responses to changes in environmental parameters such as temperature, dissolved oxygen, and ocean pH. This includes the physiological processes providing energy from food and those using that energy to support performance of the whole organism.
2. Behavioral responses at the level of the individual. This includes the avoidance of unfavorable conditions and, if possible, movement into suitable areas.
3. Changes in population dynamics as a result of alterations in the balance between rates of mortality, growth, and reproduction. This includes the dispersion of early life stages by ocean currents, which lead to the establishment of new populations in new areas or abandonment of traditional habitats.
4. Ecosystem-level changes in productivity and/or food-web interactions due to different responses by organisms at different levels of the food web. These observed impacts of global warming on fish at organismal, population, and community/ecosystem levels will be species specific and build on physiological changes at molecular, cellular, and whole-organism levels.

Ecosystem Level Phenomena: Populations and Communities

In the oceans, temperature defines the large-scale geographical distribution of marine water-breathing animals, within conditions set by geomorphology, ocean currents, water depth, and stratification or salinity. As a result of thermal specialization, warming or cooling would then drive marine fish to higher and lower latitudes, respectively. In fact, ocean warming is currently one of the main driving forces causing species-specific changes in distribution and abundance and, thus, in species composition in marine ecosystems. Shifts in geographical distribution are generally most evident near the northern or southern boundaries of the geographic range of a species. A number of studies have documented such changes within particularly well-studied ecosystems of the world's oceans including the North Sea and other parts of Europe. Furthermore, the seasonal timing of crucial events (phenology) also changes in relation to one another. For example, the timing of spawning and development of the fish larvae may shift out of phase with that of phyto- and zooplankton production such that food availability to the fish is reduced.

Meta-analysis of available data shows that there are differences in the tolerance to extreme temperatures both within species and/or between species (**see also Temperature**: Measures of Thermal Tolerance). The difference between upper and lower lethal temperatures, or the thermal window, of marine fish species varies with latitude, and is narrowest in fishes inhabiting high and low latitudes and widest for fish inhabiting intermediate latitudes where seasonal differences in temperatures are, on average, largest (**Figure 1**). Thermal window widths also vary depending on life stage. The relationship allows one to use geographical distributions of species (Arctic, Boreal, Lusitanian, Tropical, and Atlantic) as a classification scheme to evaluate expected responses.

Ecosystem effects are not only related to shifts in seasonal or annual mean temperatures. Temperature maxima or minima, as opposed to mean temperatures, also represent strong driving forces on both the cold and the warm side of the temperature spectrum. For example, extremely low winter-water temperatures cause losses in abundance of species and ecosystem changes, as seen in the German Wadden Sea. Conversely, cold-tolerant species will suffer from increasing exposure to summer heat. Observed shifts in geographical distribution of species such as Atlantic cod (*Gadus morhua*), snake blenny (*Lumpenus lampretaeformis*), and anglerfish (*Lophius piscatorius*) in the North Sea may involve the shifting frequencies of such weather conditions. In Atlantic cod, winter warming in the Southern North Sea is closely correlated with northward shifts in the population. Recruitment of cod in the North Sea falls during warming, but rises during cooling. In contrast, recruitment of the Barents Sea (North East Arctic) cod population decreases during cooling periods and increases upon warming. These trends emphasize that cod in the Southern North Sea and those in the Barents Sea live close to the upper and lower thermal limits of the species, respectively. In line with these observations, correlations between climate-associated temperature changes and Atlantic cod recruitment are stronger at the southern and northern borders of the geographical distribution area of the species in the northeast Atlantic. As a corollary, temperature defines the southern and northern distribution limits of the species.

These principles behind species specialization and sensitivity also extend to the population and community level and ecosystem functioning. Different thermal windows of species likely influence the quality and intensity as well as the seasonal timing of their interactions in an ecosystem. In general, species coexist where their thermal windows overlap, but these thermal windows are not necessarily identical. This would explain why climate sensitivity differs among species and may be one principal reason for climate-induced changes in community composition and food-web interactions. Regime shifts may result where one species replaces another one. For example, smaller copepods replaced the larger ones in the North Sea and anchovies (*Engraulis japonicus*) replaced sardines (*Sardinops melanostictus*) in the Sea of Japan. These examples emphasize that the limited thermal window of whole-organism performance capacity is key to understanding and projecting climate-induced changes in species interactions and, furthermore, in community composition.

Most commercial species produce millions of eggs, and mortality rates of early life-history (egg and larval) stages are very high. Relatively small changes in rates of mortality can have large impacts on the recruitment success and the number of fish destined to enter the adult population. As early life-history stages are likely to be more sensitive to altered environmental conditions (see **Figure 1**), global warming is expected to have a major effect on the distribution and abundance of fish through its influence on recruitment. Productivity will also be influenced by the effect of temperature on growth rate. In a comparative study of 15 Atlantic cod stocks, sevenfold differences in the productivity among stocks corresponded to the differences in the temperature of the environment. A part of this effect is related to different (growth) performance rates of cod populations at the same temperature due to specialization in local or regional climate regimes.

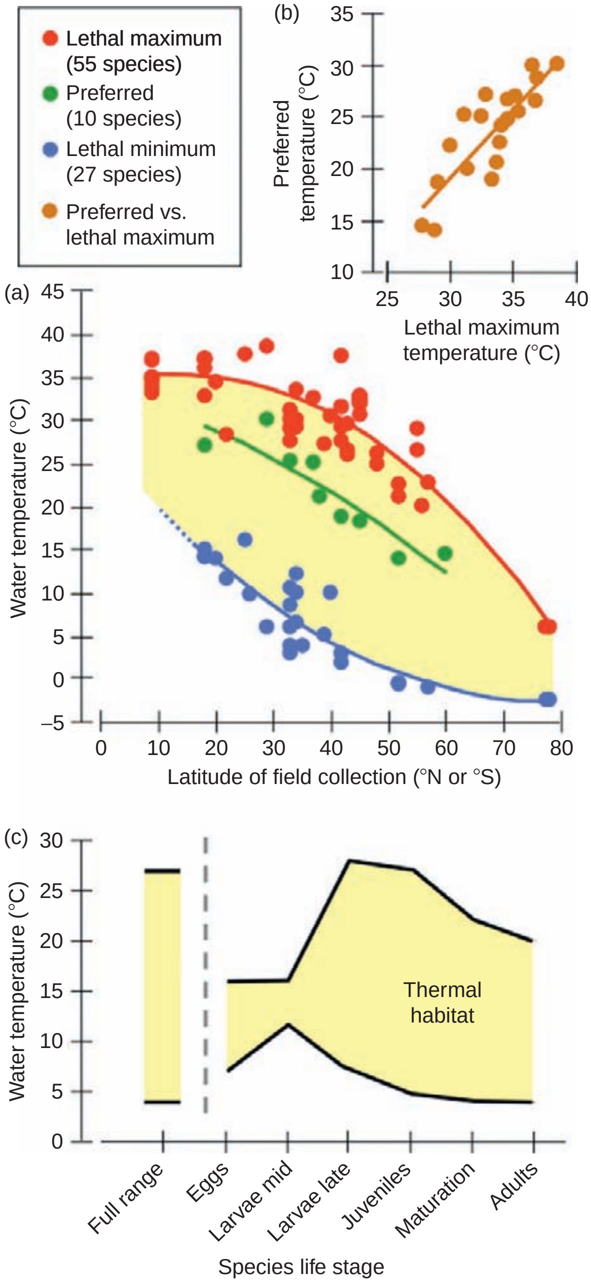


Figure 1 Thermal tolerance in marine fish species including upper lethal (blue), lower lethal (red), and preferred (green) temperatures versus latitude (°N or °S) of the fish population (a) and relationship between preferred and upper lethal temperature (b). Preferred temperatures by fish (in the laboratory) often coincide with optimal growth temperatures observed in the wild. Thermal tolerance changes with life stages (c), in this example for sole (*Solea solea*), the earliest life stages were more sensitive (had a more narrow range in thermal tolerance) than later larvae and early juveniles that can exploit the largest range in thermal habitats compared to earlier or later (adult) life stages. The tolerance range in (c) is a compilation of information from experimental work on early, pelagic life stages (eggs, larvae, and juveniles by Irvin DN (1974) Temperature tolerance of early development stages of Dover Sole, Solea Solea (L.) *Proceedings of an International Symposium on the Early Life History of Fish*, pp. 449–463. Dunstaffnage Marine Research Laboratory, Oban, Scotland, 17–23 May 1973 and Fonds M (1979) Laboratory observations on the influence of temperature and salinity on the development of the eggs and growth of the larvae of Solea solea (Pisces). *Marine Ecology Progress Series* 1: 91–99) and the range of temperatures where demersal stages of those flatfish are observed. Maturation refers to the body size at which fish become sexually mature (i.e., they first enter the adult stage) and does not refer to adult fish that are in spawning condition. Note that the lower temperature for adults is based on the lethal temperature observed during cold winters by Woodhead PJM (1964) The death of North Sea fish during the winter of 1962/63 particularly with reference to the sole, *Solea vulgaris*. *Helgolander Wissenschaftliche Meeresuntersuchungen* 10: 283–300.

As a consequence of thermal specialization, low productivity and high mortality occur when temperature conditions reach extreme values. However, additional factors interfere and interact with temperature. Mortality is elicited or enhanced during hypoxic or anoxic conditions. In some ecosystems, mass mortalities during summer have been reported in relation to harmful algal blooms. Climate change may have dramatic (negative) impacts on the productivity of fish populations by increasing the frequency of these episodic extreme events that have acute physiological consequences.

Physiological Background of Climate-Change Impacts on Ecosystems

Oxygen- and Capacity-Limited Thermal Tolerance

Specialization of marine fauna on climate-related thermal windows emerges as one overarching reason for the sensitivity of marine fauna to temperature extremes. Thermal windows differ between life stages of one species as well as between species and thereby co-define changes in higher-level processes at ecosystem level in response to climate change.

For animals, the concept of oxygen- and capacity-limited thermal tolerance (OCLT) (**Figure 2**) provides a mechanistic explanation of why, how, and at which temperature limitation occurs. It also distinguishes the thermal range of permanent, active survival from the range of time-limited passive tolerance to temperature extremes. This concept also explains the transition between those ranges. It is a suitable candidate to define the borders of the niche of functional capacity of a species with respect to temperature and species interactions.

The concept implies that oxygen supply to tissues is optimal between limits called lower and upper 'pejus' temperatures (pejus means 'getting worse'). Between pejus limits, oxygen supply can be increased to levels much in excess of maintenance demand and thereby fuel optimal performance by aerobic metabolism. The aerobic scope (the difference between the lowest and highest rates of aerobic respiration) represents the energy available for

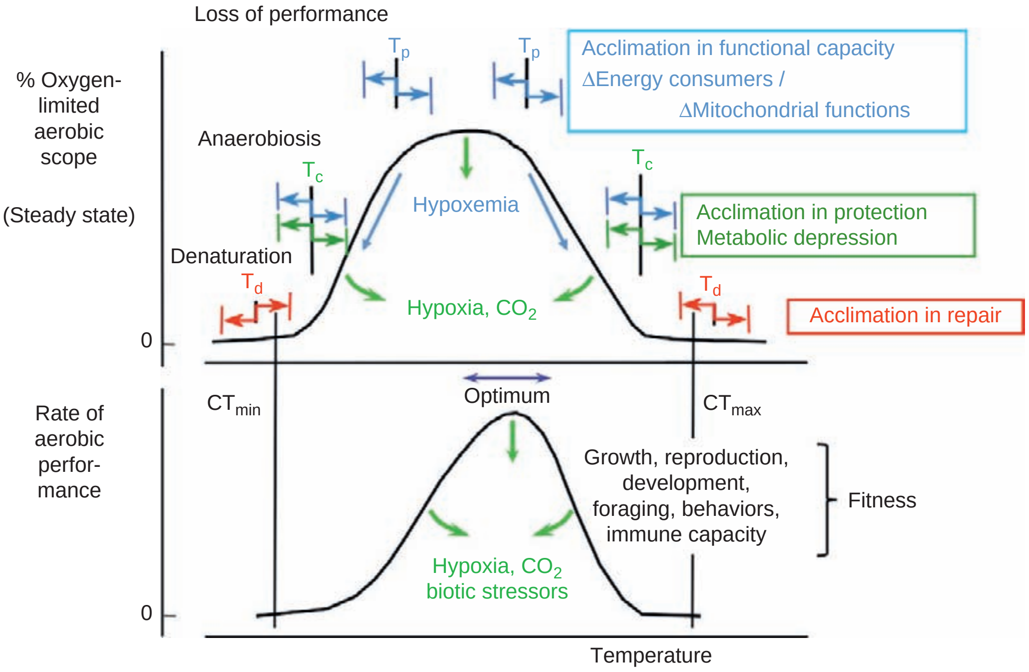


Figure 2 Conceptual model of how ocean warming, hypoxia, and acidification, as interacting stressors, shape the thermal window of performance of a species at a specific life stage, based on the concept of oxygen- and capacity-limited thermal tolerance. Oxygen supply to tissues is optimal between low and high pejus temperatures (top) and supports temperature-dependent performance capacity (bottom). Critical temperatures (T_c) characterize the onset of anaerobic metabolism and denaturation temperatures (T_d), the loss in integrity of molecular structures. Critical thermal maxima and minima (CT_{max} and CT_{min}) delineate the outermost limits where whole-organism function ceases. The kinetic stimulation of performance by warming leads to a tilted curve with a functional optimum close to the upper pejus temperature. This performance curve reflects the role or functional capacity and associated aerobic scope in ecosystem-level processes such as competition, foraging, immune response, growth, and behaviors. Ambient hypoxia and elevated CO_2 levels both cause a narrowing of thermal windows, and, possibly, lower performance optima through lower functional capacities and reduced systemic-oxygen tensions (green arrows). The graph depicts acute performance levels and limitations in response to short-term temperature fluctuations. Seasonal acclimatization causes a shift and changing widths of acute performance windows within the limits of the thermal niche of a species (horizontal arrows).

all life-sustaining processes, such as those supporting fitness and the capacity to forage, migrate, grow, or reproduce.

The kinetic slowing of performance by cooling below the optimum leads to a lower pejus limit, which is characterized by performance significantly below optimum levels. Conversely, the kinetic stimulation of performance and maintenance costs by warming causes the performance curve to be tilted to the right. Beyond the upper pejus limits, the capacity for oxygen supply becomes limiting, maintenance demand rises, and aerobic scope starts to decrease. Hypoxemia develops and whole-organism functional capacity (e.g., exercise or growth performance) declines. Once critical temperatures are reached, extreme hypoxemia sets in causing transition to anaerobic metabolism. Finally, thermal damage to the integrity of molecular structures (denaturation) may occur.

Figure 2 distinguishes between the temperature range associated with long-term survival, growth, and reproduction due to maintained performance (the active range) and the subsequent time-limited endurance of temperature extremes (passive range). The transition between the two and the loss in performance is progressive, leading to a progressively severe time limitation of thermal tolerance. The point where this transition starts is defined as the pejus temperature (T_p, the temperature at which aerobic scope begins to decline). Organisms would not survive long term at temperatures higher than T_p; time constraints become more and more severe as temperatures approach and reach beyond their critical temperature (T_c) where aerobic scope approaches zero. The product of time and heat load beyond T_p would then set the lethal temperature, which indicates the ultimate limits of passive tolerance. The lethal temperature is thus time dependent, short-term heating protocols leading to higher lethal temperatures than long-term heating protocols.

In laboratory studies, lethal tolerance limits of fishes have traditionally been determined as the onset of spasms at critical thermal maxima ($\mathrm{CT}_{\mathrm{max}}$) and minima ($\mathrm{CT}_{\mathrm{min}}$). $\mathrm{CT}_{\mathrm{max}}$ and $\mathrm{CT}_{\mathrm{min}}$ are the temperatures at which fish are definitely unable to escape conditions that will ultimately lead to thermal death (**see also Temperature**: Measures of Thermal Tolerance). These limits occur at temperatures beyond the T_c values, as defined by the OCLT concept. During exposures to extreme temperatures beyond T_p, animals progressively enhance exploitation of protective mechanisms such as the capacity of anaerobic metabolism (beyond T_c), anti-oxidative defense, and the heat-shock response. All of these contribute to extend the period of passive tolerance. The relevance of these processes varies between species and would be under strong selection in some environments where organisms are regularly exposed to thermal extremes. For example, passive extension of the range of tolerance appears crucial in thermally variable environments such as the intertidal zone.

Pejus limits reached acutely on short timescales may shift over time when the organism has the capacity to acclimate to temperature change. However, acclimation capacity is limited, such that during exposure to temperature on long timescales (as in growth studies), performance decrements occur at the limits of thermal acclimation capacity. Beyond these long-term thermal limits, the loss in fitness remains uncompensated and leads to various phenomena at ecosystem level, that is, a loss of growth performance and reduced population abundance. These were observed in eelpout (*Zoarces viviparus*) in the German Wadden Sea, where abundance falls upon exposure to extreme summer temperatures, in the same temperature range when growth performance decreases and oxygen-supply capacity by the circulatory system becomes limiting. Large individuals disappear first from the population since they are affected most by the heat stress, in line with the exacerbation of oxygen limitation at larger body sizes.

Performance Patterns and Energy Budget

Thermal specialization according to local climate conditions contributes to shape the energy budget of marine organisms. Balanced bioenergetics budgets (**see also Energy Utilization in Growth**: Energetics of Growth, **Energetic Models**: Bioenergetics in Aquaculture Settings, and Bioenergetics in Ecosystems) that include specific pathways for energy gain and loss may be a useful tool to assess the potential impacts of climate change on fish. These equations are generally written as follows:

$$C = G + R + E + F \tag{1}$$

where C is the energy gained from food consumption, G the energy gained in growth, R the energy lost via respiration, E the energy lost via nitrogenous excretion, and F the energy lost in egested fecal material. All parameters have common energetic units (e.g., joules per day). Rates of R (and E) can be subdivided to include different levels of energy loss due to basal (standard, R_S), feeding (specific dynamic action, R_{SDA}), and active (R_A) metabolism. Growth can also be subdivided to partition between gonadal (G_G) and somatic (G_S) growth. Rates of these various parameters are affected by a number of factors including water temperature, fish body size, and feeding level. Thus, balanced budgets provide a route for exploring possible impacts of environmental changes at the level of the individual. This balanced equation is most commonly used to estimate either growth or food consumption after the

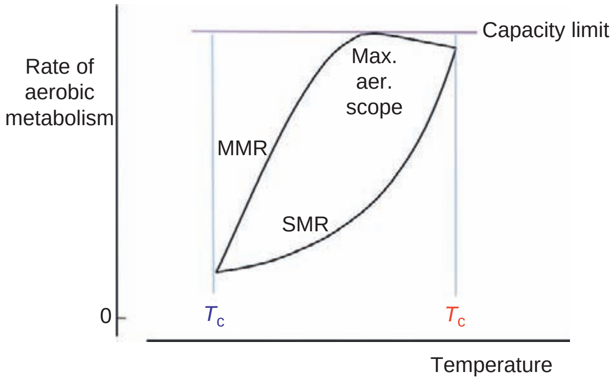


Figure 3 Conceptual model of how standard and maximal metabolic rates fit the thermal window of oxygen- and capacity-limited thermal tolerance (projected from mechanistic knowledge as in **Figure 2**). The exponential phase of standard metabolism is bordered by critical temperatures. The performance curve as in **Figure 2** results from the difference between maximum and standard metabolic rates.

other parameters are determined. For a true cause-and-effect understanding, however, the question requires consideration of which variables are dependent variables.

The effect of temperature on growth rate (*G*) follows the aerobic performance curve with net growth performance only possible above the low and below the high critical thresholds (cf. **Figures 2** and **3**). A reduced upper temperature threshold occurs when *C* is much less than *R*, *E*, and *F* (i.e., when metabolic costs cannot be met by feeding). Above the lower threshold, growth rates increase with increasing temperature until an optimal temperature is reached, when food availability is not limiting. With further increases in temperature, *G* often rapidly declines until mortality occurs even when food is available. At these suboptimal high temperatures, fish cannot consume enough food to meet increasing metabolic costs; available evidence suggests that there is not enough aerobic scope available to cover the postprandial increase in oxygen demand (cf. **Figure 2**; **see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation). Losses in appetite occur at the highest temperatures, rapidly leading to mortality.

The relationships between the levels of energy turnover, the capacities for activity, and the widths of thermal windows support an integrative understanding of specialization on climate and of sensitivity to climate change. The balance between oxygen demand for maintenance and the level of aerobic scope available for crucial performances between critical temperatures therefore appear critical for sensitivity to abiotic and biotic environmental factors (**Figure 4**). Pejus limits encompass the optimal range in temperatures (i.e., the temperatures where metabolic scope and thus performance is highest) and therefore delineate the window of highest competitiveness. In line with the predictions resulting from **Figures 1** and **2**, aerobic scope closely describes the range of performance sensitivity to environmental changes.

The range of tolerable temperatures is both species and often life-stage specific, and is a key attribute defining the observed distribution (niches and habitats utilized) by marine fish species. Global warming is not

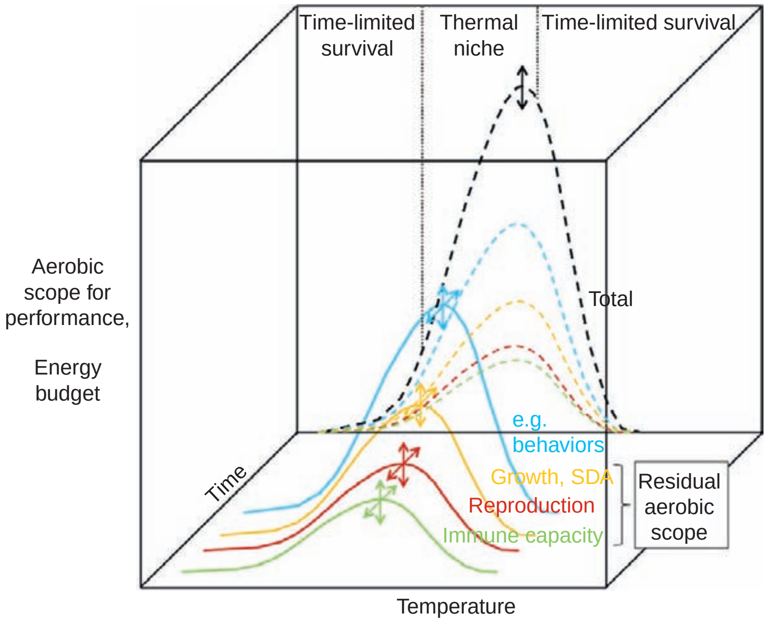


Figure 4 Energy budget for processes beyond maintenance comprising fractions of overall performance fueled by the temperature-dependent window of aerobic scope (cf. **Figure 2**). Arrows illustrate shifting fractions of various processes in the energy budget over time (order and timing arbitrary).

expected to impact all life stages of marine fishes in the same manner. For example, evidence for the common sole (*Solea solea*) and the Atlantic cod suggests that the optimum temperatures for growth decrease with increasing body size of juveniles and adults. Decreases in oxygen-limited tolerance to warm temperatures with increasing body size can be used to explain the ontogenetic shift in habitats observed in several fish species where young stages occur in shallower (warmer) waters rather than larger stages. In some species, egg and larval stages have a narrower range in tolerable temperatures than other life-history stages (particularly juveniles), making these early life stages more vulnerable to the impacts of global warming (see **Figures 1** and **5**). The sensitivity of larval stages to global warming likely relates to the ontogenetic development of convective oxygen transport during a period when diffusive oxygen supply becomes limiting. Another sensitive life stage is the mature spawner, where the egg mass may be the largest tissue to be supplied with oxygen, which narrows the tolerance range to temperature fluctuations due to the allometry of oxygen limitation. **Figure 5** outlines these patterns expected from OCLT principles.

Changes in species interactions and competitiveness at ecosystem level may also reflect these principles and may result from relative changes in performance due to different thermal sensitivities (**Figure 5**). The regime shifts described, then, not only relate to the principal effects of temperature according to thermal windows. These shifts may be accelerated by the relative changes in performance and the associated shift in competitiveness once species compete for the same resource or ecological niche.

Physiology-Based Projections of Impacts

Projections have been made of the impacts of global warming on marine fishes using a variety of methods that range from simple to complex, each of which has its own caveats and only some of which incorporate physiological mechanisms. Most projections of the impacts of global warming on fish populations, aside from simple what-if scenario tests, rely on the outputs of global climate models (GCMs).

In the most simple example, outputs from GCMs are used to adjust environmentally sensitive stock–recruitment relationships which are essentially functions estimating year-class survival (recruitment) from key population and environmental factors (e.g., spawning stock biomass and temperature, which are described by the dome-shaped relationships above). Such predictions do not reveal mechanisms, but, nonetheless, provide short-term estimates of population strength in the face of climate impacts, all other elements of the ecosystem being equal.

Bioclimatic-envelope modeling represents a second approach which is more physiology based in that it makes broad brush projections of changes in distribution based upon changes in environmental conditions correlated with the survival (or current distribution) and productivity of species. Recent modeling results for >800 commercially important marine fish species suggested that climate change could lead to numerous local extinction events by the year 2050, particularly in subpolar regions, the tropics, and semi-enclosed seas (e.g., the Mediterranean Sea), with the distribution of pelagic fish species (sardines, herring, and anchovies) and demersal

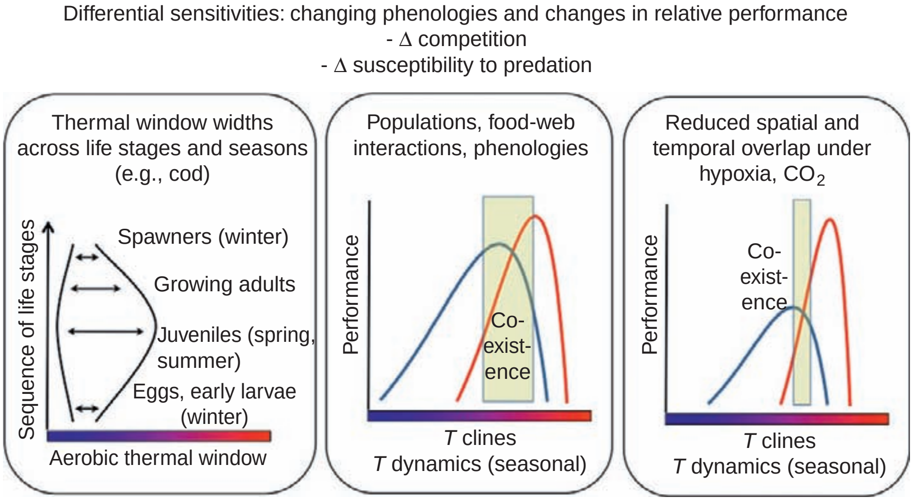

Differential sensitivities: changing phenologies and changes in relative performance
- Δ competition
- Δ susceptibility to predation
Thermal window widths across life stages and seasons (e.g., cod)
Sequence of life stages
Spawners (winter)
Growing adults
Juveniles (spring, summer)
Eggs, early larvae (winter)
Aerobic thermal window
Populations, food-web interactions, phenologies
Performance
Co-exist-ence
T clines
T dynamics (seasonal)
Reduced spatial and temporal overlap under hypoxia, CO2
Performance
Co-exist-ence
T clines
T dynamics (seasonal)

Figure 5 Ecosystem-level perspective of how thermal windows develop during ontogeny in relation to seasonal temperature dynamics (as in Atlantic cod). Furthermore, the synergistic effects of shifting temperatures, CO_2, and hypoxia levels affect species interactions through changes in temporal and thermal overlap as well as in relative performance.

species (such as Atlantic cod, haddock (*Melanogrammus aeglefinnus*), and flatfishes) moving poleward by an average of 600 and 223 km, respectively. However, similar to simple stock–recruitment relationships described above, other climate-driven changes that may occur in marine habitats, which place constraints on the survival and persistence of populations of marine fish, are not considered.

A third example of climate model-based projections combines direct and indirect impacts of global warming (e.g., on prey fields and temperature-dependent growth performance) to examine survival of single species using coupled biophysical modeling. Such models can contain different subcomponents describing the marine ecosystem (e.g., three-dimensional (3-D) hydrodynamics and productivity at lower trophic levels) and the growth physiology of individual species. Such complex models have revealed how environmental variability potentially affected historical productivity of species. However, the reliability of this modeling approach to project future impacts depends upon obtaining robust estimates of hydrographic and lower trophic-level changes resulting from global warming. The latter is a rapidly advancing field of climate-impact research. In the future, the mechanism-based knowledge as outlined above, which has shown to be closely involved in ecosystem-level processes, would need to be extended and involved in such modeled projections, both for another level of integrative and in-depth understanding and for improved reliability of those projections.

See also: **Energetic Models**: Bioenergetics in Aquaculture Settings; Bioenergetics in Ecosystems. **Energy Utilization in Growth**: Energetics of Growth. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Temperature**: Measures of Thermal Tolerance.

Further Reading

Beare DJ, Burns F, Greig A, *et al.* (2004) Long-term increases in prevalence of North Sea fishes having southern biogeographic affinities. *Marine Ecology Progress Series* 284: 269–278.

Beaugrand G, Reid PC, Ibanez F, Lindley JA, and Edwards M. (2002) Reorganization of North Atlantic marine copepod biodiversity and climate. *Science* 296: 1692–1694.

Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, and Pauly D (2009) Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10: 235–251.

Daewel U, Peck MA, Kühn W, St. John MA, Alekseeva I, and Schrum C. (2008) Coupling ecosystem and individual-based models to simulate the influence of environmental variability on potential growth and survival of larval sprat (*Sprattus sprattus* L.) in the North Sea. *Fisheries Oceanography* 17: 333–351.

Drinkwater KF (2005) The response of Atlantic cod (*Gadus morhua)* to future climate change. *ICES Journal of Marine Science* 62: 1327–1337.

Dutil J-D and Brander K (2003) Comparing productivity of North Atlantic cod (*Gadus morhua)* stocks and limits to growth production. *Fisheries Oceanography* 12: 502–512.

Farrell AP, Hinch SG, Cooke SJ, *et al.* (2008) Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology* 81: 697–708.

Fonds M (1979) Laboratory observations on the influence of temperature and salinity on the development of the eggs and growth of the larvae of Solea solea (Pisces). *Marine Ecology Progress Series* 1: 91–99.

Irvin DN (1974) Temperature tolerance of early development stages of Dover Sole, Solea Solea (L.) *Proceedings of an International Symposium on the Early Life History of Fish*, pp. 449–463. Dunstaffnage Marine Research Laboratory, Oban, Scotland, 17–23 May 1973.

MacKenzie BR and Köster FW (2004). Fish production and climate: Sprat in the Baltic Sea. *Ecology* 85: 784–794.

Munday PL, Crawley NE, and Nilsson GE (2009) Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series* 388: 235–242.

Peck MA, Buckley LJ, Caldarone EM, and Bengtson DA (2003) Effects of food consumption and temperature on growth rate and biochemical-based indicators of growth in early juvenile Atlantic cod (*Gadus morhua)* and haddock (*Melanogrammus aeglefinus)*. *Marine Ecology Progress Series* 251: 233–243.

Perry AL, Low PJ, Ellis JR, and Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science* 308: 1912–1915.

Pörtner HO (2002) Climate change and temperature dependent biogeography: Systemic to molecular hierarchies of thermal tolerance in animals. *Comparative Biochemistry and Physiology* 132A: 739–761.

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Marine Ecology Progress Series* 373: 203–217.

Pörtner HO (2010) Oxygen and capacity limitation of thermal tolerance: A matrix for integrating climate related stressors in marine ecosystems. *Journal of Experimental Biology* 213: 881–893.

Pörtner HO, Bock C, Knust R, *et al.* (2008) Cod and climate in a latitudinal cline: Physiological analyses of climate effects in marine fishes. *Climate Research* 37: 253–270.

Pörtner HO and Farrell AP (2008) Physiology and climate change. *Science* 322: 690–692.

Pörtner HO and Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95–97.

Pörtner HO, Schulte PM, Wood CM, and Schiemer F (2010) Niche dimensions and limits in fishes: An integrative view. Illustrating the role of physiology in understanding ecological realities. *Physiological and Biochemical Zoology* 83: 808–826.

Rijnsdorp A, Peck MA, Engelhard GH, Möllmann C, and Pinnegar JK (2009) Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science* 66: 1570–1583.

Takasuka A, Oozeki Y, and Aoki I (2007) Optimal growth temperature hypothesis: Why do anchovy flourish and sardine collapse or vice versa under the same ocean regime? *Canadian Journal of Fisheries and Aquatic Sciences* 64: 768–776.

RESPONSES AND ADAPTATIONS TO THE ENVIRONMENT

Hypoxia

Contents

The Expanding Hypoxic Environment
Respiratory Responses to Hypoxia in Fishes
Anaerobic Metabolism In Fish
Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia

The Expanding Hypoxic Environment

RJ Díaz, Virginia Institute of Marine Science, Gloucester Point, VA, USA

DL Breitburg, Smithsonian Environmental Research Center, Edgewater, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Importance of Oxygen and Hypoxia
Where Does Hypoxia Occur?
Rise of Anthropogenic Influence on Oxygen Budgets
Oxygen Budgets and Global Climate Change
Further Reading

Glossary

Anoxia Complete absence of dissolved oxygen in water.

Eutrophication A condition of aquatic systems caused by excessive primary production, fueled by excess nutrients that enter a system either as point discharges, such as sewage, or as nonpoint runoff from land, primarily from agriculture.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Importance of Oxygen and Hypoxia

Oxygen is necessary to sustain the life of fishes and most invertebrates. When the supply of oxygen is cut off or the consumption rate exceeds replacement, dissolved oxygen (DO) concentrations can decline below levels required to sustain most animal life. This condition of low DO is known as hypoxia and in the case of no DO it is known as anoxia. While many investigators and government regulatory guidelines define hypoxia as a concentration of DO below 2–3 mg-O_2 l^{-1} (milligrams of oxygen per liter of water) for marine and estuarine environments, and 5–6 mg-O_2 l^{-1} in freshwater, such an arbitrary limit may be unsuitable when examining potential impacts of hypoxia on any one given species. Species and life stages differ in their basic oxygen requirements, and oxygen requirements increase as energy-demanding metabolic processes are mobilized. The large number of hypoxia-tolerant aquatic species, and a wide variety of anatomical, physiological, and behavioral adaptations to hypoxia, suggest that low DO environments played an important role in the evolution of many adaptive strategies for hypoxia or even anoxia survival in fish (**see also Hypoxia**: Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia, Respiratory Responses to Hypoxia in Fishes, and **Bony Fishes**: Crucian Carp). In contrast to terrestrial systems, adaptations to hypoxia in aquatic habitats are the rule rather than the exception.

The relatively low solubility of oxygen in water combined with two principal factors leads to the development of hypoxia and at times anoxia. These factors are water column stratification that isolates the bottom water from exchange with oxygen-rich surface water and decomposition of organic matter in the isolated bottom water that reduces oxygen levels. Both factors must be at work for hypoxia to develop and persist in deeper waters. However, in some smaller shallow systems hypoxia can form in the absence of stratification particularly at night when submerged aquatic vegetation and phytoplankton are not photosynthesizing and producing oxygen, and respiration by microbes and other organisms depletes the oxygen supply. When the primary producers start again the next day, the hypoxia dissipates. This particular form of hypoxia is known as diel-cycling hypoxia.

For temperate zone lakes, factors affecting vertical water mixing such as wind, temperature, and surface ice affect seasonal changes in the DO depth profile and can lower DO in bottom waters leading to winterkills. These physical factors alter the rate at which oxygen moves into water and how rapidly well-aerated surface waters mix with deeper ones. For example, ice and snow cover on lakes and streams can also block photosynthesis and reaeration, and may lead to hypoxia. In both of these cases, respiration needs to exceed oxygen production or resupply rates. In addition, water often becomes hypoxic on floodplains. For example, in tropical freshwaters, oxygenation is greater in rainy seasons with more water flow versus dry stagnant water seasons. In tidal areas, organisms also experience physiological hypoxia along with hypercapnia during intertidal exposure.

Much of the sensitivity of organisms to variations in the level of DO stems from the fact that oxygen is not very soluble in water and that small changes in oxygen concentration lead to large differences in percent air saturation. Depending on temperature and salinity, water contains 20–40 times less oxygen by volume and diffuses about 10 000 times more slowly through water than air. In addition, oxygen solubility strongly depends on the temperature and the amount of dissolved solids in water, with air saturation declining about 1 mg-O_2 l^{-1} per 5 °C increase in temperature and about 2 mg-O_2 l^{-1} from freshwater to seawater (35 psu) at similar temperatures (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange). Thus, what appear to be small changes in DO can have major consequences to animals living in an oxygen-limited milieu. Physiologically, higher temperatures also increase metabolic requirements for oxygen and increase rates of microbial respirations and, therefore, oxygen consumption (**see also Temperature**: Effects of Climate Change).

Where Does Hypoxia Occur?

Hypoxia is a natural component of many freshwater habitats such as swamps and backwaters that circulate poorly, stratify, and have large loads of decaying terrestrial organic matter. Lakes are also prone to thermal stratification that makes them susceptible to the development of hypoxia. However, there is mounting evidence that human activities have worsened the DO conditions in many lake and freshwater systems.

Areas of naturally low DO in coastal marine systems are limited to fjord-like systems prone to water column stratification and deep depositional basins, such as Oslofjord, Norway, or the central basin of the Black Sea, currently the largest pool of naturally occurring anoxic water on the Earth. In shallow marine and freshwater systems, depending on the balance between oxygen production and respiration, a natural diel cycling of DO from supersaturation during the day to hypoxic or near anoxic during the night can occur. In highly productive systems, calm weather conditions and extended periods of cloud cover often exacerbated the problem.

The largest natural low-DO areas on the Earth are oceanic oxygen minimum zones (OMZs) that form under areas of high surface productivity, which sinks and in the process of microbial metabolism oxygen is consumed. OMZs are unusual oxygen-depleted areas in that they are widespread and stable oceanic features occurring at intermediate depths (typically 400–1000 m), particularly severe in regions of sluggish circulation, persisting for long periods of time (at greater than decadal scales), and are controlled by natural processes and cycles (**Figure 1**). Where OMZs contact the bottom, globally about 1 000 000 km^2 along the continental margins, specialized communities have evolved to survive at DO concentrations as low as 0.1 mg-O_2 l^{-1}. Upwelling areas can also develop extensive hypoxia as additional deep-water nutrients are added to surface waters increasing production that eventually sinks and decomposes. Hypoxia associated with upwelling is not as long lived and stable as that associated with OMZs. (**see also Pelagic Fishes**: An Introduction to the Biology of Pelagic Fishes).

Rise of Anthropogenic Influence on Oxygen Budgets

In contrast to freshwater, much of the hypoxia and anoxia in shallow coastal marine and estuarine areas is recent in origin, probably beginning within the last 100 years, and closely associated with human activities (**Figure 1**). Even the natural diel cycling of DO can be exaggerated by human activities. These recent hypoxic areas, commonly called dead zones, are closely correlated with anthropogenic activities and appear to develop as a result of the cumulative effects of eutrophication in seasonally stratified or stagnant systems.

Eutrophication can be defined simply as the production of organic matter in excess of what an ecosystem is normally adapted to processing. However, it is only part of a complex web of stressors that interacts to shape and direct

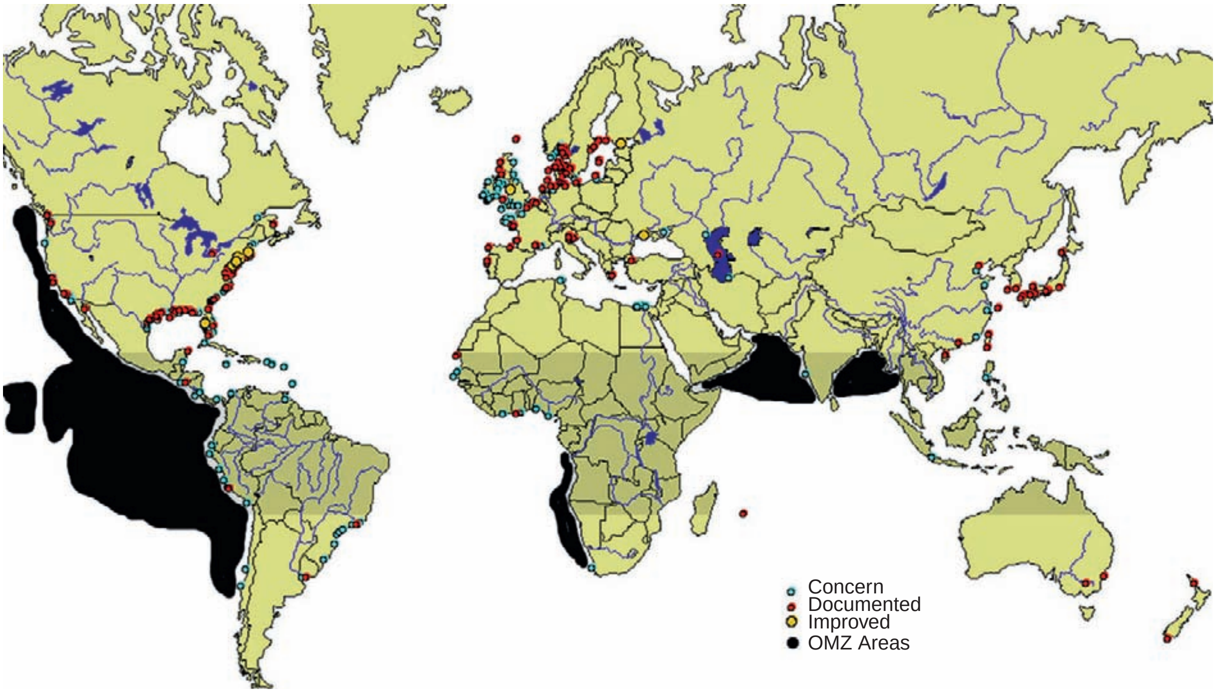


Figure 1 Global distribution of major OMZ areas and coastal hypoxic systems. Systems with documented hypoxia are red circles, areas of concern for being hypoxic are blue circles, and areas that have recovered from hypoxic conditions are yellow circles. Shading indicates the tropical regions (20 north and south of the equator) most likely to experience naturally low dissolved oxygen conditions.

ecosystem level processes. The primary driver of eutrophication in both freshwater and marine systems is excess nutrient enrichment, but physical conditions that limit reaeration are also necessary for the development of hypoxia. For freshwater systems, phosphorus tends to be the more important nutrient and for marine systems it tends to be the nitrate. This basic difference is related to the physical properties of phosphorus and nitrogen compounds, and their biogeochemical cycling through the environment.

Within the last 50 years, DO conditions of many major coastal ecosystems around the world have been adversely affected primarily by eutrophication driven by excess nutrient loading. The source of these excess nutrients is primarily agricultural runoff but also sewage and industrial discharges. As more organic matter was produced from these nutrients, more oxygen was needed to remineralize the organics, primarily through the microbial loop, and as ecosystems became overloaded DO declined. For European systems that have historical data from the early 1900s, declines in DO started in the 1950s and 1960s. However, for the Baltic Sea declining DO levels were noted as early as the 1930s in the deep central basin.

Among marine systems with long-term DO data, benthic hypoxia became a problem in the 1950s in the Baltic Sea proper, the 1960s in the northern Adriatic Sea, the 1970s in the Kattegat, and the 1980s on the Northwest continental shelf of the Black Sea. Hypoxia does not appear to be a natural condition for marine waters except for those systems previously described. Even in Chesapeake Bay where hypoxia existed even when the first DO measurements were made in 1920s in the Potomac River and 1930s in the mainstem channel, the geological record does not support the hypothesis that seasonal hypoxia is a natural condition predating European colonization. Geochronologies from the hypoxic area on the continental shelf of the northern Gulf of Mexico also indicate that the current seasonal hypoxia, which can cover over 20 000 km^2, was not present prior to the 1950s. Hypoxia was recorded with the first DO measurement made on the central Louisiana continental shelf in the summer of 1973. By 1978, hypoxia caused the first recorded mass mortality event on the Louisiana shelf. In subsequent years, hypoxia has led to extensive mortalities and low trawl catches.

Once a system arrived at the point of having hypoxia, it quickly became an annual event and a prominent feature affecting energy flow processes in many affected ecosystems. From the 1980s to the present, the distribution of hypoxia around the world has not changed appreciably in a positive way with the number of reported cases currently about 500. Only in systems that have experienced intensive regulation of nutrient or carbon inputs have oxygen conditions improved, primarily from initiation of sewage treatment or upgrades in treatment level, for example, the Hudson River in New York, the Delaware River in Pennsylvania-New Jersey, and the Mersey Estuary and Thames River in England. For example, the hypoxic zone downstream of the London's sewage input into the Thames

River was greatly reduced after upgrades to the sewage treatment plant to reduce the biological oxygen demand of the sewage being released. As a result, even hypoxia-sensitive salmonids returned to the river.

Temporary improvements have been seen in systems with changes in hydrology or nutrient inputs. In the northern Gulf of Mexico, the size of the hypoxic area responds annually to Mississippi River discharge with low flow years having less hypoxia and high flow years more. Large-scale meteorological events that disrupted stratification are also capable of reducing the area of hypoxia, as in the Gulf of Mexico and the Gulf of Finland. The northwest shelf of the Black Sea once experienced annual hypoxic events, but is now in a state of recovery largely due to the economic collapse of Eastern Europe in the early 1990s, which greatly reduced the use of fertilizer and subsequent nutrient loading in runoff. Within three years, the hypoxic area in the northwest shelf of the Black Sea went from a maximum area of about 40 000 km^2 to none. While no hypoxic events were recorded on the shelf between 1993 and 2001, a full recovery of the shallow continental shelf waters of the northwest Black Sea is far from certain. Climatic conditions caused a large hypoxic area to form during a warmer than average 2001 and the expected recovery of farming in Eastern Europe will likely lead to increased nutrient loadings.

Oxygen Budgets and Global Climate Change

The future status of hypoxia will depend upon how water column stratification is affected mainly by climate change and also by factors that affect organic matter production such as nutrient supplies that are mainly controlled by runoff and land management practices. Expanding agriculture for production of crops to be used for food and biofuels will result in increased nutrient loading and expand eutrophication effects, which in turn will expand hypoxic areas.

For climate change, general circulation models predict a decrease in the oceanic oxygen inventory through increased stratification and warming. However, the effect is several times larger than expected from warming alone and points to the importance of biology in mediating oxygen exchange. General circulation models also predict large changes in rainfall patterns. If these changes in rainfall lead to increased discharges of freshwater to coastal ecosystems, stratification and nutrient loads are likely to increase and worsen the oxygen depletion in those systems already affected and hypoxia may develop in other systems. Conversely, if stratification decreases due to increased mixing in a stormier climate, the chances for oxygen depletion will decrease. For the Mississippi River basin associated with the northern Gulf of Mexico seasonal dead zone, climate predictions suggest a 20% increase in river discharge that would lead to elevated nutrient loading, a 50% increase in primary production, a 30–60% decrease in bottom water DO, and expansion of the oxygen-depleted area.

Global warming will lead to increased water temperatures, decreased oxygen solubility, increased organism metabolism and remineralization rates, and enhance stratification. If in the next 50 years humans continue to modify and degrade coastal systems as in previous years, human population pressure will be the main driving factor in spreading of coastal dead zones, and climate change factors will be secondary (**Figure 2**). Climate forcing, however, will tend to make systems more

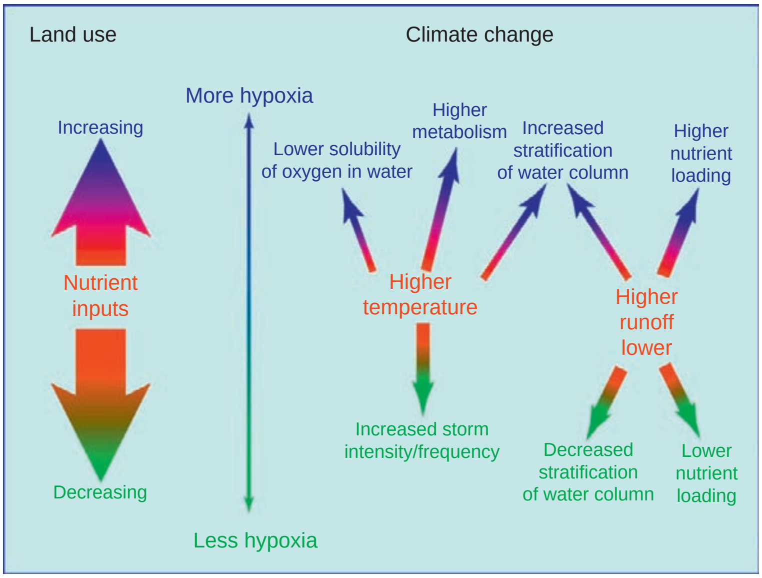


Figure 2 Relative contribution of global climate change and land use to future hypoxia. Thickness of the arrows indicates relative magnitude of effect.

susceptible to the development of hypoxia through direct effects on stratification, solubility of oxygen, metabolism, and mineralization rates.

See also: **Bony Fishes**: Crucian Carp. **Hypoxia**: Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia; Respiratory Responses to Hypoxia in Fishes. **Pelagic Fishes**: An Introduction to the Biology of Pelagic Fishes. **Temperature**: Effects of Climate Change. **Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange.

Further Reading

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology – Progress Series* 210: 223–253.

Díaz RJ and Breitburg DL (2009) The Hypoxic Environment. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology,* vol. 27, pp. 1–23. Burlington: Academic Press.

Díaz RJ and Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321: 926–929.

Helly JJ and Levin LA (2004) Global distribution of naturally occurring marine hypoxia on continental margins. *Deep-Sea Research (Part I)* 51: 1159–1168.

Jones PD (2006) Water quality and fisheries in the Mersey estuary England: A historical perspective. *Marine Pollution Bulletin* 53: 144–154.

Karlson K, Rosenberg R, and Bonsdorff E (2002) Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian and Baltic waters – a review. *Oceanography and Marine Biology: An Annual Review* 40: 427–489.

Keeling RF, Körtzinger A, and Gruber N (2010) Ocean deoxygenation in a warming world. *Annual Review of Marine Science* 2: 199–229.

Mee L (2006) Reviving dead zones. *Scientific American* 295: 78–85.

Rabalais NN, Díaz RJ, Levin LA, *et al.* (2010) Dynamics and distribution of natural and human-caused coastal hypoxia. *Biogeosciences* 7: 585–619.

Turner RE, Rabalais NN, and Justic' D (2008) Gulf of Mexico hypoxia: alternate states and a legacy. *Environmental Science and Technology* 42: 2323–2327.

Vaquer-Sunyer R and Duarte CM (2008) Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences of the United States of America* 105: 15452–15457.

Respiratory Responses to Hypoxia in Fishes

SF Perry, University of Ottawa, Ottawa, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
What Is the Hypoxic Hyperventilatory Response?
What Is the Benefit of the Hypoxic Ventilatory Response?
What Are the Pathways Mediating the Hypoxic Ventilatory Response?
Ontogeny and Plasticity of the Hypoxic Ventilatory Response
Further Reading

Glossary

Aquatic surface respiration The phenomenon whereby some fish skim relatively O_2-enriched water at the air–water interface to pass over their gills.

Bohr effect Effect of the proton concentration (pH) on the oxygen affinity of hemoglobin.

Critical P_{O_2} Defined as the environmental P_{O_2} at which fish are no longer able to maintain normal rates of aerobic O_2 consumption.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Pseudobranch A structure present in some fish, it is a reduced gill arch that lies along the inner edge of the opercular flap; its function is largely unknown.

Ram ventilation The process whereby fish are able to ventilate their gills by simply swimming forward with open mouths.

Introduction

Hypoxia, a lowering of the partial pressure of O_2 (P_{O_2}) in the ambient environment (**see also Hypoxia**: The Expanding Hypoxic Environment), occurs in aquatic ecosystems for various reasons including: (1) inadequate mixing of water, (2) vertical stratification of water columns, (3) excessive O_2 consumption in waters densely populated with organisms, and (4) a lowering of barometric pressure as occurs with increasing altitude. Fish might experience aquatic hypoxia continuously (e.g., fish living in a high-altitude lake) or alternatively they might experience hypoxia seasonally or diurnally. Good examples of species experiencing hypoxia on a diurnal basis are fish inhabiting the flood basins of the Amazon.

Depending on the severity of hypoxia, fish respond with an array of behavioral, cardiovascular, respiratory, and metabolic responses aimed at preserving homeostasis (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia, **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism, **Hypoxia**: Anaerobic Metabolism in Fish, **Bony Fishes**: Crucian Carp, and **Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P_{50}). Arguably, the most important of these responses is hyperventilation, which is defined as an increase in the volume of water flowing over the gills per unit time (minute ventilation volume, $\dot{V}_w$). Fish can hyperventilate by changing one or both of the variables that determine $\dot{V}_w$, which are breathing frequency (f_V) and stroke volume (V_{SV}). Increasing $\dot{V}_w$, however, comes at a considerable cost owing to the high energetic requirements associated with moving large volumes of water, a dense and viscous medium (at least relative to air). For this reason, there is considerable interspecific variation in the degree of hypoxia required to initiate hyperventilation. Moreover, hyperventilation, while being an appropriate strategy for mild or moderate hypoxia, may be less desirable or even inappropriate when hypoxia becomes so severe that the rising metabolic cost of increasing $\dot{V}_w$ outweighs any benefit.

What Is the Hypoxic Hyperventilatory Response?

Simply put, hypoxic hyperventilation refers to increased breathing associated with a lowering of environmental P_{O_2}. This simple definition, however, does not do justice to the impressive assortment of ventilatory responses exhibited by fish experiencing hypoxia, nor does it adequately explain the varied strategies employed by water breathers, air breathers, and bimodal breathing fish.

Water Breathers

Water-breathing fish ventilate their gills actively through the action of oscillating buccal and opercular pumps, passively by simply swimming through the water with an open mouth (a strategy referred to as ram ventilation), or by some combination of active and ram ventilation. Tunas and some shark species breathe by ram ventilation and upon exposure to hypoxia are able to increase $\dot{V}_{\mathrm{w}}$ by increasing their mouth gape. Thus, in these fish, there is relatively little cost (except that incurred by the increased hydrodynamic drag) associated with hyperventilation. On the other hand, if sufficiently severe, hypoxia will impair swimming capacity and thus $\dot{V}_{\mathrm{w}}$ may be reduced regardless of the gape of the mouth. Although there is great diversity and some exceptions, increases in $\dot{V}_{\mathrm{w}}$ in actively breathing fish are achieved predominantly through increasing V_{SV} and to a lesser extent by elevating f_{V}. This strategy is considered to be energetically less costly than relying predominantly on changes in f_{V}. In some species that normally exhibit periodic (or episodic) breathing (e.g., carp, sturgeon, eel, and zebrafish), hypoxia may cause a switch to continuous breathing (e.g., see **Figure 1**).

In some species, hypoxia may initiate a totally different manner of breathing water, termed aquatic surface respiration (ASR). During ASR, fish rise to the surface and skim the O_2-enriched water at the air–water interface which then flows across the gills. Perhaps owing to the increased predation risks associated with ASR, fish typically exploit this strategy only at severe levels of hypoxia and well after standard hyperventilatory responses are generally initiated. The risk of predation makes it less likely that smaller fish will exploit ASR than larger fish, all other things being equal.

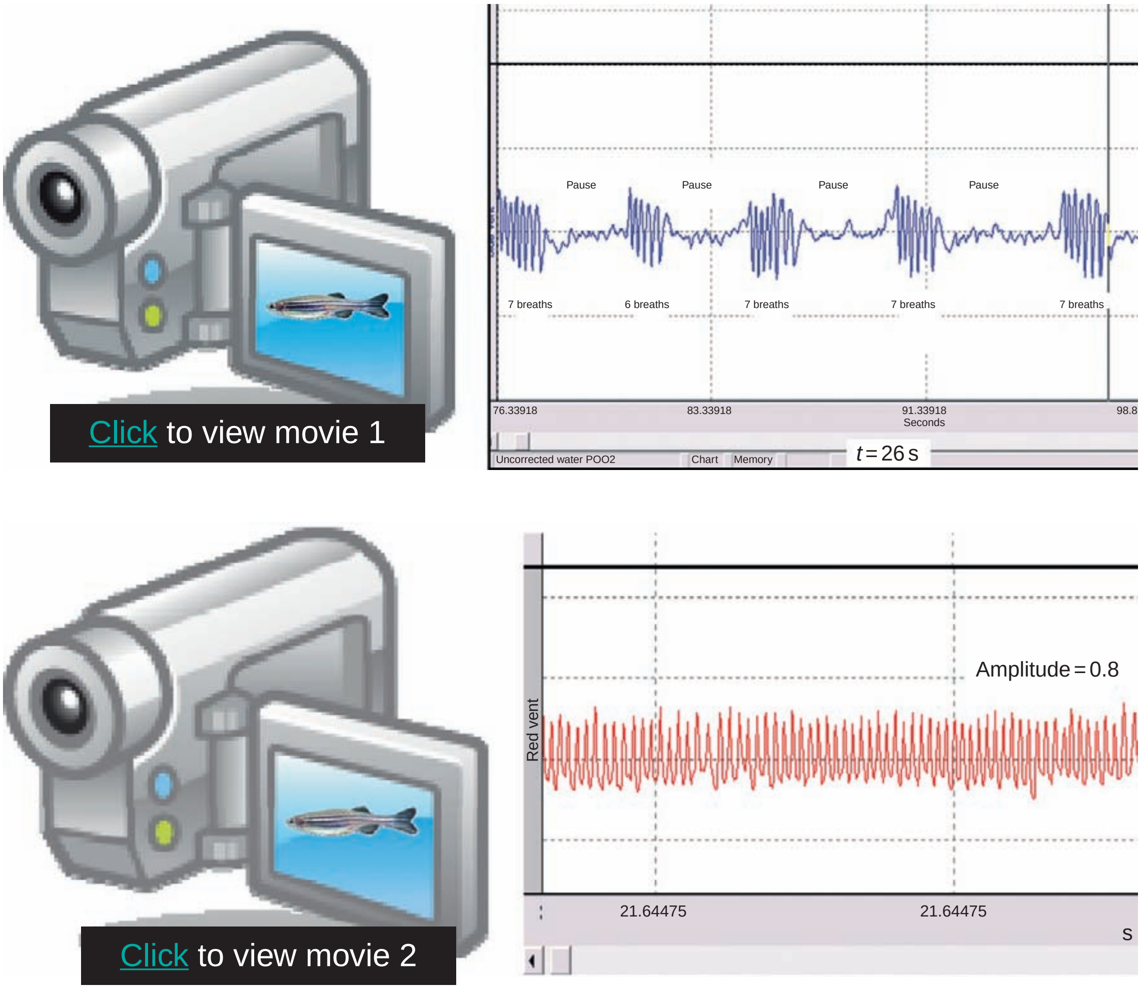


Figure 1 Representative data recordings and accompanying video images (from the same fish) demonstrating markedly different breathing patterns in zebrafish under normoxic or hypoxic conditions. The data recordings represent oscillating voltage changes recorded from the water that are associated with breathing (each peak represents a single breath). Under conditions of normoxia (**Slide 1/Video Clip 1**), zebrafish typically (although not always) display episodic breathing characterized by clusters of breaths separated by distinct pauses or periods of apnea. Under conditions of hypoxia (**Slide 2/Video Clip 2**), zebrafish exhibit continuous breathing which is characterized by a large increase in breathing frequency. These data were provided by Branka Vulesevic (previously unpublished).

Air Breathers

Two strategies for breathing air exist in fish; the facultative air breathers supplement aquatic gill breathing with episodic aerial breathing, while the obligate air breathers must continually breathe air to survive and indeed will drown if denied access to air. Although there is some uncertainty as to the response of obligate air breathers to aquatic hypoxia (i.e., there is evidence both for and against a hypoxic hyperventilatory response), the responses of facultative air breathers are well characterized. In some species (e.g., armored catfish (*Ancistrus chagresi*) and jeju (*Hoplerythrinus unitaeniatus*)), air breathing is normally absent under normoxic conditions but is initiated at levels of aquatic hypoxia usually corresponding to maximal rates of gill $\dot{V}_{\mathrm{w}}$. Similar to ASR, the predation risks associated with air breathing mean that this strategy is exploited only at severe levels of hypoxia, often near the critical $P_{\mathrm{O_2}}$ (**Figure 2**). In other species, the frequency of air breathing, already present under normoxic conditions, is increased by aquatic hypoxia.

What Is the Benefit of the Hypoxic Ventilatory Response?

The ventilatory responses to hypoxia, namely hyperventilation of the gill, ASR, and/or air breathing, represent attempts to maintain O_2 uptake in the face of declining aquatic O_2 levels. The rate of branchial O_2 transfer is governed by diffusivity, convection (ventilation and perfusion), and the blood-to-water $P_{\mathrm{O_2}}$ gradient (the driving force for O_2 diffusion). During hyperventilation, increased water flow across the gill serves to raise the mean blood-to-water $P_{\mathrm{O_2}}$ gradient. Effectively, fewer O_2 molecules can be removed from the water during the shortened duration of transit over the gill, which raises the average $P_{\mathrm{O_2}}$ of the ventilatory water and results in an elevation of arterial $P_{\mathrm{O_2}}$. Hyperventilation during hypoxia therefore serves to minimize the extent of the reduction in arterial $P_{\mathrm{O_2}}$ (and hence arterial O_2 content) that is the inevitable consequence of lowering water $P_{\mathrm{O_2}}$.

Although theory readily predicts a beneficial effect of hyperventilation on raising arterial $P_{\mathrm{O_2}}$, it is more challenging to provide direct empirical data in support especially considering that many protocols or environmental conditions known to elicit hyperventilation may simultaneously influence other factors (e.g., circulating catecholamine levels or blood flow) regulating the rate of O_2 diffusion across the gill. Hence, most experimental protocols cannot easily distinguish between the specific effects of ventilatory adjustments from other factors such as changes in blood-flow patterns and acute changes in functional lamellar surface area. Recently, investigators were able to record continuously in real time, arterial $P_{\mathrm{O_2}}$ and ventilation from goldfish (*Carassius auratus*) experiencing injections of the ventilatory stimulant NaCN into the buccal cavity. These data (**Figure 3**) clearly demonstrate a cyclical rise and fall of PaO_2 that is in phase with the increasing and decreasing $\dot{V}_{\mathrm{w}}$.

The benefit of hyperventilation becomes particularly important as arterial $P_{\mathrm{O_2}}$ approaches the P_{50} value of hemoglobin (the $P_{\mathrm{O_2}}$ at which hemoglobin is 50% saturated with O_2), where the steep slope of the O_2 equilibrium curve means that even small differences in arterial $P_{\mathrm{O_2}}$ can have a

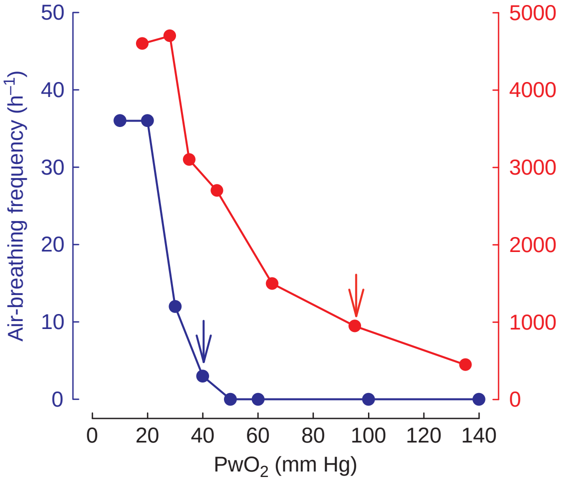


Figure 2 The effects of aquatic hypoxia on gill minute ventilation (*Vg*; red symbols and lines) and air-breathing frequency (blue symbols and lines) in the tropical fish jeju (*Hoplerythrinus unitaeniatus*). The red and blue arrows represent the onset of gill hyperventilation and air breathing, respectively. Air-breathing data are from Perry SF, Reid SG, Gilmour KM, *et al.* (2004) A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. *American Journal of Physiology* 287: 188–197, while the gill ventilation data are from Oliveira RD, Lopes JM, Sanches JR, *et al.* (2004) Cardiorespiratory responses of the facultative air-breathing fish jeju, *Hoplerythrinus unitaeniatus* (Teleostei, Erythrinidae), exposed to graded ambient hypoxia. *Comparative Biochemistry and Physiology* 139: 479–485.

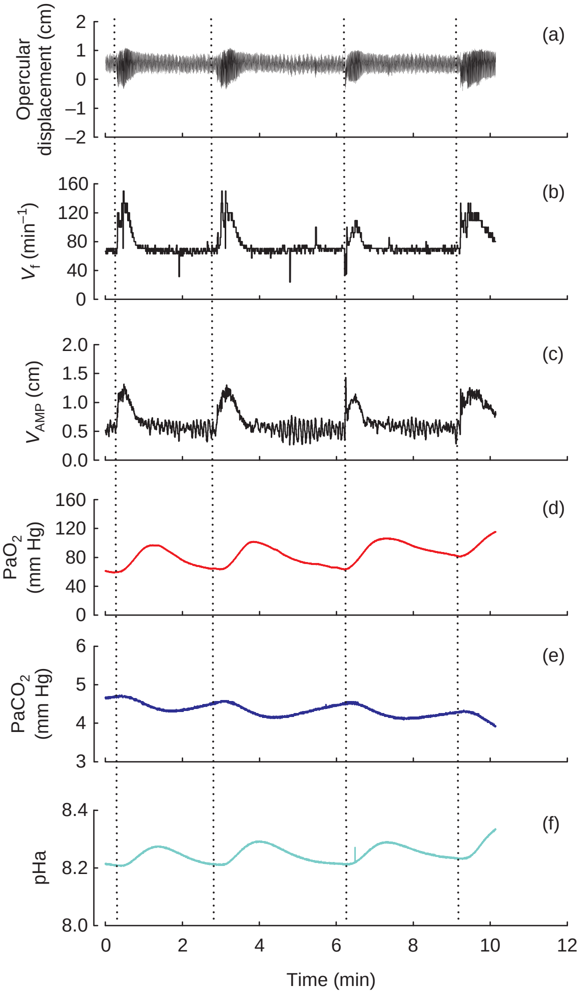


Figure 3 Representative recordings illustrating the effects of four consecutive NaCN injections (indicated by the dotted lines) into the buccal cavity of a goldfish (*Carassius auratus*) on (a)–(c) ventilation parameters and (d)–(f), arterial blood gases. The measured ventilation parameters are (a) linear deflections of the opercular flaps as measured by impedance changes, (b) ventilation frequency (f_V), and (c) ventilation amplitude (V_{AMP}) as determined from the impedance trace. Arterial blood was continuously monitored for (d) P_{O_2} (PaO_2), (e) PCO_2 ($PaCO_2$), and (f) pH (pHa). Note that each bout of hyperventilation associated with NaCN injection caused transient increases in PaO_2 and pHa and decreases in $PaCO_2$. These data were provided by Velislava Tzaneva (previously unpublished).

relatively large impact on arterial O_2 content. In addition to defending arterial P_{O_2}, hyperventilation during hypoxia produces a respiratory alkalosis as arterial PCO_2 is lowered by equilibration of the arterial blood with ventilatory water of lower mean PCO_2. Elevation of red blood cell pH following from the respiratory alkalosis can, in turn, increase the affinity of hemoglobin for O_2 via the Bohr effect, thereby aiding O_2 uptake (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange and Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder). The benefit of the hypoxic hyperventilatory response, then, is increased branchial O_2 transfer which contributes to the maintenance of metabolic rate under hypoxic conditions in oxyregulators (fish that maintain a constant metabolic rate during hypoxia).

The trade-off is that more and more water is moved across the gills as less O_2 is removed from the water per unit volume moved. Thus, the downside of hypoxic hyperventilatory response is an increasing energy expenditure on ventilation, the cost of which is high even at rest in water-breathing fish. The maintenance of metabolic rate under these circumstances eventually becomes futile when the cost of increasing ventilation to maintain O_2 uptake from an environment of reduced O_2 availability eventually exceeds the benefits of the O_2 so obtained. The switch to oxygen conforming (the strategy of lowering metabolic rate with reduced ambient P_{O_2}), often with a concomitant reduction in $\dot{V}_w$, will lower the energetic expenditure on ventilation (**see also Hypoxia**: Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia).

What Are the Pathways Mediating the Hypoxic Ventilatory Response?

Unlike birds and mammals, which possess a predominant lone site of O_2 sensing (the O_2 chemoreceptors of the carotid body), fish and other lower vertebrates may exhibit multiple sites of O_2 chemoreception. The O_2 chemoreceptors that initiate hyperventilatory responses are oriented in such a way as to sense changes in both external and internal P_{O_2} levels, although it is unclear whether the receptors comprise a single population of cells positioned to simultaneously sense both environments or separate cell populations each positioned to monitor external (via external receptors) or internal (via internal receptors) P_{O_2} levels (**see also Control of Respiration**: Oxygen Sensing in Fish).

Branchial O_2 Chemoreceptors

The most important site of O_2 chemoreception in fish is the gill with some species exhibiting a preferential distribution of O_2 receptors to the first gill arches. As predicted from the pioneering studies of Pierre Laurent and colleagues, Susanne Dunel-Erb and Yanick Bailly, the O_2-sensing cells of the gill have been identified as neuroepithelial cells (NECs) which are structurally and functionally analogous to the O_2-sensing glomus (type-1) cells of the mammalian carotid body. As many (but, not all) NECs contain an abundance of serotonin (5 hydroxytryptamine (5-HT)), they can be readily identified by immunofluorescence using anti-serotonin antibodies. There are two major populations of branchial NECs (see **Figure 4**): one

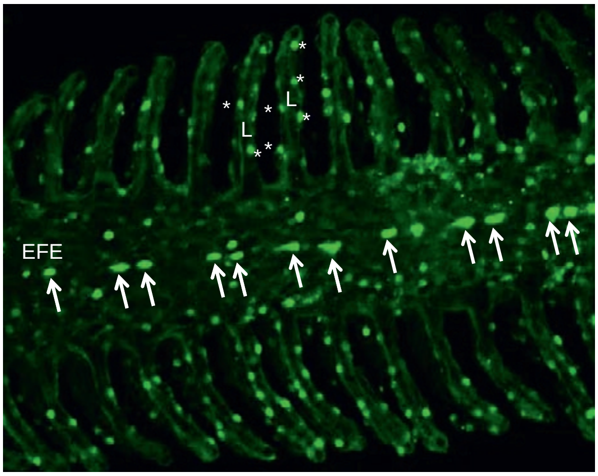


Figure 4 Whole mount immunofluorescence image of a goldfish gill treated with antibodies against serotonin to indicate (green fluorescence) the presence of two populations of neuroepithelial cells. One population (arrows) resides below the efferent filament epithelium (EFE) and above the efferent filament artery, while another (asterisks) can be found on the external surfaces of lamellae (L). This image was provided by Velislava Tzaneva (previously unpublished).

population resides below the efferent filament epithelium and above the efferent filament artery, while another can be found on the external surfaces of lamellae. Thus, it is believed that NECs of the filament are ideally suited to sense both water and blood P_{O_2} while the NECs of the lamellae most likely sense water P_{O_2} only and thus function exclusively as external O_2 chemoreceptors.

The definitive proof that NECs are O_2 chemoreceptors was provided by Michael Jonz studying zebrafish and later corroborated by Mark Burleson studying channel catfish (*Ictalurus punctatus*) (**see also Control of Respiration**: Oxygen Sensing in Fish). Using patch-clamp techniques and cultured cells, it was demonstrated that NECs experience membrane depolarization in response to hypoxia which in turn is related to an inhibition of K^+ conductance through background K^+ channels. The membrane depolarization is the trigger that ultimately leads to neurotransmitter secretion and the downstream efferent neural pathways underlying reflex hypoxic hyperventilation. Interestingly, as in the mammalian carotid body glomus cells, the NECs of zebrafish also respond to elevated CO_2 (hypercapnia) and thus likely play an important role in mediating both hypoxic and hypercapnic ventilatory responses.

Extra-Branchial O_2 Chemoreceptors

The pseudobranch (**see also Role of the Gills**: Morphology of Branchial Ionocytes) is frequently mentioned as an O_2-sensing site on the basis of its morphology and the responsiveness of *in vitro* perfused preparations to hypoxic saline. Yet, neither bilateral sectioning of pseudobranch afferent nerve fibers nor pseudobranch ablation altered the ventilatory response of trout to external hypoxia. In addition, many species known to exhibit robust ventilatory responses to hypoxia lack a pseudobranch (e.g., channel catfish). Thus, at present, there is no solid evidence to implicate the pseudobranch as an extra-branchial site of O_2 sensing. The central nervous system (brain) has also been implicated as a site of O_2 chemoreception largely on the basis of studies performed in the 1970s. More recent studies, however, employing more direct approaches have failed to provide any evidence to support the existence of central O_2 chemoreceptors in fish.

In the absence of any direct data to support a role for the pseudobranch or central nervous system in O_2 sensing, the persistence of ventilatory responses to hypoxia in fish experiencing gill denervation may reflect the presence of extra-branchial receptors within the orobranchial cavity. An alternate and not mutually exclusive hypothesis is that ventilatory responses attributed to extra-branchial O_2 receptors may, in fact, arise from the release of catecholamines (adrenaline and noradrenaline) into the circulation (**see also Hormonal Responses to Stress**: Catecholamines). The principal evidence that led to the theory of a supporting role for circulating catecholamines in stimulating breathing is that these hormones are released into the bloodstream during acute hypoxia, coupled with reports that intravascular injections of catecholamines can evoke hyperventilatory responses in European eel, *A. anguilla*. However, upon analysis of all existing data it is clear that circulating catecholamines do not play a general stimulatory role in the control of breathing during hypoxia, although in certain species it is conceivable that they play a supplementary role at very severe levels of hypoxia when O_2 chemoreceptors may be inhibited. The reader is encouraged to survey the evidence both for and against a role for circulating catecholamines in the control of breathing in comprehensive review articles listed under the section 'Further reading'.

Ontogeny and Plasticity of the Hypoxic Ventilatory Response

Zebrafish respond to hypoxia as early as 2 days post fertilization (dpf), well before the gill lamellae are developed and prior to innervation of the gill filament primordia. At such early life stages, hyperventilatory responses may be driven by O_2 chemoreceptors (perhaps NECs) residing on the external surfaces of the skin. By 7 dpf, the NECs of the developing gill filaments become innervated and this coincides with a sudden increase in the magnitude of the hypoxic ventilatory response. Given the prevailing view that the gill does not play a role in O_2

uptake until about 14 dpf, it begs the question as to the physiological significance of hypoxic hyperventilation in these young fish.

The reflex ventilatory response of adult fish to hypoxia can be markedly influenced by the environmental conditions during a critical period of embryonic and larval development. For example, zebrafish reared under conditions of hyperoxia for the first 7 dpf and then raised 3–4 months in normal water exhibit a blunted ventilatory response to acute hypoxia and the ventilatory stimulant NaCN. This phenomenon whereby adult responses can be shaped by early life experiences is termed 'developmental plasticity'.

In addition to developmental plasticity, the hypoxic ventilatory responses can be influenced by acclimatization. For example, adult zebrafish, maintained under hyperoxic conditions for 28 days, exhibit a reduced capacity to hyperventilate during subsequent acute hypoxia. In contrast to the significant effects of chronic hyperoxia, prior acclimatization of adult zebrafish to hypoxic conditions or rearing them in hypoxic water does not appear to influence subsequent acute hypoxic ventilatory responses. Yet, in catfish, acclimatization to moderate hypoxia for 7 days causes a marked increase in the capacity to hyperventilate during subsequent acute hypoxia. Clearly, it would be premature to draw any general conclusions regarding plasticity of the hypoxic ventilatory response until additional species are examined.

See also: **Bony Fishes**: Crucian Carp. **Control of Respiration**: Oxygen Sensing in Fish. **Hormonal Responses to Stress**: Catecholamines. **Hypoxia**: Anaerobic Metabolism in Fish; Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia; The Expanding Hypoxic Environment. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia. **Role of the Gills**: Morphology of Branchial Ionocytes. **Swimming and Other Activities**: Cellular Energy Utilization: Environmental Influences on Metabolism. **Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange; O_2 Uptake and Transport: The Optimal P_{50}; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder.

Further Reading

Burleson ML, Mercer SE, and Wilk-Blaszczak MA (2006) Isolation and characterization of putative O_2 chemoreceptor cells from the gills of channel catfish (*Ictalurus punctatus*). *Brain Research* 1092: 100–107.

Dunel-Erb S, Bailly Y, and Laurent P (1982) Neuroepithelial cells in fish gill primary lamellae. *Journal of Applied Physiology* 53: 1342–1353.

Gilmour KM and Perry SF (2007) Branchial chemoreceptor regulation of cardiorespiratory function. In: Hara TJ and Zielinski B (eds.) *Sensory Systems Neuroscience*, pp. 97–151. San Diego, CA: Academic Press.

Graham JB (1997) *Air-Breathing Fishes*, pp. 1–299. San Diego, CA: Academic Press.

Jonz MG, Fearon IM, and Nurse CA (2004) Neuroepithelial oxygen chemoreceptors of the zebrafish gill. *Journal of Physiology* 560: 737–752.

Oliveira RD, Lopes JM, Sanches JR, *et al.* (2004) Cardiorespiratory responses of the facultative air-breathing fish jeju, *Hoplerythrinus unitaeniatus* (Teleostei, Erythrinidae), exposed to graded ambient hypoxia. *Comparative Biochemistry and Physiology* 139: 479–485.

Perry SF, Jonz MG, and Gilmour KM (2009) Oxygen sensing and the hypoxic ventilatory response. In: Richards JG, Brauner CJ, and Farrell AP (eds.) *Hypoxia*, pp. 193–251. Amsterdam: Academic Press.

Perry SF, Kinkead R, and Fritsche R (1992) Are circulating catecholamines involved in the control of breathing by fishes? *Reviews in Fish Biology and Fisheries* 2: 65–83.

Perry SF, Reid SG, Gilmour KM, *et al.* (2004) A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. *American Journal of Physiology* 287: 188–197.

Randall DJ and Taylor EW (1991) Evidence of a role for catecholamines in the control of breathing in fish. *Reviews in Fish Biology and Fisheries* 1: 139–157.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Anaerobic Metabolism In Fish

Y Wang, Queen's University, Kingston, ON, Canada

JG Richards, The University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Overview of the ATP-Generating Pathways in Fish
The Fate of Pyruvate
Lactate and Acid
Why Use Anaerobic Metabolism?
Anaerobic Metabolism and Hypoxia Exposure in Fish
Anaerobic Metabolism and Exhaustive Exercise
Further Reading

Glossary

Krebs cycle This is a cyclic pathway located in the mitochondrial matrix that liberates electrons from carbon substrates for donation to the electron transport chain. The Krebs cycle is also known as the tricarboxylic acid cycle and citric acid cycle.

Oxidative phosphorylation A process in the mitochondrion in which the synthesis of ATP (adenosine triphosphate) is driven by (1) the transfer of electrons alone the electron transfer chain to molecular O_2 and (2) coupling to an electrochemical H^+gradient.

Reducing equivalents Molecules derived from vitamins that transfer electrons in redox reactions: mainly NADH and $FADH_2$.

Substrate-level phosphorylation A chemical reaction that results in the formation of ATP by the direct transfer of a phosphate group to ADP from a reactive intermediate.

Introduction

Most biological functions, ranging from powering a membrane-bound ion transporter to the generation of mechanical work through muscle contraction, require the input of metabolic energy in the form of adenosine triphosphate (ATP). ATP is generated by a series of metabolic biochemical pathways that transform the chemical energy stored in food molecules (fats, sugars, and proteins) into usable metabolic energy. The metabolic pathways responsible for this process fall into two broad categories: aerobic metabolism and anaerobic metabolism.

The terms 'aerobic' versus 'anaerobic' provide some initial insight into the differences in these groups of pathways. Aerobic means 'with oxygen', whereas anaerobic means 'without oxygen'. Thus, aerobic generation of ATP via aerobic metabolism, by definition, requires the input of oxygen, whereas anaerobic metabolism does not require any oxygen to generate ATP. This article briefly summarizes the metabolic pathways involved in aerobic metabolism, and then focuses on the biochemical pathways involved in anaerobic metabolism and when in fish these pathways are used.

General Overview of the ATP-Generating Pathways in Fish

Pathways of Aerobic Metabolism

For the most part, fish are similar to other vertebrates in terms of their basic metabolism. Under fully aerobic, resting conditions, more than 95% of the ATP required by a cell is derived from mitochondrial-based aerobic metabolism, referred to as oxidative phosphorylation (**see also Tissue Respiration**: Mitochondrial Respiration and Specializations in Mitochondrial Respiration of Fish). Carbon substrates, usually fats, carbohydrates, and to a lesser extent proteins, are broken down by a series of biochemical pathways whose products ultimately feed into the Krebs cycle, located inside the mitochondria. Fats, in the form of fatty acids, are broken down first by a biochemical pathway called β-oxidation, which liberates acetyl-coenzyme A (acetyl-CoA), which is then transported into the mitochondria and enters the Krebs cycle (**see also Energetics**: General Energy Metabolism). Carbohydrates, as glucose, are partially broken down by glycolysis into pyruvate, which enters the mitochondria and is converted into acetyl-CoA by the enzyme pyruvate dehydrogenase (see **Figure 1**). The pathways involved in amino acid breakdown for energy

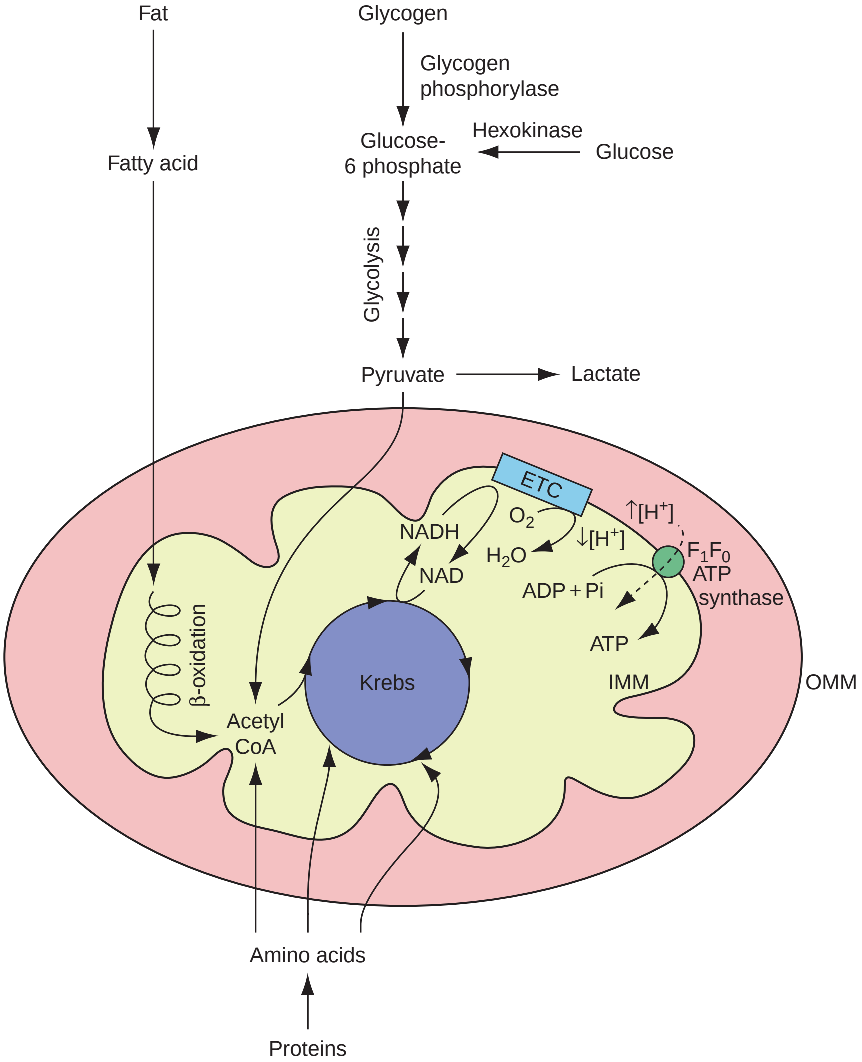


Figure 1 Biochemical map of oxidative phosphorylation and its feeder pathways ETC, electron transport chain; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; Krebs, Krebs cycle. See text for other abbreviations.

are more varied than for fat and carbohydrate oxidation, because depending on the nature of the amino acid that is catabolized, different pathways are used (**see also Energetics**: General Energy Metabolism). Ultimately, however, no matter what pathway is employed in the oxidation of amino acids for energy production, the products of the pathways are delivered to the Krebs cycle either through acetyl-CoA or through other intermediates in the pathways.

The Krebs cycle liberates electrons from carbon substrates and transfers them, as reducing equivalents to molecules such as nicotinamide adenine dinucleotide (NAD) forming reduced NAD (NADH) or flavin adenine dinucleotide (FAD) forming reduced FAD (FADH2). These reducing equivalents, along with electrons stored in the succinate generated by the Krebs cycle, then donate their electrons to a series of proteins embedded in the inner mitochondrial membrane, which are called the electron transport chain proteins (**see also Tissue Respiration**: Mitochondrial Respiration and Specializations in Mitochondrial Respiration of Fish).

As electrons pass along the electron chain proteins, protons are consumed from the inside of the mitochondria and/or transported from the inside of the mitochondria to the outside of the mitochondria. The final electron acceptor of the electron transport chain is oxygen, which accepts electrons while combining with protons forming water. In its simplest sense, the electron transport chain functions to reduce the proton concentration inside the mitochondria (increase pH) while increasing the proton concentration (decrease pH) outside the mitochondria. This generates a proton gradient across the mitochondrial inner membrane, termed the proton motive force, which serves as the driving force for

ATP synthesis. The energy stored within the proton motive force is harnessed for ATP generation though a specific protein called the F_1F_0-ATP synthase, which is an inner mitochondrial membrane-bound protein through which protons can flow. The flow of protons causes the molecular machinery of the F_1F_0-ATP synthase to turn, providing the energy to form ATP from adenosine diphosphate (ADP) and inorganic phosphate. Thus, the energy stored in the proton gradient is used to generate ATP.

The simplified biochemical equation for the aerobic oxidation of glucose by oxidative phosphorylation is

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + 38\,\mathrm{ATP} \qquad (1)$$

As can be seen from eqn (1), oxygen is an essential part of the oxidative phosphorylation and is the reason it is called 'aerobic'.

Pathways of Anaerobic Metabolism

Anaerobic metabolism, which can be defined as ATP production without oxygen (or in the absence of oxygen), occurs by direct phosphate transfer from phosphorylated intermediates, such as glycolytic intermediates or creatine phosphate (CrP), to ADP forming ATP. This process of direct phosphate transfer from a substrate to ADP forming ATP is termed 'substrate-level phosphorylation' and takes place in the cytoplasm, in contrast to oxidative phosphorylation that takes place in the mitochondria in aerobic metabolism.

Although both processes of ATP generation can occur under any physiological condition, the amount of ATP produced per mole of substrate consumed is approximately 15- to 30-fold lower using substrate-level phosphorylation than if mitochondrial-based oxidative phosphorylation is used to generate ATP.

There are two main anaerobic pathways responsible for ATP production in fish: CrP hydrolysis and glycolysis yielding lactate accumulation.

CrP hydrolysis. To exercise enthusiasts, creatine is a rather well-known compound composed of parts from three different amino acids, including arginine, glycine, and methionine. Creatine can be phosphorylated by the following reaction, which is catalyzed by the enzyme creatine phosphokinase or simply creatine kinase:

$$\text{creatine} + \text{ATP} \rightarrow \text{phosphocreatine} + \text{ADP} + \text{H}^+ \qquad (2)$$

This reaction is reversible. During periods of energy excess, when ATP is not being utilized by other processes at appreciably high rates and oxygen is plentiful (i.e., under normoxic/resting conditions), the creatine kinase reaction favors CrP formation and CrP is maintained at a high concentration in the tissue. For example, in the white muscle of rainbow trout (*Oncorhynchus mykiss*) under resting normoxic conditions, CrP levels up to 45 or 50 mM exist. In contrast, under conditions of where energy demand rapidly increases or oxygen can no longer be supplied to the tissues, CrP serves as a reserve of high-energy phosphates that can be rapidly mobilized; CrP can 'anaerobically' donate phosphate groups to ADP forming ATP. Thus, CrP serves as a kind of phosphate energy buffer in the cell and this role is most commonly observed in muscle. Although CrP levels in fish muscle seem quite high relative to the ATP concentration, which is up to 5 mM in trout muscle, the CrP reserve is rapidly exhausted during periods of intense exercise. Phosphocreatine levels are typically high in skeletal muscle and nervous tissue in fish, which have high and fluctuating energy demands, but low (often less than 5 mM) in other organs.

Glycolysis. The term 'glycolysis' is a combination of the ancient terms 'glycose' meaning sugar and 'lysis' meaning to break. Therefore, quite literally, glycolysis means the breaking of sugar. Glycolysis is a ubiquitous pathway seen in many organisms from yeast, to animals and plants, with few modifications. The main substrate for glycolysis is glucose; however, most cells keep free glucose concentrations relatively low (5 mM). Consequently, tissues that rely on glycolysis to a large extent, store glucose as a carbohydrate polymer called glycogen. Glycogen is a highly branched string of glucose molecules, each of which can be enzymatically cleaved off to feed glucose (as glucose-1 phosphate) into glycolysis.

The simplified biochemical equation for glycolysis is

$$\begin{aligned}&\text{Glucose} + 2\text{NAD} + 2\text{ADP} + 2\ \text{inorganic phosphate}\\&\quad\rightarrow 2\ \text{pyruvate} + 2\ \text{NADH} + 2\text{H}^+ + 2\text{ATP}\end{aligned}$$

Thus, a six-carbon glucose molecule is converted into two-, three-carbon pyruvate molecules plus protons, while simultaneously releasing energy to generate ATP from ADP and store reducing equivalents in the form of NADH.

The specific enzymatic steps of glycolysis are shown in **Figure 2**. Glycolysis is a sequence of 10 enzymatic reactions that can be divided into three stages. In the first stage, often called the preparatory stage, the glucose molecule is phosphorylated (the addition of phosphates) on each of its carbon ends. This process requires three enzymes (hexokinase, phosphoglucose isomoerase, and phosphofructokinase) and yields three metabolic intermediates. In the second stage, the enzyme aldolase splits the six-carbon fructose 1,6-bisphosphate into two-, three-carbon molecules (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) and then thriose phosphate isomerase converts the dihydroxyacetone phosphate into glyceraldehyde 3-phosphate (isomerase

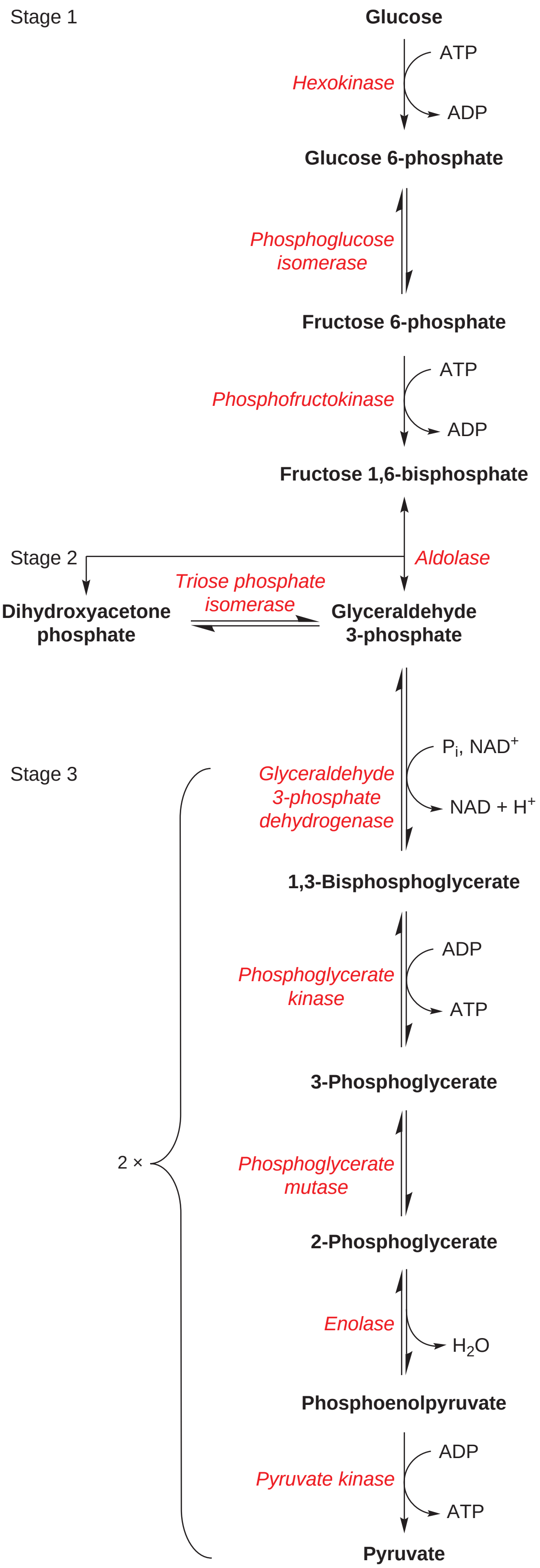


Figure 2 Glycolytic pathway showing the three main stages of glycolysis.

enzymes simply alter the molecular structure without the addition or subtraction of metabolites). Thus, at the end of the second stage there are two molecules of glyceraldehyde 3-phosphate ready to enter into the third stage of the pathway – the payoff stage. In the first step in the payoff stage, another phosphate is added to each glyceraldehyde 3-phosphate forming 1,3-bisphosphoglycerate (a three-carbon molecule phosphorylated at each end) while electrons liberated from the process are stored as NADH. The last four steps of glycolysis generate ATP by direct transfer of the four phosphates onto four molecules of ADP forming ATP.

The Fate of Pyruvate

Under aerobic conditions, the pyruvate generated by glycolysis is transported into the mitochondria through a specific transporter, the monocarboxylate transporter, and enters the Krebs cycle via the enzyme pyruvate dehydrogenase. This enzyme converts pyruvate into acetyl-CoA. At the same time, provided there is ample oxygen available and the electron transport chain is operational, the NADH generated in stage 3 of glycolysis is moved to the inside of the mitochondria through a specific shuttle system that regenerates NAD in the cytosol while supplying electrons to the electron transport chain for ATP generation. This system serves to keep NAD concentrations high and stable in the cytosol, so glycolysis can continue to function.

Under conditions when the mitochondria cannot accept the pyruvate and NADH generated by glycolysis, either because of oxygen limitation (hypoxia; **see also Hypoxia**: The Expanding Hypoxic Environment) or because pyruvate and NADH are being produced at a rate much higher than the mitochondria can accept, a mechanism to recycle NADH into NAD is needed. Without such a pathway for regenerating NAD, glycolysis will grind to a halt. Therefore, cells use the lactate dehydrogenase (LDH) reaction to convert the NADH into NAD, while simultaneously converting pyruvate into lactate, by the following reaction catalyzed by LDH:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{lactate} + \text{NAD} \qquad (3)$$

The enzyme LDH exists at high levels in most tissues, and especially those tissues that rely heavily on glycolysis for the anaerobic generation of ATP. The tongue muscle of hagfishes is reportedly to have one of the highest LDH activities (**see also Hagfishes and Lamprey**: Hagfishes).

Lactate and Acid

There is a very tight relationship between lactate formation and metabolic acid production (protons or H^+), such that as lactate accumulates during either exhaustive exercise or hypoxia exposure (discussed below), there is a decrease in cellular and blood pH. Because of the use of faulty biochemistry, it has long been thought that this tight relationship between lactate and acid production is a direct one through the formation of lactic acid. However, theoretical analysis performed over the past couple of decades clearly demonstrates that an increase in lactate concentration does not directly cause a cellular acidosis. Lactic acid is not formed because there is no proton to be liberated during lactate formation. In fact, eqn (3) clearly demonstrates that lactate production is a proton-consuming process. The acidosis is actually a product of ATP breakdown to ADP. During ATP hydrolysis, a proton is released:

$$\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathrm{H}^+ + \mathrm{Pi} \qquad (4)$$

Thus, ATP hydrolysis is the primary source of metabolic protons. During periods when ATP hydrolysis is low and met entirely by aerobic metabolism, the protons generated by ATP hydrolysis in the cytoplasm contribute to the generation of the mitochondrial proton motive force through oxidative phosphorylation. During periods of intense ATP hydrolysis, when the mitochondria cannot keep pace with the rate of ATP hydrolysis, the rate of proton production exceeds the buffering capacity of the cell, protons accumulate in the cytoplasm, and acidosis ensues. Both the lactate and hydrogen ions produced can be released into the blood, although not necessarily at the same rates or in the same amount. Therefore, the kinetics of of lactate and hydrogen ions in the blood differ during intense activity although both of them increase considerably in their concentrations.

Why Use Anaerobic Metabolism?

The answer to this question comes in part from a consideration of another question: Under what physiological conditions are anaerobic pathways used over aerobic pathways? Reliance on anaerobic metabolism to support ATP turnover occurs under two conditions, both of which are characterized by a limitation in the capacity of the mitochondria to generate ATP. First, under oxygen-limiting conditions, such as exposure to environmental hypoxia (**see also Hypoxia**: The Expanding Hypoxic Environment), the mitochondria is limited in its capacity to generate ATP because of a lack of its terminal electron acceptor, oxygen. Thus, the only way to generate ATP is to rely on pathways that do not require oxygen. Second, anaerobic metabolism is activated during periods of intense exercise, not because of an oxygen limitation, but because the rate at which ATP must be generated to support intense muscle contraction exceeds the rate at which mitochondria can produce ATP. Consequently, a faster pathway is selected (**see also Ventilation and Animal Respiration**: The Effect of Exercise on Respiration).

There is a large variation in the rate at which ATP can be generated by various metabolic pathways. For example, fatty acid oxidation by β-oxidation and mitochondrial oxidative phosphorylation can only generate ATP at a rate of ~20 μmol-ATP g^{-1} min^{-1} in mammalian muscle (**Figure 1**) The oxidation of glucose through aerobic glycolysis and oxidative phosphorylation generates ATP at a 50% faster rate, ~30 μmol-ATP g^{-1} min^{-1}. The anaerobic generation of ATP via glycolysis alone, yielding lactate production, can generate ATP at roughly twice the rate as aerobic oxidation of glucose, ~60 μmol-ATP g^{-1} min^{-1}. Even faster rates are possible with phosphocreatine hydrolysis, which can generate ATP at a rate somewhere between ~100 and 300 μmol-ATP g^{-1} min^{-1}. As a result, cells select pathways of ATP generation according to the metabolic demands the cell is experiencing (**Figure 3**). In exercising fish, for example, low-intensity exercise is primarily powered by fat oxidation and as exercise intensity increases faster and faster pathways are recruited, but the duration that the exercise can be sustained for decreases. For example, although CrP hydrolysis can generate ATP at a very high rate, it can only do so for a very short period of time because cellular CrP levels are normally kept low. In contrast, fats produce ATP at low rates, but because many fish have very large stores of fats, ATP can be generated for a very long periods of time.

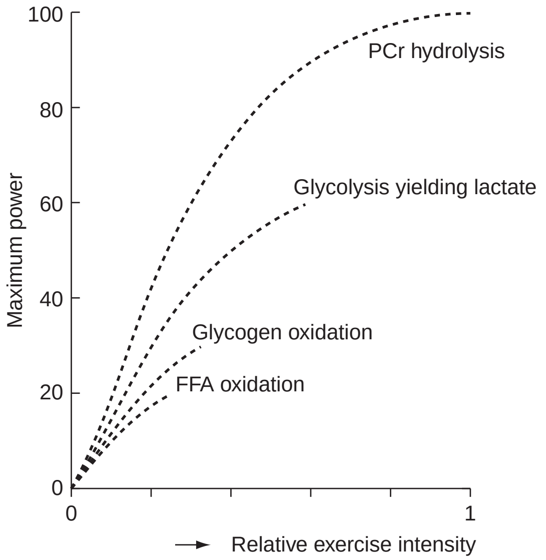


Figure 3 Relationship between relative exercise activity and fuel selection in muscle.

Clearly, the reason for using anaerobic metabolism is because ATP cannot be supplied at a sufficient rate by the mitochondria, no matter how efficient this process is. In the case of oxygen deficiency, mitochondria are shut down; in the case of intense exercise, muscular contractions are consuming ATP too fast for the mitochondria to keep up.

Anaerobic Metabolism and Hypoxia Exposure in Fish

Hypoxia exposure in fish elicits a strong activation of anaerobic metabolism, producing ATP from glycogen and CrP. Endogenous glycogen typically serves as the carbohydrate store for glycolysis. Thus, the levels of glycogen in a tissue are indicative of its capacity to support ATP turnover via glycolysis. Starvation will eventually deplete glycogen stores, limiting the period over which glycolysis can be maintained.

It has been fairly well documented that hypoxia-tolerant animals such as crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*) typically have higher levels of tissue glycogen relative to animals considered to be hypoxia sensitive (e.g., rainbow trout). Glycogen concentrations tend to be much higher in liver than any other tissues, including the heart, brain, and skeletal muscle. Liver glycogen is thought to serve as a repository of glucose that can be used by other tissues to support glycolytic ATP production during hypoxia exposure. This means that glucose is shuttled from the liver to other tissues during hypoxia, with tissues such as the brain and heart taking priority over the skeletal muscle.

The activity of the glycolytic enzymes can be altered by hypoxic acclimation. For example, in the killifish (*Fundulus grandis*), 4 weeks of hypoxia exposure at ~15% air saturation suppressed glycolytic metabolism in skeletal muscle, while several enzymes involved in glycolysis and carbohydrate oxidation increased in the liver. Fewer changes in glycolytic and glycogen enzymes were observed in the heart and brain compared with the liver and muscle, and those that did change, did so with a smaller magnitude. Interestingly, among the tissues that showed general increases in enzymes within the glycolytic pathway, the enzymes that increased were not always the same enzymes.

A small group of fish, including only the crucian carp, goldfish and bitterling (*Rhodeus amarus*), have the remarkable ability to convert lactate into ethanol and therefore excrete ethanol as their major anaerobic waste. A detailed description of this unique anaerobic end product can be found in **Bony Fishes**: Crucian Carp.

Anaerobic Metabolism and Exhaustive Exercise

Fish, particularly salmonids (e.g., the rainbow trout), have long served as a model for understanding exercise biochemistry. The reason for this popularity is twofold. First, in trout and many fish species, unlike mammals, there is a spatial separation of red and white muscle fibers allowing researchers to examine how different muscle fiber types respond during exercise. Second, methods for exercising fish are well established and there is a well-described gate transition in swimming so that the transition from slow sustainable swimming to burst nonsustainable swimming can be easily quantified. This gate transition, for the most part, is related to the switch from aerobically powered swimming to anaerobically powered swimming (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance).

From a muscle biochemistry perspective, the majority of studies with fish have focused on white muscle metabolism and the metabolic pathways involved in powering exercise and recovery. For the most part, exercising fish adhere to the classical scheme of substrate utilization during high-intensity exercise involving first CrP hydrolysis to power the first few tail flaps, then an activation of glycolysis with lactate accumulation. Over time, swimming intensity must necessarily decrease as muscle glycogen is depleted and metabolic wastes accumulate, and the fish slowly transition to more aerobic metabolism without burst activity. In rainbow trout, intense exercise elicited by chasing in a tank lasts between 1 and 5 min, and at the end of this exercise protocol the fish will have almost completely depleted muscle glycogen content, CrP and ATP content, as well as accumulate high levels of lactate and hydrogen ions, causing a severe acidosis. Numerous authors, however, have noted that wild fish are much better at intense exercise and something like a wild salmon can endure chasing much longer than domestic trout; however, the metabolic profile that characterizes an exhausted fish is not appreciably different.

Recovery from exhaustive exercise in rainbow trout has received considerable attention. During recovery, pathways must be activated to resynthesize ATP, CrP, and glycogen in preparation for another possible bout of exercise. The interesting case within salmonids is that they appear to keep most of the lactate produced in their muscle during exercise and limit the amount that is released into the blood. It is thought that lactate is retained in trout muscle so that it can serve as the direct substrate for glycogen resynthesis, by almost simple reversal of the glycolysis. This is in contrast to how mammals recover from intense exercise; they convert lactate into glucose in the liver and the muscle uses the subsequent glucose for glycogen resynthesis. Regardless of the location of glucose resynthesis, this recovery period of fish and mammals alike is accompanied by excess post-exercise O_2 consumption, which represents a stimulation of oxidative phosphorylation for ATP production to support the metabolic costs of recovery, including the replenishment of ATP, CrP, glycogen, and oxygen stores. It is now fairly well documented that the metabolic costs associated with recovery are fueled by fat oxidation.

See also: **Bony Fishes**: Crucian Carp. **Energetics**: General Energy Metabolism. **Hagfishes and Lamprey**: Hagfishes. **Hypoxia**: The Expanding Hypoxic Environment. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance. **Tissue Respiration**: Mitochondrial Respiration; Specializations in Mitochondrial Respiration of Fish. **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration.

Further Reading

Hochachka PW and Mommsen TP (1983) Protons and anaerobiosis. *Science* 219: 1391–1397.

Milligan CL, Hooke GB, and Johnson C (2000) Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *Journal of Experimental Biology* 203: 921–926.

Moyes CD and West TG (1995) Exercise metabolism of fish. In: Hochachka P and Mommsen T (eds.) *Molecular Biology of Fishes*, vol. 4, pp. 367–392. Amsterdam: Elsevier.

Richards JG (2009) Metabolic and molecular responses of fish to hypoxia. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Hypoxia*, vol. 27. San Diego, CA: Elsevier.

Richards JG, Heigenhauser GJF, and Wood CM (2002) Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. *American Journal of Physiology* 282: R89–R99.

Metabolic Rate Suppression as a Mechanism for Surviving Hypoxia

JG Richards, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Defining Metabolic Rate and Metabolic Scope
Measuring Metabolic Rate
Hypoxia Exposure Imposes a Metabolic Stress in Animals
Metabolic Scope for Survival: Suppression of Metabolic Rate Saves Precious Energy
Changes in Behavior Contribute to Metabolic Rate Suppression
Changes in Physiology Contribute to Metabolic Rate Suppression
Changes in Biochemistry Contribute to Metabolic Rate Suppression
Summary
Further Reading

Glossary

Anoxia Complete absence of dissolved oxygen in water.

Critical oxygen tension (P_{crit}) The environmental O_2 tension below which an animal cannot sustain a routine rate of O_2 uptake independent of environmental O_2 tensions. The lower a fish's P_{crit}, the better able it is to maintain normal levels of activity and, therefore, fish with lower P_{crit}'s are believed to be more hypoxia tolerant than those with higher P_{crit} values.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Hypoxia tolerance A relative indicator of an animal's ability to survive hypoxia. A hypoxia-tolerant fish is able to survive more severe hypoxia or a longer time at a set level of hypoxia than a hypoxia-sensitive animal.

Maximal metabolic rate (MMR) The maximum metabolic rate achieved by an animal. If measured as O_2 uptake, it would be termed $\dot{V}o_{2\text{max}}$.

Metabolic rate suppression A reduction in metabolic rate below RMR or SMR values typically induced in response to exposure to environmental stress. Theoretically, the lower the metabolic rate during hypoxia exposure, the longer the organism will survive with its stored fuel.

Routine metabolic rate (RMR) The metabolic rate associated with the maintenance of cellular and whole animal homeostasis as influenced by activity under experimental conditions in which movements are presumably somewhat restricted and the fish is protected from outside stimuli.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animal has had ample time to acclimate.

Introduction

Periods of environmental hypoxia or even anoxia are extremely common in aquatic systems due to natural causes as well as more recent anthropogenic causes (e.g., eutrophication; **see also Hypoxia**: The Expanding Hypoxic Environment). As a result of these oscillations in dissolved O_2, it is perhaps not surprising that among all vertebrates, fish boast the largest number of hypoxia-tolerant species. In the simplest sense, hypoxia exposure imposes a stress on metabolic energy balance, which elicits a suite of behavioural, physiological, or biochemical responses that either function to enhance O_2 uptake from the environment or permit hypoxia-tolerant fish to defend against the metabolic consequences of O_2 levels that fall below a threshold where metabolic functions cannot be maintained. This article outlines the whole-animal and cellular mechanisms used by fish to suppress metabolic rate during hypoxia exposure with the overall goal of conserving precious metabolic energy.

Defining Metabolic Rate and Metabolic Scope

Roughly 95% of the O_2 consumed by a resting, normoxic fish is used by the mitochondria to generate energy in the form of adenosine triphosphate (ATP), which supports the metabolic costs associated with the maintenance of cellular and whole-animal homeostasis. Overall, metabolic rate can be defined as the total energy turnover within an animal, which is heavily influenced by physiological state. A fish's metabolic rate is normally divided into basal or standard metabolic rate (SMR; basal metabolic rate is normally using only when referring to endotherms) and routine metabolic rate (RMR). SMR is defined as the minimum rate of metabolism determined on a resting, unstressed, adult animal in the postabsorptive state under normothermic conditions. For fish, normothermic can be defined as a temperature well within the species tolerance limits for which the animal has had ample time to acclimate. At the cellular level, SMR is composed predominately of the metabolic costs associated with the maintenance of cellular ion gradients, protein turnover (synthesis and breakdown), and other cellular processes. Based on this definition of SMR, one might expect that it is easy to measure SMR and that SMR is constant for a given animal. However, the minimum cost of survival in fish is highly dependent on environmental condition.

Under most conditions, free-living fish have a higher rate of metabolism than SMR because of the energy expended on physical activity (even if minor), digestion, and reproductive investment, to name a few processes. These are difficult to eliminate in their entirety and can easily double SMR. As a result, measurements of metabolic rate made under these somewhat more natural circumstances are collectively referred to as RMR. The maximal metabolic rate (MMR) of a fish, normally determined as the highest oxygen consumption rate during step-wise increases in swimming, represents the highest metabolic rate achievable by a fish. In this context, MMR is analogous to the $\dot{V}\mathrm{o}_{2\mathrm{max}}$ determined in mammals. The difference between SMR and MMR is aerobic scope or the metabolic scope for activity, with the difference being as much 10-fold. Aerobic scope has been measured in literally hundreds of species of fish and is related to habitat use and ecological success in many, more active species of fish.

Many fish species possess the ability to suppress metabolic rate below RMR or even SMR values as a response to environmental challenge and this is generally referred to as metabolic rate suppression or "turning down the pilot light" as referred to by the comparative physiologist Kjell Johansen. The difference between the lowest measurable metabolic rate and SMR has been referred to as the metabolic scope for survival by the comparative biochemist Peter Hochachka. Theoretically, the greater an animal can reduce its SMR or RMR in a controlled fashion when facing an environmental challenge such as hypoxia, the longer it can survive. Scope then refers to the amount of this reduction. Thus, the capacity to reduce metabolic rate in response to hypoxia/anoxia challenge has emerged as a unifying concept explaining hypoxia survival.

Measuring Metabolic Rate

Metabolic rate cannot be measured directly *in vivo*; therefore, indirect measures must be used. The two most commonly used measures for metabolic rate are O_2 uptake and heat production. Measurements of O_2 uptake are common in the scientific literature; however, O_2 uptake rate measurements only provide insight into metabolic rate under fully aerobic conditions where all of an organism's energy is provided by mitochondrial oxidative phosphorylation and O_2 use. While O_2 uptake and O_2 consumption are often used as interchangeable terms, there is a subtle yet important distinction. Consumption refers to the O_2 used by mitochondria, whereas uptake is typically what is measured in the whole animals and that O_2 is removed from the environment. While the two are equivalent over the long term, they may not be over the short term, as with the depletion or replenishment of O_2 stores following intense anaerobic exercise. Under conditions where anaerobic metabolism is used to provide cellular energy, measurements of O_2 uptake will, in most cases, drastically underestimate metabolic rate. Measurements of O_2 uptake are blind to anaerobic metabolism because this form of energy production is not linked with O_2 consumption. The best indirect measure of metabolic rate, often referred to as direct to emphasize its superiority, is the measurement of heat production by an animal.

Metabolic heat is the inevitable product of energy transduction during ATP turnover and the rate of heat production is directly proportional to ATP turnover rate and hence metabolic rate. Indeed, since about 70% of the energy transfer is lost as heat, the process is often referred to as inefficient, but then this heat is essential for endothermy (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks)! Changes in heat production can be interpreted as a change in metabolic rate (increased heat production indicates an increase in metabolic rate and vice versa) and unlike O_2 uptake rates, these measurements are not affected by the mode of cellular energy production and therefore detect changes in metabolic rate no matter whether they are fueled aerobically or anaerobically. They do, however, assume that the efficiency of energy conversion operates at a constant efficiency, which is now known not to be always true. Measurements of heat production are rare in the scientific literature compared with measurements of O_2 uptake.

Hypoxia Exposure Imposes a Metabolic Stress in Animals

The fundamental issue facing fish exposed to hypoxia or anoxia is that of the maintenance of metabolic energy balance. If environmental hypoxia leads to hypoxemia (reduced blood O_2 content) and reductions in O_2 at the mitochondria, then mitochondrial ATP production will be compromised. Under these conditions, ATP can only be generated by anaerobic or O_2-independent mechanisms such as glycolysis (**see also Hypoxia**: Anaerobic Metabolism In Fish).

Although anaerobic metabolism can generate ATP during hypoxia, the total ATP yield is roughly 15 times lower than from mitochondrial processes when the same substrate is used. For example, the full mitochondria-based breakdown of 1 mol of glucose yields ~30 mol of ATP (theoretical ATP yield should be 38 ATP, but the maximum is never quite reached because of proton leak and the metabolic costs of moving substrates across the mitochondrial membrane), while the anaerobic breakdown of glucose, involving only glycolysis, produces 2 mol of ATP, plus partially broken down metabolic wastes (e.g., lactate).

Thus, the switch from aerobic mitochondrial metabolism to anaerobic metabolism during hypoxia exposure imposes at least two metabolic stressors that may limit survival. First, the lower ATP yield from anaerobic metabolism places a substrate-limited cap on the duration of survival during hypoxia. Without O_2, the only metabolic substrates available for ATP production are glycogen and high-energy phosphates and because of the lower ATP yield of anaerobic metabolism, these fuels can be quickly used up. The second issue of relying on anaerobic metabolism is that it produces metabolic waste products such as lactate (plus an associated proton) and its accumulation can have devastating effects on tissues (**see also Hypoxia**: Anaerobic Metabolism In Fish). Overall, the lower ATP yield and accumulation of metabolic wastes during hypoxia exposure present a problem for fish, particularly hypoxia-sensitive fish which quickly succumb to hypoxia due to an inability to maintain cellular energy balance and a loss of cellular ATP.

For all fish, life without O_2 will eventually prove fatal. Thus a fundamental question is: What are the metabolic processes responsible for extending life during hypoxia exposure?

At environmental O_2 tensions where O_2 uptake by the fish is compromised (the point at which O_2 uptake transitions from being independent of environment O_2 to being dependent in terms of the critical oxygen tension or P_{crit}), the duration of survival is dictated by two, interrelated factors:

1. the ability to reduce metabolic demands through a controlled metabolic rate suppression (i.e., have a high metabolic scope for survival) and
2. the availability of substrate for O_2-independent ATP production (**see also Hypoxia**: Anaerobic Metabolism In Fish).

Figure 1 illustrates a conceptual framework to understand the relationship between these two factors. Scenario 1 in **Figure 1**, represents an exquisitely hypoxia-sensitive fish, where at O_2 tensions below P_{crit} the fish attempts to maintain metabolic rate through a large activation of anaerobic metabolism (indicated by long red arrow), which results in the rapid depletion of glycogen and accumulation of lactate and acid. In this case, the length of survival will primarily be dependent on the amount of anaerobic substrate available and the capacity of the organism to deal with the metabolic waste products. At this point, fish, and other animals, are limited in their capacity to store fermentable fuels such as glucose and glycogen because, unlike aerobic fuels such as fat, these fuels must be stored intracellularly and are hydrated. Thus, fermentable fuels occupy a greater space than an equivalent mass of fat. Therefore, without some reduction in metabolic rate, stored fermentable fuels are rapidly exhausted (and deleterious wastes accumulate equally rapidly). On the other hand, scenarios 2 and 3 in **Figure 1** represent increasing levels of hypoxia tolerance, where decreases in metabolic rate limit the need to activate anaerobic metabolism (shorter red arrows in **Figure 1**), extending the time a set quantity of glycogen or other fermentable fuel can support metabolism. The more a fish can reduce its metabolic rate in response to hypoxia exposure, the longer it will live. Of course, reductions in metabolic rate come with consequences in terms of increasing the risk of predation, if the hypoxia environment

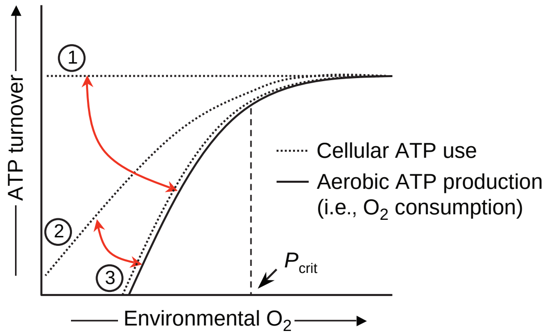


Figure 1 Metabolic responses of fish to changes in environmental O_2. A species' critical oxygen tension (P_{crit}) is the point at which O_2 uptake rate transitions from being independent of environmental O_2 levels (often referred to as O_2 regulation) to being dependent on environmental O_2 (often referred to as O_2 conforming) At O_2 levels below P_{crit}, survival is dependent upon the ability of an animal to suppress metabolic ATP use to limit the extent of the activation of O_2-independent pathways of ATP production. See text for more detail.

does not result in the exclusion of predatory species (**see also Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia).

Metabolic Scope for Survival: Suppression of Metabolic Rate Saves Precious Energy

Decreases in heat production as a response to hypoxia exposure have been measured in many fish species including goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), tilapia (*Oreochromis mossambiscus*), European eel (*Anguilla anguilla*), zebrafish (*Brachydanio rerio*), and in isolated liver cells from rainbow trout (*Oncorhynchus mykiss*). Interestingly, across this very broad range of fish species, which includes species that are considered extremely hypoxia tolerant (crucian carp, goldfish, and tilapia) and tissues from fish considered to be very hypoxia sensitive (rainbow trout), all species show the capacity to decrease metabolic rate in response to hypoxia exposure.

The precise oxygen conditions that elicit metabolic rate suppression differ between species, but with the limited number of measurements in the literature it is impossible to draw any broad meaningful conclusions about the relationship between hypoxia tolerance and the capacity to suppress metabolic rate. However, generally, the most impressive reductions in metabolic rate occurred in the goldfish, tilapia, and European eel with an ~70% decrease in metabolic rate during hypoxia. Theoretically, and all other things being the same, this reduction in RMR could extend survival time by threefold. The degree of metabolic rate suppression in response to hypoxia exposure in other species appears to be considerably less. These data strongly support the notion that metabolic rate suppression is enhanced in hypoxia-tolerant animals versus hypoxia-intolerant species; however, there is a pressing need to carefully examine the relationship between the metabolic scope for survival in and hypoxia tolerance in multiple species where phylogeny can be taken into account.

Any process that reduces whole animal or cellular energy expenditure during hypoxia exposure will conserve precious ATP and prolong life. Maximal metabolic rate is most sensitive to environmental hypoxia exposure resulting in dramatic decreases in aerobic scope for activity. These decreases in MMR and aerobic scope for activity are likely a consequence of inadequate O_2 extraction from the environment and delivery to muscle, which then imposes a decrease in muscle activity, often manifesting as changes in behavior (reduced swimming activity). Decreases in swimming activity can substantially reduce energy consumption. Fish also reduce RMR through other changes in behavior as well as physiological changes. Equally important, and perhaps more fundamental, are the cellular aspects of metabolic rate suppression and the basic mechanisms that facilitate sometimes-substantial reductions in SMR in response to hypoxia exposure. The remainder of this article outlines the processes, starting from modifications to behavior, physiology, and extending down to the cell and individual proteins that form the basis of turning down the pilot light to the bare minimal levels essential for hypoxia survival.

Changes in Behavior Contribute to Metabolic Rate Suppression

As many fish species are exposed to a progressive decreases in environmental O_2, they often display a suite of behaviors that first serve to seek out better-oxygenated areas but if these cannot be located, shifts in behavior can yield sometimes-substantial savings in metabolic energy. In response to hypoxia exposure, fish have been observed to reduce swimming activity, courtship behavior, and feeding (**Figure 2**). Swimming activity can represent a major component of a fish's energy budget and it seems reasonable to assume that fish exposed to hypoxia will decrease swimming activity to save metabolic energy. Recent reviews of the literature suggest that benthic or demersal fish, those that may experience hypoxia frequently in their natural environment, show reduced swimming activity during hypoxia. For example, in the crucian carp (*C. carassius*) exposed to anoxia, a 50% reduction in spontaneous swimming activity was observed, which is projected to yield up to a 35% decrease in energy use.

However, not all fish species decrease spontaneous swimming activity during hypoxia exposure. Active, pelagic fishes, which likely experience hypoxia in their natural environment far less frequently, attempt to sustain swimming activity and in some cases increase their swimming activity in response to hypoxia exposure. Species of tuna, particularly skipjack tuna (*Katsuwonus pelamis*), show increased swimming speed in response to progressive declines in environmental oxygen. This increase in swimming activity is likely an attempt to escape hypoxia and find better-oxygenated waters. Overall, as might be expected, any single fish species can display both increases and decreases in swimming activity in response to hypoxia exposure and the response is likely dependent upon the degree of hypoxia exposure, the rate of change in water oxygen tension, and the degree of activity in normoxia.

There is also accumulating evidence from behavioral studies that hypoxia exposure can affect courtship behaviors, mate choice, and reproductive efforts in fish and there could be potential metabolic savings from reduced reproductive activity. However, reductions in reproductive efforts, especially in terms of postfertilization parental care, must be counterbalanced with overall reductions in reproductive output.

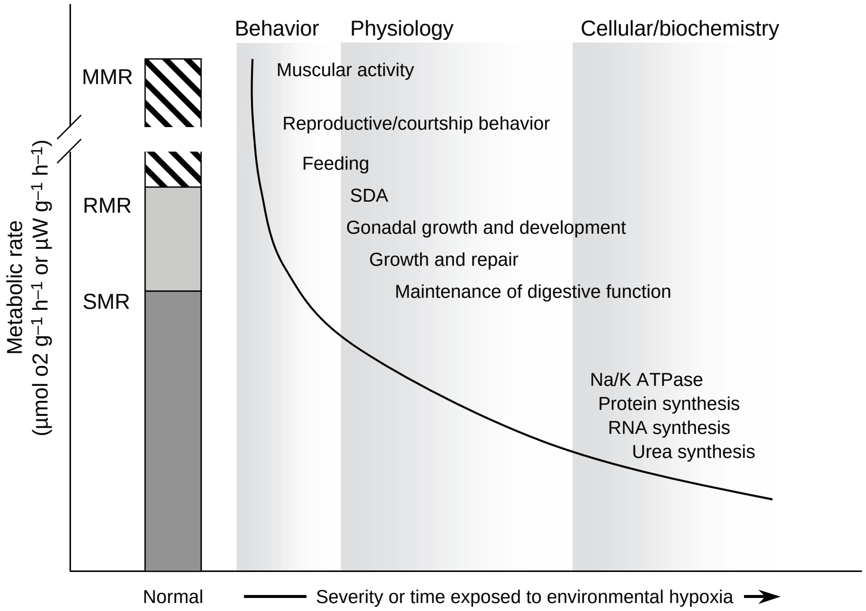

Behavior
Physiology
Cellular/biochemistry
MMR
RMR
SMR
Metabolic rate (μmol o2 g^{-1} h^{-1} or μW g^{-1} h^{-1})
Muscular activity
Reproductive/courtship behavior
Feeding
SDA
Gonadal growth and development
Growth and repair
Maintenance of digestive function
Na/K ATPase
Protein synthesis
RNA synthesis
Urea synthesis
Normal
Severity or time exposed to environmental hypoxia

Figure 2 Contributors to metabolic rate suppression in fish. During exposure to environmental hypoxia of increasing severity or duration, metabolic rate is decreased by a variety of behavioral, physiological, and cellular/biochemical adjustments. At the onset of hypoxia exposure, behavioral adjustments of decreasing muscular activity, reproductive/courtship, feeding, and other voluntary activities facilitate metabolic saving by reducing metabolic rate from a maximum metabolic rate (MMR; thus, reducing aerobic scope) toward a routine or standard metabolic rate (RMR and SMR, respectively). As the environmental challenge persists or grows more intense, physiological adjustments that include reductions in gonadal development, growth, and energy allocated for the maintenance of nonessential physiological functions (e.g., digestive function) reduce RMR toward basal SMR levels. Biochemical adjustments at the cellular level including controlled reductions in Na^+/K^+-ATPase activity, protein synthesis, RNA synthesis, and urea synthesis, to name a few, further decrease overall SMR and enhance survival.

Changes in Physiology Contribute to Metabolic Rate Suppression

There are numerous physiological strategies employed by fish to reduce their metabolic demands during hypoxia exposure, which can generally be described as switching off processes, ranging from the cessation of feeding and digestion, reducing or stopping gonadal development, stopping growth, and shifting metabolic resources from one organ to another depending on the relative importance of each organ system to survival. Although few studies have directly measured the cost-saving measures associated with these physiological shifts, it is not difficult to imagine that these shifts could yield substantial reductions in energy demand and facilitate metabolic rate suppression.

One relevant example is the potential metabolic saving associated with the cost of ventilation in fish. It has been estimated that ventilation in water represents a substantial contribution to SMR and a reduction in the need to ventilate water would facilitate metabolic rate suppression (**see also Hypoxia**: Respiratory Responses to Hypoxia in Fishes). Furthermore, hypoxia in most fish species is known to cause a significant reduction in appetite, which results in reductions in ingested food and reduced growth rate. Reductions in food intake also limit the digestion-associated increase in specific dynamic action, which is the increase in metabolic rate associated with the costs of digestion and nutrient assimilation.

Changes in Biochemistry Contribute to Metabolic Rate Suppression

The biochemical underpinnings of metabolic rate suppression have long fascinated comparative physiologists and biochemists. Unlike humans and many other homeothermic endotherms, which show little or no capacity to reduce cellular SMR or basal metabolic rate, cells from various organs from many fish species possess incredible abilities to reduce their metabolic rate.

In general, the most energetically expensive work a cell does is make proteins and maintain transmembrane ion gradients. Indeed, in an average cell, protein turnover (included synthesis and breakdown) can account for up to 55% of a cell's total ATP demand, and maintenance of ion gradients can account for up to 25% of a cell's total ATP demand. Other processes that contribute to cellular ATP demand include gluconeogensis, urea synthesis, RNA turnover, substrate cycling, and myosine ATPase for intracellular transport. The precise cellular breakdown of the important contributors to cellular SMR will depend on the cell under examination. For example, the percent of cell ATP demand devoted to the maintenance of transmembrane ion gradients will likely be much higher in neurons, whose function is more dependent on membrane ion movements as compared with, say, a liver cell, although these precise measurements have never been made.

As pointed out for the crucian carp or its relatives, the goldfish (**see also Bony Fishes**: Crucian Carp), some fish are capable of reducing their cellular metabolic rate by up to 80%. This overall reduction in metabolic rate is achieved through the dramatic downregulation of Na pumping, protein turnover, urea synthesis, and gluconeogensis. It is now well accepted that the cellular mechanisms underlying metabolic rate suppression are similar across broad taxonomic groups and the mechanisms involved in metabolic rate suppression in carp are very similar to those observed in other species, such as the anoxia-tolerant turtle (*Chrysemys picta*), which can reduce cellular metabolic rate by up to 94% during anoxia exposure. However, there are tissue-specific differences in the use of metabolic suppression among species. For example, in the heart of turtles, anoxia exposure induces metabolic rate suppression resulting in a reduction of cardiac performance to ~5% of its normal level, while in the anoxic crucian carp, heart performance remained close to routine levels because of a large capacity to anaerobic ATP generation. Despite these tissue-specific differences, whole-animal metabolic rate is still suppressed in both the crucian carp and the turtle. Nevertheless, different tissues contribute differently to metabolic rate suppression.

The suppression of protein synthesis has been described in both isolated hepatocytes and fish *in vivo* in species ranging from the crucian carp, goldfish (*C. auratus*), and the Amazonian oscar (*Astronotus ocellatus*). In the oscar, severe hypoxia exposure (10% air saturation) caused tissue-specific decreases in protein synthesis rates that varied from 27% decreases in brain to 60% decreases in heart. In the crucian carp, there is also a substantial decrease in protein synthesis rates in heart, liver, and muscle during anoxia and these decreases were, in part, mediated by decreased RNA transcription rates. In the goldfish, hypoxia exposure (<0.5% air saturation) caused a very rapid (within 0.5 h) decline (~70%) in liver protein translation rate. Combined, it is believed that these decreases in protein synthesis rates are primarily mediated through the specific phosphorylation of eukaryotic elongation factor 2, which halts protein elongation during translation.

Besides protein synthesis rates, few studies have examined how other ATP-consuming processes are modified during hypoxia exposure in fish. Some modifications to ion pumping rates have been noted. In particular, hypoxia-induced decreases in the activity of Na^+/K^+-ATPase were observed in some studies and could represent a substantial ATP savings, but results are conflicting. The crucian carp does not decrease brain Na^+/K^+-ATPase activity during anoxia exposure, despite increases in the inhibitory neuromodulators gamma-aminobuytric acid (GABA) and adenosine. This lack of an effect of anoxia/hypoxia exposure on Na^+/K^+-ATPase activity in the brain of crucian carp is unlike the response observed in turtles, which suppress the activity of Na^+/K^+-ATPase. The differential responses observed in these two champions of anoxia tolerance are probably associated with the simple fact that crucian carp remains active during anoxia exposure, unlike the turtle which becomes comatose. Furthermore, substantial decreases in gill Na^+/K^+-ATPase activity in the oscar exposed to hypoxia (~5% air saturation) have been noted, and it was speculated that this decrease was achieved by a post-translational modification to the Na^+/K^+-ATPase protein. A similar effect of hypoxia exposure was observed in isolated trout hepatocytes, where a hypoxia caused a transient downregulation of Na^+/K^+-ATPase activity and led to speculation that decreases in Na^+/K^+-ATPase activity in response to hypoxia may be accomplished by local changes in reactive oxygen species, but no precise mechanism was given.

Summary

Hypoxia survival in fishes necessitates a metabolic reorganization to reduce ATP consumption through a regulated metabolic rate suppression to match the limited capacity for O_2-independent ATP production. As outlined above, controlled metabolic rate suppression is essential to extend the length of time that can be supported by the limited levels of fermentable fuels. Modifications to behavior, physiology, and cellular biochemistry all contribute to saving precious metabolic energy during hypoxia/anoxia exposure. Thus, it appears reasonable to speculate that the degree of metabolic rate suppression and the quantity of stored fermentable fuel are likely strongly selected for in hypoxia-tolerant fishes. Furthermore, although it has not been explicitly examined, differences between hypoxia-tolerant and hypoxia-intolerant fishes could, along with the noted physiological difference discussed in other articles, be related to their ability to reset SMR to pilot light levels.

See also: **Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia. **Bony Fishes**: Crucian Carp. **Hypoxia**: Anaerobic Metabolism In Fish; Respiratory Responses to Hypoxia in Fishes; The Expanding Hypoxic Environment. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks.

Further Reading

Guppy M (2004) The biochemistry of metabolic depression: A history of perceptions. *Comparative Biochemistry and Physiology Part B* 139: 435–442.

Hochachka PW, Buck LT, Doll CJ, and Land SC (1996) Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proceedings of the National Academy of Sciences of the United States of America* 93: 9493–9498.

Hochachka PW and Somero GN (2002) *Biochemical Adaptation: Mechanisms and Process in Physiological Evolution*. New York: Oxford University Press.

Richards JG (2009) Metabolic and molecular responses of fish to hypoxia. In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Hypoxia. Fish Physiology* vol. 27, pp. 444–485. New York: Academic Press.

Vornanen M, Stecyk JAW, and Nilsson G (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology* vol. 27, pp. 397–441. New York: Academic Press.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Hagfishes and Lamprey

Contents

Hagfishes
Lampreys: Energetics and Development
Lampreys: Environmental Physiology

Hagfishes

ME Forster, University of Canterbury, Christchurch, New Zealand

© 2011 Elsevier Inc. All rights reserved.

The Fascination and a Few Misconceptions
Anaerobic Abilities
The Unique Physiology and Anatomy of Hagfishes
Further Reading

Glossary

Anaerobic processes Occurring without the need for oxygen.

Glomerular capsules The site in the kidney where the primary urine is produced as an ultrafiltrate of the blood.

Hypoxia Low partial pressures of oxygen in external or internal environments.

In-series circulation Blood passes first through the gills and then to the systemic vessels, two resistances in series. This contrasts with the divided circulation of birds, crocodiles, and mammals.

Isosmotic Having the same osmotic pressure.

Primitive or derived condition Ancestral trait or one that has evolved more recently.

Osmoconforming Where the osmotic strength of the body fluids matches that of the medium in which an animal lives.

The Fascination and a Few Misconceptions

Hagfishes have long fascinated zoologists because of their unique anatomy and physiology. Often described as the most primitive of the vertebrates, this classification is technically incorrect: hagfishes lack vertebrae, as well as jaws. They are an ancient craniate lineage, diverging from the line that leads to the vertebrates at least 300 mya. Their phylogeny is still unresolved. DNA evidence, both nuclear and mitochondrial, points to a monophyletic origin of hagfishes and lampreys, but many aspects of their morphology and physiology suggest a divergence of the two lines, as does some genetic analysis (**Figure 1**). Whichever way their phylogeny is resolved, the hagfishes remain interesting because of their early separation from the chordate lineage and their potential to shed light on the physiology of the earliest chordates. Limited information from the two described fossils, *Myxinikela* and *Myxineides*, from 305 mya, suggests that they superficially resemble the modern genera. Even so, we need to remember that the hagfishes are an ancient line with relatively few living genera, and it is possible that the living species are not representative of the ancestral forms.

A characteristic of living hagfishes is their low metabolic rate. Hagfishes live on the seafloor, with at least one species recorded at a depth of 5000 m. Abyssal species typically have low metabolic rates, which is presumed to reflect the lack of food at depth. However, even hagfishes found in shallow, productive waters, such as species of the genus

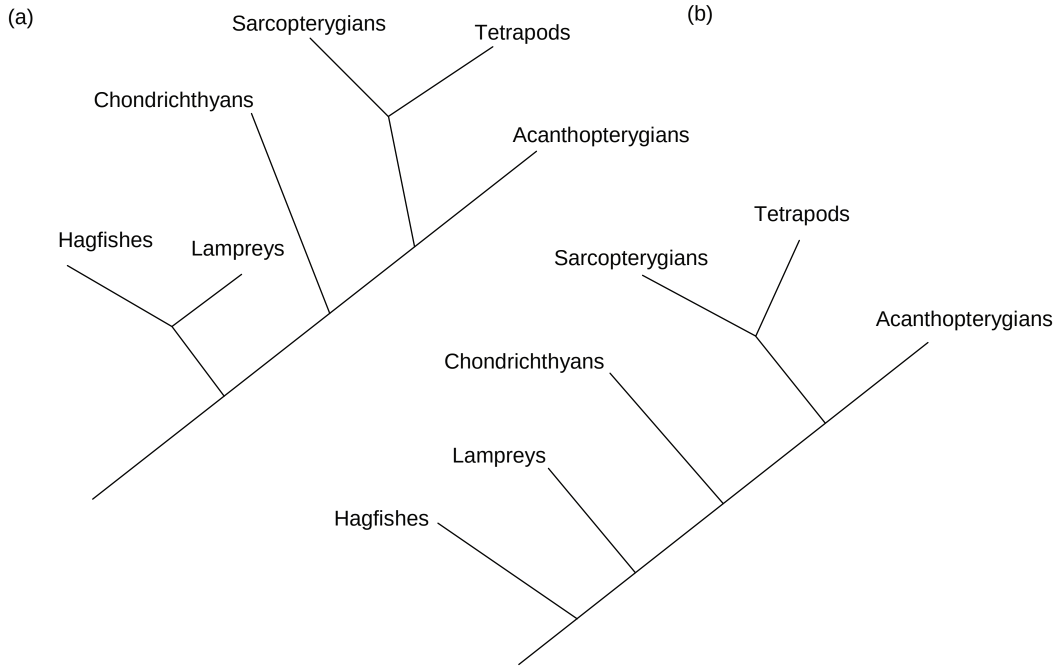


Figure 1 Alternative craniate phylogenies, showing (a) a monophyletic and (b) a diphyletic origin of hagfishes. Scenario (b) would place the hagfishes as the living craniates closest to the ancestral craniate line.

Eptatretus, have very low rates of oxygen consumption. If hagfish gas-exchange systems are efficient (see below), we would expect an animal with a low metabolic rate to tolerate low oxygen partial pressures in its environment, but hagfishes are considered to be unusually tolerant of extreme hypoxia. Early research on modern hagfishes concentrated on *Myxine glutinosa*, which lives in burrows on the Atlantic seafloor and can occur at high densities in cool waters. *Myxine* is extremely tolerant of low oxygen concentrations and is reported to survive when its single nostril is blocked and ventilation prevented. Oxygen is known to be able to enter across its skin, presumably at a rate sufficient to meet the low metabolic demand. This species has also a low body mass and relatively high skin surface area, which would facilitate cutaneous gas exchange. Other species do not live buried in anoxic mud, and one nonburrowing species, *Eptatretus stoutii* (**Figure 2**), has been recently found to survive near anoxic conditions for up to 36 h. Interestingly, and in contrast, a larger species found on the opposite, west side of the Pacific Ocean, *Eptatretus cirrhatus* (**Figure 3**), reacts to waters of low oxygen content by swimming vigorously to escape, although it too can survive hypoxic conditions.

Anaerobic Abilities

The classification of hagfishes and lampreys as jawless fishes (cyclostomes: literally round mouths) does not mean that they are unable to bite their food. Hagfishes have numerous teeth arranged on semicircular dental plates within their mouth, which are everted by the action of a protractor muscle. A large retractor muscle then closes and withdraws the teeth into the mouth, dragging material with it. Hagfishes are capable of consuming food rapidly, although this may exhaust the tongue muscles, which are unusually adapted to function anaerobically, being very poorly supplied with blood vessels. The tongue muscles are capable of accumulating extremely high concentrations of lactic acid, which is trapped, as there are

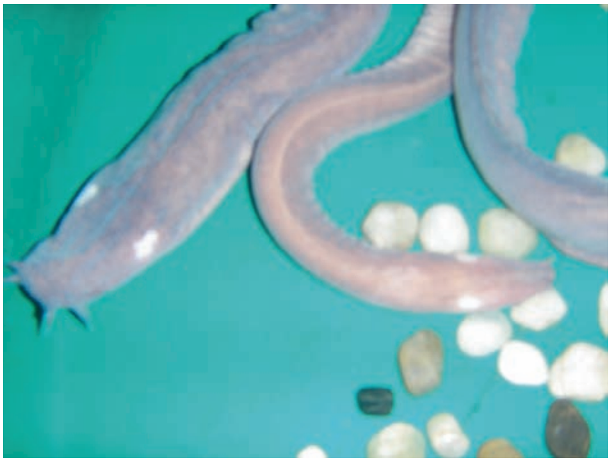


Figure 2 The Pacific hagfish of North America, *Eptatretus stoutii*. These animals average ~100 g in body mass, but the specimen on the left is an unusually large one. Photograph courtesy of Georgina Cox.

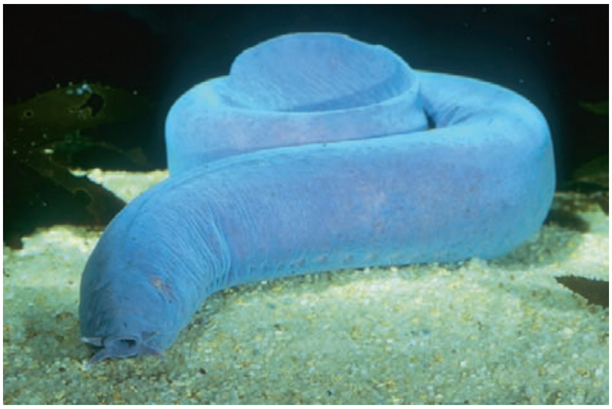


Figure 3 The New Zealand hagfish, *Epatatretus cirrhatus*, showing the single nostril, and the eye spot, and the openings of the efferent gill ducts of the animal's left side. These animals average ~1 kg in body mass. Photograph courtesy of Rufus Wells.

Table 1 Ratios of divalent ion concentrations in seawater to the concentrations in plasma of hagfishes (*M. glutinosa* and *E. stoutii*)

Ion	*Ratios (SW: plasma)*
Calcium	1.65–2.4:1
Magnesium	2.25–3.0:1
Sulfate	4.65–8.55:1

Numbers based on Robertson JD (1976) Chemical composition of the body fluids and muscle of the hagfish *Myxine glutinosa* and the rabbit-fish *Chimaera monstrosa*. *Journal of Zoology* 178: 261–277; Holmes WN and Donaldson EM (1969) The body compartments and the distribution of electrolytes. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, vol. 1, pp. 1–89. New York, NY: Academic Press and Sardella BA, Baker DW, and Brauner CJ (2009) The effects of variable water salinity and ionic composition on the plasma status of the Pacific hagfish (*Eptatretus stoutii*). *Journal of Comparative Physiology B* 177: 721–728.

few blood vessels to carry it away. With a low metabolic rate, it may well be that hagfishes need only to feed at long intervals, allowing the feeding muscles sufficient time to resynthesize glycogen from lactate, ready for the next feeding bout. Their anaerobic abilities may also enable them to burrow through the tissues of their moribund prey (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia).

Interest in exploring the anaerobic capabilities of hagfishes was influenced by the suggestion that the earliest chordates had a well-developed anaerobic swimming ability using fast, but short, bursts of activity to capture prey. However, with an efficient countercurrent exchange of oxygen between the blood and external seawater in its gill pouches, *E. cirrhatus* is capable of fuelling swimming through aerobic metabolism.

The Unique Physiology and Anatomy of Hagfishes

Osmotic and Ionic Regulation

Living hagfishes are exclusively marine, and are unique among the craniates in having extracellular body fluids with an ionic composition similar to seawater, and isosmotic with that medium. They are osmoconformers. All other craniates maintain ionic concentrations in their body fluids approximately one-third of those in seawater, whether they live in the sea, in freshwater, or on land. Maintaining ionic concentrations close to those of seawater may allow hagfishes to reduce their metabolic rate, since it reduces the amount of energy they have to expend on ion regulation. However, hagfishes do regulate divalent ions, such as calcium, magnesium, and sulfate, to plasma concentrations that are significantly less than those in seawater (**Table 1**). The kidneys, liver (bile), and gills are involved in the excretion of divalent ions and the animal can regulate calcium and magnesium concentrations even when external concentrations are raised.

There has been much discussion about the origins of the vertebrates, whether it was in freshwater, brackish waters, or seawater. The high ionic strength of hagfish tissues has influenced this debate, given that they are the surviving craniate group with the longest pedigree. Is the high ionic strength of hagfish blood a primitive or derived condition (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction)? The presence of a kidney in hagfishes has raised interest in the evolution of that organ. If the kidney's primary role is osmoregulation, then what is it doing in hagfishes, which are reportedly highly permeable to water? Any free water the kidney excretes will return passively at the gills and through other external epithelia. The structure of the kidney is also unusual in that it possesses a very few, large glomerular capsules, leading to two apparently undifferentiated archinephric ducts, the homolog of the nephron in other craniates. The tissue surrounding the archinephric duct is lipid filled, suggesting a fuel source for active transport, but to date, we have limited evidence of such processes. There are also some unanswered questions about the mechanisms by which the primary glomerular fluid is formed.

The Blood

The essentially hyperbolic oxygen equilibrium curves (OECs) (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function) of hagfishes demonstrate a very high affinity of their multiple hemoglobins for oxygen (P_{50} values of between 0.5 and 1.8 kPa (**see also Transport and**

Exchange of Respiratory Gases in the Blood: Evolution of the Bohr Effect and Hemoglobin), which might be expected in an animal with a low oxygen demand. A high-affinity hemoglobin will also improve oxygen uptake from hypoxic water.

The hemoglobins of hagfishes are unusual in that they behave as monomers in the oxygenated state, but aggregate as dimers or tetramers as they deoxygenate, which results in increased oxygen unloading. They show a weak Bohr effect. It is cooperativity between the β-chains of the tetrameric hemoglobin of fishes and tetrapods that creates a steep OEC able to unload oxygen at relatively high partial pressures of oxygen (lower affinity). Another unusual feature is that bicarbonate is carried bound to hemoglobin within the red cells of hagfishes, which facilitates carbon dioxide excretion.

The blood volumes reported for three species of hagfishes, ranging from 138 to 187 $\mathrm{ml\,kg^{-1}}$ body mass, are unquestionably the largest within the Craniata. These volumes are 3 times higher than the volume of the primary circulation of teleosts and double that of mammals. However, measurements of plasma volume in hagfishes might be overestimated to some extent, as there are problems finding appropriate indicators that can be retained within the plasma, in what is reportedly a leaky circulation.

An unusual anatomical feature of the hagfish circulatory system is the presence of a large subcutaneous sinus, which lies along the entire length of the animal beneath the dermis of the outer body wall and envelops the musculature and viscera. An astonishing ~30% of the total blood volume has been reported to lie within this sinus, whose significance is not well understood. Furthermore, the hematocrit (packed cell volume) of blood within the sinus is lower than that in the primary vessels. This means that red blood cells do not enter the sinus as readily as does plasma. In 1912, Cole published his observations on the fenestrated papillae on the branchial arteries, which connect to the sinus system, and allow plasma to move to the sinus, but make it more difficult for red cells to leave. This arrangement has parallels with the secondary circulation of bony fishes. Presumably, the low oxygen demand allows the low proportion of erythrocytes reported in the central blood (15% or less of blood volume). Fewer red cells will help keep blood viscosity low, and so reduce the force that the ventricle must develop to generate flow (see below).

Accessory Hearts

There has to be a mechanism to return sinus blood to the central circulation and it is achieved by the action of two accessory pumps. The so-called cardinal hearts are valved structures close to the velum, which are compressed and expanded as the velum beats. As such, their own muscle does not contribute to movement of fluid, which disqualifies them for consideration as hearts. In contrast, the caudal hearts, at the other end of the animal, are muscular, beating structures that empty sinus blood into the caudal vein (**see also Design and Physiology of the Heart**: Accessory Hearts in Fishes).

The Central Circulation

Basic vascular anatomy is similar to that of other fishes, particularly with respect to the branchial circulation. The so-called branchial heart of hagfishes, homologous to that in other craniates, is characterized as a high-volume, low-pressure heart, in that the stroke volume is relatively high, but the force the ventricle develops is remarkably low. Arterial blood pressures in hagfishes are considerably lower than in any other group. Hagfish hearts are only able to generate arterial pressures of up to 20 mmHg (2.7 kPa), less than half the pressure generated in other craniates. The anatomy of the heart prevents rapid, forceful contraction, with the contractile material being relatively sparsely distributed in the cardiomyocytes (**see also Design and Physiology of the Heart**: Cellular Ultrastructure of Cardiac Cells in Fishes). The conduction path for the action potential through the heart is a tortuous one, and, unusually, with the prolonged conduction time of the action potential, atrial repolarization is observed in the hagfish electrocardiogram before the ventricle depolarizes (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping and **Role of the Kidneys**: Histology of the Kidney). This probably explains their low heart rates.

The hagfish heart also lacks a controlling innervation, although it does contain, within its structure, chromaffin cells containing epinephrine and norepinephrine (**see also Brain and Nervous System**: Autonomic Nervous System of Fishes and **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). Factors controlling the release of these catecholamines and their site of action in living animals are not well understood, although blood vessels in many parts of the circulation, including central veins, can respond to rising concentrations by either contracting or relaxing, depending on the adrenergic receptor type present. It appears that the hagfish circulation can adapt to volume overload by adjusting vascular tone and preventing a large rise in blood pressures that might upset the balance of blood distribution.

Hagfishes have another accessory heart, the portal heart. This pumps blood from the posterior cardinal vein through the liver. It is not clear why hagfishes need an accessory pump to push blood through their livers, and a fully grown specimen has been found that lacked a portal heart. Certainly, the liver is the site of much metabolic activity, but other animals with an in-series circulation, including the teleost fish, have venous blood pressures in the same low range as hagfishes. Perhaps the answer will be discovered in the vasculature of the liver, which might have a greater resistance to flow than in other craniates.

Gill Structure and Ventilation

Both the gill structure and mechanism for ventilating the gills of hagfishes differ considerably from the typical fish arrangement (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy), but it is efficient. Partial pressures of oxygen in the blood leaving the gill exceed 100 mmHg, as high as recorded in other fishes. Hagfish gill pouches are discrete structures. *Myxine glutinosa* has six pairs of them, cranial to the branchial heart (**Figures 4** and **5**).

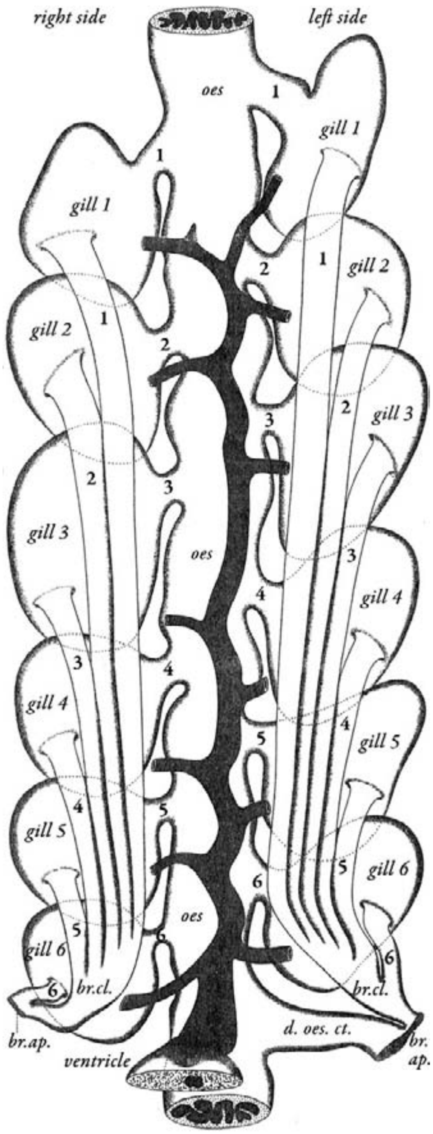


Figure 4 Cole's 1913 drawing of the gill pouches of *Myxine glutinosa*, showing the afferent branchial arteries leading off the single ventral aorta, six pairs of separate inhalant water channels from the pharynx, and the joining of the efferent water ducts of the gill pouches of each side into a single exhalant opening. *Eptatretus cirrhatus* has seven pairs of gill pouches, paired ventral aortas, and a separate efferent gill duct for each gill pouch.

Hagfishes, such as larval lampreys, move water across their gills with a velar pump. The velar chamber, an enlarged portion of the nasopharyngeal duct, bears a cartilage-reinforced membrane that projects ventrally from its roof and ends in paired horizontal membranes or leaves whose free ends lie within the duct. Using red (aerobic) muscles, the leaves can be scrolled and unscrolled. Because of their orientation, water is drawn in through the single nostril and propelled in an anterior-to-posterior direction toward the gill ducts in the wall of the pharynx. Although the gill pouches of hagfishes are muscular and can show contractions and relaxations, it is not thought that they make a major contribution to normal ventilation. Strong contractions of the gill pouch muscles probably help to expel particles, including mucus, when the gill ducts are blocked. In contrast, bi-directional, or tidal ventilation in an adult lamprey is carried out by muscles around their gill pouches that attach to a strong cartilaginous branchial basket, a structure lacking in hagfishes.

Measuring water flow rates across hagfish gill pouches is difficult, as some species do not tolerate the presence of a tube in their nostril, the site where seawater is inspired. Recent measurements suggest that flow rates can be relatively high. However, water pressures associated with gill

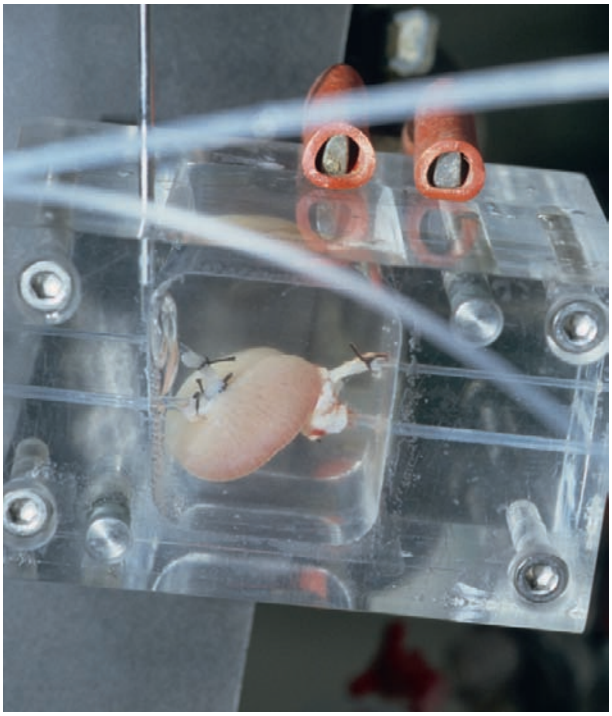


Figure 5 A gill pouch from *E. cirrhatus* with cannulated afferent and efferent branchial arteries and water ducts enclosed in a Perspex chamber. Its discrete nature makes the hagfish gill pouch a potentially useful structure for measuring active and passive exchanges between the blood and water compartments.

ventilation are likely to be low in the absence of a strong muscular pump. As the pharynx has a fixed volume, changes in inspiratory flow must result from an increase in ventilation frequency, which can increase by almost threefold in *Eptatretus* species exposed to hypoxic conditions.

A thin barrier to diffusion between blood and water maximizes gas exchange across gills. The blood-to-water distance in hagfishes is comparable to many teleost fish, being ~1.6 μm at its thinnest point. It is possible that low water pressures within the gill pouches require low blood pressures within the gill lamellae. Otherwise, higher blood pressure in the gill vessels might reduce seawater flow across the gills. This constraint might explain the relative lack of contractile material in the hagfish heart and the presence of auxillary pumps to maintain circulation in a high-volume blood system.

Swimming and Musculature

Muscle accounts for about 40% of body mass, which is not much less than a trout. The lack of a calcified vertebral column probably prevents the hagfish from supporting itself out of water. This contrasts with adult lampreys, for example.

Hagfishes lack a swimbladder, which provides buoyancy in bony fishes, but with a skeleton composed of cartilage rather than bone, they are closer to the density of seawater, which reduces the energy expenditure necessary for maintaining a position high in the water column. However, hagfishes have reasonable stamina, and it can take up to 30 min to exhaust the animals when swimming at 0.5 body lengths per second. Hagfish muscle has a low resting metabolic rate, in common with other tissues in the animal, and it is not known if this compromises its ability to generate force.

Locomotion is eel-like (anguilliform) and involves all muscles posterior to the gills. The hagfish myotome is arranged in a W shape with the two flexures backwardly directed. Within each muscle block, there is some zonation of the white, red, and intermediate fibers, the red being nearer the skin, but the fiber arrangement has been described as a mosaic. This contrasts with the myotome of most teleost fish, for example, where the red muscle is placed superficially under the lateral line. As in mammals, the proximity of anaerobic and aerobic fibers in hagfishes potentially allows the better-vascularized red fibers to process lactic acid produced in the white fibers. From this perspective, the teleost condition is seen as more specialized. Hagfish white fibers are larger, possessing fewer mitochondria and contract more rapidly than their red muscle fibres.

Defense Mechanisms

Hagfishes have a variety of common names. They are called ‘snotties’ or ‘slime hags’ because they can release prodigious quantities of mucins from glands running in two lines down the length of the body. This is sufficient to turn large volumes of seawater into a gelatinous mass. Mucin threads are ejected forcefully from the slime glands. Once outside the animal the threads trap copious quantities of water. Slime production must be an energetically expensive process, with presumed benefits to the animal. Slime can deter attackers. The material, for example, clogs the gills of predatory fish. Interestingly, fishes are not recorded as common predators of hagfishes (**see also The Skin**: Hagfish Slime).

Slime production also creates a potential problem for the hagfish itself, as it might prevent the hagfish’s own ventilation. However, they have a behavior that helps them clear this mucus from their head. This remarkable feature of the hagfish’s neuromuscular system is the animal’s ability to tie itself into a figure-of-eight knot. The animal pushes its tail through a loop it forms in its body near the posterior end. The knot so formed is then moved forward toward the head. In addition to assisting in slime removal, the knotting maneuver might also help the hagfish to withdraw its head and disengage from its food, once burrowed inside it. Alternatively, it could help tear off flesh from its prey, since the knot can be used as an anchor, to apply force.

Acid–Base Regulation

Rapid movement, facilitating prey capture or escape, involves the white muscle cells, generating lactic acid via glycolysis. Lactic acid production has the potential to alter the pH of body fluids. In 1984, Evans first showed that a hagfish, *M. glutinosa*, had the ability to be a net exporter of either acid (H^+) or base, and that these processes depended on sodium and chloride ions, respectively, as the counterions. Since that time, the presence of a sodium–proton exchanger has been confirmed in *M. glutinosa* and *E. stoutii.* In response to injection of either acid or base, hagfishes can rapidly restore the pH of their blood to normal values. Restoration of blood pH following acid injection was associated with increased expression of the sodium–proton exchange enzyme. In this respect, their acid–base regulation is comparable to that of bony fishes. The latter use these same pumps in ionic regulation, but hagfishes are osmoconformers, which suggests that the primary role of the ion-transporting proteins might be in acid–base regulation. Tongue muscle has a high buffering capacity, which contrasts with the blood, which is poorly buffered.

The Digestive System

The food of hagfishes, including dead or dying animals, does represent a high-energy diet. The gut of hagfishes is extremely simple, without a differentiated stomach. Associated with this there is no early acidic phase of digestion, which might indicate that food processing is a prolonged process. Alternatively, decaying flesh may already be partially softened! With their low metabolic rates, they can probably survive months between feeding. In an aquarium, if they are hungry, they certainly feed voraciously and can ingest a large volume of meat. Both *Myxine* and *Eptatretus* spp. are reported to burrow into the bodies of large animals, which indicates that they can tolerate hypoxic conditions at this time.

The control of digestive processes in hagfishes warrants further attention. Hagfishes possess most of the hormones found in other craniates (**see also Hearing and Lateral Line**: Lateral Line Structure, **Integrated Function and Control of the Gut**: Gut Secretion and Digestion, and **Gut Anatomy and Morphology**: Pancreas), although unsurprisingly, their molecular structure often shows differences. Hagfishes are unusual in that the islets of cells secreting insulin and somatostatin are scattered in the gut mucosa, rather than being found within Brockman bodies or a pancreas. Hagfish insulin has been characterized and its secretion rises and falls with blood sugar concentrations, as it does in other craniates.

The Nervous System

Within the chordates, we differentiate the brain from the rest of the spinal cord on the basis of its size and complexity, its anterior position reflecting the forward direction of motion of the animal. Given our anthropogenic tendencies, we imagine an increasing specialization of the brain in evolutionary development from primitive fishes to the anthropoid apes. In this respect, the brain of hagfishes is somewhat remarkable, possessing unusual features. Northcutt and his colleagues have described the telencephalon as massive and complex. The telencephalon (**see also Brain and Nervous System**: Functional Morphology of the Brains of Agnathans and Functional Morphology of the Nervous System: An Introduction) is the most anterior paired region of the forebrain, and includes both the olfactory bulbs, which occupy about 40% of its volume, and the cerebral hemispheres.

Hagfish eyes are very simple structures. They lack a lens and specialized eye muscles. The simple retina is only two cells thick, compared with the three cell layers found in the adult lamprey and jawed vertebrates. The eye probably functions much like the pineal gland of other vertebrates, responsible for sensing light and entraining circadian behaviors rather than detecting external objects. The structure of the eyes of hagfishes argues against a monophyletic origin of the two cyclostome groups.

Given their lack of eyes, we should expect hagfishes to rely heavily on their sense of smell (olfaction) to detect their food. There is a large olfactory organ positioned within a cartilaginous capsule in the dorsal wall of the nasohypophyseal duct, the tube arising from the single anterior nostril, whose current is generated by the pumping action of the velum. Numerous olfactory axons run to two parts of the brain, the olfactory bulbs and the olfactory cortex. That so much of the brain is devoted to olfaction supports the premise that the role of chemoreception is of vital importance to the animals. Experiments with amino acids and steroids demonstrated differing sensitivities to individual molecules at relatively high micromolar thresholds. In contrast to lampreys and jawed vertebrates, hagfishes have been reported to lack taste buds, aggregations of chemosensitive cells (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction), but such structures have been described in the tentacle epithelium of *M. glutinosa*. Hagfishes also have chemosensitive cells in the epithelium of the mouth and pharynx. Unusually, over the outer surface of their bodies, they have 180 000 Schreiner organs, similar in appearance to taste buds. The afferent nerve fibers from these organs occupy about 10% of the terminal field of the brain's medulla oblongata.

Given that a hagfish cannot use vision to orientate itself as it swims, and lacks paired fins for stability and a cerebellum, we might expect the vestibular system would be well developed to compensate. This is the system of semicircular canals in the inner ear, which give humans and other vertebrates information about the body's movement in three planes. Surprisingly, hagfishes have only paired, single semicircular canals rather than three and McVean has demonstrated that they have relatively poor acuity, only triggering a clear sensory nerve response to yawing movements. What other senses assist directed movement in hagfishes? There is a lateral line system around the head region of *Eptatretus* species, but it is lacking in *M. glutinosa*, perhaps reflecting *Myxine*'s burrowing habit. Anyone who handles a live hagfish becomes aware of the acute sensitivity of the skin. Histological evidence suggests the presence of numerous mechano- and proprioceptors in the skin. Clearly, the entire surface of their bodies is involved in sensing stimuli, both mechanical and chemical. Hagfishes are unusual in apparently lacking electrosensitive receptors in their skin. Hagfishes also appear to lack Mauthner neurons in their hindbrains. These neurons are involved in the startle reflex in other craniates and their activation causes withdrawal of the part of the body under potential attack from a predator.

Table 2 Features of their physiology that distinguish hagfishes from other craniates

1. Low basal metabolic rates in all taxa
2. Ionoconformers, to a large degree
3. High blood volumes
4. The subcutaneous sinus
5. Low arterial blood pressures
6. Kidney structure and function
7. Slime glands
8. Lack of a lens in their eyes
9. Single semicircular canals

This brief review has concentrated on the differences between hagfishes and other craniates. While the article has highlighted unique features (**Table 2**), it is important to note that there are numerous features of hagfish physiology that are shared with the vertebrates. For example, the same small peptide molecules and the gasotransmitters that control the rates of processes in higher vertebrates seem to be present in hagfishes, and, with few exceptions, they modulate the same processes. In this respect, the craniates are a conservative group of animals. Hagfishes occupy a pivotal position in our understanding of chordate evolution and we desperately lack information on many aspects of their physiology and development.

See also: **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction. **The Skin**: Hagfish Slime.

Further Reading

Brodal A and Fänge R (eds.) (1963) *The Biology of Myxine*, 588pp. Oslo: Universitetsforlaget.

Cole FJ (1913) Monograph on the general morphology of the myxinoid fishes, based on a study of *Myxine* – part 4 – on some peculiarities of the afferent and efferent branchial arteries of *Myxine*. *Transactions of the Royal Society of Edinburgh* 49: 215–230.

Evans DH (1984) Gill Na^+/H^+ and Cl^-/HCO_3^- exchange systems evolved before the vertebrates entered fresh water. *Journal of Experimental Biology* 113: 465–469.

Hardisty MW (1979) *Biology of the Cyclostomes*, 428pp. London: Chapman and Hall.

Jørgensen JM, Lomholt JP, Weber RE, and Malte H (eds.) (1998) *Biology of Hagfishes*, 578pp. London: Chapman and Hall.

McKenzie DJ, Farrell AP, and Brauner CJ (eds.) (2007) *Primitive Fishes, Fish Physiology,* vol. 26, 562pp. San Diego, CA: Academic Press.

Sardella BA, Baker DW, and Brauner CJ (2009) The effects of variable water salinity and ionic composition on the plasma status of the Pacific hagfish (*Eptatretus stoutii*). *Journal of Comparative Physiology B* 177: 721–728.

Strahan R (1958) The velum and respiratory current of *Myxine*. *Acta Zoologica (Stockholm)* 16: 227–240.

Lampreys: Energetics and Development

MP Wilkie, Wilfrid Laurier University, Waterloo, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Background
Life History of Lampreys
Locomotion and the Skeletal-Muscle System
Metamorphosis
Upstream Migration and Reproduction
Feeding
Growth and Metabolism
The Great Lakes Invasion by Sea Lampreys
Further Reading

Glossary

Ammocoete Alternate name for the larval stage of the lamprey life cycle.

Endostyle A tissue located in the floor of the pharyngeal cavity in lower chordates and larval lampreys that secretes mucus to trap food particles.

Lamphredin A secretion arising from the buccal glands of lampreys that promotes blood and/or tissue feeding due to its anticoagulant and cytolytic properties.

Oral disk The multi-toothed sucker-like structure surrounding the mouth formed during metamorphosis in the lamprey used to attach to prey for feeding, or to hold position to the substratum; also referred to as the sucker or buccal funnel.

Oral hood The anterior-most fleshy organ found on the head of larval lampreys encircling a series of tentacles (oral cirrhi) through which water currents are directed to the mouth.

Suctorial feeding A mode of feeding in which an animal adheres to its prey using suction, and proceeds to extract fluids or tissue for food.

Velum A muscular diaphragm-like organ that generates currents to draw water through the mouth and into the pharynx of larval lampreys, and separates the esophagus from the water tube in post-metamorphic lampreys.

Background

Lampreys (order: Petromyzontiformes), along with hagfishes (order: Myxinidae) (**see also Hagfishes and Lamprey**: Hagfishes), are the only modern-day representatives of the jawless fishes (superclass: Agnatha) that flourished in the extensive seas that covered the earth during the Paleozoic era, approximately 400–500 million years ago (mya). These jawless, scaleless, eel-like fishes have a cartilaginous skeleton and lack paired fins. Often termed ‘cyclostomes’ because of their round mouths, lampreys and hagfishes are best known for their unsavory feeding habits. Parasitic species of lampreys are suctorial feeders, using an oral disk (sucker) to latch onto fish and continuously ingest the blood and/or flesh of their prey (**Figure 1**). Hagfish are scavengers that tear flesh off of carrion, often from the inside out.

Fossil remains of the lamprey-like *Mayomyzon pieckoensis*, *Hardistellia montanensis*, and *Piipiscius zangerli* in North America suggest that lampreys date back to at least the Upper Carboniferous period, approximately 280–300 mya. The more recent discovery of *Priscomyzon riniensis* in South Africa suggests lampreys have existed in their present form at least since the Devonian (approximately 360 mya). *P. rininensis* closely resembles modern (extant) lampreys, possessing a similar body plan and an oral disk with circumoral teeth that strongly suggests that it too used suctorial feeding.

There are 41 species of living lamprey; 37 belong to the family Petromyzontidae and are found in cool waters of the Northern Hemisphere, while four species reside in the Southern Hemisphere belonging to either the family Geotriidae (one species) or Mordaciidae (three species).

Life History of Lampreys

All lampreys have a prolonged, freshwater larval phase followed by a true metamorphosis (**Figure 2**). The protracted larval phase in freshwater streams typically lasts 3–7 years (sometimes longer), during which the larvae (also called ammocoetes) subsist as filter feeders. In this phase, water is directed through an overhanging oral hood to the pharynx by water currents generated by a muscular velum located near the pharyngeal opening (**Figure 3**). Food particles are trapped by mucus (described below), while the water exits the pharynx via the branchiopores (gill slits) after passing over the gills, the main site of oxygen uptake and carbon dioxide

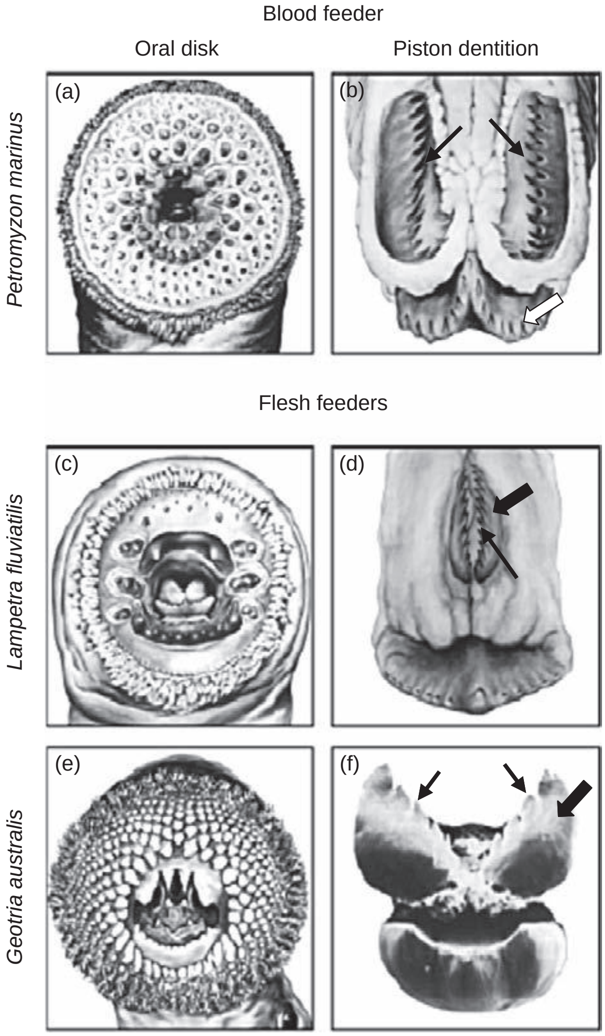


Figure 1 Design and structure of the lamprey oral disk. The multiple, inward (hook-like) facing teeth on the oral disk (a) of sea lampreys (*Petromyzon marinus*) facilitate prolonged attachment to their prey as they ingest the blood of their hosts by piercing the hide with their piston (or piston-like tongue) (b), which is studded with many fine teeth (white arrow) and fine serrations (thin arrows) on the two, widely separated cusps. In contrast, the flesh-feeding river lamprey (*Lampetra fluviatilis*) has fewer teeth studding the oral disk (c), and the piston (d) contains two large, serrated (thin arrows), cusps (thick arrow) that can interlock, allowing the animals to gouge flesh from their prey. The oral disk of pouched lamprey (*Geotria australis*) has many backward-oriented teeth (e), allowing the animal to slide along its prey as it removes chunks of flesh via a scissor action of the two, large, serrated cusps on its piston (f). Modified from Renaud CB, Gill HS, and Potter IC (2009) Relationships between the diets and characteristics of the dentition, buccal glands and velar tentacles of the adults of the parasitic species of lamprey. *Journal of Zoology* 278: 231–242.

excretion in lampreys and most fishes (**see also Hagfishes and Lamprey**: Lampreys: Environmental Physiology).

At the end of the larval phase, the ammocoetes undergo a metamorphosis that is characterized by the appearance of the eyes, and a switch from a unidirectionally ventilated to a tidally ventilated gill in which water is actively pumped in and out of gill pouches. The most notable change is the formation of the multi-toothed oral disk and a toothed piston-like tongue, which facilitates the ingestion of blood and/or tissues of fishes (**Figure 1**). Following the 8–24-month parasitic phase, sexually mature adults migrate up freshwater streams, spawn, and then die. All nonparasitic lamprey species are thought to have evolved from an identifiable parasitic lamprey ancestor, but bypass the feeding period following metamorphosis.

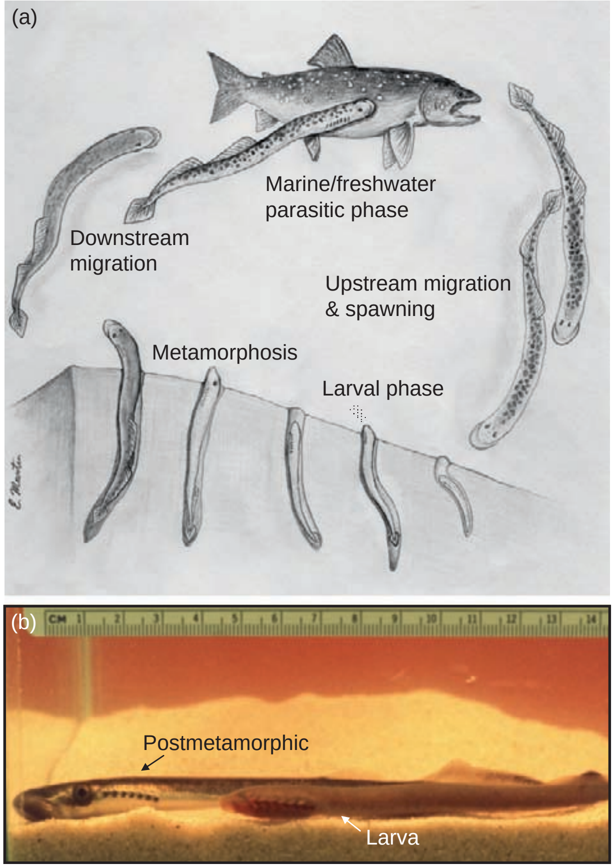


Figure 2 Life history of a parasitic species of lamprey. (a) The life history of lampreys begins with a prolonged, relatively sedentary, filter-feeding larval stage (ammocoete), followed by a 3–4-month period of metamorphosis preparing them for the more active parasitic phase which is usually 8–24 months (depending upon species). The terminal spawning phase takes place in freshwater rivers/streams. (b) Comparison of sea lampreys (*Petromyzon marinus*) in the larval (foreground) and postmetamorphic stages (background). Photo by MP Wilkie.

Locomotion and the Skeletal-Muscle System

Like other fishes, the skeletal muscle is arranged in overlapping W-shaped myotomes running the length of the body. Swimming is anguilliform, achieved by sending

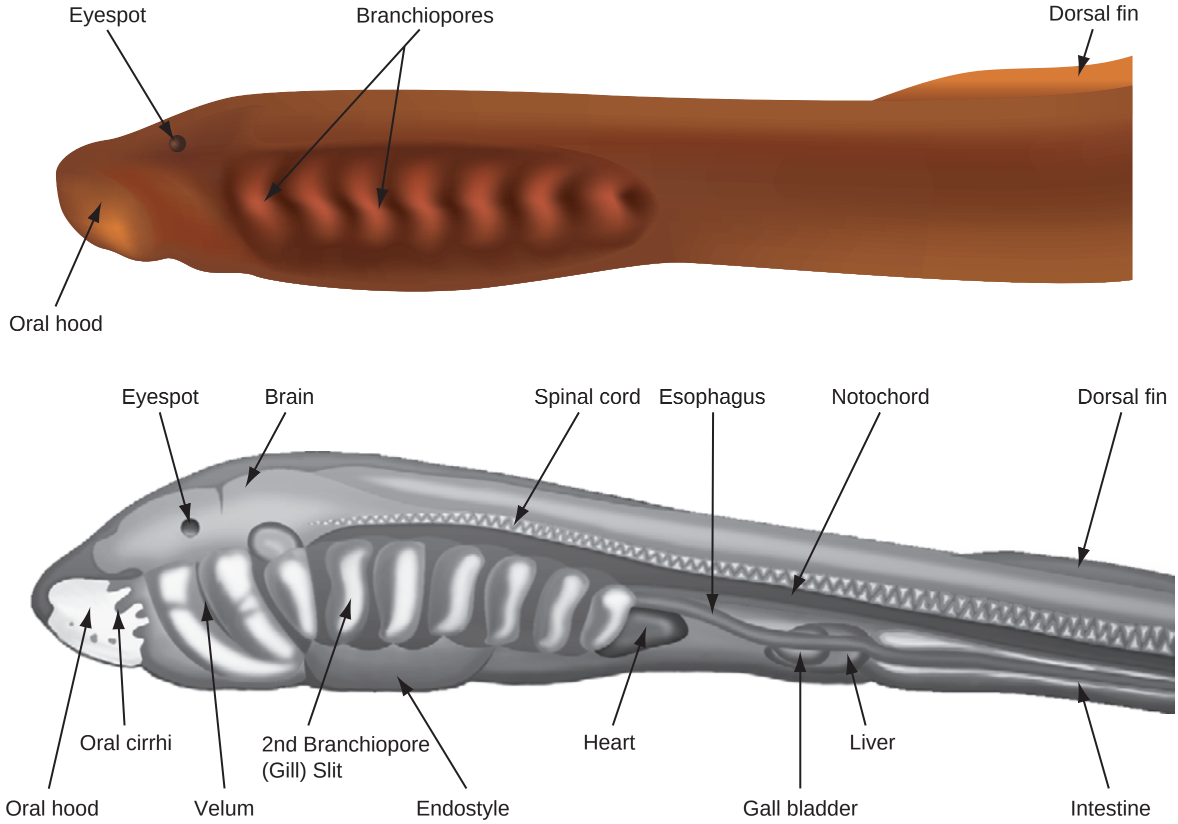


Figure 3 Longitudinal views showing the external and internal anatomy of larval lampreys. Redrawn from Hardistry MW and Potter IC (1971) The behaviour, ecology and growth of larval lampreys. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 1, pp. 85–125, London: Academic Press.

alternating waves of contractions down either side of the body that result in eel-like swimming. However, this mode of locomotion generates lateral forces that impede forward momentum, which likely explains the lower critical swimming speeds (U_{crit}) of upstream-migrant sea lampreys (*Petromyzon marinus*; 1.1–1.4 body lengths s^{-1}) versus comparably sized salmonid fishes (>2 body lengths s^{-1}) that use the more efficient carangiform or subcarangiform swimming modes (**see also Buoyancy, Locomotion, and Movement in Fishes**: Experimental Hydrodynamics and Undulatory Swimming). Although the maximum burst swimming speed attained by an adult, upstream-migrant sea lamprey is up to 390 cm s^{-1} and is comparable to speeds attained in salmonids, such bursts can only be sustained for short periods (a few seconds). Accordingly, upstream-migrant sea lampreys navigate against fast-flowing currents using brief bursts of swimming, followed by rest intervals lasting several minutes during which they attach to the substrate using their oral disk.

Lampreys, such as the pouched lamprey (*Geotria australis*), the Pacific lamprey (*Entosphenus tridentata*), and likely other species, use their oral disks to climb over rocky obstacles and waterfalls as they swim upstream. Indeed, the Pacific lamprey has been observed scaling the vertical inclines of man-made dams! In laboratory experiments, upstream-migrant Pacific lampreys can traverse inclines as steep as 45°. Climbing is achieved by sending undulations down either side of the body, culminating in a powerful flicking of the tail. The lamprey then straightens its body while simultaneously detaching and then reattaching the oral disk to the substrate to inch itself forward and upward (see **Video Clip 1**).

Burrowing, which allows larval lampreys to occupy niches with abundant food and few predators, is initiated by large lateral whip-like undulations of the body driving the head vertically into the substrate. Unlike swimming, the amplitude and the duration of undulations are greater due to the need to generate greater forward momentum to overcome the greater resistance of the substrate (usually silt, sand, or fine gravel) compared to water. Because the muscle contractions involved in burrowing are at least as intensive as those associated with vigorous swimming, burrowing is likely energetically costly.

The skeletal muscle of lampreys has a high anaerobic capacity with high amounts of phosphocreatine that are used to temporarily sustain adenosine triphosphate (ATP) supply during brief bursts of activity. High muscle glycogen stores, along with relatively high activities of lactate dehydrogenase (LDH), indicate that lampreys also have a high glycolytic capacity to fuel intense activity when ATP supply does not match ATP demand (**see also Hypoxia**: Anaerobic Metabolism In Fish). Although post-exercise recovery can take several hours in fishes such as salmonids, larval lampreys and upstream-migrant lampreys can restore phosphocreatine and glycogen stores, as well as correct accompanying acid–base disturbances, within 1 h, quickly preparing them for additional bursts of burrowing or swimming.

The rapid recovery of larval lampreys from exercise is related to an ability to increase postexercise oxygen consumption by up to sixfold. Unlike the trunk musculature of salmonids, which is mainly fast-twitch (glycolytic or white muscle) fibers, the trunk of lampreys is a mosaic of overlapping slow-twitch oxidative (red) and fast-twitch (white) fibers, which may promote shuttling of lactate from white to red muscle and immediate lactate oxidation for ATP production or glycogen replenishment during or following exercise.

Unlike teleosts, lampreys do not have a swimbladder (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes). There was likely little selective pressure for buoyancy control in burrow-dwelling larvae, or in parasitic stage animals that often latch onto fish. When not attached to fish/substrate, or during upstream migration, lampreys likely have to continually swim to avoid sinking. The relatively light cartilaginous skeleton (specific gravity ~1.1) and the large lipid (specific gravity ~0.8) reserves that accumulate during the parasitic phase likely provide some buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes) after metamorphosis.

Metamorphosis

When larval lampreys have accumulated sufficient lipid, and other environmental and endocrine factors are suitable, they enter metamorphosis. Lipid is an essential fuel reserve because lampreys cease feeding during metamorphosis. The gradual accumulation of thyroid hormone in the blood (both thyroxine (T4) and triiodothyronine (T3)) during larval life is also critical because a sudden drop in thyroid hormone concentrations following peak T3 and T4 concentrations triggers metamorphosis. A preceding rise in water temperature is also needed, but photoperiod does not appear to be important (**Figure 4**).

Seven stages of metamorphosis are recognized, mainly based on changes in the appearance of the eye, and the development of the oral disk (**Figure 5**). The gills also become tidally ventilated following metamorphosis. Greater metabolic rates, an increased height of the one or two dorsal fins, and a more elaborate lateral line system are associated with the more active life style following metamorphosis. Body coloration also changes from light or dark brown to a silvery to blue-black sheen in many species (**Figure 2**).

The major reorganization of internal organs also occurs, including the intestine and liver, which reflects the switch from a filter-feeding phase to a blood-feeding

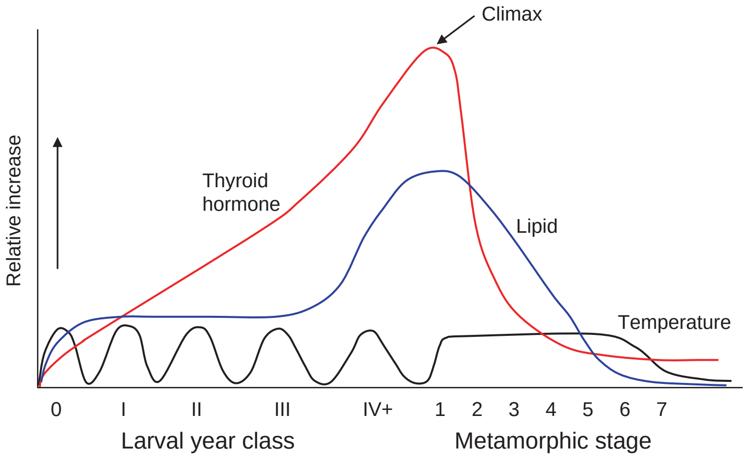


Figure 4 Prerequisites for the initiation of metamorphosis in the lamprey. Prerequisites include the gradual accumulation of thyroid hormone (solid red line) and lipid (blue line) throughout larval life, and fluctuations in ambient temperature (solid black line). Metamorphosis is initiated when thyroid hormone (T3 and T4) concentrations peak (climax), and then precipitously drop. Reproduced from Youson JH (1994) Environmental and hormonal cues and endocrine glands during lamprey metamorphosis. In: Davey KG, Peter RE, Tobe SS (eds.) *Perspectives in Comparative Endocrinology*, pp. 400–408. Ottawa: National Research Council of Canada, with permission from National Research Council of Canada.

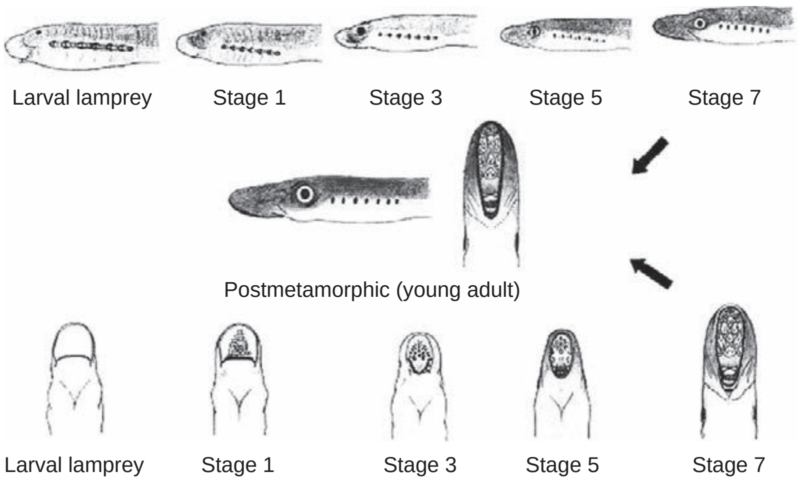


Figure 5 Lamprey metamorphosis. Lateral (upper row) and ventral views (lower row) of larval, metamorphic (stages 1, 3, 5, and 7) and postmetamorphic (juvenile adult) stages in sea lamprey showing the key changes that take place in the structure and organization of the head and feeding structures of lamprey during metamorphosis. Modified from Youson JH and Potter IC (1979) A description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. *Canadian Journal of Zoology* 57: 1808–1817.

phase of the parasitic species. A perplexing observation is the loss of the hepatic bile ducts (biliary atresia), leading to the accumulation of bile pigments (biliverdin and bilirubin) in the liver. Due to the switch from filter to suctorial feeding, the velum is no longer needed to generate feeding or respiratory water currents and is remodeled to serve as a valve to prevent food from entering the pharyngeal (water) tube, which is internally connected to the gills. The endostyle is replaced by follicles of thyroid tissue (follicular thyroid) rather than becoming the distinct thyroid gland of higher vertebrates. The conversion of the oral hood of the larva to the multitoothed oral disk is accompanied by the formation of the serrated, piston-like tongue used for piercing the hide of hosts/prey and the development of buccal glands, which are the source of a salivary secretion called lamphredin (discussed below). Pancreatic tissue, more appropriately called an enteropancreas, is comprised of a series of follicles in the ammocoete that form larger masses of pancreatic tissue following metamorphosis. The larval kidney completely degrades in the mid-posterior region of the body before being replaced by an adult structure (**see also Hagfishes and Lamprey**: Lampreys: Environmental Physiology).

Upstream Migration and Reproduction

Anadromous upstream-migrant lampreys do not home to their natal streams as migrating salmon do (**see also Fish Migrations**: The Biology of Fish Migration and Pacific Salmon Migration: Completing the Cycle). Instead, they use a most suitable river strategy in which appropriate rivers/streams are identified through the detection of bile salts secreted by resident larval lampreys. In sea lampreys, these bile salt pheromones (petromyzonamine disulfate, petromyzosterol disulfate, and petromyzonol sulfate) are very potent and can attract lamprey at extremely low (picomolar) water concentrations. Upstream-migrant male sea lampreys secrete another pheromone, 3-keto petromyzonol sulfate, which triggers aggressive mate seeking by ovulating females. The most suitable river strategy of lampreys may have co-evolved with the parasitic habits of the anadromous lampreys, which undergo wide geographic distribution while attached to their hosts during the marine phase of their life cycle.

Spawning takes place over redds, constructed by the males in gravelly to rocky riffles. The females of parasitic species are highly fecund, sometimes with >100 000 eggs, depending upon the species and their size. Egg production is lower in smaller nonparasitic species. Spent male and female lampreys die within days of spawning.

Feeding

Larval lampreys filter-feed on suspended matter in the water column, which is mainly detritus, but includes diatoms, algae, and other microorganisms. The velar pump draws water into the pharynx via the oral hood that overhangs the oral cavity. Initial filtration is by the fringe-like oral cirrhi that guard the mouth, but smaller food particles are subsequently trapped in the pharynx by tangles of mucus secreted by the endostyle and by the lateral walls of the pharynx. The food is subsequently diverted to the esophagus and intestines, while the water exits the pharynx via the branchiopores (**Figure 3**).

Parasitic lampreys may begin feeding as juvenile adults shortly after metamorphosis is completed, often as they migrate downstream. Their diet consists of blood, tissue, or both, depending upon the species. The serrated, piston-like tongue of blood feeders is equipped with many fine teeth for piercing the hide of hosts/prey. Flesh feeders contain a few stout cusps for gouging tissue (**Figure 1**). The velar tentacles are also larger in number and size in flesh feeders, presumably to prevent chunks of tissue from clogging the branchiopores (**Figure 1**). Both blood- and flesh feeders secrete lamphredin from the paired buccal glands. Lamphredin contains cytolytic enzymes and an anticoagulant to break down tissue and to prevent blood clotting, respectively, but the secretion may also have anesthetic properties.

In freshwater, parasitic lampreys attack a variety of teleosts; large salmonids such as lake trout are preferred in the Great Lakes. Less is known about marine feeding, where prey includes Atlantic salmon and Pacific salmon, swordfish, eel, mackerel, herring, and cod. Sea lampreys have been sited attached to North Atlantic right whales, and to a variety of shark species including sandbar, dusky, tiger, Greenland, and basking sharks (**Figure 6**; see **Video Clip 2**). Although actual feeding on sharks was considered doubtful due to the shark's thick dermal denticle armor, the high urea excretion rates measured in lampreys removed from a basking shark confirmed that feeding had taken place (**Figures 6**(**b**) and **6**(**c**)). Because lampreys have low urea production rates, most of the postremoval urea excreted must have originated from the shark, which retain high concentrations of urea in their blood and tissues as a counterbalancing osmolyte as in all marine elasmobranchs (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts).

Upstream-migrant lampreys do not feed, but are able to survive for months by subsisting on endogenous lipid stores and, in the final stages of their life, the proteolysis of tissue protein. Remarkably, the Pacific lamprey undergoes natural starvation for up to 14 months during its spawning migration up large rivers in British Columbia.

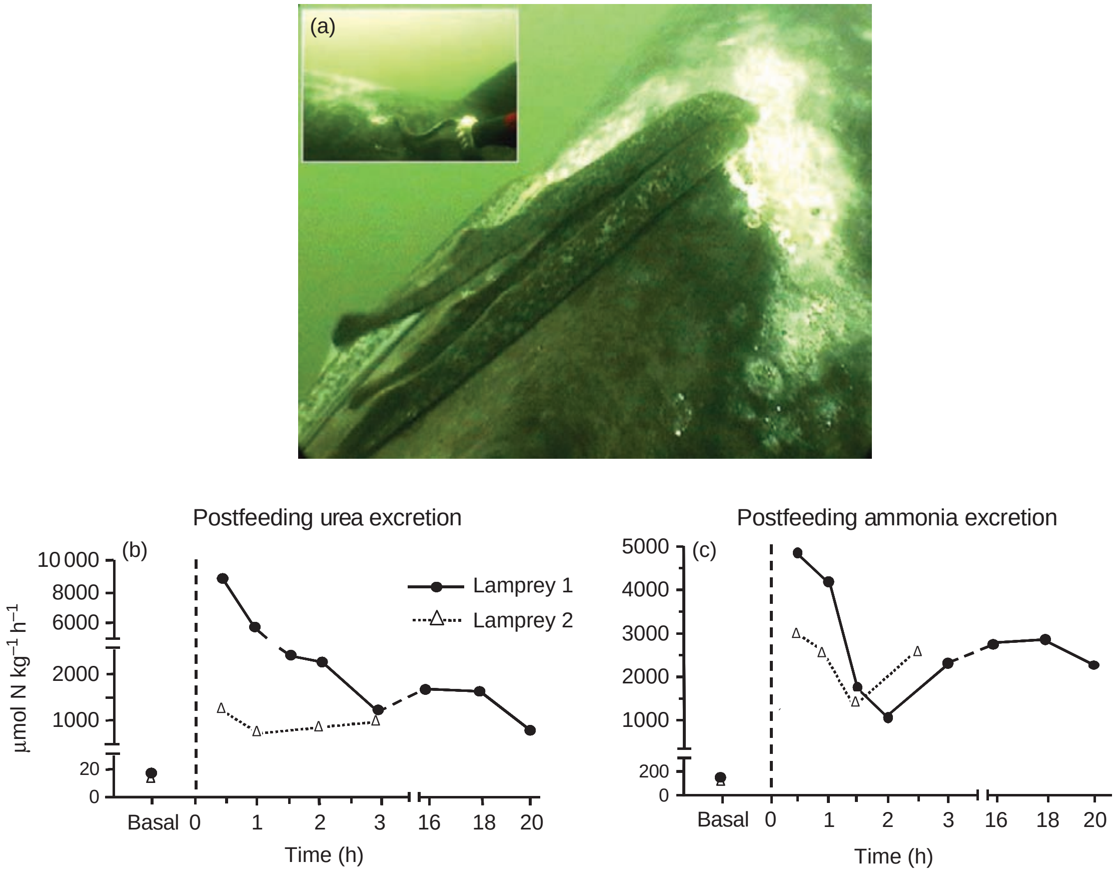


Figure 6 Sea lamprey parasitism of sharks. Sea lamprey feeding upon a basking shark (*Cetorhinus maximus*) in the Bay of Fundy, New Brunswick, Canada (a). Feeding was confirmed by marked elevations in urea (b) and ammonia (c) excretion measured immediately following the removal of lampreys from the sharks. Reproduced from Wilkie MP, Turnbull S, Bird J, *et al*. (2004) Lamprey parasitism of sharks and teleosts: High capacity urea excretion in an ancient vertebrate relic. *Comparative Biochemistry and Physiology, Part A* 138: 485–492.

Growth and Metabolism

The low nutritive value of their food and slow rates of assimilation are associated with low metabolism and slow growth, which result in the prolonged larval stage compared with other metamorphosing vertebrates (e.g., amphibians). Growth is greatest in early midsummer, when food supply is abundant, but before waters become too warm, which impairs digestion and lowers assimilation efficiency. Glycogen concentrations are maintained at relatively high concentrations in the muscle, likely to fuel burst swimming or burrowing (see above). Glycogen is also substantially higher in the meninges of the brain than in other fishes, supplying glucose to the nervous system. Glycogen stores in the very small liver (<1% of wet body mass) and the nephric folds (kidney plus surrounding adipose tissue) are limited, or in the case of muscle glycogen, unavailable for glucose homeostasis.

The fall and winter preceding metamorphosis is one of arrested growth for sea lampreys, when they stop increasing in length but continue to gain mass as they accumulate the lipid needed to endure the nontrophic (nonfeeding) metamorphic phase. An arrested-growth phase is not seen in other species of parasitic or nonparasitic lampreys, such as the pouched or American brook lamprey, which gradually accumulate lipid throughout larval life. Lipid accumulates in the nephric folds and the fat columns surrounding the notochord, with lesser amounts accumulating in the intestine, beneath the skin and among the viscera. While the liver stores lipid, its overall contribution is likely minimal due to its small size. The progressive reductions in kidney and liver triacylglycerol and other lipid stores indicate that these are main lipid fuel depots during metamorphosis. Amino acid catabolism increases when lipid stores are nearing depletion as metamorphosis is completed.

The parasitic phase is associated with rapid growth. At the completion of metamorphosis, when anadromous sea lampreys begin their downstream migration, they are less than 20 cm long and weigh a few grams. When they return to freshwater up to 24 months later to migrate upstream, they may be over 80 cm long and weigh approximately 900 g. Landlocked sea lampreys and other species are generally much smaller at this age.

During the parasitic phase, lipid re-accumulates in the nephric folds and liver. In addition, glycogen increases in the muscle, likely to fuel the bursts of swimming needed to pursue prey or evade predators. The very high concentrations of glycogen in the meninges of the brain (>100 mmol kg^{-1} wet tissue) may provide a critical glucose reserve for the nervous system when lampreys starve for long periods in the parasitic phase, or during upstream migration.

Glycogen and lipid synthesis in parasitic juvenile lampreys at sea clearly comes from protein-rich blood. Blood is ingested at high rates, 2–16% of their dry body mass per day, which can result in a nitrogenous waste (mainly ammonia and lesser amounts of urea) load up to 5 times of that reported in intensively feeding hatchery-reared salmon (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). Postmetamorphic increases in the rates of nitrogenous waste excretion and the activities of the amino acid catabolizing enzymes, such as glutamate dehydrogenase (GDH) in sea lampreys, suggest that juvenile lampreys have a high capacity to use amino acids for glycogenesis and lipogenesis when ingesting large amounts of protein-rich blood.

Like salmonids (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle), migrating lampreys rely on stored lipid to satisfy their basal energy requirements. Between the onset of migration and spawning, whole-body lipid stores decrease 50% in both males and females. Proteolysis becomes important in the terminal phase of migration just before or following spawning, when available lipids are likely nearing depletion. Not surprisingly, fat and protein content in male and female gonads are not reduced, even after periods of starvation lasting several months or more. Spawning is accompanied by further depletion of energy reserves including muscle glycogen, which likely fuels the final bouts of burst exercise associated with redd building and the spawning act. While the reduction in fuel reserves associated with the upstream migration and spawning is severe, it does not approach those levels observed immediately following metamorphosis. Thus, death following spawning is not likely the result of energy depletion in lampreys, but one of general senescence.

The Great Lakes Invasion by Sea Lampreys

Much of the original interest in lamprey physiology was due to the devastating effects that sea lamprey had on fisheries in the Great Lakes of North America. There are likely two populations of sea lampreys in North America, an anadromous population found along the Atlantic coast, and a landlocked population now thought to be indigenous to Lake Ontario, based on recent microsatellite genotyping analysis.

Niagara Falls posed an insurmountable barrier to sea lamprey entry from Lake Ontario into Lake Erie and beyond, until a deeper and wider Welland canal was constructed in mid-1800s that eventually allowed invasion of Lake Erie and the upper Great Lakes. By the 1940s, sea lamprey predation of Great Lake fishes, along with overfishing, had wiped out many economically important fishes; lake trout populations were near collapse.

In response, Canada and the United States formed the Great Lakes Fishery Commission (GLFC), a binational body given the mandate to eradicate the sea lamprey.

Sea lampreys were never eradicated from the Great Lakes. Instead, the GLFC developed and implemented an integrated pest-management plan to control sea lamprey populations. The measures include

1. *Physical barriers*. The use of traps and barriers to block the upstream migration of sea lampreys into rivers and streams that are suitable for spawning and the nurturing of larval sea lamprey.
2. *Sterilizing techniques*. Captured males may be treated with a compound (bisazir) that renders them sterile. The sterilized males are then released onto spawning beds to mate with females, without fertilizing the eggs.
3. *Pesticides*. The most widely used, and successful, method of sea lamprey control is the pesticide, 3-trifluoromethyl-4-nitrophenol (TFM), which is applied to lamprey-infested streams, sometimes in combination with low amounts (1–2%) of niclosamide, a molluskcide. TFM selectively targets larval sea lampreys, which have a low capacity to detoxify TFM compared to other resident fishes and invertebrates. By interfering with oxidative phosphorylation in the mitochondria, TFM reduces ATP production rates and eventually depletes fuel stores leading to death. TFM also undergoes rapid photodegradation, minimizing environmental persistence and reducing its nontarget impact on aquatic ecosystems. In recent years, a better knowledge of sea lamprey growth, metamorphosis, and metabolism has made it possible to predict from length and weight measurements when sea lampreys start metamorphosis. This permits targeted TFM applications in streams containing larval sea lampreys most likely to enter metamorphosis the following summer.
4. *Attractants*. The recent recognition that upstream-migrant sea lampreys rely on bile salts (pheromones) secreted by larval sea lampreys to locate suitable spawning habitat has made it possible to develop attractants that can more effectively trap upstream-migrant lampreys or divert them into unsuitable habitat.

As a better knowledge of lamprey physiology develops, more effective means of sea lamprey control will follow. However, an improved understanding of lamprey physiology might also contribute to the conservation of vulnerable lamprey species. Unfortunately, populations of these living fossils are threatened in many parts of the world.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Experimental Hydrodynamics; Swimbladder Function and Buoyancy Control in Fishes; Undulatory Swimming. **Fish Migrations**: Pacific Salmon Migration: Completing the Cycle; The Biology of Fish Migration. **Hagfishes and Lamprey**: Hagfishes; Lampreys: Environmental Physiology. **Hypoxia**: Anaerobic Metabolism in Fish. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts.

Further Reading

Beamish FWH and Potter IC (1975) The biology of anadromous sea lamprey (*Petromyzon marinus)* in New Brunswick. *Journal of Zoology, London* 177: 57–72.

Boutilier RG, Ferguson RA, Henry RP, and Tufts BL (1993) Exhaustive exercise in the sea lamprey (*Petromyzon marinus)*: Relationships between anaerobic metabolism and intracellular acid–base balance. *Journal of Experimental Biology* 178: 71–88.

Brown LR, Chase SD, Mesa MG, Beamish RJ, and Moyle PB (2009) *Biology, Management, and Conservation of Lampreys in North America*. Bethesda, MD: American Fisheries Society.

Bryan MB, Zalinski D, Filcek KB, *et al.* (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus)* in North America as revealed by microsatellite genotypes. *Molecular Ecology* 14: 3757–3773.

Gess RW, Coates MI, and Rubidge BS (2006) A lamprey from the Devonian period of South Africa. *Nature* 443: 981–984.

Johnson NS, Yun S-S, Thompson HT, Brant CO, and Li W (2009) A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps. *Proceedings of the National Academy of Sciences of the United States of America* 106: 1021–1026.

Li W, Scott AP, Siefkes MJ, *et al.* (2002) Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* 296: 138–141.

Mallatt J (1996) Ventilation and the origin of jawed vertebrates: A new mouth. *Zoological Journal of the Linnean Society* 117: 329–404.

Osório J and Rétaux S (2008) The lamprey in evolutionary studies. *Development*, *Genes and Evolution* 218: 221–235.

Potter IC and Gill HS (2003) Adaptive radiation of lampreys. *Journal of Great Lakes Research* 29: 95–112.

Potter IC, Gill HS, and Renaud CR. Petromyzontidae : Lampreys. In: Warren M and Burr B (eds.) Freshwater Fishes of North America, chap. 1. Baltimore: John Hopkins University Press (in press).

Reinhardt UG, Eidietis L, Friedl SE, and Moser ML (2008) Pacific lamprey climbing behaviour. *Canadian Journal of Zoology* 86: 1264–1272.

Renaud CB, Gill HS, and Potter IC (2009) Relationships between the diets and characteristics of the dentition, buccal glands and velar tentacles of the adults of the parasitic species of lamprey. *Journal of Zoology* 278: 231–242.

Rovainen CM (1996) Feeding and breathing in lampreys. *Brain Behaviour and Evolution* 48: 297–305.

Sorensen PW and Hoye TR (2007) A critical review of the discovery and application of a migratory pheromone in an invasive fish, the sea lamprey *Petromyzon marinus* L. *Journal of Fish Biology* 71: 100–114.

Sower SA, Freamat M, and Kavanaugh SI (2009) The origins of the vertebrate hypothalmic–pituitary–gonadal (HPG) and hypothalmic–pituitary–thyroid (HPT) endocrine systems: New insights from lampreys. *General and Comparative Endocrinology* 161: 20–29.

Waldman J, Grunwald C, and Wirgin I (2008) Sea lamprey *Petromyzon marinus*: An exception to the rule of homing in anadromous fishes. *Biology Letters* 4: 659–662.

Wilkie MP, Bradshaw PG, Joanis V, Claude JF, and Swindell SL (2001) Rapid metabolic recovery following vigorous exercise in burrow-dwelling larval sea lampreys (*Petromyzon marinus*). *Physiological and Biochemical Zoology* 74: 261–272.

Wilkie MP, Turnbull S, Bird J, *et al.* (2004) Lamprey parasitism of sharks and teleosts: High capacity urea excretion in an ancient vertebrate relic. *Comparative Biochemistry and Physiology, Part A* 138: 485–492.

Youson JH (2003) The biology of metamorphosis in sea lampreys: Endocrine, environmental, and physiological cues and events, and their potential application to lamprey control. *Journal of Great Lakes Research* 29: 26–49.

Relevant Websites

http://www.dfo-mpo.gc.ca – Description of the role of Canada's Department of Fisheries and Oceans role in controlling invasive species in Canada, including the sea lamprey.

http://www.blueworldtv.com – Episode 15, 'The Shark and the Lamprey', with more information on attempts to find and study lampreys feeding on sharks. From the PBS television show Jonathan Bird's Blue World© covering marine biology with links to educational resources and photography.

http://www.GLFC.org. – The web site of the Great Lakes Fishery Commission provides a historical account of the sea lamprey invasion of the Great Lakes, and describes ongoing efforts to control sea lamprey and other invasive species in the basin. Links to sites describing ongoing and previous research, literature, and photos.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Lampreys: Environmental Physiology

MP Wilkie, Wilfrid Laurier University, Waterloo, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Gas Exchange and Cardiovascular System
Osmoregulation
Acid–Base Regulation
Nitrogenous Waste Production and Excretion
Concluding Remarks
Further Reading

Glossary

External branchiopore One of seven external openings found on either side of the lamprey head through which exhalent water currents pass after irrigating the gills.

Gill pouch One of seven pairs of tidally ventilated gill chambers in post-metamorphic lampreys in which exhalent water is forced across the gills by active compression of the gill basket, and inhalant water is drawn into the chamber due to the elastic recoil associated with branchial muscle relaxation.

Glomus A bundle of capillaries across which primary urine (filtrate) is produced, and subsequently taken up by one of several associated kidney (renal) tubules via the renal (Bowman's) capsule.

Internal branchiopore One of seven internal openings that connect the water tube (branchial duct) in lampreys to the gill pouches.

Opisthonephros The functional kidney found in the posterior trunk of larval and post-metamorphic lampreys, which replaces the early larval pronephros.

Pronephros A kidney formed from the anterior nephric ridge during development that is comprised of a glomus that services several kidney tubules, but gradually degenerates during larval lamprey life and during embryonic development in higher vertebrates.

Velum A muscular diaphragm-like organ that generates currents to draw water into the pharynx of larval lampreys, and separates the esophagus from the water tube in post-metamorphic lampreys.

Introduction

Lampreys likely face a range of environmental challenges during their complex life cycle (**Figure 1**; **see also Hagfishes and Lamprey**: Lampreys: Energetics and Development). Larval lampreys may have to deal with fluctuations in O_2 and temperature within their burrows in the silty substrate of streams, and the increased metabolic demands and reorganization of the respiratory and circulatory systems during metamorphosis may explain why transforming lampreys move to faster-flowing, better-oxygenated waters. Anadromous parasitic-phase lampreys must also acclimatize to seawater, while upstream-migrant lampreys have to re-acclimatize to freshwater, navigate around obstacles, and/or swim against fast-flowing waters prior to the terminal, spawning phase of their life cycle. Despite such challenges, lampreys have proven very resilient, having survived in their present form since at least the Devonian (~360 million years ago (mya)). Much of the lamprey's success may be related to a variety of respiratory, cardiovascular, and renal adaptations that allow them to cope with a wide range of environmental challenges.

Gas Exchange and Cardiovascular System

Respiration

Respiration primarily takes place across the gills (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics and O_2 Uptake and Transport: The Optimal P_{50}). An extensive dermal capillary network in the adults of some species, however, may allow some gas exchange to take place across the skin. A dermal capillary network is absent in larvae, but the subcutaneous tissue below the thin dermis is well vascularized, and perhaps sufficient to allow limited cutaneous O_2 and CO_2 exchange. Migrating adults of the pouched lamprey (*Geotria australis*), Pacific lamprey (*Lampetra tridentata*), and likely other species emerge from water during upstream migration. The skin could become important for respiration if gill function is compromised under such conditions. In fact, *G. australis* can survive emersion for many hours in moist environments, but it is unclear if this is due to cutaneous respiration or a resistance to collapse of the gill pouch (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

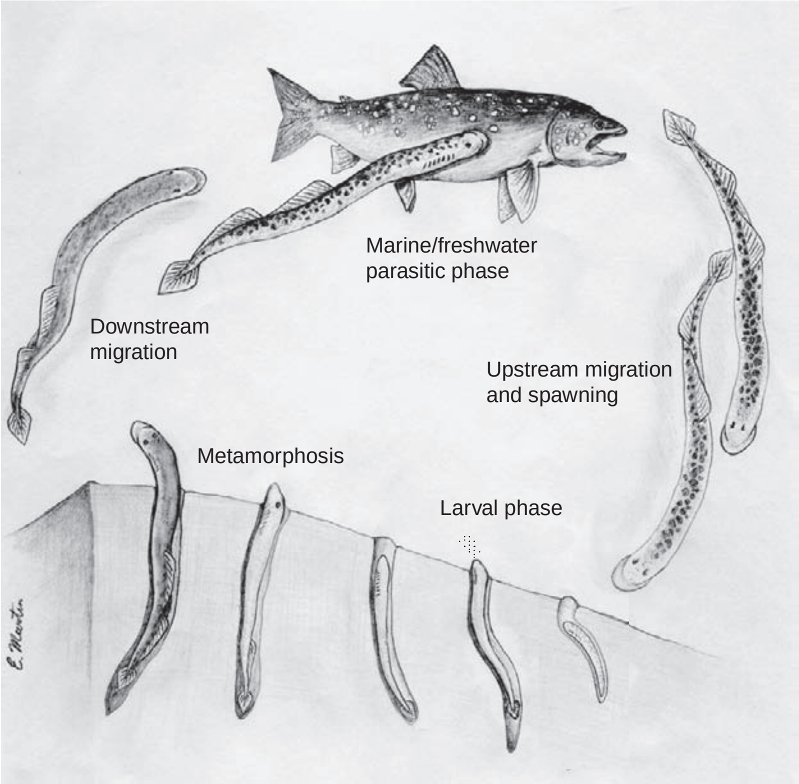

Marine/freshwater parasitic phase
Downstream migration
Upstream migration and spawning
Metamorphosis
Larval phase
E. Martin

Figure 1 Summary of the life cycle of a parasitic lamprey species.

There are seven gill slits or pores (branchiopores) leading into branchial pouches through which water passes when the gills are irrigated. The gills themselves are comprised of gill filaments studded with numerous thin, plate-like lamellae, the probable site of gas exchange, as in the teleost fishes (**see also Role of the Gills**: Morphology of Branchial Ionocytes). Unlike in teleost fishes, however, the gills are supported by a lattice-like cartilaginous skeleton (branchial basket). The outer margins of lamellae from adjacent filaments come into close contact with one another, forming a sieve-like barrier through which exhalent water flows. Blood flow through the lamellae is counter to water flow – a similar countercurrent arrangement to that of elasmobranch and teleost fishes that maximizes O_2 uptake by ensuring a steady partial pressure diffusion gradient is maintained along the length of each lamella (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics and **Transport and Exchange of Respiratory Gases in the Blood**: O_2 Uptake and Transport: The Optimal P_{50}). It is likely that blood flow through the lamellae is controlled by neural and humoral factors, but experimental studies are lacking.

Irrigation of the larval gill is unidirectional, but unlike teleosts which use a buccal–opercular pump to irrigate the gills, larval lampreys generate respiratory currents via contractions of a muscular velum (velar pump) and the branchial musculature, which is anchored to the branchial basket (**Figure 2**(**a**)). Upon inspiration, medial flaps of the velum seal together and contract in a caudal direction, increasing the volume and decreasing the pressure in the oral cavity, drawing water into the mouth (**Figure 2**(**b**)). As the velum continues to contract (velar stroke), the branchial muscles contract, squeezing the pharyngeal chamber and forcing water across the gills, and out the branchiopores. Relaxation of the velar and branchial musculature leads to elastic recoil of the pharyngeal chamber and closure of the branchial valves guards against flow reversal. The corresponding decrease in intrapharyngeal pressure continues to draw water into the pharynx via the oral cavity and the process repeats (**Figure 2**(**b**)).

Ventilation is primarily tidal after metamorphosis, which allows lampreys to breathe while attached to their prey or the substratum with their oral disk. The velum is also remodeled, primarily serving as a valve to ensure that ingested blood/tissue is directed to the esophagus, and away from the branchiopores (**Figure 3**(**a**)). During respiration, contraction of the branchial musculature forces water out of the gill pouches. Subsequent relaxation of the branchial musculature leads to elastic recoil (expansion) of

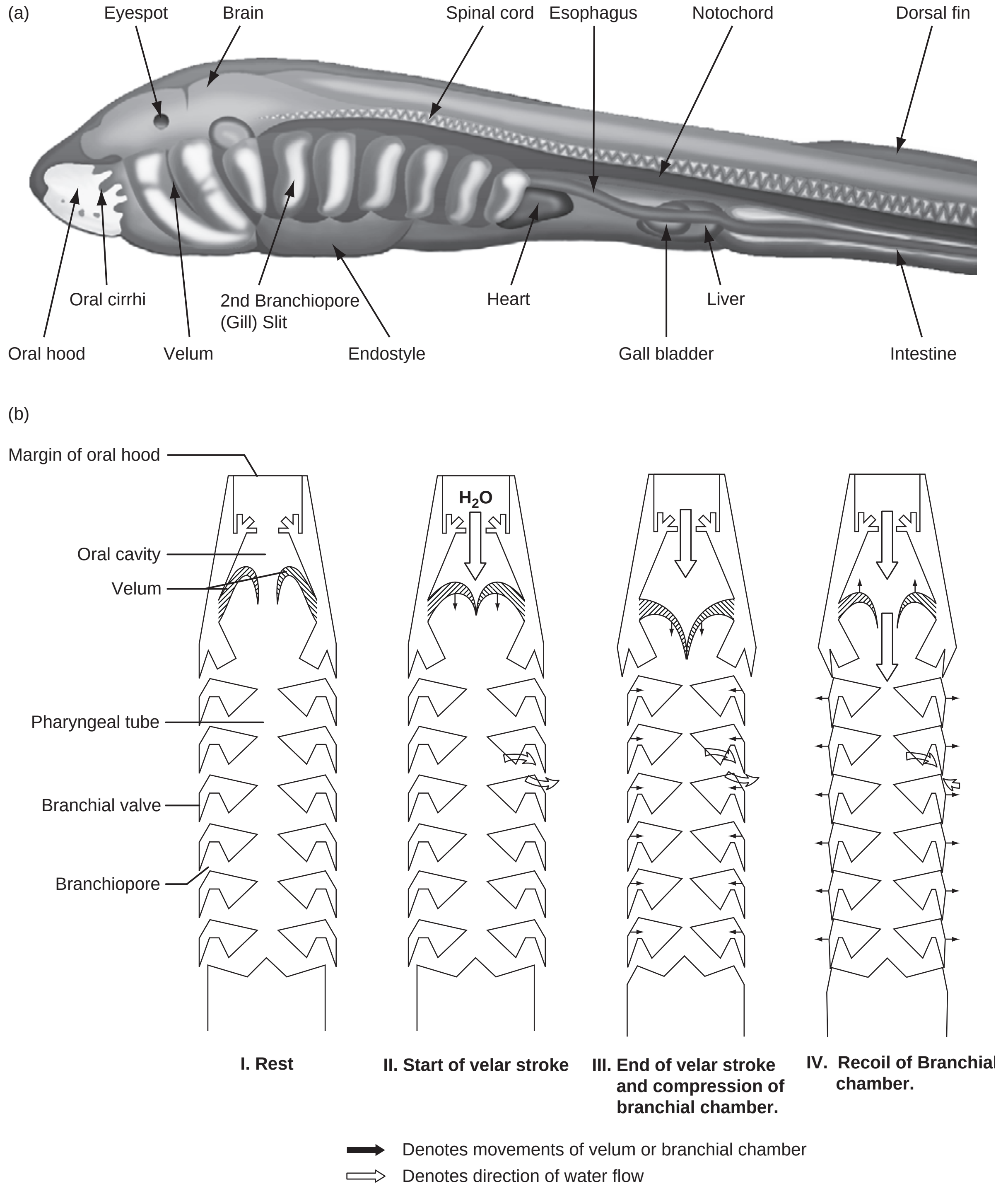

(a)
Eyespot
Brain
Spinal cord
Esophagus
Notochord
Dorsal fin
Oral cirrhi
2nd Branchiopore (Gill) Slit
Heart
Liver
Oral hood
Velum
Endostyle
Gall bladder
Intestine
(b)
Margin of oral hood
Oral cavity
Velum
Pharyngeal tube
Branchial valve
Branchiopore
H2O
I. Rest
II. Start of velar stroke
III. End of velar stroke and compression of branchial chamber.
IV. Recoil of Branchial chamber.
Denotes movements of velum or branchial chamber
Denotes direction of water flow

Figure 2 (a) Lateral view of the head region of a larval sea lamprey. Water containing O_2 and food is drawn into the oral cavity and pharynx (pharyngeal tube) via contractions of the velum before passing across and exiting via the branchiopores. Mucus secreted by the endostyle, traps food in the pharynx, which is then directed to the esophagus. (b) The velar pump. Contraction (solid arrows) of the velum initiates the velar stroke (II) which expands the volume of the oral cavity generating inward water flow (open arrows) but reduces the volume of the branchial chamber forcing water across gills. Near the end of the velar stroke (III), contraction of branchial musculature ensures continued flow across gills. Elastic recoil and relaxation of the velum (IV) results in expansion of the branchial chamber and continued unidirectional water flow, while closure of the branchial valves prevents backflow across gills. (a) Redrawn from Hardistry MW and Potter IC (1971) The behaviour, ecology and growth of larval lampreys. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 1, pp. 85–125. London: Academic Press. (b) Modified from Rovainen CM (1982) Neurophysiology. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 4A, pp. 1–136. London: Academic Press.

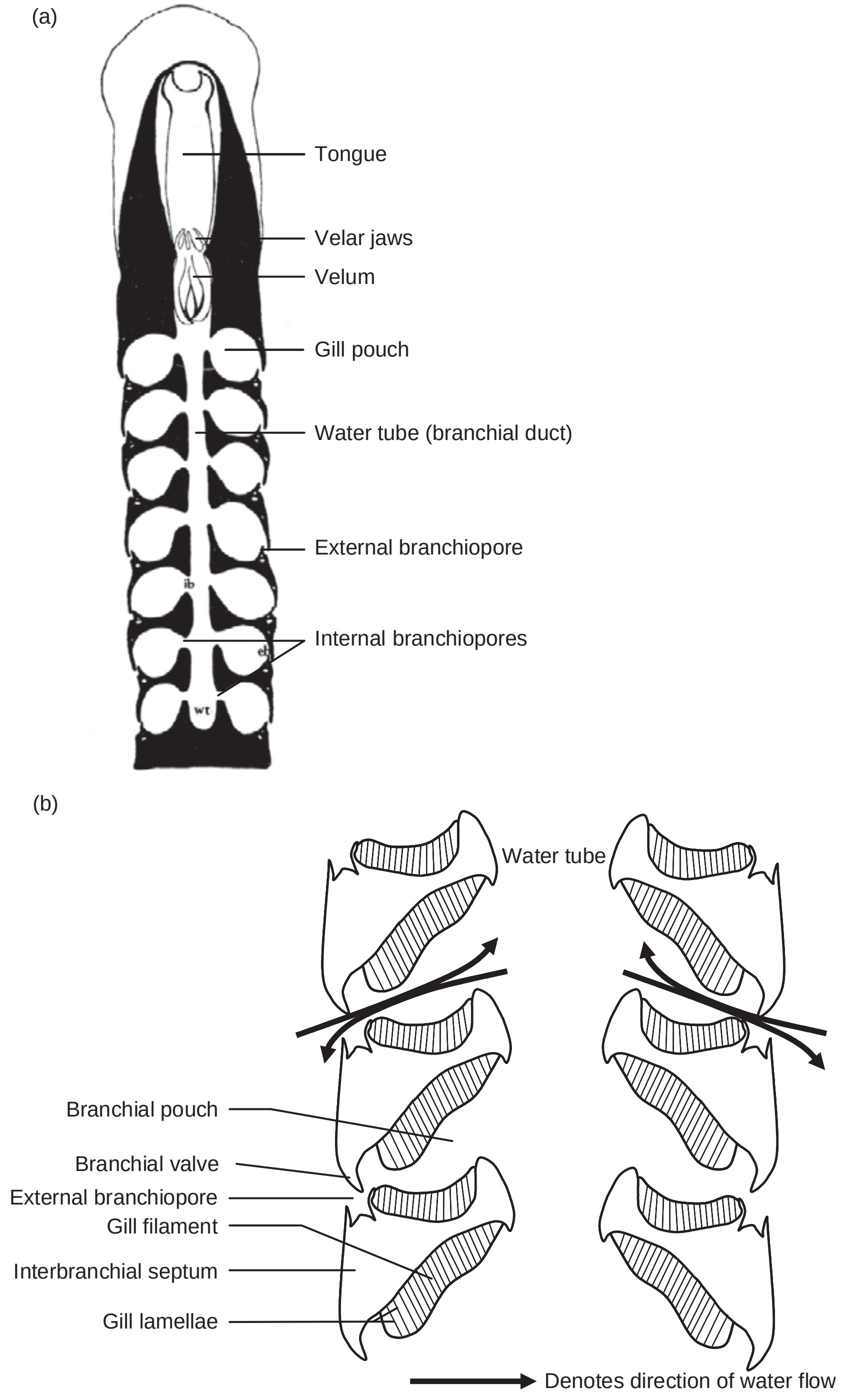


Figure 3 (a) Transverse view of head of post-metamorphic lamprey. Gills are irrigated in a tidal fashion by pumping water in and out of gill (branchial) pouches via external branchiopores. Internally, the gill pouches are connected via internal branchiopores to the water tube (branchial duct). (b) Schematic representation of ventilation in post-metamorphic lamprey gills. Contraction of branchial musculature forces water across the gills and out the external branchiopore, followed by elastic recoil which expands branchial pouch to draw in water. Redrawn with modifications from Randall DJ (1972) Respiration. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 2, pp. 287–306. London: Academic Press.

the branchial basket, creating suction to draw water into the gill pouches during inspiration (**Figure 3**(**b**)).

To promote gas exchange, more active or fast-growing fishes tend to have larger lamellar surface areas and thinner lamellae compared to more sedentary species. Despite a 2- to 2.5-fold increase in O_2 consumption following metamorphosis, the blood–water diffusion distance across the lamellar epithelium and the total gill surface area change little in lampreys. In fact, total gill surface area (14–27 $cm^2\ g^{-1}$ body mass) is comparable to that of many active teleosts (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics). Such findings might explain the relatively high metabolic scope of lampreys. Although larval lampreys have very low resting metabolic rates when burrowed, they can increase their mass-specific O_2 consumption by 5–6 times following exercise, which is comparable to measures made in salmonid fishes (**see also Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming).

Circulation

Deoxygenated blood is pumped by a heart into the ventral aorta, which subdivides into paired afferent branchial arteries. The blood then flows from the branchial arteries to afferent arterioles and then lamellar capillary beds. The blood leaves each lamella via an efferent arteriole, subsequently draining into an efferent branchial artery and the dorsal aorta, which subdivides into vessels that provide the head and trunk with oxygenated blood. Although the number is different, the branchial vessels have a similar arrangement as in teleosts (**see also Circulation**: Circulatory System Design: Roles and Principles).

Most O_2 is transported bound to hemoglobin, which is comprised of an iron-containing heme group to which O_2 reversibly binds. Unlike most vertebrates (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin), lamprey hemoglobin dissociates to form monomers (single globin chains) when oxygenated. Upon deoxgenation, however, the hemoglobins associate to form dimers (two globin chains) or oligomers (<2 chains). The O_2 affinity of the oxygenated monomer is higher than that of the oligomers, as characterized by hemoglobin-O_2 dissociation curves that are parabolic in shape with low P_{50} values (**Figure 4**(**a**)). This serves to maximize O_2 uptake by the animals, especially when O_2 is limited. In contrast, the formation of dimeric or oligomeric hemoglobin with deoxygenation is characterized by a more sigmoidal O_2-dissociation curve which is shifted to the right (**Figure 4**(**a**)). This association may promote hemoglobin-O_2 unloading at the tissues by lowering the hemoglobin-O_2 binding affinity (higher P_{50}) when O_2 is abundant in the water. Depending on the species, O_2 unloading is markedly enhanced by acidification of the blood (Bohr shift; **see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function) arising from the production of metabolic acid and CO_2.

Carbon dioxide loading at the tissues is facilitated by a pronounced Haldane effect, which increases the blood's CO_2-carrying capacity through the carbonic anhydrase (CA) catalyzed hydration of CO_2 to HCO_3^- and H^+ within the red blood cell (RBC; **Figure 4**(**b**)). Unlike higher vertebrates (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion and Carbonic Anhydrase in Gas Transport and Exchange), lampreys retain most of this HCO_3^- within the RBC (as opposed to the plasma) because they lack an erythrocytic Cl^-/HCO_3^- exchange protein. The H^+ associates with the hemoglobin, to facilitate O_2 offloading at the tissues via the Bohr effect. At the gills, oxygenation leads to the release of H^+ from the hemoglobin, leading to the CA-mediated dehydration of HCO_3^- to CO_2. The resultant increase in the P_{CO_2} diffusion gradient allows CO_2 to be excreted across the gills (**Figure 4**(**b**)).

The unique characteristics of their hemoglobin gives lampreys the dual ability to efficiently offload O_2 during vigorous exercise (e.g., swimming or burrowing) when O_2 is abundant, and to maximize O_2 uptake when O_2 is limited (hypoxia). It is difficult to establish when free-swimming, parasitic or upstream-migrant lampreys might encounter hypoxic conditions, but larval lampreys certainly face O_2 limitations at the sediment–water interface or within their burrows. In fact, larval lampreys survive indefinitely at O_2 tensions below 10 mmHg and for up to 36 h at 3–5 mmHg in cold (5 °C) water. They cannot survive anoxia. When the river lamprey (*Lampetra fluviatilis*) becomes hypoxic, hemoglobin-O_2 affinity increases due to RBC swelling, which dilutes the hemoglobin leading to less globin chain interaction. Hypoxia-induced increases in RBC pH also cause a leftward Bohr Shift and increased O_2 affinity (**Figure 4**(**a**)). Larval lampreys likely have a higher basal hemoglobin-O_2 affinity than post-metamorphic lampreys as indicated by their 80–90% lower P_{50} values.

The hypoxia tolerance of lampreys is also related to their ability to lower metabolic demands as demonstrated by marked decreases in ventilation during hypoxia. The presence of blood sinuses in the lamprey circulatory system contributes to a high venous O_2 reserve in the event of hypoxia. Despite relatively low concentrations of liver glycogen, the glycogen reserves are very high in the meninges of the brain (<100 mmol kg^{-1} wet wt), particularly in parasitic and upstream-migrant lampreys, which may provide an anaerobic energy source for the nervous system during hypoxia or glucose reservoir during prolonged starvation.

Osmoregulation

Freshwater

The body fluids of larval lampreys, landlocked post-metamorphic lampreys, and upstream-migrant lampreys are hyperosmotic to their freshwater habitats, resulting in large outward ion gradients and inwardly directed osmotic gradients (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

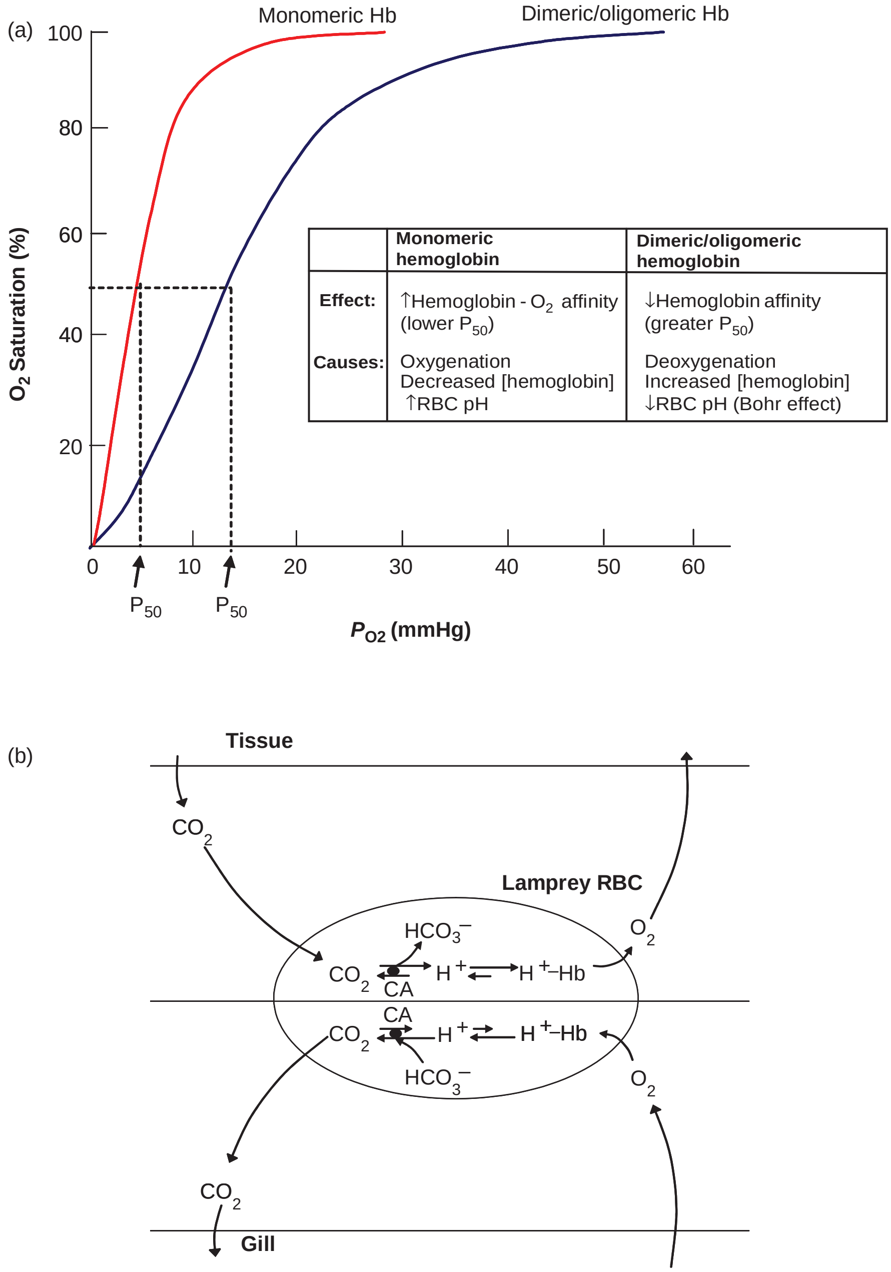


Figure 4 (a) Hemoglobin-oxygen dissociation curves of lampreys. Lamprey hemoglobin contains only a single heme group to which O_2 binds. Oxygenation causes hemoglobin to aggregate resulting in the formation of dimers or oligomers of hemoglobin, and a corresponding rightward shift in the hemoglobin-oxygen dissociation curve (greater P_{50}). (b) Carbon dioxide transport by lampreys. At the tissues (top half of figure) CO_2 diffuses into red blood cells (RBCs) down P_{CO_2} gradients maintained by the carbonic anhydrase (CA) catalyzed hydration of CO_2 to HCO_3^- and H^+ leading to H^+-hemoglobin associations that promote O_2 unloading to the tissues by decreasing hemoglobin-O_2 affinity (Bohr shift). H^+-hemoglobin association also increases intracellular pH, promoting greater HCO_3^- generation, increasing the blood CO_2-carrying capacity (Haldane effect). The HCO_3^- is retained in the RBC due to an absence of Cl^-/HCO_3^- exchange across the plasma membrane of the RBC. At the gills (lower half), the opposite occurs, where the loss of CO_2 across the gills leads to HCO_3^- dehydration and further CO_2 generation and unloading from the RBC. (b) Reproduced from Nikinmaa M, Airaksinen S, and Virkki LV (1995) Haemoglobin function in intact lamprey erythrocytes: Interactions with membrane function in the regulation of gas transport and acid–base balance. *Journal of Experimental Biology* 198: 2423–2430, with permission from Company of Biologist.

To counterbalance diffusive and renal ion losses, ions are extracted from blood as it is filtered at the kidneys and ions are taken up by the gills. There may also be some intestinal Na^+ and Cl^- uptake in larvae.

Larval lampreys have a pronephric kidney located in the anterior body, whichgradually atrophies before being replaced by a pair of opisthonephric (mesonephric) kidneys in the posterior two-thirds of the body. In both the pronephros and opisthonephros, blood is filtered across a glomus, which is a single, compact bundle of capillaries invested by several renal (Bowman's) capsules to form a renal corpuscle. The primary urine filtrate, comprised of small organic molecules (e.g., glucose and amino acids), nitrogenous wastes (ammonia and urea), and ions (Na^+, Cl^-, and Ca^{2+}), passes from the capsule into the proximal and the distal tubules, before draining into a common collecting duct and a urinary (archinephric) duct leading to the cloaca, the final site of urine excretion. The proximal and distal tubules are arranged in a loop that superficially resembles the loop of Henle in mammalian nephrons, but the functional significance, if any, is unknown (**Figure 5**(**a**)).

In freshwater, the kidneys excrete copious amounts of dilute urine to counter continual influxes of water across the gill and body surface (**see also Role of the Kidneys**: The Kidney and **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). Urine flow rates (UFRs) in larval lampreys and post-metamorphic lampreys range from 150 to 350 ml kg^{-1} d^{-1}, higher than in freshwater teleosts (~100 ml kg^{-1} d^{-1}). Thus, the gills and/or tegument of lamprey likely have high water permeability. Glomerular filtration rates

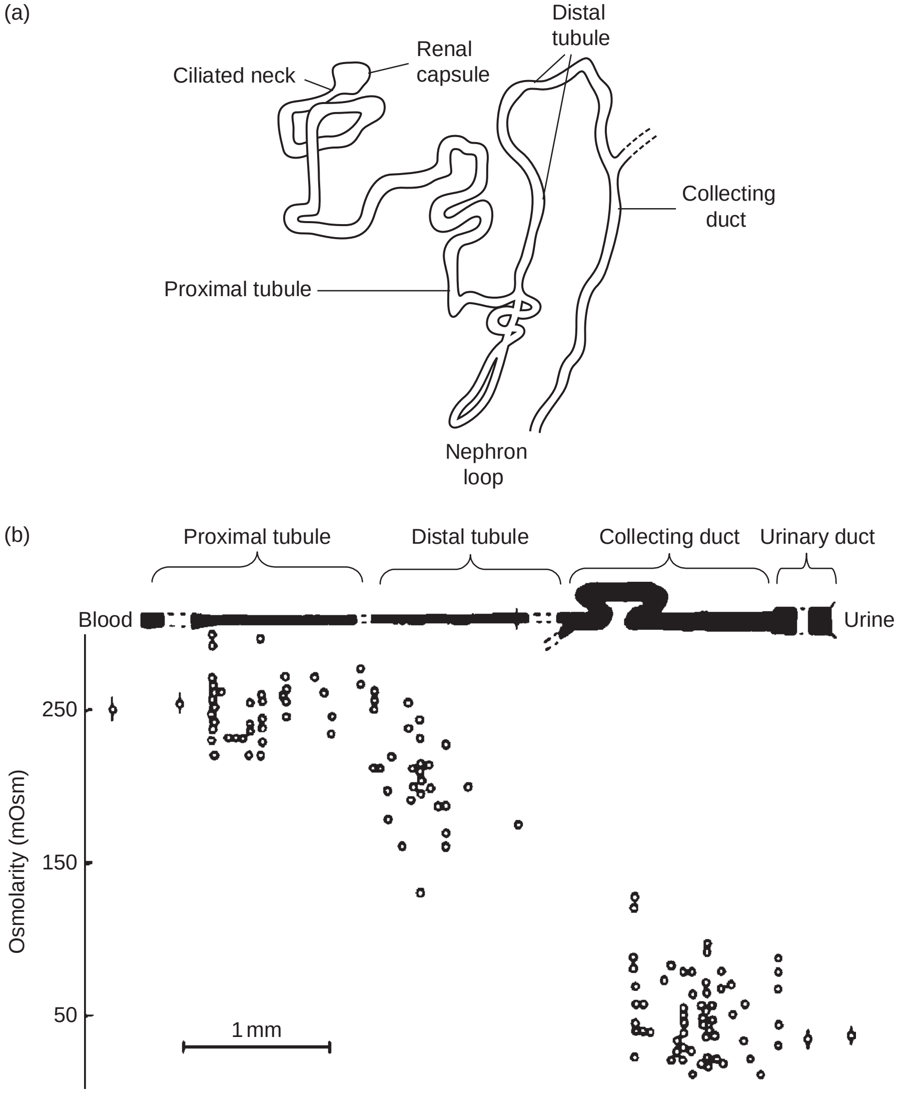


Figure 5 Lamprey renal tubule (opisthonephros) design and urine production. (a) Illustration of the lamprey renal tubule. Note the nephron loop, which superficially resembles the loop of Henle seen in the mammalian kidney. (b) Urine production in freshwater lampreys begins with formation of the primary filtrate at the renal capsule (Bowman's capsule) which empties into the proximal tubules of several renal tubules. The majority of ion reuptake in freshwater takes place at the distal tubule and the collecting segment resulting in a copious dilute urine. Reproduced from Logan AG, Moriarty RJ, and Rankin JC (1980) A micropuncture study of kidney function in the river lamprey, *Lampetra fluviatilis,* adapted to fresh water. *Journal of Experimental Biology* 85: 137–147, with permission from Company of Biologist.

(GFRs) are also high, approximately 5–7 times greater than teleost values. The dilute urine of freshwater lampreys has low concentrations of Na^+, Cl^-, and K^+ due to highly efficient renal tubular reuptake of these ions, which mainly (< 90%) takes place across the distal and collecting segments (**Figure 5(b)**). Radio-tracer studies (^{22}Na and ^{36}Cl) indicate that net urinary ion (Na^+ and Cl^-) loss is compensated by ion uptake by the gills.

As in the jawed fishes, the gills are a major site of osmotic and ion regulation, acid–base regulation, and nitrogenous waste excretion. The lamprey gill epithelium is comprised of mucus cells, pavement cells (PVCs), and mitochondrion-rich (MR) cells. There are three classifications of MR cells: ammocoete MR cells (AMRs) are found in larval lampreys (**Figure 6(a)**); intercalated MR (IMRs) cells are between PVCs in both ammocoetes and post-metamorphic lampreys (**Figure 6(b)**); and chloride cells (CC) appear after metamorphosis and disappear in upstream migrants (**Figure 6(c)**). Branchial ion uptake likely takes place via the MR cells, as in teleosts. However, the type of MR cell involved is unresolved. Because the morphology, surface area, and density of AMRs do not change following acclimation to more dilute or saline waters, their role in ionoregulation may be minimal. The IMRs (also referred to as ionocytes and ion uptake cells) have more extensive surface ornamentation and a complex tubular network in the apical region of the cell supporting a role in ion transport. The IMRs also exhibit ultrastructural alterations and increased surface area in response to altered water chemistry, further suggesting that they are sites of ion uptake. The CCs appear following metamorphosis (see below), when the AMRs are lost, but the IMRs are retained.

Immunohistochemical and molecular analyses indicate that Na^+ uptake is likely similar to that proposed for teleosts **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes) in which Na^+ crosses the apical membrane via epithelial Na^+ channels (eNac) via electrochemical gradients generated by an apical vacuolar H^+-adenosine triphosphatase (ATPase) pump and the basolateral Na^+/K^+-ATPase. The H^+-ATPase pumps H^+ across the apical membrane,

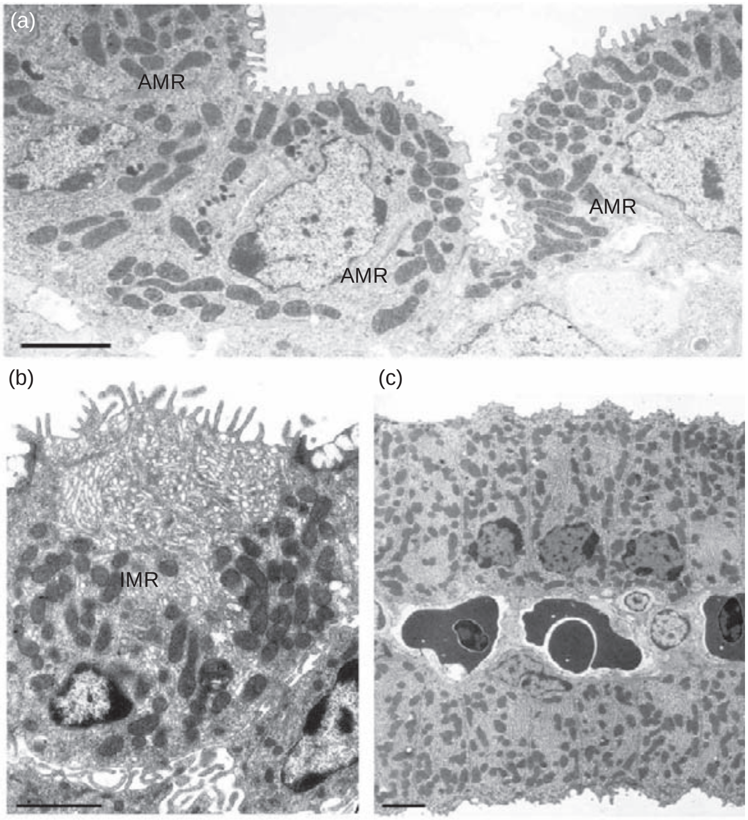


Figure 6 Ultrastructure of lamprey mitochondria-rich cells. (a) Ammocoete MR cells (AMR) cell from sea lamprey larvae, and (b) Intercalated MR (IMR) cell and (c) rows of chloride cells (CC) on either side of blood space in downstream migrant pouched lampreys. Note the extensive ornamentation and a complex tubular network in the apical region of intercalated MR cells and CCs which mediate ion uptake and ion excretion in freshwater and saltwater acclimated lampreys, respectively. Scale = 2 μm (a, b), 3 μm (c). Reproduced from Bartels H and Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462, with permission from Company of Biologist.

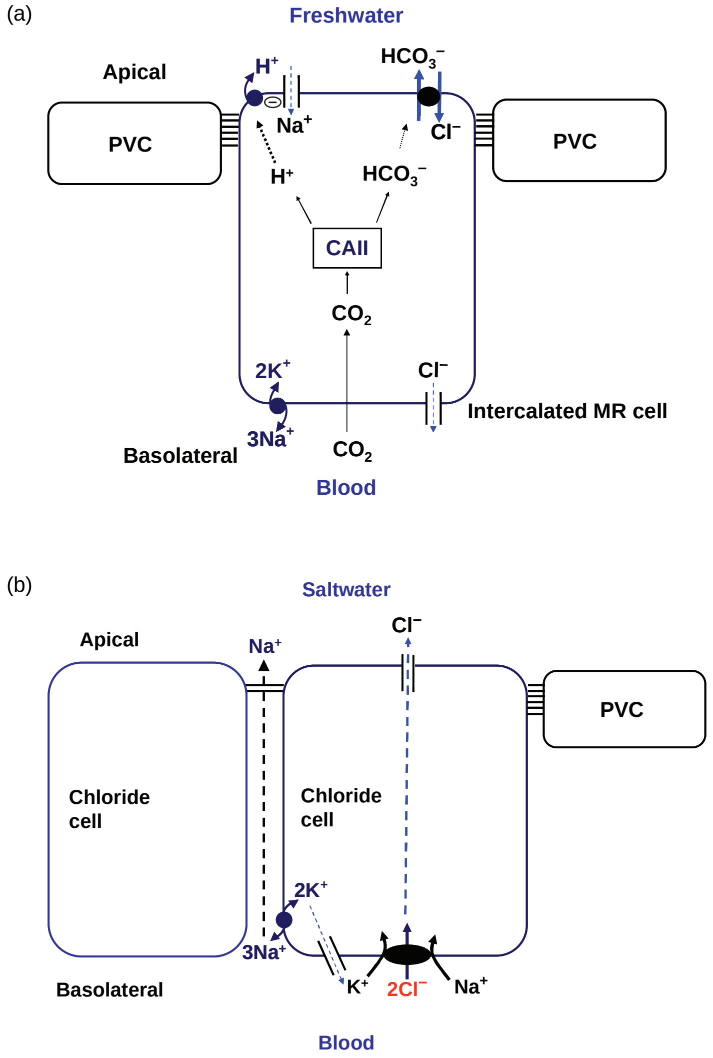


Figure 7 (a) Gill-mediated ion uptake in fresh water. The site of ion uptake is thought to be via intercalated mitochondria-rich (IMR) cells, though the precise mechanisms need confirmation. The pavement cells (PVCs) predominate in the gill but their role in ion exchange is presently thought to be minimal. (b) Gill-mediated ion uptake in saltwater. Higher rates of Na^+/K^+-ATPase activity in saltwater likely concentrate Na^+ in the paracellular spaces between adjacent rows of chloride cells (CC), generating electrochemical gradients that favor Na^+ extrusion across leaky tight junctions. Chloride excretion involves basolateral Na^+:K^+:$2Cl^-$ (NKCC) co-transport, leading to buildups of intracellular Cl^- that likely favor Cl^- excretion via apical Cl^- channels. From Bartels H and Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462.

creating an electrical gradient to draw Na^+ into the cell before it is transported to the blood via the oubain-sensitive basolateral Na^+/K^+-ATPase (**Figure** 7(**a**)). The presence of CA within the gill ICF promotes the H^+ generation needed to drive the H^+-ATPase and the HCO_3^- needed to drive Cl^- uptake, possibly via an apical anion exchange protein (Cl^-/HCO_3^- exchange) (**Figure** 7(**a**)).

Seawater

Metamorphosis prepares anadromous lampreys for their seaward migration, in addition to their parasitic lifestyle. Like anadromous teleosts (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation), sea lampreys drink seawater from which water absorption takes place in the anterior intestine down osmotic gradients generated by the uptake of Na^+ and Cl^-. UFR is reduced by more than 90% in saltwater due to decreases in GFR, and increased water uptake across the distal and collecting tubules. The urine has relatively low Na^+, suggesting that most of the Na^+ that enters the blood is excreted by branchial CCs. Yet, the urine is slightly hyperosmotic to the blood due to high concentrations of Cl^-, and toxic divalent ions such as Mg^{2+} and SO_4^{2-}.

The mode of Na^+ and Cl^- excretion by the CC is similar to marine teleosts (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts), and likely involves a basolateral Na^+:K^+:$2Cl^-$ (NKCC) co-transport system, which moves Na^+, K^+, and Cl^- into the cytosol of the CC (**Figure 7**(**b**)). The very high activities of Na^+/K^+-ATPase, on the tubular system and basolateral membranes of CCs, move the Na^+ into the paracellular spaces between the rows of adjacent CCs which likely generates an outwardly directed electrochemical gradient for Na^+ extrusion via leaky tight junctions. The K^+ passes back into the extracellular fluid (ECF) via basolateral channels. Chloride is proposed to exit the gills via apically located Cl^- channels, which have been isolated from gills of teleosts but not yet lampreys.

Hormonal Regulation

There is a lack of data on the hormonal regulation of osmo- and ionoregulation in lampreys. As in teleosts, arginine vasotocin (AVT) and the renin–angiotensin system appear to be involved. AVT injection in upstream-migrant river lampreys results in increased urinary Na^+ and K^+ excretion, and a greater UFR suggests that AVT might be involved in freshwater acclimation. Angiotensin-converting enzyme and angiotensin I have been isolated from river lamprey, indicating the presence of a renin–angiotensin system in lampreys. Angiotensin I and II injection increases blood pressure in lampreys, and the system may stimulate drinking behavior in seawater-acclimated lampreys, but this possibility requires further investigation.

Acid–Base Regulation

Because lampreys retain HCO_3^- in the RBC due to the lack of RBC Cl^-/HCO_3^- exchange, lampreys have a low capacity to buffer acid–base disturbances in the plasma (**Figure 4**(**b**)). This is compounded by a low nonbicarbonate (protein) buffering capacity, making the lamprey particularly prone to wide fluctuations in pH as a result metabolic H^+ production during exercise or environmental disturbances (e.g., low pH). Despite their vulnerability to plasma (extracellular) acid–base disturbances, larval and upstream-migrant sea lampreys quickly correct exercise-induced acidosis via a high-capacity H^+ excretion system. The presence of branchial MR cells suggests that the gills may be able to modulate H^+ excretion in response to internal and externally induced acid–base disturbances. Nothing is known about renal acid–base regulation in lampreys.

Nitrogenous Waste Production and Excretion

Ammonia

The breakdown of excess amino acids generates highly toxic ammonia (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia). At physiological pH, most ammonia is in the NH_4^+ form due to its relatively high dissociation constant (p$K \sim 9.5$ at 15 °C) which can be described by the following equation:

$$NH_3 + H_2O \leftrightarrow NH_4{}^+ + OH^-$$

Blood and tissue ammonia concentrations in lamprey tissues are comparable to measurements made in other fishes. However, larval sea lampreys are ammonia tolerant and can withstand blood ammonia concentrations (< 2 mmol L^{-1}) fatal to many other fishes. High ammonia tolerance could suit the burrow-dwelling life style of larval lampreys, where decaying organic matter could cause fluctuations in water ammonia.

Urea

In terrestrial and semiterrestrial vertebrates, ammonia is converted to less toxic urea. Most fishes (**see also Nitrogenous-Waste Balance**: Ureotelism), including lampreys, excrete urea, but it only constitutes 10–20% of the total nitrogenous waste excreted (ammonia plus urea excreted). Lampreys only increase urea excretion rates slightly when exposed to high ammonia; therefore, urea production does not likely explain the high ammonia tolerance of larval sea lampreys. Basal urea production in lampreys is primarily via the catabolism of purines via the process of uricolysis in the liver. In mammals, amphibians, and a few unique teleosts (**see also Nitrogenous-Waste Balance**: Ureotelism), urea is primarily produced using the ornithine urea cycle (OUC) to detoxify ammonia. Urea is also produced via the OUC in elasmobranchs, but retained in the blood and tissues as an osmolyte to maintain water balance in seawater.

Urea production by the OUC entails the conversion of ammonia or glutamine to carbamoyl phosphate via carbamoyl phosphate synthetase III (CPSase III), followed by a series of intermediate steps leading to arginine, which is then hydrolyzed to urea via the enzyme arginase. Although all enzymes of the OUC were detected in parasitic-phase sea lampreys, the low activities of key enzymes (e.g., CPSase III) suggest that urea production by the OUC is physiologically irrelevant. Regardless, these observations do support a long-standing hypothesis that the components of the OUC were likely present in an ancestral jawless vertebrate. Because the ability to detoxify ammonia to urea using the OUC was considered a prerequisite for the invasion of land by vertebrates, these findings support a long-standing hypothesis that this critical metabolic machinery was present at an early stage in vertebrate evolution.

Parasitic lampreys markedly increase urea excretion rates (10- to 15-fold) after feeding on teleosts, and by a remarkable 75- to 450-fold after being removed from basking sharks (*Cetorhinus maximus*) (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development). Thus, lampreys likely possess specialized mechanisms of urea transport in the gill and/or kidneys. In elasmobranchs, urea transporters in the gills are used to retain urea, but urea transporter genes have also been isolated in the gills of the gulf toadfish (*Opanus beta*) and the Lake Magadi tilapia (*Alcolapia grahami*), fishes that normally produce and excrete high amounts of urea (ureotelism) (**see also Nitrogenous-Waste Balance**: Ureotelism). Because urea concentrations in shark tissue are two to three orders of magnitude greater than in lampreys (**see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays), lampreys may need to rid their body of urea due to its potential to perturb proteins and/or disrupt osmotic balance. While sharks have adaptations to cope with high concentrations of internal urea, lampreys may not be able to as effectively deal with urea's potentially toxic effects. Elasmobranchs retain high concentrations of trimethylamine oxide (TMAO) in their blood and tissues, which counteract some of urea's perturbing effects, and may confer some protection to lampreys that ingest shark blood but this needs to be examined.

Concluding Remarks

While not as extensively studied as some teleost fishes, a surprising volume of research has been conducted on the environmental physiology of sea lampreys. Despite diverging over 500 mya from the gnathostomes, in many ways the physiology of modern-day lampreys is remarkably similar to bony fishes. While clear differences certainly exist, there are fundamental similarities in the modes of respiration, ion- and acid–base regulation, and nitrogen excretion used by extant lampreys and teleost fishes. It remains to be determined whether or not these similarities were the result of convergent evolution, or whether or not they represent traits that were present in a common ancestor to the lampreys and the gnathostomes.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Circulation**: Circulatory System Design: Roles and Principles. **Hagfishes and Lamprey**: Lampreys: Energetics and Development. **Integrated Response of the Circulatory System**: Integrated Cardiovascular Responses of Fish to Swimming. **Nitrogenous-Waste Balance**: Excretion of Ammonia; Ureotelism. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction. **Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation; Morphology of Branchial Ionocytes. **Role of the Kidneys**: The Kidney. **Transport and Exchange of Respiratory Gases in the Blood**: Carbonic Anhydrase in Gas Transport and Exchange; Carbon Dioxide Transport and Excretion; Hemoglobin; Red Blood Cell Function; O_2 Uptake and Transport: The Optimal P_{50}. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Bartels H and Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: Implications for osmoregulation in fresh and seawater. *Journal of Experimental Biology* 207: 3447–3462.

Beamish FWH (1980) Osmoregulation in juvenile and adult lampreys. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 1739–1750.

Choe KP, O'Brien S, Evans DH, Toop T, and Edwards SL (2004) Immunolocalization of Na^+/K^+-ATPase, carbonic anhydrase II, and vacuolar H^+-ATPase in the gills of freshwater lamprey. *Journal of Experimental Zoology* 301A: 654–665.

Evans DH, Piermarini PM, and Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 80: 97–177.

Fänge R (1972) The circulatory system. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 2, pp. 241–259. London: Academic Press.

Gess RW, Coates MI, and Rubidge BS (2006) A lamprey from the Devonian period of South Africa. *Nature* 443: 981–984.

Johansen K, Lenfant C, and Hanson D (1973) Gas exchange in the lamprey. *Entosphenus tridentatus. Comparative Biochemistry and Physiology, Part A* 44: 107–119.

Logan AG, Moriarty RJ, and Rankin JC (1980) A micropuncture study of kidney function in the river lamprey, *Lampetra fluviatilis,* adapted to fresh water. *Journal of Experimental Biology* 85: 137–147.

Logan AG, Morris R, and Rankin JC (1980) A micropuncture study of kidney function in the river lamprey *Lampetra fluviatilis,* adapted to sea water. *Journal of Experimental Biology* 88: 239–247.

Morris R (1972) Osmoregulation. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys,* vol. 2, pp. 193–239. London: Academic Press.

Nikinmaa M (2001) Haemoglobin function in vertebrates: Evolutionary changes in cellular regulation in hypoxia. *Respiration Physiology* 128: 317–329.

Nikinmaa M, Airaksinen S, and Virkki LV (1995) Haemoglobin function in intact lamprey erythrocytes: Interactions with membrane function in the regulation of gas transport and acid–base balance. *Journal of Experimental Biology* 198: 2423–2430.

Potter IC, Hill BJ, and Gentleman S (1970) Survival and behaviour of ammocoetes at low oxygen tensions. *Journal of Experimental Biology* 53: 59–73.

Randall DJ (1972) Respiration. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys,* vol. 2, pp. 287–306. London: Academic Press.

Reis-Santos P, McCormick SD, and Wilson JM (2008) Ionoregulatory changes during metamorphosis and salinity exposure of juvenile lamprey *(Petromyzon marinus* L.). *Journal of Experimental Biology* 211: 978–988.

Rovainen CM (1996) Feeding and breathing in lampreys. *Brain Behaviour and Evolution* 48: 297–305.

Wilson JM and Laurent P (2002) Fish gill morphology: Inside out. *Journal of Experimental Zoology* 293: 192–213.

Wright PA (2007) Ionic, osmotic and nitrogenous waste regulation. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Fish Physiology – Primitive Fishes*, vol. 26, pp. 83–318. San Diego, CA: Academic Press.

Youson JH (1980) Morphology and physiology of lamprey metamorphosis. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 1687–1710.

Youson JH (1981) The kidneys. In: Hardisty MW and Potter IC (eds.) *The Biology of Lampreys*, vol. 3, pp. 191–261. London: Academic Press.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Sturgeon

Physiology of Sturgeon

SI Doroshov and JJ Cech Jr., University of California, Davis, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Reproduction
Endogenous and Environmental Regulation of Reproduction
Growth
Respiratory Metabolism
Blood-Oxygen Affinity
Acid–Base Balance
Gut Blood Flow
Osmoregulation
Swimming Performance
Stress Responses
Further Reading

Glossary

Gymnovarian Naked ovaries with ovarian folds open to the peritoneal cavity from where the eggs ovulate.

Hypercapnia High levels of carbon dioxide in blood.

Hypometabolic rate Decreased metabolic rate.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Iteroparous Capable of reproducing more than once (usually many times) in a lifetime.

Normocapnia Dissolved carbon dioxide levels in the environment that are at, or very near, air-saturated levels.

Normoxia Normal levels of oxygen in water or arterial blood.

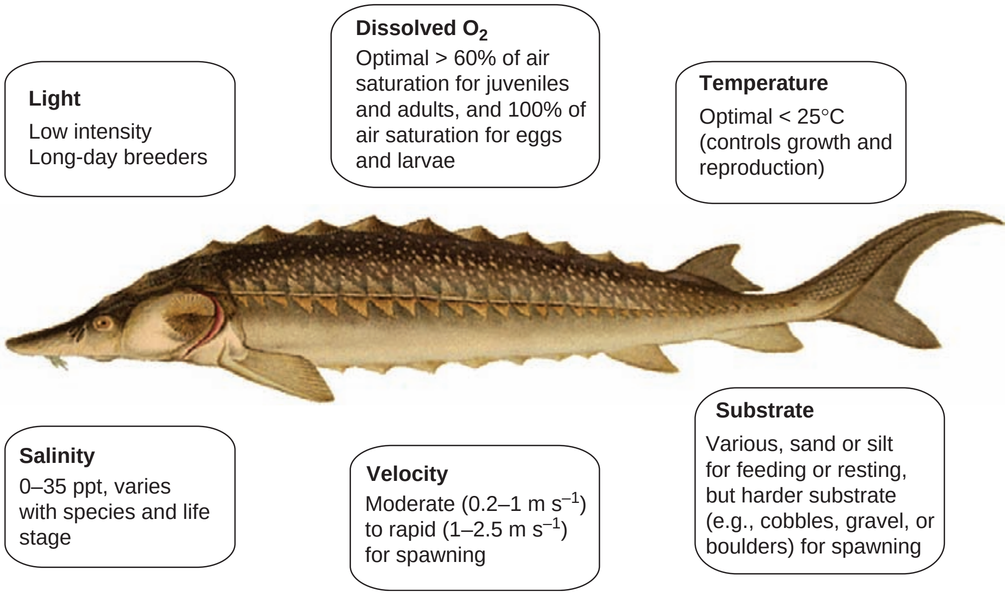
Oxyconformer Organisms that allow their metabolic rate to fall as the environment becomes progressively hypoxic.

Oxyregulator Organisms that maintain their metabolic rate as the environment becomes progressively hypoxic.

Introduction

Sturgeon and paddlefish (order Acipenseriformes) are a phylogenetically ancient clade of ray-finned fish, with fossil evidence from the Jurassic Period ($\sim 1.5-2.0 \times 10^6$ years ago). There are 25 extant species of sturgeon and two species of paddlefish living in the fresh- and saltwaters of Northern Hemisphere. Most of them are subjected to many environmental challenges and are endangered (i.e., on the verge of extinction). The lure of caviar, one of the most-expensive food products, played a major role in the dramatic decline of overfished sturgeon stocks. Today (2010), most sturgeon stocks in North America are protected from the impacts of commercial fisheries. However, they represent only a small fraction of their historic abundance, and their recovery is hampered by human impacts on their reproductive habitats.

Physiology is the science that investigates mechanisms in living organisms. It is the study of key performance pathways between an organism's phenotypic design (structure) and its patterns of resource use and survival that determine its evolutionary fitness. Sturgeon, as well as other animals, must use their (inherited and developed) structural and design (morphological and genetic) characteristics through biochemical and physiological mechanisms to achieve performance (e.g., reproduction, tolerances and adjustments to environmental changes, growth, and locomotion) levels, to survive (e.g., fighting off pathogens or evading predators), and use needed available resources (e.g., O_2, food, mates, and living and nesting spaces). The interactions between performance and patterns of resource use are often expressed in a bioenergetic relationship using energy as its currency (**see also Energetics**: General Energy Metabolism). Changes in environmental conditions (e.g., temperature, dissolved gases, and salinity) often elicit physiological responses, which may affect fishes' performance levels. **Figure 1**



Light
Low intensity
Long-day breeders

Dissolved O_2
Optimal > 60% of air saturation for juveniles and adults, and 100% of air saturation for eggs and larvae

Temperature
Optimal < 25°C (controls growth and reproduction)

Salinity
0–35 ppt, varies with species and life stage

Velocity
Moderate (0.2–1 m s^{-1}) to rapid (1–2.5 m s^{-1}) for spawning

Substrate
Various, sand or silt for feeding or resting, but harder substrate (e.g., cobbles, gravel, or boulders) for spawning

Figure 1 Some environmental requirements for a generalized sturgeon.

shows some environmental requirements for a generalized sturgeon. Whereas low-level or short-duration changes are dealt with via slight adjustments, stress-inducing water-quality changes (e.g., increased temperature, low dissolved O_2 (hypoxia), high dissolved CO_2 (hypercapnia), and increased salinity) challenge fishes, including sturgeons, and may activate a suite of metabolically costly physiological countermeasures. Understanding the time frames and limits of these responses and their potential costs (e.g., in redirecting energy from growth or reproduction to other energy-conversion categories) may help meet conservation, management, or commercial goals for protection and use of sturgeon resources. This article provides a brief overview of sturgeons' reproductive physiology and their physiological responses to hypoxic, hypercapnic, and saline environments and makes comparisons with some more-derived teleosts, which have been studied more extensively.

Reproduction

Reproduction, including sexual maturation, spawning, and early development, is a most sensitive part of sturgeons' life cycles. All sturgeons require a much longer time to reach sexual maturity than do modern teleostean fishes. The median age at first spawning varies between 10 and 20 years in most sturgeon species. Although all sturgeon are iteroparous (i.e., spawn several times during their lives), the breeding intervals, particularly in females, can be as long as 8–10 years, compared to the annual interval in the majority of the temperate-zone teleosts. In spite of their high number of eggs (up to 1 million in Atlantic sturgeon *Acipenser oxyrhynchus*) produced per spawning by a female sturgeon, late sexual maturity and long breeding intervals contribute to low overall reproductive rates in sturgeon and make them very vulnerable to overfishing and environmental changes in their reproductive habitats. Finally, all Acipenseriformes reproduce in rivers and require specific conditions for successful spawning and for the survival of their embryos: current velocity 1–2 m s^{-1}, hard spawning substrate, moderate turbidity, and river temperature ranging from 10 to 18 °C, in most species.

Sturgeon are gymnovarian (i.e., have naked ovaries with ovarian folds open to the peritoneal cavity where the eggs ovulate). Ovulated eggs are transported via the heavily ciliated Müllerian funnel into the mesonephric duct, acting as a Wolffian duct, in sturgeon females. Lake sturgeon *A. fulvescens* continued laying eggs over an 8–12-h period, with the eggs released in 1000–1500-egg batches, and their spawning behavior was polygamous, with several males fertilizing the eggs of a single female. The fecundity (number of eggs produced at single spawning) varies in different species from approximately 50 000 to more than 1 000 000, depending on the fish size and that of the ovulated eggs. For example, the average fecundity of the anadromous Atlantic sturgeon from the Hudson River, USA, was 500 000, and the eggs were 2.5 mm in diameter (smallest eggs in North American acipenserids). In contrast, the anadromous green sturgeon *A. medirostris* of the Pacific Coast had a fecundity of 160 000 with an egg diameter of 4.4 mm (largest eggs in North American acipenserids).

The spawning periodicity in the wild stocks is usually inferred from observations on tagged fish. Analysis of multiyear observations on paddlefish (*Polyodon spathula*) in the Missouri River (USA) watershed indicates breeding intervals of 2 years in males and 3 years in females. Somewhat similar intervals were observed in lake sturgeon in Lake Winnebago, USA. The most common estimates from studies of cultured sturgeons are 1 year in males and 1–3 years in females. Our observations on cultured white sturgeon *A. transmontanus* strongly suggest that endogenous reproductive cycles in fish born and reared in captivity are annual in males and biennial in females. All sturgeon species reared in modern aquaculture exhibit a dramatic acceleration of growth and early sexual maturation (e.g., approximately half of the time required for maturation, compared with wild fish).

Endogenous and Environmental Regulation of Reproduction

Puberty (i.e., the acquisition of the capacity to reproduce and the onset of the first reproductive cycle) is recognized in sturgeon by the increase in plasma concentration of sex steroids, proliferation of spermatogonia in males, and the start of vitellogenesis in females. Body size and accumulated energy play important permissive roles in the onset of the first reproductive cycle, when energy is diverted from somatic growth to reproduction. The onset of puberty and the reproductive cycle is orchestrated by environmental signals (such as photoperiod) and secretion of two pituitary gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The system is regulated by a complex feedback of the gonadal sex steroids and growth factors and is linked with other endocrine pathways. Pubertal age of wild sturgeon varies from 5 to 15 years in different species, but farmed sturgeon exhibit early puberty (3–6 years), because they are fed, continuously, on a high-energy diet and are grown at relatively high temperatures (e.g., 18–24 °C).

The endogenous regulation of the reproductive cycle in sturgeon includes hormones of the hypothalamic–pituitary–gonadal reproductive axis. In general, it is similar to the reproductive axis of modern teleosts (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). Brain peptides and neurotransmitters play the key roles in puberty and the reproductive cycle, but their functions have not been studied, systematically, in sturgeon. Gonadotropin-releasing hormone (GnRH) of sturgeon is identical in molecular structure to mammalian GnRH-1, and is effectively used as a synthetic agonist GnRHa for the induction of ovulation and spermiation in sturgeon aquaculture. The functional role of another GnRH form found in the sturgeon brain (i.e., chicken GnRH2) has not been determined. As in teleosts, two pituitary gonadotropins regulate the reproductive cycle in sturgeon. They were first described in farmed white sturgeon as stGTH1 and stGTH2, and later confirmed as molecular and functional homologs of mammalian FSH and LH in Siberian sturgeon *A. baeri*, Russian sturgeon *A. gueldenstaedti*, and Chinese sturgeon *A. sinensis*. Plasma concentrations of FSH are elevated from the onset of puberty and during gonadal growth and vitellogenesis, whereas the plasma concentration of LH rises significantly during the spawning season of sturgeon.

The FSH and LH secretions control the synthesis and secretion of gonadal sex steroids: androgen, estrogen, and maturation-inducing steroids (MISs). Biosynthesis of sex steroids takes place in the ovarian theca and granulosa cells and testicular Leydig cells. Sex steroids, in turn, control germ-cell development, sexual behavior, and secretion of GnRH, FSH, and LH via feedbacks to the brain and pituitary. Gonadal steroids with known or assumed functions in sturgeon are testosterone (T), 11-ketotestosterone (11-KT), estradiol-17β (E_2), and MIS 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P). All of these hormones are present in the circulation in both sexes, but in different concentrations. Testosterone provides the substrate for synthesis of 11-KT and E_2, and a positive feedback to the sturgeon pituitary. The 11-KT is believed, largely by analogy with teleosts, to control spermatogenesis. The E_2 stimulates secretion of the yolk precursor (vitellogenin) in the liver and is required for the maintenance of ovarian follicles in the late phase of oogenesis. The MIS 17,20β-P was found in high concentrations in ovulating females and spermiating males. Two other candidates for the MIS, progesterone (P_4) and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S), have been proposed in sturgeons. All of the different MISs, including P_4, are highly potent in stimulating egg maturation *in vitro*. Knowledge of growth factors participating in sturgeon gametogenesis is limited to recent studies on insulin-like growth factor (IGF-1) and its receptors in the sturgeon ovary, showing the important roles of IGF-1 in the differentiation of granulosa cells and regulation of vitellogenesis.

The endogenous reproductive cycle is synchronized by environmental cues, such as photoperiod and temperature. Similar to salmon, some sturgeon species have different spawning ecotypes distinguished by timing of spawning migrations and spawning seasons. Most sturgeon species spawn during the spring and summer (i.e., the long-day breeders) but some, such as Chinese sturgeon in the Yangtze River, spawn in the fall. There is some indication that the endogenous reproductive cycle can override the effect of photoperiod. For example, the majority of white sturgeon females farmed in California and reared in outdoor tanks under natural photoperiod reaches full sexual maturity during March–June, but some females reach maturity in October–November. To our knowledge, the regulatory role of photoperiod in sturgeon reproduction has not been studied in an experimental setting.

Seasonal temperature plays an important role in the gametogenesis and spawning of sturgeon. For example, maturing white sturgeon females and males undergo gonadal regression (ovarian follicular atresia and phagocytosis of sperm) if they are exposed to temperatures above 15 °C during the winter months before the spring spawning period. The fact that elevated pre-spawning temperatures induce dramatic decreases in concentrations of plasma T and E_2 before the onset of gonadal regression suggests that seasonal environmental temperature is a regulatory factor in sturgeon gametogenesis and spawning. A better understanding of the role of temperature as an environmental regulator of sturgeon reproduction is particularly important for the conservation of sturgeon.

Growth

The semi-anadromous white sturgeon (*Acipenser transmontanus*) and the anadromous green sturgeon (*A. medirostris*) are native to eastern North Pacific waters. In California, there is a coastal and inland white-sturgeon sportfishery and a growing commercial (freshwater) white-sturgeon culture industry. Because some poorly circulated inland systems may become warm and hypoxic and some high-density culture systems hypercapnic, some physiological responses, including growth rates, of these sturgeon to these environments have been measured.

As temperature increased from 15 to 20 °C, under normoxic conditions, juvenile white sturgeon specific growth rate significantly increased from 1.6% to 2.6% body weight d^{-1}. In contrast, a further temperature increase to 25 °C resulted in only a slight, nonsignificant increase (to 2.9% body weight d^{-1}). Further, mildly hypoxic conditions (at 58% of air-saturated conditions) significantly decreased growth at all temperatures. This hypoxia-induced decrease in growth appeared to result from decreased food-consumption rates. Sympatric striped bass (*Morone saxatilis*) of the same size showed growth-rate increases with each increase in temperature under normoxia, including at 25 °C, where the fastest rates of growth were shown (3.2% body weight d^{-1}). Instead of ascribing these temperature-related growth differences to evolutionary lineage, historical habitat differences constitute the more likely explanation. Striped bass were introduced to California waters in the late 1870s, and their significant growth rate increase with the 5 °C increase to 25 °C probably reflects the warmer conditions of Chesapeake Bay on the USA's Atlantic Coast, where they evolved. Growth of the young striped bass was also sensitive to hypoxia, although not significantly so at the lowest temperature (15 °C), where its growth rate is the lowest. Finally, growth rates of young juvenile green sturgeon (0.10 g wet mass) increased with an increase in temperature (to 24 °C), compared with fish at 19 °C, as long as sufficient food and oxygen were available.

White sturgeon growth performance was significantly decreased in the hypercapnic conditions (1.17% wet body mass increase d^{-1}), compared with normocapnic conditions (2.86% body mass d^{-1}). As in the growth experiment with hypoxia, the decreased growth was apparently due to a decreased food-consumption rate. In a sham treatment using HCl, the decreased pH (simulating the hypercapnic pH) did not affect growth, implying that the decreased appetite and decreased growth rate seen in the hypercapnia treatment were due to the high CO_2, but not due to the resulting pH decrease. Hypercapnia (50–60 mg L^{-1}, pH: 6.15–6.30) also decreased growth of rainbow trout (*Oncorhynchus mykiss*) after a 330-day chronic exposure. Sophisticated, high-density white-sturgeon culture systems may become hypercapnic. Such high densities of fish are possible with the carefully metered addition of pure oxygen into the water inflows, increasing the quantity of dissolved O_2 for the fish to respire. However, the high respiring biomass of the sturgeon produces much CO_2. Because CO_2 is 25–30 times more soluble in water than O_2, depending on the temperature, the CO_2 tends to stay dissolved, producing hypercapnic conditions.

Respiratory Metabolism

The respiratory metabolic rates (**see also Energetics**: General Energy Metabolism) of three ontogenetic stages of juvenile white sturgeon were examined at stage-appropriate environmental temperatures. Expressed as mg O_2 consumed per gram body weight per hour, small (0.2 g body weight at 10 °C), medium (1.9 g at 16 °C), and large (63 g at 20 °C) white sturgeon all displayed significant decreases in mean oxygen consumption (i.e., aerobic metabolic) rate with exposure to mild (51% of air-saturation levels) hypoxia. Analysis of videotapes of the experimental fish showed that hypoxia also significantly decreased activity. Therefore, the significantly decreased metabolic rate of juvenile white sturgeon was at least partially due to decreased activity. The juvenile white sturgeon did not show a compensatory increase in oxygen-consumption rate when returned to normoxic conditions, after exposure to hypoxia. This response is consistent with the hypometabolic (decreased overall metabolic) response to hypoxia, in part due to decreased activity levels in the juvenile white sturgeon, seen also in white sturgeon adults. This response may serve white sturgeon well in natural habitats where decreased activity would decrease oxygen demand, thereby conserving oxygen resources in hypoxic habitats until conditions improved. This response differs from that seen in Siberian sturgeon, which do not decrease oxygen consumption rate during hypoxia and show a compensatory rate increase after return to normoxia. This compensatory rate increase repays the oxygen debt associated with anaerobic contributions to overall metabolism (**see also Swimming and Other Activities**: Energetics of Fish Swimming). This distinctly different pattern, compared with white sturgeon, may stem from habitat-related differences in the Siberian sturgeon's life history. Finally, Adriatic sturgeon *A. naccarii* exhibited a linear decline in oxygen consumption (i.e., 'oxyconformer') with significant decreases in arterial oxygen content and increases in plasma (lactate) when exposed to progressive hypoxia under static conditions. However, when these sturgeon were allowed to swim at a low sustained speed, they could regulate oxygen consumption (i.e., 'oxyregulator') down to 37 mmHg partial pressure of oxygen (P_{O_2}). At moderate levels of hypoxia, these swimming fish exhibited no significant changes in either arterial blood oxygen content or plasma lactate.

Blood-Oxygen Affinity

Increasing CO_2 decreased white-sturgeon blood-O_2 affinity, from the arterial-type CO_2 levels, through low- and high-hypercapnic levels we would associate with the high-density white sturgeon culture (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin and O_2 Uptake and Transport: The Optimal P_{50}). This Bohr effect-type shift (**see also Design and Physiology of the Heart**: Cardiac Cellular Length-Tension Relationship) results in higher P_{50} values (i.e., lower blood-oxygen affinities) at increased P_{CO_2} values. Note that although low hypercapnic conditions (30.4 mmHg P_{CO_2}) dramatically decreased the oxygen affinity (increased P_{50}), compared with normocapnic conditions at each temperature, further P_{CO_2} increases (to 76 mmHg P_{CO_2}) do not decrease blood-O_2 affinity substantially further (P_{50} only increases by 3 and 5 mmHg at 15 °C and 20 °C, respectively). Thus, white sturgeon in high hypercapnic conditions at 15 or 20 °C are able to adequately saturate their blood with oxygen for respiratory purposes under high hypercapnia, almost as much as under low hypercapnia. Studies on rainbow trout do not show this pattern, albeit at lower P_{CO_2} levels (up to 11 mmHg P_{CO_2}). Finally, juvenile green sturgeon blood-oxygen equilibria data over the 11–24 °C range indicate that these fish tolerate moderate environmental hypoxia, moderate aerobic activity, low-to-moderate hypercapnia, and moderate temperature changes in their environment.

Acid–Base Balance

Arterial CO_2 conditions directly reflect those in the water in hypercapnia-exposed fishes. Hypercapnic exposure induced stress in white sturgeon, as shown in significantly increased plasma catecholamines (norepinephrine and epinephrine) and cortisol concentrations. Ventilatory frequency consistently increased (hyperventilation) under hypercapnia, compared with the normocapnic controls. Arterial oxygen tension showed either no difference or a slight increase in the hypercapnic fish, presumably due to the hyperventilation. As expected, arterial (plasma) pH significantly decreased in the hypercapnic fish. However, after about 2 days, arterial pH started to show a compensation toward that of the normocapnic sturgeon, due to the significant accumulation of bicarbonate ion. This response parallels that in most teleosts studied, but the white sturgeon's response (~35% of pH restoration) was more limited and slower. For example, an ~91% arterial pH restoration after a 7-day hypercapnic exposure in rainbow trout has been reported, and the marine teleost, *Conger* sp., recovered 92% of its initial pH depression only after a 30-h hypercapnic exposure. Similarly, juvenile green sturgeon show a decreased accumulation of plasma (lactate), compared with that in rainbow trout, following severe chasing stress.

Gut Blood Flow

If hypercapnia exposure decreases digestive efficiency from decreased gut blood flow, growth may be limited in high-density white-sturgeon-culture tanks. Blood flow to the splanchnic organs typically decreases in unfed exercising or struggling teleosts. Although hypercapnic exposure did not affect gut blood flow in white sturgeon, the associated struggling significantly decreased it. An increased number of struggle events occurred when white sturgeon were exposed to hypercapnia, compared to when they were exposed to normocapnia. Thus, the stress associated with hypercapnia could increase struggling, with consequent decreases in gut blood flow, under hypercapnic culture conditions. Interestingly, the relative gut blood flow (% of cardiac output) measured in white sturgeon was approximately half of that measured in Atlantic cod (*Gadus morhua*), indicating a possible reduced splanchnic perfusion scope in white sturgeon, compared with Atlantic cod.

Osmoregulation

Because of the anadromous green sturgeon's threatened-species status, physiological aspects of the juveniles' acclimation to seawater have been studied to assist in its protection. Semi-anadromous shortnose sturgeon (*A. brevirostrum*) and anadromous European Atlantic sturgeon (*A. sturio*) juveniles apparently move into low-salinity areas, and in the latter case, move into seawater after several years. Similarly, juvenile Atlantic sturgeon (*A. oxyrhinchus*) have been reported to move into estuaries and eventually to the ocean after 2–6 years. The timing of seawater entry in juvenile green sturgeon is unknown, although physiological studies and field-based evidence indicate that fish move into seawater between 6 months and 3 years of age. Laboratory-based studies showed that green sturgeon can osmo- and ionoregulate in seawater for long periods of time from as young as 6 months of age, with fish becoming better adapted with increasing age and body-size. Juvenile green sturgeon may not experience high salt concentrations prior to direct entry into seawater (e.g., because of seasonal sandbar occurrences across the mouths of spawning rivers), suggesting that these juvenile fish must be physiologically prepared to hypo-osmoregulate prior to oceanic entry.

In many salmonids (e.g., *Oncorhynchus* species), physiological preparative changes for a seawater-based existence occur prior to actual exposure to hyperosmotic salinities. Using trace-element analysis, laser ablation-inductively coupled plasma-mass spectrometry, to analyze differences in freshwater and seawater elemental signatures between calcified growth zones of pectoral fin rays, it was determined that juvenile green sturgeon from the Klamath River move into seawater between the first and second growing seasons (6–18 months of age). After the initial movement into seawater, the juvenile green sturgeon appear to remain there for at least 5 years, as indicated by the constant high Sr/Ba ratio concentrations in growth zones 2–6 of the fin-ray sections. Although field data are limited, few green sturgeon between 3 and 13 years of age are found in the Klamath estuary. Long-distance migrations are known to occur in green sturgeon, and, perhaps, juveniles move along the coast, remaining primarily in seawater.

After 7 weeks' acclimation to three salinities (<3, 10, and 33), anadromous green sturgeon (of three ages: 100, 170, and 533 days posthatch), Na^+, K^+-ATPase activities increased in the gills and decreased in the kidneys with increasing salinity, consistent with the change from ion reabsorption to excretion seen in many teleosts. Gill mitochondria-rich cells (MRCs) increased in size and decreased in relative abundance with fish size/age. Gill MRC Na^+, K^+-ATPase content (e.g., ion-pumping capacity) was proportional to MRC size, indicating greater abilities to regulate ions with size/age. In the gastrointestinal tract (GIT), Na^+, K^+-ATPase activities increased fourfold in the pyloric ceca, presumably due to increased drinking rates and salt and water absorption. The increased GIT fluids, solids, and ion concentrations were similar to those found in teleosts in hyperosmotic conditions, along with the very low fluid volumes in isomotic and hyposmotic conditions. However, a greater proportion of base (HCO_3^- and $2CO_3^{2-}$) was found in intestinal precipitates, rather than in fluids, which is different from teleosts. Overall, green sturgeon, and likely other sturgeon species, osmo- and ionoregulate with similar mechanisms to more-derived teleosts, although acclimation to hyperosmotic salinities may be more dependent on body size.

Swimming Performance

Swimming performance (e.g., as measured in cm s^{-1}, in a laboratory flume) generally increases with increasing body size as juvenile sturgeon grow. An interesting exception was noted in anadromous green sturgeon when they reached the size required for seawater tolerance (i.e., ~30 cm total length (TL)). During a critical (autumnal) season after reaching this size, the critical swimming velocity (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance) decreased with further increases in size for a period of several weeks. If their (decreased) swimming performance did not match the increased river velocities associated with autumnal rains, juvenile green sturgeon of sufficient (seawater-tolerant) size would drift downstream, presumably migrating to the ocean. Juveniles of the same size tested later (winter) failed to show this decline in swimming performance with increasing size, and these fish may not migrate out of the rivers until a later time. Interestingly, this pattern is similar to that reported in some migrating salmon juveniles (smolts). At the other end of the body-size spectrum, adult white sturgeon can burst-swim up to 2.5 m s^{-1} to ascend a prototype fishway.

Stress Responses

Overall, the physiological stress responses in sturgeon seem similar to those in teleostean fishes that have been studied. Plasma catecholamines and cortisol concentrations typically increase in fish exposed to net-confinement, air-exposure, or chasing-type stressors. Increased plasma lactate and decreased plasma pH also accompany struggling or high-activity-type stress responses in both teleosts and sturgeons. However, the freshwater sturgeon of the genus *Scaphirhynchus* show reduced stress responses, compared with teleostean species that have been investigated. Juvenile green sturgeon subjected to chronic stress for 28 days show an increased maintenance metabolic rate and a decreased scope for activity, compared with unstressed control fish.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance; Energetics of Fish Swimming. **Energetics**: General Energy Metabolism. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin; O_2 Uptake and Transport: The Optimal P_{50}; Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems.

Further Reading

Allen PJ, Cech JJ, Jr., and Kültz D (2009) Mechanisms of seawater acclimation in a primitive, anadromous fish, the green sturgeon. *Journal of Comparative Physiology* B179: 903–920.

Allen PJ, Hobbs JA, Cech JJ, Jr., Van Eenennaam JP, and Doroshov SI (2009) Using trace elements in pectoral fin rays to assess life history movements in sturgeon: Estimating age at initial seawater entry in Klamath River green sturgeon. *Transactions of the American Fisheries Society* 138: 240–250.

Allen PJ, Hodge B, Werner I, Cech JJ, Jr. (2006) Effects of ontogeny, season, and temperature on the swimming performance of juvenile green sturgeon *(Acipenser medirostris). Canadian Journal of Fisheries and Aquatic Sciences* 63: 1360–1369.

Billard R and Lecointre G (2000) Biology and conservation of sturgeon and paddlefish. *Reviews in Fish Biology and Fisheries* 10: 355–392.

Bruch RM and Binkowski FP (2002) Spawning behavior of lake sturgeon. *Journal of Applied Ichthyology* 18: 570–579.

Cech JJ, Jr. and Crocker CE (2002) Physiology of sturgeon: Effects of hypoxia and hypercapnia. *Journal of Applied Ichthyology* 18: 320–324.

Crocker CE and Cech JJ, Jr. (1998) Effects of hypercapnia on blood-gas and acid–base status in the white sturgeon, *Acipenser transmontanus. Journal of Comparative Physiology* B168: 50–60.

Crocker CE, Farrell AP, Gamperl AK, and Cech JJ, Jr. (2000) Physiology of sturgeon: Effects of hypoxia and hypercapnia. *American Journal of Physiology (Regulatory, Integrative and Comparative Physiology)* 279: R617–R628.

Dettlaff TA, Ginsburg AS, and Schmalhausen OI (1993) *Sturgeon Fishes: Developmental Biology and Aquaculture*. New York: Springer.

Doroshov SI, Moberg GP, and Van Eenennaam JP (1997) Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontatus. Environmental Biology of Fishes* 48: 265–278.

Farrell AP, Thorarensen H, Axelsson M, Crocker CE, and Cech JJ Jr. (2001) Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry and Physiology* A128: 551–563.

Gardiner BG (1984) Sturgeons as living fossils. In: Eldridge N and Stanley SM (eds.) *Living Fossils*, pp.148–152. New York: Springer.

Kaufman RC, Houck AG, and Cech JJ, Jr. (2006) Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria. *Environmental Biology of Fishes* 76: 119–127.

Lankford SE, Adams TE, Miller RA, and Cech JJ, Jr. (2005) The cost of chronic stress: Impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiological and Biochemical Zoology* 78: 599–609.

McKenzie DJ, Steffensen JF, Korsmeyer K, *et al.* (2007) Swimming alters responses to hypoxia in the Adriatic sturgeon *Acipenser naccarii. Journal of. Fish Biology* 70: 651–658.

Moberg GP, Watson JD, Doroshov SI, Papkoff H, and Pavlick RJ, Jr. (1995) Physiological evidence for two sturgeon gonadotropins in *Acipenser transmontanus. Aquaculture* 135: 27–39.

Querat B, Sellouk A, and Salmon C (2000) Phylogenetic analysis of the vertebrate glycoprotein hormone family including new sequences of sturgeon (*Acipenser baeri*) β subunits of the two gonadotropins and the thyroid stimulating hormone. *Biology of Reproduction* 63: 222–228.

Sternin V and Doré I (1993) *Caviar – The Resource Book*. Moscow: Cultura Publishing House.

Webb MAH, Van Eenennaam JP, Feist GW, *et al.* (2001) Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus. Aquaculture* 201: 137–151.

Wrobel K-H (2003) The genus *Acipenser* as a model for vertebrate urogenital development: The Müllerian duct. *Anatomy Embryology* 206: 255–271.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Chondrichthyes

Physiology of Sharks, Skates, and Rays

JS Ballantyne and JW Robinson, University of Guelph, Guelph, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

General Overview
Sensory Biology
Muscle Physiology and Swimming
Electric Organs
Buoyancy Regulation
Deep-Water Elasmobranchs
Freshwater Elasmobranchs
Respiration
Circulation
Reproduction
Osmo- and Ionoregulation/Osmoconformation
Digestion
Intermediary Metabolism
Excretion
Neurophysiology
Immune System and Disease Resistance
Endocrinology
Endothermy in Elasmobranchs
Acid–Base Physiology
Summary
Further Reading

Glossary

Antibody Proteins found in blood that are used to neutralize foreign disease organisms (e.g., bacteria, viruses, and protozoa) as part of the immune defenses of an animal.

Basolateral The part of a cell or layer of cells lying closest to the blood supply faces the internal environment.

Batoid The group of cartilaginous fish commonly known as skates or rays.

Cone The sensory cell type in the eye (photoreceptor) involved in color vision and bright light conditions.

Countercurrent Flowing in opposite directions. Used for an arrangement of vessels (e.g., blood vessels or kidney tubules) such that fluid flows in one direction in one vessel that lies close to another vessel in which the flow is in the opposite direction. This arrangement allows the transfer of molecules or heat from one vessel to another in such a way as to maintain a gradient along the countercurrent vessels.

Ketone bodies Four carbon molecules produced by the incomplete oxidation of fats or from a few specific amino acids.

Lipids Group of naturally occurring hydrophobic compounds including fats, oils, waxes, phospholipids, and others.

Na^+/K^+-ATPase (NKA, the sodium pump) This is a major ATP-consuming ion pump that, directly or indirectly, drives many ion-regulatory processes, including maintaining sodium and ion gradients across cell plasma membranes and being present at high levels in ionocytes.

Osmoconformer An organism that maintains the total concentrations of all the small molecules in its body fluid compartments similar to that of the environment.

Osmolyte Small highly soluble molecules used to control the osmotic pressure of body fluid compartments.

Paracrine A cellular signal like a hormone that is released by a cell and acts on nearby cells.

Pharynx The part of the digestive tract between the mouth and the esophagus.

Pheromone A molecule that is released outside the body to signal other members of the same species.

Proprioception The ability to sense the positions of one's own body parts.

Sphyrnid Members of the family Sphyrnidae – the hammerhead sharks.

Tympanum Ear drum.

General Overview

The sharks, skates, and rays (hereafter referred to as the elasmobranchs) belong to one of the most ancient fish groups, first appearing in the fossil record perhaps as early as the late Silurian or early Ordovician 420–450 million years ago. They represent the most ancient extant jawed fishes. Although mostly known as large predatory animals, this only encompasses a few of many species of elasmobranchs. Currently, there are about 1000 known species of elasmobranchs divided into 48 families, indicating their diversity of form. They include the largest fish in the world (the filter-feeding whale shark, *Rhincodon typus*), warm-bodied fast-swimming predators such as the great white shark (*Carcharodon carcharias*), flattened bottom dwellers (the skates), and deep-sea and freshwater species.

Elasmobranchs, in general, are long-lived species that mature late and produce few offspring. Morphologically, they are relatively simple. They have no swimbladder for buoyancy. They lack a plate covering the gills and they have a skeleton composed entirely of cartilage rather than bone. They have several novel structures such as a rectal gland for salt secretion, a spiral-valve intestine for digestion, and specialized organs for producing cells of the immune system. They are reputed to be remarkably resistant to cancer but the veracity of this has not been established. The existing forms have many physiological features that may be considered primitive and some that are almost unique. Physiologists have long regarded elasmobranchs as good models for the study of certain aspects of physiology. For example, the rectal gland has been studied for decades as a model for salt excretion. Elasmobranchs also have many unique aspects in their internal biochemistry. A defining feature of this group is the use of urea for osmotic support and this has widespread consequences for their metabolic organization. As fish with many primitive features, they can give us insights into the evolution of vertebrate physiological systems.

Sensory Biology

Elasmobranchs use one of the widest arrays of senses available to any organism. They have available all of the senses that humans have, in addition to being able to sense electrical fields, magnetic fields, and vibrations using specialized sensing cells. These senses differ in the distance at which they can gather information from the environment. This allows for long-distance detection of prey and closer fine-tuning to locate prey precisely. The brain regions involved contribute to the relatively large brain:body size ratios of elasmobranchs (**see also Brain and Nervous System**: Functional Morphology of the Brains of Cartilaginous Fishes).

Olfaction

The olfactory organs of elasmobranchs are found in sacs in the nostrils (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction). The sensitivities of these to L-amino acids such as lysine and valine are similar to those of other fishes (10^{-6}–10^{-8} M), while sensitivity to L-serine is much higher (10^{-14} M). This allows gathering information about chemical sources from great distances. The part of the brain involved in processing this information is largest in those relying most on this sense. The great white shark has the largest (18% of the total brain mass), indicative of its need to detect colonies of its prey (marine mammals). The detection of sex pheromones for reproduction also relies on these organs. Elasmobranchs are well known for being able to track minute amounts of blood from long distances.

Taste

The taste buds of elasmobranchs are structurally similar to those of other vertebrates and occur in the epithelium of the mouth and pharynx (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction).

Audition

Although sharks are not known to make sounds, they can detect low-frequency sounds (20–1000 Hz) helped in part by a connection (fenestra ovalis) of the posterior semicircular canal to the tympanum not found in other vertebrates. Although they lack a swimbladder, used in other fishes to amplify sound and detect pressure changes, some parts of the inner ear can respond to pressure change (**see also Hearing and Lateral Line**: Auditory System Morphology and The Ear and Hearing in Sharks, Skates, and Rays).

Electro- and Magnetoreception

The ability to detect low-frequency electrical events is made possible by specialized electroreceptors found below the skin (**see also Detection and Generation of Electric Signals**: Active Electrolocation and Detection and Generation of Electric Signals in Fishes: An Introduction). These are called the 'ampullae of Lorenzini' and can detect impulses as low as 5 nV cm^{-1}. They are thought to be used in detecting planetary magnetic fields for navigation and prey detection (**see also Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes). These organs have also been implicated in sensing thermal gradients due to their extreme sensitivity to temperature.

Mechanoreception

As in other fishes, the lateral-line system provides the mechanoreception needed for localizing the moving objects and currents. These are important for schooling, prey detection, and mating. The basic component of the lateral-line system is the neuromast, a group of sensory hair cells covered by a gelatinous cupola. The lateral-line system can extend over the entire body with denser arrays in the head region. Some types of neuromasts are used for proprioception.

Vision

Elasmobranchs have well-developed eyes with a nearly complete (360°) visual field found in all species (**see also Vision**: Adaptations of Photoreceptors and Visual Pigments). Relative to body size, benthic batoids and sharks have the smallest eyes, while pelagic sharks have the largest eyes. In addition to an upper and lower eyelid, a protective nictitating membrane is found in carcharhinids and sphyrnids. Most elasmobranchs have a reflective tapetum lucidum that improves sensitivity in low-light conditions. The lens shape is highly variable among elasmobranchs. Elasmobranchs have both rods and multiple cones and thus can see color. The visual pigments are adapted to the wavelengths found in the habitats of each species. Deeper-water species have pigments adapted for better reception of blue-green wavelengths. Skates have a single cell type in the retina that functions as a rod at low-light intensities and as a cone at higher intensities.

Balance and Motion Detection

Elasmobranchs may have been the first vertebrates to employ three semicircular canals for the balance and perception of motion.

Muscle Physiology and Swimming

The myotome is arranged in a cone-shaped bundle that can be isolated intact, easily, making it an excellent model for studies of muscle physiology. The muscles consist of red and white fibers as in other fish (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance and **The Skeleton**: Cartilaginous Fish Skeletal Anatomy, and Cartilaginous Fish Skeletal Tissues). The pectoral fins of sharks have limited mobility and do not play a role in propulsion, while batoids use their pectoral fins for most of their propulsion. The skin of elasmobranchs is designed to reduce drag, using scale-like denticles to minimize turbulence and turbulent shear stress. The size, shape, and spacing of the denticles are correlated with the swimming speed of the fish.

Electric Organs

In addition to sensing electric fields, some elasmobranchs can produce electricity to a significant extent. There are two types of electric organs known in elasmobranchs (**see also Detection and Generation of Electric Signals**: Electric Organs and Generation of Electric Signals). One involves the production of weak electric fields by organs along the tail for communication and prey detection. This is found exclusively in skates (Rajoidei). Another involves the production of much stronger electric impulses for confusing or immobilizing prey and perhaps for defense. This is found in members of the order Torpediniformes containing four families and about 40 species. These have appropriately named genera such as *Torpedo*, *Narcine*, and *Hypnos*. In these species, the electric organ is derived from the modified gill muscles that occupy about one-sixth of the body mass.

Buoyancy Regulation

Elasmobranchs have no gas-inflated swimbladder and must rely on a variety of factors to reduce their density and hence their energy expenditure to maintain their position in the water column (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). The lack of substantial calcification of the skeletal system (bone) eliminates a major factor in reducing buoyancy. The use of density-lowering organic solutes (urea and methylamines) in high concentrations reduces the density of the various fluid compartments. These molecules act by reducing the packing of water molecules surrounding them, resulting in negative partial molal volumes in aqueous solution. Because of this, plasma density and presumably density of most tissues are less than that of seawater.

A third major contributor to buoyancy is the large fatty liver. The liver of elasmobranchs is unusually large in deep-water species (**Figure 1**). In general, the larger the liver the more fat (oil) it contains with some species having livers with 80% oil by weight. Elasmobranchs' liver oil contains triacylglycerols (TAGs), alkyldiacylglycerols (DAGEs), wax esters, and squalene. Deep-water species tend to have either high DAGE (up to 89%) or high squalene (up to 79%) (**Figure 1**). Shallow-water pelagic and benthic species mostly use TAG (**Figure 1**). The most buoyant liver lipids (DAGEs) are spared during periods of starvation and are synthesized if needed to increase buoyancy.

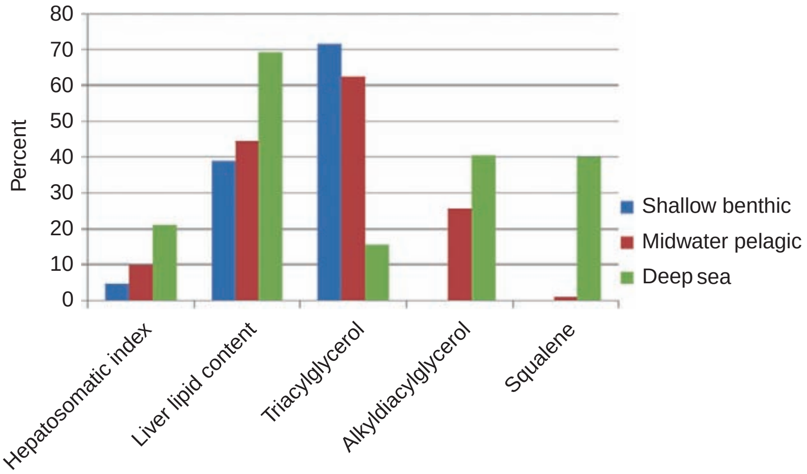


Figure 1 Hepatosomatic index (liver weight as % of body weight), liver lipid content (lipid weight as % of liver weight) and percentages of specific lipids (% of liver lipids) in shallow-water benthic, shallow-water pelagic, and deep-sea elasmobranchs. Values are estimated from multiple sources.

Deep-Water Elasmobranchs

While many elasmobranchs exist over a wide range of depths, some spend most of their lives at great depth. Adaptations to deep water include higher lipid content in the liver, higher proportions of diacylglycerols and squalene in liver lipids, and altered proportions of methylamines and urea. The altered liver lipids help maintain neutral buoyancy to reduce the energy requirements of locomotion. Higher levels of trimethylamine-*N*-oxide (TMAO) and lower concentrations of urea have been observed and these may help offset the detrimental effects of high pressure on proteins and membranes. Enzyme activities of deep-water elasmobranchs are lower than those of their surface counterparts.

Freshwater Elasmobranchs

Only a small percentage (less than 5%) of elasmobranchs can live in freshwater compared to 40% of teleost species. The basis for this has been speculated upon and may relate to the urea requirement of proteins. Some species such as the bull shark (*Carcharhinus leucas*) enter and leave freshwater while others such as some African (e.g., African freshwater stingray, *Dasyatis garouaensis*) and Southeast Asian (e.g., white-edged freshwater whipray, *Himantura signifer*) species spend their entire lives in freshwater. All of these species undergo reductions of urea levels in tissues but still maintain relatively high concentrations (80–200 mM). This may be necessary to maintain the function of urea-requiring proteins. Only one group, the members of the family Potamotrygonidae of South America, resides entirely in freshwater and maintains negligible urea levels. This group has lost the capacity for urea synthesis and those parts of the kidney responsible for urea retention. The rectal gland is also reduced or absent in this group (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes).

All freshwater elasmobranchs give birth to live young ones perhaps to avoid the osmotic challenges faced by external eggs in freshwater.

Sensory traits also evolved for life in freshwater. For example, optimal detection of electrical signals in freshwater is associated with shorter canals in the ampullae of Lorenzini. These have been termed ‘miniampullae’ as found in *H. signifer* or ‘microampullae’ as found in the Potamotrygonidae.

Because of the reduced need to direct nitrogen to urea via glutamine synthetase, levels of this enzyme are lower and glutamate dehydrogenase levels are higher in liver of freshwater species than in marine species. The reduced need for urea allows amino acids to be deaminated via glutamate dehydrogenase (GDH) and the ammonia to be eliminated. Ammonia excretion rates of freshwater elasmobranchs are higher than those of marine species (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia).

Respiration

Elasmobranchs have from five to seven gill slits, with seven being the ancestral condition. The gills have a typical countercurrent flow of blood against the water to optimize gas exchange. The popular perception that all sharks need to swim continuously to breathe is a myth. Some elasmobranchs do ram-ventilate or force water through the gills by swimming, but others including the skates and rays use muscles in the head region to pump water across the gills and do not need to swim to breathe. Under hypoxic conditions, ram-ventilating species swim faster to improve oxygen uptake. In skates and rays with mouths that are located ventrally, water enters and may exit via the spiracle.

The metabolic rates of elasmobranchs are comparable to those of teleosts when factors such as temperature and lifestyle are similar. Blood flow to the gills can be increased or decreased as needed for improved ventilation as in other fishes.

Circulation

The circulation of elasmobranchs is similar to that of the other fishes, with deoxygenated blood first going to the gills where it picks up oxygen and loses carbon dioxide (**see also Circulation**: Circulatory System Design: Roles and Principles). The oxygenated blood then flows to the rest of the body and returns to the heart. The heart has four chambers: the sinus venosus, the atrium, the ventricle, and the conus arteriosus (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping). The sinus venosus receives deoxygenated venous blood and delivers it to the atrium. Contraction of the atrium moves blood into the muscular ventricle that contracts forcing the blood into the conus arteriosus and thence to the gills. The ventricle has a compact outer layer and a spongy inner layer. There are coronary arteries supplying the conus and ventricle with oxygenated blood from the gills (**see also Design and Physiology of the Heart**: The Coronary Circulation).

Reproduction

All elasmobranchs employ internal fertilization and display one of the two types of reproduction (oviparity, i.e., egg laying, and viviparity, i.e., live birth) (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish). The male has two external intromittent organs called 'claspers' with grooved surfaces to introduce sperm into the female. Seawater is used by the muscles of the claspers to pump sperm into the female. In egg-laying species, a tough leathery egg case is deposited around the egg, and this is released to the environment soon after fertilization. Among livebearers (55% of all elasmobranch species and 100% of freshwater species), there are further two reproductive types (placental and aplacental). The embryos of aplacental forms rely on yolk reserves or eat unfertilized eggs or other embryos. Placental embryos either feed on placental milk produced by a placental analog (uterine epithelium) or form a true placenta with the maternal and embryonic tissues in close contact.

In all cases of viviparity, the embryo is released in a highly developed state and no further maternal care is given.

Osmo- and Ionoregulation/ Osmoconformation

As osmoconformers, marine elasmobranchs use an unusual combination of organic solutes to balance osmotically (actually slightly hypertonic) with seawater. Urea accounts for 40% of osmotic support, and methylamines and/or amino acids another 20% with inorganic ions making up the rest (**Figure 2**). Urea is a small molecule that diffuses almost as rapidly in water as do the water molecules themselves. This may facilitate rapid osmotic adjustment within the various body fluid compartments. Rapid movement of urea and water across membranes requires pores such as aquaporins, and these have been found in elasmobranchs (**see also Osmotic, Ionic and Nitrogenous-Waste**

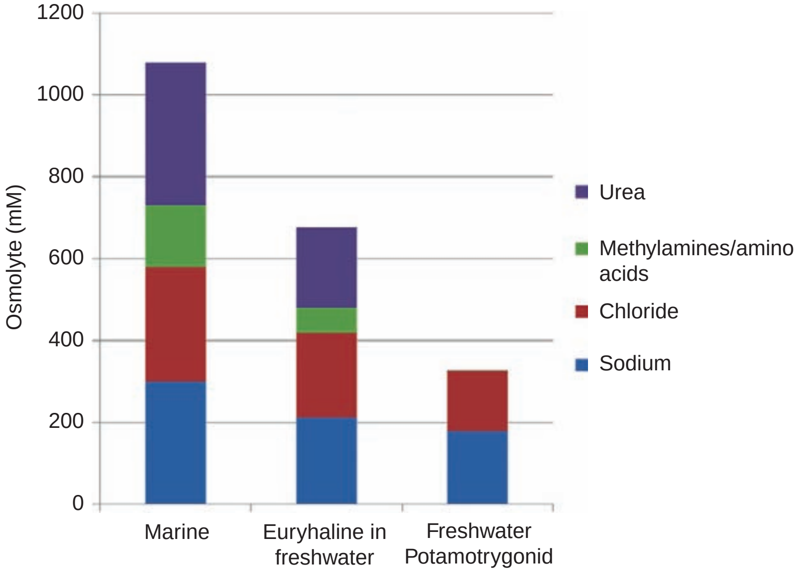


Figure 2 Major solute concentrations in plasma of marine, and freshwater elasmobranchs.

Balance: Water Balance and Aquaporin). The slightly hypertonic condition means elasmobranchs do not need to drink seawater to replace water losses. This reduces but does not eliminate the intake of salt (Na^+ and Cl^-). The activities and abundance of Na^+/K^+ adenosine triphosphatase (ATPase) in gills of marine elasmobranchs are an order of magnitude lower than those of teleost fish. Unlike teleost fishes, salt excretion is accomplished primarily by the rectal gland, instead of the gills. The rectal gland is a finger-shaped structure with a lumen emptying into the rectum (**see also Role of the Gut**: Dogfish Rectal Gland). The mechanism of action is similar to that of the chloride cells of teleost fishes. This involves lowering intracellular sodium by the action of a Na^+/K^+-ATPase on the blood side causing sodium to enter with K^+ and Cl^- via an Na^+/K^+2Cl^- symporter (**Figure 3**). The transepithelial potential generated causes Na^+ to move between the cells into the lumen. Chloride follows through a chloride channel on the lumen side. The secretion is regulated at several levels by nervous, hormonal, and paracrine signals. The fluid produced contains levels of sodium and chloride similar to that of seawater. Since marine elasmobranchs do not drink seawater, they must bring in ions such as Mg^{2+} and Ca^{2+} from food in the gut (**see also Role of the Gut**: Gut Ion, Osmotic and Acid-Base Regulation).

Digestion

As carnivores, elasmobranchs take in mainly protein and lipid. They rely on many of the same enzymes for digestion as do other vertebrates. The elasmobranchs are the most ancestral extant vertebrates capable of acid secretion in the stomach (**see also Integrated Function and Control of the Gut**: Gut Secretion and Digestion). Acid proteases, such as pepsin, act to initiate digestion of protein. Gastrin or gastrin-like peptides are the signals for acid secretion. Chitinases are found in species feeding on crustaceans. In the intestine, alkaline phosphatase, elastase, carboxypeptidases, trypsin, colipase, alpha-amylase, saccharase, and dipeptidase are secreted from the pancreas stimulated by secretin and cholescystokinin (CCK).

Lipid digestion involves bile salts and pancreatic lipase as in other vertebrates. Pancreatic colipase activates the lipase in the presence of bile salts. Bile is used for lipid solubilization using bile acids that form alcohol sulfate esters. The two main bile alcohols are chimaerol and scymnol. Bile release is induced by gastrin and CCK-related peptides. The pancreatic lipase acts primarily on the 1 and 3 positions of TAGs. This enzyme may also be involved in hydrolysis of wax esters. Digestion of a range of carbohydrates also occurs with the appropriate enzymes and many of these are localized not only in the digestive tract but also in the tissues of the immune system (spleen, Leydig's organ, and epigonal organ) for digesting bacteria. Food takes longer to pass through the gut of elasmobranchs, compared to that of teleosts, due to the relatively inefficient spiral valve intestine.

Intermediary Metabolism

The intermediary metabolism of elasmobranchs is different from that of the other vertebrates in several ways. These include aspects of nitrogen, lipid, and ketone body metabolism. All of these differences can be attributed to the heavy reliance on urea for osmotic support.

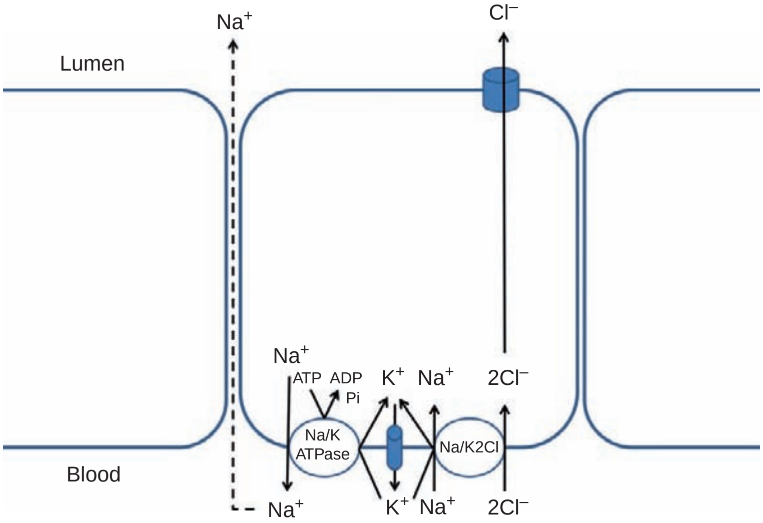


Figure 3 Ion movements for salt secretion in rectal gland of a marine elasmobranch.

Carbohydrate Metabolism

Carbohydrate metabolism of elasmobranchs is not as well developed as in more derived fishes. Elasmobranchs lack insulin-dependent glucose transporters and thus have a slow insulin response with no antagonism by glucagon. The activities of lactate dehydrogenase are lower than those of comparable teleost fish. During exhaustive exercise, glycogen is broken down and lactate is accumulated anaerobically in part because pyruvate dehydrogenase activity is low in white muscle and is not activated during exhaustive exercise or recovery, unlike the situation in teleost fish. The extent to which carbohydrate is used aerobically during nonexhaustive exercise is not known, although it is likely used in conjunction with amino acids and ketone bodies.

Nitrogen Metabolism

A major factor in the organization of nitrogen metabolism is the overriding impact of urea synthesis (**see also Nitrogenous-Waste Balance**: Ureotelism). The urea lost at the gills (although minimized) must be replaced. Unlike the situation in mammals and many other vertebrates, urea synthesis via the urea cycle requires glutamine instead of ammonia as the nitrogen donor via carbamoyl phosphate synthetase III (CPS III) (**Figure 4**). The heavy reliance on glutamine results in low circulating levels of glutamine, especially in food-deprived individuals. Dietary glutamine is insufficient to supply the needed glutamine, and a considerable recycling of ammonia from amino acid degradation is needed for glutamine synthesis. The need of nitrogen for urea synthesis means that very little ammonia is excreted compared to bony fishes. The main enzyme involved in ammonia assimilation is glutamine synthetase, and this is found in high activities in many elasmobranch tissues including muscle. The K_m for ammonia for this enzyme is very low (15 μM), allowing it to scavenge ammonia at very low concentrations. The subcellular compartmentation of urea synthesis in the urea cycle is different from that of other vertebrates (**Figure 4**), with arginase (the enzyme producing urea from arginine) being found in the mitochondrion. The importance of this has not been established, but a special transporter has been found to facilitate movement of urea out of the mitochondrion.

Urea is a disruptor of hydrophobic interactions and could disrupt protein and membrane structure if it were not for the counteracting effect of methylamines such as TMAO. A 2:1 ratio of urea to methylamines is optimal for counteraction in many cases. However, not all elasmobranch proteins are disrupted by urea, and some may be intrinsically insensitive to these solutes. Urea has a fluidizing effect on membranes, and some elasmobranch membranes may contain a higher proportion of saturated phospholipid fatty acids to compensate for this.

The counteracting methylamines, especially TMAO, are largely taken in from the diet and only members of the Carcharhiniformes and Orectolobiformes are known to have the capacity to synthesize these molecules *de novo*.

The high levels of the glutaminase in heart and red muscle of elasmobranchs have led to the suggestion that

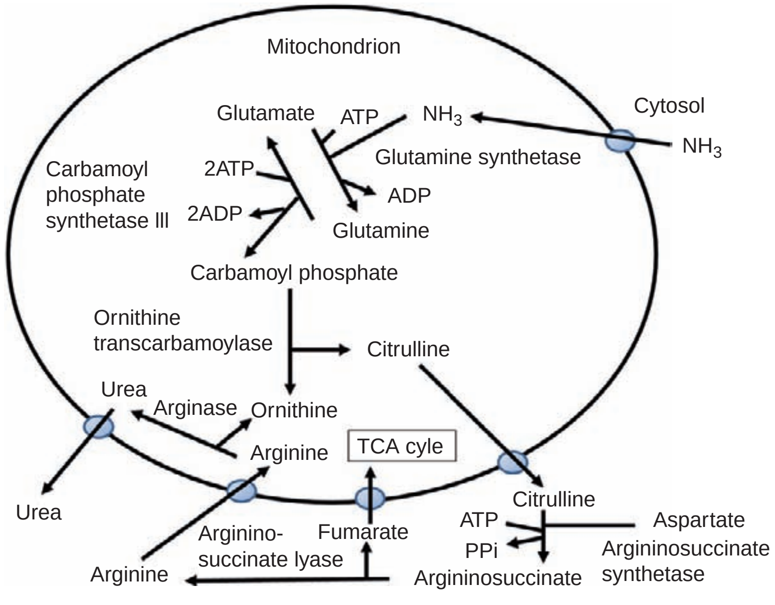


Figure 4 Compartmentation of key enzymes and metabolites in the hepatic urea cycle of a marine elasmobranch. Not all intermediates are displayed.

amino acids are important fuels for exercise. This has not yet been demonstrated.

Lipid Metabolism

The elasmobranchs do not rely on lipids as an energy source for locomotion, and the levels of circulating non-esterified fatty acids (NEFAs) in the plasma are the lowest of any vertebrate. In most vertebrates, the plasma NEFAs are used for rapid delivery of lipids to tissues such as muscle and heart. Fatty acids are not very soluble in water and must be carried on specialized proteins such as albumin. Albumin is apparently absent in the plasma of marine elasmobranchs, based on fatty acid binding studies, but the existence of the protein in elasmobranchs has not been ruled out by genome analysis. The enzymes of fat catabolism (e.g., carnitine palmitoyltransferase) are absent in the heart and muscle of elasmobranchs. The elasmobranch kidney and rectal gland have some capacity for lipid oxidation.

The transfer of lipids from reserves and dietary intake takes place via plasma lipoproteins as in other vertebrates. All the plasma lipoproteins found in other vertebrates, very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs), have been found although the HDL levels are lower than those of other vertebrates. Hydrocarbons and DAGEs are also transported on these lipoproteins especially via LDL.

Ketone Body Metabolism

Unlike the situation in most vertebrates, where ketone bodies are only used extensively during periods of starvation to replace fuels such as glucose needed by the brain, elasmobranchs use ketone bodies routinely as aerobic fuels in tissues such as heart and, perhaps, red muscle during nonexhaustive exercise. Elasmobranchs have high levels of the enzymes of ketone body metabolism in these tissues and high circulating levels of ketone bodies. This strategy allows carbon from fatty acids partially oxidized in the liver to be transported as ketone bodies without the need to transport NEFAs via albumin in the plasma. Ketone bodies are highly soluble compounds.

Excretion

Because marine elasmobranchs are slightly hypertonic, there is a net influx of water unlike the situation in hypotonic marine teleost fish. One of the roles of kidney, therefore, is to produce a hyposmotic urine (~80% of seawater osmolality) to offset the water influx. Another major role of the kidney is urea retention. As the major osmolyte, urea conservation is essential. While the gills have mechanisms to help retain urea, the kidneys must also recover urea from the filtrate before excretion takes place. The kidneys of marine elasmobranchs are complex. The kidney tubules are very long with four loops and a countercurrent arrangement of capillaries that allow them to produce urine without urea in it. Urea transporters, which are sodium-linked with very low K_m's (~0.7 mM), allow urea to be recovered to very low levels, have been identified in the kidney. The main constituents of the urine are sodium and chloride, with magnesium, phosphate, and sulfate making up most of the remainder.

Elasmobranchs lose both ammonia and urea, primarily at the gills, but at rates much lower than in marine teleost fish. This is because both the molecules are in high demand and need to be conserved. Any losses are usually due to inefficiencies in the retention and recovery systems. Only a small percent of an injected load of urea is lost per day, orders of magnitude less than that lost by other fish. This efficient retention system is due to both structural and transport-mediated phenomena. The gills of marine elasmobranchs have transporters for recovery of any urea that might escape at the gills and a high cholesterol content in the basolateral membrane of the gill that further reduces gill permeability to urea.

Neurophysiology

The osmotic and ionic environment of the nerve cells of the brain is isolated from that of the blood by a blood–brain barrier as in other vertebrates, but it is leakier than that of higher vertebrates. This is because there are no tight junctions between endothelial cells – only between glial cells in the perivascular region. The brain is enveloped in a fluid (cranial fluid, extradural fluid, and perimeningeal fluid) different in composition from the cerebrospinal fluid and both are regulated independently of the blood. The elasmobranchs are the earliest known vertebrate group to use myelin around their nerves to improve conduction velocity.

Immune System and Disease Resistance

In the absence of bone and bone marrow, much of the generation of red and white blood cells occurs in two organs unique to elasmobranchs: the Leydig's organ and the epigonal organ. The epigonal organ is found in various locations in the body cavity, often near the gonads. It contains sinuses containing developing leukocytes (white blood cells) analogous to mammalian bone marrow. The extent of erythropoiesis in the epigonal organ can be judged by the color. The more red the greater the extent of red blood cell production. The Leydig's organ is usually found in close association with the esophagus and is histologically similar to the epigonal organ. Both the organs

Table 1 Hormones and their functions in elasmobranch fishes

Hormone	*Occurrence and function in elasmobranchs*
Adrenaline	Present. Glomerular diuresis, mobilization of energy reserves, regulation of blood pressure, regulation of blood flow to gut, regulation of perfusion and ventilation of gills
Adrenocorticotropic hormone (ACTH)	Present. Regulation of production of 1-alpha-hydroxycorticosterone, hyperglycemia
Amylin	Not reported for elasmobranchs
Angiotensin I	Present. Precursor to angiotensin II
Angiotensin II	Present. Modulation of HPI axis, vasoconstriction, stimulation of drinking, stimulation of 1-alpha-hydroxycorticosterone; regulation of kidney secretion, glomerular filtration rate, and urine flow rate, inhibition of salt secretion by rectal gland (due to reduced blood flow)
Arginine vasotocin	Present. Regulation of urine flow and GFR; vasoconstriction, influence on urea reabsorption and osmoregulation in high salinities
Atrial natriuretic peptide	Not reported for elasmobranchs
Bombesin	Present. Increased blood flow to gut, inhibits rectal gland secretion
Calcitonin	Present. Regulation of calcium homeostasis during uterolactation, fetal nutrition
Cholecystokinin	Present. Stimulation of bile and pancreatic enzymes
Corticotrophin-releasing factor (CRF)	Not reported for elasmobranchs.
C-type natriuretic peptide CNP	CNP3 only reported. Increase in urine flow rate, GFR, increased clearance of urea, Na and Cl, stimulation of salt secretion in rectal gland, inhibition of drinking
Endothelin I	Present. Stimulation of salt secretion in rectal gland
Enkephalin	Met- and leu-enkephalinpresent. Function not established
17beta-estradiol	Present. Stimulation of vitellogenin synthesis in liver, stimulation of growth of oviducal gland, development of follicles, modulation of placental function, and regulation of gonad development
Follicle-stimulating hormone (FSH)	Not reported for elasmobranchs
Galanin	Present. Vasoconstriction
Gastric-inhibitory polypeptide	Not reported for elasmobranchs
Gastrin	Present. Stimulation of gastric acid secretion
Gastrin-releasing peptide	Presents. Increased blood flow to gut
Glucagon	Present. No effect on blood glucose
Glucagon-like peptides 1 and 2	Not reported for elasmobranchs
Gonadotropin-releasing hormone	Present.
Growth hormone	Present. Function not determined
1-alpha-Hydroxycorticosterone	Present. Control of rectal gland secretion, sodium and chloride retention
Insulin	Present. Decreases plasma glucose, increases muscle and liver glycogen, reduces plasma free amino acids, increases lipid uptake
Insulin-like growth factor I (IGF -I)	Present. Increased cartilage growth
Hypocalcin	Not reported for elasmobranchs
Leptin	Present. Function not determined
Luteinizing hormone	Not reported for elasmobranchs
Melanocyte-concentrating hormone	Present. Color change
Melanocyte-stimulating hormone (MSH)	Present. Color change
Melatonin	Present. Regulation of light production by photophores in deep-water elasmobranchs
Neuropeptide Y	Present. Increased blood flow to gut, inhibits rectal gland secretion
Neurotensin	Present. Function not determined
Noradrenaline	Present. Mobilization of energy reserves, increase blood pressure, reduced blood flow to gut, increased perfusion and ventilation of gills
Pancreatic polypeptide Y	Not reported for elasmobranchs
Parathyroid hormone	Parathyroid hormone-related peptide reported. Potential role in ionoregulation
Peptide YY	Present. Function not determined
Progesterone	Present. Inhibition of vitellogenin production, regulation of gonad development
Prolactin	Present. Increases GFR, stimulates melanosome dispersion
Propiomelanocortin	Not reported for elasmobranchs
Relaxin	Present. Increase in compliance of cervix, regulation of semen quality

(*Continued*)

Table 1 (Continued)

Hormone	*Occurrence and function in elasmobranchs*
Scyliorhinin I	Present. Increased blood flow to gut
Scyliorhinin II	Present. Increased blood flow to gut
Secretin	Present. Stimulation of pancreatic secretions
Somatostatin	Present. Inhibition of gastric acid production, inhibits rectal gland secretion
Stanniocalcin	Not reported for elasmobranchs
Testosterone	Present. Regulation of oviducal sperm storage, regulation of oviposition, regulation of gonad development, regulation of mating behavior
Thyroxine	Present. Regulation of urea synthesis
Triiodothyronine	Present. Regulation of urea synthesis, regulation of expression of amino acid enzymes in liver, development of embryos
Thyroid-stimulating hormone	Present.
Thyrotropin-releasing hormone	Present.
Urotensin I	Present. Blood pressure regulation
Urotensin II	Not reported for elasmobranchs
Urotensin III	Not reported for elasmobranchs
Vasoactive intestinal peptide (VIP)	Present. Increases GFR, reduces perfusion of gut and gut motility, stimulates salt secretion by rectal gland

have cells that secrete immunoglobulin. There are species-specific differences in the size of each organ with one or the other sometimes being reduced or absent. There is generally a reciprocal relationship between the sizes of these two organs such that in species lacking one, the other is larger. Elasmobranchs have lymphocytes, granulocytes, monocytes, and macrophages involved in the immune response (**see also Blood**: Cellular Composition of the Blood). Of these, lymphocytes account for almost half of the circulating leukocytes. Elasmobranchs are the first vertebrate group to develop a fully functional adaptive immune response involving antibodies, T-cell receptors, major histocompatibility proteins, and recombination-activating gene proteins. The antibody system involves a Y-shaped system with two light and two heavy chain proteins as in other higher vertebrates. To generate a large array of these, elasmobranchs have more than 100 copies each of the genes for the heavy and light chains. This contrasts with only a few copies of these genes in other vertebrates and a reliance on cutting and splicing these to generate antibody diversity.

Endocrinology

Elasmobranchs have many of the typical vertebrate hormones (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction; **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut, **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids, The Hormonal Control of Osmoregulation in Teleost Fish and **Hormonal Responses to Stress**: Catecholamines), although the number of isoforms of some hormones is less than those found in higher vertebrates. Elasmobranchs have a pancreas with both exocrine and endocrine functions unlike most teleosts where these functions are in separate structures. As in higher vertebrates, there is a functional hypothalamic–pituitary–interrenal axis with associated hormones. **Table 1** summarizes the main vertebrate hormones and their known function (if any) in elasmobranchs. Much more work is needed to establish roles for many of these hormones. Elasmobranchs have a unique stress hormone, 1-alpha-hydroxycorticosterone, which replaces cortisol in the stress response (**see also Hormonal Responses to Stress**: Hormone Response to Stress). Binding of this hormone to a receptor has been demonstrated in the gills of elasmobranchs and it may function in regulating salt and urea permeability.

Endothermy in Elasmobranchs

A few elasmobranchs have the capacity to maintain parts of their bodies above ambient temperature (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks). The extra speed made possible by warm muscles is an advantage when feeding on cold-bodied fishes or squids and on marine mammals. In endothermic sharks, the red muscle is localized in the interior of the body, close to the vertebral column unlike the peripheral location of that tissue in other sharks. Heat generated by this muscle is trapped in the muscle by a countercurrent arrangement of blood vessels called 'retia mirabilia' (**see also Design and Physiology of Arteries and Veins**: The Retia). Retia have been found above the liver (maintaining a warm stomach) in red muscle and in the head (carotid rete, keeping the eyes warm). The fast sharks of the family Lamnidae have documented higher body temperatures, and members of the Alopiidae and the some mobulids have the anatomical features needed for endothermy. The lamnids include the great white shark (*C. carcharias*), the longfin and shortfin mako sharks (*Isurus paucus* and *Isurus oxyrinchus*, respectively), the porbeagle (*Lamna nasus*), and the salmon shark (*Lamna ditropis*).

Acid–Base Physiology

The gills of elasmobranchs play an important role in acid–base regulation (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). Ion regulation and acid–base regulation are connected in that the uptake of Cl^- is coupled to the extrusion of HCO_3^- via the Cl^-/HCO_3^- exchanger, and Na^+ uptake is coupled to the extrusion of H^+ via the Na^+/H^+ exchanger. Carbonic anhydrase, which interconverts carbon dioxide and bicarbonate in maintaining acid–base balance, is found in gill cells. The kidney has a limited capacity for hydrogen-ion excretion.

Summary

Many aspects of the physiology of elasmobranchs reflect their early evolutionary position and offer insights into the pathways leading to the more sophisticated physiological systems of higher vertebrates. Their continued existence attests to the early optimization of their physiological systems. Many aspects of their physiology require more extensive investigation.

See also: **Blood**: Cellular Composition of the Blood. **Brain and Nervous System**: Functional Morphology of the Brains of Cartilaginous Fishes. **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: The Retia. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping. **Detection and Generation of Electric Signals**: Active Electrolocation; Detection and Generation of Electric Signals in Fishes: An Introduction; Electric Organs; Generation of Electric Signals. **Hearing and Lateral Line**: Auditory System Morphology; The Coronary Circulation; The Ear and Hearing in Sharks, Skates, and Rays. **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids; The Hormonal Control of Osmoregulation in Teleost Fish. **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress**: Catecholamines; Hormone Response to Stress. **Integrated Function and Control of the Gut**: Endocrine Systems of the Gut. **Nitrogenous-Waste Balance**: Excretion of Ammonia. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Role of the Gut**: Dogfish Rectal Gland; Gut Ion, Osmotic and Acid-Base Regulation; Gut Secretion and Digestion. **Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes; Water Balance and Aquaporin. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction. **Social and Reproductive Behaviors**: Sexual Behavior in Fish. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance. **The Skeleton**: Cartilaginous Fish Skeletal Anatomy; Cartilaginous Fish Skeletal Tissues. **The Skeleton**: Ureotelism. **Vision**: Adaptations of Photoreceptors and Visual Pigments.

Further Reading

Ballantyne JS (1995) Metabolic organization of thermogenic tissues of fishes. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes,* vol. 4, pp. 241–258. Amsterdam: Elsevier Science.

Ballantyne JS (1997) Jaws: The inside story. The metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology* 118B: 703–742.

Ballantyne JS, Chamberlin ME, and Singer TD (1992) Oxidative metabolism in thermogenic tissues of the swordfish and mako shark. *Journal of Experimental Zoology* 261: 110–114.

Ballantyne JS and Robinson JW (2010) Freshwater elasmobranchs: A review of their physiology and biochemistry. *Journal of Comparative Physiology* 180B: 457–493.

Bone Q and Roberts BL (1969) The density of elasmobranchs. *Journal of Marine Biological Association of United Kingdom* 49: 913–937.

Carrier JC, Musick JA, and Heithaus MR (2004) *Biology of Sharks and Their Relatives,* pp. 1–596. Boca Raton: CRC Press.

Chamberlin ME and Ballantyne JS (1992) Glutamine metabolism in elasmobranch and agnathan muscle. *Journal of Experimental Zoology* 264: 269–272.

Fines GA, Ballantyne JS, and Wright PA (2001) Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. *American Journal of Physiology* 280: R16–R24.

Finkelstein JB (2005) Sharks do get cancer: Few surprises in cartilage research. *Journal of the National Cancer Institute* 97: 1562–1563.

Hamlett WC (1999) *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*, pp. 1–515. Baltimore: The Johns Hopkins University Press.

Hazon N, Wells A, Pillans RD, *et al.* (2003) Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity. *Comparative Biochemistry and Physiology* 136B: 685–700.

Lacy ER, Reale E, Schusselberg DS, Smith WK, and Woodward DJ (1985) A renal countercurrent system in marine elasmobranch fish: A computer-assisted reconstruction. *Science* 227: 1351–1354.

Mestel R (1996) Sharks' healing powers. *Natural History* 105: 40–47.

Ostrander GK, Cheng KC, Wolf JC, and Wolfe MJ (2004) Shark cartilage, cancer and the growing threat of pseudoscience. *Cancer Research* 64: 8485–8491.

Richards JG, Heigenhauser GJF, and Wood CM (2003) Exercise and recovery metabolism in the Pacific spiny dogfish. *Squalus acanthias. Journal of Comparative Physiology* 173B: 463–474.

Shuttleworth TJ (1988) *Physiology of Elasmobranch Fishes,* pp. 1–324. Berlin: Springer.

Speers-Roesch B, Ip YK, and Ballantyne JS (2006) Metabolic organization of freshwater, euryhaline, and marine elasmobranchs: Implications for the evolution of energy metabolism in sharks and rays. *Journal of Experimental Biology* 209: 2495–2508.

Speers-Roesch B, Ip YK, and Ballantyne JS (2008) Plasma non-esterified fatty acids of elasmobranchs: Comparisons of temperate and tropical species and effects of environmental salinity. *Comparative Biochemistry and Physiology* 149A: 209–216.

Treberg JR, Speers-Roesch B, Piermarini PM, *et al.* (2006) The accumulation of methylamine counteracting solutes in elasmobranchs with differing levels of urea: A comparison of marine and freshwater species. *Journal of Experimental Biology* 209: 860–870.

Wourms JP and Demski LS (1993) The reproduction and development of sharks, skates, rays and ratfishes: Introduction, history, overview, and future prospects. *Environmental Biology of Fishes* 38: 7–21.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Bony Fishes

Contents

Zebrafish
Crucian Carp
Life in Hot Water: The Desert Pupfish
Blind Cavefish

Zebrafish

JG Richards, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Appearance
Taxonomy
Distribution and Natural History
Lifecycle and Reproduction
Zebrafish as a Model Organism for Scientific Research
Further Reading

Glossary

Forward genetics The induction of random mutations in an organism followed by examining of the resulting phenotype.

Fusiform A body shape characterized by tapering at both the anterior and the posterior ends.

Gastrulation Migration and rearrangement of cells after embryo undergoes proliferation to increase cell number (cleavage stage) to create three primary germ layers during early development.

Insertional mutagenesis Insertion of one or more bases into the genome.

Retrovirus An RNA virus that is replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome, which is then incorporated into the host's genome.

Reverse genetics Identification of gene function through selective manipulation of that gene followed by analysis of the arising phenotype.

Introduction

The zebrafish, *Danio rerio*, is now one of the mostly widely used experimental animals in biological and biomedical research. This popularity is primarily due to their reasonably fast generation time and the vast array of genomic resources available, which allow for manipulations at the smallest scale. Furthermore, they are almost transparent during the majority of their embryonic development, making them amenable to direct visualization of developmental processes. Until recently, however, their use in physiological studies has been limited and, for the most part, the vast majority of the published literature on these fish does not work toward understanding their physiology, but rather understanding basic biological processes. They are also extremely small when it comes to tissue sampling.

With the incorporation of genomics tools into physiological research, fish physiologists are now flocking to this small cyprinid to ask new and exciting questions about physiology, adaptation (**see also Responses and Adaptations to the Environment**: General Principles of

Biochemical Adaptations), and how this animal responds to environmental challenge. This article, however, focuses primarily on the natural history, development, and genomic resources available in this important biological model. Aspects of their physiology are covered elsewhere in this encyclopedia.

Appearance

The zebrafish is aptly named for the five uniform, horizontal blue or black stripes that are located on the side of the body, all of which extend from the operculum through to the caudal fin (**Figure 1**). The fish shape is characterized by tapering at both the anterior and the posterior ends (commonly referred to as fusiform) and it is laterally compressed with an upward directed mouth. Zebrafish are sexually dimorphic, meaning males and females look different. Male zebrafish are thinner and more torpedo shaped, with yellow stripes between the blue/black stripes; female zebrafish are generally larger, with white bellies and silver stripes instead of yellow ones. Adult zebrafish have been recorded to grow up to ~6.5 cm, but for the most part captive zebrafish do not exceed 4 cm in length.

Taxonomy

Zebrafish are a tropical freshwater fish belonging to the family Cyprinidae from the taxonomic order Cypriniformes. The Cyprinidae is the largest family of freshwater fish with roughly 2500 species in just over 200 genera. Of particular note, this family includes the carps (e.g., the anoxia-tolerant Crucian carp (*Carassius carassius*; **see also Bony Fishes**: Crucian Carp) and the goldfish (*Carassius auratus*) as well as the true minnows.

The genus *Danio* was at one point comprised of somewhere between 35 and 50 species, but this traditional grouping of all species under the genus *Danio* was split and now the species are divided among two different genera, *Danio* and *Davario*. An example phylogeny showing the split in species is depicted in **Figure 2** and was originally constructed in order to examine coloration patterns among different species.

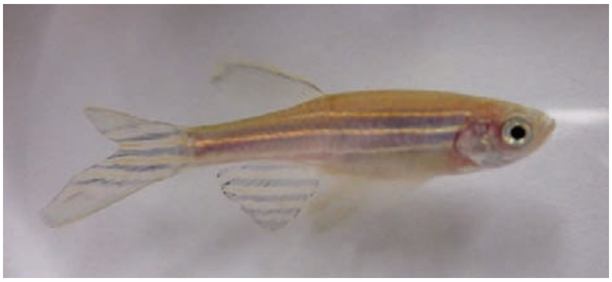


Figure 1 Photo of a male *Danio rerio.*

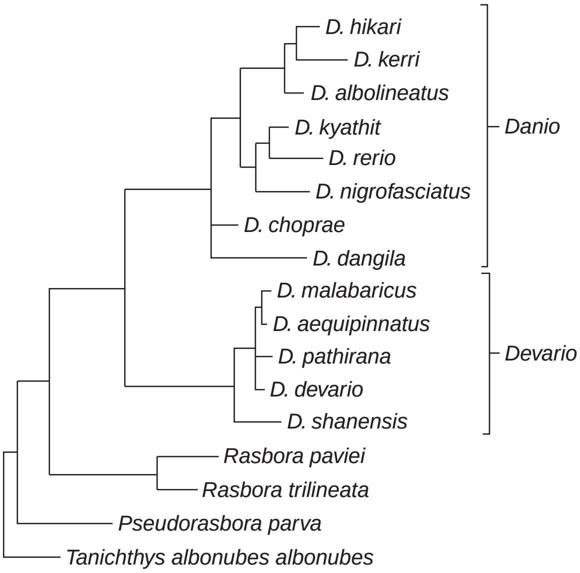


Figure 2 Phylogenetic relationship of 13 species of *Danio* and *Davario* based on maximum likelihood and Bayesian analysis of 12S and 16S ribosomal DNA sequence. Phylogeny has been modified from Parichy DM (2006) Evolution of *Danio* pigment pattern development. *Heredity* 97: 200–210.

Distribution and Natural History

Zebrafish originate from the waters from the southeastern Himalayan region, but there is some controversy over their precise natural range. Recent analysis of collection records suggest that the natural geographic range of zebrafish extends from Pakistan to the western edge of Myanmar and from Nepal in the north to the Indian state of Karnataka in the south. They are primarily found in streams, canals, ditches, rice fields, and other slow-moving bodies of water with clay, silt, or cobble substrates. The waters are typically still and clear, roughly ranging in temperature between 27 and 34 °C and a slightly alkaline pH (7.9–8.5). In these environments, they mostly feed on mosquito larvae and other small insects, but they are omnivorous and have also been observed feeding on phytoplankton. They are unlikely to experience large variations in temperature within a single body of water, although the temperatures are warm and there is likely temperature variation over their natural range. The highly vegetated nature of their water and the fact that it can be stagnant likely means zebrafish experience large variations in dissolved oxygen on a daily basis (**see also Hypoxia**: The Expanding Hypoxic Environment). Oddly, despite the fact that hypoxia is likely prevalent in their natural environment, only a small amount of work has been done on their hypoxia tolerance. For more information on hypoxia response of zebrafish, the reader is referred to **Hypoxia**: Respiratory Responses to Hypoxia in Fishes.

Lifecycle and Reproduction

The typical life span of zebrafish is roughly 2–3 years, but it can be up to 5 years under ideal conditions in captivity, but probably much shorter in the wild. The generation time from a fertilized egg to a reproductively active adult is between 3 and 4 months. Adult females are able to spawn every 2–3 days and each female can lay up to 200 eggs/week, but ovulation and spawning only occur in the presence of a male. Zebrafish mate in the morning and it is well known that light is the primary cue for mating. Within 30 min of the lights being turned on (if in the laboratory) or sunrise (if in the natural environment), a female will sometimes lay hundreds of eggs, which are subsequently fertilized by a male. At high densities in aquarium-bred zebrafish, it is typical to observe group-spawning behavior where egg fertilization is done in an indiscriminate fashion. Upon the release of eggs, embryonic development begins immediately, but if not fertilized, growth is halted after the first few cell divisions and the eggs die or are eaten.

Upon fertilization, the eggs almost immediately become transparent and development progresses quickly (see **Figure 3** for an outline of the lifecycle of a zebrafish (**see also Reproduction**: The Diversity of Fish Reproduction: An Introduction). Within 4 h, the fertilized egg has gone from a single cell to ∼1000 cells. Shortly after that, the embryo undergoes gastrulation, which is the developmental stage associated with the formation of a double-walled embryo resulting from the invagination of the blastula, which is associated with the differentiation of the ectoderm into the mesoderm and endoderm. The development of discreet organs, referred to as organogenesis, begins between 16 and 24 h postfertilization (hpf) and the precursors to all major organs appear within 36 hpf. For example, the zebrafish heart starts off as a 'heart tube' which is formed by 18 hpf and rhythmic, peristaltic contractions can be observed by 24 hpf (**see also Circulation**: The Circulatory System: An Introduction). Hatching Ultimately takes place 48–72 hpf, depending on the temperature, and feeding and swimming begin at roughly 72 hpf. Within 2–3 months, the zebrafish have matured into adults and can then begin breeding.

Although the reproductive behavior of captive zebrafish is well known, the reproductive behavior of wild zebrafish is largely unexplored. Wild-reared zebrafish

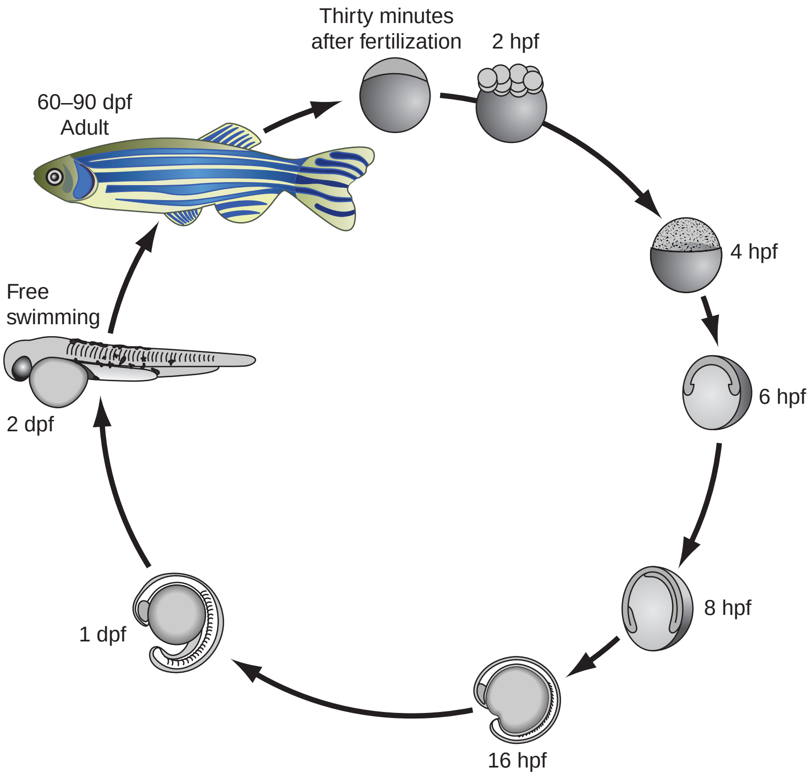


Figure 3 Lifecycle of the zebrafish. The development of zebrafish is rapid with the fertilized embryo transforming into a 1000-cell stage within 4 h postfertilization (hpf) and organs being visible at roughly 1 day postfertilization (dpf). Hatching occurs at 2 dpf and the zebrafish matures within 2–3 months. Image adapted from Wolpert L, Beddington R, Jessell T, *et al*. (2002) *Principles of Development*, 2nd edn. New York: Oxford University Press.

take up to 4–5 months to reach sexual maturity rather than the 2–3 months commonly observed in the laboratory. In one study, wild-caught zebrafish from India were observed to spawn in pairs rather than in groups as is common in aquaria. Furthermore, there was evidence for sexual selection and females taking an active role in choosing their mates. This discrimination may be primarily mediated by density in that, at low densities as seen in the wild, there may be elevated levels of sexual selection and pair formation; however, at high densities, characteristic of aquarium breeding, sexual selection and pair formation break down.

Zebrafish as a Model Organism for Scientific Research

Zebrafish have long been an important model in developmental biology, but their rise as an important experimental system in biology emerged from several major advances that have occurred since the early 1990s. First, the most important advance came in the 1990s, where several research groups induced large-scale genetic mutations in the zebrafish and subsequently screened the mutants for identifiable phenotypes. This approach of inducing random mutations and then searching for the genetic underpinnings is referred to as forward genetics. The second major advance was the start of a whole-genome sequencing project that was initiated in 2001 and is still underway. This large-scale sequencing project has facilitated the third major advance, reverse genetics, which involves modifying single genes and then examining the effects of these genetic changes on the whole-animal phenotype.

Forward Genetics in Zebrafish

The first step in the popularization of zebrafish as a model organism in biology was the mutagenesis projects initiated in the early 1990s. This work followed in the footsteps of earlier work performed on the fruit fly (*Drosophila melanogaster*) and the nematode (*Caenorhabditis elegans*). In 1996, two important works were published by Driever and Haffter and their respective co-workers that described two large-scale genetic mutation screenings, where a chemical mutagen, ethylnitrosourea, was used to induce random mutations in zebrafish sperm. Ethylnitrosourea induces point mutations in the sperm genome that could affect protein sequences or gene structure and ultimately cause, through random chance, a loss of some physiological or biochemical function. After breeding, the developing embryos were screened for visible abnormalities in muscle and other organs at several different time points and from these screens more than 6500 mutants were identified. It is important to note that the majority of the mutations were lethal if they appeared homozygous in the fish.

At the same time as the chemical mutagenesis was underway, in a separate research laboratory Gaiano and co-workers used a retrovirus to perform insertional mutagenesis, which refers to the insertion of one or more bases into the genome. The subsequent screening of these mutants resulted in the identification of roughly 500 mutations at the phenotypic level of which just over 300 have been explicitly linked to a specific genetic change. One important advantage of this technique over the chemical mutagenesis described above is that a portion of the retroviral gene sequence is inserted into the host zebrafish genome, thereby providing a genetic tag aiding in the identification of which gene is affected in the genome.

The systemic issue with forward genetics is that it is resource intensive and the direct links between phenotype and specific mutations are difficult to establish (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Fish as Model Organisms for Medical Research). Establishing these links is easier with insertional mutagenesis compared with chemical mutagenesis, but many of the direct links from these early studies have not yet been established. The genome sequencing project, started in 2001, has greatly enhanced the ability of researchers to identify where mutations have occurred and it plays an important role in advancing this field.

The Zebrafish Genome

Sequencing of the zebrafish genome was started in 2001 by the Sanger Institute and is due to be completed sometime in 2011. The zebrafish genome consists of 25 linkage groups, which are equivalent to chromosomes and the entire genome size is $\sim$1.4 gigabases. The genome is currently being annotated, which refers to the identification of genes and, once complete, this resource will be invaluable to researchers trying to understand the genetic basis of physiological and biochemical phenotypes. In addition, the genome sequencing has greatly facilitated the development of reverse genetic approaches in zebrafish.

Reverse Genetics in Zebrafish

Reverse genetics refers to the identification of gene function through selective manipulation of the gene followed by analysis of the arising phenotype. This approach attempts to identify what phenotype arises as a result of a particular gene and, for the most part, this approach involves knocking down the expression of genes of interest with subsequent examination of phenotype. These types of studies are broadly referred to as loss-of-function studies.

The most common approach for performing loss-of-function studies in zebrafish is using antisense technology.

The basic premise of this technique is that if the genetic sequence of a particular gene of interest is known, then researchers can synthesize a strand of RNA that will bind to the messenger RNA (mRNA) transcript produced by that gene. The synthetic RNA is called antisense because its nucleic acid sequence is complementary to the gene's normal mRNA sequence, which is referred to as the 'sense' sequence. The binding of the synthetic antisense RNA to the sense mRNA results in a doubled-stranded RNA molecule, which cannot be translated into protein. As a result, the incorporation of antisense RNA strands either limits or eliminates the protein coded for by the mRNA transcript.

The most common antisense approach using zebrafish reverse genetics is morpholinos. Morpholinos are synthetic oligonucleotides, in which the normal ribose or deoxyribose sugars are replaced with a morpholine ring, which are constructed to be antisense to the mRNA of interest. Normally, morpholinos are ~25 nucleotides long and are designed to bind to the native mRNA, usually near the protein translation start site. The advantage of incorporating a morpholine ring into the antisense sequence is that it makes the morpholino resistant to the normal nucleases that are present in cells and would break down unwanted RNA. The binding of the morpholino to the native mRNA causes translation blockade, where the gene of interest is silenced and very little protein is translated. It is a typical practice in the zebrafish to inject the morpholinos into the freshly fertilized egg while it is still in the one-to-eight cell stage, after which the morpholino becomes more or less uniformly distributed through the cells of the developing embryo. After this point, the efficacy of the knockdown is assessed and the resultant effects of the knockdown on phenotype are typically assessed over the first 5 or so days postfertilization.

Other commonly used methods of reverse genetics for loss-of-function studies in zebrafish are targeting-induced local lesions in genomes (TILLING) and zinc finger nucleases. TILLING combines forward genetic approaches with reverse genetics to identify specific mutations in genes of interest from a random mutagenesis screening. Specifically, this approach begins with the generation of a large number of mutated zebrafish, normally from a chemical mutagenesis screen as described earlier (**see** Section Forward Genetics in Zebrafish). DNA is then sampled from these fish and TILLING is performed. In the most basic sense, the purpose of TILLING is to look for specific mutations in specific genes in a collection of DNA samples prepared from chemically mutagenized individuals. Identification of these mutations is done using standard polymerase chain reaction (PCR) and sequencing of PCR products. Additional methods for identifying whether mutations have occurred in select areas can also be carried out to reduce the excessive sequencing costs associated with the TILLING approach.

Zinc finger nucleases represent another method for reverse genetics. Zinc finger nucleases consist of several zinc-finger DNA-binding domains plus restriction enzymes that, when combined, can be engineered to target specific DNA sequences and cut the double-stranded DNA in intact cells. The normal cellular DNA repair mechanisms then repair the broken DNA strands, but often insert nucleotides during the repair process, thus altering the genetic sequence disrupting codon arrangement.

Far less frequent are gain-of-function studies, which attempt to insert specific genes or alter genes in a way that could result in a new or enhanced phenotype. There are various techniques for inserting genes to examine gain of function, the simplest of which is microinjection of synthesized mRNA into the fertilized zebrafish eggs. These eggs then express, or, in some cases, overexpress the gene of interest and investigators can then examine the resulting phenotype. Other techniques are available for the expression or overexpression of genes in zebrafish and some of these techniques even allow researchers to target specific tissues or cells at precise developmental stages.

Overall, there is an ever-expanding collection of cell biology and genomic techniques for the zebrafish that allow manipulation of proteins and development at the most basic level. Slowly, fish physiologists are adopting this model organism and its impressive array of tools to explicitly establish links between genes and physiological attributes. Consideration of the natural habitat and use of the wide array of species available will facilitate adoption of this model system to investigate environmental adaptation and evolutionary physiology.

See also: **Bony Fishes**: Crucian Carp. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Fish as Model Organisms for Medical Research. **Circulation**: The Circulatory System: An Introduction. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes; The Expanding Hypoxic Environment. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Responses and Adaptations to the Environment**: General Principles of Biochemical Adaptations.

Further Reading

Driever W, SolnicaKrezel L, Schier AF, *et al.* (1996) A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* 123: 37–46.

Gaiano N, Amsterdam A, Kawakami K, *et al.* (1996) Insertional mutagenesis and rapid cloning of essential genes in zebrafish. *Nature* 383: 829–832.

Haffter P, Granato M, Brand M, *et al.* (1996) The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* 123: 1–36.

Parichy DM (2006) Evolution of *Danio* pigment pattern development. *Heredity* 97: 200–210.

Perry SF, Ekker M, Farrell AP, and Brauner CJ (2010) *Zebrafish. Fish Physiology,* vol. 29. San Diego, CA: Academic Press.

Crucian Carp

GE Nilsson, University of Oslo, Oslo, Norway

© 2011 Elsevier Inc. All rights reserved.

Introduction
Occurrence and Habitat
Avoiding Anoxic Energy Deficiency
Ethanol Production
Neural Depression
Heart Activity in Anoxia
Mechanisms of Metabolic Depression
Strategies to Avoid Tissue Anoxia
Conclusions
Further Reading

Glossary

Adenosine An inhibitory neuromodulator and vasodilator formed from the breakdown of phosphorylated adenosines such as ATP.

Anoxia Complete absence of dissolved oxygen in water.

Brain slices A preparation of thin slices of isolated brain tissue used for metabolic and electrophysiological studies.

Eurythermy The ability to live in a wide temperature range.

Gamma-amino butyric acid (GABA) Synapse that uses GABA as its neurotransmitter, the major inhibitory neurotransmitter across the central nervous system.

Hypoxia Low partial pressures of oxygen in external or internal environments.

***N*-methyl-D-aspartate (NMDA) receptor** An abundant receptor for the excitatory neurotransmitter glutamate in the brain of vertebrates, named after the artificial agonist *N*-methyl-D-aspartate.

P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.

Pyruvate dehydrogenase (PDH) complex A three-enzyme complex responsible for producing acetyl coenzyme A from pyruvate, thereby linking glycolysis with the citric acid cycle.

Introduction

Like virtually all vertebrates, most fishes need a constant supply of oxygen to survive. Nevertheless, there are two cyprinid fishes, the crucian carp (*Carassius carassius* L.) (**Figure 1**) and the closely related goldfish (*Carassius auratus* L.), which can survive for extended periods of time without any oxygen (anoxia). Consequently, the anoxia tolerance of these species has been the focus of intensive study. Anoxic survival is not indeterminant, however, as certain activities are suspended, including reproduction, or greatly limited, such as locomotion, and the fuel needed to survive anoxia becomes progressively depleted. The key phrase, therefore, is prolonged anoxia survival. By comparison, mammals survive interruption of the constant oxygen supply for just minutes rather than months.

Studies on goldfish have mainly used fish obtained from the aquarium trade, where this species has been cultured for more than a thousand years. Unfortunately, goldfish in the aquarium trade have been intensely selected for their ability to breed in captivity as well as for their pretty colors and odd morphology. Thus, they are likely to have lost some of their original anoxia tolerance. This article therefore focuses primarily on the crucian carp and the mechanisms that allow it to survive days to months of anoxia, depending on temperature.

The only other adult vertebrates that show a similar degree of anoxia tolerance are some North American freshwater turtles (particularly of the genera *Trachemys* and *Chrysemys*), which can survive anoxia from a few days at room temperature to several months at near-freezing temperatures. Like the crucian carp, these turtles also rely on their anoxia tolerance for overwintering at the bottom of ice-covered lakes and ponds.

Occurrence and Habitat

The crucian carp occurs in central Asia and Europe, reaching the Arctic Circle in Scandinavia. It is found both in large lakes and small ponds. They have distinct morphological types in these two environments. In large lakes, where it occurs together with numerous other fish species, including predatory ones such as pike and perch, the crucian carp is deep bodied. In small ponds, prone to seasonal anoxia and where it is the only fish species, it has a much more slender body shape. It has been suggested that the deep body form found in large lakes is the result of phenotypic plasticity induced by the smell of predators or injured crucian carp, and the function of the deep body

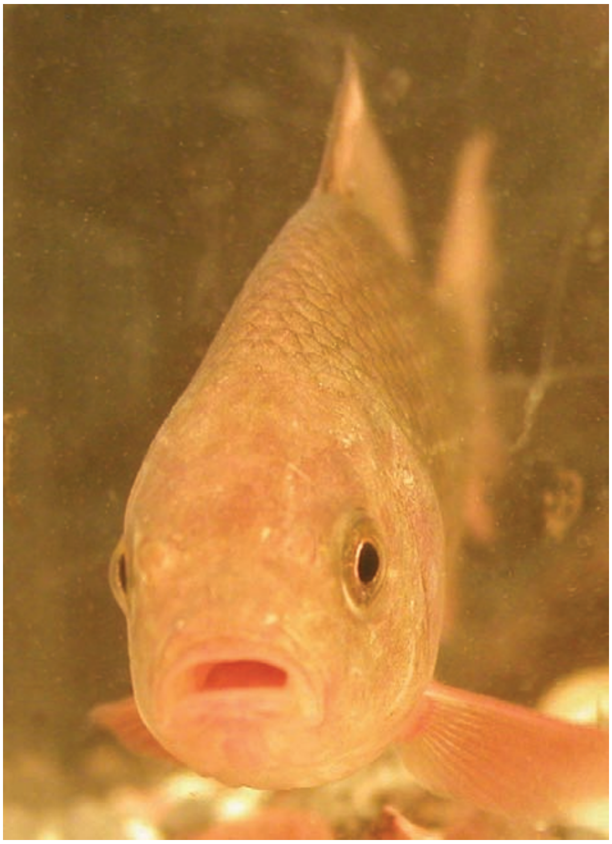


Figure 1 The crucian carp. Photo GE Nilsson.

is to make the crucian carp less likely to fit into the mouth of a predatory fish.

Extreme as this phenotypic plasticity is, equally extreme is the anoxia tolerance of the crucian carp. Anoxia tolerance can also be regarded as a predator-avoidance strategy as it allows the crucian carp to inhabit small ponds where wintertime anoxia excludes other fish species. Anoxia develops in these small ponds during wintertime because ice cover blocks oxygen diffusion from the atmosphere. In addition, limited light penetration through the ice blocks plant photosynthesis. The result is progressive hypoxia following ice formation and finally anoxia when organisms have removed all oxygen from the water. In the spring when ice melts, oxygen from the atmosphere and from photosynthesis reoxygenate the ponds, but until then, anoxia may persist for several months. The same shallow ponds also show extreme seasonal changes in water temperature. Thus, the crucian carp is exceptionally eurythermal, being able to tolerate an unusually large temperature range of 0–38 °C, with a thermal optimum of 27 °C. However, with rising temperature its anoxia tolerance falls sharply, from several months at close to 0 °C to just a day or two at 25 °C. A prime reason for this must be the temperature-dependent rise in metabolic rate.

Avoiding Anoxic Energy Deficiency

Anoxia halts oxidative phosphorylation leaving glycolysis as the only option for adenosine triphosphate (ATP) production in vertebrates. However, due to the poor ATP yield per mole of glucose by glycolysis, which is about 6–10% of the complete oxidation of glucose to H_2O and CO_2, anoxia leads to a rapid decrease in ATP levels in most vertebrates. The brain and heart are particularly sensitive to anoxia due to their very high intrinsic rates of ATP consumption and limited stores of glucose. If the ATP production in the brain and heart is not matched to ATP demand, the ATP pools of these organs will be depleted within minutes and the cells will die.

Clearly, the crucian carp has the means to avoid the anoxic catastrophe seen in tissues of other vertebrates. These mechanisms allow the crucian carp to maintain stable cellular ATP levels during anoxia, thereby avoiding energy deficiency and death. Its adaptations to anoxic survival involve: (1) producing ethanol as the major glycolytic end product, which prevents a buildup of more toxic wastes; (2) supporting glycolysis with exceptionally large glycogen stores; and (3) depressing the cellular use of ATP through increased levels of inhibitory neurotransmitters and neuromodulators in the brain and curtailing other activities. During the fall, the crucian carp will be exposed to progressive hypoxia and finally anoxia. During this initial hypoxic period, oxygen uptake is aided by an extremely high blood oxygen-binding affinity and gill remodeling to acquire a larger respiratory surface area (**see also Ventilation and Animal Respiration**: Plasticity in Gill Morphology).

Ethanol Production

Possibly the most spectacular mechanism underlying the crucian carp's impressive repertoire of adaptations for anoxia tolerance is the ability to produce ethanol as the major end product of anaerobic glycolysis. This ability is shared by the goldfish and according to one report, by another cyprinid fish, the bitterling (*Rhodeus amarus*). The main advantage of producing ethanol during anoxia is that it allows the fish to avoid a buildup of lactate and hydrogen ions, which would otherwise accumulate to very high concentrations leading to unmanageable osmotic and acid–base disturbances. The ethanol production allows the crucian carp to remain relatively active during anoxia exposure. Ethanol production, however, does present a clear energetic drawback because its release to the environment means that an energy-rich hydrocarbon is forever lost to the fish. Therefore, keeping metabolic rate low during anoxia is advantageous.

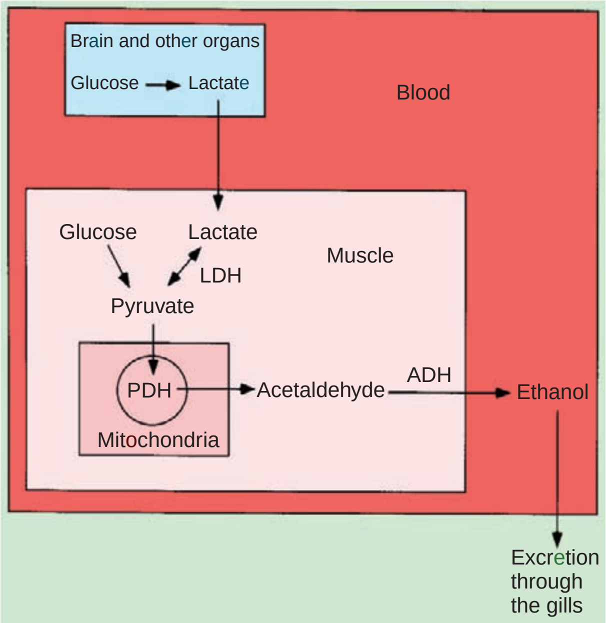


Figure 2 The ethanol-producing pathway in anoxic crucian carp that allows it to avoid lactic acidosis in anoxia. Ethanol production is confined to muscle tissue. Other organs produce lactate during anoxia. In muscle, lactate is turned into pyruvate by lactate dehydrogenase (LDH). The pyruvate is further converted to acetaldehyde by pyruvate dehydrogenase (PDH). Unlike other vertebrates, crucian carp muscle PDH appears to leak out acetaldehyde during anoxia. The acetaldehyde is turned into ethanol by alcohol dehydrogenase (ADH). Subsequently, ethanol diffuses out into the blood and finally leaves the fish over the gills.

The ethanol-producing pathway of the genus *Carassius* (**Figure 2**) is confined to the red and white skeletal muscle. Lactate produced by other tissues reaches the muscle through the blood circulation. Here, it is turned into pyruvate by the enzyme lactate dehydrogenase (LDH). Subsequently, the pyruvate is turned into acetaldehyde by one of the enzyme subunits of the pyruvate dehydrogenase (PDH) complex. The enzymes of the PDH complex normally channel substrates and intermediates from enzyme subunit to enzyme subunit, meaning that acetaldehyde is typically never released from the enzyme and is directly converted into acetyl-CoA. In *Carassius*, however, this complex must be modified to allow acetaldehyde to leak out of the PDH complex. Ethanol is finally formed from acetaldehyde by an enzyme called alcohol dehydrogenase (ADH). In crucian carp and goldfish, ADH is detected only in skeletal muscle, so all other tissues, including the brain and heart cannot make ethanol. Therefore, all tissues other than skeletal muscle produce lactate during anoxia, which they transfer into the blood. Ethanol is highly lipophilic, meaning it can readily pass through cellular membranes. Thus, ethanol produced in the muscle can easily diffuse into the blood stream and finally pass over the gills into the water. As a result, ethanol levels in anoxic crucian carp are normally below 10 mM.

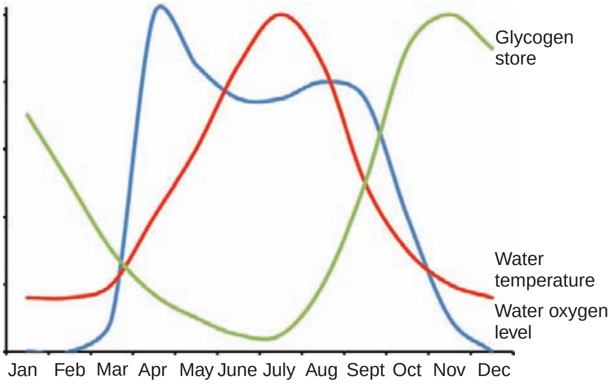


Figure 3 A normal year in the life of a crucian carp illustrated by the annual changes in the crucian carp glycogen store, shown together with changes in water temperature and oxygen level that are typical for many crucian carp habitats in Scandinavia and Finland. In this case, the crucian carp is exposed to about 2 months of anoxia from mid-December to mid-February, at which time water temperature is likely to be below 4 °C. This is also the time when most of the glycogen store is being used up. Also, note the build up of glycogen during the autumn.

To sustain long-term survival in anoxia, the crucian carp has the largest glycogen stores of any vertebrates studied and these stores peak just prior to the winter. The liver contains the largest glycogen store, and the maximal anoxic survival time depends primarily on how large the liver glycogen store is before winter. During winter anoxia, these glycogen stores are progressively depleted. In **Figure 3**, the annual changes in the crucian carp glycogen store are shown together with typical changes in water temperature and oxygen level.

Neural Depression

Although ethanol production allows the crucian carp to sustain activity during anoxia, the fish will need to suppress energy use as much as possible to avoid running out of glycogen. Under laboratory conditions, anoxic crucian carp maintain some, but limited swimming activity. They do not enter a comatose-like state as seen in anoxia-tolerant freshwater turtles which have an electrically silent brain. In goldfish, a 70% reduction in whole-body heat production occurs after 3 h in anoxia at 20 °C, indicating whole-animal metabolic depression. This is likely due to the suppression of certain metabolically demanding activities such as protein synthesis, which can be switched off or greatly turned down (**see also Hypoxia**: Anaerobic Metabolism in Fish).

In brain slices from crucian carp, anoxia results in a 30% drop in metabolic rate. Moreover, a profound suppression of the activity of the auditory nerve has been observed in anoxic goldfish. The response of the crucian carp retina (and optic tectum of the brain) to light virtually disappears in anoxic crucian carp. Thus, the crucian carp is probably temporarily deaf and blind during anoxia. Apparently, these senses are not essential for life in the cold dark anoxic water under the ice, where there are no aquatic predators, so energy is saved by shutting them down.

Heart Activity in Anoxia

In contrast to the brain, heart activity, including cardiac output, is maintained at normoxic levels during anoxia in crucian carp (**Figure 4**). Indeed, the crucian carp is the only animal known to have a heart that can function under anoxic conditions as if it was fully oxygenated. Maintenance of heart activity during anoxia is probably important for the shuttling of lactate and ethanol to and from the muscles, thereby avoiding the accumulation of ethanol and possible intoxication. It is certainly no longer needed to move oxygen! The continued function of the heart is aided by low temperature and low intrinsic energy demands.

Mechanisms of Metabolic Depression

The degree of metabolic depression in the brain of crucian carp appears to be less drastic than in turtles. At a

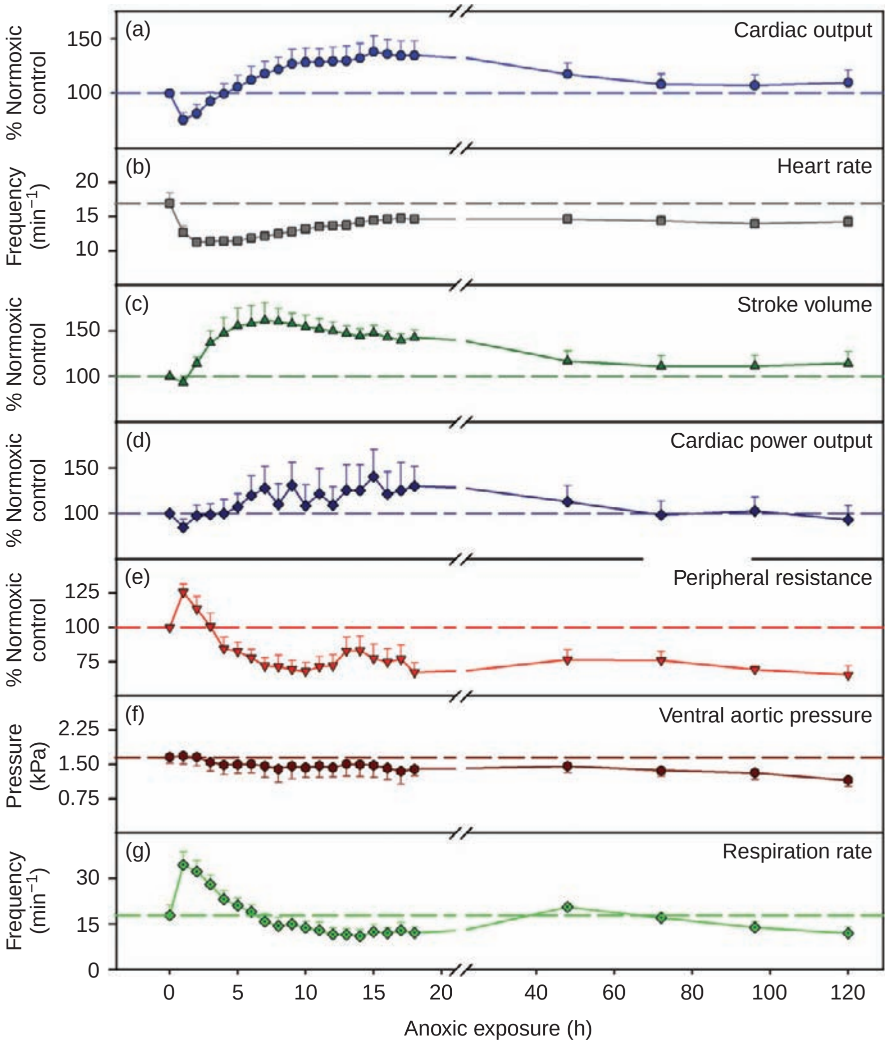


Figure 4 Changes of cardiorespiratory variables in crucian carp during 5 days of anoxia exposure at 8 °C. (a) Cardiac output, (b) heart rate, (c) stroke volume, (d) cardiac power output, (e) peripheral resistance, (f) ventral aortic pressure, and (g) respiration rate. Dashed lines indicate the control normoxic level for each measured parameter. For cardiac variables, significant differences (asterisks) are only indicated between normoxic control (time zero) and hours (48, 72, 96, and 120). For respiration (ventilation) rate, all significant differences from the normoxic control are indicated. Values are means ± SEM from 6 to 18 fish. From Stecyk JAW *et al.* (2004) Maintained cardiac pumping in anoxic crucian carp. *Science* 306: 77.

mechanistic level, the activity of Na^+/K^+-ATPase, the most energy-consuming enzyme in brain, is maintained in crucian carp brain, while it is suppressed in the brain of turtles. The sustained metabolic activity of the crucian carp brain during anoxia at 10 °C is supported by a sustained doubling in brain blood flow, which is probably needed to support the high glucose requirements of the brain when glycolysis is the only available route for ATP production.

A major difference between the crucian carp and turtles appears to be their utilization of reduced ion-channel conductivity (channel arrest) during anoxia. While reduced channel conductivity is thought to play a major role in depressing neural activity in turtles during anoxia, there is little evidence for such mechanisms in the anoxic crucian carp. Here, brain K^+ permeability appears to be unaffected by anoxia, and in crucian carp brain slices there is a lack of change in Ca^{2+} permeability, at least during the initial hours of anoxia. One exception is probably the *N*-methyl-D-aspartate (NMDA) receptor – a major excitatory glutamate receptor. While the expressions of most excitatory neurotransmitter receptors and ion channels are quite stable in the anoxic crucian carp brain, mRNA levels of some NMDA receptor subunits, including the ubiquitous NR1 subunit, fall by about 50% after 7 days of anoxia at 10 °C. Similarly, experiments using patch-clamp techniques in telencephalic brain slices from goldfish show that 40 min of anoxia causes a 40–50% fall in the NMDA receptor activity.

Protein synthesis rates are often depressed in response to hypoxia/anoxia exposure in many fish species and tissues. In the crucian carp brain, however, protein synthesis is maintained unlike the strong reduction in protein synthesis in the turtle brain. All the same, there is a 95% suppression of protein synthesis in the liver and a 50% fall in muscle and heart tissue of anoxic crucian carp, suggesting that reduced protein synthesis contributes significantly to their whole-body metabolic depression (**Figure 5**).

The most important mechanisms for reducing brain energy use in crucian carp involve neurotransmitters and neuromodulators. Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain and it is the target of many neurosuppressive drugs such as anesthetics and tranquilizers. Microdialysis measurements of fluids in the brain show that the extracellular GABA concentration doubles in the crucian carp telencephalon after 6 h of anoxia. This is a relatively modest change compared to the 80-fold increase in extracellular (GABA) that has been measured in the anoxic turtle brain, suggesting that GABA acts more like a tranquilizer than an anesthetic in the anoxic crucian carp. Moreover, there is a relatively large individual variation in how the brain GABA concentration changes during anoxia in crucian carp, ranging from a sixfold increase to no change after 6 h of anoxia exposure. However, these measurements had to be done on anesthetized fish so the GABA upregulation in awake animals could be different, possibly higher. Nevertheless, the neurosuppression brought about by the elevated GABA levels in the crucian carp brain could mediate significant energy savings especially for a tissue that has limited glycogen storage capacity. Treatment of crucian carp with either inhibitors (isoniazid or 3-mercaptopropionic acid) of the GABA-synthesizing enzyme glutamate decarboxylase or a blocker of $GABA_A$ receptors (securinine) causes the fish to release up to 3 times more ethanol to the water during anoxia (**Figure 6**), suggesting that GABA mediates a considerable inhibition of energy use also on the whole-body level.

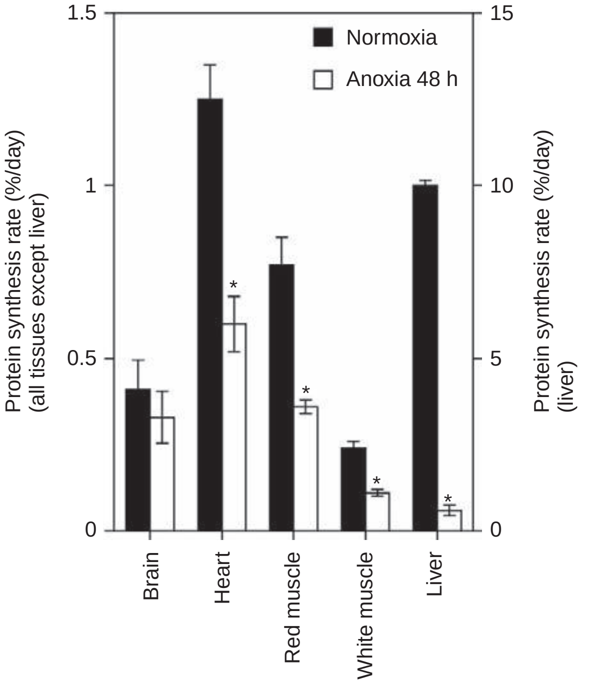


Figure 5 Rates of protein synthesis *in vivo* in tissues of crucian carp exposed to anoxia for 48 h at 9 °C. Values are means ± SEM from 12 to 16 fish. From Smith RW *et al.* (1996) Tissue specific changes in protein synthesis rates *in vivo* during anoxia in crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 271: R897–R904.

Also, the neuromodulator adenosine is probably involved in metabolic depression in crucian carp. Similar to the effect of inhibiting the GABAergic system, blocking adenosine receptors pharmacologically with a drug called aminophylline causes anoxic crucian carp to release 3 times more ethanol to the water. Moreover, the anoxia-induced increase in cerebral blood flow in crucian carp can be blocked by aminophylline treatment, suggesting that adenosine not only suppresses ATP use but simultaneously promotes ATP production also by stimulating glucose delivery to the brain, thereby helping to balance the ATP budget during anoxia. Microdialysis experiments have so far failed to detect an increase in

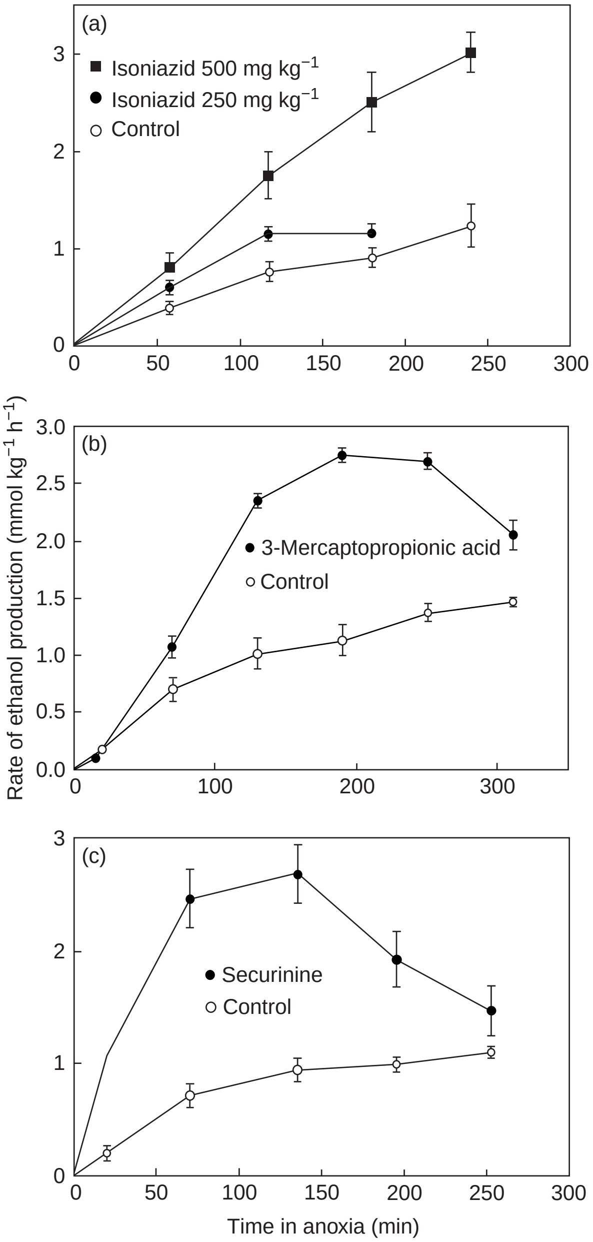


Figure 6 Evidence for a role of GABA in metabolic depression, as suggested by the increased ethanol release to the ambient water casued by three different anti-GABAergic agents. Isoniazid (250–500 mg kg^{-1} i.p.) and 3-mercaptopropionic acid (200 mg kg^{-1} i.p.) both blocks the GABA-synthesizing enzyme glutamate decarboxylase. 3-mercaptopropionic acid also inhibits neuronal GABA release. Securinine (20 mg kg^{-1} i.p.) blocks GABA$_A$ receptors. Experiments were done at 18 °C. Values are means ± SEM from 4 to 6 fish. From Nilsson GE (1992) Evidence for a role of GABA in metabolic depression during anoxia in crucian carp (*Carassius carassius* L.). *Journal of Experimental Biology* 164: 243–259.

extracellular adenosine in the brain of anoxic crucian carp. However, in freshwater turtles a rapid but temporary 10-fold rise in the extracellular adenosine level, linked to the simultaneous fall in ATP, has been measured during the early part of anoxia exposure.

Strategies to Avoid Tissue Anoxia

As mentioned, surviving anoxia by producing ATP glycolytically is energetically very expensive for the crucian carp as an energy-rich hydrocarbon, ethanol, is lost to the environment and wasted. If the entry into anoxia can be delayed, thereby reducing the time spent surviving on glycolysis alone, then the chance of the glycogen stores lasting all through the winter will be improved.

In its natural habitat, anoxia does not appear suddenly, but rather it is preceded by a long period of falling oxygen levels. The crucian carp has evolved some striking physiological adaptations that maximize oxygen uptake during severe hypoxia. First, crucian carp hemoglobins have the highest oxygen-binding affinity measured in any vertebrate ($P_{50} = 0.8$ mmHg at 10 °C). This will obviously aid in oxygen uptake during hypoxia. The high blood oxygen affinity is most likely a prerequisite for another striking adaptation to hypoxia: the ability to change the gross morphology of the gills to match its oxygen need (**see also Ventilation and Animal Respiration**: Plasticity in Gill Morphology). In cold normoxic water, the high blood oxygen affinity allows the crucian carp to have gills that lack protruding secondary lamellae (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy and **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics), possibly allowing it to reduce the energy spent on osmoregulation (**see also Role of the Gills**: The Osmorespiratory Compromise). When exposed to hypoxia, it remodels its gills by reducing the intralamellar tissue mass, which results in a sevenfold increase in the respiratory surface area. This should greatly aid its ability to take up oxygen during hypoxic periods. Interestingly, normoxic crucian carp that are directly transferred to anoxic water do not remodel their gills. This makes sense as there is no oxygen to take up and it remains to be shown if the crucian carp actually go from having gills with protruding lamellae to gills without protruding lamellae when it goes from severe hypoxia to true anoxia.

Conclusions

When the crucian carp is exposed to severe hypoxia, and even anoxia, it has a better chance of survival than probably any other fish, the reason being a set of striking physiological and biochemical adaptations. In the small ponds it inhabits, wintertime anoxia will be preceded by hypoxia. During hypoxia, it is extremely good at taking up the little oxygen there in the water by having an unusually high blood oxygen affinity and by remodeling its gills to boost the respiratory surface area. When the water becomes so depleted of oxygen that it needs to produce ATP through anaerobic glycolysis. The lactate it produces is turned into ethanol, allowing it to avoid a lactic acidosis, which is the fate of nearly all other vertebrates in anoxia. To do this, lactate produced by organs such as the brain and heart is transported to muscle, where the ethanol-producing pathway is confined. The ethanol is subsequently released to the water over the gill epithelium. Its anaerobic ATP production is supported by the largest glycogen stores found in vertebrates, which, when combined with cellular metabolic depression, is sufficient to allow anoxic survival for months at low temperatures. Even so, the anoxic crucian carp maintains some physical and neural activity in anoxia, unlike anoxia-tolerant freshwater turtles. Brain Na^+/K^+-ATPase activity and neural ion permeability remain unchanged, but there is probably a downregulation of glutamatergic NMDA receptors. The major contributors to its metabolic depression are probably inhibitory neurotransmitters and neuromodulators such as GABA and adenosine. The crucian carp is the only vertebrate known to maintain a high cardiac activity during anoxia. A maintained cardiac output is probably needed to ensure sufficient glucose delivery to tissues and ethanol release to the water, thereby avoiding self-intoxication. Thus, while this animal can survive months of anoxia, it can probably not tolerate being drunk the whole time.

See also: **Design and Physiology of Arteries and Veins**: Branchial Anatomy. **Hypoxia**: Anaerobic Metabolism in Fish. **Role of the Gills**: The Osmorespiratory Compromise. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics; Plasticity in Gill Morphology.

Further Reading

Brönmark C and Milner JG (1992) Predator-induced phenotypical change in body morphology in crucian carp. *Science* 258: 1348–1350.

Ellefsen S, Sandvik GK, Larsen HK, *et al.* (2008) Expression of genes involved in excitatory neurotransmission in anoxic crucian carp brain (*Carassius carassius*). *Physiological Genomics* 35: 5–17.

Hylland P, Milton S, Pek M, Nilsson GE, and Lutz PL (1997) Brain Na^+/K^+-ATPase activity in two anoxia tolerant vertebrates: crucian carp and freshwater turtle. *Neuroscience Letters* 235: 89–92.

Hylland P and Nilsson GE (1999) Extracellular levels of amino acid neurotransmitters during anoxia and forced energy deficiency in crucian carp brain. *Brain Research* 823: 49–58.

Johansson D, Nilsson GE, and Døving KB (1997) Anoxic depression of light-evoked potentials in retina and optic tectum of crucian carp. *Neuroscience Letters* 237: 73–76.

Johansson D, Nilsson GE, and Törnblom E (1995) Effects of anoxia on energy metabolism in crucian carp brain slices studied with microcalorimetry. *Journal of Experimental Biology* 198: 853–859.

Lutz PL, Nilsson GE, and Prentice H (2003) *The Brain Without Oxygen*, 3rd edn. Dordrecht: Kluwer Academic.

Nilsson GE (1988) A comparative study of aldehyde dehydrogenase and alcohol dehydrogenase activity in crucian carp and three other vertebrates: apparent adaptations to ethanol production. *Journal of Comparative Physiology B* 158: 479–485.

Nilsson GE (1990) Long term anoxia in crucian carp: changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *Journal of Experimental Biology* 150: 295–320.

Nilsson GE (1992) Evidence for a role of GABA in metabolic depression during anoxia in crucian carp (*Carassius carassius* L.). *Journal of Experimental Biology* 164: 243–259.

Nilsson GE (2001) Surviving anoxia with the brain turned on. *News in Physiological Sciences* 16: 218–221.

Shoubridge EA and Hochachka PW (1980) Ethanol: novel end product of vertebrate anaerobic metabolism. *Science* 209: 308–309.

Smith RW, Houlihan DF, Nilsson GE, and Brechin JG (1996) Tissue specific changes in protein synthesis rates *in vivo* during anoxia in crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 271: R897–R904.

Sollid J, Weber RE, and Nilsson GE (2005) Temperature alters the respiratory surface area of crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*). *Journal of Experimental Biology* 208: 1109–1116.

Stecyk JAW, Stensløkken K-O, Farrell AP, and Nilsson GE (2004) Maintained cardiac pumping in anoxic crucian carp. *Science* 306: 77.

Vornanen M (1994) Seasonal adaptation of crucian carp (*Carassius carassius* L.) heart: glycogen stores and lactate dehydrogenase activity. *Canadian Journal of Zoology* 72: 433–442.

Vornanen M and Paajanen V (2006) Seasonal changes in glycogen content and Na^+–K^+-ATPase activity in the brain of crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 291: R1482–R1489.

Vornanen M, Stecyk JAW, and Nilsson GE (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Richards JG, Farrell AP, and Brauner CJ (eds.) *Fish Physiology Vol. 27. Hypoxia,* ch. 9, pp. 397–441. Academic Press.

Wilkie MP, Pamenter ME, Alkabie S, *et al.* (2008) Evidence of anoxia-induced channel arrest in the brain of the goldfish (*Carassius auratus*). *Comparative Biochemistry and Physiology C* 148: 355–362.

Wissing J and Zebe E (1988) The anaerobic metabolism of the bitterling *Rhodeus amarus* (Cyprinidae, Teleostei). *Comparative Biochemistry and Physiology B* 89: 299–303.

Life in Hot Water: The Desert Pupfish

SD Hillyard, UNLV School of Dental Medicine, Las Vegas, NV, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Habitats
Physiological Studies of Desert Pupfish
Conservation
Further Reading

Glossary

Clade A group consisting of a single common ancestor and all its descendants.

Hypoxia Low partial pressures of oxygen in external or internal environments.

Phenotypic plasticity Variability in the form or function of an organism within its genetic makeup.

Primary production The total amount of carbon fixed into biomass by photosynthetic organisms.

Salinity Number of grams of salt per kilogram of water.

Introduction

New World pupfish in the genus *Cyprinodon* are classified into five distinct species groups or clades that are believed to have diverged from a common ancestor 6–7 million years ago (Mya). Periods of heavy rainfall and tectonic shifts in the landscape of southwestern North America produced a network of large lakes and interconnecting streams that allowed dispersal of pupfish, and by 3.6–3.8 Mya one of these clades appeared in the Death Valley system that includes the present-day Owens Valley, Amargosa River, Death Valley, and Ash Meadows (**Figure 1**). Climate change over the past 10 000–20 000 years has caused the lakes to dry up, the streams to become intermittent, and has resulted in isolated populations in the remaining springs and pools (**Figure 2**).

Habitats

Few fish are known to live at such high-temperature extremes as the pupfish. Water temperatures in the individual springs and pools can be relatively constant and range from approximately 28 to 34 °C, while spring outflows and associated marshes are subject to extreme environmental temperatures that range from near freezing in the winter to over 40 °C in the summer. Below is a summary of desert pupfish species and their habitats.

Cyprinodon radiosus, the Owens Valley pupfish, was historically widespread in the region and used as a food source by Native Americans as recently as the nineteenth century. They are found in 33 °C springs and have been seen under the ice during the winter.

The desert pupfish, *Cyprinodon macularius*, was historically found along the Colorado River and is closely related to *C. radiosus*, indicating a historical connection between the Owens River and Colorado River drainages. Current populations are confined to isolated springs, including those that drain into the Salton Sea in southern California where they encounter temperatures in excess of 40 °C and salinities in excess of seawater.

The Salt Creek pupfish, *Cyprinodon salinus*, is only found in Salt Creek, in the floor of Death Valley. Salt Creek originates from McClain springs with a temperature that varies seasonally between 15 and 30 °C and has a salinity of 15–20 parts per thousand (ppt; seawater is 33–34 ppt) (**Figure 3**). Downstream flows are variable and water temperatures in excess of 40 °C have been reported in the summer with salinities in excess of seawater (**Figures 4** and **5**). Winter temperatures, in contrast, have been reported to be near freezing. A subspecies, *C. salinus milleri*, is found in nearby Cottonball Marsh, so named because of white crusts of salt surrounding the water, which has a salinity of 45 ppt (**Figures 6** and **7**). Air temperatures near Cottonball Marsh have been reported in excess of 55 °C, the hottest in North America, but water temperatures are cooler, ranging from 23 to 36 °C during the course of one day in August when measurements were made. The other habitats are essentially freshwater and with the exception of *Cyprinodon diabolis* are inhabited by subspecies of *Cyprinodon nevadensis*.

The Saratoga Springs pupfish, *Cyprinodon nevadensis nevadensis*, are found in Saratoga Springs at the southern

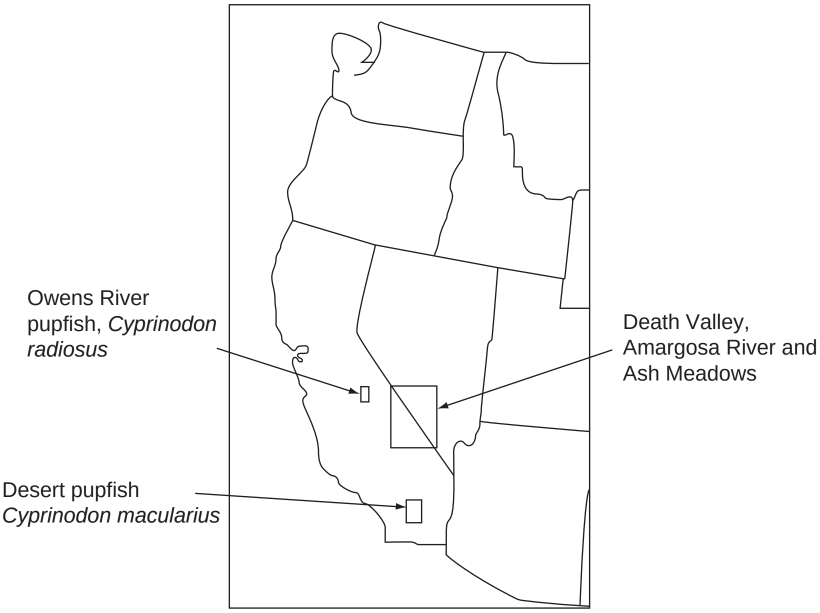

Owens River pupfish, *Cyprinodon radiosus*
Death Valley, Amargosa River and Ash Meadows
Desert pupfish *Cyprinodon macularius*

Figure 1 Location of habitats occupied by pupfish species in the Death Valley Amarogosa River and Ash Meadows region. *Cyprinodon macularius* are found in saline springs near the Salton Sea. The Owens River pupfish, *C. radiosus*, is closely related to *C. macularius*, suggesting a common historical connection with the Colorado River drainage.

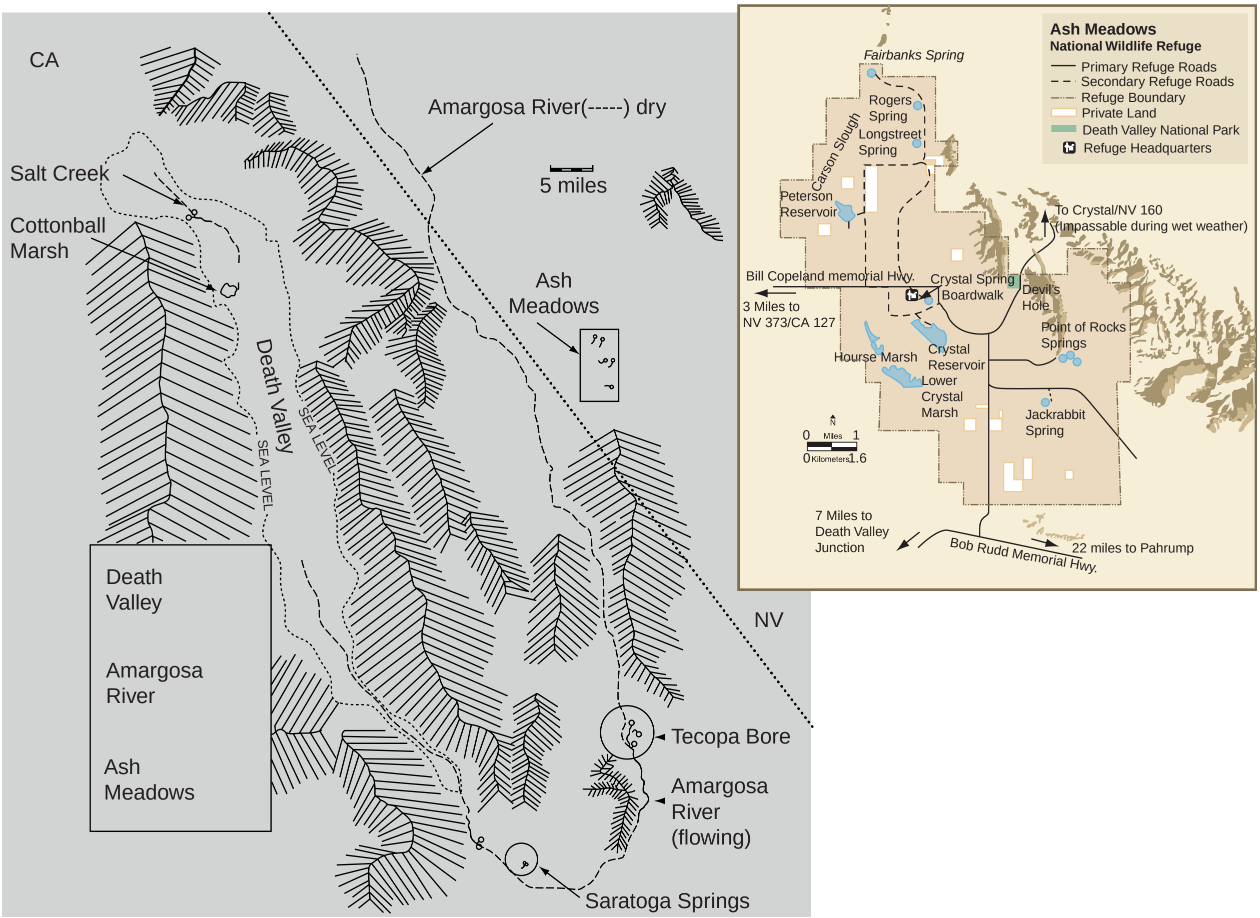


Figure 2 Details of Death Valley, Amargosa River system with inset showing springs in Ash Meadows National Wildlife Refuge. From Brown JH and Feldmeth CR (1971) Evolution in constant and fluctuating environments: Thermal tolerances of desert pupfish (*Cyprinodon*). *Evolution* 25: 390–398.

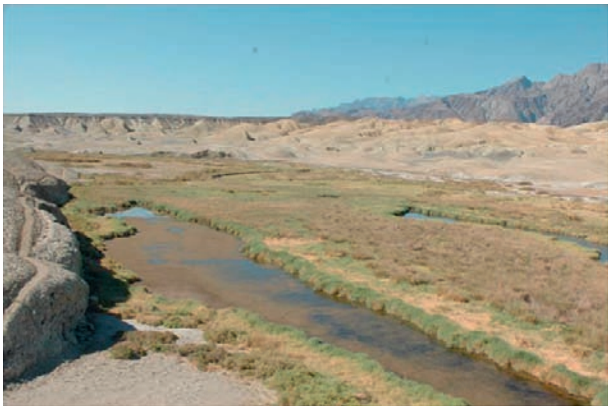


Figure 3 McClain Springs, headwater for Salt Creek, has a continuous flow of water having a salinity of about 15 ppt. Photo courtesy of the US National Park Service.

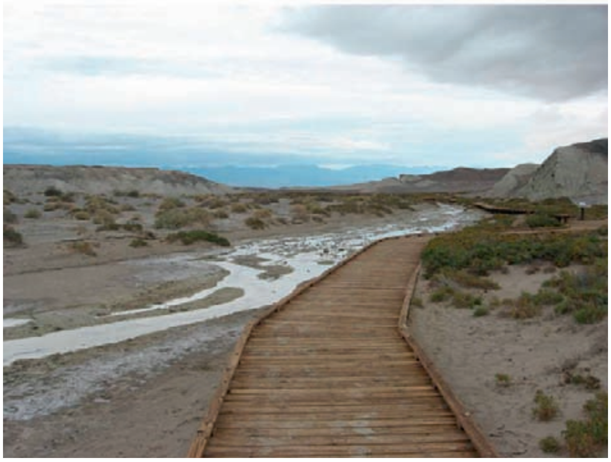


Figure 4 Downstream flow in Salt Creek declines during the summer creating isolated pools with high temperature and salinity. Photo courtesy of Frank van Breukelen.

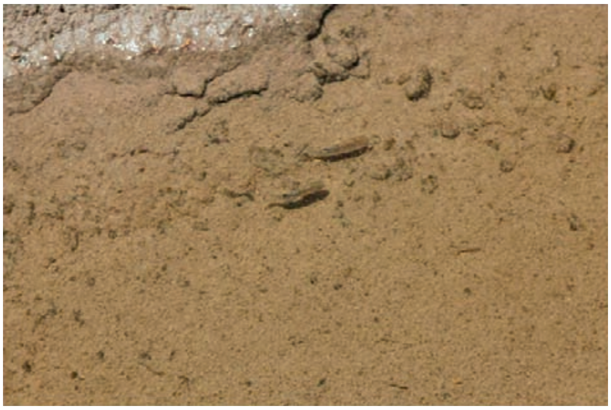


Figure 5 *Cyprinodon salinus* in a shallow pool bordered by crystallizing salt accumulated at the water's edge. Photo courtesy of Frank van Breukelen.

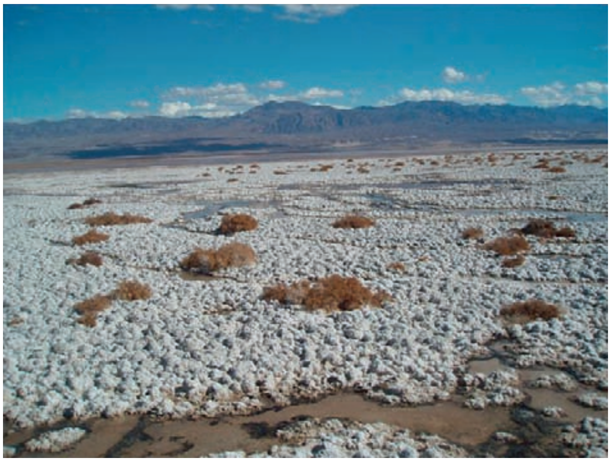


Figure 6 Cotton Ball Marsh is named for the salt deposits surrounding the spring flow. It is located near Furnace Creek in Death Valley, the hottest place in North America. Photo courtesy of the US National Park Service.

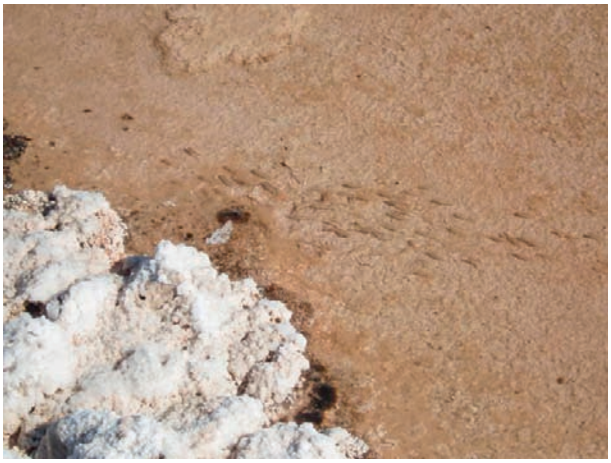


Figure 7 School of *C. salinus milleri* near salt deposit on the shore of Cottonball Marsh. Photo courtesy of the US National Park Service.

edge of Death Valley, which provides a relatively constant headwater with cooler temperatures that average 27 °C (**Figures 8** and **9**). However, the springs flow into a downstream marsh that may have temperatures in excess of 40 °C.

The Amargosa River pupfish, *Cyprinodon nevadensis amargosae*, are found in the short sections where there is flow in the Amargosa River and in hot springs near Tecopa, California. One spring, Tecopa Bore, was formed by humans drilling into the water table and has water temperatures as high as 47 °C at the headwaters. Water temperature cools as the outflow reaches downstream marshes and most fish live in water between 28 and 34 °C. Brief sections of river that flow continuously have annual variation in temperature that may be over 40 °C in the summer and near freezing in the winter.

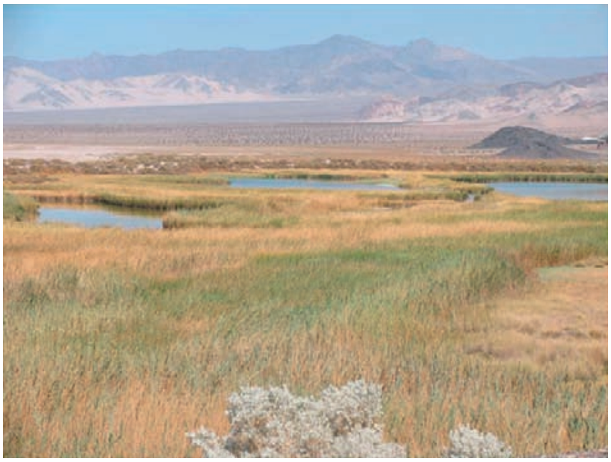


Figure 8 Saratoga Springs at the southern border of Death Valley is home to *C. nevadensis nevadensis*. Spring flow water temperature is relatively constant but downstream marshes have seasonal and daily temperature fluctuations. Photo courtesy of Frank van Breukelen.

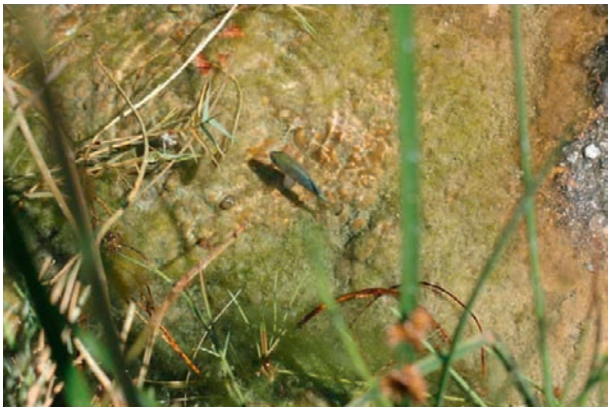


Figure 9 *Cyprinodon nevadensis nevadensis* in a shallow corner of Saratoga Springs. Photo courtesy of Frank van Breukelen.

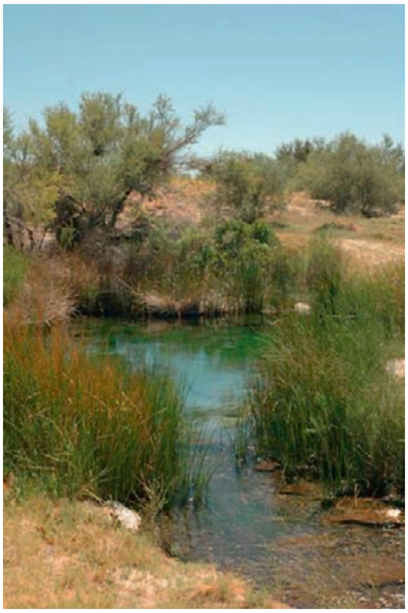


Figure 10 King's Pool at Point of Rocks in Ash Meadows, one of several springs home to *C. nevadensis mionectes*. The spring maintains a relatively constant temperature of 33–34 °C. Photo courtesy of the US National Park Service.

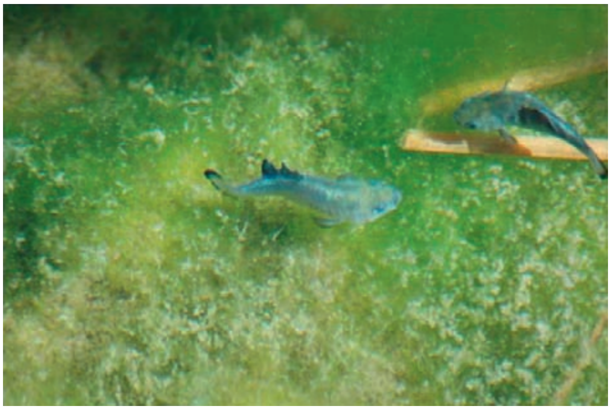


Figure 11 *Cyprinodon nevadensis mionectes* in King's Pool. Note the rich algal growth. Photo courtesy of Frank van Breukelen.

Two species of pupfish, the Ash Meadows pupfish, *C. nevadensis mionectes*, and the Warm Springs pupfish, *C. nevadensis pectoralis*, are found in Ash Meadows, which is a system of springs and pools that are fed by a large carbonate aquifer (**Figures 10** and **11**). Temperatures in the springs are relatively constant and vary among the springs from about 26 to over 33 °C.

The most unique habitat in Ash Meadows is Devils Hole, an opening into the aquifer that is believed to have resulted when the roof over a subterranean pool collapsed about 60 000 years ago (**Figure 12**). At some point after the collapse, the Devils Hole pupfish, *C. diabolis*, colonized the pool whose water level currently lies 15 m below the surrounding desert such that direct sunlight does not reach the water during the winter months, late November to early February, when air temperatures may be near freezing. Sunlight reaches the water for the remainder of the year, the most intense being in the summer when air temperatures routinely exceed 45 °C. Despite these variations, the main pool has a remarkably stable temperature of 33–34 °C. However, reproduction occurs on a shallow ledge where daily and seasonal variation in temperature and solar energy input result in water temperatures that range from <30 °C in the winter to >35 °C in the summer (**Figures 13** and **14**). The seasonal fluctuation in sunlight also limits primary production in Devils Hole and a large fraction of the total carbon input is from external sources, such as blown organic matter and matter washed in by rainfall.

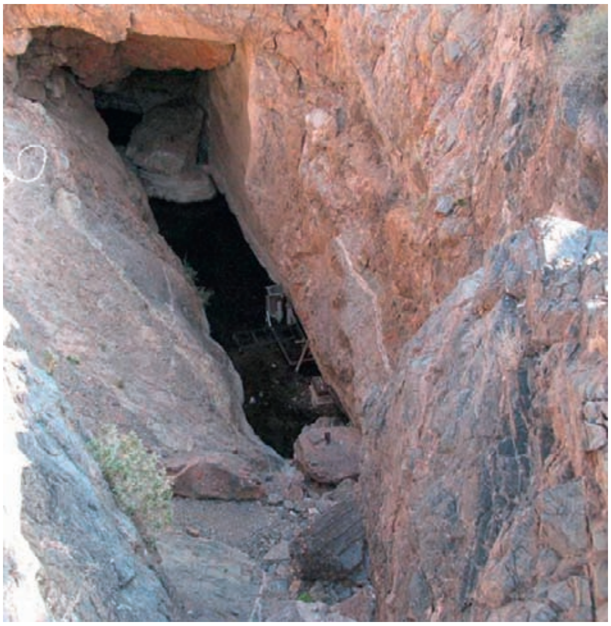


Figure 12 Devils Hole seen from the visitor platform. The water level is 15 m below the surrounding desert. Photo courtesy of the US National Park Service.

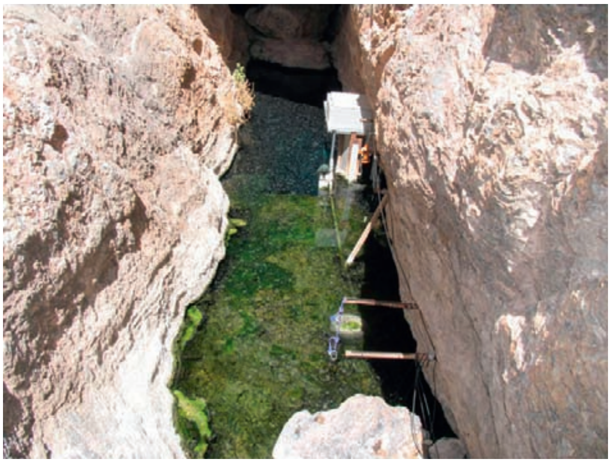


Figure 13 Closer view of Devils Hole shows algal growth on the shallow breeding ledge during times when sunlight reaches the water. Photo courtesy of the US National Park Service.

The population is greatest in the fall following breeding and recruitment that occurs in the spring and summer, while it is minimal in the spring following the dark winter months (**Figure 15**). Between 1969 and 1973, agricultural pumping drew the water level down, exposing the breeding ledge and causing a decline in the population. After a prolonged legal battle and favorable judgment by the Unites States Supreme Court, pumping was halted, the water level rose, and the population recovered to maximal levels of over 500 fish in the mid-1990s. The population then mysteriously declined until by the spring of 2006 only 38 fish were counted. In the spring of 2008, the minimum had stabilized to 45 and increased to 126 fish in the fall. A variety of hypotheses have been proposed to account for the decline, including energy limitation, thermal stress, and hypoxia. The degree that these factors, alone or in concert, contributed to the decline remains unknown. However, physiological measurements on pupfish species provide insight into the capacity of *C. diabolis* and other pupfish species to cope with stressful habitats.

Physiological Studies of Desert Pupfish

Critical Thermal Maxima and Minima

A common method for making species comparisons of thermal tolerance is the critical thermal maximum (CTMax), which is determined by placing fish in a cooler aquarium and heating the water, approximately 2 °C per minute until some marker of stress is observed, such as the inability to swim normally. The CTMax values in the literature are variable according to the rate of temperature rise and the chosen end point. When comparisons are made using the same criteria, CTMax values from species taken directly from warmer thermally stable habitats have been shown to be greater than those from cooler springs (**Figure 16**). Species that inhabit seasonally variable habitats show considerable variability in CTMax: the mean value for CTMax of *C. nevadensis amargosae* from the Amargosa River averages less than 39 °C during the winter but is near 42 °C in the spring. Laboratory experiments have similarly shown that species, regardless of native habitat, acclimated to warmer temperatures have a higher CTMax (**Figure 17**). The overall conclusion is that desert pupfish as a group display considerable plasticity in their ability to acclimate to a wide range of temperatures.

Winter temperatures in the deserts are often near or below freezing and cool temperatures may also impair physiological function. Critical thermal minima (CTMin) can be determined by cooling aquaria until coordinated swimming activity is impaired. CTMin are generally raised when pupfish are acclimated to warmer temperatures, that is, raising CTMax produces a corresponding rise in CTMin. However, when fish are acclimated to cycling temperatures that reflect minimal and maximal levels in the habitat, CTMin is lowered to give a broader scope for thermal tolerance (**Figure 18**).

Evidence has been obtained for genetic differences that may contribute to the broader scope for thermal tolerances in subspecies inhabiting thermally fluctuating habitats. *Cyprinodon nevadensis mionectes* collected from a stable 27 °C spring in Ash Meadows, and *C. nevadensis amargosae* from the more variable Amargosa River, were bred in the laboratory and first- and second-generation offspring for

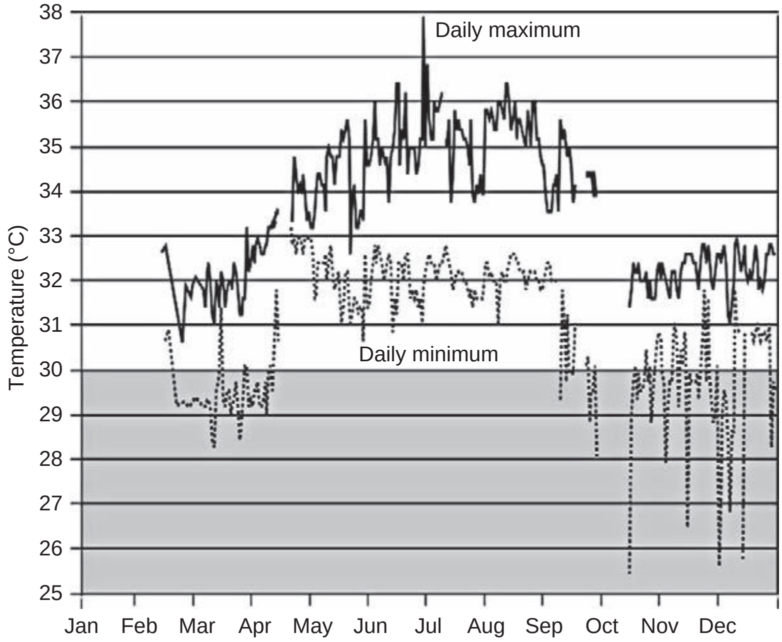


Figure 14 Temperature fluctuations on the breeding ledge recorded over a 1-year cycle in 2001. Redrawn from Riggs AC and Deacon JE (2002) Connectivity in desert aquatic ecosystems: The Devils Hole story. In: Sada DW and Sharpe SE (eds.) *Conference Proceedings. Spring-Fed Wetlands: Important Scientific and Cultural Resources of the Intermountain Region*, pp. 1–38. Las Vegas, NV, 7–9 May, 2002.

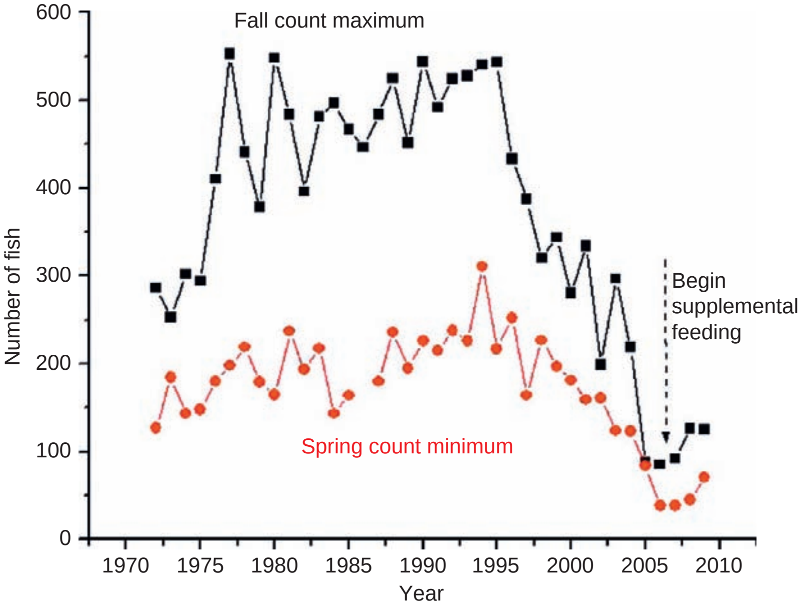


Figure 15 Maximal and minimal population estimates for *C. diabolis* based on surface counts from the breeding ledge and underwater SCUBA counts in the deeper water. Plotted from data provided by the US National Park Service.

both subspecies were maintained in the laboratory under constant (25–27 °C) temperatures. When subject to higher and lower temperatures, CTMax was greater (~41.9 °C vs. 41.6 °C) and CTMin was lower (~2.4 °C vs. 2.7 °C) in *C. nevadensis amargosae*. These small differences were statistically different suggesting selective advantage for fish in the more variable habitat.

Thermal Limits to Reproduction

CTMax and CTMin studies are generally done with adults to reflect the capacity to survive extreme temperatures. This may be advantageous for individual survival, for example, *C. nevadensis amargosae* in Tecopa Bore have been observed to move rapidly to feed on algae at

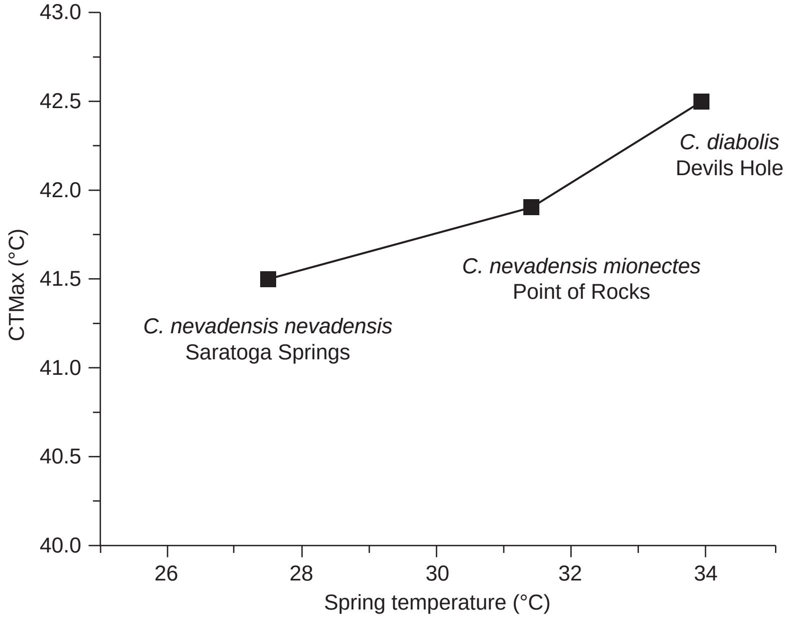


Figure 16 CTMax values are greater for species taken directly from springs having progressively warmer temperatures. Data from Brown JH and Feldmeth CR (1971) Evolution in constant and fluctuating environments: Thermal tolerances of desert pupfish (*Cyprinodon*). *Evolution* 25: 390–398.

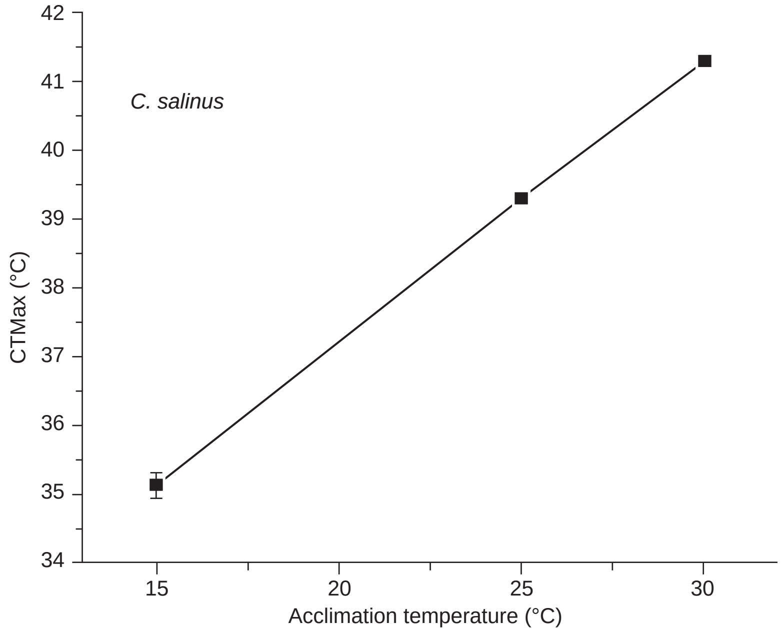


Figure 17 CTMax, shown here for *C. salinus*, increases when fish are acclimated to warmer temperatures. Data from Stuenkel EL and Hillyard SD (1981) The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish *Cyprinodon salinus*. *Copeia* 1981: 411–417.

temperatures above their CTMax before returning to cooler water. However, reproductive capacity and recruitment of young into the population are more sensitive to temperature extremes than the adults. In this regard, oogenesis appears to be the most thermally sensitive stage in pupfish reproduction. The number of eggs per spawning by *C. nevadensis nevadensis* bred in the laboratory is maximal when the fish are maintained at constant temperatures between 24 and 32 °C or fluctuating temperature regimes of 28–32 °C or 28–36 °C. Spawning activity was greatest over a slightly wider range (~24–34 °C). Hatching of fertilized eggs exceeded 50% at temperatures between 20 and

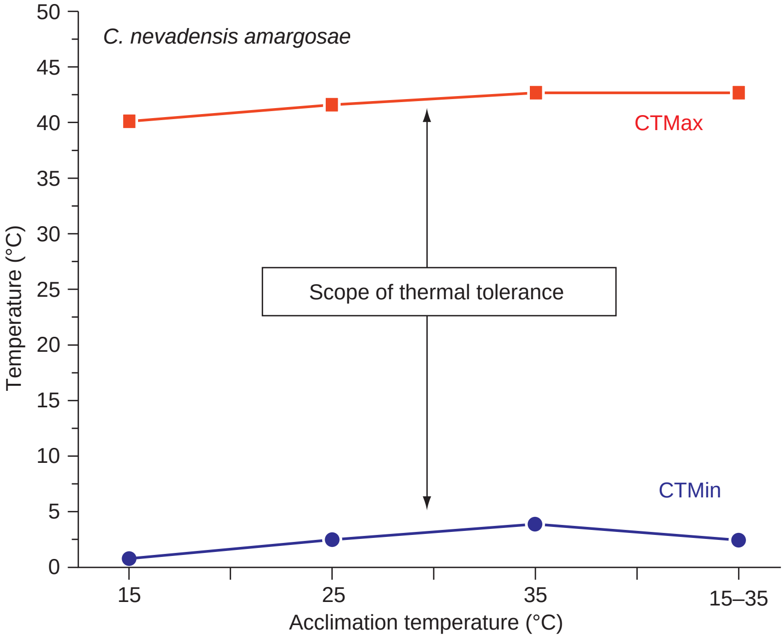


Figure 18 Increasing critical thermal maxima by warm acclimation also increases the critical thermal minima. Acclimation to fluctuating temperatures increases critical thermal maxima without raising CTMin. Data from Feldmeth CR, Stone EA, and Brown JH (1974) An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon*) to cycling temperatures. *Journal of Comparative Physiology* 89: 39–44.

36 °C, while the CTMax for newly hatched fish acclimated to 36 °C was about 44 °C, similar to that of adults.

The temperature of the main pool at Devils Hole (33–34 °C) is above the upper limit for maximal oogenesis in Saratoga Springs' pupfish. The number of eggs released per spawning is similar to that for *C. nevadensis nevadensis* at 34 °C. The greatest number of mature oocytes in female *C. diabolis* and the greatest number of larval fish (≤5 mm length) occur between early February and late May when lower ambient temperatures, cool shallow water on the breeding ledge, and sunlight begins to reach the water to increase primary production and photosynthetic oxygen production.

Metabolism

Metabolic rates, like other chemical reactions, increase with increasing temperature. This is typically evaluated as

$$Q_{10} = (R_2/R_1)^{(10/T_2 - T_1)}$$

where R_2 and R_1 are rates at increasing temperatures $T_2 > T_1$. For a 10 °C rise in temperatures, Q_{10} values for biological rates are typically about 2–3 indicating a doubling or tripling of rate (**see also Temperature**: Effects of Temperature: An Introduction). Thus, even a 1°C rise in temperature will produce a 20–30% rise in metabolic rate. An important question for desert pupfish conservation is how close to the edge are they living and how might even small increases in temperature associated with climate change approach or exceed this limit. Increasing rates for maintenance metabolism will clearly limit the energy available for growth and reproduction illustrating why reproduction is sensitive to even small increases in temperature.

The temperature sensitivity of metabolic rate, measured as oxygen consumption, can be seen for the Salt Creek pupfish, *C. salinus*. Lower rates are observed at higher acclimation temperatures to compensate for the thermal effect on metabolic reactions (**Figure 19**). Increasing temperature also reduces oxygen solubility. Thus, fish will need to pump more water over the respiratory surfaces and require additional energy expenditure for maintenance levels of metabolic function (**Figure 20**).

Desert pupfish living in stable flowing springs generally have well-aerated water and a rich supply of photosynthetic algae. Fish in more variable habitats may encounter a wider range of oxygen levels in addition to thermal fluctuation. Offspring of Ash Meadows pupfish, *C. nevadensis mionectes*, collected from a stable spring are less tolerant of hypoxic conditions (1.00–1.45 ppm) than the offspring of *C. nevadensis amargosae* collected from areas of the Amargosa River that become hypoxic during periods of low flow and elevated temperature during the summer. These results were obtained by placing fish in a hypoxic aquarium and measuring the time before all the fish in the experimental groups became disoriented, at a temperature

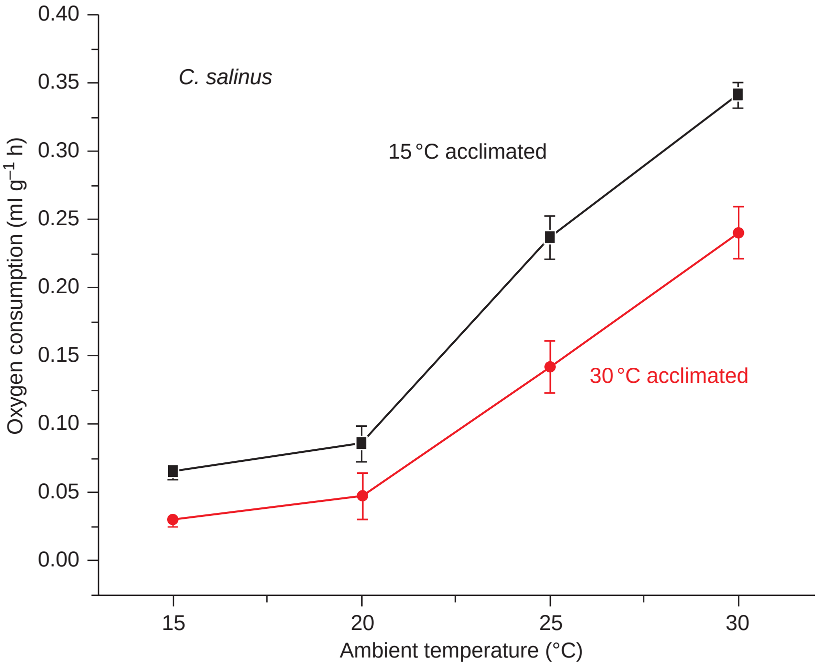


Figure 19 Metabolic rate, measured as oxygen consumption, increases with increasing temperature but is lower in fish acclimated to warmer temperatures to compensate for the thermal effect on biochemical rates. Redrawn from tabular data in Stuenkel EL and Hillyard SD (1981) The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish *Cyprinodon salinus. Copeia* 1981: 411–417.

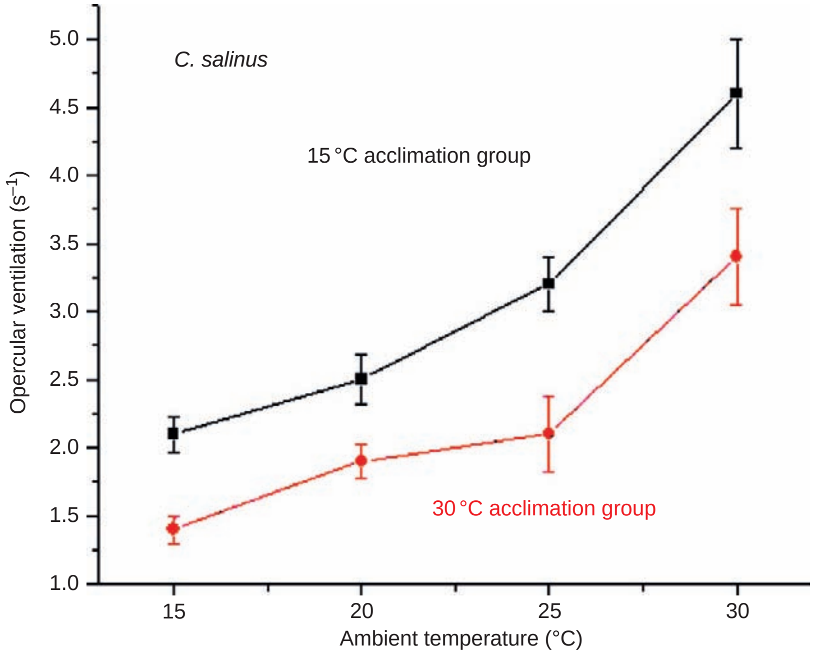


Figure 20 The rate of opercular ventilation, opercular beats per second, increases with increasing temperature to support increased metabolic rate. As with metabolic rate, opercular ventilation is lower in fish acclimated to a higher temperature. Redrawn from tabular data in Stuenkel EL and Hillyard SD (1981) The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish *Cyprinodon salinus. Copeia* 1981: 411–417.

well below the CTMax. The time course for the loss of orientation was in the order of minutes. It is obvious that a level of aerobic metabolism sufficient to maintain reproductive activity and stable population size will require greater oxygen levels, especially when the temperature approaches the upper limit for normal oogenesis.

Devils Hole presents an especially challenging combination of high temperature and low oxygen levels. Except for a west-facing wall outside of the breeding ledge, direct sunlight seldom hits the deeper levels and oxygen levels are approximately 2.3–2.4 ppm. The oxygen content of air-saturated water at the ambient altitude (~800 m) and temperature (33 °C) of Devils Hole is approximately 7 ppm and near saturation levels occur on and around the breeding ledge during the day in months when photosynthetic algae receive direct sunlight (**Figure 21**). At night and during the winter months, the saturation level declines to values similar to that of the main pool (**Figure 22**).

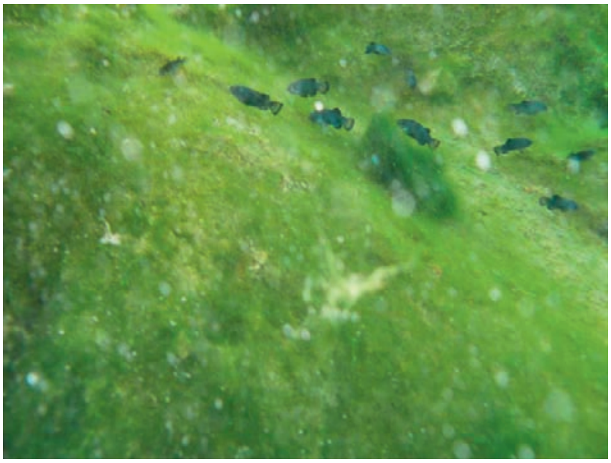


Figure 21 *Cyprinodon diabolis* on the edge of the breeding ledge during the summer where algal growth provides oxygen levels at saturated or near saturated levels. Photo courtesy of the US National Park Service.

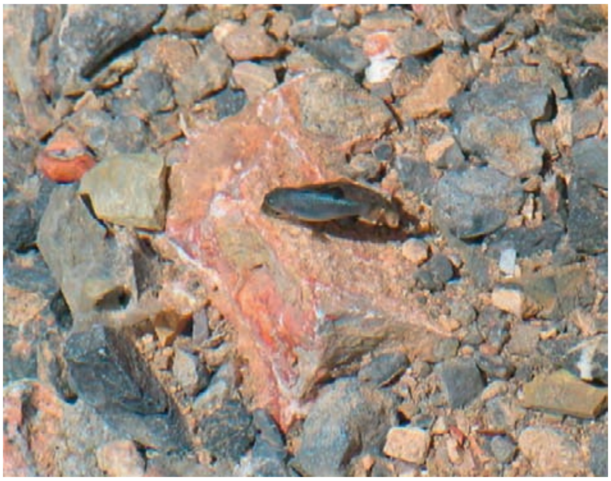


Figure 22 *Cyprinodon diabolis* on the breeding ledge with no algal growth encounter oxygen levels reported to be one-third that of saturated levels. Photo courtesy of the US National Park Service.

Below the shallow breeding ledge, there are three accumulations of rock and gravel that form deeper ledges at approximately 5, 15, and 25 m. Below 25 m, the cavern expands and has been explored to depths near 150 m but fish are not seen below the 25-m ledge. Notably, there is a 10-cm lunar tide fluctuation in the water level and earthquakes even as far away as Alaska and Mexico routinely produce water-level fluctuations in excess of 1 m that can disrupt the algal mats on the breeding ledge.

Historically, an appreciable fraction of the total population has been counted by self-contained underwater breathing apparatus (SCUBA) divers at the lower levels with the greatest number on the 5-m ledge. The fraction on the breeding ledge is consistently lower during the hot summer months even though photosynthesis may increase the oxygen content to near saturation values. In recent years, a smaller fraction of the total population has been observed on the breeding ledge at all times of the year (**Figure 23**) and the population has declined drastically. The reason for this change is not known but may be due to a warming trend in southwestern North America and a reduction in algal mats that previously covered the breeding ledge during the summer months.

Energetics

Depending on the habitat, pupfish may feed on aquatic insects, crustaceans, copepods, snails, or their own eggs and young. Three main factors determine energy available for maintaining a stable population: (1) primary production; (2) the fraction of primary production that is ingested by the fish; and (3) The efficiency with which the ingested food can be assimilated for growth and reproduction. One measure of food utilization is the conversion efficiency for fish fed in the laboratory, measured as the percentage increase in dry mass relative to the dry mass of food ingested over the same period. Conversion efficiency for *C. macularius* raised in 35 ppt salinity and fed invertebrates varied with temperature and age with the greatest efficiency at 20 °C. Efficiency declined progressively in fish reared at 25, 30, and 35 °C with a value of 7.3% for adult fish at 30 °C (**Figure 24**). Amargosa pupfish living in Tecopa Bore have been found to ingest 17.5% of the primary production, while 6.1% of the ingested food was assimilated into biomass. Thus, pupfish production corresponded to only 1.1% of the total primary production. Most of the ingested food was poorly digestible plant matter and the values obtained for *C. nevadensis amargosae* are comparable to herbivorous fishes raised in pond culture. Tecopa Bore is a productive habitat (46 296 kJ m^2 over a 1-year sampling period) that was found to support a population that varied between 13 and 196 fish m^2.

Devils Hole presents quite a different picture. Primary production between the years 1999 and 2001 was

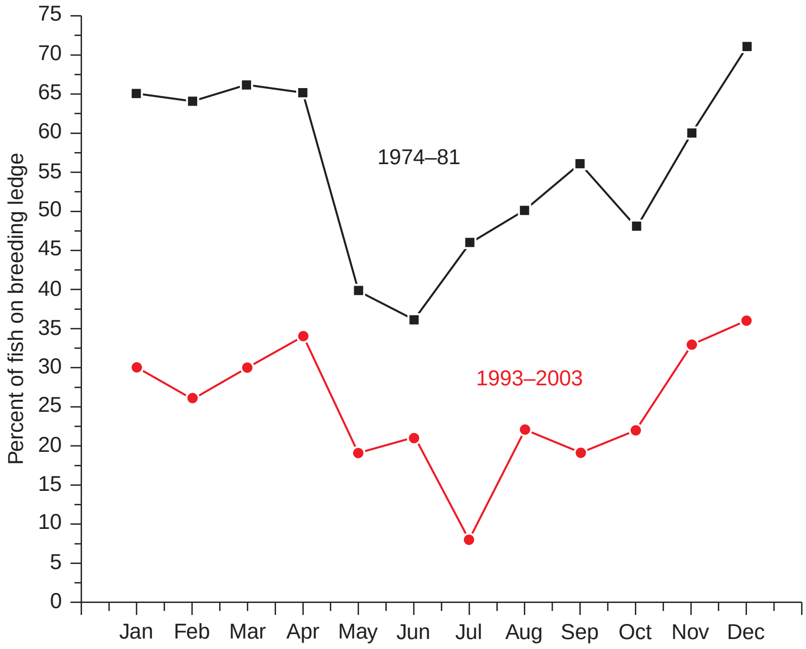


Figure 23 A smaller percentage of *C. diabolis* are counted on the breeding ledge in the hot summer months relative to the cooler winter and spring. In recent years, a smaller percentage has been counted on the ledge at all times of the year. Redrawn from Riggs AC and Deacon JE (2002) Connectivity in desert aquatic ecosystems: The Devils Hole story. In: Sada DW and Sharpe SE (eds.) *Conference Proceedings. Spring-Fed Wetlands: Important Scientific and Cultural Resources of the Intermountain Region*, pp. 1–38. Las Vegas, NV, 7–9 May, 2002.

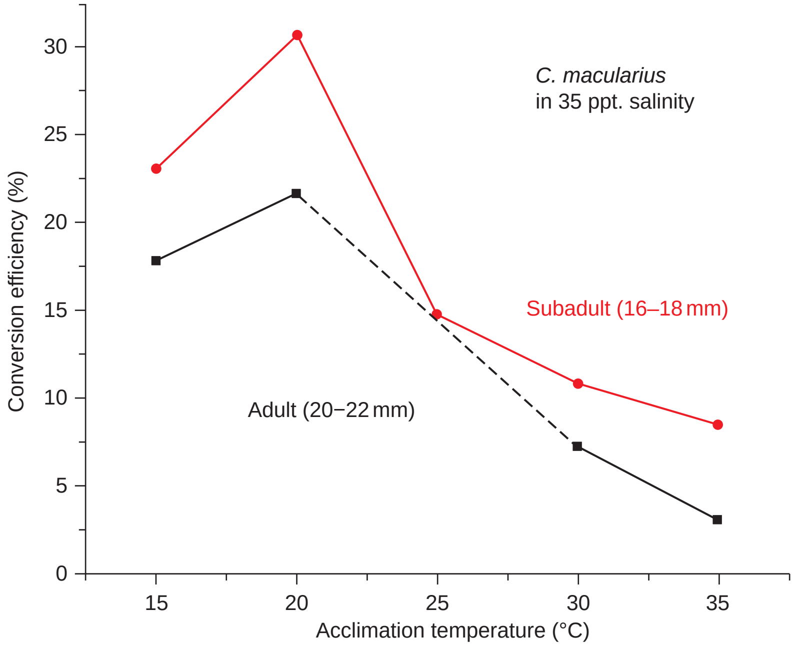


Figure 24 Conversion efficiency of young adult and adult *C. macularius* was found to decrease with increasing temperature. Redrawn from tabular data in Kinne O (1960) Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiological Zoology* 33: 288–317.

estimated to be approximately 5000 kJ yr^{-1} for the whole system at a time when the population was in rapid decline. If the ingestion and assimilation values for *C. diabolis* are comparable to those of *C. nevadensis amargosae*, even 2% of the primary production will provide only 100 kJ yr^{-1} for the fish. Beginning in winter 2007, a supplemental feeding program was initiated and resulted in the stabilization and modest recovery of the population. This suggests that the *C. diabolis* population is energy limited and raises the question of why the productivity has declined from the levels in the mid-1990s when the population exceeded 500 fish.

Conservation

Because of the precarious nature of pupfish habitats, refuge populations have been established for several of the species, including *C. radiosus*, *C. macularius*, and *C. diabolis*. Captive breeding experiments with *C. nevadensis amargosae* have shown that increased acclimation temperature and reduced food supply can produce changes in morphology that produce offspring which resemble *C. diabolis* in having lower body mass, proportionately large head and eyes, and reduction in pelvic fin development. Fish reared in this manner had lower levels of thyroid hormone, and rearing young fish in the presence of goitrogenic drugs (that inhibit endogenous thyroid hormone production) produced a similar phenotype. Thus, pupfish display plasticity in both morphological and physiological characteristics.

Refuge populations of *C. diabolis* have not fared well and, unlike other pupfish species, captive breeding has not been successful. In one refuge, near Point of Rocks Spring, *C. nevadensis mionectes* were somehow introduced and hybridized with *C. diabolis*. The hybrids reproduce well in captivity at temperatures comparable to springs in Ash Meadows (~28 °C) and Devils Hole (~33 °C). Captive populations provide the opportunity to carefully evaluate the physiological responses to environmental variables at each life stage and factors that might allow captive populations of native *C. diabolis* to be established. In conjunction with physiological and reproductive studies on the hybrids, detailed analyses of the temperature and oxygen-level profiles on the breeding ledge are being conducted, as are studies on the community structure and productivity that will be required for recovery to more sustainable levels.

See also: **Behavioral Responses to the Environment**: Temperature Preference: Behavioral Responses to Temperature in Fishes. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia. **Temperature**: Measures of Thermal Tolerance.

Further Reading

Brown JH (1971) Desert pupfish. *Scientific American* 225: 104–110.

Brown JH and Feldmeth CR (1971) Evolution in constant and fluctuating environments: Thermal tolerances of desert pupfish (*Cyprinodon)*. *Evolution* 25: 390–398.

Echelle AA (2008) The western North American pupfish clade (Cyprinodontidae, *Cyprinodon)*: Mitochondrial DNA divergence and drainage history. In: Rehis MC, Hersheler R, and Miller DM (eds.) *Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Region: Geologic and Biotic Perspectives,* Geological Society of America Special Paper 439, pp. 27–38. Geological Society of America.

Feldmeth CR, Stone EA, and Brown JH (1974) An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon)* to cycling temperatures. *Journal of Comparative Physiology* 89: 39–44.

Hirschfield MF, Feldmeth CR, and Soltz DL (1980) Genetic differences in physiological tolerances of Amargosa pupfish (*Cyprinodon nevadensis)* populations. *Science* 207: 999–1001.

Hubbs CL (1941) Fishes of the desert. *Biologist* 22: 61–69.

Kinne O (1960) Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiological Zoology* 33: 288–317.

Lema SC and Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. *Journal of Experimental Biology* 209: 3499–3509.

Naiman RJ (1976) Productivity of a herbivorous pupfish population (*Cyprinodon nevadensis)* in a warm desert stream. *Journal of Fisheries Biology* 9: 125–137.

Riggs AC and Deacon JE (2002) Connectivity in desert aquatic ecosystems: The Devils Hole story. In: Sada DW and Sharpe SE (eds.) *Conference Proceedings. Spring-Fed Wetlands: Important Scientific and Cultural Resources of the Intermountain Region*, pp. 1–38. Las Vegas, NV, 7–9 May 2002. http//:www.wetlands.dri.edu (accessed December 2009).

Shrode JH and Gerking SD (1977) Effects of constant and fluctuating temperatures on reproductive performance of a desert pupfish *Cyprinodon n. nevadensis*. *Physiological Zoology* 50: 1–10.

Stuenkel EL and Hillyard SD (1981) The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish *Cyprinodon salinus*. *Copeia* 1981: 411–417.

Wilson KP and Blinn DW (2007) Food web structure, energetics and importance of allochthonous carbon in a desert cavernous limnocrene: Devils Hole, Nevada. *Western North American Naturalist* 67: 185–198.

Relevant Websites

http://www.desertcomplex.gov – Ash Meadows National Wildlife Refuge.

http://www.nps.gov.deva – Death Valley National Park.

http://www.desertfishes.org – Desert Fishes Council.

Blind Cavefish

Y Yamamoto, University College London, London, UK

WR Jeffery, University of Maryland, College Park, MD, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Astyanax Cavefish
Troglomorphism
Eye Degeneration
Feeding Apparatus Amplification
Inheritance of Troglomorphic Traits
Evolution of Troglomorphism
Further Reading

Glossary

Albinism Absence of melanin pigment.

Apoptosis The process of programmed cell death.

Candidate gene Gene likely to be involved in a process as determined by its expression pattern and/or function.

Constructive trait Trait increased or gained during evolution.

Convergence Independent evolution of the same traits in taxa lacking a recent common ancestor.

Morpholinos Modified oligonucleotides antisense to a part of an mRNA or its nuclear precursor that are used to prevent its translation into protein.

Phenocopy Mimic of a phenotype caused by genetic manipulation.

Pleiotropy Process in which a single gene controls two or more seemingly unrelated traits.

Quantitative trait locus Chromosomal region defined by genetic linkage that contains a gene(s) responsible for a phenotypic trait.

Regressive trait Trait reduced or lost during evolution.

Sonic hedgehog Ligand initiating a key signaling pathway in vertebrate development.

Stream capture Geologic process in which surface streams and their organisms are diverted into caves in limestone areas.

Troglomorphism Suite of convergent constructive and regressive traits in cave-adapted animals.

Introduction

Blind cavefish is the common name for teleost fishes that have adapted to life in limestone caves. As a consequence of evolution in the absence of light, cavefish lost their eyesight and pigmentation and gained other traits. Cavefish exhibit troglomorphism, a convergent suite of regressive and constructive characters shared by cave-adapted animals in many different phyla. Troglomorphism has evolved many times in teleosts. At least 136 species of troglomorphic cavefish have been described belonging to 19 different families. Of the large number of known cavefish species, very few have been subjected to comprehensive study. The best studied are the poecliid *Poecilia mexicana*, the amblyopsid *Amblyopsis* (and related forms), and the characid *Astyanax mexicanus*.

A limiting factor in studying cavefish is their ability to survive in the laboratory. Accordingly, most cavefish research has been done with *A. mexicanus*, which is domesticated for laboratory study and has other attributes that make it the preferred model for investigating troglomorphism. Because of the wealth of knowledge about *A. mexicanus* cavefish, they are the subjects of this review.

Astyanax Cavefish

A. mexicanus (sometimes called *A. fasciatus*) is a single species consisting of a surface-dwelling form with normal eyes and pigmentation and a cave-dwelling form with troglomorphic features (**Figure 1**). There are 30 known *Astyanax* cavefish populations showing various degrees of troglomorphism. The evolutionary relationships and degree of isolation between these cavefish populations are complex and are not completely understood. However, it is generally agreed that some populations evolved independently from the same or similar surface fish ancestors. The surface- and cave-dwelling *Astyanax* are capable of hybridization in the laboratory, and thus considered the same species. It is also possible to hybridize different cavefish populations. The hybrids can recover ancestral traits that were lost during cavefish evolution, supporting multiple indpendent origins of troglomorphism in *Astyanax* cavefish.

The genus *Astyanax* initially spread to Central America from South America after the Isthmus of Panama was formed, and then extended its range northward into Mexico and the southwestern United States. Beginning about a million

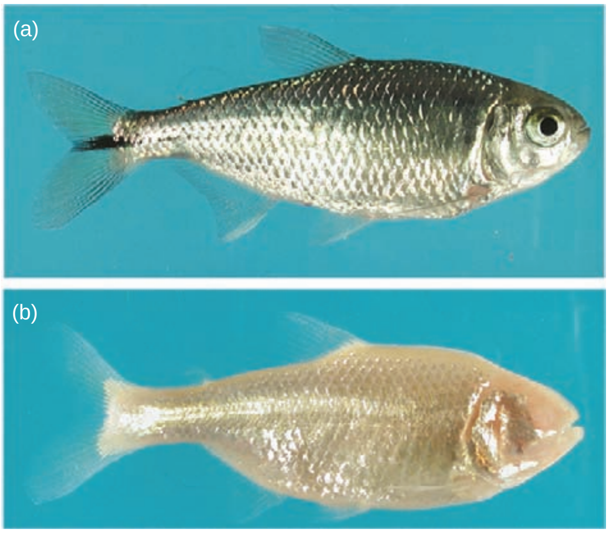


Figure 1 *A. mexicanus* surface fish (a) and Pachón cavefish (b). Note the absence of eyes and pigmentation in the latter.

years ago, *Astyanax* surface fish entered limestone caves resulting from stream capture and gradually evolved troglomorphism. The major site of *Astyanax* cavefish evolution is the Sierra de El Abra region in the states of Tamaulipas and San Luis Potosí in northeastern Mexico, which contains 29 known caves with troglomorphic populations (**Figure 2**). There is a single cavefish population in the southwestern state of Guerrero. Despite the extensive range of *Astyanax* surface fish in the vast limestone terrains of Texas, Mexico, and Central America, the cave-adapted forms are restricted to these two relatively small areas in which the conditions for cave adaptation may be ideal. *Astyanax* cavefish are named according to their cave of origin (**Figure 2**). For example, Pachón and Molino cavefish inhabit Cueva de El Pachón and Sotáno de El Molino, respectively.

The surface and cave forms of *A. mexicanus* are excellent laboratory organisms. They can be maintained on a simple diet, spawn frequently, have robust and rapidly developing embryos, and exhibit generation times of less than a year. Interbreeding can be done between the surface and cave forms, as well as among the various cavefish populations, allowing genetic analysis of troglomorphic traits. Embryological manipulations are possible and molecular technologies have been successfully developed. Finally, *Astyanax* is closely related to *Danio rerio* (zebrafish), allowing the extensive resources developed for zebrafish to be used in *Astyanax* research. For example, there is sufficient genomic synteny to allow traits mapped genetically in *Astyanax* to be identified as candidate genes on the zebrafish genome map.

Troglomorphism

Astyanax cavefish exhibit a suite of regressive and constructive traits that constitute troglomorphism in this species.

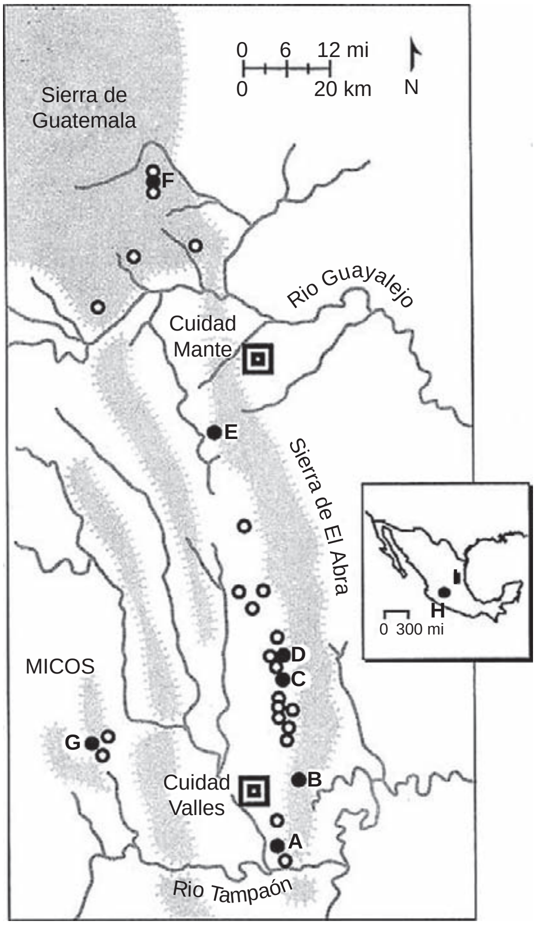


Figure 2 *A. mexicanus* cavefish distribution in northeastern Mexico. The spheres indicate the approximate positions of caves containing the following cavefish populations: A, Chica; B, Curva; C, Tinaja; D, Los Sabinos; E, Pachón; F, Molino; and G, Subterráneo. (Inset) Mexico showing the location of northeastern region shown in the larger map (shaded rectangle) and the outlying Granadas cavefish population in Guerrero (shaded sphere).

Regressive Traits

The most famous regressive traits are loss of eyes and pigmentation (**see also The Skin**: Coloration and Chromatophores in Fishes). These troglomorphic characters can be considered developmental defects because the primordia of eyes and body pigment cells (e.g., the pigmentogenic neural crest) are present in embryos, but their growth and differentiation is arrested during development. Accordingly, adult cavefish have small vestigial eyes buried beneath the skin of their orbits and melanophore precursors that remain undifferentiated throughout the body. Various parts of the cavefish eye appear to be more affected than others, suggesting a degree of independence in their troglomorphic evolution. For example, the lens shrinks to a tiny vestige, and the cornea and ciliary body are absent. By contrast, a layered retina is formed, although its growth is retarded and its layers disorganized. Neurites intially extend from the retinal ganglion cells to the optic tectum, but these connections probably decay in adults. In concert with visual decay, cavefish have a smaller optic tectum (**see also Vision**: Optic Tectum).

Craniofacial morphology also changes in cave dwelling *Astyanax*. The orbital bones surrounding the eye sockets are larger and more numerous due to their fragmentation and overgrowth into the space normally occupied by the eyes. These differences are due to the absence of a normal eye, which negatively influences orbit bone growth and fragementation. Surface fish-like craniofacial structure can be restored when the cavefish eye is rescued by lens transplantation (see below).

There are three types of body pigment cells in *A. mexicanus*: silver iridophores, orange xanthophores, and black melanophores. The pigment cell types are also subject to differential regression. Melanophores are reduced in number and in some cases melanin pigment is completely lost, causing albinsm. Pachón and Molino cavefish include a high proportion of albino individuals. The effects on iridophores and xanthophores, if any, are less severe.

Other regressive traits in *Astyanax* cavefish are (1) reduced metabolism, which is presumably adapative for survival in the cave environment, (2) reductions in the size of scales and number of fin rays, and (3) loss of one or two rib-bearing vertebrae. The purpose of scale and skeletal reductions, if any, is obscure. Finally, behavioral traits displayed by surface fish can be absent in cavefish. These behaviors include schooling behavior, which is based on vision, aggressive behavior toward conspecifics (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals and **Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?), and the scattering (or alarm) reaction, which is triggered by a secretion that warns schooling fish of predator approach (**see also Hormones in Communication**: Hormonal Pheromones). Predator absence could be a facilitator of some of these behavioral changes.

Constructive Traits

Many constructive traits are presumably related to feeding requirements in the cave environment, including larger jaws, more taste buds, and additional maxillary teeth. There is also a dramatic change in feeding behavior itself. Adult surface fish feed primarily in the water column guided by visual imputs, but in complete darkness, they search for food on the bottom, approaching the substrate at a 90° angle and ackwardly swivelling on their body axis to pick up food. In contrast, adult cavefish feed on the bottom in both light or darkness, smoothly gliding across the substrate at a 45° angle and apparently using their larger jaws to scoop up and sample detritus. An experiment was done to compare the ability of surface fish and cavefish to compete for limited food in lighted or darkened tanks. The results showed that surface fish outcompete cavefish in the light, but cavefish obtain food more successfully in the dark.

Because primary producers are lacking, food can only enter caves from the outside, and its availability may be restricted to certain seasons of the year. In northeastern Mexico, there is a wet season, when food supply may be abundant, and a dry season, when food may be minimal. Probably to facilitate survival during periods of low food availability, cavefish exhibit a higher lipid content and have more extensive fat deposits than surface fish. Clearly, a large part of adapting to life in caves may involve enhancing the ability to detect food and store energy.

Cavefish must also navigate without vision in the dark cave environment. This appears to be accomplished by increasing the size and number of motion-detecting neuromasts. As in most other teleosts, *A. mexicanus* has canal neuromasts located in the lateral lines and superficial neuromasts distributed individually in the head (**see also Hearing and Lateral Line**: Lateral Line Structure and **Hearing and Lateral Line**: Lateral Line Neuroethology). The number and size of superficial neuromasts is increased in cavefish, particularly above the empty eye sockets. In addition to assisting with navigation in the dark, the neuromast system may allow cavefish to memorize their complex physical environment. *Astyanax* perform exploratory swimming behaviors when challenged with new environments that later allow them to avoid obstacles, and this behavior seems to be enhanced in cavefish (**see also Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?).

Remarkable changes have also occurred in the cavefish brain: the ventral forebrain and hypothalamus are significantly enlarged compared to surface fish (**see also Brain and Nervous System**: Functional Morphology of the Nervous System: An Introduction). A benefit of these increases may be to enhance information processing from additional taste buds in the secondary and tertiary gustatory centers. Ventral forebrain enhancement also involves the formation of more olfactory interneurons, suggesting that increases in olfaction could be an important troglomorphic trait. This possibility is supported by the greater size, and presumably receptor capacity, of cavefish nasal areas.

Eye Degeneration

Four steps involving combinations of various transcription factors (e.g., Pax6 and Pax2) and signaling systems (e.g., Sonic hedgehog (Shh)) are important for vertebrate eye development: (1) specification, (2) commitment, (3) differentiation, and (4) growth and maintenance. Each of these steps has been modified to some extent during cavefish evolution.

Specification

From the earliest stages of eye specification, cavefish exhibit smaller pre-optic (and pre-forebrain) Pax6 expression domains are reduced in the anterior neural plate relative to surface fish. Reduction of Pax6 expression is caused by inhibitory effects of Shh, which is overexpressed along the cavefish anterior midline (**Figure 3**). The Pax6 expression domains in the lens placodes, which form in the ectoderm adjacent to the neural plate, are also reduced. Together, these changes account for specification of smaller eye primordia in cavefish.

Commitment

The commitment stage involves the formation of several different parts of the optic vesicle: the future retina, retinal pigment epithelium (RPE), and optic stalk. The optic vesicle is much smaller in cavefish than in surface fish, and the relative proportions of its parts have been changed. Continued Shh overexpression along the midline and ventral forebrain upregulates Pax2 and Vax1 in the anterior part of the optic vesicle, the optic stalk precursor, and these factors downregulate Pax6 expression in the posterior optic vesicle, which becomes the retina and RPE (**Figure 3**). Thus, the cavefish optic stalk is increased at the expense of the optic cup (retina and RPE). This is one of several developmental trade-offs revealed by studies of cavefish eye development.

Differentiation

By the beginning of the differentiation stage, lens vesicles have been induced from surface ectoderm on each side of the optic cups. In accordance with smaller optic cups, smaller lens vesicles are induced in cavefish relative to surface fish. The two parts of the lens vesicle, the lens epithelium and the zone of primary fiber cells, are formed in cavefish but further lens development is gradually arrested and apoptosis begins, eventually resulting in the virtual disappearnce of the cavefish lens. The *aA-crystallin* gene, which encodes an anti-apoptotic factor that is

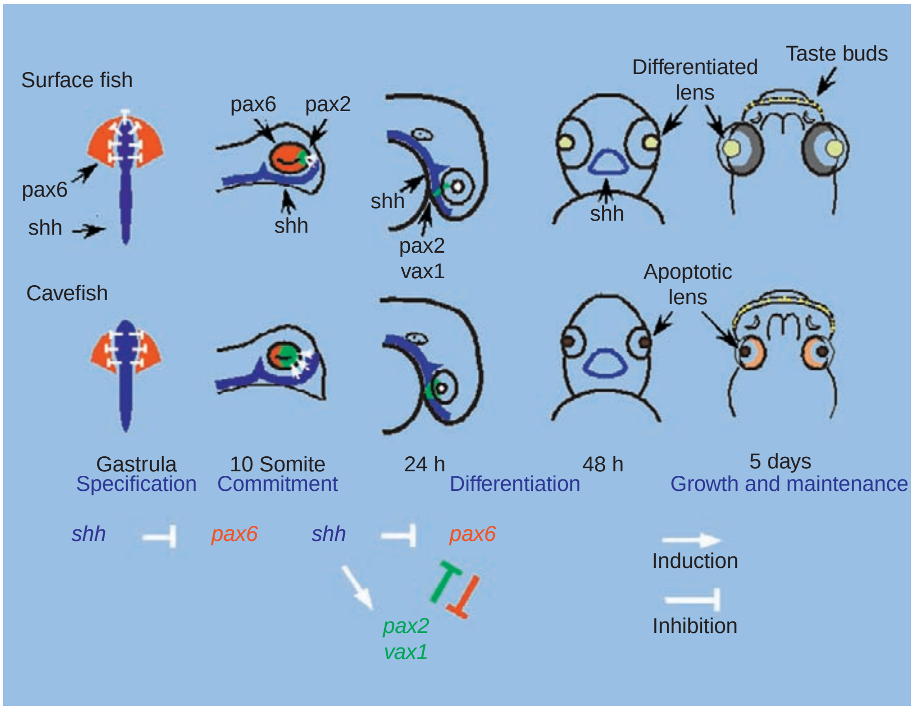


Figure 3 Summary of events during the pleiotropic trade-off between eyes and jaws/taste buds in cavefish. Shh is enhanced at anterior midline, reducing Pax6 expression in the anterior neural plate of cavefish gastrula. Later, *shh* expression is also enhanced in the ventral head region, inducing expanded *pax2* expression in the anterior optic cup. The *pax2* expression domain becomes optic stalk. Cavefish have enhanced the optic stalk at the expense of the ventral optic cup. Cavefish have a small optic cup with a tiny lens. In surface fish, the lens differentiates and accumulates crystallin proteins, but in cavefish the lens undergoes apoptosis. At the same stage, Shh is expressed at the edge of the mouth, which is larger in cavefish, and later in the taste buds. During the larval stage, cavefish have small eyes and wider jaws with increased numbers of taste buds.

downregulated in the cavefish lens, and *heat shock protein 90α* gene (*HSP90α*), which encodes a putative pro-apoptotic factor that is upregulated in the cavefish lens, are two of the factors likely to be responisble for lens apoptosis.

Meanwhile, the parts of the retina begin to differentiate. Although total cell numbers are less, ganglion, amacrine, horizontal, bipolar, and Muller glial cells are produced in the cavefish retina. Rod photreceptor cells appear transiently, but subsequently undergo apoptosis and are lost. The transient photoreceptor cells appear to be abnormal, having shortened outer segments. Eventually, apoptotic bodies also appear within the layered retina, and the layers become disorganized. Pigmentation appears in the RPE of some cavefish populations, although not in albinitic Pachón and Molino cavefish. Later, the RPE also may undergo cell death.

Growth and Maintanence

In teleosts, growth of the eye continues through adulthood, with new cells added at the margin of the lens and the outer edges of the retina/RPE, a region called the ciliary marginal zone (CMZ). The proliferation rate of cavefish lens cells is apparently not sufficient to replace the cells removed by apoptosis, and thus the lens gradually becomes smaller. In contrast, there is a healthy rate of cell division in the cavefish CMZ, the same or even perhaps greater than its surface fish counterpart. After the early stages of development, however, the cavefish retina does not increase in size at the same rate as in surface fish. This is attributed to complete turnover of newly born retinal cells by apoptosis after they leave the CMZ, a process unique to the cavefish retina. Surprisingly, cavefish relentlessly form new cells at the CMZ, only to destroy them before they can contribute to the retina. Thus, net growth is arrested in the cavefish eye, which is eventually overgrown by adjacent head tissues and sinks into the orbit. The final size of the vestigial eye is variable both between and within *Astyanax* cavefish populations.

Rescue and Phenocopy

There are three ways in which the cavefish eye has been rescued during development: (1) genetic rescue, (2) pharmacological rescue, and (3) rescue by lens replacement. Furthermore, cavefish phenocopies with degenerating eyes can be produced in surface fish. It is important to consider both rescue and phenocopy because they provide important clues about the mechanisms of eye degeneration.

Genetic rescue

In some cases, eyes and vision have been rescued in hybrids produced by crossing two different cavefish populations. Genetic rescue shows that different genes are responsible for eye degeneration in various cavefish populations.

Pharmcologial rescue

Pharmacological rescue depends on the inhibition of HSP90 function with the drug radicicol. When cavefish embryos are treated with radisicol prior to lens apoptosis, cell death does not occur and a larger lens is formed. However, HSP90 is also important for other events in embryogenesis, such as muscle development, and thus cavefish with rescued lenses do not survive. However, pharmacological rescue shows that the program leading to lens death is reversible.

Rescue by lens replacement

Early defects in the cavefish lens suggest a role in eye degeneration. To test this possibility a cavefish lens was removed from the optic cup during early development and replaced with a surface fish lens. Remarkably, the transplanted lens survived and was able to recuse other eye parts, including the cornea, ciliary body, and retinal photoreceptor cells. Moreover, the retina did not undergo apoptosis after lens replacement, and a large eye was eventually formed in cavefish. Whether the rescued eye is functional in vision remains to be shown, but the lens replacement experiments indicate that eye degeneration is controlled largely by the lens and that rescued eye parts contain the intrinsic information required to build a complete eye.

Phenocopy of Eye Degeneration

In vertebrates, the midline signaling molecule Shh has an important role in bilateral separation of the eyes. When Shh was overexpressed in surface fish, either by injecting into eggs an excess of *shh* mRNA or a DNA expression construct containing Shh driven by a heat shock promoter, the resulting larvae showed eye degeneration. Just as in cavefish, Pax2 is expanded and Pax6 depressed in the optic vesicles, resulting in large optic stalks and small optic cups. Importantly, Shh overexpression also induced apoptosis in the cavefish lens. The phenocopy studies show that Shh overexpression causes eye degeneration through inducing lens apoptosis in *Astyanax* cavefish.

Feeding Apparatus Amplification

Cavefish larvae have a feeding apparatus consisting of larger and wider jaws with more taste buds on their lips relative to surface fish larvae. Taste buds are a chemosensory organ important for finding food (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction). Thus, these modifications could be associated with changes in feeding behavior. Surface fish larvae find a food item using visual information and bite off pieces of the food. In contrast, cavefish larvae stop swimming when food is near, open their mouths widely, and appear to suck in the food. As described above, the wider jaw with enhanced taste buds may help cavefish feed in a dark cave.

Shh is also involved in feeding apparatus development. When the mouth develops, *shh* is expressed along its edges, and later in taste buds. To test whether Shh activity is required for producing the feeding apparatus, morpholinos were used to downregulate *shh* mRNA translation. The injected embryos developed small jaws with reduced taste bud numbers. In contrast, Shh overexpression results in larger jaws with increased taste buds. Importantly, conditional expression of the Shh DNA construct revealed a period during gastrulation in which Shh is required both for eye loss and feeding apparatus enlargement, defining a linkage between these regressive and constructive traits via the Shh signaling pathway.

Inheritance of Troglomorphic Traits

In *Astyanax* cavefish, eye degeneration is controlled by mutations in as many as 12 different genes, each playing a small role in the loss of vision. Quantitative trait locus (QTL) analysis with microsatellite markers has been used to map traits involved in eye regression and identify candidates for the mutated genes based on synteny with zebrafish. One of the eye candidate genes is *aA-crystallin*, whose downregulation may affect the survival of the cavefish lens.

The reduction in melanophore number is also controlled by multiple genes with additive effects. Similar to the case of eyes, the genes responsible for melanophore reduction can be different in various cavefish populations. Accordingly, pigmentation can be rescued in F1 hybrids. Eighteen different QTLs affect melanophore pigmentation. Among the many QTLs and predicted pigmentation genes, there are some traits with simple inheritance. Considerable progress has been made in identifying the genes governing two of these simple traits: albinism and the brown eye phenotype.

Cavefish albinism is inherited as a simple Mendelian trait controlled by a single recessive gene. The responsible gene is *oculocutaneous albinism2* (*oca2*), which also has a major role in mouse and human albinisms. Loss of *oca2* function has occurred in both Pachón and Molino cavefish, but the mutations are distinct: they are large deletions encompassing different regions of the protein-coding sequence. Thus, the *oca2* gene has been a repeated target for mutation, resulting in independent origins of albinism during cavefish evolution.

Mutations in the *brown* gene decrease the number of melanophores and change eye pigmentation from black to brown. Hybridization of different cavefish populations indicates that this gene is responsible for brown pigmentation in the (degenerate) eyes of many different cavefish populations. Genetic analysis predicted a single gene responsible for the *brown* phenotype, and as expected only one QTL was identified. The *brown* gene encodes melanocortin 1 receptor (Mc1r). The Mc1r ligand is melanosome stimulating hormone-*a* (MSH-*a*), which is involved in pigmentation changes in many different vertebrates. Similar to albinism, different mutations in the same *Mc1r* gene are known to cause the brown eye phenotype in various cavefish populations. In some cavefish populations, loss of function mutations are found in the *Mc1r*-coding region, whereas in others, the mutation is likely to be in gene regulatory regions. Thus, *Mc1r* is another example of a gene that has been repeatedly mutated to generate the same troglomorphic phenotype.

In contrast to the large number of genes involved in eye and pigment degeneration, only three gene loci, one major gene and two modifiers, are required to change feeding behavior. This is surprising because of the complexity assumed to underlie behavioral traits. Candidate genes for feeding behavior have yet to be identified.

Evolution of Troglomorphism

It is generally agreed that constructive troglomorphic traits have evolved by natural selection, but the evolutionary forces controlling regressive traits are less clear. Two major hypotheses have been proposed: (1) accumulation of neutral mutations under conditions of relaxed selection and (2) selection against the lost trait based on either energy conservation and/or pleiotropy.

In the case of eye loss, selection seems to be supported over neutral mutation by the following arguments. All of the eye QTLs decrease eye size, which is unexpected if random neutral mutations are occurring. The restoration of eyes by lens transplantation suggests that downstream eye genes are functional. The genes involved in eye development identified thus far have not mutated to loss of function. Most genes with modified expression patterns, such as those in the Shh signaling pathway and *HSP90α*, increase rather than decrease their activity during eye degeneration. Eye loss may be a result of indirect selection involving a developmental trade-off: reduction of eyes allows for enhancement of adaptive feeding traits (wider jaws and more taste buds) controlled by the pleiotropic *shh* gene. In contrast, loss of pigmentation seems to be caused by neutral mutation. Genetic analysis shows that individual QTL can increase or decrease melanophore abundance, supporting a random process. Loss-of-function mutations in *oca2* may be permitted if this gene is not pleiotropic and its disruption does not affect other important developmental pathways. These results suggest that a combination of different evolutionary forces may have contributed to the evolution of troglomorphism in *Astyanax* cavefish.

See also: **Brain and Nervous System**: Functional Morphology of the Nervous System: An Introduction. **Hearing and Lateral Line**: Lateral Line Neuroethology; Lateral Line Structure. **Hormones in Communication**: Hormonal Pheromones. **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals; Spatial Orientation: How do Fish Find their Way Around?. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction. **The Skin**: Coloration and Chromatophores in Fishes. **Vision**: Optic Tectum.

Further Reading

Alumni A, Menuet A, and Candal E, et al. (2007) Developmental mechanisms for retina degeneration in the blind cavefish *Astyanax mexicanus. Journal of Comparative Neurology* 506: 221–233.

Borowsky R (2008) Restoring sight in blind cavefish. *Current Biology* 18: R23–R24.

Dowling TE, Martasian DP, and Jeffery WR (2002) Evidence for multiple genetic lineages with similar eyeless phenotypes in the blind cavefish, *Astyanax mexicanus. Molecular Biology and Evolution* 19: 446–455.

Gross JB, Borowsky R, and Tabin CJ (2009) A novel role for Mc1r in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus. PLoS Genetics* 5: e10000326.

Gross JB, Protas M, Conrad M, *et al.* (2008) Synteny and candidate gene prediction using an anchored linkage map of *Astyanax mexicanus. Proceedings of the National Academy of Sciences of the United States of America* 105: 20106–20111.

Jeffery WR (2009) Evolution and development in the cavefish. *Astyanax. Current Topics in Developmental Biology* 86: 191–221.

Jeffery WR (2009) Regressive evolution in *Astyanax* cavefish. *Annual Reviews of Genetics* 43: 25–47.

Mitchell RW, Russell WH, and Elliot WR (1977) Mexican eyeless characin fishes, genus *Astyanax:* Environment, distribution, and evolution. *Special Publications/The Museum, Texas Tech University* 12: 1–89.

Protas M, Hersey C, Kochanek D, *et al.* (2006) Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nature Genetics* 38: 107–111.

Protas M, Tabansky I, Conrad M, *et al.* (2008) Multi-trait evolution in a cavefish. *Astyanax mexicanus. Evolution and Development* 10: 196–209.

Proudlove G (2006) *Subterranean Fishes of the World*. Moulis, France: International Society for Subterranean Biology.

Rétaux S, Pottin K, and Alunni A (2008) Shh and forebrain evolution in the blind cavefish *Astyanax mexicanus. Biology of the Cell* 100: 139–147.

Sharma S, Coombs S, Patton P, and Burt de Perera T (2009) The function of wall-following behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra *(Astyanax). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 195: 225–240.

Strickler AS, Yamamoto Y, and Jeffery WR (2001) Early and late changes in *pax6* expression accompany eye degeneration during cavefish development. *Development Genes and Evolution* 211: 138–144.

Strickler AS, Yamamoto Y, and Jeffery WR (2007) The lens controls cell survival in the retina: Evidence from the blind cavefish *Astyanax. Developmental Biology* 311: 512–523.

Wilkens H and Strecker U (2003) Convergent evolution of the cavefish *Astyanax* (Characidae, Teleostei), genetic evidence from reduced eye size and pigmentation. *Biological Journal of the Linnean Society* 80: 545–554.

Yamamoto Y, Byerly MS, Jackman WR, and Jeffery WR (2009) Pleiotropic functions of embryonic *sonic hedgehog* expression link jaw and taste bud amplification with eye loss during cave fish evolution. *Developmental Biology* 300: 200–211.

Yamamoto Y and Jeffery WR (2000) Central role for the lens in cavefish eye degeneration. *Science* 289: 631–633.

Yamamoto Y, Stock DW, and Jeffery WR (2004) Hedgehog signalling controls eye degeneration in blind cavefish. *Nature* 431: 844–847.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Air-Breathing Fishes

Contents
The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction
Respiratory Adaptations for Air-Breathing Fishes
Circulatory Adaptations for Air-Breathing Fishes

The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction

JB Graham, University of California at San Diego, La Jolla, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
The Diversity of Fish Air Breathing
The Types of Air-Breathing Fishes
The Evolution of Air Breathing
Air Breathing and Natural History
Summary
Further Reading

Glossary

Air-breathing organ (ABO) A structure or body surface of a fish having the specialized structural capacity for aerial gas exchange; utilized by air-breathing fishes to supplement the normal respiratory mode which is aquatic respiration using gills.

Amphibious air breathing The mode of air breathing when a fish is out of water.

Aquatic surface respiration (ASR) A hypoxia-driven behavior in which a fish surfaces and places its the mouth as close to the water surface as possible in order to ventilate the gills with the upper few millimeters of water that remains well oxygenated because of atmospheric diffusion.

Autapomorphy A specialization unique to one group and thus not useful for establishing relationships.

Bimodal respiration The capacity to exchange respiratory gases in both air and water and to do so either simultaneously or sequentially.

Continuous air breathing The frequent and regular occurrence of air gulping, even when aquatic oxygen levels are sufficient to sustain aquatic respiration. This behavior often indicates the use of ABO gas for functions other than respiration such as buoyancy.

Facultative air breathing Emergency aquatic air breathing initiated in response to a diminished capacity for aquatic respiration usually associated with aquatic hypoxia or some other stress factor.

Hypoxia-inducible factor (HIF-1α) Integrated hypoxia response that activates genes whose protein products either increase O_2 transfer (i.e., erythropoiesis, Hb affinity increases, angiogenesis, etc.) or upregulate metabolic adaptation by regulating anaerobiosis and O_2 consumption rate. HIF-1α induction is an ancient adaptation that first appeared in eukaryotic cells and thus long preceded the origin of metazoans.

Lung The principal aerial respiratory organ of all vertebrates and some primitive air-breathing fishes. An outpocketing along the vertebrate digestive tube having a vascular epitheilial surface that functions for aerial gas exchange through its connection to the body surface by a duct through which air can be inhaled and exhaled.

Obligatory air breathing A high degree of physiological dependence on air breathing for the maintenance of basal oxidative processes or to prevent suffocation. This is usually associated with a reduction in gill surface area that occurs with progressive specialization for air breathing.

Physoclistous gas bladder A gas-filled structure within the body of the fish that has lost its connection to the body surface and thus cannot function for gas exchange but retains functions for buoyancy and either or both sound production and sound reception.

Physostomous gas bladder A gas-filled structure within the body of a fish that is connected to the body surface by the pneumatic duct that allowing inflation or deflation. In different fishes, functions of this organ may include aerial respiration as well as buoyancy and both sound reception and production.

Placoderm Armored Paleozoic fish that were the first vertebrates to have jaws.

Plesimorphy A primitive trait.

Pneumatic duct Tube linking the pharynx to either the lung or physostomous gas bladder which functions for the passage of air for either air breathing, volume control, or both.

Synapomorphy A specialized trait shared by two or more groups, which implies that an ancestral group also possessed this trait.

Tetrapodomorpha The group of vertebrates consisting of the tetrapods (four-limbed vertebrates) and their closest sarcopterygian (lobefin fish) relatives which invaded the land during the Devonian period.

Introduction

All of the over 28 000 living fish species use gills to exchange O_2 and CO_2 with water (**see also Gas Exchange**: Respiration: An Introduction). However, some fishes are also bimodal breathers, that is, they have the capacity to respire aerially as well as aquatically. This most usually happens when the water in which a fish lives becomes hypoxic (i.e., lower than atmospheric O_2 partial pressure). Because air has both a greater and less variable quantity of oxygen than water, some species have evolved the capacity to use aerial oxygen. Air breathing is an auxiliary respiratory mode that enables a fish to live in hypoxic waters or to become amphibious and to perhaps further utilize the mechanical processes integral to this adaptation in the augmentation of functions such as buoyancy, sound reception or production, and reproduction.

Air breathing is an ancient trait that first evolved in fishes and has played a significant role in vertebrate evolution. Not only were fishes the first vertebrates to breathe air, it was an air-breathing, lobe-finned fish that gave rise to the Tetrapodomorpha – a group that lived in shallow water, developed an amphibious capacity, invaded the land in the late Devonian period (360 million years ago, mya), and became the ancestors of all tetrapods. While air breathing is an ancient fish trait, it has also evolved in a number of derived fish groups, including the modern teleosts.

The objective of this article is to develop a biological synthesis of fish air breathing that examines the evolution and diversity of this adaptation and the ways it has contributed to the survival, radiation, and natural history of different species. This perspective sets the stage for subsequent articles on the structure and function of fish air-breathing organs (ABOs; **see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes) and on how the blood circulation of air-breathing fishes is modified for the transport of respiratory gases between the body tissues and both aerial and aquatic gas-exchange surfaces (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes, **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns, Branchial Anatomy, **Circulation**: Circulatory System Design: Roles and Principles, and **Design and Physiology of the Heart**: The Coronary Circulation).

The Diversity of Fish Air Breathing

Phylogeny and Diversity

An understanding of the evolutionary relationships of fishes is required to comprehend the evolution and diversity of air breathing as well as the structure and function of fish ABOs. The phylogenetic relationship of the families of air-breathing fishes is shown in **Figures 1(a) and 1(b)**. Air breathing occurs only in the bony fishes (Osteichthyes) where it is found in nearly 400 species distributed among approximately 140 genera in 50 families and spanning 18 orders. The Osteichthyes have a 400-million-year-old history and air breathing was present in many, if not all, of the earliest bony fishes and predated the Silurian (438–408 mya) separation of this group's two subdivisions: the Sarcopterygii (lobefins) and Actinopterygii (rayfins).

The broad phyletic distribution of the air-breathing fishes suggests that this adaptation is autapomorphic, meaning that it evolved independently in many groups. Assuming that the lack of an air-breathing synapomorphy (the absence of this specialization in the common ancestors of many of the groups that have it) indicates the independent origin of air breathing; then this adaptation has evolved independently at least 38 times in the bony fishes. Or, if it is further assumed that air breathing evolved independently among many of the species that

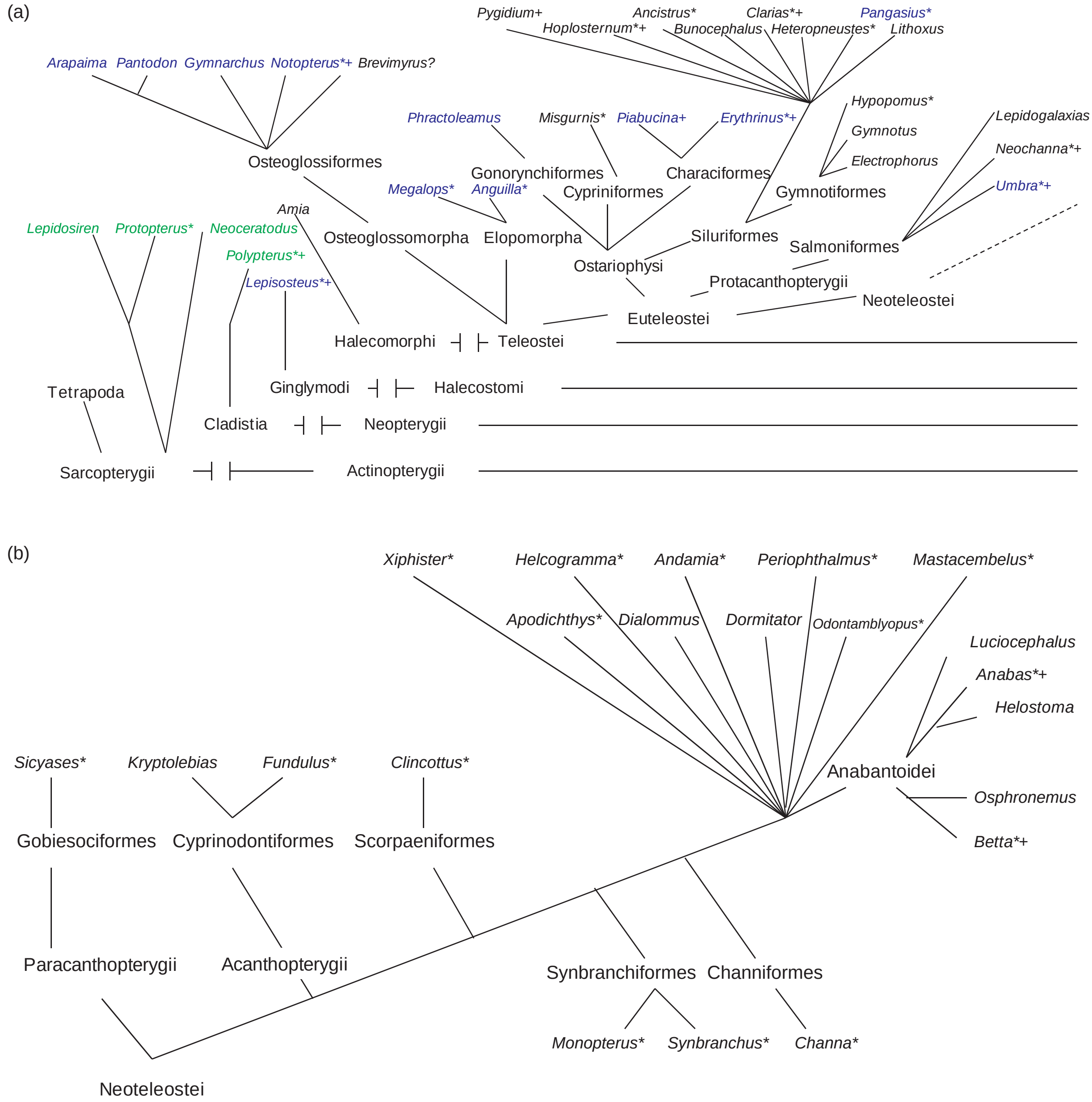


Figure 1 Phylogenetic arrangement of the 50 air-breathing fish families within the class Osteichthyes: (a) phylogeny from the subclass Sarcopterygii to the teleost infradivision Euteleostei, (b) infradivision Neoteleostei. (Note: Family names are replaced by the name of a genus representative of that family.) Asterisk indicates the presence of more than one air-breathing species in the specified genus. Cross indicates more than one genus of air breather in the family. Question mark indicates newer and incomplete data. Colors indicate ABO type: red, lung; blue, gas bladder; black, other structure. Modified from Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press.

use either a lung or a gas bladder as an ABO (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes), then this adaptation could have independently evolved as many as 70 times.

Air breathing is not known to occur in any fish group other than bony fishes. It may occur in jawless fishes as several species of lampreys make occasional amphibious excursions during upstream migration and laboratory studies with one of these, New Zealand lamprey *Geotria australis* show it can respire in air and endure prolonged aerial exposure. There are no known chondrichthyan (sharks and rays) air breathers, although one species, the sand tiger shark, *Odontaspis taurus*, does swallow and discharge air in order to adjust its buoyancy. The only suggestion of air breathing at an earlier stage of vertebrate evolution than the Osteichthyes is the report of paired lung-like structures in fossils of the Devonian (408–360 mya) placoderm *Bothriolepis canadensis*.

The interpretation of these structures as lungs has remained controversial for nearly 70 years and should be resolved using high-resolution X-ray computed tomography on some of the many well-preserved *Bothriolepis* fossils (there are over 70 species) residing in many museum collections. Because placoderms are ancestral to the chondrichthyans, the report of lungs in *Bothriolepis* and knowledge that no air-breathing structures occur in living chondrichthyans prompted speculation that air breathing evolved in placoderms, was lost in chondricthyans, and reappeared in the osteichthyans. Support for this hypothesis has never been strong; however, in view of the extent to which the independent origin of air breathing occurs among osteichthyans, the presence of this specialization in a placoderm or an early jawless fish is not unexpected. *Orthacanthus* (at least three known species), a large, eel-like shark that lived in freshwater swamps during the mid- to late Paleozoic (400–225 mya), would seem a likely candidate for air breathing.

Phylogeny and the ABO

The vertebrate lung originated in fishes and is present in both the sarcopterygians (e.g., lungfish) and the actinopterygians (**Figures 1**(**a**) and **1**(**b**)). In nearly all instances tetrapod evolution is linked to increased lung efficacy; however, for actinopterygians the evolutionary history of the lung took a quite different course; it became gradually transformed, first into a physostomous (remotely linked to the air via a tube, the pneumatic duct, connection to the pharynx) respiratory gas bladder, then to a nonrespiratory physostomous gas bladder, and finally to a physoclistous (no duct connection) gas bladder primarily specialized for sound reception or production, buoyancy control, or several of these (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes and **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes). The transition from lung to gas bladder closely paralleled the adaptive radiation of actinopterygians; paired lungs occur in the primitive Cladistia (bichirs, *Polypterus* (*P. senegalus* and about 13 other species) and the ropefish, *Erpetoichthys calabaricus*), while respiratory gas bladders occur exclusively in the other primitive groups (Ginglymodi, gars *Lepisosteus occulatus*, *Atractosteu* sp., and about seven total species; Halecostomi, *Amia calva*) and even in some teleosts (**Figure 1**(**a**)).

Among the Euteleostei, which includes the most highly derived bony fishes, the respiratory gas bladder is limited to the most primitive superorders Ostariophysi and Protacanthopterygii (**Figure 1** (**a**)). The ostariophysan order Siluriformes (catfishes) illustrates the manner in which the evolutionary canalization of the gas bladder structure and function (e.g., in some cases being embedded in bone to augment sound reception) had proceeded to the point where this organ could not be converted, through natural selection, into an ABO in cases where environmental or climate changes or a group's ecological radiation placed it in a habitat necessitating air breathing. Only one silurid, *Pangasius* (*P. sutchi* and at least three other species) has a respiratory gas bladder featuring a lung-like vascular or pulmonoid epithelium. *Pangasius* is in the family Pangasiidae which, as seen in the silurid cladogram (**Figure 2**), is not a basal group, is one of eight siluriform families with air-breathing species, but the only one of these having a respiratory gas bladder. This indicates the apomorphy of the pulmonoid gas bladder in *Pangasius* as well as the other siluriform ABOs, which occur in many genera and species in these other families and range in diversity from the buccal chamber (*Bunocephalus amaurus*) to the stomach (*Ancistrus* (= *Liposarcus*) *chagresi*, *Hypostomus plecostomus*, *Pygidium striatum*, and *Lithoxus lithoides*), the intestine (*Misgurnis anguillicaudatus*, *Hoplosternum thoracatum*, *Callichthys asper*, and *Corydoras aeneus*), and a suprabranchial chamber with gill-derived dendrites and fans (*Clarias batrachus* and several other species) having extended pharyngeal pouches (*Heteropneustes fossilis*) (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes).

Thus, and commencing with the Ostariophysi, adaptive specialization of the gas bladder for functions discordant with bimodal respiration led to natural selection for novel ABOs in all of the higher teleosts, especially the Neoteleostei (**Figure 1**(**b**)). Correlations between phylogeny and bimodal breathing morphology extend to the patterns of blood circulation, to the mechanisms for and the control of ABO ventilation, and to morphological specializations (body size and shape, gill structure, and fin position) favoring amphibious life (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes and Circulatory Adaptations for Air-Breathing Fishes).

The Types of Air-Breathing Fishes

Initial categorizations of air-breathing fishes were based on ABO structure (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes) but these had limited utility because they did not account for phylogeny, were not based on the fine-scale ABO structure, and were not inclusive of the many neoteleost air breathers lacking a well-developed ABO. A categorization based on the functionality of air breathing is an efficient way of incorporating these important features and circumscribing the range of physical and ecological factors likely affecting aerial and aquatic respiration.

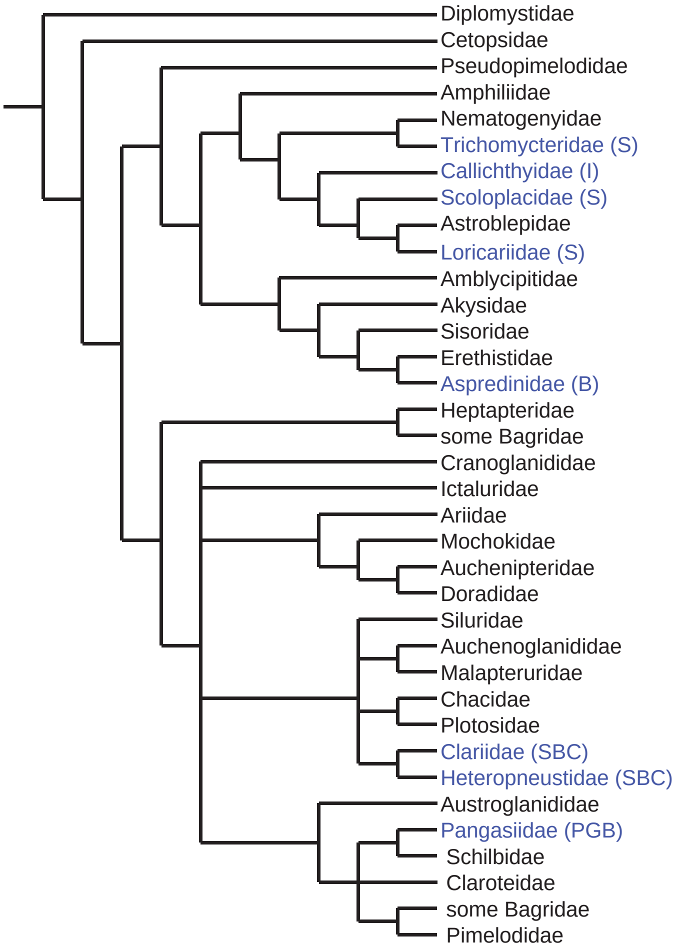


Figure 2 Phylogenetic relationships within the order Siluriformes showing the families in which air breathing occurs (blue) and their ABO type in parentheses: B, buccal chamber; I, intestine; PGB, pulmonoid gas bladder; S, stomach; SBC, suprabranchial chamber. Modified from Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge University Press.

Aquatic Air Breathers

From a functional standpoint, fish air breathing is readily classified as either aquatic or amphibious. Aquatic air breathers remain in water (although some species doing this can also be amphibious air breathers) and there are two types, facultative and continuous. Facultative air breathers normally respire aquatically and use air breathing only when it is required by environmental conditions, principally hypoxia. Functional hypoxia caused by intense exercise or other stress factors can also elicit facultative air breathing. Included among many facultative air breathers are the Australian lungfish *Neoceratodus forsteri*, nearly all of the genera of the South American suckermouthed armored catfishes (Loricariidae, e.g., *Liposarcus chagresi* and many other species) and other Amazon catfishes (*Gymnotus carapo*, *Hypopomus brevirostris*, and *H. occidentalis*), the swamp eel (*Synbranchus marmoratus*), the salmoniforms (*Umbra limi* and three other species and *Dallia pectoralis*), the estuarine goby (*Gillichthys mirabilis*), and the Asian eel goby (*Odontamblyopus lacepedi* and other species), which switches to air breathing only when its burrow water becomes hypoxic at low tide.

Some aquatic air breathers do this regularly even in normoxic water and are termed continuous air breathers. Continuous air breathing is common in species living in habitats where hypoxic water is either a chronic or frequent occurrence, and in many cases continuous air breathing is not solely for respiration but also functions to maintain the volume and wall tension of the ABO in order to serve additional and potentially multiple purposes such as buoyancy and either or both sound production and reception (e.g., featherbacks *Notopterus notopterus* and two other species, also *Papyrocranus afer* and *Xenomystus nigri*).

Obligate Air Breathers

Although most aquatic air breathers are continuous air breathers, some are also obligatory air breathers, meaning they completely depend on and require access to aerial oxygen and, even if they are in water that is well oxygenated, they will have a reduced aerobic scope or possibly even drown if prevented from air breathing. Examples of obligatory air breathers include the four African lungfish (*Protopterus atheiopicus*, *P. amphibius*, *P. annectens*, and *P. dolloi*), the South American lungfish (*Lepidosiren paradoxa*), the Amazon osteoglossid (*Arapaima gigas*), the African butterfly fish (*Pantodon bucholzi*), the Atlantic tarpon (*Megalops atlanticus*), the silurid (*Pangasius sutchi*), some species of clariid catfishes (but results vary, *C. batrachus, C. lazera, C. gariepinus, C. macrocephalus*), the armored catfish *H. thoracatum*, the monotypic Amazon electric knifefish (*Electrophorus electricus*), several anabantoids including *Anabas testudinus*, *Osphronemus goramy*, *Trichogaster trichopterus* and *T. pectoralis*, and *Colisa fasciatus*), the swamp eel (*Monopterus cuchia*), and probably the seven other species in this genus), and probably some of the 12–14 species of snakehead (*Channa*), but this has not been studied.

Obligatory air breathing appears to be the consequence of developing such a proficient level of air-breathing specialization that the capacity for aquatic respiration using the gills is compromised (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes, **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns, Branchial Anatomy, **Circulation**: Circulatory System Design: Roles and Principles, **Design and Physiology of the Heart**: The Coronary Circulation, and **Ventilation and Animal Respiration**: Plasticity in Gill Morphology). An ABO that develops within the branchial chamber can, for example, occlude the gills or restrict their size. Reductions in gill area can also be caused by the functional conflict between the in-series circulation typical of fishes and the positioning of the ABO. In most aquatic air breathers, oxygenated blood exits the ABO via the venous circulation and passes through both the heart and gills before entering the systemic circulation. The exposure of oxygenated blood to hypoxic water in the gills increases the potential for the trans-branchial loss of O_2 and this is often countered by reduction in gill surface area or the permanent shunting of blood through nonexchange surfaces within the gill.

Amphibious Air Breathing

There are three grades of amphibious air breathers: species that endure seasonal exposure in drying mud, those that endure brief stranding by a receding tide, and those that volitionally emerge from the water. Fishes subject to long-term exposure in drying mud include the African and South American lungfish (*Protopterus* and *Lepidosiren*), swamp eels (Synbranchidae), armored catfish (*Liposarcus*, *Hypostomus*, and *Callichthys*), anabantoids (*Anabas* and *Ctenopoma*), snakeheads (*Channa*), clariid catfishes (*Clarias*), and many other species, including non-air-breathers inhabiting lowland tropical streams that stagnate and disappear during the dry season. Many intertidal fish species including *Fundulus majalis* (and other species in this genus) can become trapped on land during low tide and fishes residing in congested littoral tidepools that become progressively hypoxic during low tide may, under extreme conditions, emerge from the water (e.g., the sculpins *Clinocottus globiceps* and *Oligocottus snyderi*). The estuarine mudsucker goby *G. mirabilis* also does this. The mangrove killifish (*Kryptolebias* (=*Rivulus*) *marmoratus* and other species in this genus) occupies unique, above-the-water-line habitats such as in leaf litter and termite burrows in floating logs. While amphibious air breathing is often associated with hypoxic stress and disappearance of water from a habitat, the natural behavior of a number of tropical and subtropical intertidal fishes such as the rockskipper *Dialommus* (=*Mnierpes*) *macrocephalus*, numerous blennies (*Blennius pholis*, *Entomacrodus nigricans*, *Alticus kirki*, and *Andamia tetradactyla*, there several species in each of these genera), and the mudskippers (*Scartelaos histophorus*, *Boleophthalmus pectinirostris*, *Periophthalmus modestus*, and *Periophthalmodon schlosseri*, there are numerous species in each genus) make frequent terrestrial sojourns for the purpose of exploiting resources above the water line.

The Evolution of Air Breathing

For the majority of air-breathing fishes a close link exists between this specialization and environmental hypoxia, suggesting that natural selection for air breathing has been driven largely by the cyclic occurrence, over geologic time, of severe environmental hypoxia (about 30–40% air saturation of water). For each group in which air breathing has evolved, selection has operated on a specific combination of factors that included genetic variation and the potential advantages in terms of inter-specific competition and ecological radiation through niche expansion afforded by increased hypoxia tolerance and air breathing. The potential interaction of these factors over time is illustrated in **Figure 3**.

The important consequence of selection for air breathing has been to preserve or open ecological access to habitats that would otherwise be inaccessible to non-air-breathing fishes. In terms of the mechanism for selection, the initial steps toward air breathing probably emerged from hypoxia adaptations common to non-air-breathing fishes. A key behavioral response to hypoxia is searching for nearby water having a higher O_2 concentration, such as more shallow areas or at the water surface. Many non-air-breathing fishes use aquatic surface respiration (ASR), a behavior in which the mouth is positioned as close to the water surface as possible in

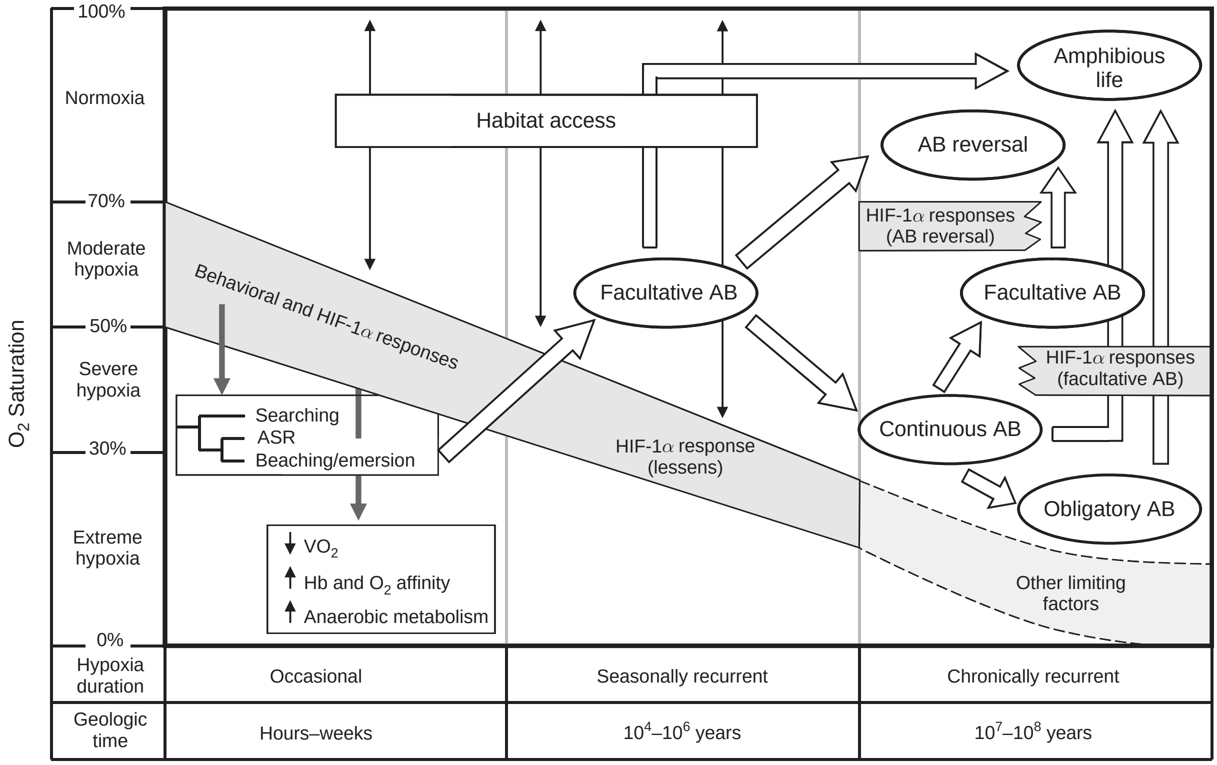


Figure 3 Integrated aspects of environmental hypoxia adaptation and the selective mechanisms operating over different periods of time leading to the evolution of air breathing and terrestriality in different fish groups and its effect of opening limited habitats for occupation and promoting radiation and diversification. Behavioral responses such as ASR that takes a fish close to the surface increased the potential for inadvertent air gulping may have been a major selective factor in the origin of air breathing. Specialization for hypoxia and air breathing would lead to down regulation of HIF-1α response mechanisms. Modified from Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge University Press.

order to ventilate the gills with the upper few millimeters of water that remains O_2 rich because of atmospheric diffusion. A further refinement in ASR, one that directly relates to the origin of air breathing, is gulping air which is done to increase buoyancy and place the mouth in a better position for surface ventilation. Whether air is gulped intentionally or inadvertently, the O_2 it contains has the potential to enrich the O_2 supply and could contribute to aerobic metabolism if the bubble contacts an absorptive epithelial surface in the mouth or other area, or if it is held in and oxygenates the ASR ventilatory stream. In this way, initial selection for gulping to aid ASR could, in recurrent conditions of environmental hypoxia extending over at time frame of perhaps 10^4–10^6 years or longer, select for adaptations leading to the use of aerial O_2.

Activation of the hypoxia-inducible factor (HIF-1α), an ancient eukaryote adaptation predating the origin of vertebrates, is another key hypoxia response. HIF-1α induction activates genes promoting O_2 transfer (i.e., erythropoiesis, Hb affinity increases, and angiogenesis) and upregulates metabolic adaptation through expression of genes controlling anaerobiosis and O_2 consumption rate (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function). The efficacy of HIF-1α has been demonstrated for both water-ventilating and air-breathing fishes and, as illustrated in **Figure 3**, it would play a key adaptive role during irregular and brief (i.e., hours–weeks) periods of aquatic hypoxia experienced by a non-air-breathing fish. Under such conditions, HIF-1α expression would be continually reinforced by natural selection (i.e., individuals having an effective HIF-1α response would survive and propagate the next generation), and, among populations of species experiencing chronic recurrences of severe hypoxia (i.e., 30–40% air saturation) over an extended time, both the suite of adaptive responses encompassed by HIF-1α and the threshold of its onset would likely change (**Figure 3**).

Both the increase in blood hemoglobin (Hb) concentration and the reduction of the quantity of intraerythrocytic nucleoside triphosphates (e.g., adenosine and glutamine triphosphate) resulting in a left-shift (increased) Hb-O_2 affinity in facultatively air-breathing loricariid catfish (*L. chagresi*, *Pterogoplicthys multiradiatus*, and other species) are signature features of HIF-1α induction. This affinity increase enables a fish to bind more O_2 in hypoxic water, lessens the potential for trans-branchial loss, and reduces critical oxygen partial pressure (P_{O_2}) and thus increases its proficiency for aquatic respiration in hypoxia. Moreover, the annual dry-season increase in loricariid Hb concentration that occurs in advance of either drying or hypoxia suggests that there has been selection for a preadaptive change that will mitigate these conditions if they occur.

Strong selection for more proficient air breathing, occurring over geologic time and driven by both the environmental change and the diversification and radiation of groups into different, and more variably oxygenated habitats, would have also contributed to the origin of continuous air breathing and ushered in morphological and physiological changes, such as a reduction in gill area to lessen the potential for O_2 loss, leading to obligatory air breathing (**Figure 3**). The respiratory specializations of different groups, brought on by regular exposure to environmental hypoxia over periods of probably 10^7–10^8 years, concomitantly reduce the importance of an aquatic-hypoxia-induced HIF-1α response; *Lepidosiren* and *Protopterus*, for example, are obligatory air breathers that are so proficient at air breathing that they are largely insensitive to the effect of aquatic conditions such as low P_{O_2} that control respiration and trigger air breathing in most other aquatic air breathers. The high degree of respiratory independence from water conditions afforded by well-developed air breathing expands potential habitat access to the point where it becomes limited by factors such as the absence of prey and adverse conditions such as temperature, drying, and lack of cover and H_2S.

Air Breathing and Natural History

Adaptive Radiations

Air breathing appears to have been an important factor in the radiation and diversification of certain groups. Many of the species in the families Callicthyidae and Clariidae, and all of the species in the five families comprising the anabantoid suborder (Anabantidae, Belontiidae, Helostomatidae, Osphronemidae, and Luciocephalidae), breathe air. Taken together, these groups comprise a large fraction of the total air-breathing fish diversity (about 14% of the families, 16% of the genera, and 39% of the species). Air breathing permits populations to expand into habitats where, because of adverse conditions, ecological success is assured through limited competition from non-air-breathers. In this way, formerly contiguous populations may become reproductively isolated and undergo genetic divergence into separate species. The degree of diversification present among the anabantoids illustrates that, despite the requirement to sustain air breathing, extensive adaptive radiation can result in dramatic modifications in dentition, jaw structure, and diet, as well as body shape and size. These functional changes have not altered ABO respiratory function; however, dependence on air breathing seems to have become more relaxed in some of the anabantoids that are not obligatory air breathers (*Sandelia capensis*, *S. bainsi*, *Trichogaster* sp., and *Colisa lalia*).

Another pattern of adaptive radiation is seen in the African clariid catfishes, many of which have evolved into habitats in which air breathing is not required. The ABO has regressed in species of *Dinotopterus* occurring in open waters and depths of Lakes Malawi and Tanganyika. Several other regressed genera (*Xenoclarias*, one species; *Clariallabes*, 16 species; *Gymnallabes*, three species; and *Taganikallabes*, one species) are small and anguilliform with reduced fins. Most of these fishes have taken up life in swift streams where they burrow in rubble and even make amphibious excursions and feed out of water (*Channallabes apus*), while others live in lakes at considerable depth. With their independence from habitat conditions, the fully bimodal clariids achieved a broad distribution across both Africa and Asia, whereas the regressive clariids are largely confined to a narrow central African distribution where they have diversified into unfilled niches. A parallel occurs in the two species of the anabantoid genus *Sandelia* which, having radiated into more oxygenated habitats of southern Africa, have a highly reduced ABO.

An additional aspect of aquatic air breathing now demonstrated for numerous species is synchronous air breathing, a behavior characterized by the closely timed and nearly simultaneous air gulping by a group. This behavior is a form of temporal schooling that reduces the danger of predation during surfacing for air.

The transition to land is another dimension of fish air breathing. Amphibious air breathers must respire without water contact and thus risk desiccation as well as the interruption of the normal functions of the gills in water such as CO_2 release, ion regulation, acid–base balance, and nitrogen excretion (**see also Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange, **Circulation**: Circulatory System Design: Roles and Principles, and **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System). Amphibious air-breathing fishes are seldom far from water; however, air breathing does permit littoral-zone exploitation and is reflected in attendant biochemical, physiological, morphological, and behavioral adaptations for terrestriality. In the case of mudskippers, which are the most terrestrial teleosts, other facultative air breathing but mainly nonamphibious species (*Pseudapocryptes lanceolatus*, *Apocryptes bata*, and others) are members of the same clade (the goby subfamily Oxudercinae); however, for mudskippers the vacant niches of the mudflat proved to be the catalyst for amphibious life. Mudskipper amphibious adaptations include fin and body modifications for terrestrial crawling (crutching), skipping, jumping, and climbing. They also have prominent eyes and a good visual acuity in air. Although their lateral line is reduced, mudskippers can hear airborne sound (250–600 Hz). Also, their physiology is specialized to limit desiccation, to allow osmoregulation in different salinities, and to modify nitrogen excretion for water conservation. Some species can also sense the O_2 content of the air in their burrow. While most mudskippers retain the capacity for aquatic respiration, at least one species in the genus *Periophthalmodon* (*Pn. schlosseri*) is an obligatory air breather. Amphibious life poses a problem for vision because of the refractive differences of air and water. Solutions include, for some mudskippers, reduction of lens curvature to compensate for the added corneal refraction in air or, in *Dialommus macrocephalus* and some blennies (*E. nigricans*), flat corneas reduce aerial astigmatism and allow emmetropia in air without compromising aquatic accommodation. Amphibious fishes have the capacity to orient to water by using known reference points or possibly sun compass. Pit traps set at night near tropical streams demonstrate that some aquatic air breathers also leave the water. *Clarias lazera* was discovered to leave water at night to feed on stored grain while the amphibious, regressive air breather *Channallabes apus* is able to capture prey on land using a snapping (not suction) jaw action.

Ancillary Air-Breathing Functions

Aspects of air breathing have become integrated into functions unrelated to respiration. In *N. forsteri*, *Piabucina festae*, *H. thoracatum*, *Umbra limi*, *H. plecostomus*, and others, the ABO contributes to static lift (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). The loricariids have a stomach ABO and in some species thick, air-filled diverticulae that branch off from the esophageal–stomach junction and encircle the stomach contribute to near-neutral buoyancy which is important for foraging on vertical surfaces, submerged roots, or tree branches. Buoyancy control may also take precedence over

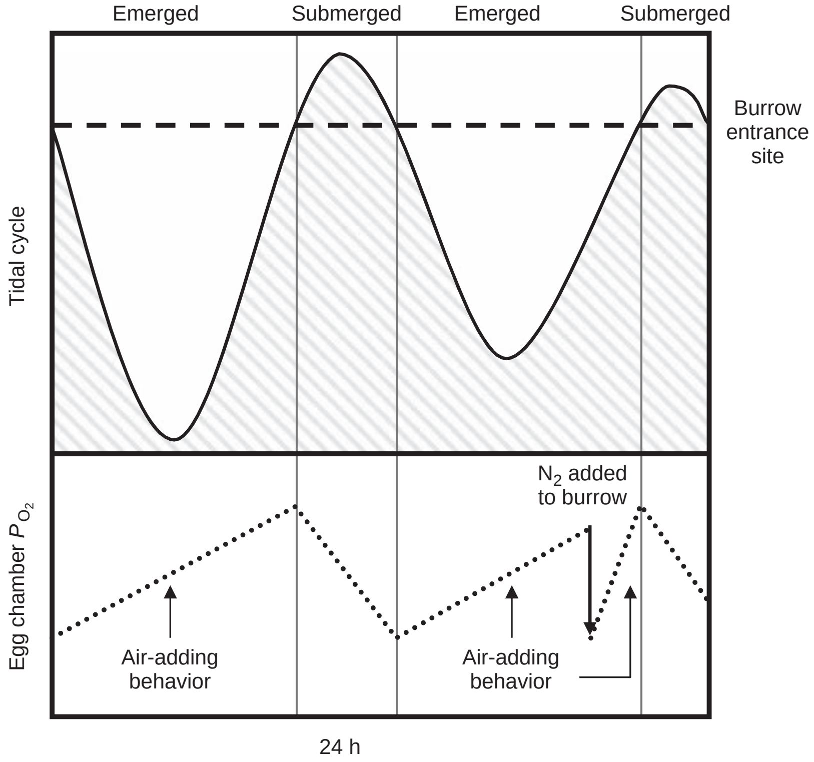


Figure 4 Tidal-cycle effects on the P_{O_2} of the egg chamber in the mudflat burrow of the mudskipper, *P. modestus*. The male mudskipper guards the intertidal mudflat nest and attends to the eggs, which hatch about 7 days postfertilization. During high tide, the burrow entrance is submerged and egg respiration combined with the reducing environment of the anoxic mud and possibly the respiration of the guarding fish reduce the egg chamber O_2 content. When the tide level recedes below the burrow opening, the male uses air-gulp transportation to raise egg-chamber O_2 content in preparation for the next high tide. The experimental injection of N_2 gas into the egg chamber causes the male fish to increase its frequency of air-deposition behavior in order to raise burrow P_{O_2} before the next incoming tide covers the burrow entrance. Modified from Ishimatsu A, Yoshida Y, Itoki N, *et al.* (2007) Mudskippers brood their eggs in air but submerge them for hatching. *Journal of Experimental Biology* 210: 3946–3954; and Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge University Press.

respiratory needs in some fishes; species of *Lepisosteus* and *A. calva* are known to regulate ABO volume for buoyancy purposes, and when in water mudskippers inflate their branchial chambers with enough air to allow them to float with their eyes in air. In anabantoids, bubbles derived from air breathing have a role in territoriality, courtship displays, and bubble-nest construction (*Betta splendens* and other species), and the ABO of these fishes is used to make sounds important in courtship behavior. *H. thoracatum* and other callichthyids also build bubble nests; the diffusive properties of these structures remain uninvestigated. Some anabantoid species (*Macropodus, Betta*) are mouth brooders, as is *A. gigas* and some species of *Chann*a; how this is coordinated with air breathing is unknown.

Air breathing plays a role in nest oxygenation. In male *Lepidosiren*, aerially obtained O_2 is exuded into the nests via gill-like filaments that form on their paired fins. (These were early studies that were controversial and more data are needed. Also, accounts differ as to whether the gill filaments form only on the pelvic fins or on both paired fins.) A comparable function has been suggested for the skin of *Synbranchus* and for cirri on the heads of male loricariids. Mudskippers incubate eggs in air-filled chambers within their littoral-zone mud burrows. An egg-tending male *P. modestus* can sense the P_{O_2} in its air chamber. During high tide when the burrow is submerged, chamber P_{O_2} decreases and during low tide the fish restores chamber P_{O_2} by bringing in gulps of fresh air. If air-chamber P_{O_2} is lowered by pumping in N_2, the fish responds with a greater rate of air transport (**Figure 4**).

Summary

Air breathing is an ancient fish adaptation that has independently evolved in many groups over 400 million years. The diversity of these fishes and their different types of ABOs and air-breathing behaviors all signify the efficacy of natural selection in achieving a nearly perfect solution to the common occurrence of environmental hypoxia in shallow water habitats throughout the long history of fish evolution. Diverse air-breathing groups demonstrate the central role of aquatic hypoxia in driving the independent origin of air breathing and for, over the expanse of geologic time, either intensifying or relaxing selection for air breathing and related adaptations, all in concert with changes in environmental factors, with shifts in the patterns of expansion and ecological radiation and morphological changes in a particular group. Specifically, the capacity of fishes to breathe air was an important precondition for the evolution of amphibious life, both in the tetrapodomorphs and in many extant air-breathing fishes such as the mudskippers. Although air breathing seemingly presents the perfect answer to shallow-water environmental hypoxia, not all fishes exposed to this environmental challenge answer it with air breathing. However, for the majority of air breathers this capacity enables them to remain in and better exploit an aquatic habitat in which they could not otherwise survive and associated with this are the examples of how air breathing has secondarily influenced other aspects of natural history. Because the independent evolution of air breathing is viewed as an ongoing process, it is likely that some fishes, because of environmental change or their potential for ecological radiation into less favorable habitats, may now be undergoing the initial selective processes leading to the acquisition of this capacity.

Acknowledgments

Research for this paper was supported by the UCSD Academic Senate and by NSF0922569.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; Respiratory Adaptations for Air-Breathing Fishes. **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Swimbladder Function and Buoyancy Control in Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; Branchial Anatomy. **Design and Physiology of Capillaries and Secondary Circulation**: Circulatory Fluid Balance and Transcapillary Exchange. **Design and Physiology of the Heart**: The Coronary Circulation. **Gas Exchange**: Respiration: An Introduction. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function. **Ventilation and Animal Respiration**: Plasticity in Gill Morphology.

Further Reading

Burggren WW (1982) "Air gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish, *Carassius auratus*. *Physiological Zoology* 55: 327–333.

Carter GS (1957) Air breathing. In: Brown M (ed.) *Physiology of Fishes*, pp. 65–79. New York: Academic Press.

Chapman LJ and Liem KF (1991) Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. *Environmental Biology of Fishes* 44: 183–197.

Clack JA (2002) *Gaining Ground: The Origin and Early Evolution of Tetrapods*. Bloomington, IN: Indiana University Press.

Farrell AP (2007) Cardiovascular systems in primitive fishes. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Primitive Fishes, Fish Physiology, vol.* 26, pp 53–120. San Diego, CA: Elsevier.

Gonzales TT, Katoh M, and Ishimatsu A (2006) Air breathing of the aquatic burrow-dwelling eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae). *Journal of Experimental Biology* 209: 1085–1092.

Graham JB (1976) Respiratory adaptations of marine air-breathing fishes. In: Hughes GM (ed.) *Respiration of Amphibious Vertebrates*, pp. 165–187. London: Academic Press.

Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press.

Graham JB (2006) Aquatic and aerial respiration. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 85–117. Boca Raton, FL: CRC Press.

Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge University Press.

Greenwood PH (1987) The natural history of African lungfishes. In: Bemis WE, Burggren WW, and Kemp NE (eds.) *The Biology and Evolution of Lungfishes*, pp. 163–179. New York: Liss.

Ishimatsu A, Aguilar NM, Ogawa K, *et al.* (1999) Arterial blood gas levels and cardiovascular function during varying environmental conditions in a mudskipper, *P. schlosseri*. *Journal of Experimental Biology* 202: 1753–1762.

Ishimatsu A, Yoshida Y, Itoki N, *et al.* (2007) Mudskippers brood their eggs in air but submerge them for hatching. *Journal of Experimental Biology* 210: 3946–3954.

Janvier P (2007) Living primitive fishes and fishes from deep time. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Primitive Fishes*, Fish Physiology, vol. 26, pp. 1–51. San Diego, CA: Elsevier.

Liem KL (1989) Respiratory gas bladders in teleosts: Functional conservation and morphological diversity. *American Zoologist* 29: 333–352.

Randall DJ, Burggren WW, Farrell AP, and Haswell MS (1981) *The Evolution of Air Breathing in Vertebrates*. Cambridge: Cambridge University Press.

Sayer MDJ (2005) Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries* 6: 186–211.

Taylor DS, Turner BJ, Davis WP, and Chapman BB (2008) A novel terrestrial fish habitat inside emergent logs. *American Naturalist* 171: 263–266.

Wassenbergh SV, Herrel DA, Hiysentruytt F, Devaeret S, and Aerts P (2006) A catfish that can strike its prey on land. *Nature* 440: 881.

Wells NA and Dorr JA (1985) Form and function of the fish *Bothriolepis* (Devonian; Placodermi, Antiarchi): The first terrestrial vertebrate? *Michigan Academician* 17: 157–173.

Respiratory Adaptations for Air-Breathing Fishes

JB Graham, University of California at San Diego, La Jolla, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Types
The Lung and Respiratory Gas Bladder
Other ABOs
Head Region
Digestive Tube
Gills
Skin
ABO Ventilation
Further Reading

Glossary

Air-breathing organ (ABO) A structure or body surface of a fish having the specialized structural capacity for aerial gas exchange; utilized by air-breathing fishes to supplement the normal respiratory mode which is aquatic respiration using gills.

Bimodal respiration The capacity to exchange respiratory gases with either air or water and to do so either simultaneously or sequentially.

Ediculae A terminal gas-exchange unit in the honeycombed aerial respiratory structure offish lungs and respiratory gas bladders characterized by being wider than they are deep.

Faveolae A terminal gas-exchange unit in honeycombed aerial respiratory structure of fish lungs and respiratory gas bladders characterized by being deeper than they are wide.

Lung The principal aerial respiratory organ of all vertebrates and some primitive air-breathing fishes. An outpocketing along the vertebrate digestive tube having a vascular epitheilial surface that functions for aerial gas exchange through its connection to the body surface by a duct through which air can be inhaled and exhaled.

Parenchyma The essential and distinctive tissues of an organ.

Respiratory epithelium A specialized respiratory surface consisting of a single or few layers of epithelial cells ranging from <1 to 15 μm thickness and covering a network of capillaries (also referred to as a vascular epithelium).

Surfactant A chemical that stabilizes mixtures of oil and water by reducing the surface tension at their interface. As in their mammalian counterpart, the surfactant in fish lungs and respiratory gas bladders, and other gut derived ABOs serves to reduce the surface tension of fluids on these surfaces and promotes tissue-elastic properties.

Type I cells Squamous epithelial cells associated with a respiratory exchange surface.

Type II cells Cuboidal epithelial cells usually associated with a respiratory surface and characterized by the presence of lamellate (double membrane) layers.

Introduction

Another article has detailed the phylogenetic relationships among the air-breathing fishes and provided a functional classification for air breathing, emphasizing both the mode (aquatic or amphibious air breathing) and the level of dependence (facultative, obligate) of a species for aerial O_2 (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). The objective of this article is to survey the structure and function of all air-breathing organs (ABOs), including the organs and tissues having functions other than aerial respiration. This article also presents a classification system for ABOs that incorporates position, structure, and function within a phylogenetic framework and describes the different mechanisms used for ABO ventilation.

Types

Most early accounts of air breathing classified fish ABOs on the basis of body position but this approach provided little comparative morphological data, did not account for phylogenetic influences, and was not in most cases inclusive of species that breathe air without a specialized ABO. A comprehensive classification of the ABO based on structure, position, and degree of development results in three groups:

I. Derivatives of the digestive tube including
   a. the lung and a physostomous gas bladder having a respiratory function and b. other sections of the digestive tube including the esophagus, pneumatic duct, stomach, and intestine.

II. Areas in and near the head, such as the buccal, pharyngeal, and opercular chambers having a dense covering of respiratory epithelium and, in most cases, an enhanced surface area, due to either or both an added chamber volume and the projection into it of branchial-arch extensions having a covering of respiratory epithelium.

III. Other organs such as the gills and skin, having structural modifications promoting aerial gas exchange.

Evolution

The three ABO categories reflect the sequence of changes in air breathing that occurred with the progressive evolution of the bony fishes (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction; **Figure 1**). Basal to group Ia is the lung, the plesiomorphic vertebrate ABO, which occurs in the three lungfish genera (*Neoceratodus forsteri*, *Lepidosiren paradoxa*, *Protopterus* (four spp., **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) and in the polypterids (*Erpetoichthys calabaricus* and *Polypterus* about 14 species). Also in this group are 18 actinopterygian genera, including several teleosts, having a physostomous respiratory gas bladder which also often functions for buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes) and for either or both sound reception and sound production (**see also Hearing and Lateral Line**: Psychoacoustics: What Fish Hear and Sound Source Localization and Directional Hearing in Fishes). As emphasized in **Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction, many of these gas bladder respiratory functions evolved independently.

Natural History

With few exceptions, groups I and II include most of the freshwater aquatic air-breathing fishes. Groups Ib, II, and III encompass the phase of novel ABOs in which, due its evolutionary specialization, the gas bladder was no longer suited for recruitment, through natural selection, for ABO function. The course of teleost radiation into many diverse ecological niches provides examples of groups in which the gas bladder was lost, became physoclistous (i.e., no pneumatic duct), or was embedded in bone to increase sound sensitivity. Group III consists mainly of

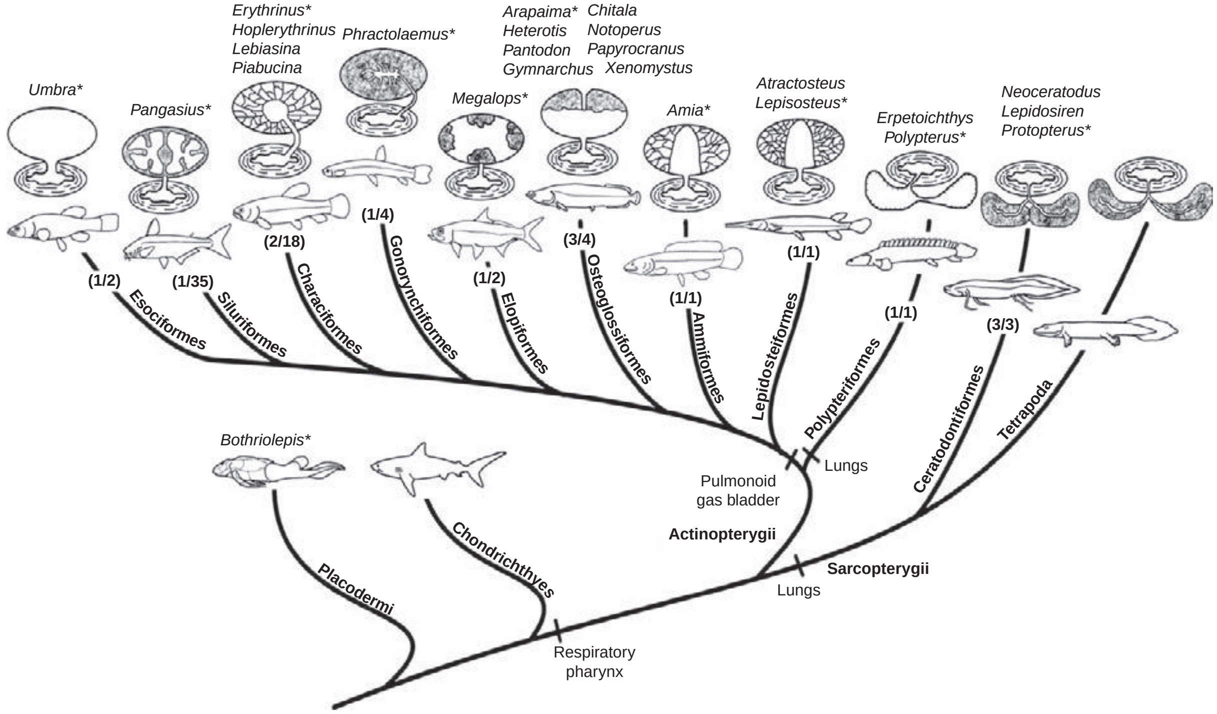


Figure 1 Occurrence and structural diversity of the lungs and respiratory gas bladders in 10 orders of extant fishes and the tetrapods. Shown for each fish order is the relative number of families having this type of ABO and a list of genera with the organ. Head-end views of transverse sections show the gut (thick with dashed lines) and the relative complexity of the ABO parenchyma. Pneumatic duct details for *Neoceratodus* are not illustrated (see **Figure 2**). Reproduced from Fig. 6.2 in Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge Press.

amphibious fishes, which take up O_2 and remove CO_2 via their skin or gills, and, in some cases, also have a well-developed respiratory epithelium in their buccal, pharyngeal, branchial, or opercular chambers.

Function

For most species in groups I and II, the ABO is an enclosed space into which air is inspired; aerial O_2 is absorbed across a respiratory epithelium (a thin layer of cells under which is a network of capillaries) and becomes bound to hemoglobin (Hb) in erythrocytes for circulation to the tissues (**see also Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin). When most of the O_2 in the inspired breath has been absorbed, this air is expelled and a new breath is taken.

Although a range of ABO structures is represented by categories I and II, these share two common features: (1) being located in close proximity to the digestive tract which provides fairly direct contact with air taken in by the mouth and (2) close proximity to circulatory network surrounding the digestive tract, an essential feature for O_2 removal from the ABO. Another common feature of ABOs of the aquatic air breathers is that these organs function mainly for the uptake of O_2 and not for the release of CO_2, which remains the province of gills and other surfaces contacting water. This is in contrast to amphibious air breathers which are capable of a high aerial carbon dioxide release rate during aerobic respiration ($\dot{V}_{CO_2}$).

The Lung and Respiratory Gas Bladder

Figure 1 shows the phyletic distribution of lungs and respiratory gas bladders among the vertebrates. Lungfishes not only breathe air with a lung but also use their gills and skin for aquatic respiration, especially *Neoceratodus*. While not an air breather, the coelacanth, *Latimeria chalumnae*, a primitive lobefin related to lungfish and the Tetrapodomorpha, has a vestigial, fat-invested lung-like organ supplied by a pulmonary circulation (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes). Among actinopterygians only the polypterids (the many species *Polypterus* and *E. calabaricus*) utilize lung respiration. The actinopterygians using a respiratory gas bladder are dispersed among eight orders, 11 families, and 18 genera. Furthermore, they include both primitive nonteleosts such as the gars (*Lepisosteus* and *Atractosteus*, about seven species in total), the bowfin (*Amia calva*), and six teleost orders (**Figure 1**). Among these only *Amia*, a primitive species considered the common ancestor of the group that gave rise to the teleosts, has a respiratory gas bladder; however, this species also has a pulmonary circulation (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

Morphology

The four morphological features distinguishing the fish lung are:

1. formed as a ventral outpocketing or budding of the posterior embryonic pharynx;
2. paired or bilobed tube extending into the posterior, mainly ventral part of the peritoneal cavity;
3. tubular connection (pneumatic duct) with the pharynx, guarded by the glottal valve; and
4. perfusion by a pulmonary circulation – the arteries of this originate from the efferent arteries of either or both branchial arches 3 and 4, while a pulmonary vein returns O_2-rich blood to or near the heart (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

The four contrasting features of the physostomous respiratory gas bladder are:

1. formed as a budding of the dorsal or lateral wall of the posterior embryonic pharynx;
2. single or bilobed tube occurring within the dorsal mesentery and occupying the upper region of the peritoneum;
3. long or short pneumatic-duct connection with the top or side of the pharynx that is generally without a glottis; and
4. blood circulation that in most cases is nonpulmonary – the arterial supply is by a branch from the dorsal aorta and return to the heart is via the hepatic portal system (or the postcardinal vein; **see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

Evolutionary Relationship

In the *Origin of Species*, Darwin pointed to what he assumed had been a gradual transformation of a fully functional organ for fish buoyancy regulation into a fully functional lung as an example of descent with change. This would have entailed a vertical shift in organ position and a 180° rotation in the site of attachment of the pneumatic duct. Over a century ago, Bashford Dean illustrated this progression by arranging a sequence of species (unfortunately, without regard to precise anatomical detail or phylogeny) having different degrees of pneumatic duct migration (**Figure 2**). Later, as phylogenetic evidence showed that the lung and not the gas bladder was the primitive organ, Dean's illustration still served to illustrate the evolutionary progression, however, in the opposite direction!

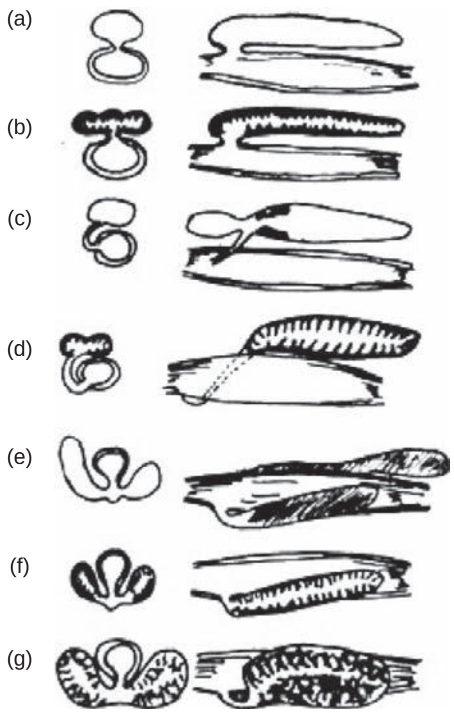


Figure 2 Modified version of Bashford Dean's illustration of the evolutionary progression of gut and lung or gas bladder position in fishes and tetrapods. Double-layered structure is gut. Dark or striated areas are respiratory epithelium. (a) Sturgeon (no aerial respiration). (b) *Lepisosteus* and *Amia.* (c) *Erythrinus;* pneumatic duct (note its left side origin). (d) *Neoceratodus;* ventrally originating pneumatic duct ascends right side. (e) *Polypterus*; note smaller left lung. (f) *Lepidosiren* and *Protopterus.* (g) Tetrapods. Reproduced from Fig. 3.4 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

The relationship between the lung and gas bladder of fishes remains controversial. **Figure 1** demonstrates the lung's basal function in respiration and shows the evolutionary trend for the gas bladder's transition from (1) respiratory to nonrespiratory (although a number of groups such as the pangasiids have secondarily returned to a respiratory gas bladder (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) and (2) physostomous (pneumatic duct present) to physoclistous (no duct). The classical view is that aerial respiration is a basal osteichthyan character and that there was a single origin of the osteichthyan lung, which is homologous with the tetrapod lung and the lung of early actinopterygians. However, **Figure 1** suggests that, while the lungs of sarcopterygians and tetrapods are homologous, polypterid lungs may have evolved independently. While similarities in ultrastructure, cell type, and surfactant properties of the two organs are expected based on their common site of embryonic differentiation (posterior respiratory pharynx immediately behind the gill arches), the many structural differences between the two organs argue against the idea of single origin of the lung or for a sequential (by means of vertical movement and rotation) transformation of a functional lung to a functional gas bladder. The suggestion that the site of the embryonic pouch formation also determines the organ's pattern of blood flow (i.e., a pulmonary circulation formed with lungs and an in-series circulation formed with a respiratory gas bladder) is not supported by *A. calva*, which has a respiratory gas bladder supported by a pulmonary circulation (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

Epithelial and Structural Complexity

The respiratory surfaces of lungs and gas bladders have type I (squamous) and II (cuboidal) epithelial cells and chemically similar surfactant proteins. Surfactant, which is important for ABO inflation, is formed by membrane-bound lamellar bodies contained within type II cells and contains a mixture of surface-active lipids and proteins that lower the surface tension of the fluid lining the respiratory surface. Surfactant occurs in the epithelial lining of the pharynx and alimentary canal as well as in fish lungs, both respiratory and nonrespiratory gas bladders, and in ABO stomachs and intestines.

Despite underlying similarities, the gross morphology of lungs and respiratory gas bladders differs markedly among species (**Figures 1–5**). Lungfish lungs have a highly complex respiratory surface comprised of parenchymal septa containing smooth muscle and subdividing the lumen into a honeycomb of small alveolar-like respiratory chambers or units termed 'ediculae' (wider than deep) and faveolae (deeper than wide) into which is invested a dense capillary bed lying within a few micrometers of the air surface. Structural differences among the three lungfish genera correlate with differences in air-breathing capacity. The lung in the facultative air-breathing *N. forsteri* is unpaired, occurs entirely in the dorsal part of the body cavity, and has both a smaller respiratory surface area and a much longer pneumatic duct, which arises on the ventral pharynx and extends dorsally to the lung (**Figure 2**(**d**)). In contrast, the paired lungs of *Protopterus* and *Lepidosiren*, which are similar to one another, have greater septation. Also, these lungs are fused at the front where they form a common chamber below the pharynx to which they are connected by a short, vertical, and valved pneumatic duct (**Figures 1** and **2**(**f**)). Posterior to this fusion, the lungs separate as they project dorsally and extend nearly to the end of the coelom.

Polypterid lungs (**Figures 1–3**(**a**)) are paired but the left lobe, which has a ductal connection to the right lobe and not the pneumatic duct, is also truncated by the stomach. These lungs lack the structural complexity of lungfishes; the respiratory epithelium lines the walls but

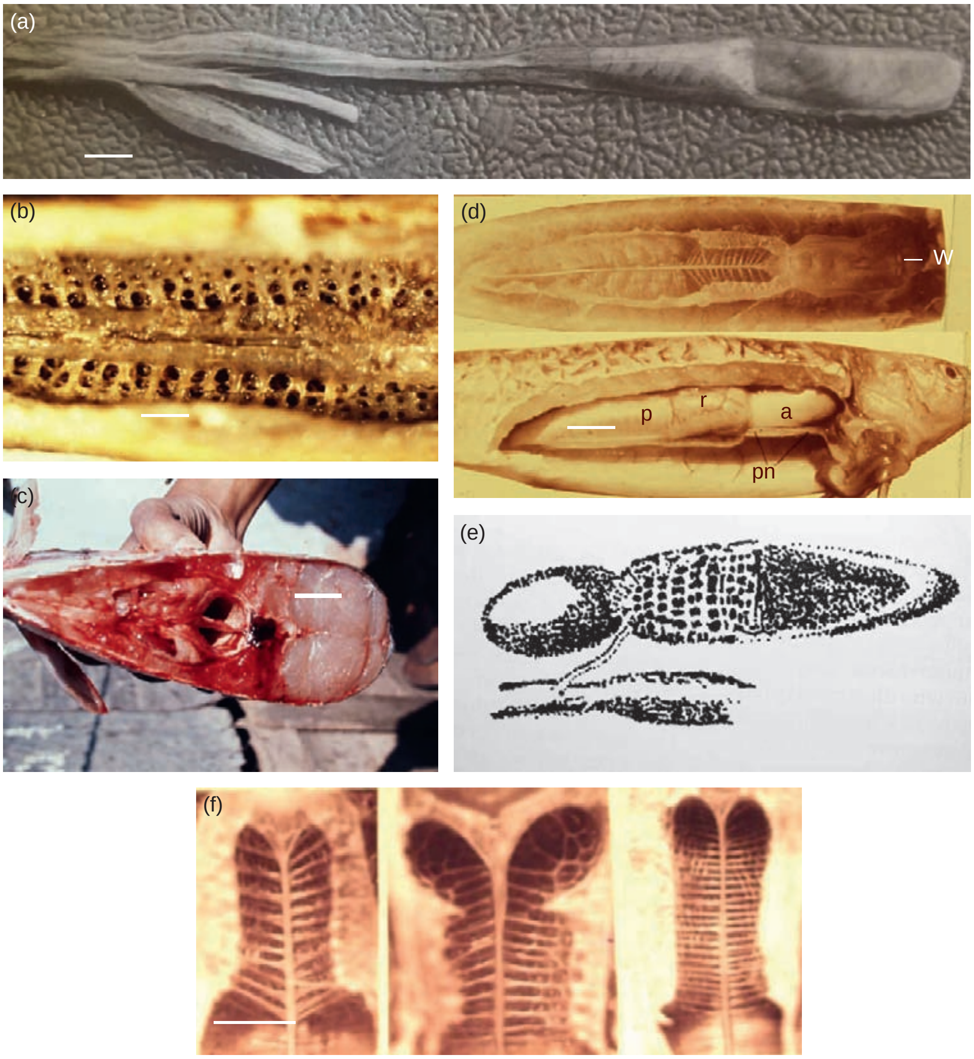


Figure 3 Lungs and respiratory gas bladders of some air-breathing fishes. (a) *Polypterus* sp.; note shorter left lung and expanded posterior section of right lung. Scale = 1 cm. (b) *Arapaima gigas*, ventral view; note kidneys contained within the gas bladder. Scale = 0.5 cm. (c) *N. notopterus*; note vertical septum in the respiratory region. Scale = 2 cm. (d) *Piabucina festae* showing side and ventral (cut away) views of the three chambers of the respiratory gas bladder; p, posterior; r, respiratory; a, anterior; pn, pneumatic duct. Note Webberian ossicles, W attached to dorsal wall of anterior chamber. Scale = ~2 cm. (e) Copy of drawing of the respiratory gas bladder in *Lebiasina bimaculata* by W.S. Rowntree in 1903. (f) Comparison of the respiratory sections of *Piabucina* (left), *E. erythrinus* (center), and *Hoplerythrinus unitaeniatus* (right). Scale = 1 cm. Reproduced from Figs. 3.7, 3.10, 3.13, 3.14, 3.16, and 3.17 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

does not extend into the lumen and there are prominent concentrations of ciliated cells in and among the type I and II cells (**Figure 4**(**a**)).

Variations in the structural complexity of the respiratory surfaces of gas bladders are detailed in **Figures 1–5**. Highly compartmentalized and septate ediculae occur in the respiratory gas bladders of *Arapaima*, *Amia*, *Lepisosteus*, *Megalops*, *Phractolaemus*, *Piabucina*, *Erythrinus*, *Hoplerythrinus*, *Pangasius*, and other genera. The retroperitoneal expansion of the gas bladders of *Arapaima* (**Figure 3**(**b**)) and *Pantodon bucholzi* encompasses the kidneys and in *Pantodon*, which floats at the surface, air spaces penetrating the vertebral column add buoyancy. The respiratory gas bladders of the characins *Erythrinus*, *Hoplerythrinus*, *Lebiasina*, and *Piabucina* (**Figure 3**(**d**)–**3**(**f**)) have three chambers: the complex honeycomb of the respiratory section differs markedly among the genera, the posterior section has linear tendons that aid in organ contraction for exhalation, and the anterior chamber, which is partially divided from the respiratory section, attaches to the Webberian ossicles for transmission of sound vibrations to the brain. Like characins, the anterior section of the gas bladder in *Megalops* (two species) contacts the brain's sound-reception center (**Figure 4**(**c**)). In

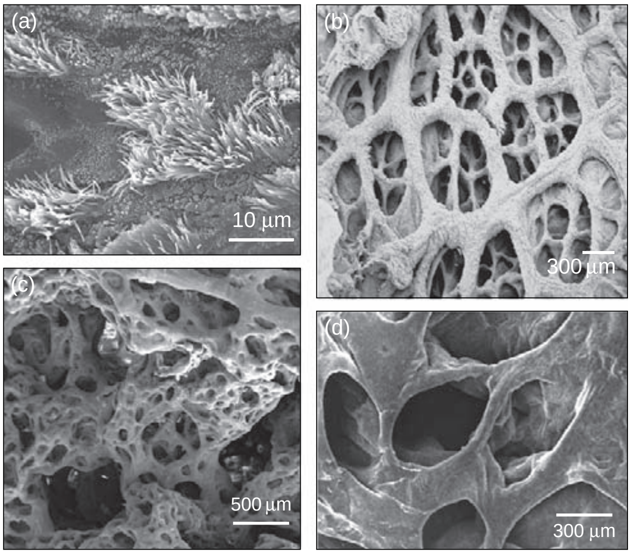


Figure 4 Scanning electron microscope (SEM) images of respiratory surfaces showing alveolar-like parenchyma with a respiratory matrix. (a) *Polypterus senegalus,* detailing cilia and small folds in the respiratory epithelium. (b) *Protopterus* lung (image courtesy of Dr. John Maina). (c) *Megalops* respiratory gas bladder. (d) *Pangasius* gas bladder (image courtesy of Dr. Dagmara Podkowa). Reproduced from Figs. 6.4 and 6.5 in Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life With and Without Oxygen*, pp. 174–220. Cambridge: Cambridge Press.

addition to its respiratory section, the gas bladder of the featherback (*Notopterus* and other genera in this family, **Figures 1** and **3(c)**; **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) has buoyancy and both sound-reception and production functions. By contrast, the respiratory gas bladder of *Umbra limi*, one of the most derived teleosts using a respiratory gas bladder, has relatively simple epithelium that does not project into the lumen (**Figure 1**).

Other ABOs

The evolution of the physoclistous gas bladder occurred independently in many teleosts; it eliminated the pneumatic duct and largely ended gas-bladder utility for air breathing. However, as teleosts continued their radiation, additional requirements for amphibious and aquatic air breathing resulted in novel ABOs and, while diverse, these share common features of an enclosed space that can be ventilated and a respiratory epithelium with ample blood supply.

Head Region

The simplest ABO in the head region forms through augmentation of the respiratory function of the buccal, pharyngeal, branchial, or opercular chambers with a covering of respiratory epithelium. The next complexity is to add more air volume, done by the addition of accessory chambers, sacs, or pouches, usually in the branchial chamber. Further refinements involve reshaping of the ventral skull bones to allow an even greater air volume, and further augmenting these spaces by filling them with multiply branched or faceted structures covered by respiratory epithelium.

Modified Chambers

Species in several families have independently converged on the use of the mouth, branchial chamber, and opercular chamber as ABOs and all of these fishes have relatively large buccopharyngeal chamber volumes (**Figure 6**). *Electrophorus electricus* has reduced gills and is an aquatic, obligate air breather. An extensive respiratory epithelium covers nearly the entire interior of its mouth, from the jaw to the pharynx, and also covers the branchial arches and the inner walls of the operculum. The mouth epithelium is formed into rows of vascularized tufts that extend from both the dorsal and ventral sides. When the mouth is closed, these rows are interdigitated and fill the buccal space with an absorptive epithelium.

All of air-breathing gobies hold air in their mouths. *Gillichthys mirabilis* is both a facultative and amphibious air breather. It has vascular surfaces on the roof of its mouth and tongue. All four mudskipper genera (*Periophthalmus*, *Periophthalmodon, Scartelaos,* and *Boleophthalmus*, about 25 species total; **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) have exceptionally large (up to 17% relative to mass) buccopharyngeal chamber volumes. In these and other closely related species

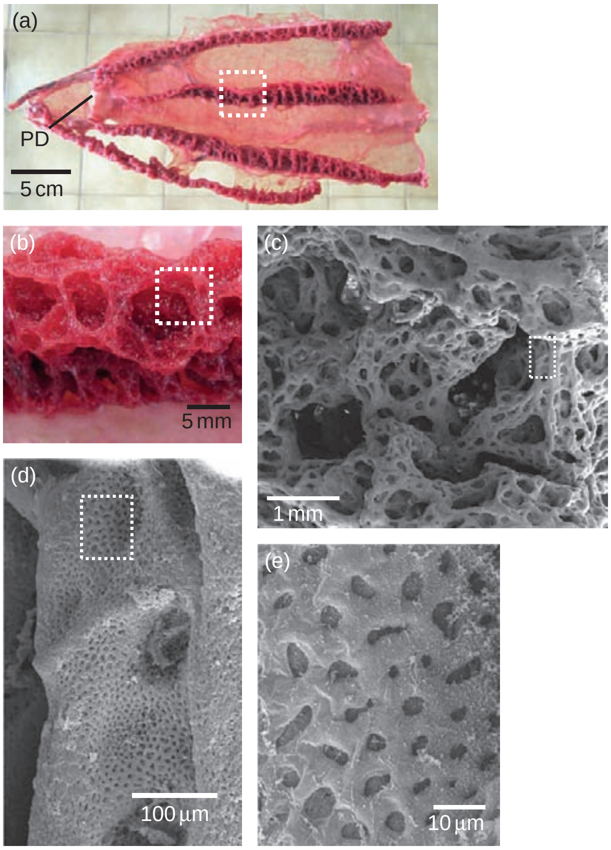


Figure 5 (a) Respiratory gas bladder of a 13.7-kg *Megalops atlanticus* opened along its dorsal side to reveal the internal surface and show the four ridges of respiratory tissue extending along its length. The dorsal ridge (at bottom) extends anterior of the pneumatic duct (PD) into the organ's narrow projection under the skull (augmenting sound reception). (b) Enlarged section of the box in (a) showing the faveolate surface surfaces of the respiratory ridge. (c) SEM of the box in (b) showing interconnection of septa forming faveolae and ediculae of different size. (d) Magnification of the box in (c) showing the respiratory surface of a septum. (e) Enlargement of the box in (d) showing the respiratory epithelium. Reproduced from Fig. 1.6 in Seymour RS, Wegner NC, and Graham JB (2008) Body size and the air-breathing organ of the Atlantic tarpon *Megalops atlanticus*. *Comparative Biochemistry and Physiology* 150A: 282–287.

(e.g., *Pseudapocryptes lanceolatus*) in the subfamily Oxudercinae, a vascular epithelium lines the surfaces of the mouth, pharyngeal, branchial, and opercular chambers. The relative area of these surfaces has not been determined and this would need to be known to determine the relative contribution of each to O_2 uptake. The expanded branchiostegal membrane and reduced opercular opening of mudskippers help to ensure air-breath retention.

Air held in the buccal chamber of the eel goby (*Odontamblyopus lacepedii*) envelopes the gills and contacts vascular beds on the inner wall of each operculum and other buccopharyngeal surfaces, all of which are functional for aerial O_2 uptake. A facultative, aquatic air breather, *Odontamblyopus*, gulps a large enough air volume to float its head and anterior body, and completely empties its ABO during exhalation.

The swamp eel, *Synbranchus marmoratus*, is both an aquatic and an amphibious air breather. A respiratory epithelium extends throughout the buccal and opercular chambers; however, this fish has a complete set of gills and is a facultative air breather. Its buccopharyngeal chamber is large and during air breathing it also holds sufficient air to float its head and upper body. The opercular openings are combined and reduced to a single ventral port which aids in air retention.

Suprabranchial Chambers

A sac or space extending from the top of the branchial chamber, the suprabranchial chamber (SBC), enhances the ABO volume and surface area. The SBC has independently evolved in species of four different families. The swamp eel, *Monopterus cuchia*, a close relative of *Synbranchus marmoratus* (above), is also an amphibious and aquatic air breather with a large buccopharyngeal chamber covered by a respiratory epithelium. However, *Monopterus* differs from *Synbranchus* in having a pair of small, respiratory epithelium-covered SBCs extending in a dorsal-posterior direction from the branchial chamber (**Figure 6**). *Monopterus* is similar to *Electrophorus* in having no gills on its branchial arches and unlike *Synbranchus* it is an obligate air breather.

Species in three clariid catfish genera (*Clarias, Heterobranchus,* and *Dinotopterus,* about 40 species in total, **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) have large, paired SBCs extending from the skull to beyond the pectoral girdle (**Figure 7**). Contained within these are respiratory trees or dendrites that emerge from the epibranchials of two gill arches. In addition, gill filaments at the dorsal end of all four gill arches are fused to form fans that partially seal the lateral side of the SBC and serve as valves for ventilation. The gill epithelium covering the SBC, the fans, and the entire surface of the dendrites is formed by gill filaments that unfold, lose their cartilaginous supports, and undergo modification in aspect ratio, but retain their signature pillar cells (**Figure 7**). A muscle sheet surrounding the posterior SBC compresses it for exhalation.

The Indian catfish (*Heteropneustes fossilis*) also has large, paired SBCs bordered by gill fans (**Figure 8**). No dendrites are present but SBC area is expanded by paired tubes that extend into the body musculature to a distance of about one-half body length. The respiratory epithelium covering the SBC and the inner surface of the fans are also formed by modified gill lamellae. Contraction of muscle sheets surrounding the SBC projections causes exhalation.

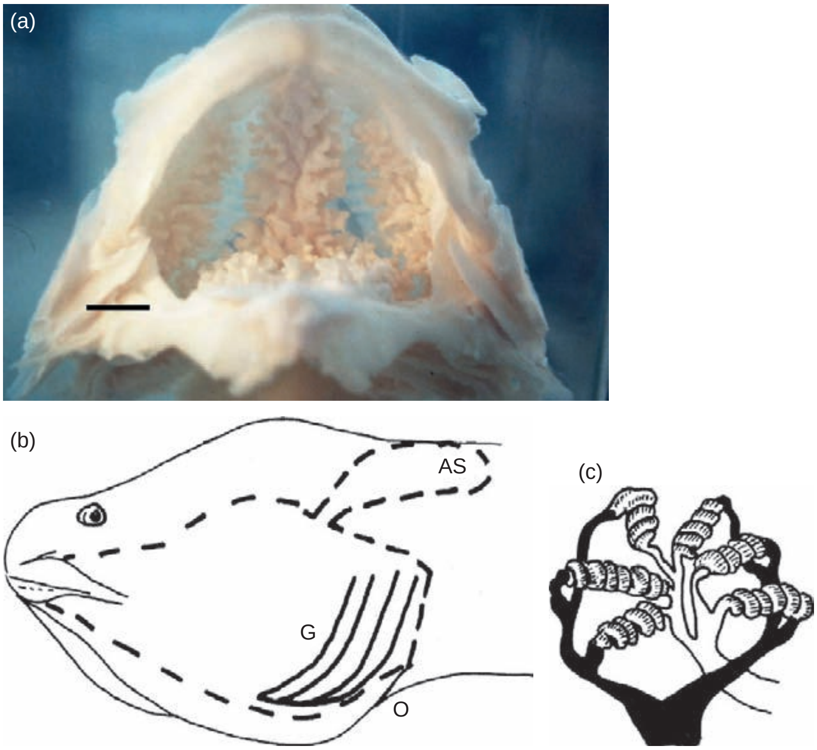


Figure 6 (a) Dorsal view of the lower jaw epithelium of *Electrophorus electricus.* Scale = 1 cm. (b) Side view of *Monopterus cuchia* with inflated ABO showing relative positions of the branchial chamber air sacs (AS), gill arches (G), and the position of the reduced opercular opening (O). (c) Capillary rosette detail. Reproduced from Figs. 3.20 and 3.25 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

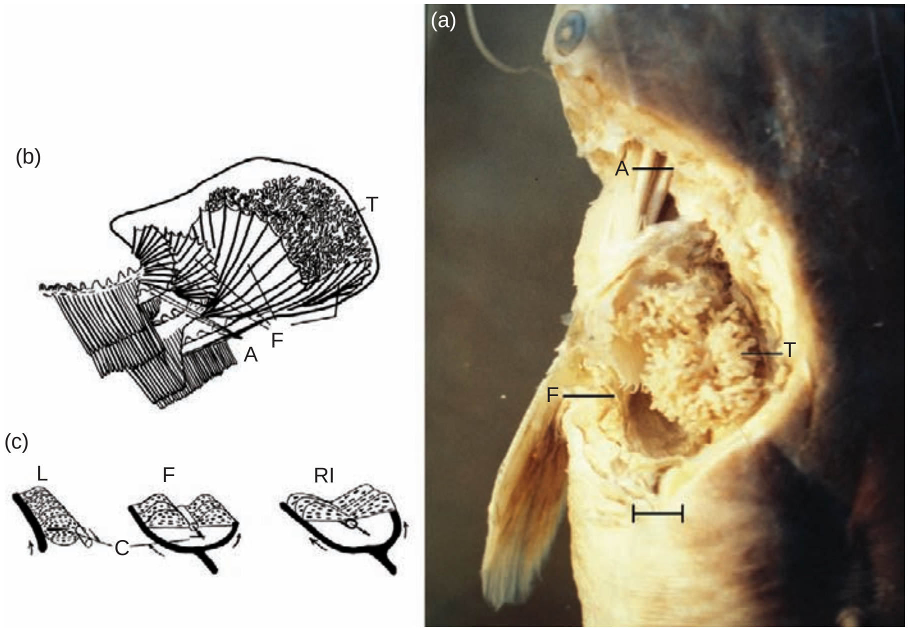


Figure 7 (a) Dorsal view of the SBC of a 35-cm *Clarias gariepinus* showing the two respiratory trees (T), gill fans (F), and gill arches (A). Scale = 2 cm. (b) Side view of the same features. (c) Probable stages in the transition of clariid gill lamellae (L) into gill fans (F) and the respiratory epithelium rows occurring on the dendrites, fans, and SBC walls. Reproduced from Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

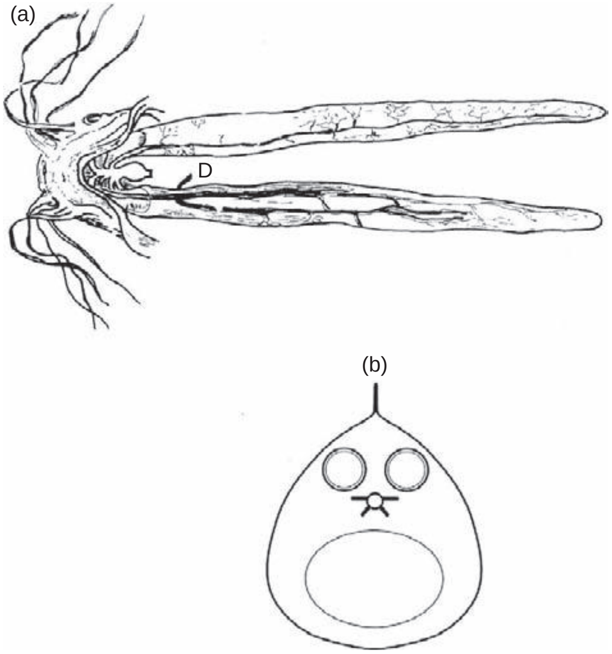


Figure 8 (a) Modified drawing of the SBC of *Heteropneustes fossilis* made by J. Hyrtl in 1854. (b) Transverse body section showing air-sac penetration into the body myotomes. Reproduced from Fig. 3.30 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

The large, paired SBCs of the anabantoids extend from behind the eye to the pectoral girdle. Protruding into each is the labyrinth, a bony element formed by the epibranchial of gill arch 1 (**Figure 9**). Labyrinth size and complexity relate to air-breathing capacity. In the obligatory air breather *Anabas testudineus*, this structure fills the SBC and has ridges that amplify its surface (**Figure 9**). The labyrinth and SBC form a rigid structure that cannot be compressed for exhalation. However, like the threads on a screw, the labyrinth's surface is continuous with the SBC contour, which means that water pumped through the chamber will not stagnate or pool. This is important for ABO ventilation, which is done by forcing water through the chamber to displace stale air and then refilling it with fresh air. The respiratory epithelium covering the labyrinth and the SBC forms parallel rows of capillaries having ring-shaped endothelial cells. Although similar to the respiratory surface in clariids and *Heteropneustes*, this epithelium does not have pillar cells.

The large channid SBC is covered by a respiratory epithelium as are bony surfaces extending into it from the first branchial and hyomandibular arches and the parasphenoid bone (**Figure 10**). The vascular structure of this epithelium, however, differs from the anabantoids; it forms islets, featuring rosettes of spiraling capillary loops, different segments of which emerge into the ABO. This epithelial structure is similar to *Monopterus* (**Figure 7**). The channid SBC is similar to the anabantoid SBC in being largely incompressible, thus requiring water flow for ventilation.

Digestive Tube

Esophagus

The amphibious blenny *Lipophrys* (=*Blennius*) *pholis* holds air in its esophagus, which has longitudinal folds containing many capillaries. There are no respiration data.

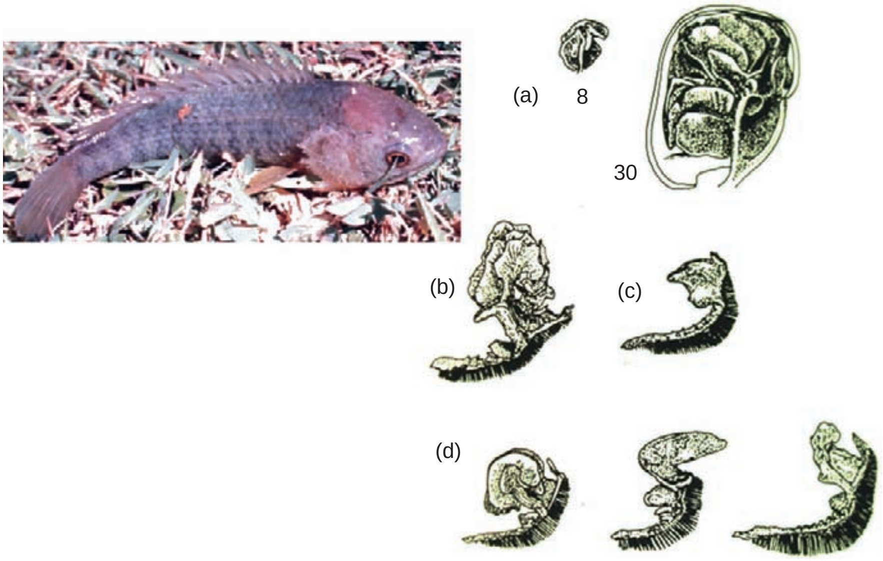


Figure 9 (Top left) *Anabas testudineus* and drawings of the labyrinth structure of several anabantoids. (a) *Osphronemus goramy* 8 cm and 20 cm total length; (b) *Anabas*; (c) *Sandelia*. (d) Three species of *Ctenopoma*: *C. nigropannosum,* left; *C. nanum,* center; *C. kingsleyae,* right. Reproduced from Figs. 2.11 and 3.32 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

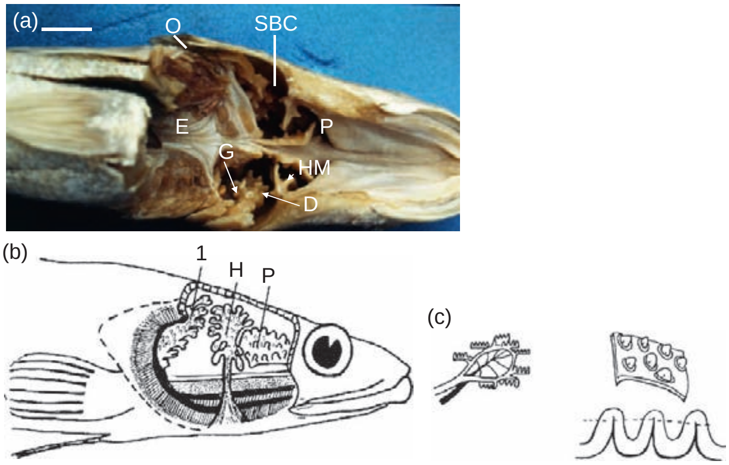


Figure 10 (a) Ventral view of the SBC of a 30-cm *Channa micropeltes*. Head was sectioned at level of the esophagus (E) and roof of mouth to show openings into the SBC. O, operculum; G, gill arches; D, dendrites on arches 1 and 2. Dendrites also on hyomandibular (H) and parasphenoid (P) bones. Scale = 1 cm. (b) Side view showing dendrite penetration into the SBC. (c) Detail for the capillary rosettes and spiraling. Reproduced from Figs. 3.23 and 3.24 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

The esophagus of the blackfish, *Dallia pectoralis*, is richly endowed with capillaries positioned within 1 μm of the surface along area-increasing micro-ridges formed by cuboidal epithelium. *Dallia* has a gas bladder that does not function for air breathing. Compression of striated muscle surrounding the esophagus and gas bladder drives exhalation by this fish.

Pneumatic Duct

The gas bladder of the eel *A. anguilla* (also other species) is physostomous; however, it also has a gas gland, the vascularized area within the gas-bladder wall that slowly secretes O_2 into the organ for buoyancy. While *Anguilla* has a pneumatic duct, this structure's esophageal opening is gated by a sphincter valve, which may limit air ventilation. The pneumatic duct is richly vascularized, and absorbs O_2 from the gas bladder, which supplies respiration. While out of water *Anguilla* makes use of this oxygen supply and also breathes through its gills and skin.

Stomach

Stomach ABO function is documented for many loricariid species. Air is gulped and swallowed and, once O_2 is depleted, the breath is burped out and exits from the operculae. Volume measurements of expired air confirm that the stomach ABO is completely emptied after each air breath. Anatomical studies show a stomach ultrastructure comparable to lungs and gas bladders in having a large number of capillaries but relatively few digestive cells near the inner surface. In *Liposarcus spinosus* (= *Ancistrus* sp.), a dense capillary mesh forms in the corpus region but not in either the cardiac or pylorus regions (**Figure 11**). A survey of over 40 loricariid genera indicates graded character states in stomach size and, in some species, accessory air chambers that function for buoyancy surround the respiratory stomach and always contain air. Stomach air breathing has also been confirmed in two other South American catfish families (Trichomycteridae, *Pygidium striatum*; Scoloplacidae, *Lithoxus lithoides*; **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction).

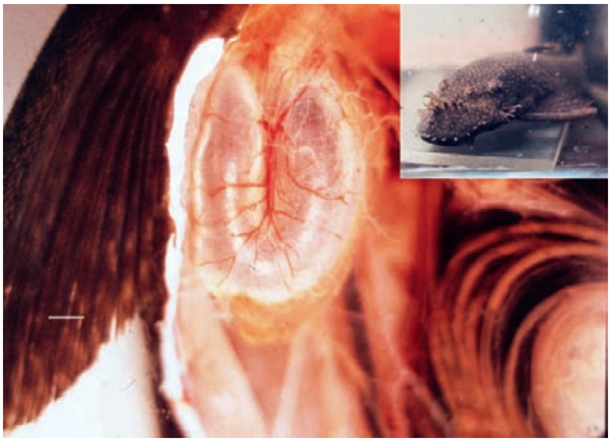


Figure 11 Air-filled stomach ABO of *Liposarchus* (= *Ancistrus*) *spinosus* on the right side of the abdomen. Note right pectoral fin and spiral intestine. Scale = 0.5 cm. (Inset) Fish exhaling air. Reproduced from Figs. 2.6 and 3.37 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

Intestine

Intestinal air breathing evolved independently in two groups, the New World catfishes (Callicthyidae, *Callichthys asper, Hoplosternum thoracatum*) and the Old World loaches (Cobitididae, *Lepidocephalichthys guntea*, *Misgurnis anguillicaudatus*). In both the groups, swallowed air is passed through the digestive intestine and into the posterior, respiratory section. Once O_2 is removed from this gas, it is ejected from the anus, usually at the same instant that new air is gulped. In both the families, the digestive intestine has the important added function of compressing and then enclosing the feces in mucus clumps to minimize their size and maximize air contact in the respiratory surface. The respiratory intestine, normally inflated in both these groups, has thin, complaint walls and a high capillary density with diffusion distances ranging from less than 1 to 3 μm. In *H. thoracatum*, the respiratory intestine is perfectly aligned with the body axis and functions in buoyancy control. During air exposure, the intestine of the amphibious marine Chilean clingfish (*Sicyases sanguineus*) contains air and the vessels of this organ are engorged with blood; there are no respiratory data.

Gills

Respiration

Although the walls of the branchial chambers of the amphibious blennies (*Entomacrodus nigricans*) and rockskippers (*Dialommus macrocephalus*) lack a prominent respiratory epithelium, oxygen partial pressure (P_{O_2}) measurements indicate that branchial oxygen consumption rate ($\dot{V}_{O_2}$) takes place in air, which implicates the gills in this process. The contribution of gills to aerial respiration cannot be quantified because most species that hold air in their branchial chambers also have a respiratory epithelium lining that chamber's surface.

Modifications Related to Air Breathing

Respiratory modifications of gills for amphibious air breathing include larger cartilaginous support rods in the filaments, which are often curved to reduce contact with adjacent filaments and thus lessen the chance of coalescence and collapse out of water (**Figure 12**). Relative to other species, mudskippers have a smaller gill area, which is compensated by the respiratory epithelium contained in their expanded buccopharyngeal chambers. This is important for *Periophthalmodon*, in which a cell matrix covering the interlamellar spaces of its filaments greatly reduces gill respiratory function and makes it an obligate air breather (**Figure 12**).

Among aquatic air breathers, reductions in gill area resulting from the diminution or the complete absence of filaments from some branchial arches are a specialization that increases air-breathing efficiency and is most common in species that use continuous aquatic air breathing or are obligate air breathers. In *Electrophorus*, *Monopterus*, and anabantoids, the lungfish *Lepidosiren* and *Protopterus*, and other species, some or all of the branchial arches extending from near the heart (ventral side of the body) to the dorsal aorta (which supplies the systemic circulation from the dorsal side of body) do not have functional gill filaments and function solely as circulatory conduits (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

On the other hand, the gill area of many facultative air breathers is not reduced and comparable to the gill areas of non-air-breathing species. Among loricariids, the gill areas of a 100-g *Rhinelepis strigosa* (20 000 mm^2) and a 100-g *Hypostomus plecostomus* (9000 mm^2) are both within the range of most non-air-breathing freshwater fishes and higher than values for most air-breathing fishes. Similarly, the facultative Australian lungfish (*Neoceratodus*) has gills on all branchial arches and its estimated total gill area of about 2500 cm^2 in a 6-kg fish (= 417 $cm^2\ kg^{-1}$) is comparable to that of non-air-breathing freshwater fishes (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics).

Skin

Cutaneous respiration is documented for most of the major fish groups and only a few differences distinguish the air-breathing species (**see also Gas Exchange**: Respiration: An Introduction). Many developing fishes breathe exclusively through their skin prior to gill development. Larval *Monopterus* respire through extensive subepithelial capillary networks. Posthatch *Neoceratodus* have a ciliated respiratory epithelium covering their body surface.

Fish skin is a less effective gas-exchange organ than either the gills or ABO because of its greater thickness, the added diffusion barriers of scales and mucus, and low perfusion and ventilation potentials. Among species shown to have cutaneous respiration, water–blood diffusion distances range from 50 to 400 μm, and there is no consistent relationship between features such as scales, epidermal thickness, or amount of vascularization, and the rate of cutaneous O_2 transfer. For example, a cutaneous $\dot{V}_{O_2}$ of 32% of total $\dot{V}_{O_2}$ was measured for the heavily scaled *Erpetoichthys calabaricus*. Fish skin is metabolically active; the epidermis contains a living epithelium as well as sensory and secretory cells, all of which receive nutrition via dermal capillaries. Cutaneous respiration may serve the skin but not deeper aerobic

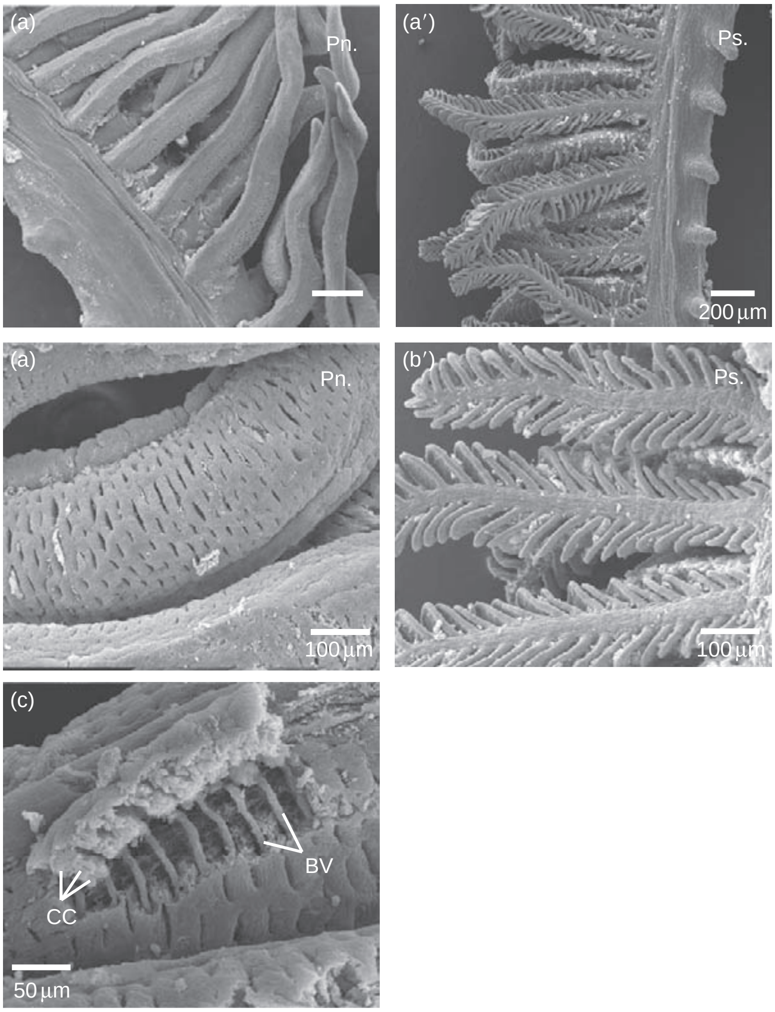


Figure 12 (a) SEM images of the gill arch-filament regions in: (a) Pn. *Periophthalmodon schlosseri* (185 mm, 54.0 g, 70×) and (a′) Ps. *Periophthalmus modestus* (71 mm, 3.0 g, 150×).(b) Pn. 400×. (b′) Ps. 400×. (c) Pn. image at 800× showing blood vessels (BV) beneath the lamellar matrix. CC, chloride cell. Reproduced from Figs. 14.2, 14.3, and 14.6 Graham JB, Lee HJ, and Wegner NC (2007) Transition from water to land in an extant group of fishes: Air breathing and the acquisition sequence of adaptations for amphibious life in oxudercine gobies. In: Fernandes MN, Rantin FT, Glass ML, Kapoor BG (eds.) *Fish Respiration and Environment*, pp. 255–288. Enfield, NH: Science Publishers.

requirements. Such a function would, however, be limited in hypoxic or stagnant water, which minimizes diffusion.

Specializations for skin respiration in amphibious air-breathing fishes include the presence of epidermal capillaries (reduce air–blood diffusion distance) along the dorsal body surface (this area is readily exposed to air and makes less contact with the substrate). In mudskippers, which obtain about half of their O_2 via the skin, air–blood diffusion distances can be less than 5 μm along the dorsal-body surface but as much as 150−200 μm at other sites. In *Kryptolebias* (= *Rivulus*) *marmoratus*, which is totally reliant on skin respiration in air, capillaries on its dorsal body surface are within 1 μm of the body surface. Among aquatic air breathers, the most dramatic application of cutaneous respiration occurs in the eleotrid, *Dormitator latifrons*. In hypoxic water, this fish hyperinflates its physoclistous gas bladder and becomes positively buoyant, thus emerging its forehead to expose a dense capillary network that is engorged with blood and functions for aerial respiration (**Figure 13**).

ABO Ventilation

Ventilation of lungs and respiratory gas bladders involves the combined actions of the buccal force pump and swallowing for inhalation, and a combination of hydrostatic pressure and the contraction of muscles within the wall of the ABO or around it for exhalation. Many lung breathers exhale prior to inhaling. However, many gas-bladder air breathers inhale first and then exhale, which results in some mixing of the new and

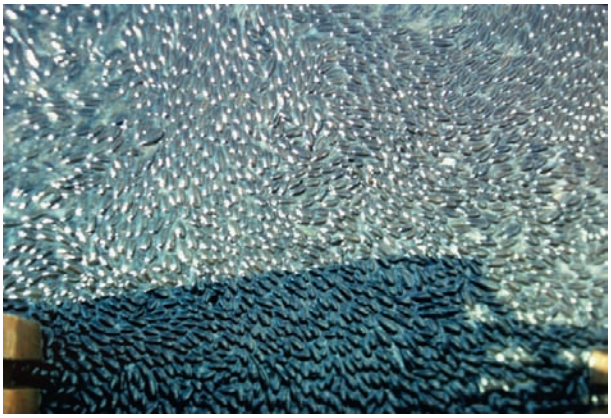


Figure 13 Overhead view of a large number of *Dormitator latifrons* aggregated at a dam in Panama. Each fish is positively buoyant and has its aerial-respiratory frontal skin patch exposed to air. Reproduced from Fig. 2.9 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

stale air. In the case of the *Hoplerythrinus* (**Figure 3(f)**), new air is sequestered into the anterior chamber of the gas bladder and only after O_2-depleted air in the respiratory and posterior sections is ejected is the new air pushed into these chambers.

Polypterids are distinguished from all other lung or gas-bladder air breathers in using recoil aspiration in which contraction of muscles within the lung forces exhalation and also causes inward flexion of the thickly scaled body walls as well as the generation of a negative intraperitoneal pressure. Inhalation is then driven by the vacuum generated by the recoil of the body walls. New data from this laboratory document that aspiratory air breathing by *Polypterus* is coupled with the use of the spiracles for air intake. These structures, which are vestiges of the hyomandibular gill opening, are covered by spiracle bones that function as valves and are opened during the few milliseconds when the fish is at the surface for an air breath (**Figure 14**). This finding has implications for the early evolution of breathing mechanisms in the Tetrapodomorphs, which also had spiracles.

Both gulping and swallowing are required for the sequestration of air in the stomach and intestinal ABOs. For loricariids, ABO emptying is via emesis followed by the release of air bubbles from the operculae; this ensures complete turnover of the ABO volume. Nonmixing of the old and new air is also achieved by intestinal air breathers, which rely on the indelicate action of flatulence to vent old air. While discretely masked by simultaneous occurrence with a new air gulp, this is also a necessary contingency for buoyancy control.

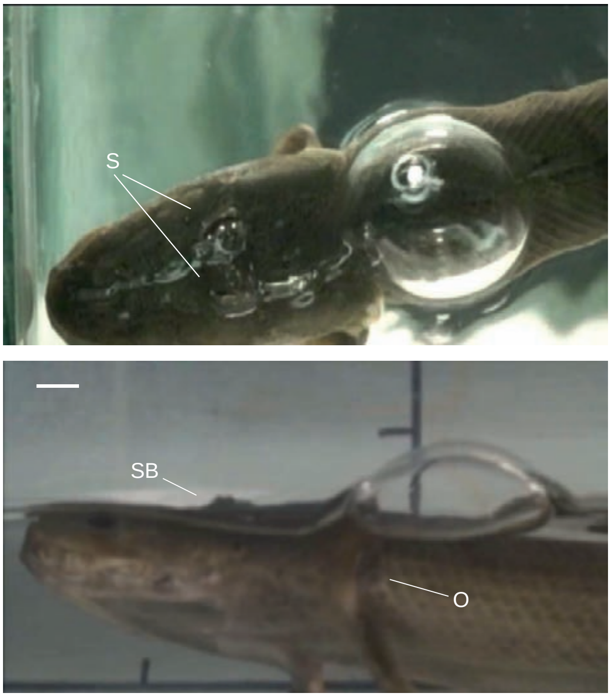


Figure 14 Top and side views of *Polypterus delhezi* at the instant in the air-breathing cycle when it is at the surface with the spiracles open for recoil-aspiration filling of the lungs. Spiracles (S) appear as dark crescents in top view. Side view showing erect spiracle bone (SB), the valve that covers the spiracle. Bubble is previous breath just released from the operculum (O). Scale = 1 cm.

The SBCs of clariids and *Heteropneustes* are filled by aspiration and buccal force and readily emptied by the action of muscles enclosing the posterior sectors of these chambers. In contrast, the SBCs of anabantoids and the channids are situated high above the gills, are intimately connected to the skull bones, and are noncompliant. The purging of an exhausted air breath from these organs requires the complex action of reversed water flow, analogous to the fish coughing reflex used to void debris from the gills. Reversed and fast water flow through the SBC, orchestrated by as many as four different hydraulic pulses initiated using different combinations of jaw and opercular actions, effectively floods out the old air and then displaces the water with new air, also resulting in a 100% breath replacement.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Gas Exchange**: Respiration: An Introduction. **Hearing and Lateral Line**: Psychoacoustics: What Fish Hear; Sound Source Localization and Directional Hearing in Fishes. **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Armbruster JW (1998) Modifications of the digestive tract for holding air in loricariid and scoloplacid catfishes. *Copeia* 1998: 663–675.

Cruz AL Pendretti ACE, and Fernandes MN (2009) Stereological estimation of the surface area and oxygen diffusing capacity of the respiratory stomach of the air-breathing armored catfish *Pterygoplichthys anisitsi* (Teleostei: Loricariidae). *Journal of Morphology* 270: 601–614.

Gonzales TT, Katoh M, and Ishimatsu A (2006) Air breathing of the aquatic burrow-dwelling eel goby, *Odontamblyopus lacepedii (Gobiidae: Amblyopinae). Journal of Experimental Biology* 209: 1085–1092.

Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press.

Graham JB (2006) Aquatic and aerial respiration. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 85–117. Boca Raton, FL: CRC Press.

Graham JB, Lee HJ, and Wegner NC (2007) Transition from water to land in an extant group of fishes: Air breathing and the acquisition sequence of adaptations for amphibious life in oxudercine gobies. In: Fernandes MN, Rantin FT, Glass ML, and Kapoor BG (eds.) *Fish Respiration and Environment*, pp. 255–288. Enfield, NH: Science Publishers.

Graham JB and Wegner NC (2010) Breathing air in water and in air: The air-breathing fishes. In: Nilsson GE (ed.) *Respiratory Physiology of Vertebrates: Life with and without Oxygen*, pp. 174–220. Cambridge: Cambridge Press.

Ishimatsu A, Aguilar NM, Ogawa K, *et al.* (1999) Arterial blood gas levels and cardiovascular function during varying environmental conditions in a mudskipper, *Periophthalmodon schlosseri. Journal of Experimental Biology* 202: 3946–3954.

Kramer DL (1978) Ventilation of the respiratory gas bladder in *Hoplerythrinus unitaeniatus* (Pisces, Characoidei, Erythrinidae). *Canadian Journal of Zoology* 56: 931–938.

Liem KL (1988) Form and function of lungs: The evolution of air breathing mechanisms. *American Zoologist* 28: 739–759.

Liem KL (1989) Respiratory gas bladders in teleosts: Functional conservation and morphological diversity. *American Zoologist* 29: 333–352.

Maina JN (2002) *Functional Morphology of the Vertebrate Respiratory System*. Enfield, NH: Science Publishers.

Ong KJ, Stevens ED, and Wright PA (2007) Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. *Journal of Experimental Biology* 210: 1109–1115.

Palzenberger M and Pohla H (1992) Gill surface area of water-breathing freshwater fish. *Review of Fish Biology and Fisheries* 2: 187–216.

Perna SA and Fernandes MN (1996) Gill morphometry of the facultative air-breathing loricariid fish, *Hypostomus plecostomus* (Walbaum) with special emphasis on aquatic respiration. *Fish Physiology and Biochemistry* 15: 213–220.

Podkowa D and Goniakowska-Witalinska L (1998) The structure of the air bladder of the catfish *Pangasius hypophthalmus* Roberts and Vidthayanon 1991 (previously *P. sutchi* Fowler 1937). *Folia Biologica (Krakow)* 46: 189–196.

Podkowa D and Goniakowska-Witalinska L (2002) Adaptations to air breathing in the posterior intestine of the catfish *Corydoras aeneus*, Callichthyidae): A histological and ultrastructural study. *Folia Biologica (Krakow)* 50: 69–82.

Podkowa D and Goniakowska-Witalinska L (2003) Morphology of the air-breathing stomach of the catfish *Hypostomus plecostomus. Journal of Morphology* 257: 147–163.

Seymour RS, Wegner NC, and Graham JB (2008) Body size and the air-breathing organ of the Atlantic tarpon. *Megalops atlanticus. Comparative Biochemistry and Physiology* 150A: 282–287.

Zhang J, Taniguchi T, Takita T, and Ali AB (2003) A study on the epidermal structure of *Periophthalmodon* and *Periophthalmus* mudskippers with reference to their terrestrial adaptation. *Ichthyological Research* 50: 310–317.

Circulatory Adaptations for Air-Breathing Fishes

JB Graham, University of California at San Diego, La Jolla, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Defining the Problem
Patterns of ABO Circulation: Lungs and Respiratory Gas Bladders
Circulation Patterns for Other ABOs
Coronary Circulation and Air Breathing
Conclusions
Further Reading

Glossary

Bimodal respiration The capacity to exchange respiratory gases with either air or water and to do so either simultaneously or sequentially.

Bradycardia A slowing of normal heart rate. In fish, this condition is often associated with hypoxia (low environmental oxygen levels).

Circulatory shunt Characterizing the presence of vessels that permit blood to bypass a particular organ. The term 'gill shunt', for example, indicates that conditions can occur in which blood normally destined for the gills bypasses that organ.

Diffusing capacity The rate of transfer, expressed in terms of the partial pressure diffusion gradient, of the respiratory gases O_2 or CO_2 between an external medium such as air or water and the blood.

Ductus arteriosus The blood vessel connecting the pulmonary artery and the dorsal aorta that, when dilated, shunts blood from the lung to the systemic circulation.

Enforced unsaturation A reduction in blood P_{O_2} caused by the convergence of two vessels, one carrying oxygenated blood and the other deoxygenated blood.

Pulmonary vasomotor segment (PAVS) Vasoactive section of the lungfish pulmonary artery that, through either vasoconstriction or vasodilation, regulates blood flow into the lung.

Respiratory sinus arrhythmia Central control mechanism integrating heart rate with the respiration cycle.

Tachycardia Increased heart rate.

Transbranchial deoxygenation A phenomenon associated with in-series circulation in which, during the transit of blood through the gills, the oxygen acquired in the ABO undergoes outward diffusion to ambient, hypoxic water.

Introduction

This article examines the patterns of blood flow to and from the air-breathing organs (ABOs) of fishes. It builds on information about the diversity and the phylogenetic relationships of the bimodally breathing fishes (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) and on their ABO structure (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes). The independent origin of air breathing resulted in a diversity of ABO types as well as a range of patterns for the flow of blood to and away from these organs (**Figure 1**). Fishes have an in-series circulation pattern in which blood flows in a single circuit from the heart to gills to the systemic circulation and back to the heart (**see also Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns). Because all air-breathing fishes have functional gills, the addition of an ABO to the in-series flow introduces problems for both the conservation and effective delivery of the aerially acquired O_2 to the systemic circulation and for ensuring sufficient contact between the blood and water in the gills needed for the vital ancillary gill functions of ion regulation, nitrogen excretion, and the release of respiratory CO_2 (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion, **Gas Exchange**: Respiration: An Introduction, and **Control of Respiration**: The Ventilatory Response to CO_2/H^+).

Since the earliest studies of air-breathing fish circulation nearly 200 years ago, emphasis has been given to identify the afferent and efferent blood vessels of the ABO. This standard was established by the nineteenth-century anatomist and physiologist Johannes Müller who, with knowledge of the blood flow pattern of light and dark blood in mammals, noted the paradoxical flow for some fishes possessing air-filled organs that were thought to function for auxiliary respiration. Müller observed that these organs often received blood that had just passed through the gills and wondered how additional oxygenation would be possible. He also noted that blood leaving a putative auxiliary breathing organ went back to the heart, mixing along the way with other venous streams of deoxygenated blood also headed to the heart. Müller reasoned that, if this blood had in fact become more oxygenated in an air-filled auxiliary breathing organ, then it would surely be deoxygenated when it mixed with the venous blood streams coming from the systemic organs. Müller's paradox and his use of the comparative approach to mammal and fish circulation serve as the starting points for this article.

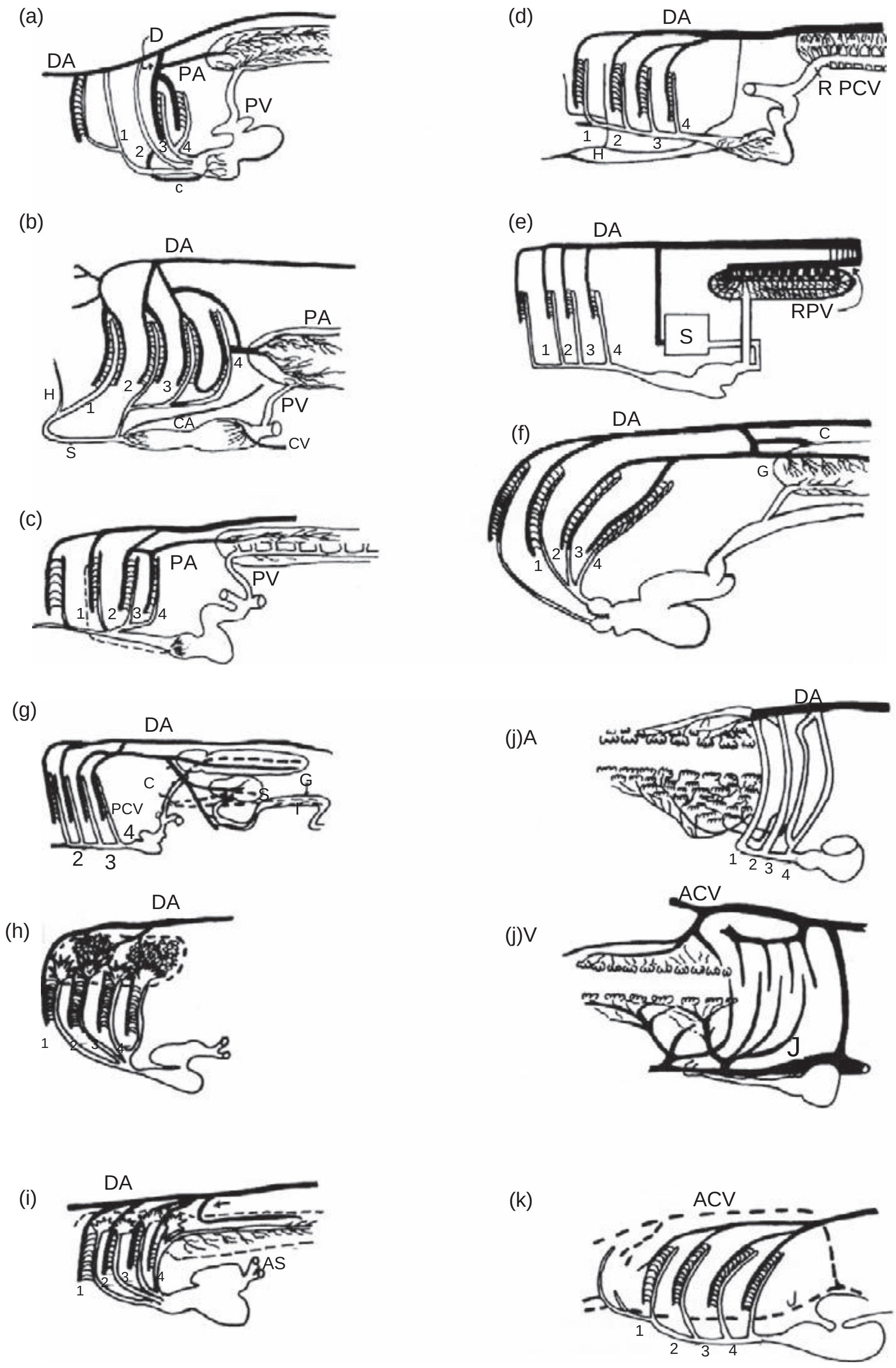


Figure 1 (Continued)

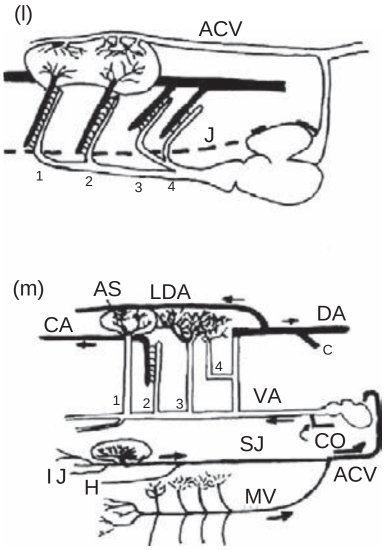


Figure 1 Circulation patterns of air-breathing fishes viewed from left side and showing tracts of major vessels to and from the ABO and their relationships to the branchial arches (1–4) and the heart (H). (a) *Protopterus*. Note pulmonary circulation and absence of gill filaments on arches 1 and 2. Coronary artery (C) branches from the hypobranchial which originates on arch 2. DA, dorsal aorta, D, ductus arteriosus; Pa, pulmonary artery; and PV, pulmonary vein. (b) *Polypterus*. Arches 3 and 4 contribute to PA formation. S is approximate position of the ventral aortic strap (**Figure 5**). Note coronary artery (CA) origin from the subclavian artery and vein (CV) drainage from ventricle. (c) *Amia*. PA originates from arches 3 and 4. PV enters left side of duct of Cuvier. Coronary supply from hypobranchial and efferent side of arch 2. (d) *Lepisosteus*. ABO drainage to right post cardinal vein (RPCV). Both subclavian (SC) and hypobranchial (H) arteries form coronary artery. (e) *Arapaima.* Afferent supply via renal portal vein. S, systemic circulation. (f) *Gymnarchus*. Note anastomoses between DA and celiac (C) and gas bladder (G) arteries (see **Figure 6**). (g) Generalized ostariophysan inter-renal vein tracks (dashed line) showing connections from the gas bladder, stomach, and intestinal (G, S, I) ABOs to the PCV. (h) *Clarias*. Dashed line marks the periphery of the ABO. Note the presence of fans on each branchial arch and that ABO efferent flow is into DA. (i) *Heteropneustes.* Dashed line marks periphery of the ABO. Note gill fans on each branchial arch and modified bulbous (**Figure 7**). Afferent 4 goes to the air sac (AS = SBC) and efferent drainage (arrow) is into the DA. (j) *Electrophorus*. A. Branchial arch arteries give rise to small superior and inferior vascular epithelial branches but are essentially ventral-dorsal conduits. V. Venous drainage is into the systemic circulation via the jugular (J) and anterior cardinal (ACV). (k) *Boleophthalmus*. Branchial circulation similar to most non-air-breathing fishes, with systemic capillaries being the site of O_2 uptake and venous return (dashed lines) occurring via the ACV and J. (l) *Anabas* and *Channa*. Efferent branchials 1 and 2 go into the ABO and ABO drainage is systemic (ACV and J, dashed line). Note reduced gills on arches 3 and 4. (m) *Monopterus.* Arrows show flow direction. (Top part of diagram) Arterial outflow from heart through elongate ventral aorta (VA) to branchial arches. Arch 1 serves SBC air sac (AS), arch 2 traverses a small gill and forms the carotid artery (CA). Arches 3 and 4 branch into the respiratory epithelium (note that arch 4 forms as a side branch of the ventral-dorsal aortic conduit). Blood supply to head is also via paired lateral dorsal aortae (LDA). The coronary artery (CO, curved arrow) emerges from the ventral aorta just beyond the bulbus. C. celiac. (Lower diagram) Venous return to the heart is via the superior jugular (SJ), and median (MV) veins into the ACV. Reproduced from Fig. 4.2 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation.* San Diego, CA: Academic Press, with permission from Elsevier.

Defining the Problem

Comparative Circulation: Mammal and Fish

Mammals (and birds) have two separate circulatory loops (pulmonary and systemic) serially linked at the heart, which is subdivided to form parallel left and right pumps (**Figure 2**(**a**)). The oxygenated blood exiting the lungs enters the left side of the heart from which it flows into the systemic circulation and returns to the right heart from where it is transported to the lungs. The respiratory and systemic circulations are then said to be in parallel. In contrast, fish circulation is but a single loop in which flow exiting the heart goes to gills and the systemic tissue beds before returning (**Figure 2**(**b**)). The respiratory and systemic circulations are then said to be in series (**see also Circulation**: Circulatory System Design: Roles and Principles).

The ABO Problem

Figure 2(**c**) shows the diverse ways that the insertion of an ABO (boxes with diagonal lines) complicates an in-series flow in which gills are also present. If, as Müller had observed, O_2 gained in the ABO enters the venous circulation, then it is at risk of forced partial unsaturation caused by mixing with other venous (hypoxic) blood streams. In the case of ABOs near the gills, if ABO blood flow bypasses the gills, this potentially eliminates the pre-ABO contact between blood and water that takes place in the gills, a step that is

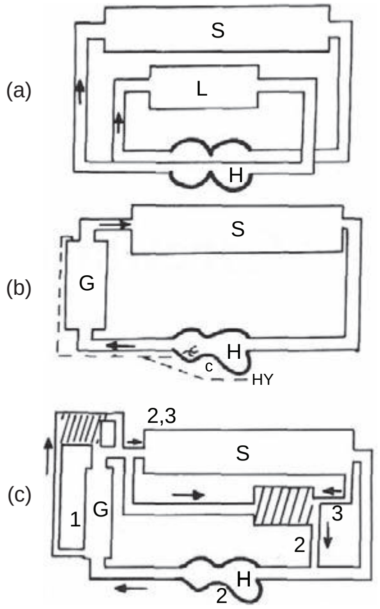


Figure 2 (a) Double-loop circulation of mammals. (b) In-series circulation loop of fishes. Dashed line shows efferent branchial origin of hypobranchial (HY) and coronary (C) arteries. (c) Three potential consequences of ABO (box with diagonal lines) placement: 1. O_2 loss across gills; 2. enforced partial unsaturation; 3. pressure drop. Arrows indicate flow direction. S, systemic circulation: G, gills; L, lung; H, heart. Reproduced from Fig. 4.1 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

generally regarded as important for increasing respiratory efficiency by lowering blood CO_2 prior to its entering the ABO (**see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion, **Gas Exchange**: Respiration: An Introduction, and **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System). Alternatively, if blood flow is to both the gills and the ABO, then a larger, stronger ventricle might be required for perfusion; however, if the efferent flows from these two organs mix, forced partial unsaturation would occur and result in a lower blood partial pressure of oxygen (P_{O_2}) to drive diffusion into the tissues.

Assuming that the water contacting the gills lacks sufficient O_2 for respiration, then **Figure 2(c)** shows that the first part of Müller's paradox (i.e., the apparent redundancy of double oxygenation, first in the gills and then in the ABO) is not a problem because blood in the gills would not be saturated with O_2 and an auxiliary ABO would be required. However, this solution immediately raises the inverse problem about gill function: If O_2-saturated blood passes through gills bathed in hypoxic water, what prevents the diffusive loss, termed 'transbranchial deoxygenation', of this blood?

Solutions

The recurring theme of this article is that the potential for transbranchial O_2 loss, together with the second part of Müller's paradox – the downstream mixing of ABO blood with deoxygenated venous blood results in forced partial unsaturation which lowers systemic P_{O_2} – remains central issues influencing the design and regulation of aquatic air-breathing fish circulation. This article further shows that the solutions to the related problems of conserving and effectively utilizing aerially acquired O_2 involve a suite of independently evolved adaptations to establish and modulate the degree of isolation between the ABO flow stream and other flows, and permit the strategic shunting (i.e., the detouring of blood from its normal destination to an alternative one) from one or the other respiratory organ in phase with the air-breathing cycle.

Structural and regulatory features known to play a role in blood-flow separation and shunting in different air-breathing fishes are summarized in **Table 1**.

Specializations that lessen contact between the ABO and other venous streams and decrease the potential for transbranchial O_2 loss are signature features of the heart, gill structure, and circulation patterns of two extant lungfish genera (*Lepidosiren* and *Protopterus*) and a few actinopterygian genera (however, none to the same extent, morphologically as lungfish) including *Polypterus* and *Erpetoichthys* (bichirs and ropefish), *Gymnarchus* (African knifefish), *Amia* (bowfin), and the teleosts *Heteropneustes* (Indian catfish), *Anabas* (climbing perch), *Channa* (snake head).

Major among these modifications is shunting to enhance separation of the oxygenated and deoxygenated streams (e.g., flow from the lungfish pulmonary vein into the left atrium) and the sequestration of O_2-rich blood from hypoxic water in the gills (e.g., using recessed lamellar vascular channels isolated from branchial water flow). Reducing total gill area also lessens the potential for transbranchial O_2 loss; some continuous air breathers have such small gill areas that they are obligatory air breathers.

Another O_2-conserving mechanism involves modulation of the relative rates of gill water flow ($\dot{V}$) and blood flow ($\dot{Q}$) through the gills during the period that air is held. Over the air-breathing cycle, many fish display considerable heart rate variability (HRV), as well as a prominent respiratory sinus arrhythmia (RSA, an integrated heart-rate (HR) response linked to the breathing cycle) characterized by a significant increase (tachycardia) in HR just as or slightly before an air breath is taken, a gradual HR reduction during the time the breath is held, and an abrupt drop in HR (bradycardia) when the breath is exhaled. HRV is often accompanied by inverse fluctuations in $\dot{V}$. Thus, as air-breath tachycardia increases $\dot{Q}$ a reduced gill ventilation frequency lowers $\dot{V}$ and the resultant drop in the $\dot{V}$:$\dot{Q}$

Table 1 Structural modifications in the circulatory anatomy of air-breathing fishes, possibly related to the shunting or selective perfusion of the ABO, systemic, or branchial vascular beds during bimodal respiration

Genus	*Structural feature*	*(Possible?) Action*
Protopterus, Lepidosiren	Ductus arteriosus	Lung shunt to dorsal aorta
	PAVS	Regulate pulmonary artery flow
	Afferent branchial arch shunts	Regulate branchial flow and pulmonary perfusion
	Incomplete ventricular septum	Balance pulmonary and systemic pressures
	Spiral and ventrolateral folds in bulbus cordis	Guide flow to systemic and branchial arches
Polypterus	Analog to ductus arteriosus	Lung shunt?
	Ventral aortic strap	Anterior branchial shunt?
Amia	Analog to ductus arteriosus	Allow ABO shunt?
	Recessed lamellar channels	Gill shunt
Gymnarchus	Dorsal aortic-efferent branchial arch junction	ABO shunt?
	No ventral aorta; four separate valved arteries	Selective branchial arch perfusion?
	Muscle insertions on pericardium	Heart torsion related to arch shunting?
Arapaima	Valve on systemic veins near heart entrance	Control venous mixing?
	Thick layers of smooth muscle in afferent and efferent branchial arteries	Control branchial circulation?
	Valved and discrete lamellar blood channels that are recessed	Control branchial shunting?
Luciocephalus	Vessel connecting pre-branchial ventral aorta to intestine	Branchial shunt?
Hoplerythrinus	Ductus arteriosus analog junction between the dorsal aorta and coeliac artery	ABO shunt
	Vasoactive branchial resistance	Regulation of branchial shunts and ABO perfusion
Clariidae	Separated ventral aortic branches	Branchial arch shunts?
Heteropneustes	Manifold bulbus with separate ventral aortic openings and dead-end sac extension	Selective branchial perfusion? Pressure compensation?
Loricariidae and Callichthyidae	Inter-renal vein	Hepatic-portal vein shunt
Channa, *Anabas,* and other anabantoids	Anterior and posterior ventral aortae. Reduced gill lamellae on arch 4	Optimal routing of ABO and systemic flows. Branchial shunt
Channa	Vessel connecting arch 2 suprabranchial artery to lateral aorta	ABO shunt
Electrophorus and *Monopterus*	Ventral–dorsal aortic conduits	Branchial shunts

Modified from Table 4.2 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

ratio would minimize transbranchial O_2 loss by rapidly pushing O_2-rich blood through under-ventilated gills. (**see also Gas Exchange**: Respiration: An Introduction, **Brain and Nervous System**: Autonomic Nervous System of Fishes, **Hypoxia**: Respiratory Responses to Hypoxia in Fishes, **Design and Physiology of the Heart**: The Coronary Circulation, and **Control of Respiration**: Generation of the Respiratory Rhythm in Fish).

This article discusses how the vascular network potentially combines with HRV to improve respiratory efficiency. It will also show how the phylogenetic progression of air breathing, from lungfish to the most derived teleosts, has been accompanied by the development of mechanisms allowing fine-scale and short-term regulation of both HR and peripheral vascular resistance, all in phase with the respiratory cycle. The sarcopterygian and primitive actinopterygian air breathers alter flow using structural features and use both hormonal and cholinergic neural regulation to alter HR and vascular resistance. By contrast, rapid changes in adrenergic and cholinergic tone associated with each air breath are distinguishing features of the teleostean air breathers and these bring about abrupt changes in both HR and vascular resistance (**see also Design and Physiology of Arteries and Veins**: Physiology of Resistance Vessels) and may to some extent supersede reliance upon the more obvious structural or mechanical features for controlling blood flow in lungfishes (**see also Control of Respiration**: Generation of the Respiratory Rhythm in Fish, **Hormonal Responses to Stress**: Catecholamines, **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System, and **Brain and Nervous System**: Autonomic Nervous System of Fishes).

Patterns of ABO Circulation: Lungs and Respiratory Gas Bladders

Lungfish

Relative to all other air-breathing fishes, the African (*Protopterus*, four species; **see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) and South American (*Lepidosiren paradoxa*) lungfishes most closely approach the separate, in-parallel pulmonary and systemic circulation pattern of mammals (**Figures 1**(**a**) and **3**). In these fishes, the pulmonary vein passes though the sinus venosus and empties into the left atrium of a nearly completely divided heart that, in combination with the septated and spiral bulbus cordis, ensures the distribution of (1) most of the oxygenated blood into branchial arches 1 and 2, which lack gills and are conduits to the systemic circulation, and (2) most of the deoxygenated blood into the pulmonary arteries after passing through arches 3 and 4, which have distinctive gill filaments. P_{O_2} measurements at strategic sites in the circulation, as well as the paths taken by India ink and contrast agents, confirm that, just after an air breath is taken, a remarkable 90% of the blood in the pulmonary vein enters the systemic circulation. Later, as lung P_{O_2} lessens, this fraction declines. Thus, while not anatomically separated the two circulations are functionally isolated.

Key to achieving this functional separation are structural elements such as the return of the pulmonary blood to the left atrium, the partial anatomical separation of the atrium and ventricle into right and left chambers, and the septated spiral bulbus cordis, which directs the pulmonary and systemic streams (**Table 1**). Regulating this are vasoactive sites controlling circulation in phase with the

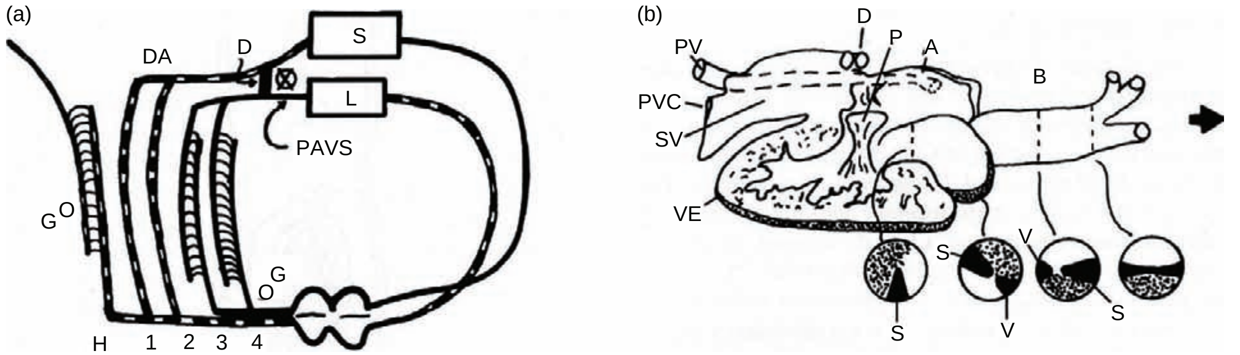


Figure 3 (a) Lung (L) and systemic (S) circulatory loops of *Lepidosiren* and *Protopterus*. Oxygen-rich blood (alternating black and while lines) leaves L, enters left side of the heart, and goes into the branchial arches (hyoid, 1, and 2) and then to either the head or the DA. Deoxygenated blood from the S loop (solid black line) goes through the right side of the heart to arches 3 and 4 and to the lung. Diagram shows location of the shunts (circles) and their configuration during air breathing: gills and PAV shunts are open and ductus arteriosus (D) is closed. (b) Right-side section view of the heart of *Lepidosiren*. Pulmonary vein (PV): dashed lines track PV advance to the anterior left atrium (A) which is obscured by the septum dividing it. PVC, posterior vena cava enters heart via the sinus venosus (SV). Circles are cross sections through the bulbus at different positions showing how the rotation of the spiral (S) and ventrolateral (V) folds affect the distribution of oxygenated blood (stippled area). Note that the two folds become fused at the end of the bulbus (arrow = rostral direction). D, ductus Cuvier; P, atrioventricular plug; VE ventricle; B, bulbus cordis (extending from VE to the arterial branches). Reproduced from Figs. 4.3 and 4.4 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

air-breathing cycle. When a breath is taken, vasodilation (controlled by circulating catechols and vagal tone) increases pulmonary blood flow by channeling more blood through arches 3 and 4 and the pulmonary vasomotor segment (PAVS), and closing the ductus arteriosus (**Figure 3**(**a**)). *Lepidosiren* and *Protopterus* hearts lack adrenergic nerve fibers and HR is regulated by vagal tone (parasympathetic, cholinergic neurons) in combination with the level of circulating catecholamines (released from tissues and blood sinuses). (Because catecholamine levels are also stress related they can increase lungfish HR to a level where a lessening of vagal tone does not further increase HR; this may explain why in some experiments tachycardia is not always associated with taking a breath. In addition, unlike the situation in teleosts, catecholamines do not affect the hydration state of lungfish erythrocytes in a manner that alters hemoglobin (Hb)–O_2 affinity; (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect, Red Blood Cell Function, **Detection and Generation of Electric Signals**: Development of Electroreceptors and Electric Organs, and **Hormonal Responses to Stress**: Catecholamines.)

The gill circulation patterns of *Protopterus* and *Lepidosiren* were first detailed in the nineteenth century and all subsequent accounts consistently reported that the anterior gill arches (1 and 2) are totally devoid of gills and function as conduits for the passage of O_2-rich blood from the bulbus cordis to the dorsal aorta and on to the systemic circulation (**Figure 3**(**a**)). However, Marcos de Moraes and colleagues recently reported that *Lepidosiren* has small gill filaments on arches 1 and 2 as well as on arches 3 and 4, but that the total gill area and both the O_2 and CO_2 diffusing capacities (i.e., the transfer rate per mean effective partial pressure gradient between the external medium and blood) of this species are too small to significantly contribute to respiration. If confirmed, these facts contradict earlier reports for *Lepidosiren* gill structure and its similarities with *Protopterus*. Furthermore, if the gills of *Lepidosiren* do not contribute to aquatic respiration, then the perception that arches 3 and 4 play an important role in preconditioning deoxygenated venous blood (by removing CO_2 and equilibrating acid–base status) before it enters the lung is in doubt, as would be the mechanism by which *Lepidosiren* releases its respiratory CO_2 into water.

Relative to the obligate air-breathing *Protopterus* and *Lepidosiren*, the circulatory specializations of the facultative air breathing Australian lungfish (*Neoceratodus forsteri*) are less refined; it has functional gills on all of its branchial arches (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes) and its pulmonary vein enters an undivided sinus venosus. Septa within the atria and ventricle of *Neoceratodus* are also less complete and the septum within the spiral bulbus is smaller.

The Polypterids and *Amia*

In *Polypterus*, *Erpetoichthys*, and *Amia*, pulmonary arteries branch from the efferent side of branchial arch 4 (in some species arch 3 also contributes; **Figures 1**(**b**) and **1**(**c**)). Also present in these fish is a connection between the pulmonary artery and the dorsal aorta which, like the ductus arteriosus in lungfish, may act as an ABO shunt. A tendonous strap across the ventral aorta proximal to aortic arch 1 in *Polypterus* (**Figures 2**(**b**) and **4**) may play a role in phasically shunting flow away from arch 1. On the venous-return side, the degree of ABO and systemic separation is far less than in either *Lepidosiren* or *Protopterus* (**Figure 5**). In polypterids, the pulmonary and hepatic veins combine to form the hepatopulmonary which enters the sinus venosus. In *Amia*, the single pulmonary vein flows into the left duct of Cuvier. Thus, blood leaving the ABO would thus be subject to both enforced partial unsaturation as well as transbranchial O_2 loss. The hearts of these fish also lack the medial septation of lungfish hearts.

Gars and Primitive Teleosts

Inflow to the respiratory gas bladder of gars (*Lepisosteus* and *Atractosteus*; **Figure 1**(**d**)) is from multiple branches of the dorsal aorta and venous return is via the right post cardinal vein (**Figure 5**). There is no heart septation.

Osteoglossiforms

The Osteoglossiforms present three distinct patterns of circulation to their respiratory gas bladders. In *Notopterus* and presumably the other featherbacks (*Xenomystus* and *Papyrocranus*), the pattern is generally similar to most other teleosts; the afferent supply is via branches

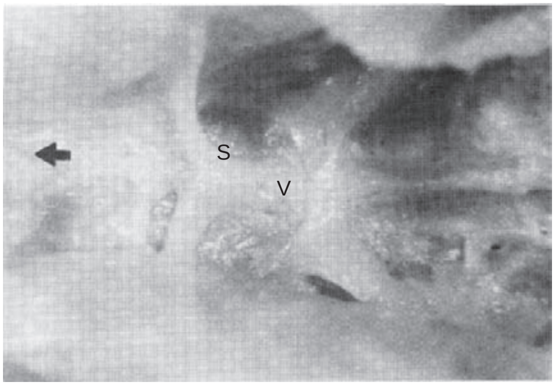


Figure 4 Ventral view of the strap (S) underlying the ventral aorta (VA) of *Polypterus bicher* (arrow = rostral direction). (**Figure 1**(**b**) shows strap position relative to anterior branchial arches.) Reproduced from Fig. 4.6 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

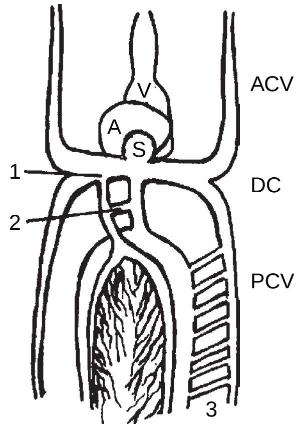


Figure 5 Generalized diagram of central venous return to the heart for three primitive actinopterygians 1. *Amia*: pulmonary vein (PV) empties into left duct of Cuvier (DC). 2. Polypterids: PV flows into hepatic vein. 3. Gars: PV flows into right post-cardinal vein (PCV). ACV, anterior cardinal vein; S, sinus venosus; A, atrium, V, ventricle. Reproduced from Fig. 4.5 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

emanating from the dorsal aorta and venous return is into the post-cardinal vein.

In *Arapaima*, *Pantodon*, and *Heterotis*, the gas bladder completely encloses the kidneys and blood supply to the organ is via the renal portal system. Because virtually all of the blood returning to the heart from the posterior body flows through the renal portal system, having the ABO in series with this flow offers the potential advantage of oxygenating a large volume of the venous return and also lessening the proportion of deoxygenated blood that could mix with it to force unsaturation. Correspondingly, ventral aortic P_{O_2} measurements in *Arapaima* are high (45–50 torr; 80–90% saturation). Moreover, and unlike most other air-breathing fishes, *Arapaima* displays no air-breath-induced changes in dorsal-aortic pressure, pulse pressure, or HR; in most other species, these factors change over the time air is held in the ABO. This may be an experimental artifact or reflect the minimal effects that HR and blood pressure augmentation would have on the more remote venous system. Flow from the ABO of these three genera is more into the left than the right post-cardinal vein.

A remarkable series of circulatory specializations for air breathing in *Gymnarchus niloticus* (**see also Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes) were documented over a century ago by Richard Assheton (**Figures 1**(f) and **6**). The honeycombed respiratory gas bladder of *Gymnarchus* is supplied by pulmonary arteries arising from branchial arches 3 and 4 and venous return from this organ is via the left post-cardinal vein to the duct of Cuvier (flow from the anterior left side also enters here), which connects to the left side of the septated atrium (**Figure 6**). The heart structure of *Gymnarchus* is unique in featuring divided atria and, rather than a single ventral aorta with afferent branchial branches, there are four short, distinct ventral aortae, each with its own mini-bulbus capacitance chamber. The separate branches likely play a role maintaining the flow separation needed to enhance aerial respiration; however, there are no physiological studies with *Gymnarchus*.

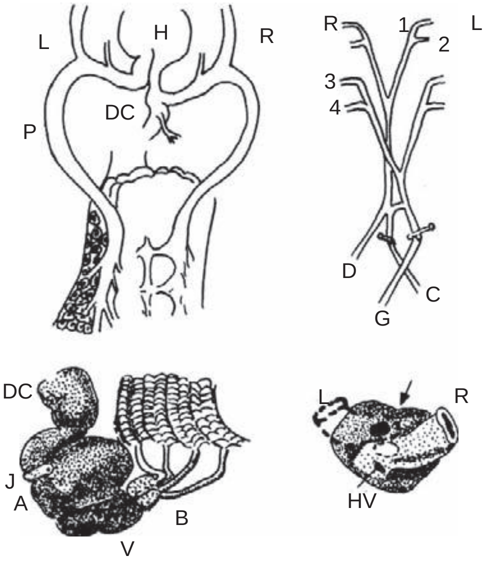


Figure 6 Redrawn detail of R. Assheton's (1907) illustrations of *Gymnarchus* blood flow. (Top left) Dorsal view of the central venous return showing the separated duct of Cuvier. H, heart (note the partial atrial septum); P, post-cardinal vein; L, left side; R, right side. ABO drainage is via the left P and into the left atrium. (Top right) Ventral view of the efferent branchial arteries (1–4) showing their connections to the dorsal aorta (D), the gas bladder (G), and the celiac (C) arteries. Note anastomoses between the three vessels. (Lower left) Right-side view of the heart showing the right ductus Cuvier (DC), jugular vein (J), the atrium (A), ventricle (V), and two of the four bulbus chambers (B) that lie at the base of each root artery. (Lower right) Posterior view of the atrium showing the left (L) and right (R) halves of the duct of Cuvier. The left duct is indicated by the dashed line and its entry port is shown as a black hole. The right duct is drawn and its entry port is shown as a white area outline by a dashed line. Note that the hepatic vein enters the right duct. Arrow shows position of the atrial septum dividing the two entry ports. Reproduced from Fig. 4.7 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

Other Teleosts Having Respiratory Gas Bladders

For other species using a respiratory gas bladder such as the characins (*Lebiasina*, *Piabucina*, *Hoplerythrinus*, and *Erythrinus*) and other genera (*Phractolaemus*, *Pangasius Gymnotus*, and *Umbra*), afferent ABO circulation is generally via the dorsal aorta, celiac artery, or a branch of these, and venous return is most often into the post-cardinal vein (**Figure 1**(g)).

The characin *Hoplerythrinus unitaeniatus* has been the subject of several studies attempting to demonstrate the role HRV in facilitating aerial O_2 uptake. Flow measurements verify that postbreath tachycardia corresponds to increased flow into the ABO just after an air breath is taken. Studies using pharmacological agents to abolish inhalation tachycardia verify that both adrenergic and cholinergic tones increase simultaneously prior to each air breath, and then decrease, and that modulation of inhibitory cholinergic tone is responsible for the major proportion of HRV. However, that these interventions did not affect either air-breathing frequency or total oxygen consumption rate ($\dot{V}O_2$) indicates that even more intricate experiments of this type, in which additional factors such as the $\dot{Q}:\dot{V}$ ratio and transbranchial O_2 loss are also measured, will be needed to comprehend the functional significance of HRV for air breathing (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping, The Coronary Circulation, and **Hypoxia**: Respiratory Responses to Hypoxia in Fishes).

Circulation Patterns for Other ABOs

Mouth Breathers

The synbranchids *Monopterus* and *Synbranchus*, the electric knifefish *Electrophorus* (**Figure 1**(**j**) and **1**(**m**)), and several other species (*Hypopomus*) breathe air by holding it in their mouths and this reduces the problem of forced unsaturation and eliminates transbranchial O_2 loss. Whether or not the gills function in air breathing, surrounding them with air rather than hypoxic water ensures the accrual of O_2. Specifically, O_2 absorbed from air by the buccopharyngeal respiratory epithelium would flow into the venous circulation and quickly pass through the heart and gills to the dorsal aorta and then to the systemic circulation. Because the P_{O_2} of the air surrounding the gills is high, there would be not be transbranchial O_2 loss from the blood passing through the gills (moreover, this is a non-problem for the obligate air-breathing *Monopterus* and *Electrophorus* which do not have gills on their branchial arches). While, as shown in **Figure 2**(**c**), there is a potential for unsaturation due to the mixing of O_2-rich venous blood exiting the ABO and the less oxygenated venous return from the systemic circulation, the net result of an extended breath hold would be to elevate blood P_{O_2} throughout the body.

The downside of this air-breath-holding mode is that the interruption of gill contact with water interrupts the aquatic loss of respiratory CO_2 which, because of its effect on Hb–O_2 affinity, could adversely affect O_2 binding and delivery. The necessary solution is alternating periods of air holding, which oxygenates tissues, with periods of gill ventilation in water, which drives off blood CO_2 and conditions the blood for the next air breath. Another solution would be to have a relatively high blood Hb concentration (i.e., >12 g% in Synbranchids and *Electrophorus*), which would allow the storage of more O_2 and also buffer the acid effect of a rising blood P_{CO_2} on Hb–O_2 affinity.

Stomach and Intestinal Air Breathers

In species with ABOs located in the stomach or intestine, modified routes of venous return appear to lessen the effects of enforced partial unsaturation and circumvent the hepatic portal circulation, which is an O_2 sink. In *Hoplosternum thoracatum*, *C. callichthys* and *Lipophrys* (=*Ancistrus*) *spinosus*, and probably many other loricariid catfish, flow out of the ABO is into the inter-renal vein and then to the post-cardinal vein and thus bypasses the hepatic portal vein (**Figure 1**(**g**)). While the inter-renal and other vascular shunts potentially heighten the handling efficiency of ABO-obtained O_2, these modifications do not eliminate the problems of enforced unsaturation due to venous mixing, nor do they lessen the potential for transbranchial O_2 loss. Nevertheless, even with ABO drainage into the hepatic portal vein, species such as *Gymnotus carapo*, *Misgurnis anguillicaudatus*, and *Cobitis barbatula* breathe air effectively. Finally, a vessel transporting blood from the ventral aorta to the respiratory intestine has been reported for *Lepidocephalus guntea* but there are few details.

Suprabranchial Chambers

No fish, including the air breathers, can function without a gill circulation (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System and **Gas Exchange**: Respiration: An Introduction). However, as seen in **Figure 2**(**c**), some air-breathing species appear to lessen the separate problems of transbranchial O_2 loss and vascular-pressure depletion by arranging the ABO and gills as in-parallel circulations, and shunting blood to either one or the other organ in phase with air breathing. As shown earlier, species that hold air over their gills can, provided they hold it long enough, eventually increase the P_{O_2} of their entire circulation.

ABO circulation is different in species such as the clariids and *Heteropneustes* where the gills and the suprabranchial chamber (SBC) have a parallel circulation (i.e., both receive a fraction of the cardiac output). In clariids a fraction of the oxygenated SBC blood continues via the dorsal aorta into the systemic circulation, and in *Heteropneustes* most of the efferent SBC blood takes this course (**Figures 1**(**h**) and **1**(**i**)). For these fishes, downstream mixing of the gill and SBC blood will occur. It can be expected that the relative flow through these different circulatory patterns is regulated in phase with air breathing; however, there are no data for shunting or selective ABO perfusion for the clariids or *Heteropneustes*.

A possible mechanism for branchial shunting in these fish takes the form of their modified ventral aorta, which divides into separate branches serving the different arches (**Figures 1**(**h**) and **1**(**i**)). Detail for this in *Heteropneustes* shows that a highly modified bulbus arteriosus forms three ventral aortae (**Figure 7**). The smallest and most anterior-ventral of these originates on the left side of the bulbus and forms gill arch 1. The middle branch gives rise to arch 2, and the third and largest branch divides into arches 3 and 4. The side view (**Figure 7**) of this structure suggests that, with expansion, the bulbar area at the base of arches 3 and 4 can compress arches 1 and 2 to send more blood into the SBC. While partial occlusion of branchial arch 1 would appear to affect brain perfusion, this could be compensated by retrograde flow through the dorsal aorta.

In both *Channa* and the anabantoids (**Figure 1**(**l**)), circulation approaches the double-loop ideal. There are two ventral aortae: outflow from one passes through the two anterior gill arches and then into the ABO, while flow from the other circulates directly to the body via branchial arches having minimal respiratory surface area. For *Channa* small P_{O_2} differences in the two streams (i.e., less O_2 in the flow headed toward the gills) suggest there is ABO and systemic flow separation in the heart. This flow sequence also enhances ABO function by preconditioning blood (i.e., removal of CO_2 to water) for maximum O_2 absorption; however, efferent ABO blood also enters the venous circulation and, in the absence of a medially septated heart, maintenance of separate high and low O_2 blood streams remains problematic. Thus, optimal function of both the clariid and *Channa*-type circulations is dependent upon streamlined flow in the great vessels and heart, as well as on the neural-control capacity to selectively regulate branchial and ABO perfusion in phase with the air-breathing cycle.

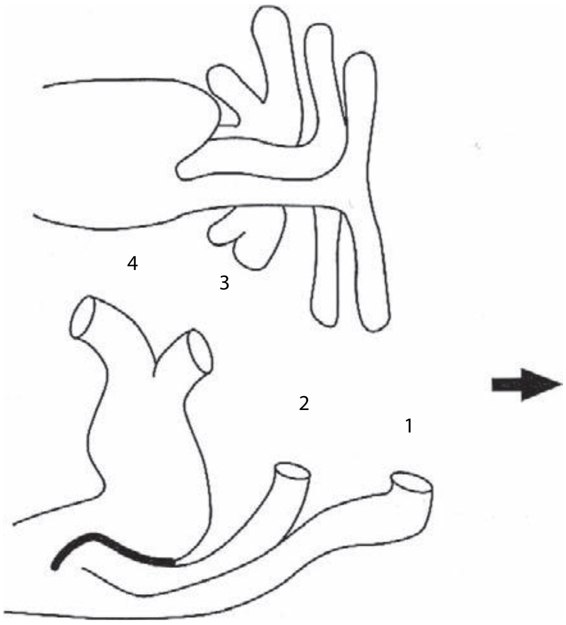


Figure 7 Bulbus arteriosus of *Heteropneustes*. (Top) Ventral view showing the displaced and tiered emergence of three ventral aortae. (Bottom) Right-side view showing how the lower bulbus section (thick line) giving rise to branchial arches 3 and 4 may compress aortae 1 and 2 to cause partial branchial shunting (arrow = rostral direction). Reproduced from Fig. 4.8 in Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press, with permission from Elsevier.

Coronary Circulation and Air Breathing

In most fishes, coronary perfusion supplies the outer compact myocardium layer, whereas the inner spongy endocardium is nourished by diffusion from venous blood circulating through the heart chambers (**see also Design and Physiology of the Heart**: The Coronary Circulation). The extent of coronary perfusion into the compact layer correlates with species activity. Little is known about how the epi- and endocardial layers of air-breathing fish hearts may differ from other species.

It has been suggested that air breathing evolved as a mechanism to oxygenate the endocardium. However, from a phylogenetic standpoint, this is not supported by the presence, in sharks, rays, and most non-air-breathing fishes, of a coronary artery that branches from the hypobranchial artery which originates on the efferent (oxygenated) side of, most usually, branchial arch 2. However, this is the pattern in *Neoceratodus* and both *Lepidosiren* and *Protopterus* have the same coronary origin; these fish lack gills on arch 2 which, due to separate pulmonary loop, contains oxygenated blood (**Figure 1**(**a**)). With few exceptions, the hypobranchial origin of the coronary artery is the most common source of ventricle oxygenation in most bony fishes. Two notable exceptions for coronary origin among the air-breathing fishes are the polypterids and gars (**Figures 1**(**b**) and **1**(**d**)) in which the subclavian artery is the sole (polypterids) or a partial (with the hypobranchial) contributor to the coronary circulation. While details are lacking for most air-breathing species, the arch 2 origin of the coronary circulation also occurs in *Channa* and this is noteworthy because the efferent branchial blood has yet to enter the ABO and is therefore not enriched with O_2. This implies that there is always sufficient O_2 within the venous circulation to sustain the heart and this is reasonable in view of the potential for axial-flow mixing and the presence of a significant ABO drainage into the systemic venous return (**Figure 1**(**l**)).

In *Monopterus*, the coronary artery originates on the ventral aorta just distal to the heart (**Figure 1**(**m**)). That blood at this site is sufficiently oxygenated to sustain the heart is attributable to the diffuse branchial circulation of *Monopterus* which feeds O_2-rich blood into the venous return.

Little data exist to show how the coronary circulations of most other air-breathing species might be modified to augment O_2 delivery during air breathing. Comparative data for the synbranchids having functional gills and for *Arapaima* and other osteoglossids having a large venous ABO flow can provide information on the roles of blood pressure and O_2 content in coronary perfusion.

Conclusions

Johannes Müller's contrast of fish and mammal circulation 170 years ago retains heuristic importance because of the focus it brings to the functional importance of ABO placement and the factors driving selection for adaptations to ensure the effective management of bimodally obtained oxygen. The vast phylogenetic gulf separating mammals and the fish-like Tetrapodomorpha of the Devonian (**Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction) is filled with many examples of the progressive radiation of circulatory specializations among the vertebrate classes. In the case of mammals (and birds), selection for heightened metabolic performance and aerobic capacity resulted in the gold standard for optimal O_2 delivery featuring a double-looped and double-pumped continuous flow that separates the deoxygenated and oxygenated streams and re-pressurizes the latter prior to its dispersal into the systemic circulation.

Fishes occupy the base of the tetrapod phylogeny and were the first lunged vertebrates. It is likely that in its earliest iteration the lung operated within the limits of an in-series circulation. Lungfish are the closest living relatives of the tetrapodomorphs and the structural and functional similarities between the lung-circulation patterns of *Lepidosiren* and *Protopterus* (e.g., pulmonary return to the left atrium and the high degree of left–right heart flow separation) closely track the basic tetrapod features. However, no air-breathing fish has a completely separated left and right heart or circulation, nor can any species function without gills. The circulatory specializations occurring in the lungfish *Neoceratodus*, which is not an obligatory air breather, and the diverse but convergent circulatory modifications apparently contributing to oxygenated and deoxygenated flow separation in several teleosts (polypterids, *Amia*, *Gymnarchus*, *Heteropneustes*, *Channa*, *Anabas*, and others), suggest there was a strong selective premium placed on the separation, either or both in space or time, of the ABO and systemic flows of fish air breathing in water.

The presence of a higher capacity for right–left separation in *Lepidosiren* and *Protopterus* may reflect an even stronger selection for these features owing to the extreme environmental conditions in which they occur. Although these fishes are capable of living in mud without water and sustaining a low $\dot{V}O_2$ for long periods (*Protopterus* can estivate), these are not amphibious fish and their behavior is in no way comparable to that of terrestrial amphibians. This indicates that flow separation was driven by the respiratory inefficiencies of in-series circulation to both gills and an ABO during aquatic air breathing. It further implies that improving respiratory efficiency in water was a more likely causal factor for the structural changes in lungfish and that such changes preceded and likely facilitated the tetrapodomorph land transition in the Devonian.

See also: **Air-Breathing Fishes**: Respiratory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Brain and Nervous System**: Autonomic Nervous System of Fishes. **Circulation**: Circulatory System Design: Roles and Principles. **Control of Respiration**: Generation of the Respiratory Rhythm in Fish; The Ventilatory Response to CO_2/H^+. **Design and Physiology of Arteries and Veins**: Anatomical Pathways and Patterns; Physiology of Resistance Vessels. **Design and Physiology of the Heart**: Physiology of Cardiac Pumping; The Coronary Circulation. **Detection and Generation of Electric Signals**: Development of Electroreceptors and Electric Organs. **Gas Exchange**: Respiration: An Introduction. **Hormonal Responses to Stress**: Catecholamines. **Hypoxia**: Respiratory Responses to Hypoxia in Fishes. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Evolution of the Bohr Effect; Red Blood Cell Function.

Further Reading

Assheton R (2007) The development of *Gymnarchus niloticus*. In: Kerr JG (ed.) *The Work of John Samuel Budgett*, pp. 293–421. London: Cambridge University Press.

Bugge J (1961) The heart of the African lungfish, *Protopterus. Videnskabelige Meddelelser Dansk Naturhistorisk Forening Kjobehavn* 123: 193–210.

Carter GS and Beadle LC (1931) The fauna of the swamps of Paraguayan Chaco in relation to its environment II. Respiratory adaptations in the fishes. *Journal of the Linnaean Society of London Zoology* 37: 327–368.

de Moraes MFPG, Holler S, da Costa OTF, *et al.* (2005) Morphometric comparison of the respiratory organs in the South American Lungfish

Lepidosiren paradoxa (Dipnoi). *Physiological and Biochemical Zoology* 78: 546–559.

Farrell AP (1978) Cardiovascular events associated with air-breathing in two teleosts. *Canadian Journal of Zoology* 56: 953–958.

Farrell AP (2007) Cardiovascular systems in primitive fishes. In: McKenzie DJ, Farrell AP, and Brauner CJ (eds.) *Primitive Fishes, Fish Physiology, vol. 26*, pp. 53–120. San Diego, CA: Elsevier.

Fishman AP, DeLaney RG, Laurent P (1985) Circulatory adaptation to bimodal respiration in the dipnoan lungfish. *Journal of Applied Physiology* 59: 285–294.

Gonzales T, Katoh M, and Ishimatsu A (2008) Respiratory vasculatures of the intertidal air-breathing eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae). *Environmental Biology of Fishes* 82: 341–351.

Graham JB (1997) *Air Breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press.

Graham JB (2006) Aquatic and aerial respiration. In: Evans DH and Claiborne JB (eds.) *The Physiology of Fishes*, 3rd edn., pp. 85–117. Boca Raton, FL: CRC Press.

Hyrtl J (1870) Über die Blütgefass der aussern Kiemendeckelkieme von *Polypterus lapradei* Steindachner. *Sitzungsberichte der Wiener Akademie der Wissenschaften* 60: 109–113.

Icardo JM, Brunelli E, Perrotta I, *et al.* (2005) Heart inflow tract of the African lungfish *Protopterus dolloi*. *Journal of Morphology* 265: 43–51.

Ishimatsu A and Itazawa Y (1983) Differences in blood oxygen levels in the outflow vessels of the heart of an air-breathing fish, *Channa argus*: Do separate blood streams exist in a teleostean heart? *Journal of Comparative Physiology* 149: 435–440.

Johansen K (1970) Air breathing in fishes. The circulatory system of air-breathing fish. In: Hoar WS and Randall DJ (eds.) *Fish Physiology IV*, pp. 361–411. New York: Academic Press.

McKenzie DJ, Campbell HA, Taylor EW, Micheli M, Rantin FT, and Abe AS (2007) The autonomic control and functional significance of changes in heart rate associated with air breathing in the jeju, *Hoplerythrinus unitaeniatus*. *Journal of Experimental Biology* 210: 4224–4232.

Müller J (1842) Beobachtungen über die Schwimmblase der Fishce, mit Bezue auf einige neue Fischgattungen. *Archiv fur Anatomie, Physiologie und wissenschftliche Medicin* 307–329.

Munshi JSD, Hughes GM, Gehr P, and Weibel ER (1989) Structure of the air-breathing organs of a swamp eel, *Monopterus cuchia*. *Japanese Journal of Ichthyology* 35: 453–465.

Olson KR (1994) Circulatory anatomy in bimodally breathing fish. *American Zoologist* 34: 280–288.

Satchell GH (1976) The circulatory system of air-breathing fish. In: Hughes GM (ed.) *Respiration of Amphibious Vetebrates*, pp. 105–123. London: Academic Press.

Satchell GH (1991) *Physiology and Form of Fish Circulation*. Cambridge: Cambridge University Press.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Pelagic Fishes

Contents

An Introduction to the Biology of Pelagic Fishes
Physiology of Tuna
Endothermy in Tunas, Billfishes, and Sharks

An Introduction to the Biology of Pelagic Fishes

D Bernal, University of Massachusetts, Dartmouth, MA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
General Adaptations for Life in the Pelagic Realm
Do Pelagic Fish Have a Depth Preference?
What May Limit the Vertical Movements of Pelagic Fishes?
Physiological and Morphological Adaptations That Allow Some Pelagic Fishes to Undergo Rapid and Repeated Vertical Movements in the Water Column
Further Reading

Glossary

Aerobic Processes occurring without the need for oxygen.

Aerobic scope The capacity of an animal to increase its level of aerobic metabolism beyond that which is required for maintenance alone and thereby support activities such as muscular exercise, growth, or reproduction; it sets the capacity for oxygen-consuming physiological processes that an animal may perform at a given time. Aerobic scope is the difference between minimal and maximal oxygen consumption rate.

Anaerobic Processes occurring without the need for oxygen.

Anoxia (anoxic) Complete absence of dissolved oxygen in water.

Aphotic Conditions where ambient light is not present.

Arterial Adjective pertaining to blood that has been oxygenated.

Benthic Organisms living on, or near, the seafloor; lowest level of a body of water.

Buoyancy, positive and negative A positively buoyant organism is less dense than the surrounding fluid, so tends to rise to the surface. A negatively buoyant one is denser than the fluid and tends to sink.

Burst swimming Short-duration (s), high-energy swimming behavior used during predator avoidance or prey capture and powered by the white myotomal muscles using anaerobic metabolic pathways.

Cardiac output (V_b) Volume of blood pumped per unit time from the ventricle; the product of heart rate and stroke volume.

Closed-system temperature change A condition where blood-oxygen content remains constant, but the partial pressures of oxygen and carbon dioxide change with temperature.

Continuous swimming Long-duration (days–years) swimming behavior used during migrations or extended movements throughout the water column powered by the red myotomal muscles using aerobic metabolic pathways.

Coronary vessels Arterial vessels usually arising from the gills and directly irrigating the myocardial tissues with oxygenated blood.

Diel Pertaining to a 24-h period.

Drag (frictional and form) Resistance to the relative motion of an object through a fluid. Frictional drag refers to the resistance proportional to the wetted surface area of the fish and form drag refers to the resistance proportional to the cross-sectional area of the fish in contact with the water.

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Elasmobranch The subclass of fishes (Elasmobranchii) found within the class Chondricthyes that includes sharks, skates, and rays, making up the Chondricthyes or cartilaginous fishes. Their bones are made up of cartilage rather than calcium found in all other vertebrates.

Endotherm An animal that uses its own metabolism as the major source of heat to maintain its body temperature greater than that of the surrounding environment.

Excitation–contraction (EC) coupling The physiological process of converting an electrical stimulus (an action potential) into a mechanical response (muscle contraction).

Hemoglobin A (usually) tetrameric, red blood cell-associated protein containing an iron core capable of reversibly binding oxygen.

Hypoxia (hypoxic) Conditions where dissolved oxygen is present at low levels.

Lactate Metabolic end product resulting from the catabolism of glucose under anaerobic conditions.

Muscle contraction When a muscle cell shortens and generates force.

Myocardium A general term for the cardiac muscle of the heart, which comprises several subtypes, including the compact myocardium and the spongy myocardium of fishes.

Myocyte An individual cardiac muscle cell.

Myoglobin An oxygen-binding protein in muscle that resembles hemoglobin in function, and enhances the rate of oxygen diffusion.

Myotomal muscle Segmented muscles along the body of a fish used for whole-body undulatory swimming.

Open-system temperature changes A condition where the partial pressures of oxygen and carbon dioxide are constant but the blood-oxygen content changes.

Oxygen minimum zone A zone in the water column (usually between 400 and 2000 m) where oxygen saturation is at its lowest.

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Photic Conditions where ambient light is present.

Ram ventilation Ventilation of the gills by swimming forward with the mouth open while suspending activity in the respiratory muscles.

Red myotomal muscle Slow-twitch muscle fiber type with a high aerobic capacity, mitochondrial density, and myoglobin concentration. Powers repeated, slow movements, such as continuous swimming.

Rete mirabile (pl. retia mirabilia) A vascular network, usually consisting of a parallel array of both arterial and venous vessels with countercurrent flow. Retia facilitate transfer of heat or oxygen between the arterial and venous vessels. Rete is Latin for ‘net or network’, mirabile for ‘wonderful’ or ‘miracle’.

Specific dynamic action The additional amount of oxygen or energy consumed above SMR as a result of feeding, measured during the period after feeding until metabolic rate returns to SMR; SDA represents the energetic cost of digestion, assimilation, and protein turnover.

Standard metabolic rate (SMR) The minimum metabolic rate of survival. Typically, SMR is measured on resting, unstressed adult animals in the post-absorptive state under normothermic conditions. For fish, normothermic is defined as a temperature well within the species tolerance limits for which the animals have had ample time to acclimate.

Stroke volume The volume of blood ejected into the aorta by a single contraction of the ventricle(s).

Teleosts A major group of ray-finned bony fish, including the most common fish.

Thermocline Zone of high rate of temperature change that separates a shallow, less dense, warm water layer (epilimnion) from a deeper, more-dense, colder-water layer (hypolimnion) in lakes, seas, and estuaries.

Thermal rate coefficient (Q_{10}) A unitless quantity that represents the factor by which the rate of a reaction is affected by a 10 °C change in temperature. A temperature-independent reaction would have a Q_{10} of 1.0 and a reaction rate that increases with temperature increase will have a Q_{10} greater than 1.0.

Upper uniform-temperature surface layers The surface layer of the open ocean that is a uniformly warm, well-mixed layer with abundant light penetration during the daylight hours and is limited vertically by the thermocline.

Ventricle A thick-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Venous Related to deoxygenated blood.

Water column The representative three-dimensional column of water in the ocean environment spanning the surface to the sediments.

White myotomal muscle Fast-twitch muscle fiber type of large diameter with a high anaerobic capacity, large fiber size, low mitochondrial density, and myoglobin concentration; powers short-duration, high-power movements such as burst swimming.

Introduction

Fishes live in all ecosystems within the planet's oceans, ranging from shallow intertidal waters to the deep, abyssal oceanic plains and trenches (**see also Deep-Sea Fishes**), as well as in the vast open ocean. Pelagic fishes differ from other species (e.g., benthic or coastal species) in that they live in a three-dimensional environment without any discrete boundaries (i.e., not associated with bathymetric or coastal structures) that impede their horizontal and vertical movements through the water column. In general, the open-ocean pelagic environment occupies the majority of the volume of the oceans, and is characterized as being a vast, nutrient-poor expanse with distinct stratification of temperature, dissolved oxygen, and light intensity. In contrast to the neritic zone that is commonly associated with the continental shelf and thus is relatively shallow (<200 m), the pelagic zone includes all other open waters that are deeper than 200 m, where the bottom (bathymetry/structure) seldom has an effect on the surface layers and the water masses stratify due to their thermohaline properties (**Figure 1**). Moreover, the bathymetry of the pelagic zone does not have a pronounced influence on local oceanography (e.g., currents, thermal fronts, and upwelling) and light penetration does not have a direct effect on the benthos.

Many pelagic fishes often occupy the same geographic areas of the open ocean, but they really occupy largely separate ecosystems based upon their capacity to move vertically through the water column and tolerate different temperatures, oxygen concentrations, and light levels, which taken together result in resource partitioning. For pelagic fishes, the open ocean does not offer physical places to hide, and they must swim in order to avoid predators and find prey. For this reason, pelagic fishes have evolved a suite of morphological and physiological adaptations that (1) enhance their ability to swim continuously for extended periods of time while searching for prey or favorable oceanographic conditions and (2) retain their capacity for rapid bursts of speed for feeding or escaping. Overall, there are species-specific adaptations that allow pelagic fishes to inhabit overlapping yet distinct ecosystems within the pelagic realm. Several pelagic species have evolved physiological specializations that allow them to effectively pursue prey that are associated with the deep, cold, and hypoxic water layers. Others have the capacity to temporality venture into these deep layers, while still others lack such specializations and tend to remain in the upper uniform-temperature surface layers of the ocean. Taken together, pelagic fishes defy a strict categorization, having a wide diversity of morphological forms, physiological capabilities, and varied ecological preferences. In the pelagic realm, there are plenty of exceptions to the rules; however, all pelagic fish successfully exploit an environment that is often sparse in food and offers limited protection from predators.

With the exception of the high-latitude polar zones, the open ocean has two distinct water masses that separate the pelagic realm into two oceanographically (and potentially ecologically) discrete vertical zones: the warm surface layers and the cold deep waters (**Figure 1**). The surface layer is a uniformly warm,

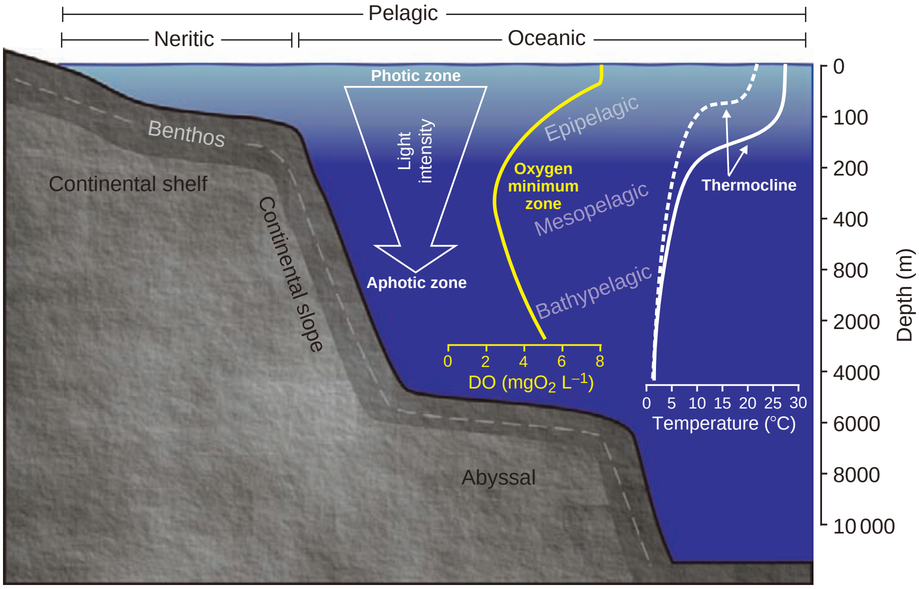


Figure 1 Classification of the pelagic environment showing the general depth-related patterns of light intensity, dissolved oxygen, and temperature in the oceanic zone. Note the oxygen-minimum zone is present at ~400 m and that the temperature–depth profiles vary by location (southern California (dashed line) and central equatorial Pacific (solid line)).

well-mixed layer of the ocean with abundant light penetration during the daylight hours and is limited vertically by the thermocline (an abrupt decrease in water temperature over a relatively small change in depth). The temporal persistence and absolute depth to which this warm, well-mixed layer penetrates vary due to local bathymetry, latitude, wind patterns, seasons, and thermohaline circulation. For example, between spring and fall, southern California waters have a thermocline between 30 and 60 m depth, whereas in the waters off Hawaii the thermocline is commonly as deep as 200 m and persists through most of the year. By contrast, the deep-water layer remains permanently and uniformly cold (2–8 °C) with very little, if any, light penetration (**see also Deep-Sea Fishes**). In addition, the lack of any measurable photosynthetic-based primary production and sparse vertical mixing in the deep-water layer (except during wind-driven upwelling events in coastal areas) results in the persistent decline in dissolved-oxygen levels leading to a marked hypoxic (i.e., oxygen-minimum) zone usually at mid-water depths (800–2000 m) (**Figure 1**).

Although the species diversity of fish inhabiting the upper 200 m of the pelagic realm (the epipelagic) represents a small fraction (~2%) of the approximately 28 000 described species, they are the most abundant fishes in the oceans. Pelagic fishes are morphologically diverse and range broadly in size (from the small anchovies Engraulidae to the whale shark *Rhincodon typus*, the largest fish in the ocean), body shape (from the elongate oarfish *Regalecus glesne* to the streamlined tunas Scombridae), coloration (from the whitish mola *Mola mola* to brilliantly colored mahimahi *Coryphaena hippurus*), swimming behavior (from schooling sardines Clupeidae to solitary oceanic whitetip sharks *Carcharhinus longimanus*), and feeding ecology (from the predatory swordfish *Xiphias gladius* to the planktivorous basking shark *Cetorhinus maximus*) (**Figure 2**). The ecology of pelagic fishes is also diverse, as some species spend only a portion of their life swimming in the open ocean (e.g., American eels *Anguilla rostrata*), others undergo diel migrations from the deep mesopelagic to the epipelagic (e.g., lanternfishes Myctophidae), while others are permanent residents of the epipelagic (e.g., tunas and jacks Carangidae). While some large pelagic species are strong swimmers and are known for their repeated, long-distance transoceanic migrations, other smaller species (e.g., American eel) also migrate over large distances (**Figure 3**) (**see also Fish Migrations:** The Biology of Fish Migration, Eel Migrations, and Tracking Oceanic Fish). It is impossible to paint an all-encompassing, generic portrait of the traits shared by all pelagic fishes, because, as always, the great diversity in fishes leads to exceptions to any biological generality. Nonetheless, the aim of this article is to provide the reader with a broad overview of the general morphological and physiological adaptations that make pelagic fishes distinct from their less-mobile, benthic, and coastal counterparts.

Numerous pelagic species co-inhabit the same geographical areas of the open ocean, but their depth distributions and vertical movement patterns vary significantly. This allows pelagic species to have a similar spatial distribution but results in marked differences in their diel utilization of the water column. We have just begun to understand the complex vertical and horizontal movement patterns of pelagic fishes and how these are influenced by regional oceanographic parameters (e.g., water temperature and light availability) and other factors such as reproduction and interspecific interactions. This article provides the reader with background information on the general adaptations that are present in pelagic fishes and then focuses on several unique physiological adaptations that allow some fishes to undergo large vertical (e.g., diving) movements through the water column.

General Adaptations for Life in the Pelagic Realm

Body Shape

Because pelagic fishes are not able to use fixed structures (benthic or otherwise) as a means of attachment or refuge, they must swim to escape predators and capture prey. The degree to which pelagic fishes swim ranges widely from relatively minimal swimming in nearly neutrally buoyant species to extensive swimming in negatively buoyant species. The latter commonly rely on continuous forward swimming to generate hydrodynamic lift (i.e., counteract their denser-than-seawater specific gravity) and to ram ventilate their gills. Due to their constant forward motion, most pelagic fishes have evolved morphological specializations that decrease both frictional and form drag (proportional to the surface area and cross-sectional area in contact with the water, respectively). These specializations include body streamlining (**Figure 2**), a high-aspect-ratio caudal fin, narrow caudal peduncles with one or two pairs of lateral keels, which decrease drag by reducing the turbulent flow across the stiff, nearly symmetrical caudal fin during the thrust-producing undulatory (side-to-side) movements, small finlets lining the dorsal and ventral margins along the posterior part of the body that help prevent flow separation at high speeds, and retractable fins. In general, pelagic fishes also possess large pectoral fins that provide additional hydrodynamic lift and a large dorsal fin that acts to minimize body rolling during swimming (**Figure 2**, **see also Buoyancy, Locomotion, and Movement in Fishes:** Maneuverability, and Undulatory Swimming).

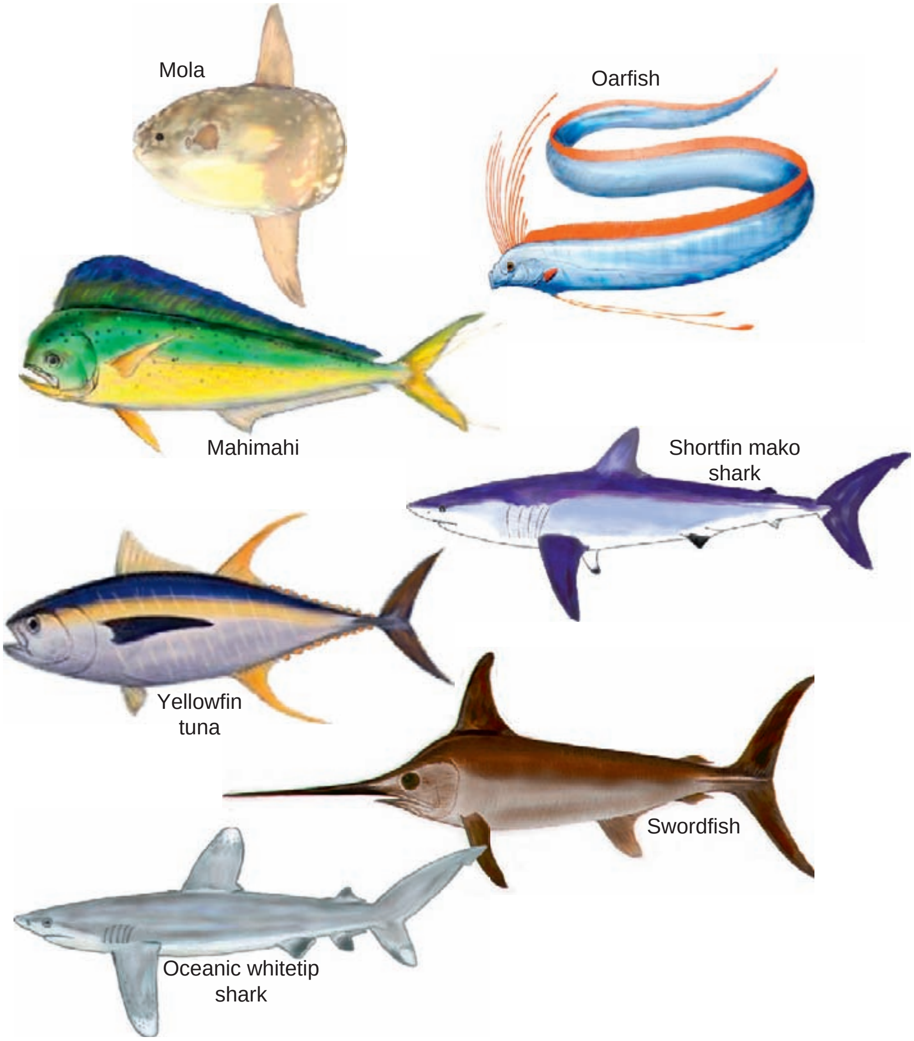


Figure 2 Representative examples of pelagic fishes showing the diversity of body shapes and colorations. Note the highly streamlined bodies and nearly symmetrical caudal fins of continuously swimming species (mahimahi, shortfin mako shark (*Isurus oxyrinchus*), yellowfin tuna (*Thunnus albacares*), oceanic whitetip shark, and swordfish).

Body Coloration

The lack of physical hiding places in the pelagic realm means that both predators and prey are easily visible in all three dimensions. Although a potential solution to this would be to have a transparent body, an adaptation present in many pelagic invertebrates and larval fishes, the combination of a large size and the use of muscles to power swimming limits this adaptation and renders pelagic fishes visible. Some fishes have evolved a laterally compressed body, making the body contour of these fishes, when observed from above or below, appear small. Other pelagic fishes that inhabit the well-lit surface waters have vibrant colors (mahi mahi) and dappling or barred patterns (mola, whale shark) that disrupt the body contour when viewed from the side. A more widely observed adaptation in pelagic fishes that inhabit the photic waters of the open ocean is to have cryptic countershading coloration in which the dorsal section of the body is darker (blue or green) than the much lighter (white) ventral side of the body (**Figure 2**). This allows the body to blend in with the blue or green water when seen from above and with the light surface waters when seen from below (**see also Vision**: Physiological Optics in Fishes, and Color Vision and Color Communication in Reef Fish).

Sensory Mechanisms

The perception of external stimuli by pelagic fishes is, as in other fishes, a complex assemblage of the visual, auditory/mechano, and olfactory systems (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction, **Hearing and Lateral Line**: Fish Bioacoustics: An Introduction, and **Vision**: Vision in Fishes:

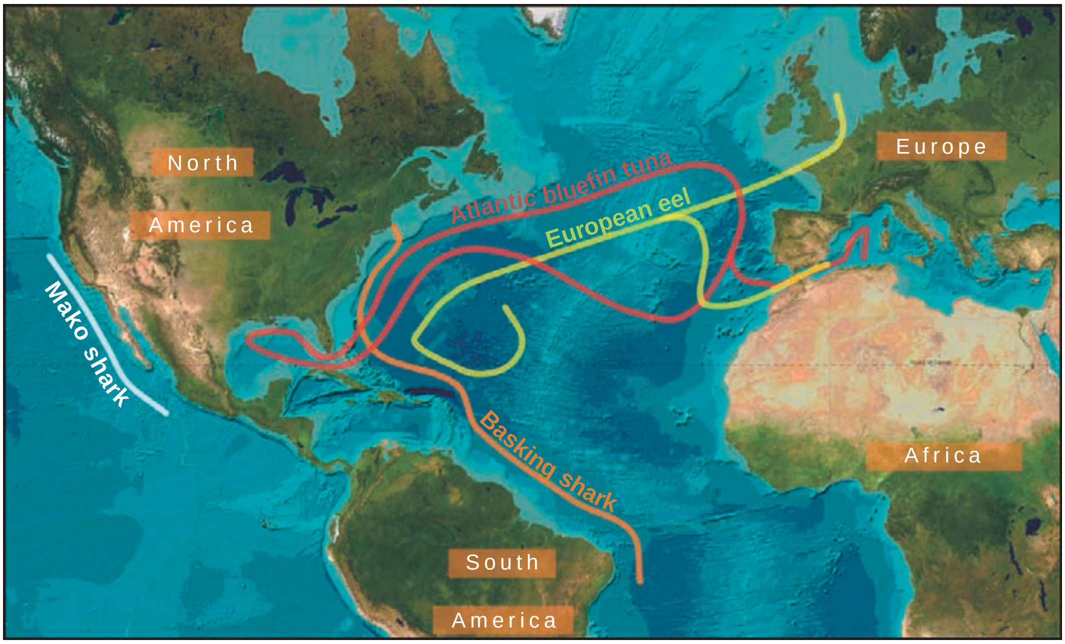


Figure 3 Horizontal movement patterns of selected pelagic fishes showing their long, trans-oceanic migratory patterns.

An Introduction). The ample light penetration during the daylight hours in the upper, photic layer of the pelagic zone has led most pelagic fishes to become visual predators. Thus, most have well-developed eyes that during the daylight hours, are light-adapted (allowing for vision in bright light). However, several pelagic species (e.g., swordfish and bigeye thresher shark *Alopias superciliosus*) that spend the daylight hours at depth (>300 m) and rarely encounter bright-light conditions have relatively larger eyes that remain constantly dark-adapted (allowing for vision under low-light levels). The extensive propagation of sound in the ocean has led pelagic fishes to evolve a complex auditory system capable of detecting sounds and vibrations across a wide range of frequencies that assist in finding prey and avoiding predators. Many small pelagic fishes form schools, which may allow hydrodynamic drafting (i.e., decreasing the metabolic costs of locomotion, through swimming in water that has been accelerated forward by preceding fish in the school) while serving as an antipredatory mechanism. This behavior relies extensively on the lateral-line (i.e., mechanoreceptor) sensory pathway, which detects pressure waves from surrounding conspecifics (**see also Hearing and Lateral Line**: Lateral Line Neuroethology). The lateral line is thus critical for the maintenance of a tight spatial formation during this grouping behavior. Unlike the keen sense of smell characteristic of benthic fishes, or those that inhabit environments with low or no light penetration (e.g., highly turbid waters, the aphotic zone) where vision is often impaired, pelagic fishes possess a modest olfactory capacity. A very small and select group of pelagic fishes (i.e., tunas, billfishes Istiophoridae, and lamnid sharks Lamnidae) have evolved the capacity for cranial endothermy, which allows for elevation of brain and eye temperatures (**see** section Regional Endothermy). This unique adaptation significantly influences the sensory capabilities of these species and increases the overall ability to locate and catch prey and avoid predation (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction, **Hearing and Lateral Line**: Fish Bioacoustics: An Introduction, and **Vision**: Vision in Fishes: An Introduction).

Buoyancy

In general, the bodies of pelagic fishes are made mostly of myotomal (i.e., swimming) muscles, bone or cartilage, and skin, which is usually covered with heavy scales. This results in an overall specific gravity (i.e., a measure of density) that is higher (~1.05–1.09 g cm^{-3}) than that of seawater (1.024 g cm^{-3}), resulting in negative buoyancy. Fishes have evolved adaptations to offset their high specific gravity allowing them to reach near-neutral buoyancy and ultimately decrease the potential cost of locomotion (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). The most obvious of these mechanisms in bony fishes is the swimbladder, which acts as a gas-filled flotation device. However, unlike the swimbladder of some shallow-water fishes, which have a pneumatic duct that is directly connected to the esophagus (physostomes), most pelagic fishes do not possess this duct (physoclists). In this case, the swimbladder is filled by transfer of dissolved gases in the blood through the gas gland and associated rete mirabile network (**see also Design and Physiology of Arteries and Veins**: The Retia). Interestingly, a swimbladder is not an essential structure for all pelagic fishes; for example, some closely related tuna genera have swimbladders and others do not. Nonetheless, the continuous rapid swimming in tunas allows the pectoral fins and body to produce hydrodynamic lift to offset the negative buoyancy. An additional benefit to the lack of the swimbladder in some of these species may be the ability to move freely up and down the water column without any adverse effect of a changing swimbladder volume, hence avoiding the overexpansion of this organ during a rapid ascent (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes and Buoyancy, Locomotion, and Movement in Fishes: An Introduction).

Another adaptation in pelagic fishes that increases their buoyancy is the presence of a large quantity of low-density lipids throughout their bodies (e.g., swimming muscles, body cavity, subdermal layer, and liver). In some species, there is a positive correlation between the overall amount of lipids and body size; that is, there may be a higher overall relative amount of lipids in the body as specific gravity increases due to their larger body mass. Unlike bony fishes, elasmobranchs do not have a swimbladder, and a principal mechanism to offset their negative buoyancy is to have a very large, oil-rich liver that decreases their overall specific gravity. In pelagic sharks, liver size ranges between 10% and 15% of body mass, but in some species (e.g., basking shark) the liver may be as large as 25% of body mass and occupy 95% of the visceral space. In addition, benthic sharks and rays have relatively smaller livers and are less buoyant than pelagic sharks, which allows them to sink and remain at the bottom.

Cardiorespiratory System

The ecology of many actively swimming pelagic fishes demands continuous locomotion during long-distance horizontal migrations and throughout repeated vertical oscillations. Because sustained swimming is powered by aerobic metabolism, the cardiorespiratory system needs to deliver ample supplies of oxygen and metabolic substrates (i.e., fuels) to the working tissues at all times. Although there are several marked differences between the cardiorespiratory systems of teleosts and elasmobranchs, continuously swimming pelagic fishes (e.g., compared with less-active species) have morphological and physiological specializations that enhance the transfer of oxygen across the gills (e.g., larger gill surface area and shorter blood–water diffusion distances), increase the quantity of oxygen transported by the blood (e.g., higher hemoglobin concentrations), elevate the delivery rate of oxygenated blood to tissues (e.g., increased capillary density and a complex capillary-muscle fiber geometry, resulting in an increased ratio of capillary surface area to muscle-fiber volume – **see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis), and facilitate the intracellular transport of oxygen to the mitochondria (e.g., increased myoglobin levels). In addition, relative to other fishes, the hearts of continuously swimming pelagic fishes have larger, more muscular ventricles that are perfused by an elaborate coronary blood supply providing the heart tissues with sufficient oxygen and metabolic substrates to sustain elevated aerobic activities (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping, and The Coronary Circulation). Pelagic fishes also have a more pyramidal-shaped ventricle that is hypothesized to enhance the effectiveness of blood outflow during systole. Taken together, these cardiorespiratory features appear to allow pelagic fishes to supply the continuously active, aerobic locomotor muscles with sufficient oxygen and metabolic substrates to sustain the high rates of intracellular adenosine triphosphate (ATP) production that power locomotor-muscle contraction during continuous swimming (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes, **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics, **Transport and Exchange of Respiratory Gases in the Blood**: Hemoglobin, and O_2 Uptake and Transport: The Optimal P_{50}).

Metabolic Rates and Swimming

Continuous swimming in pelagic fish requires a sustained supply of oxygen and metabolic substrates to fuel the aerobic myotomal muscles, and has resulted in a suite of morphological and physiological adaptations that augment not only the uptake and delivery of oxygen, but also the overall oxygen demand (i.e., aerobic metabolic rate). Although, in general, the aerobic metabolic rates of pelagic, obligate ram-ventilating fishes are usually elevated when compared to most other less-actively swimming fishes (e.g., benthic or mesopelagic), several species stand out even among pelagic fishes. For example, the swimming metabolic rates (e.g., including the maximum metabolic rates (MMRs)) of tunas and lamnid sharks (two groups of fish known for their highly migratory, and often rapid, swimming behavior) are 2–6 times higher than those measured in other actively swimming fishes (e.g., salmonids and carcharhinid sharks). These high metabolic rates have been commonly associated with the elevated maintenance costs (i.e., standard metabolic rate (SMR)) of the specialized cardiorespiratory system that allows for high rates of energy turnover as well as with the concomitant thermal effects of regional endothermy. (Although regional endothermy may increase metabolic processes (e.g., aerobic, red myotomal muscle contraction rates, and digestion), the morphological structures involved in heat retention only harness metabolically produced heat and by themselves they do not necessarily reflect any additional metabolic cost (excluding species that have thermogenic tissue); **see** section Regional Endothermy). However, the aerobic scope (i.e., MMR–SMR) is a more biologically relevant indicator than are the absolute values of metabolic rate and represents the potential capacity to handle multiple simultaneous aerobic demands (e.g., continuous swimming, recovering from oxygen debt, somatic growth, digestion of prey, and assimilation of nutrients). For example, in tunas, the MMR can be up to 18 times higher than the SMR, a markedly higher aerobic scope when compared with many other pelagic species. Similarly, the aerobic scope of lamnid sharks (e.g., MMR up to 6 times higher than SMR) is higher than that of other actively swimming pelagic shark species. Overall, the heightened aerobic scope in continuously swimming pelagic predators reflects their need for the capture of fast-moving prey and the physiological consequences (e.g., rapid recovery from burst activity and rapid digestion) of their swimming behaviors. In addition, some tropical, pelagic species (e.g., mahimahi and billfishes Istiophoridae) also have high MMRs, but this may reflect their rapid somatic and gonadal growth, a scenario not common for sharks, which have relatively low growth rates, mature later in life, and give birth to relatively few pups. (**see also Gas Exchange**: Respiration: An Introduction, **Ventilation and Animal Respiration**: The Effect of Exercise on Respiration, **Food Acquisition and Digestion**: Cost of Digestion and Assimilation, **Energetics**: General Energy Metabolism, and **Swimming and Other Activities**: Energetics of Fish Swimming).

The capacity to capture prey or escape predators is powered, largely, by the white myotomal muscles and fueled by anaerobic metabolism. Burst swimming relies on the rapid mobilization of muscle energy stores and results in the accumulation of lactate and protons. It is no surprise that pelagic fishes that routinely capture fast-moving prey not only have a higher capacity to burst swim, but also show a remarkable capacity to recover quickly from these anaerobically powered activities. Tunas, for example, have the highest known white-muscle lactate levels among vertebrates ($>100\,\mu\text{mol g}^{-1}$) and also have both high muscle and blood buffering capacities, which allows them to tolerate times of elevated proton loads during the time needed to process lactate to pre-exercise levels, a mechanism they can undergo more quickly than can other fishes. This is a consequence of tunas' cardiorespiratory system being able to deliver oxygen and metabolic substrates at rates far above those needed at routine activity levels. These adaptations appear to be a direct result of the selective pressures in the pelagic environment where food resources are aggregated, but widely scattered, and where no refuge exists for animals to hide and recover from a bout of strenuous activity.

Do Pelagic Fish Have a Depth Preference?

Many studies have hypothesized that there are species-specific depth preferences in pelagic fishes. However, it is unclear how fishes can perceive depth *per se*. That is, there is no known sensory mechanism that would permit a fish to detect the exact depth (e.g., 20 or 200 m) it is occupying. When fishes change depth, the swimbladder volume is altered, and this may be detected by the stretch receptors lining the swimbladder walls. However, it appears that these receptors only respond to acute changes in volume, and it remains to be seen whether they are able to determine the absolute depth. By contrast, elasmobranchs, which lack a swimbladder, therefore neither are subject to any volume-expansion limitations during their vertical movements nor can they rely on a change in gas volume to detect variations in hydrostatic pressure. Therefore, fish may not target a specific depth, but may associate with a depth range based on one or more environmental variables that correlates with depth, such as light, temperature, and prey availability. When viewed from an ecological perspective, it is difficult to perceive how depth itself can be relevant to pelagic fishes that inhabit an environment with dynamic oceanographic conditions such as the revolving water masses in the pelagic realm affect light penetration and water clarity, water temperature, deep-water mixing, and nutrient availability, and dissolved oxygen concentrations throughout the water column. For this reason, it is hard to associate any of these biotic and abiotic parameters to a particular depth because they are in constant flux throughout the open oceans. Because these oceanographic features appear to have a profound influence on the prey and predator distributions throughout the water column, it is clearly not the absolute depth that a pelagic fish targets. Rather, it is specific favorable environmental conditions and the associated organisms that influence the vertical (depth) movements of fishes.

Nonetheless, it is possible that the vertical movements themselves, not depth preference *per se*, may be physiologically constrained. In fishes that have a swimbladder, the range of depths they can occupy and the speed at which they can ascend or descend are limited by the capacity of this organ to adjust its volume (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). Because any pressure change during the vertical movements will affect bladder volumes, it is critical that the time required for gas secretion (bladder filling) and resorption (bladder emptying) is less than that of the actual time of the vertical movement through the water column. If not, the effect of hydrostatic pressure on swimbladder volume could limit the extent of the vertical movements by, for example, the overexpansion of the swimbladder during rapid ascents. A solution to this would be that if pelagic teleosts, which undergo repeated and rapid movements up and down the water column, maintain a swimbladder volume that is equivalent to its maximum capacity when at the top of their vertical ranges, which would result in near-neutral buoyancy after the ascent.

An additional constraint exists as well. A direct effect of very high hydrostatic pressures (>1000 m in depth) is the alteration of the three-dimensional shape of enzymes and other proteins, which may ultimately affect biochemical function (**see also Deep-Sea Fishes**). However, few species of pelagic fishes dive to these extreme depths, and when they do, it is only for short periods of time (min–h). Hence, the overall vertical movements and depth ranges occupied for substantial periods of time by almost all pelagic fishes are above these potentially critical depths.

What May Limit the Vertical Movements of Pelagic Fishes?

There is a growing body of literature documenting how the vertical movements of predatory pelagic fish are influenced by the vertical movements of their prey. In order to decrease their exposure to predation, many prey species (e.g., cephalopods and crustaceans) have evolved greater relative physiological tolerances that allow them to diurnally invade deeper waters that have lower water temperatures, decreased dissolved oxygen levels, and lower light availability. Therefore, any pelagic predator that wishes to benefit from the concentration of the deep, hard-to-reach prey will have to maintain not only a functional suite of sensory systems associated with prey detection (e.g., sight, smell, and hearing) while in the deep, cold, hypoxic, and relatively dim-lit waters, but also sustain the swimming capabilities involved in the continuous search for prey and the bouts of burst swimming associated with prey capture. Because these environmental parameters appear to impose important limitations on the vertical movement patterns of large pelagic fishes, future work should focus on how oceanographic dynamics affect species-specific physiological adaptations to these conditions.

The vertical movements of pelagic fishes appear to be influenced not only by local oceanographic parameters that affect environmental conditions, but also by several biotic factors such as reproduction, interspecific (e.g., predator–prey) interactions, and ontogeny. Although how these factors influence vertical migrations within the water column is unknown, largely, the general movement patterns of pelagic fish still can be differentiated by comparing their diel vertical and thermal distributions. Specifically, during the daylight hours, when the water column has a distinct photic zone, many species reveal discrete vertical distributions due to their physiological tolerances to the depth-related reduction in temperature, oxygen level, and light intensity. By contrast, during the night when the entire water column is exposed to low light availability, many pelagic fishes migrate to or remain within the upper, warm, well-mixed surface layer resulting in a substantial overlap of their vertical distributions. This diel shift in the vertical distribution is most likely associated with the vertical migration of prey species associated with the deep-scattering layer.

Because the different physiological capacities of pelagic fishes to tolerate large and rapid changes in temperature, dissolved oxygen, and light levels are associated with their ability to move between the surface and deep-water layers, it is possible to group pelagic fishes into three broad categories that reflect their short-term (days–weeks) general vertical-movement patterns and hence their utilization of the water column:

- Group 1. These fishes are consistently found in the upper layers of the ocean where the water column is well mixed into a homogeneous thermal layer limited vertically by the thermocline. Although the depth within this layer that each fish routinely penetrates is species specific, the unifying theme for this group is that they rarely descend to water temperatures below 20 °C and thus are distributed both day and night in the relatively warm, photic, and well-oxygenated layers of the ocean. Some examples of genera in this group are: skipjack tuna *Kastuwonus pelamis*, wahoo *Acanthocybium solandri*, mahimahi, sailfish *Istiophorus*, silky shark *Carcharhinus falciformis*, oceanic whitetip shark, sunfish (*Mola*), whale sharks, and manta rays *Manta* (**Figure 4**).
- Group 2. Fishes in this group venture below the warm, surface layer and undergo regular, but short-lived, diurnal excursions into depths at or below the thermocline. Although the frequency and duration of these brief diving excursions are species specific, they may vary from a single event per day (e.g., striped marlin *Tretrapturus audax*, blue marlin *Makaira nigricans*, and black marlin *M. indica*) to several excursions per day (e.g., yellowfin tuna *Thunnus albacares*, shortfin mako shark *Isurus oxyrinchus*, and blue shark *Prionace glauca*) (**Figure 5**). Relative to group 1 fishes, this group has a broader vertical range resulting in their exposure to colder waters (even below 10 °C), lower levels of dissolved oxygen, and decreased light levels during the day.
- Group 3. Relative to all other pelagic fishes, this group (best exemplified by the bigeye tuna *T. obesus*, swordfish, and bigeye thresher shark (*Alopias superciliosus*)) exhibits distinct diurnal behavior patterns, making regular deep descents during the day and remaining in the upper uniform-temperature surface layer at night (**Figure 6**).

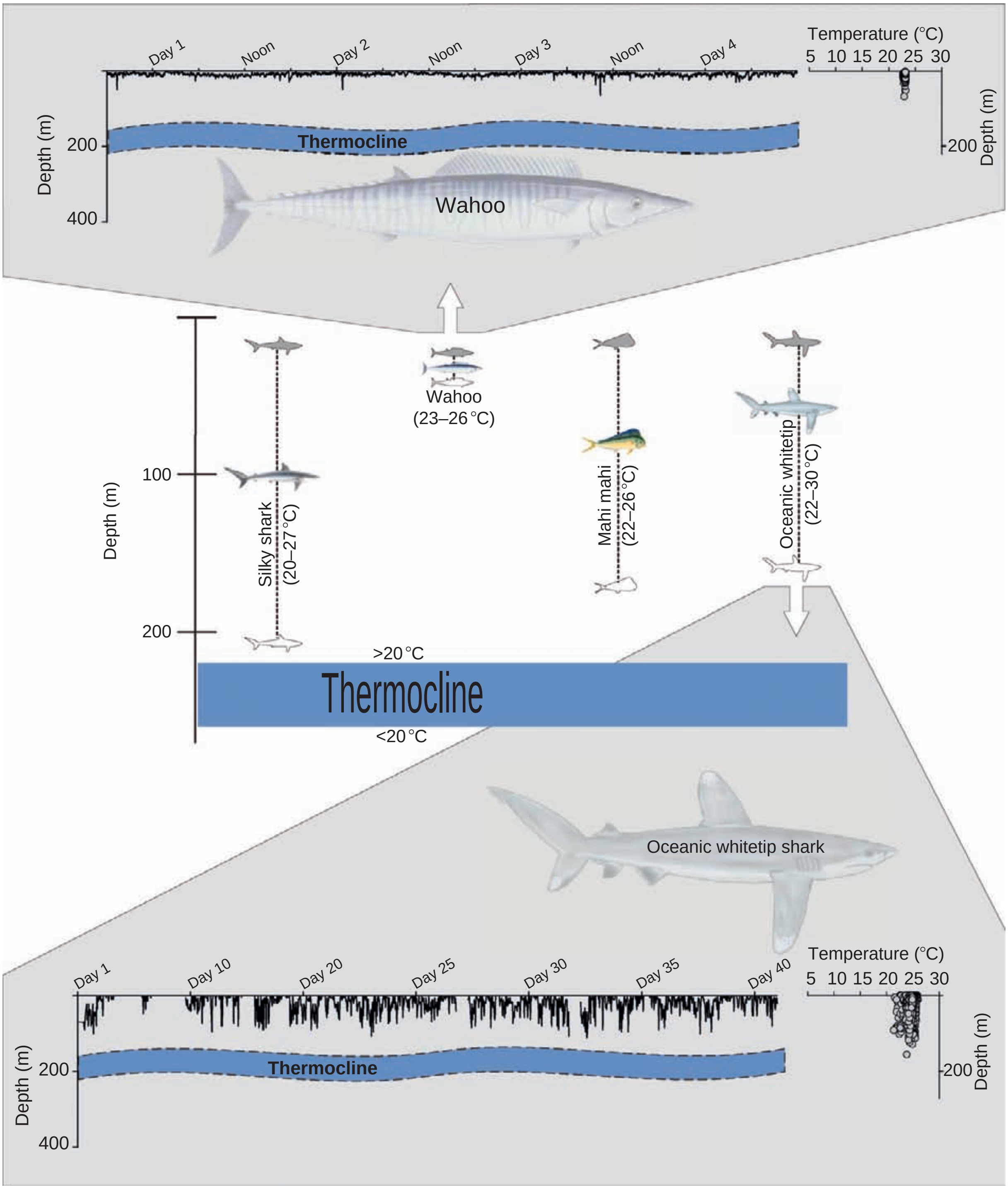


Figure 4 Representative vertical-dive profiles for group-1 pelagic fishes. Color fish images represent the average depth (combined night and day) for each species. Open and shaded fish outlines are the depths at which each species spent 95% of the time during the day and night, respectively. Values next to the name show the overall temperature ranges encountered. Blue-shaded bar represents the thermocline, separating water column into the upper warm, surface layer (>20 °C) and cooler deeper waters (>20 °C). Insets show the vertical movement patterns and temperature-depth profiles of wahoo and oceanic whitetip sharks. Day marks indicate midnight of that day.

This group has the widest thermal range and routinely descends to ~500–800 m, where water temperatures are below 5 °C and dissolved oxygen levels are low (<1 mg-O_2 L^{-1}) (**Figure 1**). In addition, this group experiences continuous low-light levels because they generally remain deep during daylight and occupy the upper water column at night. There are other species that also belong to this group because they are capable of

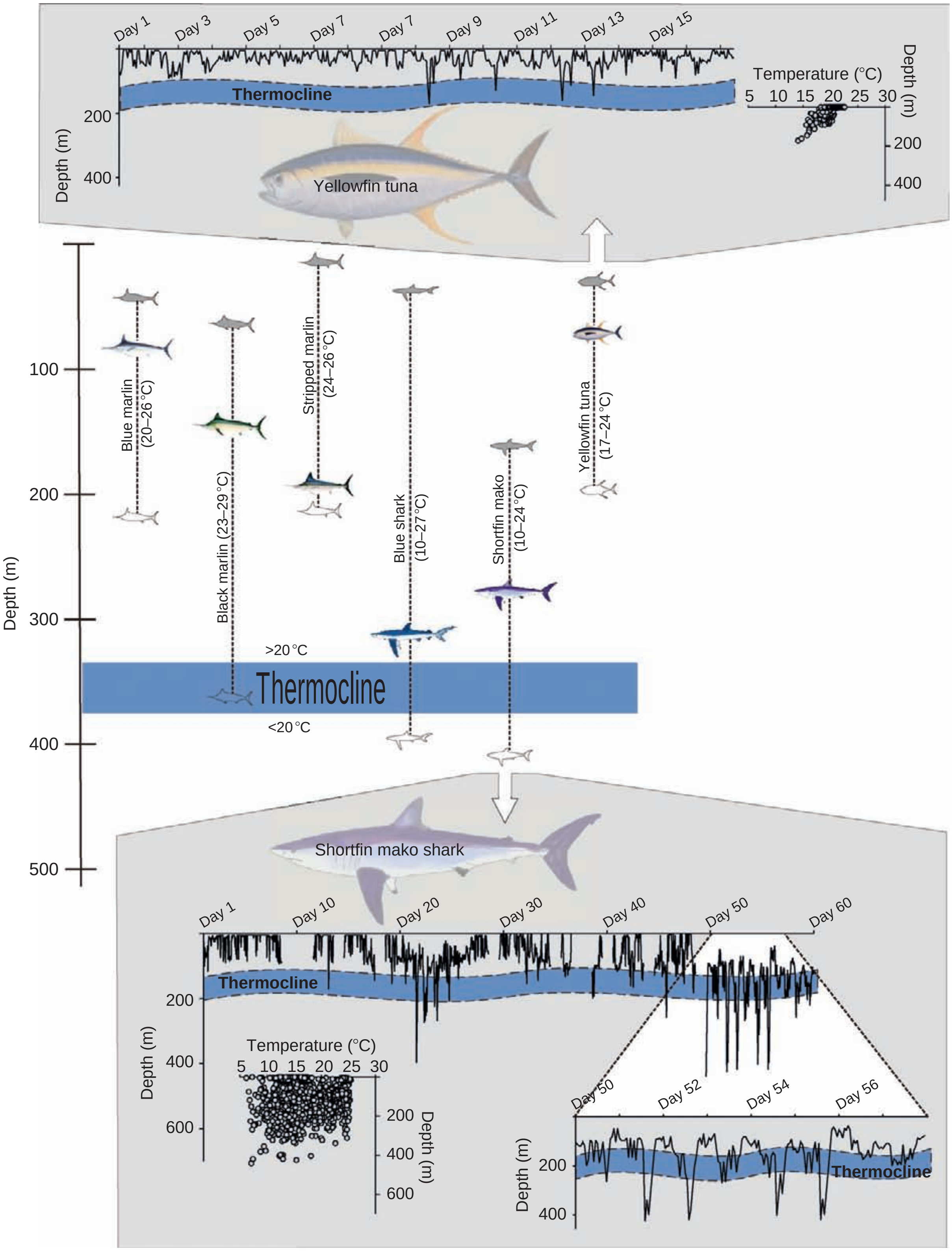


Figure 5 Representative vertical dive profiles for group-2 pelagic fishes. Color fish images represent the average depth (combined night and day) for each species. Open and shaded fish outlines are the depths at which each species spent 95% of the time during the day and night, respectively. Values next to the name show the overall temperature ranges encountered. Blue shaded bar represents the thermocline, separating water column into the upper warm, surface layer (>20 °C) and cooler deeper waters (<20 °C). Insets show the vertical movement patterns and temperature-depth profiles of yellowfin tuna and shortfin mako shark. Additional inset in the mako shark shows a finer timescale. Day-marks indicate midnight of that day.

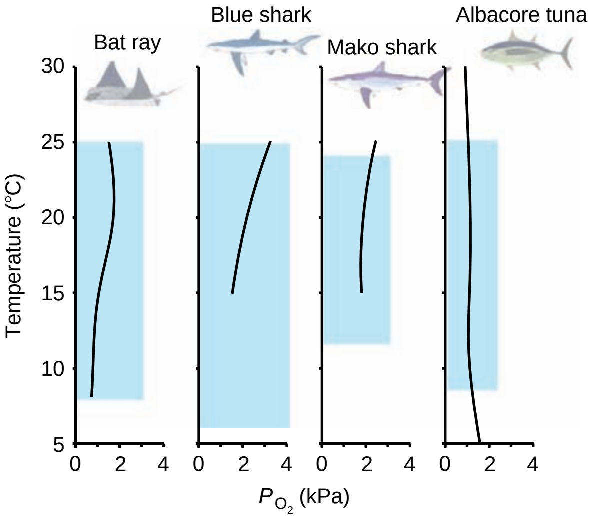


Figure 6 Comparison of the thermal effects on the oxygen-binding affinity of arterial blood at 50% saturation (P_{50}) during open-system temperature changes. Light blue shade shows the general range of ambient temperatures during their vertical movement patterns in the eastern Pacific Ocean. The bat ray (*Myliobatis californica*) migrates daily between 14 and 20 °C.

penetrating cold water during their extensive migrations to high latitudes (e.g., Atlantic bluefin tuna *T. thynnus*, porbeagle shark *Lamna nasus*, and salmon shark *L. ditropis*, basking sharks).

Physiological and Morphological Adaptations That Allow Some Pelagic Fishes to Undergo Rapid and Repeated Vertical Movements in the Water Column

Numerous species co-inhabit the same geographical areas in the pelagic realm; however, their depth distributions and vertical movement patterns vary significantly (**see** section What May Limit the Vertical Movements of Pelagic Fishes?). Thus, many pelagic species that have a similar spatial distribution have marked differences in their diurnal utilization of the water column, and hence, occupy distinct oceanographic ecosystems. For example, bigeye tuna and the yellowfin tuna, two closely related species, are often found, and targeted by the recreational and commercial fisheries, in the same geographical areas although their prey selection is clearly different (the former consumes prey from the deep layer, and the latter mostly feeds on species from the surface layer).

Pelagic fishes that undergo extensive vertical movements following their prey need to have physiological adaptations that allow for the uptake, and delivery of dissolved oxygen and metabolic substrates at rates that match whole-body metabolic oxygen demands. That is, these fishes need to be able to meet their metabolic demands during times of prey capture and predator avoidance, recovery from burst exercise, and digestion and assimilation, all the while being exposed to deep, cold, and potentially hypoxic waters. Because large pelagic fishes experience large and sudden changes in ambient water temperature during the rapid (~minutes) sojourns from the warm surface layers to the deep cold layers, they are unable to undergo temperature acclimation (e.g., synthesis of isozymes and modification of cell membrane structure). Thus, the reduction of ambient temperature and oxygen will undoubtedly alter cardiorespiratory function and ultimately restrict the swimming ability of the fishes and its capacity to undergo vertical movements through the water column.

Cardiac Function during Acute Temperature Changes

The hearts of all fishes receive blood from either the systemic venous system or the coronary circulation (**see also Design and Physiology of the Heart**: The Coronary Circulation, and Cardiac Anatomy in Fishes), which are at, or close to, thermal equilibrium with ambient temperature. Therefore, the temperature of the heart closely mirrors that of ambient conditions and immediately reflects changes in ambient temperature. Work on tunas has shown that a rapid 10 °C decrease in temperature lowers both heart rate and cardiac output, as stroke volume appears to be nearly fixed (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature). This change in heart rate appears to be independent of parasympathetic stimulation, which normally increases heart rate in fish, and thus the decline in heartbeat frequency appears to be the major contributor to the change in cardiac output. Recent work on Pacific bluefin tuna (group 3) found that a decrease in temperature from 25 to 10 °C lowered heart rate (thermal rate coefficient, $Q_{10} = 2.1$) to a lesser degree than that observed in other group 2 tunas ($Q_{10} = 3.1$) and that the heart rate, albeit low (<0.3 Hz), of bluefin tuna was maintained at temperatures as low as 2 °C, far below 10 °C at which other tuna hearts ceased normal function. This suggests that as tuna dive into colder waters at or below the thermocline, their slowing heart rate will result in a concomitant decrease in cardiac output and ultimately impair cardiorespiratory function. While tunas and other pelagic fishes still may meet their oxygen delivery requirements during slow swimming in colder temperatures, it is poorly understood how cardiac output is elevated in order to accommodate for the marked increases in oxygen demands associated with an increased swimming speed, recovery from burst swimming, and during digestion and assimilation.

Recent work on the excitation–contraction (EC coupling) dynamics in myocardium of group 3 (i.e., bluefin tuna and salmon sharks) and group 2 pelagic fishes has shown that, in the latter, a decrease in ambient temperature slows active calcium extrusion from the myocytes and prolongs relaxation times. Taken together, it appears that maintaining cardiac Ca^{2+} kinetics is critical to sustaining physiologically relevant heart rates (i.e., cardiac output) at low temperatures (**see also Design and Physiology of the Heart**: Physiology of Cardiac Pumping, **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels, **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature, and **Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux.)

Regional Endothermy

Rapid and repeated vertical movements and movements across thermal fronts are a common trait of pelagic fishes, and regionally endothermic species are able to maintain a more thermally stable operating environment for their tissues (e.g., aerobic locomotor muscles, eye and brain, and viscera). Thus, for these unique pelagic fishes, regional endothermy provides an operating temperature that safeguards physiological function when exposed to cold ambient conditions.

The steady-state body temperature of most fishes is in equilibrium with that of ambient water temperature and all metabolically produced heat is rapidly lost either by convective transfer through the blood to the gills or by thermal conduction across the body surface. The capacity for aerobic myotomal muscle and visceral endothermy, defined as the capacity to conserve metabolic heat and maintain steady-state tissue temperatures that are above ambient, has evolved independently in several pelagic fishes (i.e., tunas and lamnid sharks). Regional eye and brain endothermy, some of which results from the transformation of eye muscles into thermogenic organs that warm the brain and eyes, has also been documented in the billfishes, opah *Lampris guttatus*, and possibly butterfly mackerel, *Gasterochisma melampus*.

Unlike the central circulation pattern in most fishes where the major vessels (i.e., dorsal aorta and the postcardinal vein) are found directly ventral to the vertebral column, the central circulatory pathway of some pelagic fishes (tunas, lamnid sharks, and the common thresher shark (*Alopias vulpinus*)) is reduced and blood flow to and from the myotomal muscles relies principally on a lateral circulation that gives rise to myotomal muscle-associated vessels. These vessels act as countercurrent heat exchangers and enable the conservation of metabolically produced heat allowing the aerobic myotomal muscles to maintain temperatures that are significantly above that of the ambient water (i.e., regional endothermy). In addition, these regionally endothermic fishes may have the ability to exert physiological control over routes and rates of heat transfer resulting in maintaining power output at high cycle (i.e., tail beat) frequencies when penetrating cooler waters. In addition, it has also been hypothesized that maintenance of elevated muscle temperatures allows these unique pelagic fishes to swim faster and to recover faster from anaerobic activity (**see also Design and Physiology of Arteries and Veins**: The Retia, **Pelagic Fishes**: Physiology of Tuna, and Endothermy in Tunas, Billfishes, and Sharks.)

The ability to maintain an elevated temperature of the central nervous system conserves sensory and integrative functions while in cooler and darker deep water, and warming of the brain and eye region enhances physiological processes (e.g., synaptic transmission and temporal resolution). For example, recent work on the swordfish eye shows that it is highly sensitive (Q_{10} of 5.1) to temperature changes and that warming the retina significantly improves temporal resolution. This suggests that a warm eye increases the fish's capacity to detect and capture fast-moving prey at both low temperatures and decreased light levels.

The capacity for visceral endothermy has been documented in all lamnid sharks and cold-water tunas, and is suspected in the common thresher shark. Although the structural specializations that allow for visceral heat conservation differ between these fishes, the unifying theme appears to be that heat produced from digestion is used to maintain elevated visceral temperatures. Data on free-swimming Atlantic bluefin tuna show that stomach temperatures increase by as much as 20 °C above ambient after prey is consumed and that this temperature excess

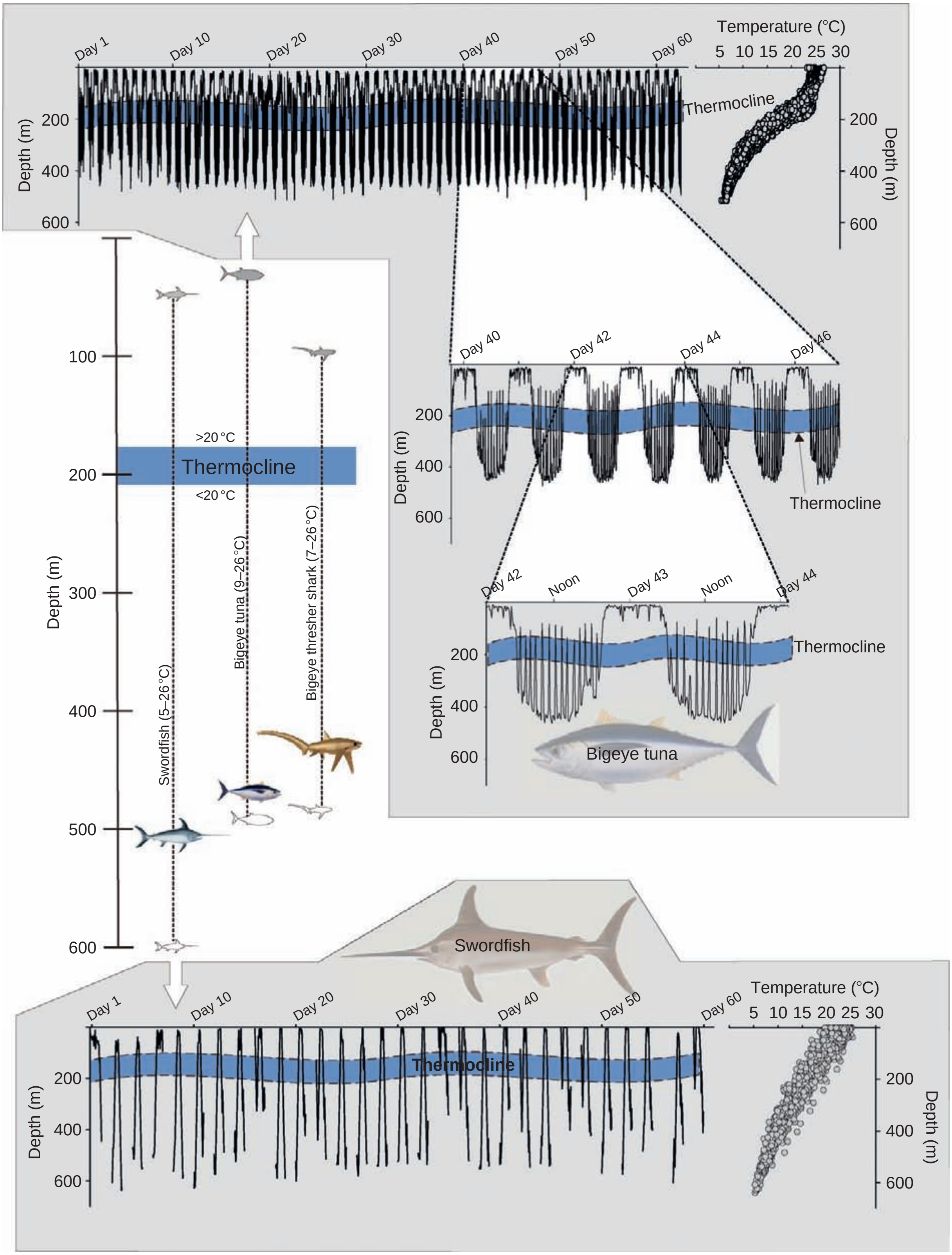


Figure 7 Representative vertical dive profiles for group-3 pelagic fishes. Color fish images represent the average depth (combined night and day) for each species. Open and shaded fish outlines are the depths at which each species spent 95% of the time during the day and night, respectively. Values next to the name show the overall temperature ranges encountered. Blue-shaded bar represents the thermocline, separating water column into the upper warm, surface layer (–>20 °C) and cooler deeper waters (<20 °C). Insets show the vertical movement patterns and temperature-depth profiles of bigeye tuna and swordfish. Additional insets in the bigeye tuna show finer timescales. Day-marks indicate midnight of that day.

slowly decreases back to the pre-feeding values over the next 20–30 h. By contrast, the stomach temperature of lamnid sharks remains continuously elevated (4–14 °C above ambient) and appears to be independent of ambient temperature. Regardless of the exact mechanism, heat production results from digestion and assimilation processes, and the presence of elevated visceral temperatures in these pelagic fishes is a potential mechanism to enhance specific dynamic action after prey is consumed.

Hypoxia and the Thermal Effects on Blood-Oxygen Binding

The extent and duration of the vertical movements in group 3 fishes routinely exposes them to low oxygen levels ($<1.5\ \text{ml-O}_2\ \text{L}^{-1}$) that appear to limit the vertical movements of fishes in groups 1 and 2. A comparison between yellowfin tuna (group 2) and bigeye tuna (group 3) shows that, in the latter, the onset of hypoxia-induced cardiorespiratory adjustments occurs at lower ambient oxygen levels (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia). In general, tolerance to low-oxygen conditions by any tuna seems to undermine their elevated metabolic rates (**see** section Metabolic Rates and Swimming), because the majority of hypoxia-tolerant fishes are generally characterized by sluggish behaviors and low metabolic rates. In addition, hypoxia-tolerant fishes usually have a high blood-oxygen-binding affinity, which could ultimately reduce oxygen off-loading and rates of oxygen delivery to the tissues, relative to blood with lower oxygen affinity (typically found in fish that need more oxygen delivery to their muscles). Not surprisingly, in bigeye tuna, the routine exposure to hypoxia has resulted in the blood having a higher oxygen affinity relative to yellowfin tuna; however, the metabolic rates of bigeye tuna are not lower than those of yellowfin tuna. Thus, bigeye tuna have evolved a unique ability that allows them to maintain elevated aerobic metabolic rates while remaining tolerant of low ambient oxygen levels (i.e., hypoxia, **see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion, Hemoglobin Differentiation in Fishes, and Hemoglobin).

During a sojourn into deeper waters, the blood in the gills of pelagic fishes in groups 2 and 3 are subject to rapid open-system temperature changes where the partial pressures of oxygen and carbon dioxide are constant, but the blood oxygen content changes. For this reason, blood passing through their gills must maintain its capacity to upload oxygen even when being exposed to a wide range of temperatures. In most fishes, an open-system change in temperature results in a lowering of oxygen affinity during warming and an increase in oxygen affinity during cooling. However, the blood of both tunas and lamnid sharks shows little or no shift in oxygen affinity when subjected to *in vitro* open-system temperature changes (**Figure 6**). These blood-oxygen binding properties are suggestive of the capacity to enable these unique pelagic fishes to be exposed to waters of very different temperatures without the adverse effect of changing the functional oxygen-binding properties of its hemoglobin.

The presence of regional endothermy in the aerobic myotomal muscles of tunas results in reduced rates of heat transfer with the ambient water that significantly slows the cooling of this tissue during rapid vertical movements. This elevated aerobic myotomal muscle temperature, which in some tuna can be as much as 20 °C above ambient, subjects the blood to a marked closed-system temperature change (i.e., blood oxygen content remains constant, but the partial pressures of oxygen and carbon dioxide change with temperature). As at the gills, the blood is at ambient temperature but quickly warms as it passes through the vascular countercurrent heat exchangers en route to the aerobic myotomal muscles. It is under these conditions that the blood of bigeye tuna (group 3) differs from that of closely related tunas in groups 1 and 2. *In vitro* studies using whole blood from bigeye tuna subjected to thermal conditions (i.e., warming) similar to that occurring *in vivo* during passage between the gills and aerobic myotomal muscles show that as the blood of the bigeye tuna is warmed, its oxygen affinity decreases to the extent that it closely matches that of other tunas in group 2.

Taken together, for bigeye tuna, the closed-system temperature changes induced a decrease in oxygen affinity, ensuring that oxygen off-loading in the swimming muscles is not compromised while the open-system thermal effects on blood-oxygen binding at the gills (i.e., high oxygen affinity) are maintained. However, this scenario leading to a unique hypoxia tolerance (enhanced oxygen uptake at the gills in hypoxic conditions and ample oxygen delivery to the warm myotomal muscles) requires the maintenance of elevated aerobic myotomal muscle temperatures and may explain bigeye tuna's regular excursions between the warm surface layers (where warmer ambient ocean temperatures will aid in whole-body warming) and cold, deep layers of the ocean (**Figure 7**).

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Buoyancy, Locomotion, and Movement in Fishes: An Introduction; Maneuverability; Undulatory Swimming. **Deep-Sea Fishes**. **Design and Physiology of Arteries and Veins**: Physiology of Capacitance Vessels; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping; The Coronary Circulation. **Energetics**: General Energy Metabolism. **Fish Migrations**: Eel Migrations; The

Biology of Fish Migration; Tracking Oceanic Fish. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Gas Exchange**: Respiration: An Introduction. **Hearing and Lateral Line**: Fish Bioacoustics: An Introduction; Lateral Line Neuroethology. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Hypoxia; Integrated Responses of the Circulatory System to Temperature. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks; Physiology of Tuna. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction. **Swimming and Other Activities**: Energetics of Fish Swimming. **Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion; Hemoglobin; Hemoglobin Differentiation in Fishes; O_2 Uptake and Transport: The Optimal P_{50}. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics. The Effect of Exercise on Respiration. **Vision**: Color Vision and Color Communication in Reef Fish; Physiological Optics in Fishes; Vision in Fishes: An Introduction.

Further Reading

Bernal D, Dickson KA, Shadwick RE, and Graham JB (2001) Analysis of the evolutionary convergence for high performance swimming in lamnid sharks and tunas. *Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology* 129: 695–726 (review).

Bernal D, Sepulveda C, Musyl M, and Brill R (2009) The eco-physiology of swimming and movement patterns of tunas, billfishes, and large pelagic sharks. In: Domenici P and Kapoor D (eds.) *Fish Locomotion – an Etho-ecological Perspective*, pp. 436–483. Enfield: NH: Science Publishers.

Block BA and Finnerty JR (1994) Endothermy in fishes: A phylogenetic analysis of constraints, predispositions, and selection pressures. *Environmental Biology of Fishes* 40: 283–302.

Block BA and Stevens ED (2001) *Fish Physiology, Vol. 19: Tuna: Physiology, Ecology, and Evolution*. San Diego, CA: Academic Press.

Brill RW (1996) Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comparative Biochemistry and Physiology A* 113: 3–15.

Carey FG, Teal JM, Kanwisher JW, Lawson KD, and Beckett JS (1971) Warm bodied fish. *American Zoologist* 11: 135–143.

Dickson KA (1995) Unique adaptations of the metabolic biochemistry of tunas and billfishes for life in the pelagic environment. *Environmental Biology of Fishes* 42: 65–97.

Dickson KA and Graham JB (2004) Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology* 77: 998–1018.

Evans DH (ed.) (1993) *The Physiology of Fishes*, Boca Raton, FL: CRC Press.

Evans DH (ed.) (1998) *The Physiology of Fishes*, 2nd edn. Boca Raton, FL: CRC Press.

Evans DH and Claiborne JB (eds.) (2006) *The Physiology of Fishes*, 3rd edn. Boca Raton, FL: CRC Press.

Farrell AP (1991) From hagfish to tuna: A perspective on cardiac function in fish. *Physiological Zoology* 64: 1137–1164.

Helfman GS, Collette BB, Facey DR, and Bowen BW (2009) *The Diversity of Fishes*, 2nd edn. Hoboken: Wiley–Blackwell.

Hoar WS and Randall DJ (eds.) (1978) *Fish Physiology, Vol. 7, Locomotion*. New York: Academic Press.

Hoar WS, Randall DJ, and Brett JR (eds.) (1979) *Fish Physiology, Vol. 8. Bioenegetics and Growth*. New York: Academic Press.

Hoar WS, Randall DJ, and Farrell AP (eds.) (1992) *Fish Physiology, Vol. 12A. The Cardiovascular System*. San Diego, CA: Academic Press.

Hochachka PW and Somero GN (eds.) (1984) *Biochemical adaptation*. Princeton, NJ: Princeton University Press.

Moyes CD and West TG (1995) Exercise metabolism in fish. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes*, vol. 4, pp. 367–392. New York: Elsevier.

Sharp GD and Dizon AE (eds.) (1978) *The Physiological Ecology of Tunas*. New York: Academic Press.

Shadwick RE and Lauder GV (eds.) (2006) *Fish Physiology, Vol. 23, Fish Biomechanics*. San Diego, CA: Academic Press.

Physiology of Tuna

KA Dickson, California State University Fullerton, Fullerton, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Continuous Swimming
High Metabolic Rates and Aerobic Scopes
Specializations Associated with High Aerobic Capacity
Physiological Consequences of Regional Endothermy
Physiological Consequences of Diving in Tunas
Further Reading

Glossary

Aerobic scope The capacity of an animal to increase its level of aerobic metabolism beyond that which is required for maintenance alone and thereby support activities such as muscular exercise, growth or reproduction; sets the capacity for oxygen-consuming physiological processes that an animal may perform at a given time. Aerobic scope is the difference between minimal and maximal oxygen consumption rate.

Anaerobic Processes occurring without the need for oxygen.

Arterial Adjective pertaining to blood that has been oxygenated.

ATPase A protein whose activity is fueled by the hydrolysis of ATP. Key ATPases involved in excitation–contraction coupling are Ca^{2+} pumps and myosin ATPases.

Atrium A thin-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Bohr effect Modification of the hemoglobin-oxygen affinity by the proton concentration (pH).

Buoyancy, positive and negative A positively buoyant organism is less dense than the surrounding fluid, so tends to rise to the surface. A negatively buoyant one is denser than the fluid and tends to sink.

Cardiac output The volume of blood pumped by the heart per unit time; it is the product of heart rate and stroke volume.

Cardiomyocyte Fundamental contractile cell of the working fish myocardium.

Clade A group consisting of a single common ancestor and all its descendants.

Citrate synthase Mitochondrial enzyme that catalyzes the first reaction of the citric acid cycle; citrate synthase activity correlates with mitochondrial density and is used as an index of a tissue's aerobic metabolic capacity.

Endotherm An animal that uses metabolic heat to achieve a body temperature greater than that of the surrounding environment.

Fast-glycolytic muscle fiber Muscle fiber type with fast isoforms of myosin, large diameter, high anaerobic capacity, and low mitochondrial density; it powers short-duration, high-power movements such as rapid acceleration.

Fork length Distance from the tip of the snout to the fork in the tail or caudal fin.

Gastric evacuation rate The rate at which the stomach is emptied postfeeding.

Hemoglobin An iron-containing oxygen-transport protein in the red blood cells of vertebrates and the tissues of some invertebrates.

Mitochondrion A cellular organelle that acts as the 'powerhouse', of the cell and produces the chemical energy source adenosine triphosphate, using oxygen as the final electron acceptor.

Muscle contraction When a muscle cell shortens and generates force.

Myocardium A general term for the cardiac muscle of the heart, which comprises several subtypes, including the compact myocardium and the spongy myocardium of fishes.

Myocyte An individual cardiac muscle cell.

Myoglobin An oxygen-binding protein in muscle that resembles hemoglobin in function, and enhances the rate of oxygen diffusion.

Net cost of transport The amount of energy above SMR required to move a given mass a given distance: [(swimming $\dot{M}_{O_2}$ – SMR)/(swimming speed × fish mass)].

Partial pressure The pressure that a component of a gas mixture would have if it alone occupied the same volume at the same temperature as the mixture.

Ram ventilation Ventilation of the gills by swimming forward with the mouth open while suspending activity in the respiratory muscles.

Rete mirabile (pl. retia mirabilia) A countercurrent system in which each arterial blood vessel is surrounded by several venous vessels and vice versa.

Ryanodine receptor Mediates the release of Ca^{2+} from the sarcoplasmic reticulum.

Sarcomeres The functional elements of a muscle cell responsible for shortening during a contraction.

Sarcoplasmic reticulum A specialized form of endoplasmic reticulum that acts as a storage site for Ca^{2+}.

Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) An energy-consuming Ca^{2+} pump located on the sarcoplasmic reticulum membrane that concentrates Ca^{2+} inside the sarcoplasmic reticulum.

Slow-oxidative muscle fiber Muscle fiber type with slow isoforms of myosin, high aerobic capacity, and high mitochondrial density; it powers repeated, slow movements such as sustained swimming.

Specific dynamic action (SDA) The additional amount of oxygen or energy consumed above SMR as a result of feeding, measured during the period after feeding until metabolic rate returns to SMR; SDA represents the energetic cost of digestion, assimilation, and protein turnover.

Standard metabolic rate Oxygen consumption rate or aerobic metabolic rate measured in resting, fasting individuals; it represents maintenance costs.

Stroke volume The volume of blood ejected into the aorta by a single contraction of the ventricle(s).

Teleosts A major group of ray-finned bony fish, including most common fish.

Ventricle A thick-walled chamber of the heart that is primarily comprised of cardiac muscle tissue.

Introduction

Tunas have been termed energy speculators because they require a high rate of energy input to support their high routine metabolic costs while gaining high energetic returns to allocate to activity, growth, and reproduction. There are five genera and 15 described species of tunas, which, according to morphological and some molecular data sets, comprise a monophyletic group within the family Scombridae (**Figure 1**). Tunas are active, pelagic predators and are important members of oceanic communities worldwide. They are specialized for high performance, with high aerobic and anaerobic metabolic capacities, high respiration rates, large branchial surface areas (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics) and large, muscular hearts (**see also Design and Physiology of the Heart**: Cardiac Anatomy in Fishes). In this respect, tunas are analogous to race cars; their high-performance level depends upon large quantities of high-octane fuel. Tunas are also among the very few fish species that can conserve metabolic heat using heat-exchanging retia mirabilia (**see also Design and Physiology of Arteries and Veins**: The Retia) to elevate the temperature of certain tissues above water temperature (i.e., regional endothermy; **see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks). This article summarizes key aspects of tuna physiology related to a high turnover rate of energy and the physiological consequences of both regional endothermy and diving behavior. It also emphasizes the key role that comparative studies of tunas and non-tuna scombrids – mackerels, Spanish mackerels, and bonitos (**Figure 1**) – have in identifying the unique physiological features of tunas.

Continuous Swimming

The life of a tuna centers on continuous swimming. Their ram-ventilation respiratory mode requires tunas to swim to force water over the gills. Furthermore, because tunas are negatively buoyant, swimming is needed to generate lift for hydrostatic equilibrium. Feeding also depends upon swimming because food resources are patchily distributed in both space and time in the open ocean where they live. Tunas necessarily swim long distances, including repeated dives in search of prey with some species making extensive migrations (**see also Fish Migrations**: Tracking Oceanic Fish). Once located, prey are consumed using a ram-feeding mode of capture (**see also Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics), coupled with bursts of high speed, powered by aerobic and anaerobic metabolism, respectively. Swimming is also a feature of tuna schooling, migratory, and spawning behaviors.

Continuous swimming is reflected in many morphological features of tunas that contribute to swimming efficiency, including a streamlined body shape, slots, or grooves into which fins can be retracted to produce a smooth body contour and reduce drag, a rigid, high-aspect-ratio caudal fin propeller, bony caudal keels that reduce resistance as the tail moves laterally, and a well-developed tendon system linking the locomotor muscle to the skeleton (**Figure 2**). Tunas swim using the unique Thunniform mode in which lateral undulation of the body is reduced and the caudal fin, moving at high frequencies, produces most of the thrust. Cruising at speeds with low transport costs or using kick-and-glide swimming behavior can also reduce energetic expenditures.

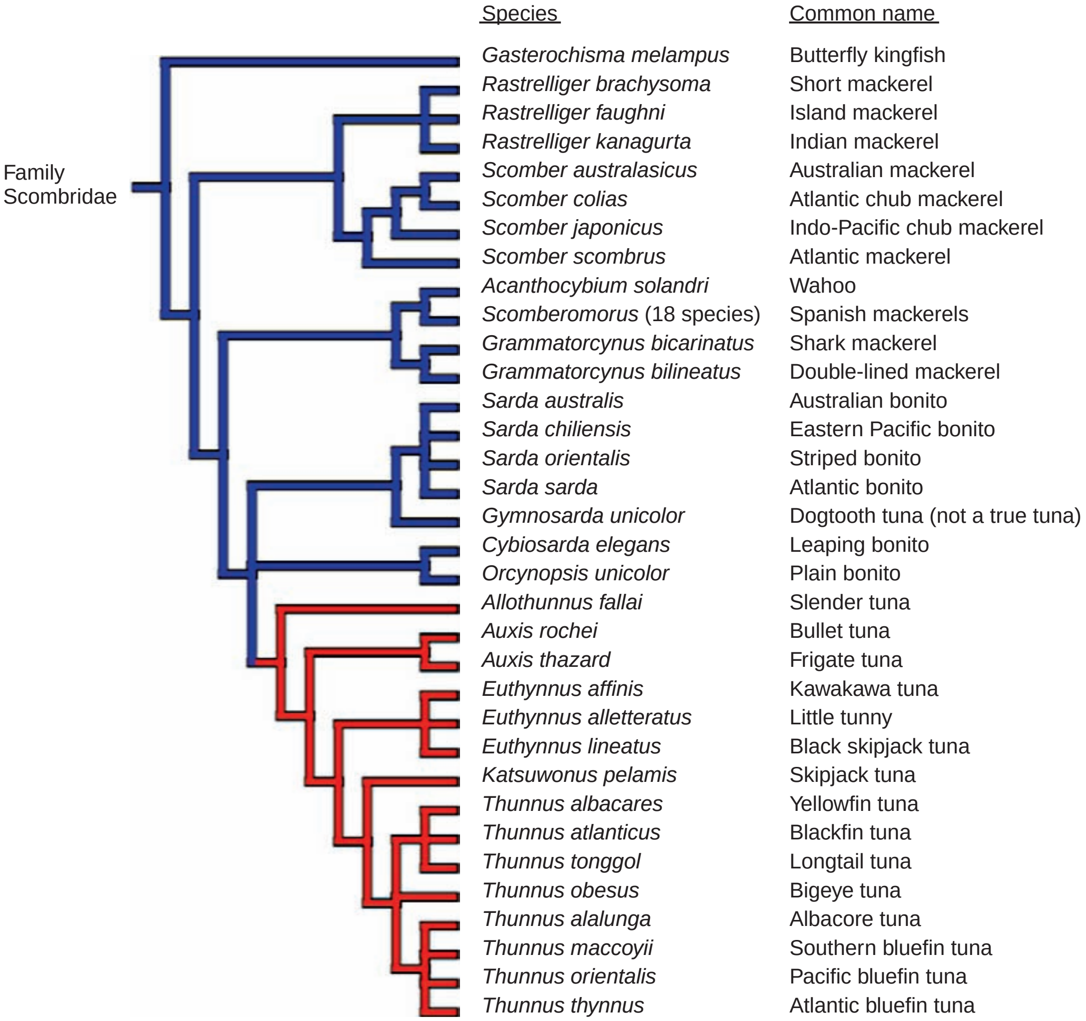


Figure 1 Cladogram showing hypothesized relationships among the 51 species in the family Scombridae, based primarily on *Fish Physiology*. All species except the 18 species of the genus *Scomberomorus* are listed, and common names are from *FAO Species Catalog*. In the text, species are referred to by scientific name. Red represents the clade of 15 tuna species and blue signifies non-tuna scombrids. Unresolved nodes of the cladogram are presented, with species listed alphabetically, if there is no published evidence to resolve relationships at the species level or if morphological and molecular data give conflicting or inconsistent results. Data from Colette BB, Reeb C, and Block BA (2001) Systematics of the mackerels and tunas (Scombridae). In: Block BA and Stevens ED (eds.) *Fish Physiology,* vol. 19, pp. 1–33. San Diego, CA: Academic Press and Collette BB and Nauen CL (1983) *FAO Species Catalog, Volume 2: Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos, and Related Species Known to Date*. Rome: Food and Agriculture Organization of the United Nations.

High Metabolic Rates and Aerobic Scopes

In fish that never stop swimming, standard metabolic rates (SMRs), which represent maintenance costs, are determined by measuring oxygen consumption rate ($\dot{M}_{O_2}$) in immobilized individuals or by extrapolating $\dot{M}_{O_2}$ versus swimming speed relationships to zero velocity. SMRs measured in tunas using both methods are greater than in non-tuna scombrids when compared at similar sizes and temperatures (**Figure 3**). Tunas also have high routine and maximal oxygen consumption rates, and their capacity for exceptional performance is reflected in high aerobic scopes (the difference between maximum $\dot{M}_{O_2}$ and SMR), which allow several aerobic processes to occur simultaneously. How the maximal recorded $\dot{M}_{O_2}$ in tunas (2500–2700 $mgO_2\,kg^{-1}\,h^{-1}$ at 25 °C) can be allocated among several different activities is illustrated in **Figure 4**.

Cost of Continuous Swimming

The net cost of continuous swimming, powered by the contraction of the slow-twitch oxidative muscle, ranges from approximately 0.5 to 3.5 times SMR in tunas, depending on swimming speed and fish size. The net cost of transport is similar in tunas and in comparably sized

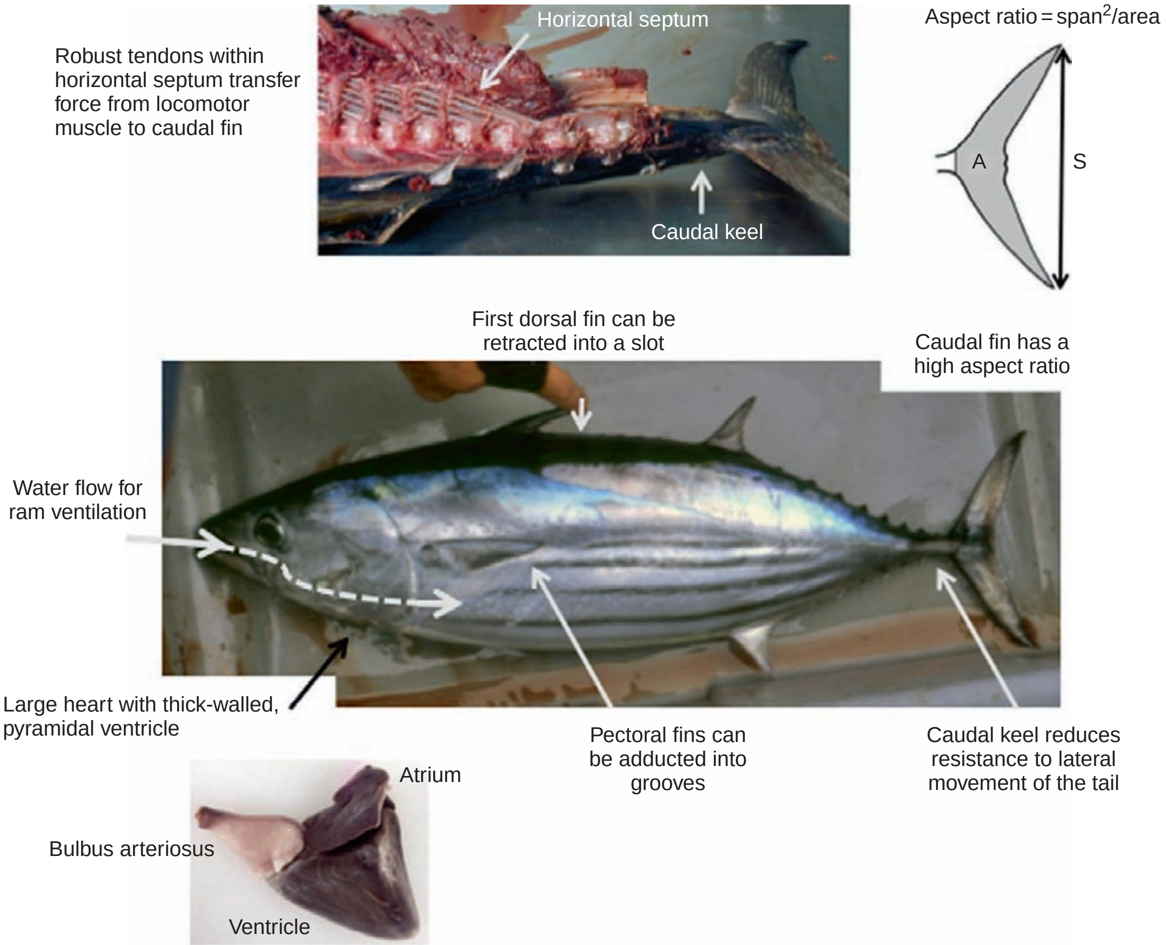

Robust tendons within horizontal septum transfer force from locomotor muscle to caudal fin
Horizontal septum
Caudal keel
Aspect ratio = span^2/area
A
S
First dorsal fin can be retracted into a slot
Caudal fin has a high aspect ratio
Water flow for ram ventilation
Large heart with thick-walled, pyramidal ventricle
Pectoral fins can be adducted into grooves
Caudal keel reduces resistance to lateral movement of the tail
Atrium
Bulbus arteriosus
Ventricle

Figure 2 Photograph of a *K. pelamis* approximately 80 cm in length showing morphological features that affect swimming performance and aerobic capacity, including a streamlined body shape, fins that can be retracted into slots or adducted into grooves to produce a smooth body surface, high-aspect-ratio caudal fin, and well-developed caudal keel. Also shown are the heart removed from *Thunnus alalunga* magnified 3× relative to the fish, and a dorsal view of the horizontal septum of a *E. affinis* showing the tendons that transfer contractile force from the locomotor muscle to the vertebrae and caudal fin to power swimming.

Sarda chiliensis and *Scomber japonicus* at the same speeds and water temperatures (**Figure 5**). Even at maximal continuous swimming speeds, $\dot{M}_{O_2}$ in tunas is less than the total aerobic scope, allowing other aerobic activities to occur while swimming maximally (**Figure 4**), something that even salmonids are apparently unable to do.

Cost of Burst Swimming

Bursts of high speed used in prey capture, courtship, and reproduction require high rates of adenosine triphosphate (ATP) production via anaerobic metabolic pathways within the fast-glycolytic locomotor muscle (**see also Hypoxia**: Anaerobic Metabolism In Fish). Compared with that of non-tuna scombrids, the fast-glycolytic muscle of tunas has higher activities of the enzymes creatine phosphokinase and lactate dehydrogenase, which are required for anaerobic ATP production. This muscle comprises 46–55% of body mass in tunas and, during bursts, can accumulate and buffer large quantities of lactate (up to 100 μmol g^{-1} in *Katsuwonus pelamis*).

The aerobic cost of burst swimming occurs during the postburst recovery period when resynthesis of creatine phosphate from creatine and of glycogen from lactate occur within the fast-glycolytic muscle. Both processes require aerobic metabolism, elevating $\dot{M}_{O_2}$ by up to 840 mgO$_2$ kg^{-1} h^{-1} during the recovery period. Recovery from maximal burst activity takes less time in tunas than in other fishes (<2 h vs. 4–24 h), but nothing is known about recovery rates in non-tuna scombrids. Although the fast-glycolytic muscle of tunas has very low or no measurable activity of key enzymes required to convert lactate to glycogen, it does have a high aerobic capacity, which may contribute to rapid recovery.

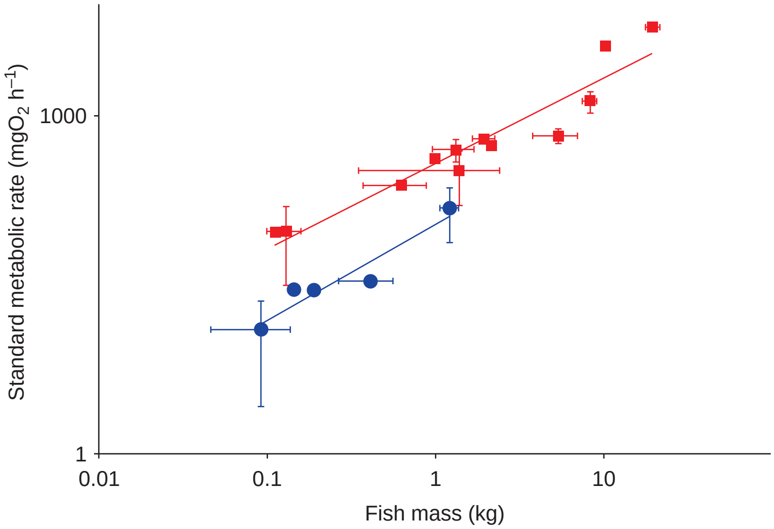


Figure 3 SMR (mgO$_2$ h^{-1}) at 25 °C as a function of fish mass (kg) in tunas (red) and non-tuna scombrids (blue), showing that SMR is greater in the tunas. If SMR was not measured at 25 °C, the reported value was adjusted to 25 °C using a Q_{10} of 1.8. Error bars represent ±1 standard deviation. Data are from six tuna species (*E. affinis*, *K. pelamis, T. albacares, T. alalunga, T. maccoyii,* and *T. orientalis*) and three non-tuna scombrids *(S. chiliensis, S. japonicus,* and *S. scombrus*). The best-fit power curves are: SMR = $372M^{0.76}$ for the tunas ($R^2 = 0.91$) and SMR = $108M^{0.85}$ for the non-tuna scombrids ($R^2 = 0.90$).

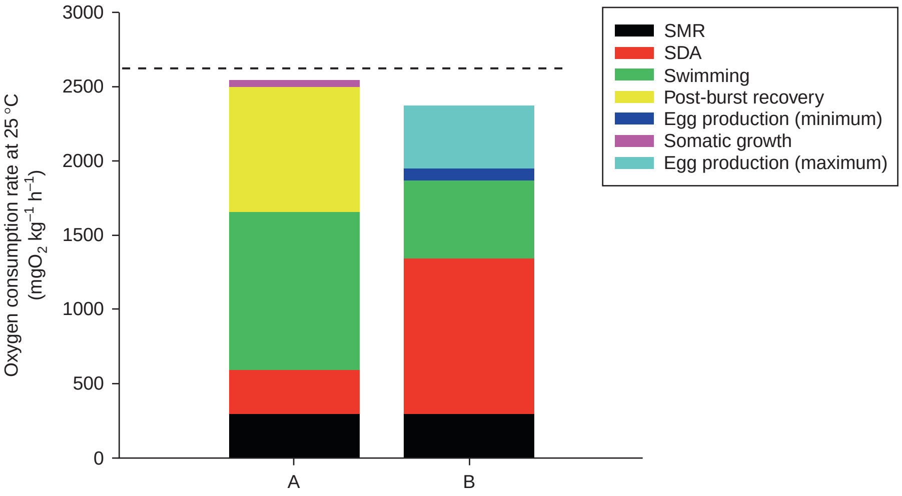


Figure 4 Two possible ways that tunas can partition aerobic scope to accomplish several different aerobic activities simultaneously. The maximal recorded $\dot{M}_{O_2}$ in tunas (2500–2700 mgO$_2$ kg^{-1} h^{-1} at 25 °C) is indicated by the dashed line, and other costs (see section High Metabolic Rates and Aerobic Scopes) were adjusted to 25 °C using a Q_{10} of 1.8, if necessary. A. Conditions within 1–2 h of feeding, with aerobic capacity supporting maximal postburst recovery costs, swimming at maximal sustainable speed, and the initial portion of SDA. B. SDA is the maximum value recorded in *T. maccoyii* at 3–7 h after consuming a large meal of sardines equivalent to 6–8.5% of body mass, postburst recovery is completed, and the costs of swimming at a speed of 1 length s^{-1} and of gamete production can be accommodated. Note that all activities cannot be conducted simultaneously at their maximal rate.

Cost of Feeding

Metabolic rate increases postfeeding due to recovery from any burst activity and as a result of specific dynamic action (SDA), the energetic cost of digestion, assimilation, and protein turnover (**see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation). Tunas consume large quantities of fishes, crustaceans, and cephalopods and have high rates of gastric evacuation and digestion and a high SDA. Gastric evacuation rates have been measured in three tuna and one mackerel species, and are greater in the tunas. The stomach was emptied within 12.0 and 10.4 h postfeeding at ~24 °C in *K. pelamis* and *Thunnus albacares*, respectively, compared with 30 h at 19 °C in *S. scombrus*; stomach contents were 90% evacuated at 4.5–6.5 h at 25 °C in *Euthynnus lineatus* versus 22 h at 19 °C in *S. scombrus*. In *T. maccoyii,* peak postfeeding metabolic rate was as high as 1290 mgO$_2$ kg^{-1} h^{-1} at 20 °C and

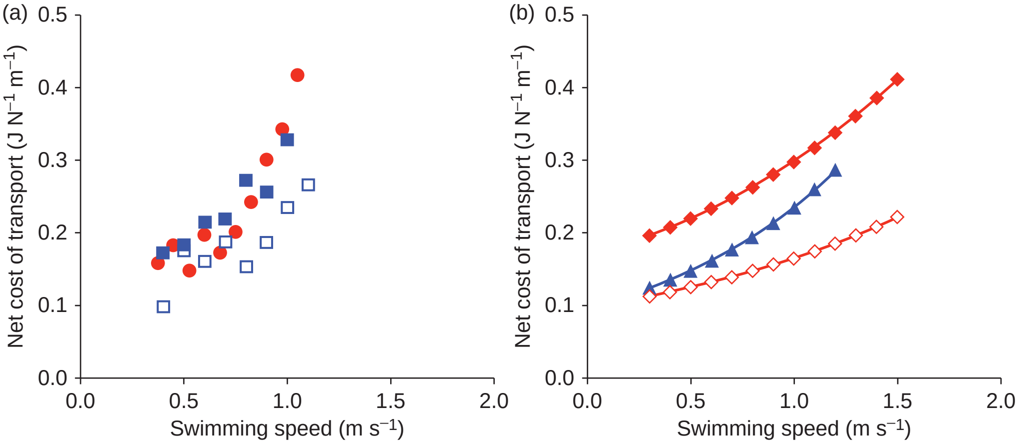


Figure 5 The net cost of transport at 24 °C (J N^{-1} m^{-1}) as a function of swimming speed (m s^{-1}), in (a) *E. affinis* and *S. japonicus* and (b) *T. albacares* and *S. chiliensis*, of approximately the same size. Data in (a) are derived from Sepulveda and Dickson for a 145 g, 22.0 cm FL *E. affinis* (solid red circles) and two *S. japonicus* 147 g, 23.4 cm FL (solid blue squares) and 156 g, 24.0 cm FL (open blue squares). Data in (b) are from Dewar and Graham for 51.3 ± 3.0 cm FL, 2.17 ± 0.5 kg *T. albacares* (open red diamonds) and 41.6 ± 2.7 cm, 1.1 ± 0.2 kg *T. albacares* (solid red diamonds), measured at 24 °C, and from Sepulveda *et al.* for 47.3 ± 1.8·cm, 1.24 ± 0.14·kg *S. chiliensis* (solid blue triangles); the bonito data were recorded at 18 °C and adjusted to 24 °C using a Q_{10} of 2.0. Data from (a) Sepulveda C and Dickson KA (2000) Maximum sustainable speeds and cost of swimming in juvenile kawakawa tuna (*E. affinis*) and chub mackerel (*S. japonicus*). *Journal of Experimental Biology* 203: 3089–3101; and (b) Dewar H and Graham JB (1994) Studies of tropical tuna swimming performance in a large water tunnel. I. Energetics. *Journal of Experimental Biology* 192: 13–31 and Sepulveda C, Dickson KA, and Graham JB (2003) Swimming performance studies on the eastern Pacific bonito *S. chiliensis*, a close relative of the tunas (family Scombridae). *Journal of Experimental Biology* 206: 2739–2748.

total SDA was 35% of the energy ingested, higher than the 5–20% reported for other teleost fishes.

A daily food consumption of 3.5% of body mass would be required to meet the energetic demands associated with maintenance metabolism plus the maximum SDA measured in *T. maccoyii*. Net energy gain requires a greater food intake, which is possible if a tuna fills its stomach once a day, given the stomach capacity of 7% of body mass (in *K. pelamis*) and maximum intakes of 8.5–8.6% of body mass per feeding measured in captive tunas. However, feeding rate also depends upon food availability. Feeding frequencies in tunas, estimated from visceral temperature changes recorded by data-logging tags, ranged from 0 to 10 meals per day in *T. orientalis* (mean + SD of 1.8 + 1.4 d^{-1}) and averaged 0.89 + 0.08 d^{-1} in *T. maccoyii*. It may be necessary for tunas to feed more frequently when food is plentiful and store the excess energy to mobilize during periods of low food availability. Tunas can store large quantities of lipid and glycogen, but how energy storage and mobilization are regulated is unknown for any scombrid fish.

Costs of Growth and Reproduction

Tunas have high rates of growth and reproduction, which can occur only if energy intake exceeds the energetic requirements of maintenance, continuous swimming, and feeding. Juvenile tunas grow rapidly, at rates similar to those of non-tuna scombrids. Early juvenile growth rates are up to 7.9 mm d^{-1} in laboratory-raised *E. lineatus* and 4.3 mm d^{-1} in field-caught *K. pelamis*, compared with 6.2 mm d^{-1} in *S. orientalis* and 3.7 mm d^{-1} in sierra Spanish mackerel, *Scomberomorus sierra*. Rapid juvenile growth rates require high feeding and food-processing rates. While high rates of feeding may increase predation risk, the resultant increase in growth rate reduces the time that juvenile fish are vulnerable to many predators as well as the time it takes to reach a size at which tissue temperatures can be elevated. Tunas can grow to very large sizes, although the maximum size of several tuna species is less than that of several non-tuna scombrids. The tuna that attains the largest maximum size (at least 315 cm and 685 kg), the *T. thynnus*, reaches a size of approximately 60 cm and 4 kg within the first year. The minimum energetic cost of this first-year growth, estimated from tuna caloric density, is ~50 mgO_2 kg^{-1} h^{-1}. As sexual maturity is reached, tunas allocate less energy to somatic growth and more to reproduction. Most tuna species have very high female batch fecundities, producing millions of eggs in a single spawning event, and spawning frequency ranges from daily to every 6 days. The cost of gamete production in tunas has been estimated to be 0.31–1.7% of body mass per day for females and 0.33% of body mass per day for males. Other reproductive costs, including those associated with migration and courtship, have not been quantified in any tuna.

Specializations Associated with High Aerobic Capacity

The large aerobic scope of tunas allows many, but not all, of the above aerobic activities to be conducted simultaneously (**Figure 4**) and depends on numerous physiological specializations for rapid rates of oxygen uptake and delivery to active tissues. Enhancing oxygen uptake at the gills, tunas have the highest gill surface areas, resulting from large numbers of gill filaments and lamellae, and the shortest diffusion distances reported for any fish group (**see also Ventilation and Animal Respiration**: Gill Respiratory Morphometrics). Tuna gills are structurally reinforced to withstand high-velocity ram ventilation, and the pathway for blood flow through the gill lamellae reduces vascular resistance and should enhance gas exchange (**see also Design and Physiology of Arteries and Veins**: Branchial Anatomy). Although resulting in high osmoregulatory costs (**see also Role of the Gills**: The Osmorespiratory Compromise), these adaptations allow tunas to process large ventilatory volumes while maintaining relatively high oxygen utilization rates (∼50%).

Tunas have thick-walled, pyramidal hearts (**Figure 2**) with a high percentage of compact myocardium and a well-developed coronary circulation (**see also Design and Physiology of the Heart**: The Coronary Circulation and Cardiac Anatomy in Fishes), as do other scombrid species. Heart mass as a percentage of body mass is high in tunas (0.28–0.81%), but values overlap with those of other scombrids (0.12–0.40%), and among scombrid species correlates with the percentage of body mass composed of slow-oxidative locomotor muscle (**Figure 6**). Tunas' muscular hearts produce high cardiac outputs (heart rate × stroke volume) and high blood pressures to propel blood through the gill and systemic circulations. In tunas, modulation of cardiac output while swimming and when exposed to hypoxia occurs primarily by altering heart rate (i.e., tuna hearts are frequency modulated), rather than stroke volume, because stroke volume is typically near maximal. At routine swimming speeds of 1–2 body lengths s^{-1}, heart rates in tunas were 62–97 beats min^{-1} at 25 °C in 1–2 kg *K. pelamis* and *T. albacares* and 20–50 beats min^{-1} at 18–19 °C in free-swimming, 10–21 kg *T. maccoyii*, compared with 80–140 beats min^{-1} in 0.4–0.6 kg *S. japonicus* at 16.1–21.8 °C. The highest reported heart rates for scombrids are >250 beats min^{-1} in *K. pelamis* and *E. affinis*, and 150 beats min^{-1} in *S. japonicus*.

A rapid heart rate requires rapid calcium cycling and ATP turnover (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element), and several characteristics related to these processes differ between tunas and other scombrids, reflecting the exceptional cardiac performance of tunas. In scombrids, the increase in intracellular [Ca^{2+}] required for cardiac muscle contraction results from Ca^{2+} release from the sarcoplasmic reticulum through ryanodine receptors, in addition to extracellular Ca^{2+} influx via L-type voltage-gated Ca^{2+} channels. For relaxation

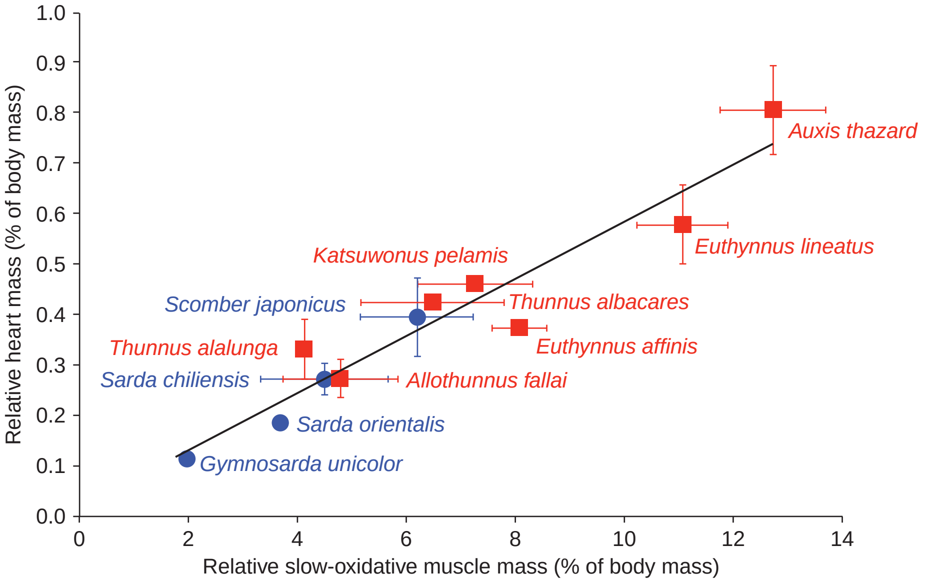


Figure 6 Relative heart mass (heart mass as a percentage of total body mass) correlates with relative mass of slow-oxidative locomotor muscle (muscle mass as a percentage of total body mass) in tunas (red) and non-tuna scombrids (blue). Error bars represent ±1 standard deviation. Black line is the best-fit linear regression ($y = 0.0567x + 0.0188$; $R^2 = 0.91$).

prior to the next heart beat, Ca^{2+} is rapidly removed by the sarcoplasmic–endoplasmic Ca^{2+}ATPase, SERCA2. Recent studies have compared these characteristics in the tunas *T. albacares*, *T. alalunga*, *T. obesus*, and *T. orientalis* and in the mackerel *S. japonicus*. Ca^{2+} influx via the L-type channel in myocytes isolated from the atria, but not ventricles, was greater in *T. orientalis* than in *S. japonicus*. Expression of both ryanodine receptor and SERCA2 proteins, and SERCA2 Ca^{2+} uptake rates and ATPase activity in microsomes prepared from both atria and ventricles, were greater in tunas compared with the mackerel. Cardiomyocyte mitochondrial densities are high in tunas (**see also Design and Physiology of the Heart**: Cellular Ultrastructure of Cardiac Cells in Fishes) and, although the activity of the mitochondrial citric-acid cycle enzyme citrate synthase is similar, activities of two key lipid oxidation enzymes are greater in tuna ventricles than in those of non-tuna scombrids.

Several features ensure that oxygen and fuels are supplied to all tissues at high-enough rates to meet their metabolic demands. Blood volume is not exceptional in tunas, but hemoglobin concentrations are higher in tunas than in non-tuna scombrids, meaning that more oxygen can be bound at the gills and transported to active tissues. Off-loading of oxygen from hemoglobin at active tissues is facilitated by a larger Bohr effect in tunas than in other active fishes (**see also Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect). In the slow-oxidative muscle that powers continuous swimming, capillary density is higher in tunas than in non-tuna scombrids and, in tunas, capillary tortuosity increases the surface area of contact between capillaries and muscle fibers (**see also Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis). The concentration of myoglobin, which facilitates the diffusion of oxygen from capillary blood to muscle mitochondria, is greater in tunas than in *S. chiliensis* and *S. japonicus*. However, slow-oxidative muscle mitochondrial densities, and citrate synthase activity measured at a common temperature, overlap in tunas and non-tuna scombrids. A key difference in tunas is that the slow-oxidative locomotor muscle is in a more anterior-medial position than in other fishes, and it is maintained at an elevated temperature (**Figure 7**), which results in higher *in vivo* enzyme activities. The diameter of slow-oxidative muscle fibers is restricted because these cells depend upon oxygen diffusing from capillary blood, and it may not be possible to increase the volume occupied by mitochondria without compromising contractile function. Maintaining this tissue at an elevated temperature will increase muscle contraction rate in tunas, and the higher myoglobin content would facilitate the resulting greater oxygen flux.

Physiological Consequences of Regional Endothermy

As regional endotherms, tunas maintain the temperature of the locomotor muscle and cranial region above ambient water temperature. Stomach, intestine, and cecal mass temperatures are also elevated in five species (*T. obesus*, *T. alalunga*, *T. maccoyii*, *T. orientalis*, and *T. thynnus*). Regional endothermy contributes directly to the high metabolic rates of tunas, but only by the amount associated with increased cellular metabolism due to warming of the specific tissues. Additional heat generation is not required for endothermy in tunas because the heat-exchanging retia retain heat that is produced by normal metabolic activity of the tissues and would otherwise be lost to the environment via conduction and convection (**see also Design and Physiology of Arteries and Veins**: The Retia). The only exception would be cranial endothermy in *Allothunnus fallai*, in which there is evidence that the four pairs of extraocular rectus muscles are modified to form a heater tissue that specifically generates heat.

Elevation of tissue temperatures increases rates of biochemical and physiological processes within those tissues (**see also Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks), but these rates do not always exceed those of non-tuna scombrids at *in vivo* temperatures. For example, contraction rate and power output of slow-oxidative muscle isolated from *T. albacares* increase with increasing temperature, but power is not higher in *T. albacares* than in *S. chiliensis* under *in vivo* conditions. Similarly, although digestive enzyme activities increase with temperature in tunas, they are no greater in *T. alalunga* or *T. albacares* than in *S. chiliensis* or *S. japonicus* at *in vivo* temperatures. Regional endothermy does stabilize tissue temperatures and physiological functions as environmental temperature changes rapidly during dives, and may have allowed some tunas to expand their thermal niche into cool waters at high latitudes and below the thermocline.

Tunas have some capacity to thermoregulate physiologically by reducing the rate of heat exchange with the environment in cool waters and increasing it when activity increases, especially in warm waters, as documented during repeated dives in *T. obesus* (**Figure 8**). The thermal coefficient in *T. obesus* was reduced during dives into cool water below the thermocline, thereby increasing heat conservation. In warm surface water, by contrast, the thermal coefficient was increased. In combination with vertical movements (behavioral thermoregulation) and thermal inertia, this physiological control of thermal conductance allows *T. obesus* to maintain internal temperatures above ~17–18 °C even though most of the day is spent in much cooler water (**Figure 8**). Thermal

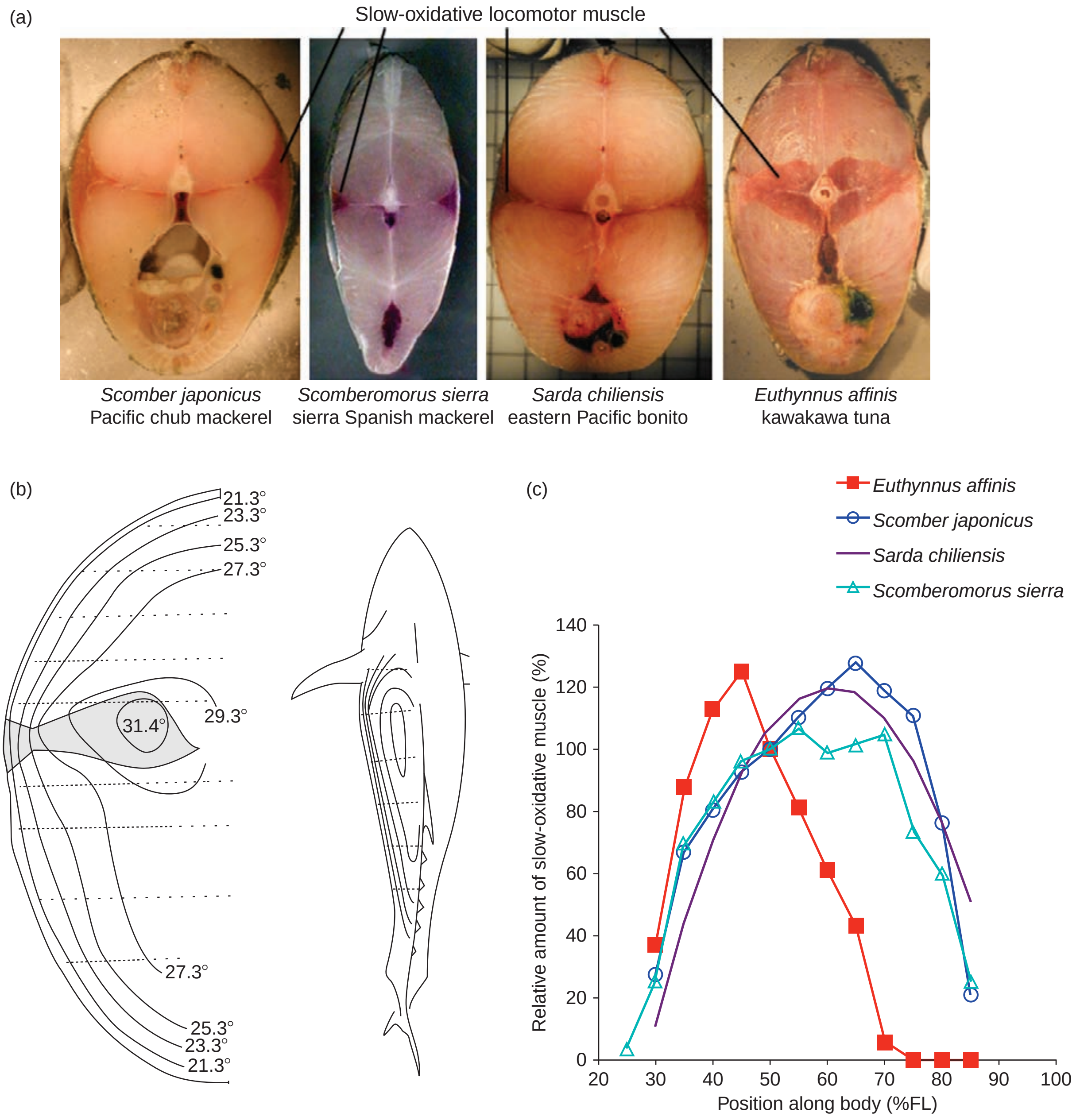


Figure 7 (a) Transverse sections, taken at a position along the body corresponding to 45% of fork length (FL), in representatives of four major scombrid groups (mackerels (*S. japonicus*), Spanish mackerels (*S. sierra*), bonitos (*S. chiliensis*), and tunas (*E. affinis*)). The unusual axial position of the slow-oxidative locomotor muscle (red tissue) in the tuna contrasts with the typical teleost distribution as a lateral wedge just beneath the skin in the non-tuna scombrids. (b) Thermal profiles of the locomotor muscle in a *T. thynnus* in cross section and frontal section from Carey and Teal. Temperature is highest (31.4 °C) in the core of the slow-oxidative locomotor muscle (stippled) and is up to 12.1 °C warmer than the ambient seawater (19.3 °C). (c) The relative amount of slow-oxidative muscle (as a percentage of the amount at 50% of FL) as a function of position along the body (% of FL) for the four scombrid species in (a). Because muscle amounts were not measured at exactly 50%FL in *S. chiliensis*, the position closest to 50%FL (50.3–52.6%FL) was assigned a relative slow-oxidative muscle value of 100%, and the curve represents the best-fit second-order polynomial for data from four individuals. The position of the slow-oxidative locomotor muscle is more anterior in the tuna than in the three non-tuna scombrids. (b) From Carey FG and Teal JM (1969) Regulation of body temperature by the bluefin tuna. *Comparative Biochemistry and Physiology* 28: 205–213.

conductance is most likely modulated in tunas by altering blood flow using vasoactive control mechanisms that involve sympathetic and endothelial-derived chemical messengers, as in other teleosts (**see also Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System), but this aspect of thermal physiology has not been investigated in tunas.

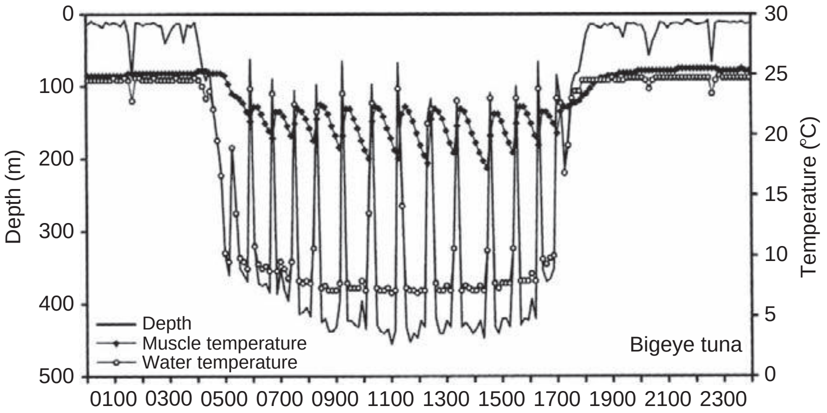


Figure 8 Repeated diving behavior in *T. obesus* documented in an archival tag record of depth, muscle temperature, and water temperature measured simultaneously over a 24-h period. Muscle temperature varies much less than does water temperature and is maintained above 17–18 °C by a combination of physiological and behavioral thermoregulation. The slope of the muscle temperature vs. time tracing is proportional to the thermal coefficient (*k*), which represents the rate of heat exchange with the environment. As the fish dives into cool water, *k* decreases, allowing heat retention, whereas *k* increases as the fish ascends into warm surface water. From Brill RW and Bushnell PG (2001) The cardiovascular system of tunas. In: Block BA and Stevens ED (eds.) *Fish Physiology,* vol. 19, pp. 79–120. San Diego, CA: Academic Press.

As a consequence of regional endothermy, blood in a heat-exchanging rete rapidly warms within the rete arterioles as it flows toward the warmed tissue and rapidly cools as it leaves the tissue within rete venules. Normally, increasing temperature decreases the binding affinity of hemoglobin for oxygen (increases the P_{50} value, the partial pressure of oxygen (P_{O_2}) at which the hemoglobin is 50% saturated with O_2), which facilitates delivery of oxygen to active tissues (**see also Transport and Exchange of Respiratory Gases in the Blood**: Red Blood Cell Function and Hemoglobin). If this happened during the closed-system temperature changes within the rete arterioles, oxygen would be released from hemoglobin before it reached the tissue. The effects of closed-system changes in temperature on hemoglobin-oxygen binding affinity in whole blood differ interspecifically among tunas. Such changes have no effect on hemoglobin-oxygen binding affinity in *K. pelamis*, *T. thynnus* at $P_{O_2} < P_{50}$, and *T. maccoyii* at 23–36 °C, thus preventing premature off-loading of oxygen within the rete arterioles. The blood of *T. alalunga, T. thynnus* at $P_{O_2} > P_{50}$, and *T. maccoyii* at 10–23 °C shows a reversed temperature effect, in which increasing temperature increases binding affinity. That response would ensure that oxygen is tightly bound to hemoglobin at the gills in warm waters and remains bound as blood traverses the rete arterioles and reaches tissue capillaries, making it necessary for other mechanisms, such as a large Bohr effect, to release sufficient oxygen at warm, active tissues. However, the typical response to temperature occurs in *E. affinis*, *T. albacares*, and *T. obesus*, and these species must possess other mechanisms to ensure adequate oxygen delivery to the tissues that are perfused by heat-exchanging retia (**see also Transport and Exchange of Respiratory Gases in the Blood**: Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange).

Regional endothermy also results in thermal gradients within tissues (e.g., in locomotor muscle; **Figure 7**) and different tissues within the body experiencing different rates of temperature change and operating at different temperatures. For example, heart temperature rapidly equilibrates with water temperature, while temperatures of the warmed tissues can be as much as 20 °C greater and change much more slowly. Heart rate decreases with temperature (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature), but the heart must pump sufficient blood to support high rates of aerobic metabolism in the warmed tissues even in cold waters. Heart function may, therefore, limit performance of other aerobic tissues during dives, may limit the time a tuna can remain in cool water, and may explain interspecific differences in thermal ranges and diving behavior among tunas.

Physiological Consequences of Diving in Tunas

Tagging studies have documented long-distance migrations and repeated diving in tunas (**Figure 8**; **see also Fish Migrations**: Tracking Oceanic Fish), with dives to depths exceeding 1000 m recorded in several species. During migrations, there is time for acclimatization to environmental conditions, but during dives tunas must tolerate rapid fluctuations in water temperature, oxygen content, hydrostatic pressure, and light levels. Foraging in deep waters or diving repeatedly requires a number of physiological specializations, including sensory mechanisms to locate and capture prey, tolerance of low temperature and rapid temperature change in tissues such as the heart and gills that are not served by heat-exchanging retia, and coordinating the function of tissues operating at different temperatures. As blood flows through the gills, open-system temperature changes, in which O_2 and CO_2 can exchange with seawater, may occur, but this type of temperature change results in little to no effect on hemoglobin-oxygen binding in all tunas that have been studied. This response allows sufficient uptake of O_2 at the gills despite the rapid temperature changes that tunas experience during dives (**Figure 8**).

Heart function and molecular determinants of cardiac muscle Ca^{2+} cycling differ among tunas, reflecting interspecific differences in thermal ranges (**see also Design and Physiology of the Heart**: Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux and Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element). The heart of *T. orientalis* continues to beat and pump blood, albeit at a reduced rate, at temperatures as low as 2 °C. Cardiac muscle in *T. orientalis* has the highest levels of SERCA2, and greater rates of SERCA2 Ca^{2+} uptake than in *T. alalunga*, which are greater than in *T. albacares* and *T. obesus*. The Q_{10} for heart rate is lower in *T. orientalis* than in *T. albacares*, reflecting the greater thermal range of *T. orientalis*, but the temperature sensitivities of Ca^{2+} uptake and ATP hydrolysis by SERCA2 did not differ significantly among the tuna species studied. Some tunas, most notably *T. obesus*, dive into and spend significant amounts of time in waters of low oxygen, and thus must also tolerate hypoxia. The very high hemoglobin-oxygen binding affinity of *T. obesus* allows sufficient oxygen binding at the gills in hypoxic water. The ventricle of *T. obesus* has the highest proportion of compact myocardium (73.6%) measured in any fish, and thus can produce high contractile force. However, heart metabolic enzymes and myofibrillar ATPase in *T. obesus* did not show any apparent adaptations to temperature or hypoxia in comparison to those of *T. albacares* and *K. pelamis*. Interspecific differences in physiological capacities and tolerances are only beginning to be understood, but are thought to be the basis for different vertical mobilities and thermal niches, and for resource partitioning among tuna species inhabiting the same geographic area.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Feeding Mechanics. **Design and Physiology of Arteries and Veins**: Branchial Anatomy; The Retia. **Design and Physiology of Capillaries and Secondary Circulation**: Capillaries, Capillarity, and Angiogenesis. **Design and Physiology of the Heart**: Cardiac Anatomy in Fishes; Cardiac Excitation-Contraction Coupling: Calcium and the Contractile Element; Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Cellular Ultrastructure of Cardiac Cells in Fishes; The Coronary Circulation. **Fish Migrations**: Tracking Oceanic Fish. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Hypoxia**: Anaerobic Metabolism In Fish. **Integrated Control and Response of the Circulatory System**: Integrated Control of the Circulatory System. **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature. **Pelagic Fishes**: Endothermy in Tunas, Billfishes, and Sharks. **Role of the Gills**: The Osmorespiratory Compromise. **Transport and Exchange of Respiratory Gases in the Blood**: Evolution of the Bohr Effect; Hemoglobin; Red Blood Cell Function. **Ventilation and Animal Respiration**: Gill Respiratory Morphometrics.

Further Reading

Bernal D, Dickson KA, Shadwick RE, and Graham JB (2001) Review: Analysis of the evolutionary convergence for high performance swimming in lamnid sharks and tunas. *Comparative Biochemistry and Physiology* 129A: 695–726.

Bernal D, Sepulveda C, Musyl M, and Brill R (2009) The eco-physiology of swimming and movement patterns of tunas, billfishes, and large pelagic sharks. In: Domenici P and Kapoor D (eds.) *Fish Locomotion – An Etho-Ecological Perspective*, pp 436–483. Enfield, NH: Science Publishers.

Block BA (1991) Endothermy in fish: Thermogenesis, ecology, and evolution. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes* vol.1, pp. 269–311. New York, NY: Elsevier.

Block BA and Stevens ED (2001) *Fish Physiology, Vol. 19: Tuna, Physiology, Ecology, and Evolution*. San Diego, CA: Academic Press.

Brill RW (1996) Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comparative Biochemistry and Physiology* 113A: 3–15.

Carey FG, Teal JM, Kanwisher JW, Lawson KD, and Beckett KS (1971) Warm-bodied fish. *American Zoologist* 11: 137–145.

Collette BB and Nauen CL (1983) *FAO Species Catalog, Volume 2: Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos, and Related Species Known to Date*. Rome: Food and Agriculture Organization of the United Nations.

Dickson KA (1996) Locomotor muscle of high-performance fishes: What do comparisons of tunas with ectothermic sister taxa reveal? *Comparative Biochemistry and Physiology* 113A: 39–49.

Dickson KA and Graham JB (2004) Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology* 77: 998–1018.

Graham JB and Dickson KA (2004) Tuna comparative physiology. *Journal of Experimental Biology* 207: 4015–4024.

Joseph J, Klawe W, and Murphy P (1988) *Tuna and Billfish – Fish without a Country*. La Jolla, CA: Inter-American Tropical Tuna Commission.

Korsmeyer KE, Dewar H, Lai NC, and Graham JB (1996) The aerobic capacity of tunas: Adaptations for multiple metabolic demands. *Comparative Biochemistry and Physiology* 113A: 17–24.

Magnuson JJ (1978) Locomotion by scombrid fishes. In: Hoar W and Randall D (eds.) *Fish Physiology* vol. 7, pp. 239–313. New York, NY: Academic Press.

Sharp GD and Dizon AE (eds.) (1978) *The Physiological Ecology of Tunas*. New York, NY: Academic Press.

Endothermy in Tunas, Billfishes, and Sharks

BA Block, Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Systemic Endothermy
Cranial Endothermy
Heater Organ Tissue
How Fish Have Built a Furnace Out of Muscle
Telemetry of Brain Temperature
Why Be Warm?
Further Reading

Glossary

Ectotherm (adj. ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioral mechanisms to regulate body temperature.

Endotherm An animal that uses its own metabolism as the major source of heart to maintain its body temperature greater than that of the surrounding environment.

Rete mirabile (pl. retia mirabilia) A vascular network, usually consisting of a parallel array of both arterial and venous vessels with countercurrent flow. Retia facilitate transfer of heat or oxygen between the arterial and venous vessels. Rete is Latin for net or network, mirabile for ‘wonderful’ or ‘miracle’ (plural, retia mirabilia).

Thermogenic tissue A specialized heat-producing tissue.

Introduction

Powerful ocean predators such as swordfish, marlins, tunas, and some pelagic sharks are unique among fish for their capacity to maintain elevated temperatures of certain tissues even when swimming in cooler waters. Most fish are ectotherms, that is, their entire body temperature is the same temperature as the water in which they swim, primarily because they breathe with a gill. Body heat loss occurs as blood passes through the gills due to their large surface area optimized for gas exchange. Fish are surrounded by cooler ambient water, which has a much higher heat capacity than air. Thus, the core tissue temperatures of ectothermic fish are governed by the surrounding environment, not by metabolically derived heat production. Heat is rapidly and efficiently drawn out of the fish into the surrounding water, the gills acting as a convective cooling system.

Endothermy, the ability to maintain elevated body temperature by metabolic means, has been documented only within about 30 of the over 25 000 species of fishes. These remarkable examples illustrate the challenges of overcoming the physical constraints and are restricted to large, predatory, open ocean fishes – one assemblage among the teleosts the Scombroidei, which includes mackerels, tunas, and billfishes, and the other assemblage among the sharks of the family Lamnidae.

The evolutionary transition from ectothermy to endothermy required both an increase in heat production by elevating metabolic capacity plus the evolution of mechanisms to reduce the rate of heat loss. Among these endothermic fish, two distinct strategies for elevating tissue temperatures have evolved: systemic and cranial endothermy. Examples of systemic endothermy occur in the sharks of the family Lamnidae (white shark, makos, salmon shark, and porbeagle) and tunas (bluefins, albacore, and skipjack). Both groups have species that have expanded their range into northern waters, often swimming in frigid seas but can have regions of their body at temperatures 20 °C or more above ambient water temperature (**Figure 1**). An example of cranial endothermy is the monotypic swordfish (*Xiphias gladius*), where highly specialized extraocular muscles are modified into a thermogenic organ that warms just the brain and eyes up to 15 °C above ambient water temperatures.

Some of the literature refers to these fish as heterotherms rather than endotherms. Their point is that the entire body temperature is not constant (some tissues may be warm while others are cold) or temporally (the stomach may be warm only after a large meal). The use of endothermy herein stresses that the source of the heat is metabolic heat produced by the fish rather than from the environment.

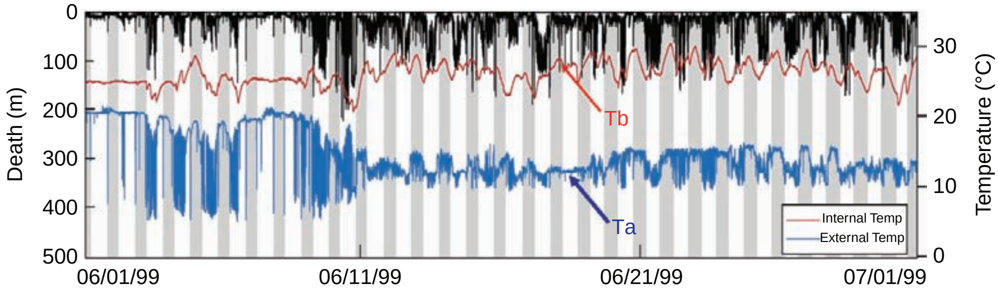


Figure 1 An archival tag record demonstrating endothermy over a 3-week period in an Atlantic bluefin tuna swimming in the North Atlantic. Modified from Walli A, Teo SLH, Boustany A, et al. (2010) Seasonal movements, aggregations and diving behavior of Atlantic bluefin tuna (Thunnus thynnus revealed with archival tags. *Plos ONE* 4(7): e6151 (doi:10.1371/journal.pone.0006151).

Systemic Endothermy

Endothermy in fish evolved in groups that attain large body size. A requirement of endothermy is an internal heat source, which occurs in some species as an increase in metabolic rate, or evolution of high aerobic capacity within a tissue. Countercurrent heat exchangers or retia mirabilia are required to conserve the metabolic heat (**see also Design and Physiology of Arteries and Veins**: The Retia). Tunas (Scombridae, Tribe Thunnini (*Katusuwonus, Euthynnus* and *Auxis*)) and sharks (Lamnidae) are unique among fishes because of their unique ability to maintain elevated tissue temperatures above ambient water temperatures in their locomotory muscle, viscera, brain, and eye muscles. Dr. Frank Carey of Woods Hole Oceanographic Institution was the first scientist to measure tissue temperatures in free-swimming tuna, and subsequently made major contributions to our understanding of endothermy in fishes. Some tuna species, similar to birds and mammals, have an elevated standard metabolic rate and numerous specializations associated with increased oxygen delivery to the tissues. The heat generated by this elevated metabolic rate is retained within these tissues with vascular countercurrent heat exchangers embedded in the systemic circulation.

Cranial endothermy in the tunas and lamnid sharks occurs through a heat exchanger retia located in the circulation to the eyes and brain. To date, there has not been a heater organ-like tissue for thermogenesis identified in tunas or sharks; the heat sources are the highly aerobic extraocular muscles as well as the brain, which thus far have been shown to occur in the bluefin tuna group only. Heat conservation mechanisms involving vascular retia are found in the lamnid sharks as well as closely related thresher sharks of the family Alopidae.

In tunas and lamnid sharks, endothermy in locomotory muscle is associated with two distinct anatomical changes: (1) the movement of the red slow-twitch aerobic swimming musculature internally and forward in the body plan and (2) the insertion of a complex countercurrent heat exchanger in the circulation to the oxidative muscle mass to reduce conductive and convective heat loss. Strong convergence in structure and function is seen in these two lineages. Both have a very streamlined shape and a shift in the body plan such that the red muscles that power endurance swimming have a central position near the vertebral column (**Figure 2**). This differs from most teleosts that have the aerobic red muscle located laterally near the surface of the fish, just beneath the skin. The central position of the aerobic muscle source facilitates heat retention as the red muscle fibers are continuously recruited, contracting and generating heat during the sustained locomotion characteristic of these lineages. The central positioning of the red muscle in tunas and lamnid sharks has received considerable attention as a result of its role in endothermy; however, it primarily reflects the evolution of a unique locomotor style. This stiff-bodied thunniform and lamniform swimming is more efficient for drag reduction (**see also Buoyancy, Locomotion, and Movement in Fishes**: Functional

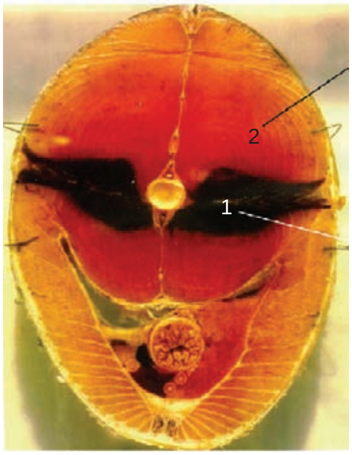


Figure 2 A cross section through a tuna that illustrates the internal location of the aerobic muscle that powers endurance swimming. This location differs from most teleosts that have the aerobic red muscle located laterally near the surface of the fish, just beneath the skin. 1, aerobic red muscle; 2, white muscle.

Properties of Skeletal Muscle: Work Loops and Undulatory Swimming). These fish generate thrust exclusively via the tail and restrict their propulsive movements to the caudal region rather than bending the whole body. Evolution of this locomotory form may be tightly coupled with the movement of the central aerobic muscle mass internally in addition to the use of this metabolic source of heat for endothermy.

In addition to the muscle heat, in some species of tunas and lamnid sharks the metabolic heat of digestive processes is conserved as well. Energy is expended (and heat is produced) during ingestion, digestion, absorption, and assimilation of a meal. When bluefin tunas digest a meal, they have a metabolic response that includes elevation of oxygen uptake called specific dynamic action (SDA) and the associated post-feeding thermogenesis (**Figure 6**; **see also Food Acquisition and Digestion**: Cost of Digestion and Assimilation). By having visceral retia mirabilia, the tunas and sharks retain the heat and warm the tissues involved in digestion of the meal. The entire muscle mass buffers visceral tissue from the surrounding aquatic heat sink to reduce conductive heat loss. Often, the muscle below the gut contains a very high fat content that probably adds to its insulative value.

An unusual aspect of endothermy in fishes is the requirement for a robust cardiac physiology as the hearts of all tunas operate at ambient water temperatures because they are not protected by retia mirabilia. The unusual physiological arrangement of endothermy in some tissues is unique among vertebrates and can result in a cold heart supplying the metabolic demands of warm tissues. In response to this, bluefin tunas and lamnid sharks have a higher capacity to cycle calcium ions in the heart cells that helps maintain beat-to-beat contraction at reduced temperatures. Results from molecular, structural, and physiological data indicate a significant role of mammalian-like calcium-induced calcium release in the cardiac myocytes of tunas and sharks. Thus, endothermic fish can protect their cardiac function in frigid seas using mechanisms that increase the capacity of the heart to function, ensuring delivery of oxygen to the metabolically active tissues required for warming the muscles, visceral, and cranial tissues.

Cranial Endothermy

The elevation of cranial tissue temperatures has the same two main requirements: (1) a heat source based on aerobic metabolism plus (2) vascular countercurrent heat exchangers to retain the heat. The main source of metabolic heat in the cranium is the extraocular muscles, the muscles that move the large eyes of these visual predators.

Cranial endothermy in tunas and sharks is not associated with a specific thermogenic tissue but with conservation of heat from continuously active red aerobic swimming muscle (lamnid sharks) or large extraocular muscles (tunas). In the case of the sharks, an unusual arrangement occurs whereby the vein coursing from the aerobic swimming muscle travels to the orbital sinus behind the eyes to bathe an orbital retia mirabilia with warm venous blood, the heat from which can be retained in the cranial region. That is, metabolic heat produced in the swimming muscle is used to warm the brain. Less complex is the situation in tunas, where the aerobic eye muscles are disproportionately large relative to eye size for ectothermic species. These extraocular muscles are among the fastest contracting muscles in vertebrates, and fiber populations are enriched with the cellular machinery for superfast contraction: the sarcoplasmic reticulum (SR) and proteins that cycle calcium. The presence of aerobic muscle fibers within the extraocular muscles, already specialized for superfast contraction, provides this muscle with relatively high thermogenic capacity and underlies the evolution of heat retention mechanisms in the circulation to the brain and eyes. The extraocular muscle of billfishes is similarly large and fast contracting, but has an additional feature, a heater organ.

Heater Organ Tissue

Some fish have evolved a heater organ for cranial endothermy. This thermogenic (heat producing) tissue, which warms only the brain and eyes, is found beneath the brain of all billfishes: swordfish, *X. gladius* (family Xiphiidae), marlins (*Makaira* sp.), spearfish *(Tetrapturus* sp.), and sailfish (*Istiophorous* sp.)(family Istiophoridae) and in one species of Scombrid fish, the butterfly mackerel (*Gastrochisma melampus*). It has also been reported in a monotypic genus, the slender tuna (*Allothunnus fallai*). The moonfish or opahs (Lampridae) have also been reported to have a countercurrent heat exchanger behind the eye, but it remains unresolved as to whether thermogenic cells are involved. Cranial endothermy in these species involves the presence of a specialized thermogenic heater organ tissue that develops from extraocular muscle and is located beneath the brain and near the eyes. The thermogenic capacity has been shown by direct telemetry in the swordfish to warm the cranial cavity up to 15 °C above ambient water temperature. The appearance of cranial endothermy independently of warming any other portion of the body indicates that there is a strong selective pressure for warming the brain and eyes.

The billfish heater organ is comprised of a mass of thermogenic tissue that is deep red in color with the appearance and texture of liver (**Figure 3**). It is associated with the superior rectus eye muscle in billfishes and with the lateral eye muscles in the butterfly

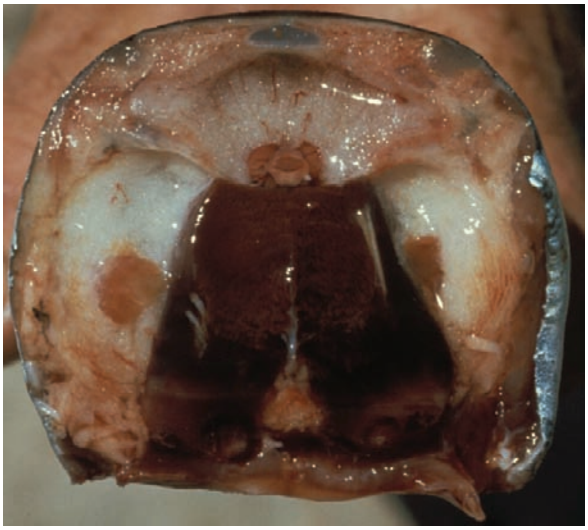


Figure 3 Transverse section through the head of a swordfish showing the position of the heater tissue beneath the brain and within the orbit. The head is packed with fat to reduce conductive heat loss.

mackerel, where modified muscles from both sides of the orbital cavity converge at the base of the braincase. In the swordfish, the brain is embedded in the dorsal surface of the heater, at the basisphenoid bone that forms the base of the braincase, which has been reduced to a membranous sheath. Large amounts of adipose tissue surround and insulate the brain, eyes, and heater organ. The major difference between heater organs in the billfishes is that of size; the heater organ is largest in the swordfish – the total mass of heater organ tissue exceeds the mass of the heart. The eyes of the swordfish are equivalent in size to small grapefruits. Both the eyes and the heater organ are surrounded by insulating fat.

In addition to the adaptation of the eye muscle to generate heat, these fish also have well-developed retia to reduce heat loss. At its base, a countercurrent heat exchanger is formed from the carotid artery and the venous return from the heater to retain the heat produced by the heater organ. Billfishes have two separate blood supplies to the brain and eyes: one supplying oxygen and the other retaining heat. The carotid circulation divides into numerous capillaries that bathe the oxidative heater cells with blood. In addition to this warm blood supply to the retina, the billfishes, like most teleost fishes, have a separate oxygenated source of blood to the retina by way of the choroid retia mirabilia. The choroid retia is extraordinarily enlarged in the marlins and other Istiophorid billfishes, and is supplied with blood via a pathway separate from the heater organ insuring a well-oxygenated blood supply to the eye. These fish have a separate circulation to the retina because the blood coursing through the heater organ is depleted in oxygen by the time the warm blood gets to the retina.

How Fish Have Built a Furnace Out of Muscle

The cells that make up the heater organ are modified skeletal muscle cells that are often called muscle fibers because they are multinucleate. They lack myofibrillar contractile proteins and are no longer involved in force generation but have an extremely high content of mitochondria and SR. In vertebrate skeletal muscle, contraction and relaxation are regulated by the concentration of calcium in the cytosol, which, in turn, is controlled by the SR and transverse tubule membranes. The SR of skeletal muscle cells is a highly organized intracellular membrane system that stores and releases Ca^{2+} in a highly regulated fashion. The SR has release channels (termed ryanodine receptors) that, when opened, allow Ca^{2+} to flow from the SR down its concentration gradient into the cytosol. Ca^{2+} is pumped back into the SR by the calcium ATPase (calcium pump protein), the major protein constituent of the SR bilayer. The calcium pumping consumes adenosine triphosphate (ATP) and heat is produced as a by-product of ATP production. If $Ca^{2+}$is cycled more than normal between the cytosol and the SR, then more ATP is consumed and more heat is produced. This is a key feature of the heater organ. In heater tissue and lacking extensive contractile protein, a thermogenic process is associated with Ca^{2+} cycling involving excitation–thermogenic coupling unlike the excitation–contraction coupling that normally occurs between the T-tubule, SR, and protein components of regular skeletal muscle.

Consistent with this role, certain morphological features stand out in all high power microscope pictures (electron micrographs) of heater tissue. The mitochondria of the heater cell are among the most densely packed in any cells in any animal, with as much as 63% of the cell volume being mitochondria. Also, located throughout the cytoplasm and in between the mitochondria is an extensive smooth membrane system comprised of tubules and membranous stacks. The tubules and membranous stacks are components of the two internal membrane components of muscle usually involved in excitation-contraction coupling, the SR. The very high oxidative capacity of the heater tissue also is reflected in high myoglobin concentration (400 μmol kg^{-1} wet weight) and in very high aerobic metabolic enzyme profiles.

Heater cells are innervated by a branch of the oculomotor nerve, and large clusters of acetylcholine receptors on the plasma membrane indicate that the cells are under nervous control similar to that in skeletal muscle. It is hypothesized that upon entering cooler water, nerve impulses to the heater cells result in depolarization of their cell membrane and that depolarization leads to Ca^{2+} release from the SR internal heater cell membrane

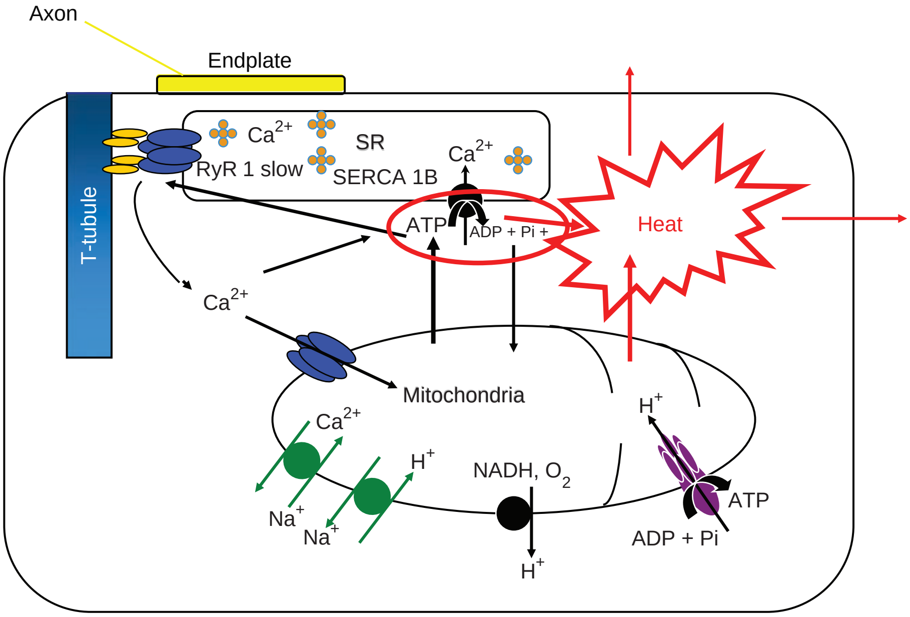


Figure 4 A model of excitation–thermogenic coupling in heater cells. Thermogenesis in heater cells is proposed to occur via depolarization-induced Ca^{2+}-release pathways. Nervous stimulation mediated by acetylcholine receptors at the endplate results in heater cell depolarization and DHPR–RyR1-mediated Ca^{2+} release from the SR. Increased cytoplasmic Ca^{2+} stimulates Ca^{2+} transport and ATP turnover by SERCA 1B and mitochondrial influx and efflux pathways. The physiological properties of the RyR1-slow isoform expressed in heater cells may facilitate prolonged channel openings under these conditions (high Ca^{2+}and the presence of adenine nucleotides) and promote further release of Ca^{2+} in a futile cycle that results in thermogenesis. Abbreviations: T-tubule, transverse-tubule; SR, sarcoplasmic reticulum; SERCA, sarco/endoplasmic reticulum Ca^{2+}ATPase; RyR, ryanodine receptor. Modified from Morrissette JM, Franck JPG, and Block BA (2003) Characterization of ryanodine receptor and Ca^{2+}-ATPase isoforms in the thermogenic heater organ of blue marlin (*Makaira nigricans*). *Journal of Experimental Biology* 206: 805–812.

system in much the same manner as occurs in a typical skeletal muscle through the Ca^{2+} release pathway (**Figure 4**). That is, depolarization leads to a conformational change in the voltage-sensitive dihydropyridine receptors (DHPRs) or L-type Ca^{2+} channels, which in turn induces the opening of SR Ca^{2+} channels and release of Ca^{2+}into the cytosol. Ca^{2+} release initiates a cycle of calcium-induced Ca^{2+} release. Similar to skeletal muscle, uptake of Ca^{2+} back into the SR requires energy in the form of ATP produced by the numerous mitochondria. The majority of energy used in generating ATP (and consumed by the Ca^{2+} pump) is released as heat – it is this heat that is used to heat the brain and eyes.

Telemetry of Brain Temperature

The best evidence for physiological regulation of brain temperatures comes from experiments with free-swimming swordfish conducted in the 1980s by Dr. Frank Carey. Swordfish have remarkable diel behavior, swimming to depths as deep as 600 m during the day in search of squid but spending most of the evening hours at the surface. During these vertical excursions, which primarily occur at dusk and dawn, the fish encounters rapid water temperature changes up to 19 °C in a short period. Measurements of cranial temperature provide direct evidence that the fish are able to warm the cranial cavity. Temperature in the cranial cavity remained about 28 °C for 36 h while the fish encountered water temperatures that ranged from 13 °C to 17 °C (**Figure 5**).

Why Be Warm?

The repeated evolution of endothermy in the scombroid fishes and lamnid sharks indicates that there is strong evolutionary selection for this energetically costly metabolic strategy. All endothermic lineages have species that have expanded their ranges into cool temperate waters and, in some cases, subpolar seas (Atlantic bluefin tunas and salmon sharks). This supports a niche expansion argument for the evolution of endothermy in fishes. There are several hypotheses on the advantages to warming of the brain, eyes, muscle, and visceral tissues.

In cranial endotherms such as swordfish, the warming of the brain and eye regions, the central nervous system and retina, is of key importance. Physiological processes such as synaptic transmission and the conduction of nerve impulses are temperature sensitive. Studies on the sensitivity of the retina indicate that vision and flicker frequency, as well as temporal resolution of a light flash,

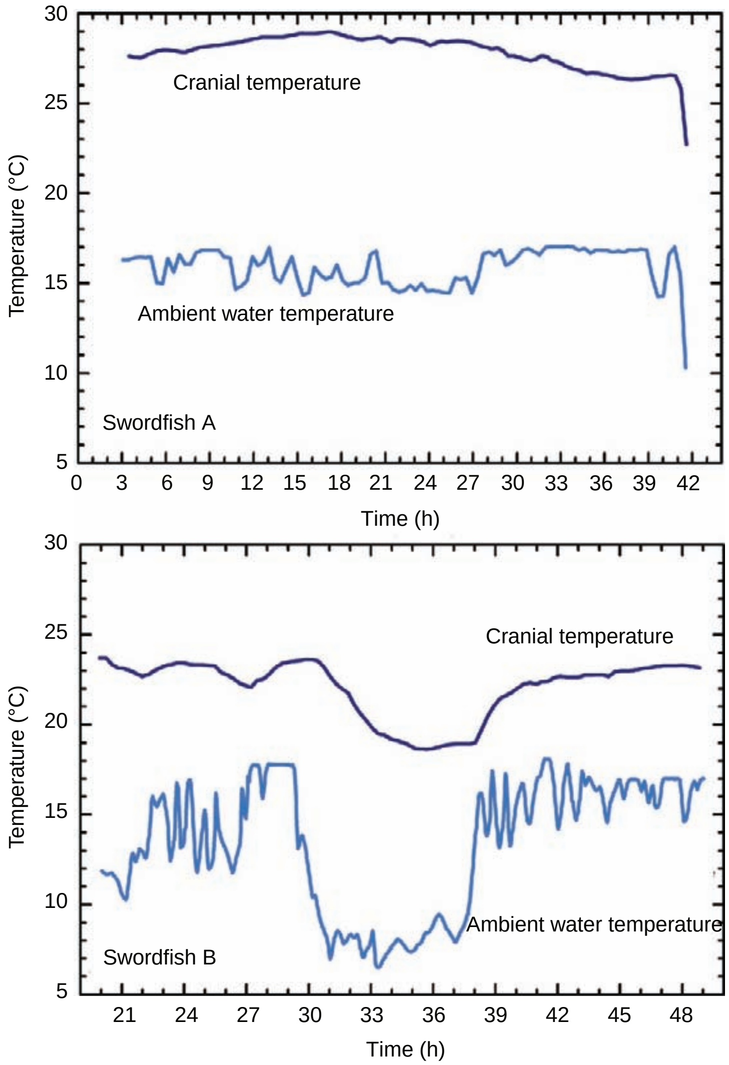


Figure 5 Telemetry record of cranial temperature and ambient water temperature from a free-swimming swordfish. Zero on the *x*-axis is midnight, top line is cranial temperature, and bottom line is water temperature. Swordfish swim to depths as deep as 600 m, typically at dusk and dawn. Modified from Carey FG (1990) Further observations on the biology of the swordfish. In: Stroud RH (ed.) *Planning the Future of Billfishes*. Savannah, GA: National Coalition for Marine Conservation.

are thermally sensitive. The warming of the eye and specifically the retina has been shown experimentally to significantly improve visual acuity, specifically the temporal resolution of nervous signals by the retina. Warming of the retina may improve the ability to detect prey in the dimly lit mid-waters (light penetration ceases around 1000 m; (**see also Deep-Sea Fishes**). Thus, fish with warm eyes can detect the rapid motion of prey species better than fish with cold eyes. Because these fish are fast-swimming predatory fishes, improvements in the temporal resolution mean that the swordfish eyes are operating faster than prey at the same temperature as the surrounding water. The convergence of cranial endothermy in several species indicates that there is strong selection for warming of the eyes and brain. Cranial endothermy has evolved multiple times in large predatory fish that experience cooler waters at high latitude distributions (temperate or subpolar seas) or at depth. Thus, warming of sensory structures in the cranium appears to be key to occupation of the colder (both at high latitude and at depth) as well as dimly lit environs.

Studies in tuna also indicate that warming of the swimming muscle permits them to generate more power that results in enhanced locomotory performance because power output of muscle is highly temperature dependent. A countercurrent heat exchanger that could maintain a 10 °C temperature differential would typically double maximum muscle power output and the frequency at which maximum power is generated. The deep slow muscle of a tuna operates at higher temperatures than slow muscle from ectothermic sister taxa such as bonito and has been shown to generate more power. However,

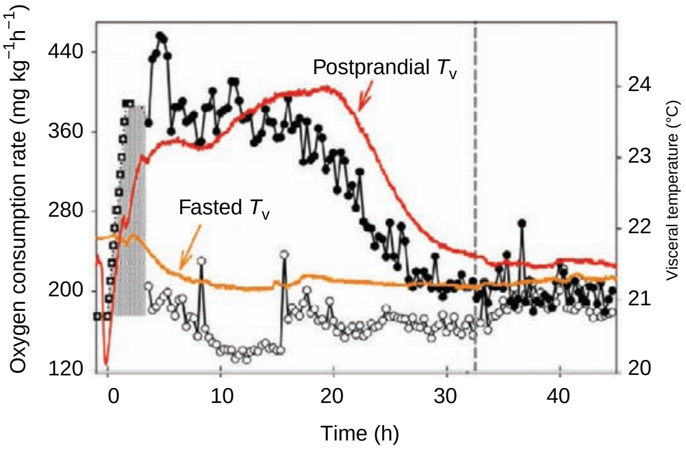


Figure 6 Traces of fasted and digesting oxygen uptake rate (MO_2; open and closed circles, respectively) and visceral temperature (T_v) from Pacific bluefin tuna (*Thunnus orientalis*) swimming at 1 BL s^{-1} at 20 °C. Spikes in MO_2 for the fasted trace resulted from short bursts of unsteady swimming. Modified from Clark TD, Brandt WT, Nogueira J, *et al.* (2010) Postprandial metabolism of Pacific bluefin tuna (*Thunnus orientalis*). *Journal of Experimental Biology* 213.

internal warm muscles are more thermally sensitive to temperature changes than more superficially located swimming muscles in tuna. This suggests evolutionary specialization for operation at higher, but also in relatively stable temperatures for function.

Digestion, like vision and muscle power, is temperature sensitive. An example of a tuna warming its viscera after a meal is shown in **Figure 6**. Food resources are ephemeral in the open ocean and often spread over large distances. Acquiring a meal quickly, digesting, and being able to take on more energy quickly is a matter of survival. Increasing the temperature of the viscera after a meal hastens digestion, largely because the action of enzymatic degradation of the meal is very temperature sensitive. Thus, when a tuna swimming in 10 °C water warms its gut from 10 to 30 °C after a meal, it decreases the time to digest the meal about threefold. Considered over a longer time period, for example, a week or a month, a tuna that warms its viscera can eat more if it can digest faster, , and thus grow more rapidly than if it did not warm its gut. That is, the advantage of the warm cecum is that protein is digested in about one-third the time so that these tuna can process about three times as much food per day.

In summary, these large, fast, oceanic predators have a number of specializations that permit them to achieve tissue temperatures higher than ambient water temperatures. Most important are the retia to conserve the heat, and an increase in metabolic heat production. Unique to a few of them is the evolution of a tissue specialized for the generation of heat to achieve high brain and eye temperatures, the heater organ.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Functional Properties of Skeletal Muscle: Work Loops; Undulatory Swimming. **Deep-Sea Fishes**. **Design and Physiology of Arteries and Veins**: The Retia. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation.

Further Reading

Altringham JD and Block BA (1997) Why do tuna maintain elevated slow muscle temperatures? Power output of muscle isolated from endothermic and ectothermic fish. *Journal of Experimental Biology* 200: 2617–2627.

Block BA (1991) Endothermy in fish: Thermogenesis, ecology and evolution. Hochachka PWH and Mommsen T (eds.) Biochemistry PWH and Molecular Biology of Fishes, vol. 1, pp. 269–311. Amsterdam: Elsevier.

Block BA (1986) Structure of the brain and eye heater tissue in marlins, sailfish, and spearfishes. *Journal of Morphology* 190: 169–189.

Block BA, Finnerty JR, Stewart AF, and Kidd J (1993) Evolution of endothermy in fish: Mapping physiological traits on a molecular phylogeny. *Science* 260: 210–214.

Carey FG (1982) A brain heater in the swordfish. *Science* 216: 1327–1329.

Carey FG (1990) Further observations on the biology of the swordfish. In: Stroud RH (ed.) *Planning the Future of Billfishes*, pp. 109–122. Savannah, GA: National Coalition for Marine Conservation.

Carey FG and Lawson KD (1973) Temperature regulation in free-swimming bluefin tuna. *Comparative Biochemistry and Physiology A* 44: 375–392.

Carey FG and Teal JM (1966) Heat conservation in tuna fish muscle. *Proceedings of the National Academy of Sciences of the United States of America* 56: 1464–1467.

Clark TD, Brandt WT, Nogueira J, *et al.* (2010) Postprandial metabolism of Pacific bluefin tuna (*Thunnus orientalis*). *Journal of Experimental Biology* 213: 2379–2385.

Dickson KA and Graham JB (2004) Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology* 77: 998–1018.

Morrissette JM, Franck JPG, and Block BA (2003) Characterization of ryanodine receptor and Ca^{2+}-ATPase isoforms in the thermogenic heater organ of blue marlin (*Makaira nigricans*). *Journal of Experimental Biology* 206: 805–812.

Stevens ED and McLeese JM (1984) Why bluefin tuna have warm tummies: Temperature effect on trypsin and chymotrypsin. *American Journal of Physiology* 246: R487–R494.

Walli A, Teo SLH, Boustany A, *et al.* (2010) Seasonal movements, aggregations and diving behavior of Atlantic bluefin tuna (*Thunnus thynnus* revealed with archival tags. *Plos ONE* 4(7): e6151 (doi:10.1371/journal.pone.0006151).

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Fish Migrations

Contents

The Biology of Fish Migration
Tracking Oceanic Fish
Eel Migrations
Pacific Salmon Migration: Completing the Cycle

The Biology of Fish Migration

TR Binder, Hammond Bay Biological Station, Millersburg, MI, USA

SJ Cooke, Carleton University, Ottawa, ON, Canada

SG Hinch, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

What is Migration?
Classifying Migrations
Orientation and Navigation
Energetics of Migration
Environmental Factors That Influence Migration
Anthropogenic Impacts on Migration
Further Reading

Glossary

Amphidromy An uncommon subcategory of diadromy, in which there is a brief excursion from freshwater to seawater during the juvenile stage, but the majority of feeding and growth and spawning occur in freshwater.

Anadromy Life-history strategy entailing reproduction and early rearing in freshwater followed by a significant growth phase in seawater.

Catadromy A subcategory of diadromy, in which the majority of feeding and growth occurs in freshwater and the fully grown adult fish migrate to saltwater and reproduce.

Crepuscular Occurring during twilight (dusk and dawn). Daily pattern with two peaks of activity centered around dusk and dawn, though not excluding some activity in between these periods. From Latin ‘crepusculum’ (twilight).

Diadromy A category of migration, in which all migratory activity crosses the seawater/freshwater boundary.

Diel Pertaining to a 24-h period.

Fluvial Relating to a river, stream, or other flowing water.

Iteroparity A reproductive strategy whereby an individual reproduces more than once within its lifetime.

Lateral line Sensory structure consisting of a series of mechanoreceptor cells on the surface of the body of fishes and for detection of water motion relative to the body and low-frequency sound.

Migration A regular directed movement of a group of animals.

Navigation A mechanism whereby fishes plot a course to a particular location.

Oceanodramy A category of migration, in which all migratory activity occurs in saltwater.

Orientation A mechanism whereby fishes position themselves in a particular direction in response to an external stimulus.

Potamodromy A category of migration, in which all migratory activity occurs in freshwater.

Rheotaxis A directional preference to flowing water.

Semelparity A reproductive strategy whereby an individual reproduces only once within its lifetime.

What is Migration?

It is sometimes the case in fishes that the life history needs of a population (e.g., foraging and reproduction) cannot be met by a single habitat. This is due to variability in the habitat conditions (e.g., temperature), or to the changing needs of the population itself (e.g., foraging habitat vs. spawning habitat). In such cases, the fitness of individuals benefits from movement to an alternate habitat. As a result, many fishes have evolved a life history that includes coordinated movement from one habitat to another. This synchronous, directed movement of part or all of a population between discrete habitats is called 'migration'.

Approximately 2.5% of all fish species undertake migrations. The physical scale of migrations is highly variable and can range from hundreds of meters, as in some coastal and stream dwelling fishes, to thousands of kilometers, as in eels (*Anguilla* spp.; **see also Fish Migrations**: Eel Migrations) and tunas (*Thunnus* spp.; **see also Fish Migrations**: Tracking Oceanic Fish). The timing of migration typically occurs on a seasonal scale, though some species display coordinated daily movements (e.g., vertical or tidal migrations). According to some authors, this represents migratory activity and, to others, these movements are a specialized form of foraging. In this article, we focus on longer-distance migrations, not because short-distance and vertical migrations are ecologically or evolutionarily less important, but because long-distance migrations typically impose greater behavioral and physiological challenges on fishes than short ones.

Classifying Migrations

Fish migrations are typically grouped into three categories, based somewhat arbitrarily on their relationship to the seawater/freshwater boundary (**Figure 1**).

1. Oceanadromous migrations, such as those performed by tunas, white sharks (*Carcharodon carcharias*), and plaice (*Pleuronectes platessa*), occur entirely within seawater.
2. Migrations that occur entirely within freshwater are classified as potamadromous. Potamadromous migrations can occur solely in lakes (e.g., lake trout, *Salvelinus namaycush*), in rivers and streams (e.g., brook lampreys, *Lampetra* spp.), or can span both lake and fluvial habitats (e.g., white suckers, *Catostomus commersoni*).
3. Migrations that cross the seawater/freshwater boundary (e.g., Pacific salmonids, *Oncorhynchus* spp.) are classified as diadromous. There are three subcategories of diadromy – anadromy, catadromy, and amphidromy:

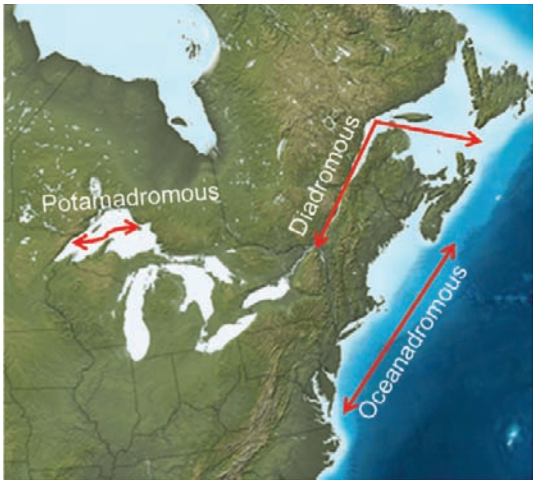


Figure 1 Map of the northeast coast of North America displaying example migratory patterns for the three major categories of fish migration. Migrations are categorized based on their relationship to the seawater/freshwater boundary – see text for details. Sources of map image: http://oversights.org.uk/.

   (a) Anadromy occurs when most feeding and growth occurs in saltwater and fully grown adults move back into freshwater to spawn (e.g., Pacific salmon).
   (b) Conversely, catadromy occurs when most feeding and growth occur in freshwater and the fully grown adults move into saltwater to spawn (e.g., eels).
   (c) The last subcategory, amphidromy, occurs when there is a brief excursion from freshwater to seawater during the juvenile stage, but the majority of feeding and growth and spawning occurs in freshwater. This last subcategory is most common in fishes inhabiting islands in the tropics and subtropics (e.g., sicydiine gobies, *Sicydium* spp.)

While it might be convenient to classify migrations, the above categories and subcategories do not provide any insights into the behavioral or physiological challenges involved in migrations, with the notable exception that diadromous migrants must contend with an osmoregulatory challenge as they move between the freshwater/seawater environments (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). As a result, this article focuses on the requisite properties for long-distance migration, rather than their categories.

Orientation and Navigation

There is a long history of interest in orientating and navigating mechanisms in fishes that stems from the discovery in the 1930s that salmon return from the ocean to their natal streams to spawn (termed 'homing'). Since then, homing has been demonstrated for numerous fish species. Originally, it was believed that the phenomenon involved a spectacular navigation mechanism. However, despite intensive research on the mechanisms of homing in fishes, no precise navigation ability has yet been demonstrated. This prompted researchers to test the hypothesis that homing is accomplished through random search. Not surprisingly, a random search model alone was insufficient to explain homing in salmon. However, when slight directional bias (orientation) was added to the model, the predicted return rate was similar to the observed return rate for these fish.

Several orienting mechanisms have been proposed, and it is likely that several different cues might be involved in orientation at various stages of migration. In particular, orientating behavior differs markedly between open water and fluvial migrations. In open water, fishes may migrate in any one of 360° of direction. By contrast, fluvial migrants are confined within a relatively narrow channel plus they have the added cue of migrating either with or against the current, termed negative or positive rheotaxis, respectively. Because of this dichotomy, we discuss the possible orienting mechanisms for open water and fluvial migrations separately.

Orienting in Open Water

Stemming from pioneering work by Hasler in the late 1950s, there is now considerable evidence that fishes use solar cues to orient during open water migrations. Fishes may orient using information derived from changes in the sun's azimuth (angle of the sun in the horizontal plane) and/or altitude (angle of sun in the vertical plane) (**Figure 2**). However, because these measures change on both a diel and seasonal scale, fishes that orient using the sun must possess an internal biological clock and calendar to compensate for these changes. For salmon that spawn on very precise dates of the year, the idea of an internal biological clock is not an unreasonable expectation. Indeed, laboratory experiments in a variety of species have demonstrated that fishes may be trained to use an artificial sun to orient in a particular compass direction. Moreover, fishes trained to orient in this manner can correctly compensate for diel and seasonal variation in the position of the sun.

The sun itself is often obscured and, thus, is not always a reliable orienting cue. Nonetheless, fishes may continue to orient when the sun is obscured by using polarized light as a directional cue. The ability to detect and discriminate planes of polarized light has been demonstrated in numerous species. However, there is some evidence that polarized light would be a reliable orienting cue only during crepuscular periods (dawn and dusk) when the percentage of polarized light is as high as 60–70%. During the day, the percentage is far lower (<40%), and may in fact be below the detection threshold of most fishes.

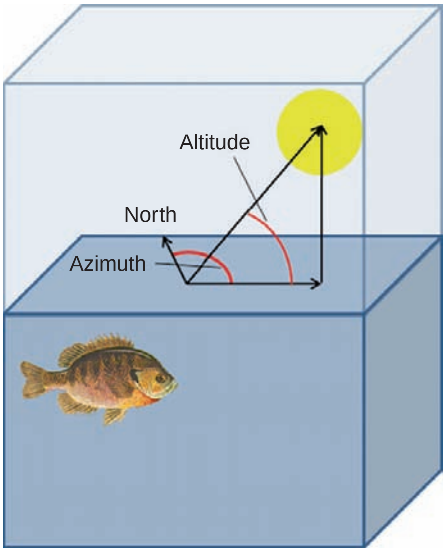


Figure 2 Solar information used by fishes for orientation in open water. Directional information is derived from changes in the angle of the sun in both the vertical (altitude) and horizontal (azimuth) planes. Fish possess a biological clock and calendar that allows them to compensate for diel and seasonal changes in these parameters.

There is a growing body of support for geomagnetic orientation, particularly in fishes such as Pacific salmon and eels (**see also Fish Migrations**: Eel Migrations), which undergo long-distance oceanic migrations. When water moves across the Earth's magnetic field, it induces a weak electric current that may be detected by fishes. In rainbow trout (*Oncorhynchus mykiss*), changes in the intensity of the Earth's magnetic field are detected by discrete magnetoreceptor cells located in the olfactory epithelium. These cells, which contain chains of magnetite crystals (biogenic Fe_3O_4) that transduce the induced electric current into a sensory signal, are innervated by a branch of the trigeminal nerve. There are, however, opponents to the theory of geomagnetic orientation in migrating fishes. The two main arguments against this type of orientation are that: (1) temporal and spatial variations in the magnetic field would make it difficult to form a useful magnetic map; and (2) there is as yet no evidence that fishes possess the ability to memorize such a map, even if they were capable of forming it (**see also Sensory Systems, Perception, and Learning**: Magnetic Sense in Fishes). Nevertheless, salmon can memorize the complex odors of a natal stream for their entire seaward migration, which can last several years.

The tendency to orient in a current is called rheotaxis. In open water, large-scale currents substantially influence the direction and speed of some migrations. For example, the ocean migration pattern of Pacific salmon from British

Columbia (Canada) is largely determined by interannual variations in the strength of the Alaskan Gyre, a counterclockwise current in the Gulf of Alaska. Smaller-scale currents can also be important. For example, models of the migratory behavior of Pacific salmon that encounter the Sitka Eddy (mesoscale eddy located northwest of the Queen Charlotte Islands, BC, Canada) suggest that salmon can exploit the rotational current of the eddy to increase speed and moderately reduce the energetic costs of migration. More recently, it has been hypothesized that even subtle currents may be important during the open water phase of migration of salmon to their natal streams.

Differences in water temperature and salinity create vertical layers and each layer has a distinct origin, presumably with its own unique home stream 'olfactory bouquet', which plays a dominant role during the final stages of locating the natal stream (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). Behavioral studies have shown that both Pacific and Atlantic salmon tend to migrate within a single vertical layer, but make brief periodic excursions into adjacent layers. Salmon may use these excursions to determine the direction of the layer's origin by detecting the acceleration that occurs as they cross between adjacent layers. It is unlikely that the lateral line is sensitive enough to detect the subtle differences in velocity between adjacent layers. However, the otolith organs are capable of detecting minute accelerations and, depending on how quickly the fish move between adjacent layers, could be capable of detecting velocity differences as small as $1\ \mathrm{mm\,s^{-1}}$.

Orienting in Fluvial Habitats

Navigation in fluvial habitats is less complicated than in open water. Here, water current is the primary orienting cue – migration is the result of the tendency of animals to orient with the current (negative rheotaxis) or against it (positive rheotaxis). Studies that employed a combination of Co^{2+} and aminoglycoside antibiotics (streptomycin and gentamicin) to block various components of the mechanosensory lateral line system have demonstrated that the lateral line is involved in mediating rheotactic behavior. Specifically, a single class of lateral line organs located on the surface of the skin, superficial neuromasts, appears to be important. A second class of lateral line organs, canal neuromasts, are located within canals beneath the surface of the skin and may contribute to navigation in complex flows (**see also Hearing and Lateral Line**: Lateral Line Neuroethology). Nevertheless, the lateral line is incapable of mediating rheotaxis on its own. Rheotaxis depends on a fixed frame of reference from which the fish can determine both the speed and direction of flow. This is accomplished using either visual cues or through periodic contact with the substrate.

There is overwhelming evidence that olfactory orientation is an important component of navigation in fluvial migrants. The olfactory basis of upstream migration has been most extensively studied in Pacific salmon and sea lampreys (*Petromyzon marinus*). Upstream-migrating salmon home to a stream-specific set of olfactory cues that are imprinted prior to their emigration from the stream as smolts (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). In sea lampreys, the situation is somewhat different. Spawning phase sea lampreys do not home to their natal stream. Instead, they are drawn to streams with suitable spawning habitat by a multicomponent bile acid pheromone released by larval lampreys, which they can detect at extremely low concentrations (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and Lampreys: Environmental Physiology). Interestingly, while these navigation strategies differ significantly, the mechanism by which olfaction mediates upstream migration appears to be the same.

The general consensus is that olfactory cues induce upstream migration in fishes through olfactory-mediated rheotaxis. This conclusion is based on two main observations. First, fishes tend to display significantly greater upstream activity (positive rheotaxis) when the correct olfactory stimulus is present than when it is not. Second, upstream migrants that no longer detect the olfactory stimulus, such as when the stimulus is pulsed, the animal is anosmic, or when the animals pass upstream of the stimulus source, display significantly greater movements in the downstream direction (negative rheotaxis). Downstream migrations, like those of salmon smolts, could similarly be induced by olfactory-mediated rheotaxis in response to conspecific olfactory cues. However, there is as yet no experimental evidence to support this assertion (**see also Sensory Systems, Perception, and Learning**: Spatial Orientation: How do Fish Find their Way Around?). Clearly, aquatic pollutants that impair olfaction (**see also Toxicology**: The Effects of Toxicants on Olfaction in Fishes) could have catastrophic effects for these fishes.

Energetics of Migration

Long-distance migrations, especially upstream migrations in fluvial systems, are energetically demanding, and feeding during long-distance migrations is rare. The reason for this is that feeding imposes several constraints on migration, including foraging time and the reduced metabolic scope available for migratory activity when energy is reallocated for foraging and digestion. As a consequence, most species rely heavily on energy reserves that they accumulate in the months preceding migration. In fact, in semelparous species such as Pacific salmon and lampreys, the alimentary canal and digestive organs undergo atrophy so that precious energy reserves are not wasted in maintaining these inactive tissues. In fishes that do not feed during migration, the energy available for migratory activity is finite; thus, energy management is a primary factor determining the success of a migrant.

The degree to which fishes deplete their energy reserves is highly variable both among species, and among different populations within a species. In some anadromous spawning migrations, energy depletion can be remarkably high, ~85% of total energy stores. There is a correlation between the degree of energy depletion during migration and the prevalence of semelparity among fish populations – the more energy they deplete, the less likely they are to be repeat spawner. For example, there are no repeat spawners among American shad (*Alosa sapidissima*) migrating up the St. Johns River (Florida, USA), which deplete as much as 75–82% of their total stored energy reserves. However, American shad migrating up the more northern Connecticut River (New England, USA), which deplete only 35–60% of their stored energy, do repeat spawn. Nonetheless, while exhaustion of energy resources probably contributes to mortality in semelparous populations, it is not the only factor. This is evident by the fact that some semelparous populations migrate only a relatively short distance – not nearly far enough to exhaust their energy reserves, indicating that there are other genetic and/or environmental factors involved. Indeed, the mechanism of post-spawning death in semelparous species appears to involve a breakdown in glucocorticoid regulation, resulting in highly elevated levels of circulating corticosteroids and a consequent disruption of immune function. This may be part of a true but rapid senesence program.

Energy for migratory activity is stored as proteins, carbohydrates, and lipids. Lipids are the most energy dense form of energy storage, having an approximately twofold greater energy density (9.45 kcal g^{-1}) than both carbohydrates and proteins (4.1 and 4.8 kcal g^{-1}, respectively). Unlike higher vertebrates, which store lipids mainly in adipose tissue, fishes distribute lipid reserves among a variety of tissues, including adipose-like tissue in the viscera, muscle tissue, and the liver. Many fishes also possess a subcutaneous layer of fat. Studies of proximate tissue composition have demonstrated that both lipids and proteins are depleted during long-distance migrations. Carbohydrate levels tend to remain high, but this is because carbohydrates (glycogen and glucose) are used mainly to fuel short bursts of activity, and are then subsequently replenished through breakdown and conversion of lipids and/or protein.

The majority of energy mobilized during migration (excluding that used for gonadal development during spawning migrations) is used by the muscles for locomotor activity. In general, fishes have two types of muscles that are both anatomically and functionally distinct. Red muscle is organized into a thin longitudinal band beneath the lateral line and is ideally suited for powering slow and intermediate speed, high endurance swimming – the swimming that comprises most of a fish's routine activities. High-speed swimming is powered by white muscle, which comprises the bulk of the muscle mass, despite the fact that most fish species only occasionally invoke high-speed (aka burst) swimming. White muscle is fueled mainly by glycolytic (anaerobic) metabolism and is prone to fatigue (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance). Moreover, during anaerobic swimming fish accumulate the metabolic by-product lactate, which can cause acidosis and even death. Reconversion of lactate to glycogen and rebalance of acid–base requires aerobic metabolism and can take as long as 24 h.

Environmental Factors That Influence Migration

Understanding how environmental variables influence migration is a popular theme in the study of migratory biology. The greatest challenge in studying how environmental factors influence migration is the fact that environmental variables are often highly correlated with one another, making it difficult to know which variables are the most relevant for influencing a migration. For example, in temperate climates there is a high degree of correlation between the length of day (photoperiod) and water temperature. Moreover, the relative importance of each environmental factor may be dependent on the local characteristics of the habitat in which the migration is occurring. Nonetheless, despite these challenges, a number of environmental factors have repeatedly been shown to influence migration in fishes.

Changing light levels are an important environmental stimulus for migratory activity. On a seasonal scale, changes in photoperiod provide calendar information that is used to initiate and synchronize the migratory activity of individuals within a population. Long-duration migrations such as the spawning migrations of Pacific salmon and Pacific lampreys (*Lampetra tridentata*) appear to be particularly dependent on photoperiod for synchronizing migratory activity. This probably reflects the fact that other environmental factors (e.g., temperature) that vary on a seasonal scale are more variable, and, therefore, less reliable. The daily (diel) alternation between night and day synchronizes migratory activity. Diel activity patterns fall into three categories – diurnal, nocturnal, and crepuscular. It is not clear for most fishes whether the diel pattern of migratory activity is a true endogenous circadian rhythm (**see also Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish) or a direct response to changing light levels. There is, however, evidence that fishes will adapt their diel activity to variations in light level. For example, nocturnal fishes often become active during the day when there is cloud cover, or when turbidity is high. Thus, the diel period in which a fish migrates appears to be related to its perceived risk of falling prey to visual predators.

Temperature can trigger and synchronize migratory activity in fishes. When temperature acts as a trigger, migration can be viewed as a form of behavioral thermoregulation (**see also Behavioral Responses to the Environment**: Temperature Preference: Behavioral Responses to Temperature in Fishes). This can occur under two conditions. First, in thermally heterogeneous environments, temperature may fluctuate beyond the range of thermal tolerance for a given population. Thus, the fish are forced to seek out a new thermal habitat. Second, the thermal requirements of the population may change. For example, the optimal temperature for growth may not be the same as for reproduction. In this example, temperature is the cause of the migration to spawning grounds. However, temperature is also a well-known synchronizing cue during spawning migrations. This is particularly true for migrations that are relatively short in duration. A good example of this is the springtime upstream migration of sea lampreys (*P. marinus*) in the Laurentian Great Lakes. Typically, the migration does not begin until temperatures in the streams exceed ~10 °C. Thus, the migration begins later in cooler than average springs and earlier in warmer than average springs. This type of thermal threshold for migratory activity in spawning phase fishes is common and seems to have evolved in response to the strict thermal requirements of the developing embryos. A related temperature effect is slowed growth at low temperature, which causes more northern populations of Pacific salmon to rear in freshwater for another year before migrating to the ocean.

In fluvial habitats, discharge-related events can also have a marked influence on migratory behavior. The effect of discharge on migration can sometimes be difficult to interpret because changes in discharge are often associated with changes in other environmental factors, such as temperature and turbidity. Nonetheless, there is evidence that periodic increases in discharge, such as those that occur during the spring snow melt and after rain events, are commonly associated with peaks in downstream migratory activity (e.g., Atlantic salmon smolts, *Salmo salar*). Perhaps more surprisingly, increases in discharge are also commonly accompanied by peaks in upstream migratory activity (e.g., spawning migrations of white suckers, *C. commersoni*, and brown trout, *Salmo trutta*). In these instances, the benefits of higher water levels (e.g., greater protection from visual predators and easier access to headwater rearing sites) seem to outweigh the greater energetic costs of swimming against greater velocity. Moreover, it has been shown in adult Pacific salmon that some fish can utilize small-scale reverse flow vortices to draft up-river with minimal swimming effort and thus can conserve high levels of energy during the migration. By contrast, in species with relatively poor swimming ability (e.g., American eels, *Anguilla rostrata*, and brook lampreys, *Lampetra planeri*), the cost-to-benefit ratio is apparently greater and increases in discharge may inhibit upstream migration.

There is fairly strong support for lunar phasing in some migrating fishes. In many cases, changes in migratory activity appear to be a direct response to variations in the intensity of moonlight. For example, upstream migratory activity in lampreys and eels is reportedly highest on nights with little moonlight. However, there is also evidence that some species may entrain to the lunar cycle. For instance, in masu (*Oncorhynchus masou*) and coho (*Oncorhynchus kisutch*) salmon smolts, circulating levels of the thyroid hormone thyroxin (T_4) peak during the new moon. This peak in T_4 is, in turn, a stimulus for downstream migratory activity. Lunar phasing has also been widely reported in diadromous species as they cross the seawater/freshwater boundary. However, in these cases, the relationship is generally believed to be indirect, and is likely a response to tidal variations, rather than changes in moonlight.

Anthropogenic Impacts on Migration

Human activities have a long history of interfering with fish migration. Probably, the most obvious way in which our activities disrupt migration is through the construction of dams and other structures (e.g., weirs and culverts) that act as barriers to migration in streams and rivers. Barriers disrupt stream continuity making it difficult, if not impossible, for fishes to reach upstream habitat (**Figure 3**). Even when a fishway is present at a barrier,

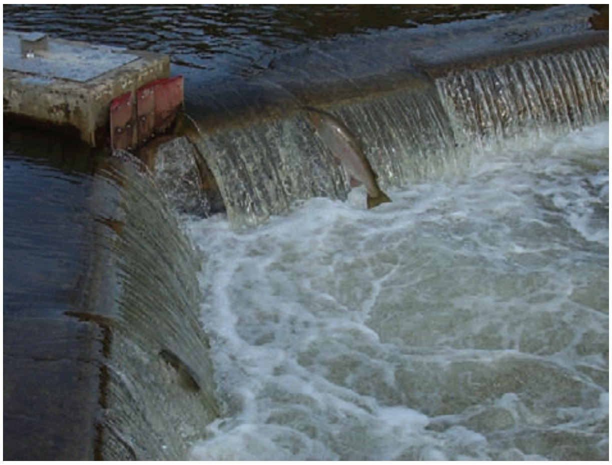


Figure 3 Upstream-migrating rainbow trout (*O. mykiss*) attempting to surmount a lowhead dam located approximately 500 m upstream of Lake Ontario in Shelter Valley Creek (Grafton, ON, Canada). Photo courtesy of Thomas Binder.

it is typically selective in the species that it passes, and those fish that do navigate the fishway usually incur high costs, in terms of both time and energy (**see also Hagfishes and Lamprey**: Lampreys: Energetics and Development and Lampreys: Environmental Physiology). In addition, barriers can drastically alter the physical and chemical characteristics of a stream, and, thus, may reduce the abundance and quality of suitable stream habitat.

Toxicants and other chemical contaminants can also directly impact the migratory success of fishes. Contaminants may mask the odors that some fishes use to identify the home stream (**see also Toxicology**: The Effects of Toxicants on Olfaction in Fishes). Moreover, some toxicants cause physical damage to the sensory cells that mediate orientation. For instance, the olfactory and lateral line organs are particularly susceptible to heavy metals. Contaminants can also interfere with metabolism. Many toxicants have been shown to reduce swimming performance in fishes by limiting oxygen uptake and shuttling energy away from the swimming muscles. This increased metabolic load can also lead to premature mortality.

The final anthropogenic impact we will discuss is climate change. It is clear that the Earth is undergoing a relatively rapid change in its temperature regime and this will have an impact on migration success. First, higher temperatures, especially in temperate regions, are reducing the abundance of suitable habitat for many species, limiting the range over which migration can occur. Second, populations whose migrations are synchronized by photoperiod, rather than temperature, will experience higher temperatures throughout their migration. These higher temperatures will put greater metabolic demands on stored energy reserves and increase disease prevalence, resulting in a greater incidence of en-route migration mortality. Indeed, there is already evidence of this occurring in populations of Pacific salmon (**see also Fish Migrations**: Pacific Salmon Migration: Completing the Cycle). Third, higher temperatures could impact the reproductive success of spawning phase migrants. Embryonic and larval development is exquisitely sensitive to temperature. Even slight deviations from optimal temperatures can have drastic effects on development, including physical deformations and a reduction in the proportion of embryos that develop into juveniles. There is a paucity of information available on the long-term effects of small-increment increases in temperature on fishes, so it is difficult to predict to what extent individual species will be able to adapt their migration strategies to the new temperature regime. It appears, however, that species that undergo long-distance migrations may be most at risk (**see also Temperature**: Effects of Climate Change).

See also: **Behavioral Responses to the Environment**: Temperature Preference: Behavioral Responses to Temperature in Fishes. **Fish Migrations**: Eel Migrations; Pacific Salmon Migration: Completing the Cycle; Tracking Oceanic Fish. **Hagfishes and Lamprey**: Lampreys: Energetics and Development; Lampreys: Environmental Physiology. **Hearing and Lateral Line**: Lateral Line Neuroethology. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction. **Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish; Magnetic Sense in Fishes; Spatial Orientation: How do Fish Find their Way Around?. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance. **Temperature**: Effects of Climate Change. **Toxicology**: The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Dingle H and Drake VA (2007) What is migration? *BioScience* 57: 113–121.

Dittman AH and Quinn TP (1996) Homing in Pacific salmon: Mechanisms and ecological basis. *Journal of Experimental Biology* 199: 83–91.

Døving KB and Stabell OB (2003) Trails in open waters: Sensory cues in salmon migration. In: Collin SP and Marshall NJ (eds.) *Sensory Processing in Aquatic Environments*, pp. 39–52. New York: Springer.

Glebe BD and Leggett WC (1981) Temporal, intra-population differences in energy allocation and use by American shad (*Alosa sapidissima*) during the spawning migration. *Canadian Journal of Fisheries and Aquatic Science* 38: 795–805.

Gross MR (1987) Evolution of diadromy in fishes. *American Fisheries Society Symposium* 1: 14–25.

Harden Jones FR (1968) *Fish Migration*. London: Edward Arnold.

Hasler AD, Horrall RM, Wisby WJ, and Braemer W (1958) Sun-orientation and homing in fishes. *Limnology and Oceanography* 3: 355–361.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP (2006) Behavioural physiology of fish migrations: Salmon as a model approach. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Fish Physiology, Volume 24: Behaviour and Physiology of Fish*, pp. 239–295. New York: Academic Press.

Jonsson N (1991) Influence of water flow, water temperature and light on fish migration in rivers. *Nordic Journal of Freshwater Research* 66: 20–35.

Lucas MC and Baras E (2001) *Migration of Freshwater Fishes*. Oxford: Blackwell Science.

Leggett WC (1977) The ecology of fish migrations. *Annual Review of Ecology and Systematics* 8: 285–308.

McCleave JD, Arnold GP, Dodson JJ, and Neill WH (1984) *Mechanisms of Migration in Fishes*. New York: Premium.

McDowall RM (1988) *Diadromy in Fishes: Migrations between Freshwater and Marine Environments*. Portland, OR: Timber Press.

McKeown BA (1984) *Fish Migration*. Portland, OR: Timber Press.

Tracking Oceanic Fish

BA Block, Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Acoustic Tags
Early Satellite Transmitters
Further Reading

Glossary

Acoustic tag A telemetry device that transmits data as a series of sounds (called pings) to a receiver.

Archival tag A telemetry device that records animal and environmental data on the device and then must be retrieved from the animal to get the data.

Chat tag An acoustic tag that stores the data and then sends it to an underwater receiver; it is only useful if the animal stays or returns to a site where the receiver is located.

Pop-up satellite tag A telemetry device that records animal and environmental data and then is released from the animal to pop up to the surface and send its data to a satellite, where it is then downloaded to scientists.

Satellite tags A telemetry device that sends data to Earth orbiting satellites.

Wildlife telemetry A special application of telemetry that records or sends data regarding an animal, that is, in its natural environment to allow remote monitoring and tracking of the animal.

Introduction

The pelagic ocean is one of the largest biospheres on the Earth, yet the patterns of species distribution, diversity, and abundance remain enigmatic. Intensive exploitation has led to dramatic changes in the structure of pelagic ecosystems due to the systematic removal of numerous species. The movements of top marine predators, such as tunas, billfishes and sharks, and hot spots (i.e., areas that attract large aggregations of one or more species), have been challenging to document due to the vast expanse of the oceanic environment and the difficulty of following animals in this undersea world. The physical and technological challenges of following large, fast, mobile marine predators such as tunas and sharks have been limited until recently. Consequently, the mechanisms linking physical forcing, zooplankton production, prey abundance and distribution, and top predator physiology, behavior, population structure and ecology remain elusive due to a lack of appropriate tools and approaches to investigate these processes. The influence of climate variability on the distribution of top predators is poorly documented and will require biological observations that span decades.

The tracking of marine fishes presents one of the most challenging problems in wildlife telemetry. Continuous tracking of water breathers submerged beneath the surface of the sea requires new techniques. Until recently, most knowledge of how large pelagic fishes use marine environs was derived from acoustic tracking tag technology. Pioneers in this research were Frank Carey at the Woods Hole Oceanographic Institute and AB Stasko at the Biological Station in St. Andrews, Canada as well as Fred Voegli of the University of New Brunswick, Canada. Acoustic tracking tags provide detailed information about vertical and horizontal movements. However, most tracks only provided a glimpse lasting several days of the routine behaviors or physiology of a fish because the scientist had to follow the animal to stay within range of the tag (i.e., up to 3 km) to detect and record the acoustic signal. The tracking of oceanic animals with an acoustic tag to learn behavior was labor intensive and required shipboard applications. More recently, the electronic tagging community, or biologging scientists, has applied increasingly sophisticated acoustic, satellite, and archival tracking techniques that have rapidly increased the data acquisition on horizontal and vertical movements, thermal physiology and oceanographic preferences.

In the past decade, there has been a rapid increase in the use of satellite-based tracking tags to study marine animals (**Figure 1**). The tags work most reliably on animals that breathe air (e.g., sea turtles, seals, and whales) since they regularly come to the surface. When the tag body is exposed, it can transmit to an Earth orbiting ARGOS satellite. The ARGOS system uses transmitters or position tracking terminals (PTTs) that send short radio signals at a frequency of about 401.650 mHz to polar orbiting satellites.

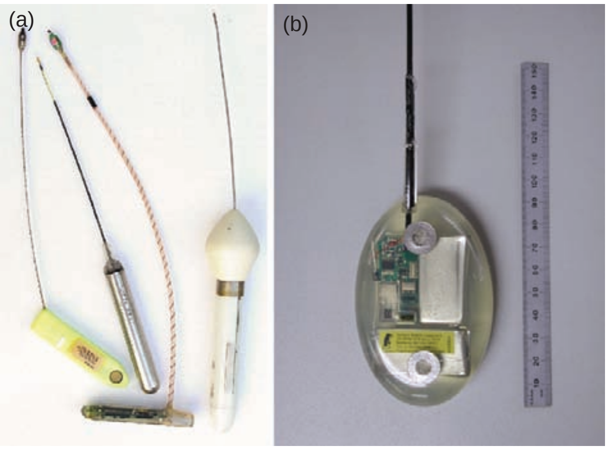


Figure 1 Electronic tags used for telemetry. (a) Left to right are archival tags that record data on light, temperature, and pressure and a pop-up satellite archival tag. (b) Single position only satellite tag.

The location of the transmitter on the animal is calculated using the Doppler shift in the frequency of transmissions received by the satellite as it makes a single pass over the tracking subject. By using this methodology, vertebrate animals have been tracked for thousands of kilometers. In the marine realm, the diving behavior of animals limits the use of the ARGOS system, especially when tracking water-breathing animals. By necessity, fishes and sharks are submerged in the water, making transmission of radio signals challenging. However, significant improvement in tag design and transmission capabilities specialized for fishes, including direct telemetry from towed tags or tags attached to shark dorsal fins as well as pop-up satellite archival tags, have made satellite tracking a powerful tool in the twenty-first century.

Despite these obvious challenges in the past two decades, the advancement of satellite tag technology for tracking fish has elucidated new information about the distributions, long-distance migrations, and population structure of marine fishes that typically have high dispersal rates (tunas, billfish, and sharks). Rapid advancement in satellite archival and direct uplinking tags has helped to unravel the migratory movements, diving behaviors and thermal preferences of a wide range of teleosts and elasmobranchs in an unprecedented decade of tagging advancements. In recent years, the capacity to keep satellite tags attached for longer periods (1–4 years) has led to the recording of philopatry behavior, and has helped to identify key foraging hot spots, as well as breeding and parturition areas. By archiving diving behaviors, the vertical habitat utilization of numerous marine pelagic species has been rapidly elucidated (**Figure 2**). These

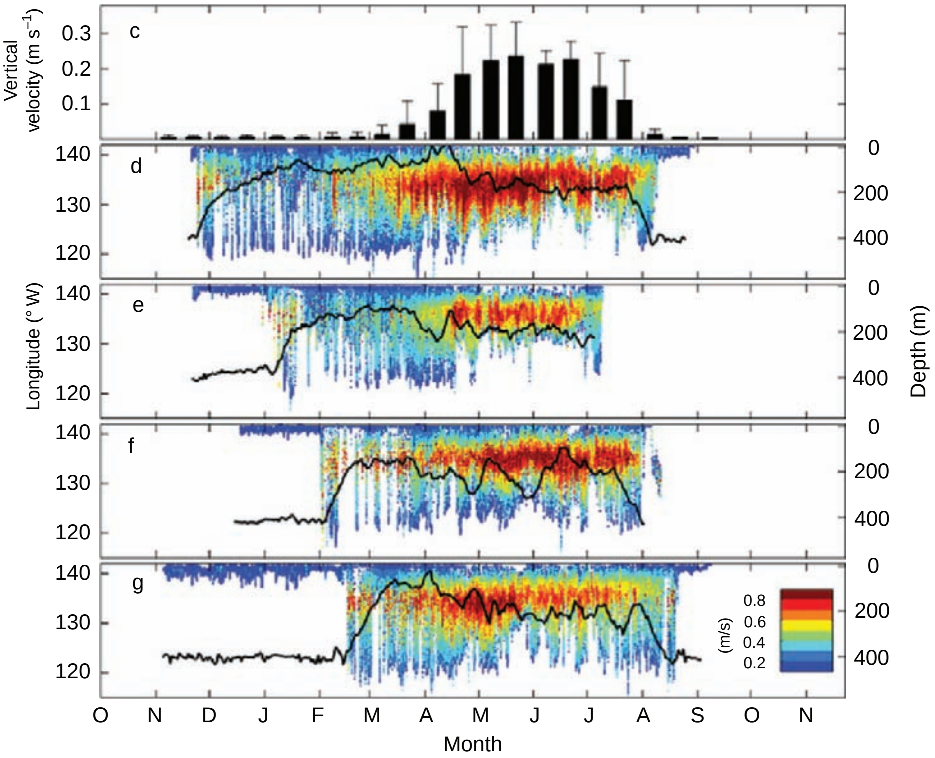


Figure 2 Extraordinary oscillatory diving by white sharks in the "White Shark Café," an offshore region half way between North American and Hawaii, revealed by using pop-up satellite archival tags attached to individuals. From Jorgensen SJ, Reeb CA, Chapple TK, *et al.* (2009) Site fidelity, homing and population structure in eastern pacific white sharks. *Proceedings of the Royal Society of London B*, doi:10:1098/rspb.2009.1155.

advancements, in combination with evolving genetic tools, are providing the opportunity for examining population structure in marine pelagic ecosystems which has rapidly advanced the prospects for conservation in the pelagic realm.

Recent developments in telemetry and biologging technologies also have greatly enhanced our ability to enlist apex marine predators to sample the marine environment, measuring ambient water temperature and chlorophyll concentration. These ‘animal oceanographers’ identify critical foraging habitat, migration corridors, and regions of high occupancy in global oceans. Enlisting animals is also relatively inexpensive compared to oceanographic research vessels and provides coverage in unique places such as under the ice at the poles (elephant seals) and in trans-oceanic corridors.

Acoustic Tags

Acoustic tags permit collecting data (most often individual identification codes, depth or temperature) from a single fish equipped with an ultrasonic tag that generates sound by driving an annular ceramic transducer at its resonant frequency. As electronic circuitry controlling tag function has been miniaturized, first with surface-mount and then with integrated circuit technology, tags have become small enough so that salmon fry can carry these tags. The constraints of transducer size and battery volume remain when large range or extended transmission life is required. Used in combination with real-time measurements of the physical and biological environment by the tracking vessel, acoustic tags have become powerful tools in examining environmental preferences of tuna. The tags encode the data as a series of ultrasonic pulses transmitted through the water column to a hydrophone and receiver on the ship. The use of multi-directional hydrophone arrays coupled with independent acoustic receivers and computers has improved estimation of direction and range of fish relative to the tracking vessel.

‘Listening stations’ are an exciting new acoustic technology. They are acoustic receivers that are moored at set locations and are able to communicate with, and log details of, tags that come within close range (250–500 m) over extended periods of time. Extensive tracking with listening stations is possible and is being used extensively with salmonids to study movements in riverine systems as well as enclosed embayments to examine migratory behavior and residency of fish. Acoustic listening stations installed at hot spots have also been used to track the movements of great white sharks (**Figure 3**).

The success of listening stations has spawned the development of ‘chat tags’, which combine many of the features of data storage, or archival tags (see below), with

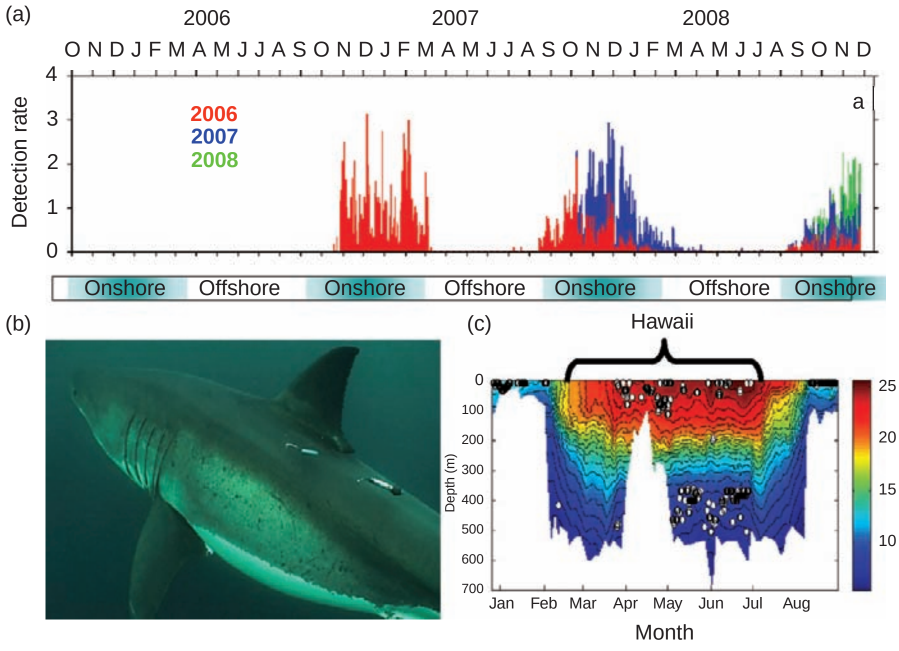


Figure 3 (a) Acoustic tags attached externally to white sharks provide presence and absence data in the vicinity of receivers placed in central California. Tags provide information on occurrence and residency. (b) A large white shark carrying two types of telemetry, an acoustic tag (forward) and pop-up satellite archival tag. (c) Pop-up satellite archival tags store data on depth and temperature that can be used to reconstruct the behavior of the shark during the period it carried the tag. In this example, the shark swam from California coastal waters to the waters off Hawaii. From Jorgensen SJ, Reeb CA, Chapple TK, *et al.* (2009) Site fidelity, homing and population structure in eastern pacific white sharks. *Proceedings of the Royal Society of London B*, doi:10:1098/rspb.2009.1155.

acoustic transmission of data when a fish comes close enough to a receiver. The receiver used with chat tags regularly send signals out to search for and identify tags within its range. Having received a positive response from a fish/tag, it then sends a command enabling the tag to download data via an acoustic signal. Chat tags have great potential in areas where fish are likely to return regularly to topographical or aggregating features. Mobile predators carrying receivers and chat tags yield the promise of a wired ocean community where predators and prey listen and detect one another. Importantly, listening stations and chat tags can record signals from multiple species allowing broad collaborations among scientists to cover the costs of the receivers.

Early Satellite Transmitters

Dr. Monty Priede made the first successful tracking of a gill-breathing animal using the ARGOS satellite system on a basking shark (*Cetorhinus maximus*) in 1984. The shark was tracked using a tethered tag over a period of 17 days. The basking shark was equipped with a ultrahigh frequency (UHF) radio transmitter and followed off the west coast of Scotland. The animal surfaced frequently and displayed rather localized movements. Simultaneous imagery from NOAA 7 AVHRR allowed for integration of the movements with sea-surface temperature data. The shark was following a frontal area.

Towed satellite tags were deployed in 1998 and 1999 to monitor the paths and habitat utilization of whale sharks *Rhincodon typus*, off South Africa (Indian Ocean) and in the waters off Honduras (Caribbean Sea). A Telonics satellite transmitter was packaged in a cylindrical flotation unit, and the tether employed a 1.5–7 m piece of airplane wire. Five whale sharks were tagged in this fashion by Scott Eckert, and diving profiles as well as horizontal movements were obtained. Animals were tagged by divers using tag anchors placed in the skin or musculature of the shark. Tethered tags provide real-time data about the habitat use and diving profiles of whale sharks, and may be recovered if they detach prematurely from the host animals. The whale sharks dove regularly to depths of >320 m, which may have contributed to premature detachment of the tags due to drag, and in later units the hydrodynamics of the tag were refined. Sharks tagged off the coast of KwaZulu-Natal, South Africa, traveled northwards. One shark tagged off the coast of Utila Bay Islands, Honduras, traveled to the Swan Islands, then moved along the Yucatan Peninsula and into the Mexico Basin, whereas the second shark tagged off Utila traveled to the coast of Belize. This study confirmed that tethered satellite tags are effective tools in monitoring travel paths and habitat use of whale sharks when real-time data are needed.

Towed tags were also placed on blue marlin (*Makaira nigricans*) by Drs. Heidi Dewar and Barbara A. Block in 1994. This package consisted of a Telonics transmitter that was placed in a streamlined tow body and was attached to the marlin with a 3–4 m tether made of airplane wire. This attachment and tag succeeded, providing several hits of low quality from marlin that traveled offshore from the point of tagging and release off Kona, Hawaii. However, overall few hits were obtained, and thus there was little ability to track the animals. After eight deployments using this tag configuration, no further attempts were made with this technology, and the scientists made a commitment to develop a detachable satellite tag.

PAT Pop-Up Satellite Archival Transmitting Tags

Pop-up satellite (tags often abbreviated with PSAT or PAT) were first placed on Atlantic bluefin tuna (*Thunnus thynnus*) in January of 1997. This novel tag technology allowed for a mobile predator to be tracked beneath the sea with the tag storing data on light, temperature, and pressure. The tag's shape and flotation were initially designed so the tag would be towed off the tapered portion of the bluefin's ventral region. At a preprogrammed time, an electrical impulse burned the wire that attached the dart to the tag, and the tag released from the fish to transmit its data. Original tags provided an endpoint and temperature data. As the tag evolved, a more sophisticated algorithm was developed that enabled position estimations that were based on light level-derived geolocation and sea-surface temperature-enhanced measurements. Because of the limitation of data transmission by satellite due to battery requirements, the logged data are compressed prior to transmission. The temperature and depth data are synthesized in the form of histograms and temperature depth profiles.

The PSAT or PAT tag has been extremely successful with thousands of deployments on tunas, sharks, turtles, and even marine mammals. The tags are unraveling many of the journeys of once secretive animals as they course thousands of kilometers under the sea. They are limited currently to about a year maximum deployment, primarily due to the challenges of keeping the tags on the animal.

Satellite Tags on Fishes and Sharks

The first directly attached fin tag was developed by Dr. Francis G. Carey, who designed a fixed position, dorsal fin-mounted, satellite tag that was placed on blue sharks (*Prionace glauca*) in 1993 in collaboration with the Sea Mammal Research Unit of the UK. The experiment was highly successful with high-quality locations obtained from sharks surfacing regularly enough to track the sharks along the Gulf Stream. The tag was robust and straddled the dorsal fin. The tags measured pressure (and thus calculated depth), speed, and position on a half dozen sharks in the North Atlantic. The longest track was over 2 months.

Direct telemetry off the dorsal fin of sharks provides an opportunity to enhance the capability of near real time tracking, data collection, and ocean observation. Most recently, this technique was applied to five species of sharks in the Tagging of Pacific Pelagics program (TOPP). Salmon sharks (*Lamna ditropis*) are large in body size and surface regularly due to behavioral thermoregulation in cold subpolar seas, making it easy to attach satellite tags. Tracks of over 3 years are routine on this shark, and makos have been followed for over 2 years.

Salmon sharks are apex predators in Alaskan ecosystems, yet little is known about their movements or population dynamics in Alaskan waters. Tagging through TOPP has been critical for documenting the extensive oceanic migrations of salmon sharks and enabled the characterization of their habitat use on oceanic scales (**Figure 4**). Tagging has demonstrated that salmon sharks utilize the inshore and nearshore waters of Prince William Sound, Cook Inlet, the Alaskan Peninsula, and southeastern Alaska shelf habitat throughout the year.

The same techniques utilized for studying the salmon shark were rapidly integrated with multiple species within the TOPP program. To date, over 10 species of sharks have been tagged with the single position only transmitting or SPOT tags with extremely successful tracking in species such as shortfin mako sharks (*Isurus oxyrinchus*), blue sharks and juvenile white sharks (*Carcharodon carcharias*). have also been tracked with limited success (Galapagos sharks *Carcharhinus galapagensis*, silky sharks *C. falciformis* and oceanic whitetip sharks *C. longimanus*).

Most teleost fish do not have appendages that break the surface of the water and can serve as a fixed attachment point for a tag as is possible with a shark's first dorsal fin. However, recently Holdsworth and colleagues obtained high-resolution satellite locations from striped marlin (*Tetrapturus audax*) using Argos transmitters attached to the upper lobe of the caudal fin. Twenty-six striped marlin were tagged off New Zealand (2005–07) and tracked as far as the central Pacific Ocean. Remarkably, the caudal fin mounted Argos tags generated

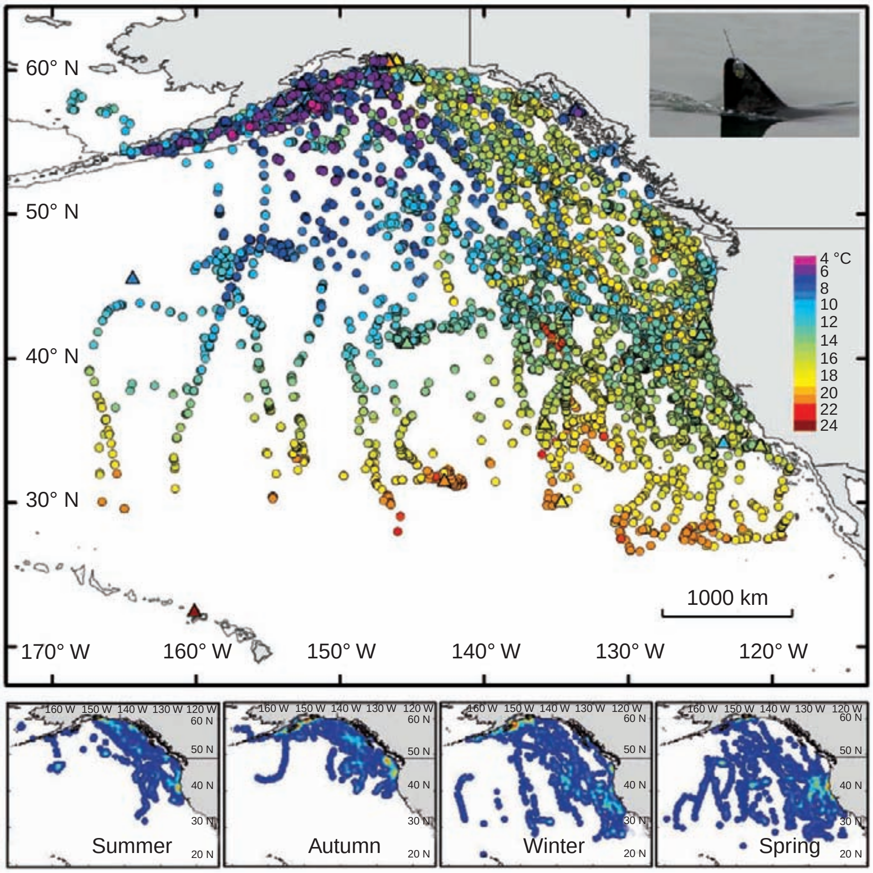


Figure 4 Single Position Only tags (SPOT) provide positions of salmon sharks, *Lamna ditropis*, carrying the tags. Sharks were tagged in Alaskan waters while foraging. Sea-surface temperature data experienced by the sharks are shown in color. From Weng KC, Castilho PC, Morrissette J, *et al.* (2005) Satellite tagging and cardiac physiology reveal niche expansion in salmon sharks *Science* 310: 104–106.

a significant number of high accurate positions of ±1 km or better. The caudal fin attachment methodology and antenna configuration were adjusted each season to improve transmission life and data quality, with the best results obtained in the last year of deployments (2007). The longest track duration was 102 days, with a total displacement of 4959 km and a total track distance from all locations received of 6850 km. Tag shedding and antenna failure appear to have limited the duration of SPOT tracks on fish. The high temporal and spatial resolution data revealed behaviors not previously observed in striped marlin, including associations to sub-surface bathymetric features. High-resolution location data will be valuable for generating robust behavioral state-space and habitat selection models.

Archival Tags

Archival tags are miniature computers, incorporating microprocessors, memory, and a suite of environmental and physiological sensors. They are low power tags, allowing data to be collected over many years (**Figure 5**). Specifications vary among manufacturers; however, the most advanced tags have light sensors capable of measuring variation in moonlight at depths of 100 m, 125 Mb or more of flash (nonvolatile) RAM memory, depth sensors providing accuracy of <1 m over a range of 1000 m, and thermistors capable of measuring temperature to within 0.1 °C over a range of 40 °C. They use 16-bit microprocessor technology and allow data to be collected from three to four sensors every few minutes for periods of up to 5 years (**Figure 6**). The nonvolatile memory allows data to be retrieved even after the tag's battery has run down.

Perhaps the most critical feature of an archival tag is the collection of data that can be used in estimating the position of the tagged animal. Researchers have used a variety of algorithms that combine the light measurements, water temperature, and depth data collected by archival tags to enhance the estimation of position. Estimating position from variation in light levels involves a suite of concepts and algorithms developed by Roger Hill augmented by sea-surface temperature enhancements by several authors including Steve Teo and B. A. Block (**Figure 7**). If fish remained on the surface and did not migrate long distances in short periods, estimating their position from archival tag data would be relatively straightforward. Unfortunately, most fish tend to make very rapid dives, often at sunrise and sunset – the times most critical for estimating latitude – and all are capable of rapid movement over large geographic scales. Thus, when using archival tags to estimate the position of tunas, we can expect systematic and random errors resulting from: equinoxes, light attenuation, water clarity, weather patterns, precision of astronomical algorithms, clock errors, resolution of light sensor, and behavior of the animal. Ingenuity has allowed scientist to overcome these difficulties, and using archival tags Atlantic bluefin have been tracked for as long as 5 years and 3-year tracks now exist in the Pacific Ocean (**Figure 8**). The capacity to record internal body temperature (tags are surgically placed in the peritoneal cavity), in addition to position and environmental variables, allows records of foraging, behavior, and environmental preferences. The duration of track and suite of data that can be obtained positions this type of tag as one of the most powerful in unraveling the mysteries of fish biology.

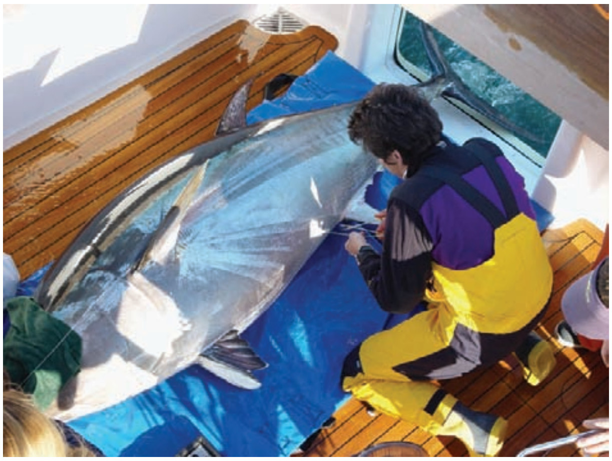


Figure 5 The author implanting an archival tag surgically in a giant bluefin, *Thunnus thynnus* off Nantucket, Massachusetts.

Multi-Species Tagging

The Census of Marine Life (COML) has enabled two large electronic tagging programs on the west coast of North America, Tagging of Pacific Pelagics (TOPP) and Pacific Ocean Shelf Tracking (POST). The two programs have shown the capacity to track multiple species simultaneously – putting tags into the peritoneal cavity or attaching them externally to track marine animals including salmon smolts, rockfishes, tunas, sharks, sturgeon, cetaceans, pinnipeds, sea turtles and squid over long distances and for multiple years, as they move along the North American continental shelf. TOPP and POST currently operate two of the largest undersea data acquisition networks, inclusive of ~4000 extant mobile tag platforms associated with predators that use the North American continental shelf and Pacific Ocean realms. TOPP has tested global positioning system (GPS) and conductivity, temperature, depth (CTD) tags, and has developed new algorithms for sampling the ocean environment (e.g., estimating *in situ* chlorophyll from tag measurements of light attenuation with depth). The programs have collected millions of ocean observations from COML animals, and maintain 300 permanent acoustic

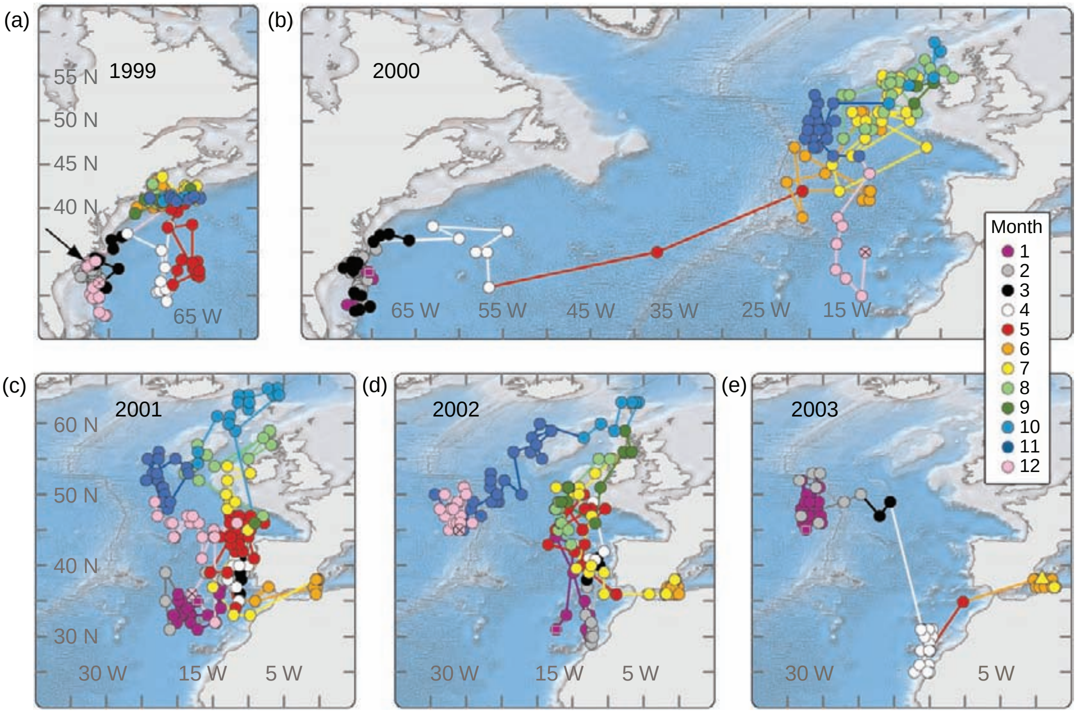


Figure 6 A 5-year record of a single bluefin tuna released with an archival tag off the coastal waters of North Carolina. Each lettered panel (a–e) shows a year in the life of the bluefin tuna and colors indicate positions in a monthly scheme. From Block BA, Teo S, Walli A, *et al.* (2005) Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* 434: 1121–1127.

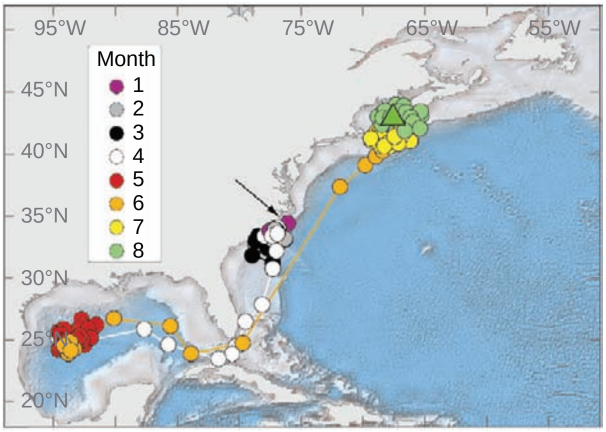


Figure 7 A single year record of a bluefin tuna, *Thunnus thynnus* making a breeding migration from the western North Atlantic into the Gulf of Mexico. Geolocation by light level and sea-surface temperature provides daily positions (colored circles). From Block BA, Teo S, Walli A, *et al.* (2005) Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* 434: 1121–1127.

receivers deployed in cross-shelf listening lines, island listening stations, or estuary and river basins.

TOPP identifies the spatial distribution of multiple species in an ecological context. To date, TOPP researchers have mapped common migration corridors for sharks, albatross, and tunas across the Pacific, and located multi-species hot spots for species utilizing the eastern Pacific. TOPP data have quickly revealed the seasonal and interannual variations in the patterns of movements of pelagic species throughout the North Pacific. Understanding the environmental cues underlying these patterns of habitat utilization is requisite for predicting patterns of predator distributions, diversity, and population structure.

The integration of the biological and oceanographic data sets generated from the electronic tagged animals elucidates how marine predators use physical features in oceanic ecosystems (i.e., mesoscale eddies and convergence zones). Tag data also allow TOPP scientists to observe behavioral responses of apex predators to climate-driven processes and have helped identify transitory hot spots in a dynamic ocean environment.

Animals as Ocean Sensors

Animals profile the water column as they move and provide *in situ* data much as man-made probes such as CTDs are capable of doing. Electronic tagging has the capacity to deliver ocean observations from undersea networks of animals in the open sea. The integration of tag-collected animal tracking data with environmental data from satellites and *in situ* oceanography has enabled scientists to study marine predator

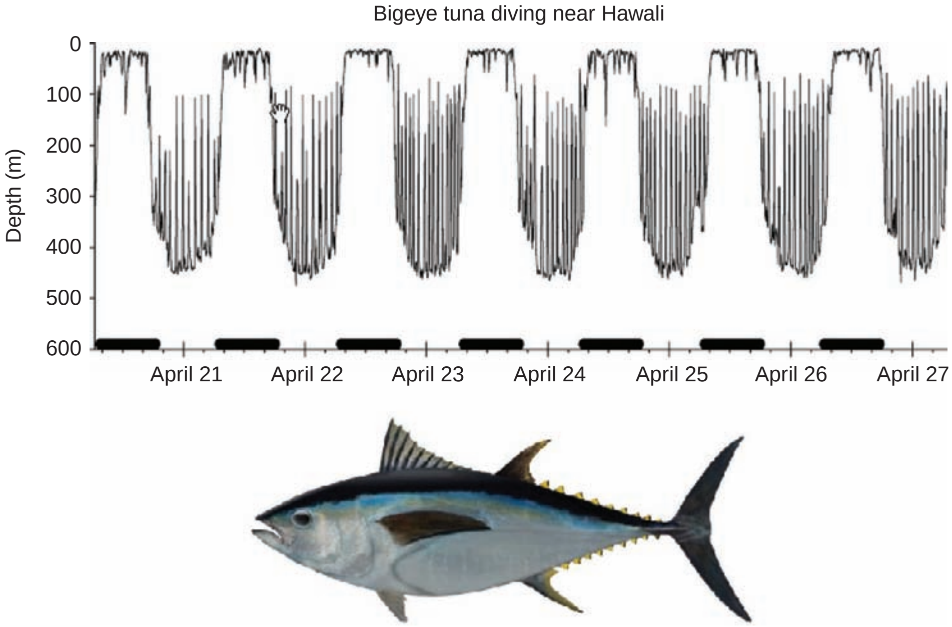


Figure 8 The diel behavior of a bigeye tuna revealed with an implantable archival tag in the waters off Hawaii.

habitat utilization, chart biological hot spots and movement corridors, and identify key areas and processes that concentrate coastal and pelagic fishes, sea turtles, marine mammals, and seabirds (**Figure 9**). These data on abundance and distribution of animals in a variable ocean environment are essential for designing ecosystem-based management plans in coastal and shelf habitats.

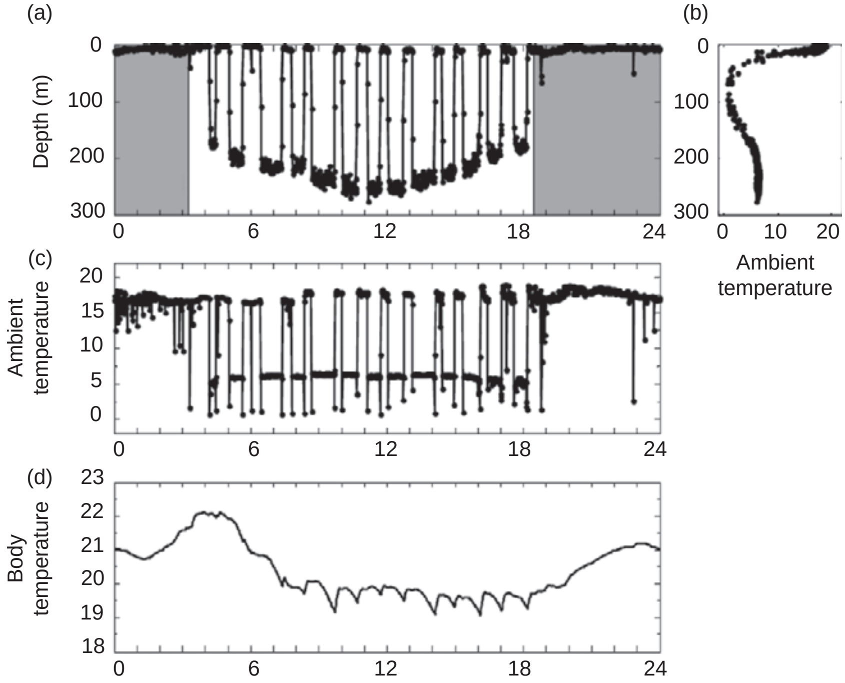


Figure 9 A day in the life of an Atlantic bluefin tuna, *Thunnus thynnus,* diving in a frigid sea. Panels (a–d) reveal various aspects of the bluefin's biology and thermoregulatory capacity with an internally placed archival tag. (a) Bounce diving during a foraging bout; (b) depth and temperature profile of the water column revealing extraordinary conditions of a cold inversion; (c) ambient temperatures; and (d) body temperatures. Lawson GL, Castleton MC, and Block BA (2010) Movements and diving behavior of Atlantic bluefin tuna *Thunnus thynnus* in relation to water column structure in the northwestern Atlantic. *Marine Ecology Progress Series* 400: 245–265.

Further Reading

Block BA, Teo S, Walli A, *et al.* (2005) Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* 434: 1121–1127.

Block BA, Dewar H, Williams T, *et al.* (1998) A new satellite technology for tracking the movements of Atlantic bluefin tuna. *Proceedings of the National Academy* 95: 9384–9389.

Boustany AM, Matteson R, Castleton M, Farwell C, and Block BA (2010) Movements of Pacific bluefin tuna (*Thunnus orientalis)* in the eastern North Pacific revealed with archival tags. *Progress in Oceanography* 86: 94–104.

Carey FG and Lawson KD (1973) Temperature regulation in free-swimming bluefin tuna. *Comparative Biochemistry and Physiology A* 44: 375–392.

Gunn J and Block BA (2001) Advances in acoustic, archival and satellite telemetry. In: Block BA and Stevens ED (eds.) *Tuna: Physiology, Ecology and Evolution*, pp. 167–224. Oxford: Academic Press.

Hill RD (1994) Theory of geolocation by light levels. In: LeBoeuf BJ (ed.) *Elephant Seals,*, pp. 227–236. Los Angeles, CA: California Press.

Holdsworth J, Sippel T, and Block BA (2008) Near real time satellite tracking of striped marlin (*Kajikia audax)* movements in the Pacific Ocean. *Marine Biology,* doi: 10.1007/s00227-008-1104.

Jorgensen SJ, Reeb CA, Chapple TK, *et al.* (2009) Site fidelity, homing and population structure in eastern pacific white sharks. *Proceedings of the Royal Society of London B,* doi:10:1098/rspb.2009.1155.

Priede IG (1984) A basking shark (*Cetorhinus maximus).* tracked by satellite together with remote sensing. *Fisheries Research* 2: 201–216.

Stasko AB and Pincock dG (1977) Review of underwater biotelemetry, with emphasis on ulatrsonic techniques. *Journal of the Fisheries Research Board of Canada* 34: 1261–1285.

Teo S, Weng K, Boustany A, Walli A, and Block BA (2004) Validation of geolocation on archival tags: Light level estimations versus ARGOS. *Marine Ecological Progress Series* 283: 81–98.

Teo S, Boustany A, and Block BA (2007) Environmental correlates of breeding biology of Atlantic bluefin tuna, *Thunnus thynnus*, on their Gulf of Mexico breeding grounds. *Marine Biology* 152: 1105–1119.

Weng KC, Castilho PC, Morrissette J, *et al.* (2005) Satellite tagging and cardiac physiology reveal niche expansion in salmon sharks. *Science* 310: 104–106.

Relevant Websites

www.topp.org – Tagging of Pacific Pelagics program (TOPP).

www.gtopp.org – Global Tagging of Pelagic Predators (GTOPP).

www.tagagiant.org –Tag-A-Giant Foundation.

Eel Migrations

DA Righton and JD Metcalfe, Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

Crown Copyright © 2011 Published by Elsevier Inc. All rights reserved.

Introduction
Larval Migration: The Early Years
Migration from the Coastal Shelf: Life as a Glass Eel
Life in freshwater
The Downstream Migration: Back to the Sea
The Spawning Migration: Into the Big Blue
Concluding Thoughts
Further Reading

Glossary

Catadromous A fish born in the sea that moves into freshwater to grow and mature, before returning to the marine environment to spawn.

Conspecifics Other individuals of the same species.

Leptocephalus The leaf-like larva of an eel.

Neuromasts The sensory cells of the lateral line system in fish responsible for detecting movement.

Osmoregulation The physiological process of regulating the water content of bodily fluids and tissues.

Otoliths Small naturally formed stones found in the inner ear of most teleost fish.

Semelparous An organism that reproduces only once and then dies.

Introduction

The migration of European eels (*Anguilla anguilla*) to and from their spawning grounds in the subtropical, west Atlantic Ocean is one of the most remarkable feats of animal migration and also one of the most mysterious. Ever since the great Greek philosopher and scientist Aristotle first mused that eels “arose from the bowels of the earth” in the fourth century BC, generation after generation of biologists and oceanographers have become fascinated by the unique and, in many ways, exceptional life history of eels.

European eels, like all other anguillid eels, have a catadromous life, that is, they are born in marine waters, migrate to freshwater to grow and mature, before they return to the marine environment and migrate (typically 5000–7000 km, up to 9800 km from the furthest point in the eastern Mediterranean, **Figure 1**), spawn, and start a new generation. Like Pacific salmon and some other fish species, European eels are semelparous, that is, they only spawn once and then die.

To successfully complete their life cycle, eels undergo a number of transformations as they grow from egg to reproductively mature adult, and each stage involves its own adaptations that maximize the chances of successfully migrating to a new environment or location and reaching the next stage (**Figure 2**). In the following sections, we describe the main features of the migration of each of the stage of the European eel.

Larval Migration: The Early Years

Although the capture of eel larvae (called leptocephali, meaning leaf like, **Figure 3**) in the Mediterranean in the mid-nineteenth century led to the early belief that eels spawn there, the location of European eel spawning grounds is now known to be the Sargasso Sea, an area of over 1 million square kilometers located between 20–30° N and 48–79° W (**Figure 1**). This fact was established in the 1920s, when the Danish oceanographer Johannes Schmidt published his classic accounts of his surveys for the larvae of eels. He showed that the larvae of eels occurred at increasing size as the distance from the Sargasso Sea increased. In addition, the very smallest larvae, those that could be considered to be newly hatched from eggs, were only found in an area centered at 26° N, 60° W (**Figure 1**), predominantly during the months of April and May. The center of this bull’s-eye showed where the spawning grounds of the European eel must be. Schmidt also discovered that the earliest larvae of the American eel (*Anguilla rostrata*), as well as the European eel, were found in the same area in the Sargasso Sea, albeit slightly earlier in the year. More recent surveys have shown that American eel larvae are concentrated slightly further to the west than European eels. The location of the spawning sites poses the question of how European and American eel leptocephali migrate to their continental habitats up to 9000 km away (in the extreme case of European eels in the Mediterranean;

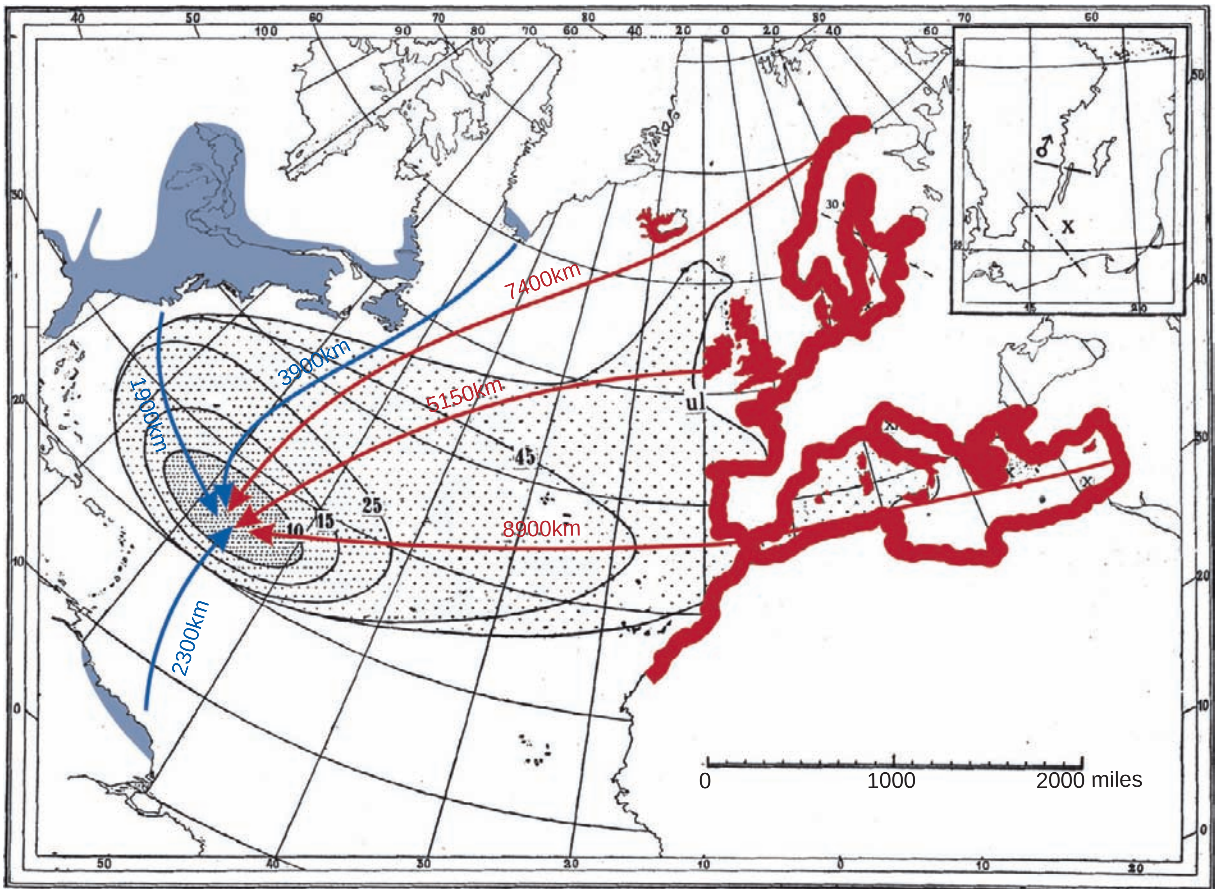


Figure 1 European eel. Spawning places (bounded by the innermost – 10 mm – curve); larval distribution (dotted area) and adult distribution (black strip) along the coasts of Europe and north Africa. The curves show the limits of occurrence; i.e., larvae less the 15 mm have only been found inside the '15' curve, larvae less the 25 mm have only been found inside the '25' curve, etc. The outermost curve (ul) denotes the limit of unmetamorphosed larvae. For the Baltic, '♂' and '30 cm' denote the limit of male eels and of eels less than 30 cm, respectively. 'X' denotes eastern-most limit of unpigmented elvers (Baltic and Mediterranean). The red arrows (added by the present authors) indicate the approximate migration distances for silver eels from northern Norway, western Ireland, and the eastern Mediterranean. Modified from Fig. 1 in Schmidt J (1923) Breeding places and migrations of the eel. *Nature* 111: 51–54.

Figure 1), as well as the transport mechanism that sends American eels west, and European eels to the east.

While there are still many details left to resolve, analysis of the ocean currents of the north Atlantic has shown that the leptocephali are transported principally by the Gulf Stream and North Atlantic Drift (**Figure 4**) to the European continental shelf over a period of between 1 and 3 years. The leaf-like shape of the leptocephali is thought to increase the effectiveness of this drift, but they are not just passive travelers on the ocean currents. Based on the fact that leptocephali spend much of their time in depths between 50 and 300 m, and therefore significantly deeper than the wind-driven surface currents, it is likely that orientation and directed swimming are also important factors in getting the larval eels to their destination. In fact, behavior is a significant factor that accounts for the separation of the paths of European and American eels; the American eels, which grow faster than their European cousins, are able to supplement the drift they receive from the Gulf Stream and arrive at the continental shelf on the eastern seaboard of the USA within 1 year of hatching. By contrast, the journey of the European eel larvae is much less clear, and is the subject of considerable debate. Based on modeling studies, as well as examination of the structure of otoliths (**Figure 5**), the leptocephali are thought to arrive at European coasts between 6 months and 3 years after hatching. This would put their travel speed somewhere between 10 and 50 km day^{-1}, based on a larval migration distance of 8500 km. How much of the migration distance is achieved via passive drift and active swimming is unknown. A 5-cm leptocephalus swimming at 2–3 body lengths per second would cover 8–13 km day^{-1}, and thereby considerably reduce the larval journey time by drift alone.

More recently, the location of the spawning area of the Japanese eel (*Anguilla japonica*) has been identified in the north-west Pacific Ocean in an area between the Philippines and the Mariana Islands. This discovery suggests a similar role for the North Equatorial and

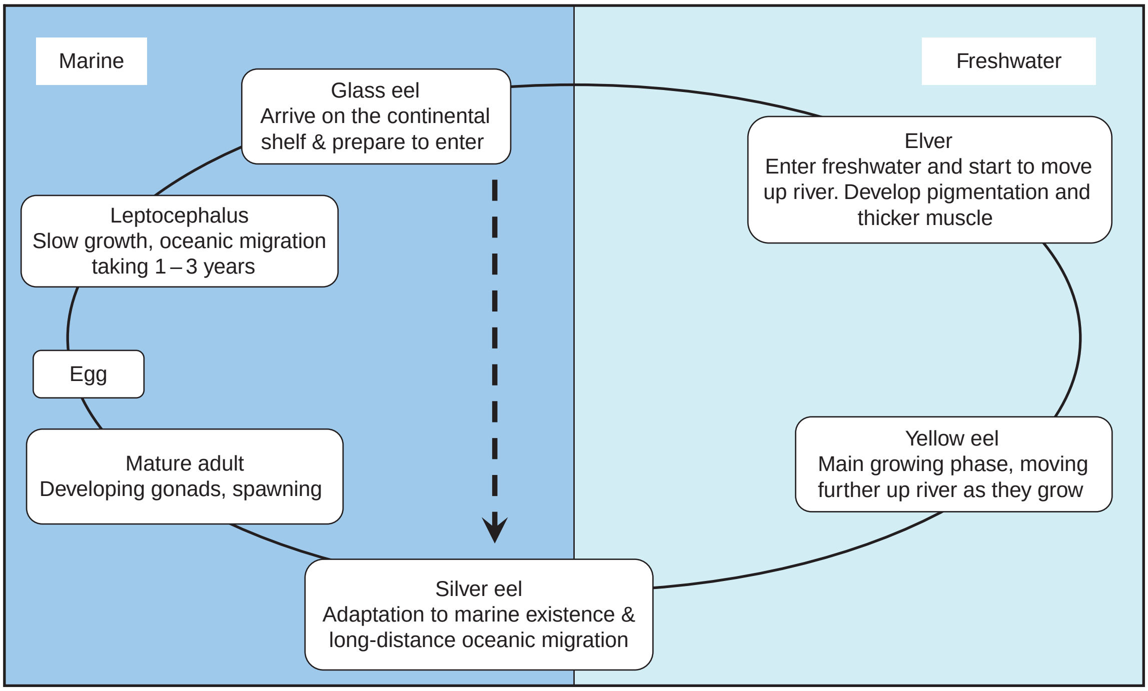

Marine
Freshwater
Glass eel
Arrive on the continental shelf & prepare to enter
Elver
Enter freshwater and start to move up river. Develop pigmentation and thicker muscle
Leptocephalus
Slow growth, oceanic migration taking 1 – 3 years
Egg
Mature adult
Developing gonads, spawning
Yellow eel
Main growing phase, moving further up river as they grow
Silver eel
Adaptation to marine existence & long-distance oceanic migration

Figure 2 The stages in the lifecycle of the European eel.

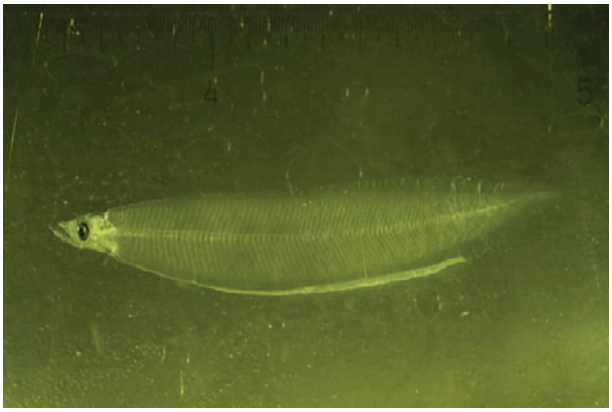


Figure 3 An eel larva (leptocephalus).

Kuroshio Currents (**Figure 4**) as a means of transporting leptocephali of this species to the coastal waters of eastern Asia, where the adults originate. Thus, the role of ocean currents in the movements and dispersal of the larvae of many eel species would appear to be a fundamental component of their life histories.

Migration from the Coastal Shelf: Life as a Glass Eel

It is estimated that much less than 1% of eel larvae successfully reach the European coast, and the arrival of leptocephali at the edge of the European continental shelf (typically defined as the 1000 m contour at the western edge of Europe) between September and February marks the end of this first and clearly perilous phase of life and the start of the next. Here, the leptocephali undertake their first metamorphosis into glass eels, so called because they are almost completely transparent.

Glass eels gather together in large shoals and migrate into shallower water and toward the coast. It is here that humans first detect them, usually in November, first at the southern edge of their distribution in Portugal (~40° N), which is the closest point to the spawning grounds.

Leptocephali have to travel a lot further to reach the more distant shelf edge further North, so monitoring stations start to report the arrival of glass eels at different times in France (January–March), and southern England and Ireland (February–April). Leptocephali that pass through the Straits of Gibraltar and into the Mediterranean, or that travel to the north of the UK toward Norway (April–May), are the last to reach shallow water, so they can only metamorphose and begin their inshore migration in June. As a result, the age of a cohort of glass eels varies a lot across Europe, being youngest in the south, and oldest to the north and east. Investigations into the age of glass eels using otoliths are still inconclusive, and put glass eels at between 6 months and 3 years old at first arrival.

The metamorphosis from leptocephalus to glass eel results in the development of a number of physiological and behavioral traits that enable the glass eels to find suitable growth habitat. Importantly, glass eels are able to identify the direction of the coast through a sensitivity to salinity and differences in odor between fresh and marine

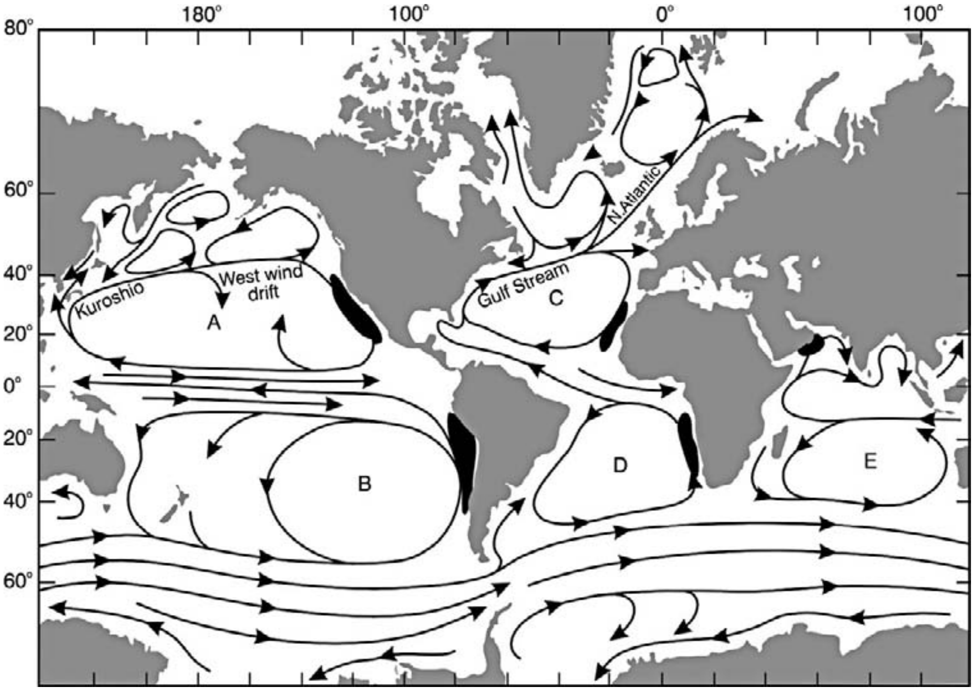


Figure 4 A simplified chart of the surface ocean currents of the world oceans. Main subtropical gyrals: A, North Pacific; B, South Pacific; C. North Atlantic; D, South Atlantic; E, South Indian. Important areas of upwelling in the eastern boundary currents (California, Peru, the Canary, and Benguela) and off Arabia are indicated in black.

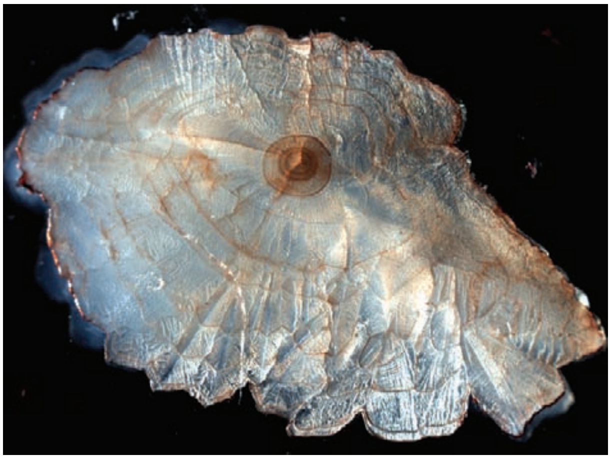


Figure 5 An eel otolith, stained to show the annual deposition of growth rings. Photo by Anders Asp, supplied by Håkan Wickstrom, Swedish Board of Fisheries.

waters. This ability is coupled with other behaviors that allow them to reach the coasts quickly and with minimal risk of predation. First, glass eels move from open water to the seabed, reducing the risk of predation. This also provides them with the opportunity to refuge from currents that would take them back out to sea. Glass eels also reduce their activity during the day, when predation risk is at its greatest. As they journey toward freshwater, glass eels need to be capable of tolerating water of gradually diminishing salt content. In adult eels, this is achieved in the gills (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts), which appear to be capable switching between excreting excess salts in seawater and extracting them in freshwater. It is not known, however, whether the same mechanisms operate in glass eels. Nonetheless, it is evident that these behaviors and sensitivities provide them with the means to locate the mouths of estuaries and reduce the risk of being eaten, while at the same time preadapting them for life in freshwater. So it is that, by converging toward freshwater sources, glass eels gather together in the company of thousands upon thousands of their conspecifics, and enter the lower reaches of rivers and begin their transition to the freshwater environment. This feature of their behavior is exploited by glass eel fisheries in most European countries of the Atlantic Arc, which scoop up the eels by the million for the restaurant or aquaculture trade. In recent years, however, catches of glass eels have declined dramatically, leading many to the conclusion that the sustainability of eel stocks in Europe is threatened.

Recently, evidence has emerged that all eels are not necessarily catadromous. A significant proportion of glass eels do not enter freshwater, but instead remain in the marine environment for their entire lives. This discovery was made by analyzing otoliths, or ear stones, of adult silver eels caught at sea. These structures are present in all bony fish, and grow by the incremental deposition of layers of aragonite (a crystalline form of calcium carbonate), which also traps trace elements present in the water in which the fish live. Studies in Japan showed that the otoliths of some eels did not contain the chemical signature typical of freshwater at all, only that of seawater. This discovery has since been repeated for other eel species, including the European eel, and demonstrates that the lifecycle and biology of eels is even more flexible and complex than previously thought. However, at the present time, while we know of their existence, almost nothing else is known about the biology or the marine eels behavior.

Life in freshwater

By the end of the glass eel phase, a new generation (cohort) of eels will have redistributed from the Sargasso Sea to most of the river systems in Europe. In fact, European eels are found in all European rivers draining to the Mediterranean, the North and Baltic Seas, and those rivers that drain to the Atlantic as far south as the Canary Islands. Eels can also be found in some rivers in Northern Africa and Asia that drain to the Mediterranean. Where eels are not found naturally, such as northern Scandinavia, eastern Europe, and even land-locked countries such as Austria, glass eels have often been translocated or stocked to rivers and lakes to provide a fishery for adult eels.

Once glass eels have entered freshwater and migrate up-river into their new habitat, and at about 6–7 cm in length, they undergo another metamorphosis and become elvers. By now, they are feeding on whatever suitably sized prey they can find, such as small invertebrates and this results in the development of pigmentation and of thicker bands of muscle. The goal of the elvers is to reach a habitat suitable for the growing phase of their lives. Thus, elvers migrate further and further from the estuaries that they entered, through the further development of the behavioral traits that enabled them to find their growth habitat. Hence, elvers continue to swim against the flow of the river, but with the tide (if within the tidal reach), restricting their activity to the hours of darkness. In fact, the compulsion to migrate against the flow is very strong and there are many stories of eels crossing barriers to their progress, such as man-made vertical drops or across fields, that would seem to be insurmountable to less able or determined fish. That they can do so relies in part upon this compulsion, and also in the fact that eels are able to absorb oxygen through their skin, thereby enabling them to remain out of water for long periods. During this time, the gills collapse, but then recover their normal form when the eel re-enters water. This feature of their physiology also comes in useful in the latter stage of their lives, when the compulsion to migrate down-river to the sea is equally strong.

The elver phase lasts until the complete freshwater form is attained, usually called the yellow eel, (but sometimes the green or brown eel) at approximately 30 cm in length.

Yellow eels can be found in almost all freshwater habitats, and generally avoid strong currents, moving little. Tracking studies, using implanted acoustic or radio tags, have shown that eels in freshwater may range no more than a few tens or hundreds of meters per day, within a limited home range of, at most, a few kilometers. (Eels living in marine waters seem to have similar habits: local, rather than extensive, movements.) They retain this habit as they age, but tend to relocate their home range further up-river. In consequence, larger eels are usually found further from the sea, and at a lower density. They may remain in freshwater for many years: the oldest European eel ever captured in freshwater was estimated to be >90 years old and the largest ever captured was 147 cm and several kilograms in weight. In general, however, they complete their growth phase within 20 years or less, and at 35–65 cm for males and 50 to >130 cm for females. The factors that trigger the transition from the growth phase to the maturation and marine migration phases are not well known, because the range of size and age at which yellow eels enter the silver eel phase is so wide.

The Downstream Migration: Back to the Sea

Despite their potential longevity and large size, eels in freshwater should not really be considered to be adults in the biological sense because their sexual organs only develop fully once their spawning migration is underway. While this is an unusual strategy, the capacity to have a long growth phase (if necessary) provides the flexibility for eels to attain the size and condition required for them to return successfully to the Sargasso Sea and have a chance to spawn. With this flexibility, eel populations are probably more resilient to changes in the productivity in their growth habitat than would otherwise be the case.

However, just attaining the right size and body condition is not enough, because before they can migrate downstream and escape to the sea to spawn, eels must undertake their second major metamorphosis. This process is called silvering, so called because eels change color from yellow (or brown/green) to silver (actually, the belly becomes silvery-white while the sides and back become a dark bronze color). However, despite its name, silvering is not just a superficial change in color (**see also The Skin**: Coloration and Chromatophores in Fishes), but a fundamental suite of physiological and morphological changes that preadapts eels to the next stage of their life: the oceanic spawning migration.

The counter-shading coloration that eels adopt is typical of oceanic pelagic fish, and reduces the risk of detection by predators, but there are several other major adaptations.

Perhaps the most obvious external change is the enlargement of the eyes that, along with an increase in the number of rods on the retina (**see also Vision**: Extraretinal Photoreception), adapt eels to seeing even at very low light levels. Other morphological changes include the development of thicker skin (and more mucus to protect it), a darkening of the pectoral fins, and the appearance along the lateral line of neuromasts, which increase the sensitivity of the eel to movements in the water around them. These morphological changes, while distinct, are nothing compared to the dramatic changes to the physiology and structure that are taking place internally.

Most importantly, the fat content of eels increases considerably during the silvering process, and reaches between 25% and 30% of body mass, about 2–3 times that of farmed salmon. This energy store is fundamental for the long migration ahead. Sufficient fat stores are required because eels, once silver, do not feed and the gut, or alimentary canal, ceases to function in digestion. Without sufficient fat, eels may be unable to complete the migration at all or they may not be able to produce enough good quality eggs (or sperm) at the end of their journey to the spawning grounds. Without a digestive function, the gut decreases in length and its walls become thinner, and its only remaining function is to absorb water and help maintain the osmoregulatory balance (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts).

Secondly, the specialized organ that controls buoyancy, the swimbladder (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes), also becomes more vascularized, and its walls become thicker. These adaptations enable better gas secretion and retention, so that any required changes in buoyancy, while in the ocean, can be achieved quickly and efficiently.

Finally, eels must adjust their ionoregulatory and osmoergulatory processes for a life in seawater rather than freshwater. Teleost fish regulate the osmotic concentration of their body fluids at about 30–40% of the level of oceanic seawater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). Fish in freshwater, therefore, must deal with the osmotic entry of water and diffusional loss of salts via the gills and gut. To compensate, they produce large volumes of dilute urine. Marine fish have the opposite problem of osmotic loss of water and diffusional entry of salts. As a consequence marine fish drink seawater and excrete excess salts. While most fish species are adapted to live only in one environment or the other (stenohaline) and will die if transferred, a few species are euryhaline and able to move between seawater and freshwater. Some euryhaline species (e.g., salmon) have to make substantial physiological adaptations to survive such a change of environment and are only able to move between seawater and freshwater at specific points in their life cycle. However, eels (such as flounder) are able to move into seawater at any time, despite their longevity in freshwater habitats. This ability to move between seawater and freshwater relies on the capabilities of the organs that regulate water balance (the gills, gut, and kidney) and the tissues (principally the adrenal gland) that produce the hormones that control them.

Clearly, these changes are as considerable as they are necessary. In some ways, silvering can be likened to puberty because, at its heart, silvering transforms eels from their adolescent yellow eel form to full adulthood. Like puberty, silvering involves a series of complex hormonal changes that are only complete once the eel reaches full sexual maturity. However, it is not yet clear what factor or factors trigger silvering because the process is quite gradual and intermediate stages can be identified. Silver eels can be all sizes and ages, but generally tend to be smaller and younger to the south, and males are smaller and younger than females. Indeed, the exact definition of a silver eel is still, after many decades of study, somewhat elusive. However, there is now some evidence that somatic growth plays at least a part in the silvering process. Eels at the migratory stage show a higher growth rate than resident eels, regardless of their origin, and somatotropin (a natural animal hormone that stimulates growth and cell production) has been found to be highest in premigrant eels. There is also evidence that the cortisol (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids) is important in the initiation of migration. This hormone may play a part in the mobilization of energy stores in preparation for the long journey ahead and is also thought to be involved in increasing Na^+, K^+-ATPase activity (an important osmoregulatory enzyme found in the chloride cells of the gill epithelium).

Nonetheless, it is clear that for an eel to begin its spawning migration, it must at least be partially silvered and therefore preadapted to marine life. Once so, eels are then able to undertake the first stage of the spawning migration: escapement to the sea. Similarly to the silvering process, the cues that eels use to choose when to start their downstream migration appear to vary and are dependent upon a number of different factors. Whatever the cause, each autumn (August through December), hordes of eels start to migrate downstream and escape to the sea. In this process, it appears that decreasing (rather than absolute) temperature and shortening day length are key seasonal factors that trigger the onset of migration.

The escapement occurs at night, usually during the period of the new moon, when light is at a minimum, and typically during periods of high rainfall. Progress of eels downstream can be rapid, with eels moving many kilometers each night, but their migration may also be interrupted by natural obstacles, such as pools or dead-ends, or man-made blockages, such as weirs or hydropower stations. In consequence, and because there are only 3 or 4 days of each phase of the moon when eels will migrate, it may take eels up to 3 months (or perhaps more) to travel downstream to the sea. The further away from the Sargasso Sea that the eels reside, the earlier that the downstream migration occurs, a phenomenon assumed to ensure that the arrival of eels from different locations at the Sargasso Sea is synchronized. Thus, most eels leave Norwegian and Swedish rivers as early as August, whereas the peak season for eel escapement in Ireland and France is October through December. However, should the eel get the timing wrong, or the eel get trapped on the downstream migration, all is not lost. Until the eels escape to the sea, and become fully marine, the changes in morphology and physiology caused by silvering are reversible, and provide another chance at escapement in later years.

The Spawning Migration: Into the Big Blue

On leaving their freshwater homes, ahead is a 6-month journey of up to 9000 km or more, across an ocean that reaches over 5000 m in depth. Depending on the location and month of departure, this requires an average swimming speed of between 0.3 and 0.6 m s^{-1}. Although this may not sound too much of an effort, consider that silver eels vary in size from 35 cm (the smallest males) to 1.3 m (the largest females). Thus, the smallest males (0.3 m long) will be required to swim at least 1 body length per second, on average, for 6 months – no mean feat. The largest females will have to swim at up to 0.5 body length per second for 4 months. Even though the largest females do not need to move proportionately as fast as males, with a cross-sectional area far greater than the smallest males, they will have to expend more than 8 times as much energy to overcome the drag as they move through the water.

The journey ahead is long however, and the immediate challenge to eels when they leave their home rivers is to navigate through the relatively shallow waters (generally <200 m) of the coastal shelf to reach the open Atlantic Ocean. The difficulty of this task varies with the starting location. From countries that border the Atlantic, such as France, Ireland, or Norway, the journey is relatively simple. From countries bordering the Mediterranean or Baltic Seas, the journey is more arduous and requires the negotiation of one or more passages (e.g., the Gibraltar or Dover Straits), which are likely to slow the progress and increase the duration of the coastal phase of the spawning migration.

The coastal phase of the spawning migration was initially studied through the use of mark-recapture experiments using simple tags. The recapture, usually by fishermen, of tagged eels at a point distant from their original release, can reveal the direction and routes of migration and provide some ideas of the speed at which migration progresses. Generally, eels migrate in coastal waters in a fairly directed way. For example, eels tagged on the eastern Swedish coast in the Baltic Sea migrate west at a speed of ~20 km day^{-1} toward the junction between the Baltic Sea and the Kattegat (the gateway to the North Sea). Recent use of either acoustic tags that send a signal to a tracking vessel, or electronic tags that record data and are recovered through eel fisheries have increased the sophistication of such studies, adding speed and directional data that can reveal behaviors. What they show is that eels have a diel vertical migration: they move into deeper water, or to the seabed, in the day, and into shallow water at night. The purpose of these movements is unclear, but may relate to predator avoidance (by hiding on the seabed during the day) and/or navigation (searching for currents or migratory cues). It is unrelated to feeding activity, however, because silver eels lose their ability to digest food once they have metamorphosed.

Much less is known about the behavior of eels once they leave coastal waters and move into deeper, oceanic waters, for several reasons. First, the migration routes of European eels are unknown, and as a consequence all attempts to capture eels in the ocean have failed. This includes several concerted attempts to capture eels at their spawning grounds in the Sargasso Sea. Determining the migration routes (and depths) therefore requires either following eels from the time that they escape from rivers, or capturing silver eels in rivers and transporting them to the ocean before tagging and releasing them. However, because the practical difficulties of following eels for extended periods are so great, these types of studies have succeeded only in recording the migration for relatively short periods of time. In the first case, studies using acoustic tags have tracked eels for up to several days and over distances of a few hundred kilometers, while studies using electronic tags have succeeded for recording data for up to 71 days (in the Baltic Sea), but in neither of these cases did the eels enter open oceanic waters. In the second case, the pioneering translocation studies of Freidrich W Tesch in the late 1970s and early 1980s stand out. Tesch caught silver eels in the Baltic and then transported them aboard ship to various locations, including the Mediterranean and Sargasso Sea, before implanting acoustic tags and releasing them again. The data showed that eels could dive to depths >700 m and travel at speeds of up to 0.8 m s^{-1}. As for eels in coastal waters, diel vertical migrations were a notable feature of the behavior. Despite these valuable results, empirical evidence on the oceanic spawning migration of European eels remains elusive. More recently, the development of small satellite communicating electronic tags has revived hope that the spawning migration can be recorded. Successful application of the technology to the long-finned eel (*Anguilla dieffenbaachi* L.) has now been repeated for the European eel, and the preliminary results, while falling short of revealing the spawning grounds, suggest that it may not be too long before the full migration is described.

While empirical data on migratory behavior may currently be lacking, a considerable body of evidence has been collected on the capability of European eels to undertake lengthy migrations. Chief among these have been swim tunnel experiments that have revealed the endurance and efficiency of long-distance swimming. The experiments, undertaken at the University of Leiden in Belgium, showed that female eels have an optimal speed of approximately 0.6 m s^{-1} and that they are capable of continuous swimming at 0.5 m s^{-1} for at least 173 days (which would allow them to cover 5500 km). This achievement resulted in a surprisingly small weight loss – eels of ~900 g lost only 60 g of fat This in part can be attributed to the fact that eels have a mode of swimming that is between 2 and 4 times as efficient as that of other species of teleost fish such as salmon. Another remarkable feature of the results was that the eels used up muscle reserves of carbohydrate and protein at the same rate as fat. This not only ensures that the buoyancy of the eel tissue remains unchanged but also maximizes the reserves of fat left at the end of the migration for conversion into the next generation of eels.

Concluding Thoughts

The lifecycle of the European eel has fascinated scientists, fishermen, and natural historians for many centuries. While many aspects of their behavior and biology during their freshwater phase have been elucidated, our understanding of the marine migrations, both as larvae and subsequently maturing adults, continues largely to remain a mystery. However, concern over the substantial decline in eel recruitment of the past two decades has rekindled interest in eel biology and, with the help of new tagging technologies, science is now on the brink to solving one of the last great mysteries in natural history. We can only hope that, when combined with other knowledge of the impacts and threats eel face during the freshwater phase of their lives, this new understanding will allow us to formulate and implement conservation measures that will ensure their continued survival.

The migration is also a risky endeavor, and yet European eels have survived through ice ages and changes to ocean circulation patterns. However, current trends in eel stocks and eel recruitment point to a serious decline in numbers over the last 30 years or so. The new threats and cause of the decline have not been established and is likely to be the accumulated effects of a number of possible factors that include: overfishing, loss of habitat, climate change, barriers to migration (upstream or downstream), disease (viruses and parasites), and industrial pollution. Of these, the latter four are particularly pertinent to eel migration, each in a different way. Climate change, for example, is likely to affect eels at all stages of their lifecycle, by increasing development rates of eggs and larvae (potentially increasing starvation mortality) and by modifying the direction and speed of the oceanic currents that help to transport leptocephali. Similarly, industrial, man-made pollutants such as polychlorinated biphenyls (PCBs) are very resistant to degradation and accumulate easily in the body fat, which is very high in silver eels. PCBs through their endocrine effect and by reducing the resistance to infectious agents could easily compromise adult migration and the survival and development of eggs and larvae.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts. **The Skin**: Coloration and Chromatophores in Fishes. **Vision**: Extraretinal Photoreception.

Further Reading

Aarestrup K, Økland F, Hansen MM, *et al.* (2009) First empirical results on the oceanic spawning migration of the European eel (*Anguilla anguilla). Science* 30: 1660.

Bertin L (1956) *Eels, a Biological Study*. London: Cleaver-Hume.

Fort T (2003) *The Book of Eels*. London: Harper Collins.

Jones FRH (1968) *Fish Migration*. London: Edward Arnold.

Kettle AJ, Bakker DCE, and Haines K (2008) Impact of the North Atlantic Oscillation on the trans-Atlantic migrations of the European eel (*Anguilla anguilla*). *Journal of Geophysical Research* 113(G3): C03004.1–C03004.26.

Kettle AJ and Haines K (2006) How does the European eel (*Anguilla anguilla)* retain its population structure during its larval migration across the North Atlantic Ocean? *Canadian Journal of Fisheries and Aquatic Sciences* 63: 90–106.

Kettle AJ, Heinrich D, Barrett JH, Benecke N, and Locker A (2008) Past distributions of the European freshwater eel from archaeological and palaeontological evidence. *Quaternary Science Reviews* 27: 1309–1334.

Schmidt J (1923) Breeding places and migrations of the eel. *Nature* 111: 51–54.

Tesch F-W (2003) *The Eel*, 5th edn. Oxford: Blackwell Science.

Tsukamota K (1992) Discovery of the spawning area for Japanese eel. *Nature* 356: 789–791.

van den Thillart G, Dufour S, and Rankin CJ (eds.) (2009) *Spawning Migration of the European Eel*. New York: Springer.

van Ginneken VJT and Maes GE (2005). The Euroean eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: A literature review. *Reviews in Fish Biology and Fisheries* 15: 367–398.

Westerberg H, Lagenfelt I, and Svedäng H (2007) Silver eel migration behaviour in the Baltic. *ICES Journal of Marine Science* 64: 1457–1462.

Relevant Websites

http://www.eeliad.com – Eeliad.

http://www.ices.dk – International Council for the Exploration of the Sea (ICES), Eel Stocks Dangerously Close to Collapse.

http://www.iucnredlist.org – The IUCN Red List of Threatened Species, *Anguilla anguilla.*

Pacific Salmon Migration: Completing the Cycle

SJ Cooke, Carleton University, Ottawa, ON, Canada

GT Crossin, Simon Fraser University, Burnaby, BC, Canada

SG Hinch, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Larval Developmental Migration
Migration to Larval Feeding Grounds
Smolting and Migration to Sea
Migration to Ocean Feeding Grounds
Directed Migration to the Coast and Natal River
Adult Upriver Migration to Natal Stream
Anthropogenic Threats Involving Salmon Migrations
Conclusions
Further Reading

Glossary

Anadromy Life-history strategy entailing reproduction and early rearing in freshwater followed by a significant growth phase in seawater.

Natal homing The phenomenon whereby animals return to the site where they were born to reproduce themselves.

Osmoregulation The maintenance of consistent cellular or organismal fluid composition and volume.

Pacific salmon Fish of the genus *Oncorhynchus* in the family Salmonidae.

Semelparity A reproductive strategy whereby an individual reproduces only once within its lifetime.

Smoltification Series of physiological and morphological changes a fish may undergo when migrating from freshwater to seawater.

Introduction

Pacific salmon (*Oncorhynchus* spp.) are well known for their large-scale migrations that can take them thousands of of kilometers from inland freshwater systems, where they begin their lives, to rich and fertile ocean habitats for feeding. Ultimately, they return to their natal stream to reproduce, before this lifecycle is repeated by their offspring (see **Figure 1**).

The migratory strategy of reproducing in freshwater, yet feeding primarily in marine waters is known as anadromy. Of the ~35 000 species of fish, ~2.5% are known to undertake migrations, and of that small percentage only 16% participate in anadromous migrations. Unlike Atlantic salmon (*Salmo salar*), Pacific salmon are semelparous, meaning that they die after spawning (**Figure 2**). The dead adults provide energy and nutrients for stream ecosystems, which benefit their own offspring after they hatch and begin to feed independently. This means that a fish that fails to mature and reach spawning grounds and reproduce will have zero fitness, contributing no genetic information to future generations. Indeed, not all fish that hatch return to spawn. Typical success rates are less than 1% for alevins. Even during the upriver migration phase, significant mortality can occur (e.g., up to 90% mortality from river entry to spawning grounds for some stocks in some years).

As described in **Fish Migrations**: The Biology of Fish Migration, migration represents a complex interplay of behavior and physiology. This interplay is perhaps best exemplified by the Pacific salmon (genus *Oncorhynchus*) given the many natural challenges that these fish encounter during migration including predators, dynamic river flows and ocean currents, diseases, parasites, variable temperatures, and dramatically variable salinities. In addition, migrating salmon face additional anthropogenic challenges, such as fisheries exploitation, habitat alteration, and physical barriers (e.g., dams, climate change, and other ecosystem alterations).

There are three aspects of Pacific salmon migration that make them particularly remarkable. The first is their navigational ability, which enables them to migrate from the high seas to the exact steams where they themselves were spawned. Such natal spawning fidelity is rare among fish and among other animals. Even so, fidelity for home stream does vary among Pacific salmon species. Sockeye salmon (*O. nerka*), for example, tend to exhibit low rates of straying (<1%) whereas pink salmon (*O. gorbuscha*) tend to show less preference for natal sites.

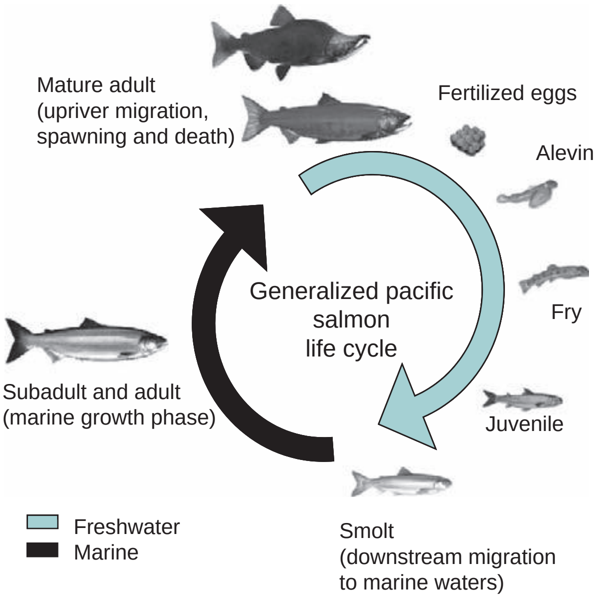


Figure 1 Generalized life cycle for Pacific salmonids.

Figure 2 Dead sockeye salmon on spawning grounds. After reproduction, most Pacific salmonids die, a characteristic of semelparity. The carcasses provide nutrients for the developing offspring as well a wide variety of plant and animal life (e.g., bears, bald eagles).

The second remarkable aspect is their ability to transition from freshwater to saltwater during their seaward migration in a process known as smoltification. When they return as adults, they reverse the process, transitioning from saltwater to freshwater. Many fish species would die when exposed to such a challenge, yet this is the norm for Pacific salmon, despite the need to remodel gill functions as well as their entire osmoregulatory apparatus (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

The third remarkable feature is the homeward river migrations of spawning adult salmon. Before they enter the river, they stop feeding and then complete a freshwater migration, sometimes in excess of 1000 km, using stored body energy, principally fat. Not only is this body fat used to fuel the entire spawning migration, but the energy must also be in sufficient quantities to support the maturation process: development of secondary sexual characteristics, the production of sperm and eggs (**see also The Reproductive Organs and Processes**: Vitellogenesis in Fishes and Regulation of Spermatogenesis), and the act of reproduction itself. Given these challenges, it is remarkable that any individual Pacific salmon is successful. Yet, every year, millions of Pacific salmon continue returning to natal spawning grounds throughout the north Pacific rim, extending from Japan and Russia in the western Pacific, northwards to Alaska, the Yukon, British Columbia, and south into Washington, Oregon, and California. Pacific salmon, however, are not found south of the equator except as an introduced or cultured species.

Although the most remarkable and notable migrations are the large-scale seaward and spawning migrations, Pacific salmon make their first migrations within hours of hatching! To fill their swimbladders for the first time, they must migrate from the gravel, where they are hatched to the water's surface to take this first gulp of air, otherwise they die. Other small-scale migrations include the day–night vertical migrations taken by juvenile salmon in rivers, lakes, and coastal seawater, usually for foraging and predator evasion. There are many other examples of specific small- and large-scale migrations made by Pacific salmon, but because there is immense variation within and among different species, we will generalize the various migratory phases of salmon and focus on what we will term the 'generic Pacific salmon'. By so doing, we will describe seven principal migratory phases that occur throughout the lifetime of a generic salmon.

Given that the vast amount of research that has focused on the downstream smoltification migration and the upriver spawning migration, we provide an in-depth description of these two particular phases. Just because the bulk of research efforts have been directed toward the larger-scale and relatively conspicuous migrations such as upriver migrations, this does not mean that the smaller, less conspicuous migrations are not important. All migratory phases of salmon are important for the survival of the species. Indeed, for an animal with only one life-time opportunity to reproduce, all migratory phases could perhaps be considered essential, irrespective of scale. Furthermore, sockeye, coho (*O. kisutch*), Chinook (*O. tschawytscha*) and pink salmon are the most well-studied salmon, particularly with respect to migration and physiology. However, other Pacific salmon including chum and masu salmon, as well as the Pacific salmonid steelhead (henceforth regarded as a Pacific salmon), make the same migrations during their lifetime and should not be overlooked. Although we have structured this article around the migratory life history of a generic Pacific salmon, we will provide examples and data from all species where available.

Larval Developmental Migration

The fertilized eggs of all Pacific salmon (including steelhead) hatch into young alevin in the gravel and rocky substrates where the mother laid them. These small salmon fuel early development using endogenous energy stored in their yolk sac. As swimming muscles develop and the yolk sac becomes nearly depleted, the alevin must forage. However, the first step is to obtain neutral buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes) and to do so they must migrate from the safety of the gravel to the surface to fill their swimbladder. From the moment this first migration begins, the fish are exposed to a variety of threats including swift water currents and various predators.

Although the timing of this migration is critical, researchers know little about the specific environmental cues or physiological processes that mediate the initiation of this first migration. The endocrine system, and in particular the thyroid hormones, appear to be associated with photic sensitivity, which may serve as a proximate control for the timing of migration; alevins typically attempt the migration at night rather than day to minimize exposure to predators. Gas exchange occurs almost exclusively at the skin surface rather than at the gills during this phase of life (**see also Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions), facilitated by dense capillary networks surrounding the yolks sac and a high surface area to body mass ration. The primary role of the gills at this phase, before they eventually are used for gas exchange, is for ionoregulation (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction).

Migration to Larval Feeding Grounds

River spawning is, by far, the most common strategy among salmonids. Alevins of many species of Pacific salmon then migrate to a nursery area (the river itself or a nursery lake) by either passive drifting or sometimes volitional swimming. During this period, they are termed fry. Sockeye salmon more typically migrate to a nursery lake, unless they were spawned in a lake.

The motivation for migration by sockeye fry to a lake-nursery environment is to take advantage of the growth potential offered by the productive lake environment, but getting there successfully from the natal stream requires important behavioral decisions to minimize energy use and avoid predators while migrating. Several physiological and behavioral adaptations facilitate this process, which vary depending upon whether the young fry move downstream or upstream to reach the nursery lake (i.e., moving with or against the water flow). The locomotory capabilities, muscle development, and anaerobic capacities of salmon fry can differ based on whether they migrate upstream to larval feeding grounds or downstream; upstream migrants have better swimming performance, muscle development, and anaerobic capacity. With regard to navigation to the lake environment, it is likely that rheotaxis (i.e., orientation against the water current) is the dominant orienting mechanism by which fry find their way upstream to a nursery lake, although olfaction may also play a role in fry, which must cross a tributary on the way to the nursery lake, or when fish must first swim down one river and then up another to reach the lake. These migrations generally occur at night, presumably to further minimize predation risk. Furthermore, life in nursery lakes involves diel vertical migrations associated with prey searching.

Smolting and Migration to Sea

The transition by salmon from freshwater to seawater is undoubtedly the best studied of all fish migrations. The principal motivation for seaward migration is the massive feeding and growth potential afforded by the nutrient-rich, open ocean compared with the nutrient-limited freshwater systems where salmon spawn. The smoltification (i.e., the transition physiologically into seagoing forms) process is an inherently stressful and risky period in the life cycles of salmon, and this phase is often considered a survival bottleneck, in which mortality is high. To survive this period, the smolts must carefully time this event to arrive at the ocean at an optimal time when ocean currents are favorable for their movement into coastal areas and when food resources are available. They must also overcome the challenges associated with the transition between hypoosmotic freshwater and hyper-osmotic marine environments, which imposes significant physiological stress (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). The smolt process involves a suite of physiological, morphological, biochemical, and behavioral changes, which all require sufficient food energy intake as well as efficient energy use. Superimposed on all of this are new predation risks, as there are many animals that feed on young salmon in the new marine environment. All of these factors contribute to the survival bottleneck effect.

Increasing photoperiod in spring seems to be the key environmental factor that synchronizes the endogenous changes occurring seasonally. Long before smolting takes place, individual fry must make a decision as to when they are going to smolt. Most Pacific salmon species complete smolting within a few months after hatching. However, some species such as sockeye wait up 1 or 2 years after hatching. The factors controlling the subsequent downriver migration, however, are not well understood. Current evidence suggests that an innate, endogenous circannual rhythm is the primary controlling factor, one that is trigger by changes in photoperiod. Temperature regulates the rate of physiological processes and is also involved as a modulating factor, just as food availability is. What is clear, however, is that the process of smoltification is directly linked with initiating this migration.

Smolts tend to eat little during the downstream migration. In fact, upon entering the ocean, smolts are often malnourished and in a catabolic state, a condition that is similar to adult salmon during upriver migrations to spawning areas. There is some evidence that the size of the heart increases disproportionately during this time, which may enhance metabolic capacity and efficiency. Although downstream migrants tend to drift passively with the flow, they can and do actively swim, which enables them to attain remarkable migration speeds of 10–20 km or more each day. In river systems, such as in the Columbia River, hydro-electric and other dams can prolong migration by several weeks, and such delays can increase energetic costs and physiological disturbances, thus exacerbating patterns of mortality at a time when mortality is already high (i.e., the survival bottleneck) (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance).

Smolting is a prerequisite to anadromy and must be regarded an important component of the downstream migration. The associated changes in body shape and metabolism facilitate active downriver migrations, and complex changes to gill, kidney, and intestinal functions occur to optimize ion- and water-exchange systems (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes). The suite of endocrine processes (**see also** The **Hormonal Control of Metabolism and Ionic Regulation**: The Hormonal Control of Osmoregulation in Teleost Fish) that underlie these physiological restructurings for a life in saltwater also have many co-evolved effects of migratory behavior. These include a migratory restlessness, which occurs in the days and nights before downriver migration begins, which is important for initiating the migration, as well as increases in general activity level (i.e., increased metabolic rate). The hypothalamus and possibly the pineal gland likely receive information about environmental light and temperature levels, which provide important information about the time of year (i.e., seasonality), and via the pituitary gland, a cascade of hormones are then released to direct physiology, behavior, and morphology along migratory lines. Hormones also elevate metabolic rate and alter the alertness of the fish to different stimuli, thus bolstering the probability of surviving the many challenges of migration.

Experimental studies have provided clues to the roles of many different hormones. During the pre-migratory season, some species of fry are negatively rheotatic, but experimental treatment with the thyroid hormone (T3) made them positively rheotactic. Thus, the natural surge of thyroxine (T4, a derivative of T3) that occurs prior to downstream migration is likely a key trigger for downstream migration. In addition to thyroxine, growth hormone, insulin-like growth factor, and cortisol are other hormones, which increase during smoltification and contribute to the development of gill chloride cells. Chloride cells on the gills represent the primary site for ion regulation in fish in saltwater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction). When fish first enter seawater, cortisol concentrations in the blood increase widely and ion concentrations are temporarily elevated. It is worth noting that not all smolts successfully adapt to seawater. In fact, mortality rates among salmon are generally highest during this migration phase, largely due to osmoregulatory unpreparedness. Furthermore, one important hormone for saltwater preparedness is cortisol, which is also a principal stress hormone (**see also Hormonal Control of Metabolism and Ionic Regulation**: Corticosteroids and **Hormonal Responses to Stress**: Hormone Response to Stress). Though the transition to saltwater is inherently stressful, undue stress or stress beyond some threshold level can provide a conflicting physiological motivation, which can adversely affect migratory behavior and survival. For example, high cortisol could lead to early entry to the marine area, before physiological systems have had a change to fully prepare for saltwater, which would put the smolt at risk of mortality. Moreover, additional stressors can lead to further elevations in cortisol, and fluctuations in hematocrit and ionic status also correlate with mortality. All of this information serves to highlight the sensitivity of smolts to a number of factors during the fresh-to-salt water transition, which ultimately affect migration, survival, and fitness.

Migration to Ocean Feeding Grounds

Pacific salmon undertake directed coastal migrations to seek out rich food sources. Some species congregate in sheltered bays, where they exploit food resources that concentrate as a result of eddies and tidal shear forces. Consequently, their speed of migration varies greatly among species. Thus, salmon migrate from near-coast environments to the open ocean after several months to a year.

Many Pacific salmon feed in the Alaskan Gyre, a productive region of the northeast Pacific Ocean (i.e., Gulf of Alaska). Modeling studies suggest that the swimming speeds required for fish to override the influence of ocean currents in this region are prohibitively costly. Consequently, sockeye salmon, for example, are known to complete two large counter-clockwise loops of the northeast Pacific during 2 years, riding on the Gyre currents, while attaining a mature size and mass prior to homeward migrations. Nonetheless, most of the Gulf of Alaska is considered unfavorable for fish growth based on temperature suitability and prey availability. Salmon prefer cold water, and even small increases in surface water temperatures can dramatically reduce growth potential. To obtain sufficient energy to fuel the homeward migration to natal streams and enable reproduction, salmon in the ocean must feed optimally (i.e., near maximum ration) and continually, searching for zones of positive growth potential. To locate and exploit patchy food resources and areas with optimal thermal conditions, perhaps avoiding areas of high temperature, salmon must have finely tuned physiological systems that enable them to detect and evaluate subtle differences in growth potential conditions. Few physiological studies have been undertaken on Pacific salmon in the high seas to examine these mysteries.

Directed Migration to the Coast and Natal River

Pacific salmon start their impressive migration toward natal streams after 1–3 years of feeding in rich ocean waters. They typically gain about half of their adult mass in their last 6 months at sea and therefore continue feeding as they migrate towards the coast. What triggers salmon to make the decision to initiate homeward migrations is poorly understood, but is presumably linked to reproductive maturation and the endocrine system (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). When researchers manipulate gonadotropin-releasing hormone (GnRH) in salmon, it triggers the growth of gonadal tissue and such growth is associated with a shift from foraging to homing in salmon.

Testosterone is an end-product of GnRH release and this hormone is believed to be the direct link to migration behavior. Masu salmon that were castrated to eliminate testosterone production stopped migrating, but resumed migration when subsequently injected with testosterone. Testosterone also influences the speed at which salmon return to their natal rivers – higher testosterone levels lead to faster swimming into natal rivers. The trigger for initial for GnRH release is likely associated with both exogenous factors such as changing photoperiod (i.e., changing seasons) and endogenous factors such as nutritional condition and body size. It is possible that GnRH will only trigger homeward migrations if fish which exceed certain growth or energetic thresholds. Thus, sometimes some Pacific salmon species vary in their abundance from year to year, sometimes spending an extra year in the ocean (with the exception of pink salmon which have a 2-year life cycle; **see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction and **The Reproductive Organs and Processes**: Vitellogenesis in Fishes).

After triggering a homeward migration, immense navigational challenges ensue as fish must find their way from the Alaskan Gyre to their natal coastal river. Natal homing represents one of the most remarkable characteristics of Pacific salmon migrations and one of the greatest feats of any animal. There is still much debate about the sensory cues and physiological systems used by salmon to direct homeward migration. In general, it is believed that while in the open oceans, salmon should swim with small vertical and horizontal oscillations, which would enable them to sample odors from different water layers. It is believed that salmon are able to recognize four major odorants (amino acids, bile salts, steroid hormones, and prostaglandins) at minute concentrations (**see also Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes). Collectively, these odorants from natal streams create what is known as the 'home stream olfactory bouquet', and these are apparently detectable even in the open ocean. Beyond detecting natal odors, salmon must use other sensory systems to help fish move toward natal streams. Vision seems to be of paramount importance for migratory salmon, and they may use a sun compass, polarized light patterns, or even landmarks to guide them homewards (**see also Vision**: Adaptations of Photoreceptors and Visual Pigments and Behavioral Assessment of the Visual Capabilities of Fish). Studies have revealed that even experimentally blind salmon can find their way home, slowed by days of random swimming. Presumably, the fish rely solely on olfactory cues when visual cues are unavailable. Water temperature and salinity levels (i.e., less saline waters) may also serve as orientation cues, particularly as they approach the river mouth.

Physiologically, preparation for transition to freshwater and reproduction also occur during homeward migration. Timing is critical. Salmon must position themselves at the mouth of the river at a time that coincides with an appropriate level of osmoregulatory preparedness and when river conditions such as flow and temperature are suitable for initiating upriver migration. Physiological sampling of fish between 700 and 250 km away from river entry shows that they are pre-prepared for the transition to freshwater well before arrival at the river mouth. For example, gill Na^+/K^+-ATPase, an important ionoregulatory enzyme (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts and Mechanisms of Ion Transport in Freshwater Fishes), is downregulated in preparation for freshwater despite the fish still being in full strength seawater.

In addition, reproductive hormone concentrations are also elevated (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction). At some point during this phase, salmon stop feeding, with the exact time and location varying by stock and species.

Adult Upriver Migration to Natal Stream

One of the most conspicuous, well-studied animal migrations is that of the upriver migrations of Pacific salmon. These migrations are perhaps the best known because they present a rare opportunity to visibly see large numbers of salmon, and because the numbers of returning salmon can reach into the millions. The distances that salmon swim to reach spawning areas vary among populations within species, and among species, and can range from as few as 10–100 km to well over 1500 km. At times, salmon must leap over barriers (**Figure 3**). Regardless of the relative difficulty of a migration, energy reserves are limited and must be allocated carefully and conserved wherever possible. Energy stores are needed to fuel migration, complete sexual maturation, and support behaviors and morphological changes associated with courtship and spawning. At the onset of upriver migration, salmon populations differ in their somatic energy store in an adaptive way; long-distance migrants starting the migration with higher somatic energy (their gas tank is fuller for the journey ahead). A trade-off for long-distance migrants is that they have fewer and smaller eggs than short-distance migrants. This type of reproductive trade-off is common in other animals that make arduous migrations. To conserve energy further, long-distance migrants have smaller and more compact bodies making them more hydrodynamic when swimming. Furthermore, because long-distance migrants often encounter more difficult river hydraulics, they typically have a higher maximum metabolic rate.

As in coastal migrations, high testosterone levels are associated with fast river migration speeds, and also with high levels of aggression in spawning fish. Aside from its influence on behavior, testosterone and other sex steroids (e.g., estradiol, 11-ketotestosterone) are responsible for the production of gametes and the development of secondary sexual characteristics – the morphing from a sleek chrome colored ocean salmon into the hooked nosed, humped backed, and brightly colored form found on spawning grounds (**Figure 4**). Gamete production and morphological change generally occur over a period of weeks to months, and reach their peaks toward the end of upriver migration, which is necessary because both processes negatively affect hydrodynamic efficiency in homing salmon. Once migration is finished and salmon are on spawning grounds, swim efficiency is no longer important and spawning forms can be modified for reproductive combat.

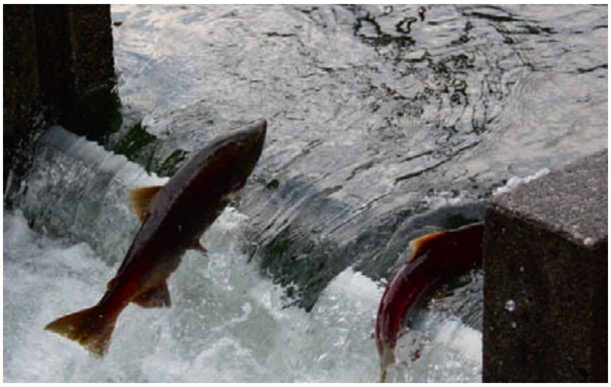


Figure 3 Jumping sockeye salmon en route to spawning grounds. Pacific salmon have remarkable jumping abilities needed to negotiate natural and artificial barriers encountered during spawning migration.

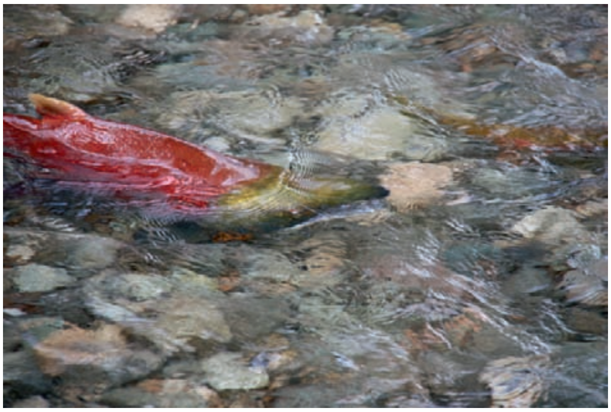


Figure 4 Mature male sockeye salmon on spawning ground. The pronounced hump on the back, curved jaw (known as the kype) and red colouration are reflective of sexually selected traits that are desired by females.

Anthropogenic Threats Involving Salmon Migrations

Because of their migratory life style, Pacific salmon face a myriad of threats to their existence. Barriers to migration represent the single largest problem faced by anadromous Pacific salmon because an inability to physically proceed to a subsequent life stage can, in a short time, extirpate a population. Large dams and associated water diversions to store and divert water for hydroelectricity, agriculture, land reclamation, and human consumption have caused widespread extinctions. For example, in the Columbia River basin, 35% of Pacific salmon stocks are extinct and 40% are endangered. Some dams have been fitted with bypass structures (e.g., fish ladders or fishways; see **Figure 5**) to enable smolts to migrate downstream and adults upstream. Small barriers are also problems. The residential and industrial development has annihilated most salmon in the lower mainland in and around

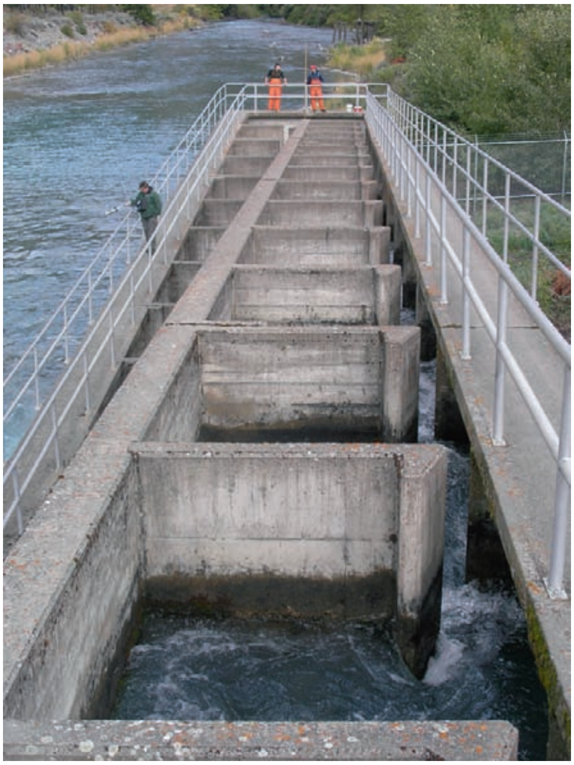


Figure 5 Fish passage facility to enable Pacific salmon to pass a dam in the Fraser River basin. Fish passages (also known as fishways or fish ladders) are engineered structures built to facilitate passage of fish around natural or artificial barriers.

Vancouver and bordering on Canada's greatest salmon watershed, the Fraser River. The most common type of stream crossing in the Pacific Northwest are corrugated pipe culverts which number in the hundreds of thousands on salmon bearing streams. Many have been installed improperly and are undersized resulting in streambed erosion at the downstream end and the culvert outfall perched above the stream thereby isolating upstream areas from downstream ones effectively eliminating adult migrants from many small creeks and streams. The replacement of perched culverts with larger diameter or open natural bottom arch culverts has shown to be an effective response to this problem (**see also Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance).

Mortality can also occur due to physiological stress, delay, and physical injury. Migrations are inherently stressful periods of life, particularly those involving transitions between freshwater and saltwater. Anthropogenic stresses add to this.

Exacerbating these stresses are the environmental challenges associated with climate change. Warming rivers are now pushing some populations of adult salmon beyond their optimal temperature such that they have reduced or no aerobic scope for migration. In addition, disease and illness have become more apparent in these warmer migratory environments affecting spawning fish. Less productive and warmer oceans are reducing growth rates and energy reserves and changing migration timing (**see also Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Temperature and **Temperature**: Effects of Climate Change).

Because adult salmon migrate in high numbers and at predictable locales and times, humans have been able to effectively exploit this resource. For centuries, First Nations relied on salmon for food, trade, and as part of their mythology and spirituality. European settlers established coastal communities in North America, which relied heavily on the fishery. Though overfishing has led to several stock declines in the past, large fisheries still exist today. Depending on the type of fishing gear, large numbers of fish can escape from capture or are released after capture. Though research is limited, stress and delayed mortality of escaped or released fish can be high, especially in warm rivers. Thus, fisheries and a warming climate may put Pacific salmon in a double jeopardy.

Conclusions

Pacific salmon are a relatively well-studied group of animals compared with other fishes, and their migrations have been focus of considerable investigation. Researchers have learned that migrations are a complex interplay of behaviors and physiology, involving an integration of sensory and locomotory systems, mediated by endocrine and osmoregulatory systems. Pacific salmon have also served as key models for addressing questions related to fish orientation, mating systems, and evolutionary biology. Yet, there remain many large gaps in our knowledge. For example, we know relatively little about the biology of Pacific salmon in the ocean. In addition, even what appears as simple questions are difficult to address: e.g., why do some individuals die and some survive migrations, why do all migrants die after spawning? New technology such as the use of biotelemetry transmitters and archival data loggers, and the integration of these approaches with physiological bioassays show promise for illuminating the biology of salmon throughout their lives, but particularly in the ocean. In addition, functional genomics has the potential to dramatically improve our understanding of the physiological basis for different migratory strategies. Given the anthropogenic threats faced by Pacific salmon during their migrations, improving our understanding of the behavior and physiology of their migration will be essential to ensure appropriate management and conservation strategies.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Fish Migrations**: The Biology of Fish Migration. **Hormonal Control of Metabolism and**

Ionic Regulation: Corticosteroids; The Hormonal Control of Osmoregulation in Teleost Fish. **Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress**: Hormone Response to Stress. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes; Osmoregulation in Fishes: An Introduction. **Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes. **Swimming and Other Activities**: Applied Aspects of Fish Swimming Performance. **The Reproductive Organs and Processes**: Regulation of Spermatogenesis; Vitellogenesis in Fishes. **Ventilation and Animal Respiration**: Respiratory Gas Exchange During Development: Respiratory Transitions. **Vision**: Adaptations of Photoreceptors and Visual Pigments; Behavioral Assessment of the Visual Capabilities of Fish.

Further Reading

Dingle H (1980) Ecology and evolution of migration. In: Gauthreaux SA, Jr. (ed.) *Animal Migration, Orientation, and navigation*, pp 1–101. New York: Academic Press.

Farrell AP (2009) Environment, antecedents and climate change: Lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology* 212: 3771–3780.

Groot C, Margolis L, and Clarke WC (1995) *Physiological Ecology of Pacific Salmon*. Vancouver: University of British Columbia Press.

Healey MC (2000) Pacific salmon migrations in a dynamic ocean. In: Harrison P and Parsons T (eds.) *Fisheries Oceanography: An Integrative Approach to Fisheries Ecology and Management*, pp 29–60. Oxford: Blackwell.

Hinch SG, Cooke SJ, Healey MC, and Farrell AP (2006) Behavioural physiology of fish migrations: Salmon as a model approach. In: Sloman A, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish. Fish Physiology Series*, vol. 24. London: Academic Press.

Hoar WS (1988) The physiology of smolting salmonids. In: Hoar WS and Randall D (eds.) *Fish Physiology*, vol. XIB. New York: Academic Press.

Høgåsen HR (1998) Physiological changes associated with the diadromous migration of salmonids. Canadian Special Publication on Fisheries and Aquatic Sciences 127.

McKeown BA (1984) *Fish Migration*. London: Croom Helm Publishers.

Northcote TG (1984) Mechanisms of fish migration in rivers. In: McCleave JD, Arnold GP, Dodson JJ, and Neil WH (eds.) *Mechanisms of Migration in Fishes*, pp 317–355. New York: Plenum Press.

Quinn TP (2005) *The Behavior and Ecology of Pacific Salmon and Trout*. Seattle: University of Washington Press.

Welch DW, Boehlert GW, and Ward BR (2002) POST – the Pacific Ocean Salmon Tracking Project. *Oceanologica Acta* 25: 243–253.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Deep-Sea Fishes

AP Farrell, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

What Is the Deep Sea?
Feeding among the Deep-Sea Fishes
Effects of Pressure
Summary
Further Reading

Glossary

Benthic Organisms living on, or near, the seafloor; lowest level of a body of water.

Deep sea Ocean depths greater than 1000 m.

Demersal Pertaining to fish that live near the bottom of oceans and lakes.

Hydrostatic pressure Pressure due to the height of a column of fluid directly above the reference point. This pressure exists in both static and flowing fluids and can be either added to, or subtracted from, the hydraulic pressure, where the reference point is the heart.

Pelagic Pertaining to fish that live in the water column of oceans and lakes.

Swimbladder A gas-filled chamber in the abdominal cavity of most bony fishes. The swimbladder is used in buoyancy control in most species; it also subsumes roles in hearing and sound production in some species.

What Is the Deep Sea?

Seawater covers the majority (71%) of the surface of the Earth (**Figure 1**). The average depth is nearly 4000 m. The deep ocean is characterized as occupying depths greater than 1000 m. Deep-sea fishes are those that occupy these depths for some or part of their life history. At such depths, the hydrostatic pressures exerted on fishes are at least 100 times greater than the atmospheric, plus there is no sunlight to support algal photosynthesis. Remarkably, abyssal depths (3000–6000 m; **Figure 2**) cover 51% of the Earth's surface, resulting in the average ocean depth being 3800 m. Hadal depths (<6000 m) cover only <2% (**Figure 1**). The deepest part of the oceans, the Marianus Trench, reaches over 11 000 m, deeper than the height of Mt. Everest. Water pressure at these extreme depths is more than 1000 times greater than that at the sea surface, and 3000 times higher than on the top of Mt. Everest! Remarkably, fishes live at these extreme depths.

Deep ocean water has a stable, cold temperature. This situation arises because, as seawater cools, its density increases up to its freezing point of about −1.9 °C (freshwater is at its most dense at 4 °C). Therefore, cold seawater tends to sink, taking dissolved oxygen with it. Additionally, when seawater freezes, the ice is virtually free of salt, which results in the remaining water having a higher salinity and density.

The boundary between the bathopelagic and overlying mesopelagic zones is set at 1000 m (**Figure 2**) and is generally viewed as the lower limit for the diel migration of micronekton. In the absence of light, organic matter is mostly transferred down to these depths by sedimentation. With a limitation of organic resource input, the biomass at 1000 m is just 10% of the euphotic zone and a mere 1% at 4000 m.

Remarkably, a zone of maximum species richness exists around 1000 m. Therefore, despite the challenges and constancy of deep water, deep-sea fishes show remarkable diversity, none more appealing than the diverse morphologies that have been described as anything from grotesque to unbelievable. Such was the fascination with the visual appearance of deep-sea fishes that they were used for the frontispiece of the high school textbook produced by Haldane and Huxley in 1927 (**Figure 3**).

Our knowledge of the deep sea and its fishes is limited, but is continuously growing. The limited knowledge base simply reflects immense technological challenges associated with such studies. Fishes brought to the surface

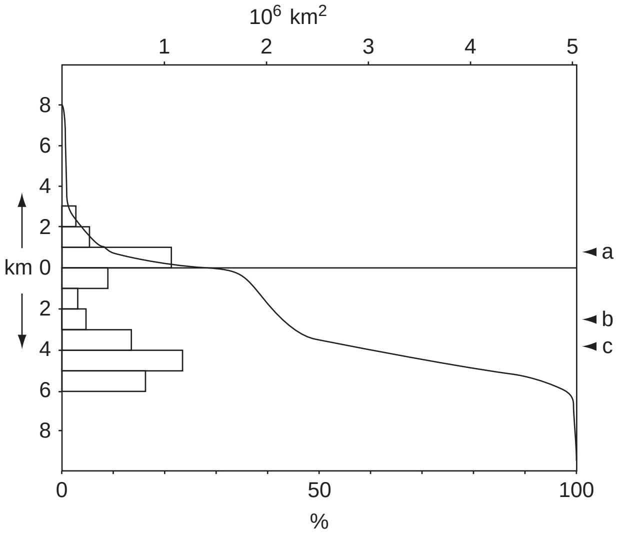


Figure 1 Percentages and areas of the Earth covered by land of various elevations and water of various depths. *a*, mean land elevation (840 m); *b,* mean sphere water depth (2440 m); and *c*, mean ocean depth (3800 m). Reproduced from Figure 1 in Angel MV (1997). What is the deep sea? In: Randall DJ and Farrell AP (eds.) *Deep-Sea Fishes*, pp. 1–41. San Diego: Academic Press, with permission from Elsevier.

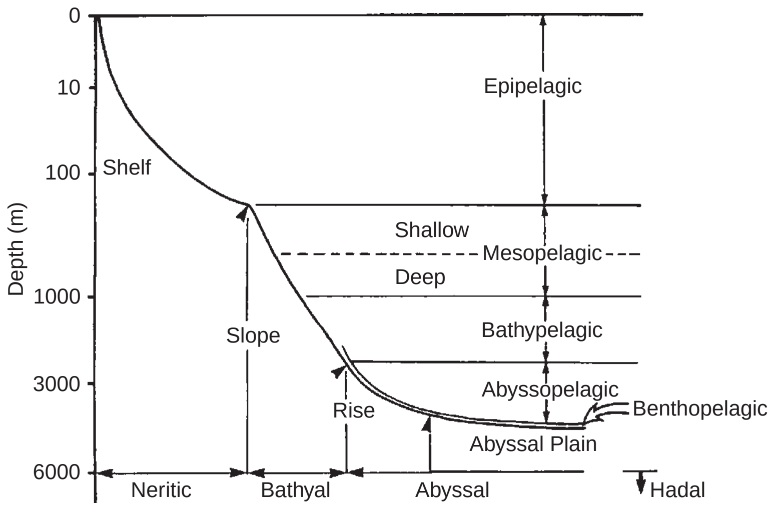


Figure 2 A schematic representation of the zonation of the oceans as a function of depth. Reproduced from Figure 4 in Angel MV (1997). What is the deep sea? In: Randall DJ and Farrell AP (eds.) *Deep-Sea Fishes*, pp. 1–41. San Diego: Academic Press, with permission from Elsevier.

from a depth may partially disintegrate under reduced surface pressure, that is, if they do not die or get eaten in the trawls that, take several hours to retrieve from such depths. To observe deep-sea animals in their own elements requires sophisticated feats of engineering so that the equipment can withstand extreme hydrostatic pressure and can be remotely operated kilometers away.

The deep sea represents the final frontier of discovery on the Earth. The challenges that scientists must overcome to observe deep-sea fishes are some of the very same ones that deep-sea fishes adapted to during evolution. This article is about some of these remarkable adaptations.

Evolutionary adaptation to the deep sea was not a single event. Deep-sea fishes belong to 22 orders of fishes. Remarkably, 10–15% of the 25 000 or more fish species are found in the deep sea, and more are being discovered every year. Even so, the Perciformes, which radiated so spectacularly in shallow waters, have very few deep-sea representatives. Species diversity decreases with depth beyond the range of 400–2000 m, which is the depth range that has the most diverse species. Some deep-sea fish species have very relatively vertical distributions of a few hundred meters, whereas others such as *Coryphaenoides rupestris* and *Synaphobranchus kaupi* vertically distribute over thousands of meters.

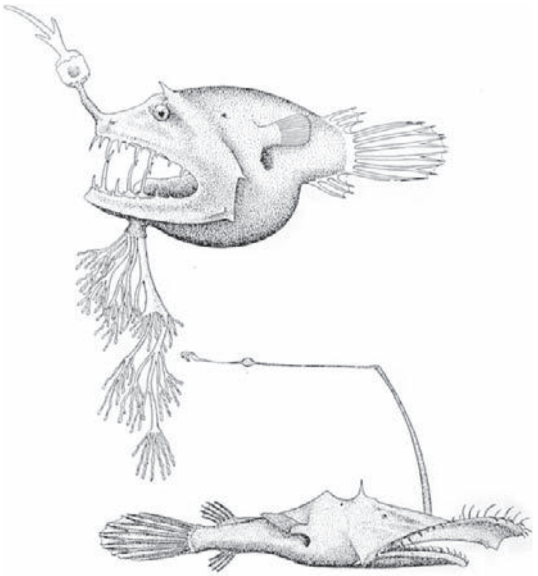


Figure 3 Illustrations of two deep-sea fishes (A. *Linophryne arborifer* and B *Lasiognathus saccostomus*) used in an introductory cover plate for Haldane's and Huxley's 1927 textbook on Animal Biology for high school biology.

Feeding among the Deep-Sea Fishes

Despite the relative constancy of the deep-sea environment, deep-sea fishes show seasonal cycling of reproduction. This is almost certainly tied to seasonal food abundance. Accompanying each spring and summer bloom of primary productivity in the euphotic surface waters of the ocean, comes a rain of sinking dead, organic material, which provides most of the energy input for the deep sea and its fishes.

With a low density of animals, no ambient light, and the need to feed in an environment where the biomass available as energy is less than 5% of that in surface waters, it pays to confuse or be invisible to potential predators. Therefore, deep-sea fishes are remarkably well endowed with effective lures and capture methods for prey (**Figure 3**), as well as visual adaptations and bioluminescence (**see also Vision**: Visual Adaptations to the Deep Sea and **The Skin**: Bioluminescence in Fishes).

Diets range widely from zooplankton, gastropod mollusks, polychaete worms, crustaceans, and gelatinous organisms (such as the salps and larvaceans belonging to the Urochordata) through to fishes. Trying to understand exactly what deep-sea fishes eat using traditional methods of species capture and stomach analysis has been fraught with technical difficulties. For example, few specimens come up in a trawl. Many of the pelagic fishes are small (<300 mm) making both capture and visual observation in submersibles difficult. Large predators often have empty stomachs. Over-inflated and everted swimbladders may empty the stomach. Of course, opportunistic feeding within a trawl net for several hours could cause bias. In addition, soft-bodied and gelatinous prey are difficult to identify.

The jaw and gill morphology provide good clues to food habits. Ambush predators are characterized by a large jaw equipped with numerous long, sharp, and depressible teeth. Short gill rakers point to piscivory, whereas long, closely set rakers suggest small prey items. A simple, short, straight gut likely indicates that large prey is ingested as a whole.

Some benthic deep-sea fishes actively forage; they arrive within minutes at baited cameras placed at a depth. They likely use odors to locate the bait (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction). These species include the abyssal macruourids, chimaeras, morids, synaphobranchids, and sharks. Ultrasonic transmitters placed on fishes similarly support the importance of active foraging among deep-sea fishes, rather than just sit-and-wait predation. All the same, the species that are seen at baited cameras may not be the same species caught by trawl in the same region, clearly suggesting that not all deep-sea fishes are active scavengers.

Trophic guilds are used to characterize a fish's feeding habit. For the demersal deep-sea fishes, several hundred species are assigned to 10 trophic guilds.

Guild 1: Piscivores that often have large teeth and lures. Included are subguilds for sit-and-wait predators (the lizard fish *Bathysaurus ferox*) and for active predators (the large macroruids of the genus *Coryphaenoides*).

Guild 2: Macronektonic foragers (macroruids of the upper and middle slopes such as *C. rupestris* and *Bathygadus melanobranchus*). These fishes may be found in schools and have large, well-developed gill rakers. Bioluminescence may be used to communicate.

Guild 3: Micronectonic/epibenthos predators (the morid *Lepidon eques* and the macroruid *Coelorhinchus coelorhinchus*). These abundant dermersal species likely use olfaction, tactile sense, and lateral line sense to detect prey.

Guild 4: Benthivorous infaunal predators (e.g., members of the eelpout family) that eat mollusks and polychaetes. Few deep-sea species use this feeding habit.

Guild 5: Microphagous epifaumal browsers (species of the genus *Polycanthonotus*). They use a slender snout and ventral mouth to feed on small benthic crustaceans and polychaetes.

Guild 6: Megafaunal croppers and browsers. A few species such as *Notacanthus chemnitzi* selectively crop anemones, corals, and bryozoans.

Guild 7: Macroplanktonivores (slickheads such as *Conocara fiolenti*). These feed on slow-moving gelatinous prey, and may drift themselves.

Guild 8: Specialist necrophages (*Synaphobranchus kaupii* and hagfishes). These fishes are strongly attracted to baited traps.

Guild 9: Necrophagivores (*Paraliparis bathybius*). These fishes are often gelatinous themselves.
Guild 10: Detritivores (*Conocara macropterum*).

The deep-sea pelagic fishes are assigned to three trophic guilds.

Guild 1: Micronecktonivores are either active foragers, with a large mouth, teeth and stomach, or sit-and-wait predators that typically use lures. The *Ceratioidei* families belong to the piscivorous subguild and *Crytopsarus couesi* belongs to the cephalopod mollusk predators.
Guild 2: Zooplanktivores typically have numerous fine teeth (members of the family Nemichthyidae).
Guild 3: Generalists (the bathypelagic gulper eel family Eurypharyngidae).

Effects of Pressure

Deep-sea fishes face immense hydrostatic pressure, at least 100 times and up to 1100 times greater than that experienced at the sea surface. Such high pressures have important consequences to buoyancy and to basic biochemical reactions.

Buoyancy

Fishes tend to sink in seawater (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes). This is because most of their tissues are denser than seawater ($\sim$1.026 kg L^{-1}). The two solutions used to solve this problem involve resting on the benthos for benthic fishes and continuous swimming for pelagic fishes. In addition, fishes in general have evolved mechanisms make their bodies neutrally buoyant, allowing them to avoid the energetic cost of continuous swimming or a benthic life. Therefore, adaptations for neutral buoyancy among deep-sea fishes are to be expected, especially given the limitation on energy resources in deep sea.

Fishes, as a whole, use three primary strategies to attain neutral buoyancy (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes).

1. They use lipids to lower their overall density.
2. They use a swimbladder (**see also Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes) to lower their overall density.
3. They decrease the amount of high-density tissue such as bone (1.3–1.5 kg L^{-1}) by either decreasing the size or thickness, or reducing the mineral content (e.g., calcium).

All three strategies are in evidence in deep-sea fishes, although the last strategy is relaxed for benthic species, especially ambush predators that need large jaws and teeth.

Swimbladder

A gas-filled chamber called a swimbladder can account for 5–6% of the size of certain fishes. While this added volume increases the drag of a fish when it moves through water, it is a very effective means of achieving neutral buoyancy because of the large difference in density of water and air. Even so physiological specializations are required to deposit gases into the swimbladder (**see also Design and Physiology of Arteries and Veins**: The Retia, **Buoyancy, Locomotion, and Movement in Fishes**: Swimbladder Function and Buoyancy Control in Fishes, **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems, and Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder) and to remove them, should the fish rise in the water column (otherwise the fish become positively buoyant as the gas expands under reduced pressure).

Gas-filled swimbladders are found in deep-sea fishes to depths of 5000–7000 m (e.g., myctophids). At these depths, the density of gases is greatly increased (0.6–0.7 kg L^{-1}) compared with the surface, but a substantial difference still remains relative to the density of seawater (1.027 kg L^{-1}). Oxygen is the primary gas found inside the swimbladder of deep-sea fishes. It is thought that a depth limitation exists for the swimbladder that is related to the ever-decreasing ability to unload gas from hemoglobin and into the swimbladder using the Root effect as fish move deeper. One suggestion is that a total pressure of 10 atm is the upper limit for gas deposition, but others suggest 140 atm. Furthermore, the walls of the swimbladder must have a greater gas impermeability compared with shallow-water teleosts because of the higher partial pressure of the gases they are containing.

Lipid inclusions

Deep-sea fishes include lipid deposits in their swimbladders as well. Lipids are typically less dense (0.78–0.93 kg L^{-1}) than seawater and only increase in density by about 1% per 100 atm. While they are denser than gas, they are not subject to volume changes if a deep-sea fish migrates vertically. Unlike the swimbladder of *Coelacanth latimeria*, which is completely filled with wax esters, mid-water deep-sea fishes, such as *Antimora rostrata*, have sheets of bilayered membranes, which have a high cholesterol content. Paradoxically, cholesterol has a higher density (1.067 kg L^{-1}) than seawater, but it offers the advantage of a high oxygen solubility, which may aid oxygen deposition to and retention in the swimbladder at high ambient pressure.

High concentrations of lipids are found in sharks (**see also Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes and **Chondrichthyes**: Physiology of Sharks, Skates, and Rays) and *C. latimeria*, especially in the liver. Eggs of deep-sea fishes (e.g., *Macropodus opercularis* and *Ophiocephalus argus*) also use oil droplets to achieve neutral buoyancy.

Watery Fluids and Gelatinous Tissues

Because muscle is denser (1.05–1.07 kg L^{-1}) than seawater, some deep-sea fishes compensate by reducing their muscle mass (which necessarily reduces locomotory ability), whereas others have watery muscles, for example, *Acanthonus armatus*. The gelatinous tissues of *Bathylagus* species have an extremely high water content (96% vs. a more typical 80%), likely aided by the inclusion of hygroscopic glycosaminoglycans. Although plasma is already less dense than seawater, *A. armatus,* for example, has hypoosmotic fluids that have reduced concentrations of sodium to decrease its plasma density to 1.008 kg L^{-1}.

Biochemical Structures and Reactions

Exposure of shallow-water fishes to high pressure typically results in hyperactivity, convulsions, torpor, and even death. Some of these responses occur at pressures as low as 50 atm, well below the pressures routinely experienced by deep-sea fishes. Knowledge on the pressure tolerance is limited, but some of this tolerance must lie at the biochemical level of biological organization.

Pressure exerts its effects through volume changes. Application of pressure shifts chemical equilibria to a lower volume. Therefore, pressure will tend to disrupt three-dimensional structure, which is often so critical for the proper functioning of biological molecules, such as proteins, and biological membranes, which are crucial for regulating the internal environment of all cells. For example, most enzymes are inhibited at high pressure. However, this inhibition is not a result of protein denaturation, at least in the pressure range experienced by deep-sea fishes.

Proteins

Studies on protein function for deep-sea fishes are limited in number. However, findings for the ubiquitous and highly expressed structural protein actin illustrate a more general finding deep-sea fishes evolved pressure-insensitive forms of proteins and enzyme, rather than forms that tolerate a specific range of high pressure.

F-Actin is a polymer of the G-actin monomer found in most cells, and the volume change that accompanies polymerization, therefore, should be pressure sensitive. This is certainly the case for actin isolated from the shallow-water *C. acrolepis*, where the volume change is 60 mL mol^{-1}. (Volume changes may be twice as high as this value for polymerization of actins isolated from terrestrial species.) By contrast, polymerization of actin isolated from *C. armatus*, which occurs at depths of 2000–5000 m, is little affected by pressure, likely because the volume change associated with polymerization is considerably smaller (<10 mL mol^{-1}). The reason for this pressure insensitivity is unclear, but it may be related to relative incompressibility of the G-actin monomer.

The tetrameric enzyme lactate dehydrogenase provides another example of pressure insensitivity among deep-sea fish proteins. Enzyme isolated from the deep-sea *Sebatolobus altivelis* and the shallow-water S. *alascanus* had identical kinetics when tested at atmospheric pressure. However, the concentration of one of its substrates (NADH – nicotinamide adenine dinucleotide) to produce half the maximal rate was doubled at 70 atm for *S. alascanus* and continued to increase up to 200 atm. By contrast, the required concentration for *S. altivelis* increased by only 33% at 70 atm and no further at higher pressures. Maximum activity of this enzyme was unaffected by pressure, independent of the species being tested. Similar results for these two fish species were found for the isolated enzymes glyceraldehyde 3-phosphate and malate dehydrogenase. Likewise, the signal transduction enzyme, adenylyl cyclase, isolated from *A. rostrata* was pressure insensitive. The molecular basis of pressure insensitivity is still being studied.

Cell membranes

The composition of cell membranes is also altered to compensate for lateral compression at high pressure. Compression would have made the membrane less fluid, similar to the overall effect of cold temperature (**see also Temperature**: Effects of Temperature: An Introduction and Temperature and Excitable Membranes). In fact, the effect on membrane fluidity of 11 000 atm at 4 °C is equivalent to that at −20 °C at atmospheric pressure. Not surprisingly, cell and mitochondrial membranes of deep-sea fishes show increased content of unsaturated fatty acids to reduce their fluidity, just like cold-adapted and cold-acclimated animals. Such changes have already been shown to be important for the function of membrane-bound proteins. For example, lipid substitution in membranes has revealed that at least a part of the pressure insensitivity of the enzyme Na^+/K^+-ATPase of a deep-sea fish is due to increased membrane fluidity.

Metabolic rate

A curious and unexplained aspect of deep-sea fishes is their low metabolic rate. It is reported that metabolic rates of deep-sea species found at 1000 m or below can be 10 to 15 times lower than surface species. Such large differences cannot be attributed to the relaxed selection for locomotion and the sit-and-wait predatory lifestyles. Nor can they be explained by reduced enzyme concentrations in the muscle tissue. While low temperature is an obvious contributing factor to low metabolism, other factors must feature. One possibility is that protein turnover rates are much lower in deep-sea fishes. While protein synthesis can represent

30% of metabolic rate in surface fishes, such determinations have not been made in deep-sea fishes.

Summary

Many species independently evolved to take advantage of the deep-sea environment, despite the challenges of extreme pressure, cold temperature, absence of light, and very low energy input. To do so, they have adopted a wide range of feeding habits, showing extreme morphological and physiological adaptations to both lure prey and avoid being captured. Their protein structure, cell membrane composition, metabolic rates, and buoyancy mechanisms appear to be well adapted for life at high pressure, cold temperature, and limited food. Nevertheless, much remains to be discovered for this fascinating group of fishes, provided the technical challenges for studying deep-sea fishes can be overcome.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes; Swimbladder Function and Buoyancy Control in Fishes. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Design and Physiology of Arteries and Veins**: The Retia. **Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction. **Temperature**: Effects of Temperature: An Introduction; Temperature and Excitable Membranes. **Transport and Exchange of Respiratory Gases in the Blood**: Root Effect: Molecular Basis, Evolution of the Root Effect and Rete Systems; Root Effect: Root Effect Definition, Functional Role in Oxygen Delivery to the Eye and Swimbladder. **Vision**: Visual Adaptations to the Deep Sea.

Further Reading

Angel MV (1993). Biodiversity of the pelagic ocean. *Biological Conservation* 7: 760–772.

Angel MV (1997). What is the deep sea? In: Randall DJ and Farrell AP (eds.) *Deep-Sea Fishes*, pp. 1–41. San Diego: Academic Press.

Gibbs A (1995) Temperature, pressure and the sodium pump. The role of homeoviscous adaptation. Environmental and ecological biochemistry. *Biochemistry and Molecular Biology of Fishes* 5: 197–212.

Haldane JBS and Huxley J (1927) *Animal Biology,* 344pp. London: Oxford University Press.

Marshall NB (1979) *Deep-Sea Biology, Development and Perspectives,* 566pp. London: Blandford.

Priede IG, Bagley PM, Armstrong JD, Smith KL , Jr., and Merrett NR (1991) Direct measurement of active dispersal of food-falls by deep-sea dmersal fishes. *Nature* (London) 351: 647–649.

Priede IG, Smith KL, and Armstrong JD (1990). Foraging behavior of abyssal grenadier fish: Inferences from acoustic tagging and tracking in the North Pacific Ocean. *Deep-Sea Research* 37: 81–101.

Randall DJ and Farrell AP (eds.) (1997) *Deep-Sea Fishes*. 388pp. San Diego: Academic Press.

Swezey RR and Somero GN (1982) Skeletal muscle actin content is strongly conserved in fishes having different depths of distribution and capacities of locomotion. *Marine Biology Letters* 3: 307–315.

Torres, JJ, Belma BW, and Childress JJ (1979) Oxygen consumption rates of midwater fishes as a function of depth of occurrence. *Deep-Sea Research* 26: 185–197.

Torres, JJ and Somero GN (1988) Vertical distribution and metabolism of Antarctic mesopelagic fishes. *Comparative Biochemistry and Physiology B* 90: 521–528.

PHYSIOLOGICAL SPECIALIZATIONS OF DIFFERENT FISH GROUPS

Intertidal Fishes

Intertidal Habitats

PM Schulte, University of British Columbia, Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Tidal Cycles
Types of Intertidal Habitats
Adaptations to Changing Salinity
Adaptations to Changing Oxygenation
Adaptations to Changing Temperature
Other Environmental Factors
Further Reading

Glossary

Abiotic factors Nonliving chemical and physical factors in the environment (e.g., temperature and salinity).
Compatible solutes A variety of organic compounds (including sugars, polyols, amino acids, and their derivatives) that animals use to balance their internal osmolarity relative to external osmolarity without disrupting cellular functions. Also known as osmoprotectants.
Estuary A partially enclosed body of water formed where freshwater from rivers and streams mixes with seawater as the tides advance and recede.
Euryhaline Animals that physiologically adjust to a wide range of water salinity are considered euryhaline (eury 'broad'; haline 'salt'), in contrast to stenohaline animals.
Eurythermal Able to live over a wide range of temperatures.
Hypoxia Low partial pressures of oxygen in external or internal environments.
Hyperoxia Higher than normal partial pressure or content of oxygen in the external environment.
Mangrove swamp Coastal wetlands of tropical and subtropical regions (from ~25° N to 25° S) dominated by salt tolerant shrubs and trees, often (but not exclusively) mangroves of the genus *Rhizophora*.
Normoxia Normal oxygen partial pressure or content in the external environment. For aquatic systems, this is generally around 130 mmHg or similar to the oxygen content of air-saturated water at a particular temperature, salinity, and atmospheric pressure.
Osmolarity The total concentration of osmotically active particles in a solution.
Oxyconformer Organisms that allow their metabolic rate to fall as the environment becomes progressively hypoxic.
Oxyregulator Organisms that maintain their metabolic rate as the environment becomes progressively hypoxic.
Saltmarsh A shallow brackish or saltwater intertidal habitat dominated by salt-tolerant grasses and shrubs.
Stenohaline Describes an organism that cannot live in environments that undergo large changes in salinity.
Stenothermal Describes an organism that cannot live in environments that undergo large changes in temperature.
Vertical zonation A pattern observed in rocky intertidal habitats where different communities of animals, plants, and algae are found at different tidal heights, forming distinctive horizontal bands.

Introduction

Intertidal habitats are not only among the most productive in the world, but also among the most challenging for the fishes that live there. As the name suggests, these habitats are found in areas that are 'between the tides'. Because they are subject to the influence of the tides, intertidal habitats are highly variable in both space and time, and undergo large changes in a host of abiotic environmental variables including temperature, salinity, and oxygenation. In addition, fishes living in these habitats can be exposed to extremely turbulent water flows and may be periodically exposed to air as the tides recede. Because of their coastal location, intertidal habitats are also strongly impacted by human activities and can experience high levels of pollution due to urban or agricultural uses.

Tidal Cycles

Tides, the rhythmic rise and fall of sea levels, result from the competing actions of centrifugal force from the Earth's rotation and the gravitational forces of the Earth and the Sun. In most places on Earth, tides are semidiurnal, with two high tides and two low tides each day (**Figure 1**(**a**)). Semidiurnal tides are common on the Atlantic coast of North America, Europe, and Africa. There are a few locations (such as the Gulf of Mexico and parts of Southeast Asia) where tides are diurnal, with a single high tide and low tide each day (**Figure 1**(**b**)). Mixed tides, in which there are two high tides and two low tides each day each having a different magnitude, are common on the Pacific coast of North America (**Figure 1**(**c**)). **Figure 2** shows the distribution of these different kinds of tides around the globe.

The height of the tide varies depending on the phase of the Moon, the relative positions of the Moon and Sun, and among locations. Approximately twice each month, the Earth, Moon, and Sun align. At these times, which occur shortly after the new moon, the gravitational effects of the Moon and Sun reinforce each other and cause a higher high tide and a lower low tide. These recurring tidal extremes are called spring tides. The most modest tides, which occur when the Moon and the Sun are at 90° relative to the Earth, are called neap tides. Neap tides occur just after the first and last quarter moons. Although the heights of spring and neap tides differ within any given location, this variability is small relative to the huge differences in tidal heights in different locations around the globe. The difference in height between high tide and low tide (or the tidal range) can be as small as 0.3 m in some parts of the Mediterranean Sea to as great as 17 m in the Bay of Fundy, between Nova Scotia and New Brunswick on the Atlantic coast of Canada and in Ungava Bay on the Arctic Coast of Quebec in Northern Canada.

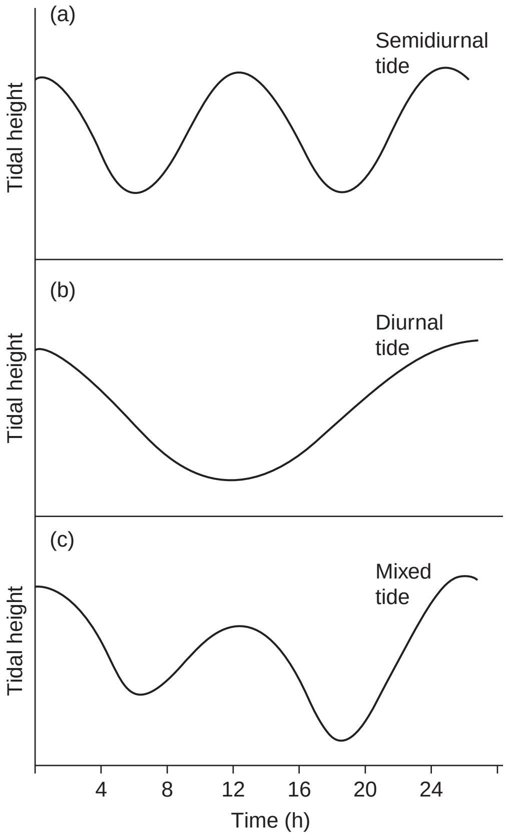


Figure 1 Types of tides. Semidiurnal tides (a) have two high tides and two low tides each day. Diurnal tides (b) have one low tide and one high tide each day. Mixed tides (c) have two high tides and two low tides each day, but their magnitude differs.

Types of Intertidal Habitats

Intertidal habitats include rocky shores, mudflats, salt marshes, and estuaries. The various types of intertidal habitats can be distinguished based on the substrate over which they occur (such as rock, mud, or sand). Rocky intertidal habitats are found in wave-swept areas, where there is sufficient turbulence to prevent sediments from accumulating on the rocky substrate. Rocky intertidal habitats are often steeply sided so the rise and fall of the tides result in a vertical gradient of the amount of time that an area spends out of the water. At the high end of the rocky intertidal, animals may be exposed to air through much of the tidal cycle, whereas at the low end of the rocky intertidal, animals may be submerged in seawater for much of the time. These differences in emersion time result in extreme differences in abiotic conditions over as little as a few meters of vertical distance. In the high intertidal, animals must cope with large changes in abiotic factors such as temperature, salinity, and oxygenation, whereas abiotic conditions are much less variable in the low intertidal. This difference in the variability of abiotic factors between the low and the high areas of the rocky intertidal results in a phenomenon known as vertical zonation (**Figure 3**), that is characterized by distinct horizontal bands composed of different communities of organisms that have different ranges of tolerance. For example, many fishes that live in the low intertidal zone are incapable of surviving the harsh conditions of the high intertidal, and only a few species of fishes are specialized for the extreme conditions of the very high rocky intertidal.

Most soft-substrate intertidal habitats occur in estuaries. The word estuary is derived from the Latin word *aestus*, meaning tides. An estuary can be defined as a partially enclosed body of water formed where freshwater

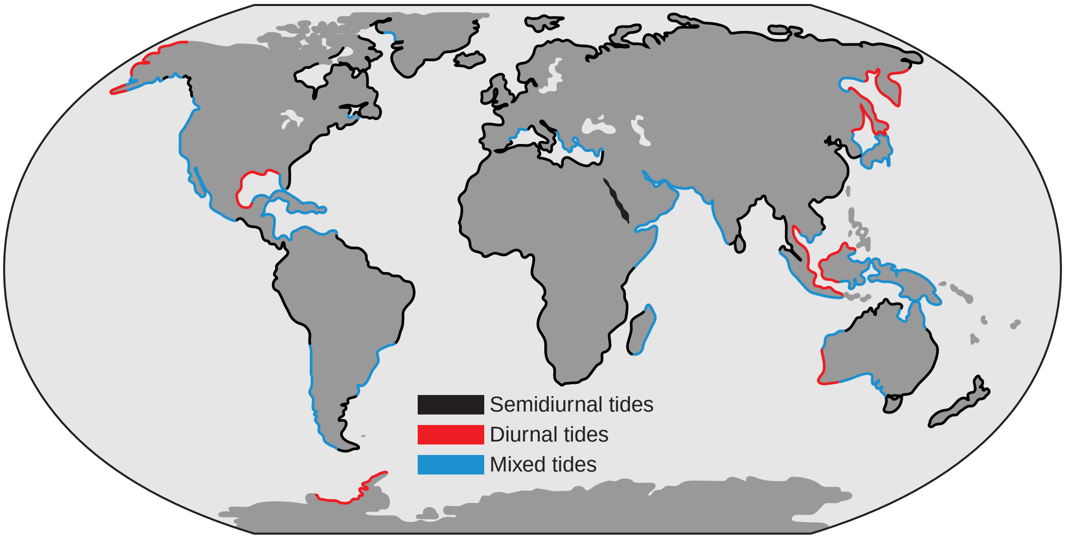


Figure 2 Global distribution of tidal types.

from rivers and streams mixes with seawater as the tides advance and recede. The substrate in an estuary is generally soft, because estuaries are typically sufficiently protected from the direct action of ocean waves to allow sediments to accumulate on the bottom in the form of mud or sand. Estuaries can be divided into a variety of sub-habitats. At the river end of the estuary, there is a zone that is not directly influenced by saltwater, but is indirectly influenced by the tides as incoming saltwater from the tides pushes the freshwater of the river upstream. Thus, these areas have an intertidal zone in which animals can be alternately submerged in freshwater or emerged in air, or in which animals are exposed to changing conditions of water flow. The middle portions of estuaries are generally submerged throughout the tidal cycle, but undergo substantial changes in salinity as seawater moves in and out of the estuary and mixes with freshwater as the tides advance and retreat. In temperate zones, these regions typically consist of a complex patchwork of salt marshes and mudflats (**Figure 4**). Salt marshes have a soft substratum, but have conditions that are suitable for the establishment of plants such as marsh grasses. Salt marshes can be important habitats for juvenile fish. In tropical regions, marsh grasses are replaced by mangroves, forming a mangrove swamp. Mudflats are areas with soft substrates that lack vascular plants, and are instead coated with blue-green algae and diatoms.

Mudflats, salt marshes, and mangrove swamps can be high in hydrogen sulfide (H_2S), a colorless, but extremely strong-smelling gas that is produced by sulfate-reducing bacteria that live in the anoxic regions of the sediment. This gas gives these habitats their characteristic 'rotten egg' smell. Hydrogen sulfide is extremely toxic because it binds tightly to iron molecules. It thus binds to and disrupts the function of critical molecules such as hemoglobin and the mitochondrial cytochromes,

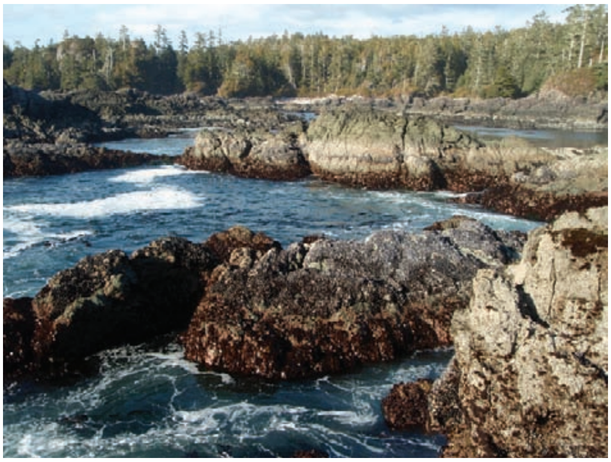


Figure 3 Vertical zonation in the rocky intertidal near Ucleulet, British Columbia, Canada. Photograph courtesy: Dr. Chris Harley.

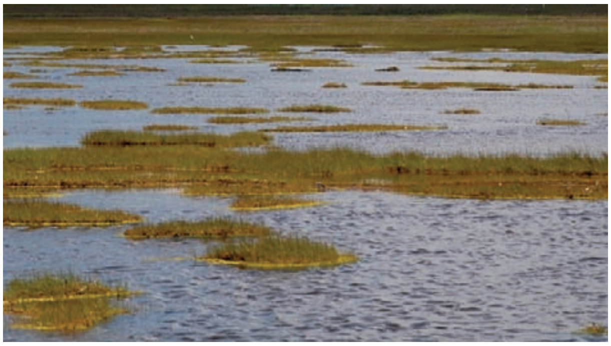


Figure 4 A salt marsh on the Atlantic coast of North America near Tuckerton New Jersey, USA. Photograph courtesy: Kristen Haakons.

preventing both oxygen delivery and oxygen utilization. In fact, hydrogen sulfide is even more toxic than cyanide, and can cause 50% inhibition of cytochrome oxidase at concentrations as low as $1\,\mu\mathrm{mol\,L}^{-1}$. Many mudflat and mangrove swamp fish remain on the surface of the sediment and so are not exposed to toxic levels of this gas. However, some mudflat fishes, such as gobies, dig burrows deep into the mud, which can result in exposure to appreciable concentrations of hydrogen sulfide. For example, hydrogen sulfide levels in the burrows of goggle-eyed mudskippers (*Boleophthalmus boddaerti*), a mangrove swamp goby, can be as high as 0.3 mM. This species possesses efficient mechanisms for detoxifying hydrogen sulfide within its tissues. In contrast, other mangrove swamp dwellers, such as the mangrove killifish (*Rivulus marmoratus*), will typically emerge from water into air when they encounter high levels of hydrogen sulfide.

Adaptations to Changing Salinity

Salinity is the term used to describe the total concentration of dissolved salts in water. Biologists and oceanographers typically express salinity in units of parts per thousand (ppt), which roughly translates to the number of grams of salt per liter of water. Full-strength seawater has a salinity of approximately 35, while distilled water has a salinity of 0. The salinity of the water in intertidal habitats can vary from that of full-strength seawater at high tide to that of nearly freshwater when there is substantial input of freshwater from rivers, streams, or rainwater. Intertidal habitats can even become hypersaline (more salty than seawater) as a result of evaporation of water in isolated pools at low tide.

Changes in salinity are extremely challenging to animal life because the salt concentration of the external medium affects the distribution of water across cell membranes. When in freshwater, fish tend to gain water and lose ions as a result of passive movements along concentration gradients, which must be compensated for through active ion uptake across the gills and intestine (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Ion Transport in Freshwater Fishes). When in saltwater, fish tend to lose water and gain ions, which must be compensated for by drinking and active ion extrusion (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). Thus, fish must use very different osmoregulatory mechanisms when in freshwater and in seawater. In fact, most fish are stenohaline, and are unable to alter their osmoregulatory mechanisms when experimentally transferred between freshwater and saltwater. Only a relatively small number of fish species are euryhaline and can survive and thrive across a range of salinities. Because of the changing salinities that are characteristic of intertidal habitats, most intertidal fishes are euryhaline.

Most elasmobranchs are stenohaline marine species with an extracellular fluid and cytoplasm osmolarity similar to that of seawater by retaining the compatible solutes urea and trimethylamine oxide (TMAO; **see also Chondrichthyes**: Physiology of Sharks, Skates, and Rays). Only 13 species of truly euryhaline elasmobranchs are known (there are about 35 species of obligate freshwater elasmobranchs and about 100 species that can enter brackish water for brief periods). Euryhaline elasmobranchs allow their body osmolarity to fall from ~1000 mOsM in seawater to ~600 mOsM in freshwater by decreasing urea retention. Note, however, that plasma osmolarity remains substantially higher than the osmolarity of the external medium, and thus euryhaline elasmobranchs in freshwater will tend to passively gain water, which must be removed by producing large amounts of dilute urine.

In contrast, teleost fishes are osmoregulators that maintain their tissue osmolarity relatively constant independent of environmental osmolarity. Although limited data are available for intertidal fishes, it has been suggested that these species have low gill and/or skin permeability to water and ions, which would reduce the costs associated with osmoregulation in a changing environment. In addition, at least some intertidal and estuarine fish, including mudskippers and killifish, are capable of rapidly (30 min to 3 h) and reversibly remodeling the structure of their osmoregulatory tissues in response to changes in external salinity, and changing the way that ions are transported across these tissues on very short timescales, which may help these species cope with their fluctuating environments.

Adaptations to Changing Oxygenation

The level of oxygen in water is typically described using the terms normoxia, hypoxia, and hyperoxia. Normoxic waters are air-saturated and have a partial pressure of oxygen of approximately 130 mmHg. Hypoxic waters have lower oxygen partial pressures, while hyperoxic waters have higher partial pressures. At high tide, rocky intertidal habitats have oxygen concentrations that are similar to the nearby seawater, and are generally normoxic or slightly hyperoxic. In contrast, at low tide, rocky intertidal habitats can have exceptionally variable oxygen partial pressures, particularly high in the rocky intertidal, where the pools are cut off from ocean water for long periods of time (**Figure 5**). During the day, when algae are photosynthesizing and producing oxygen, oxygen partial pressures in the water can exceed 400 mmHg (or 400% air saturation). In contrast, at night, when algae and other intertidal organisms are respiring, oxygen partial pressure

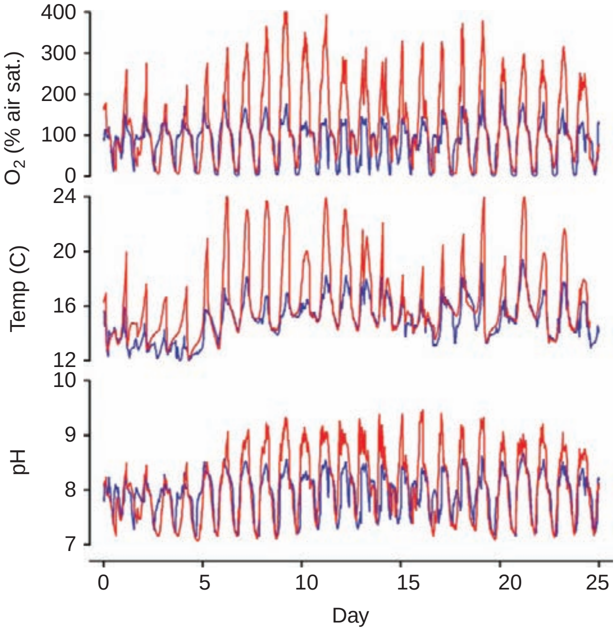


Figure 5 Variation in oxygen, temperature, and pH in tidepools in Bamfield, British Columbia, Canada, at two tidal heights during the tidal cycle. Red lines are data for a high intertidal pool. Blue lines are data for a low intertidal pool. Data courtesy of Dr. Jeffrey Richards.

can drop to near zero. Similar fluctuations in oxygenation occur in salt marshes and mangrove swamps.

Relatively little is known about the behavioral and physiological responses of intertidal fishes to hyperoxia, although hyperoxia is known to cause reductions in ventilatory rate and increases in the production of oxygen-free radicals and free-radical scavenging enzymes such as glutathione peroxidase. In contrast, responses to aquatic hypoxia have been studied intensively in intertidal fishes. Fishes can be divided into two broad groups with respect to their responses to aquatic hypoxia. Oxyconformers allow their metabolic rate to fall as environmental oxygen decreases. Oxyregulators maintain their metabolic rate more or less constant as oxygen falls up to a point called the critical oxygen tension (P_{crit}), when metabolic rate starts to decline. Note, however, that these two groups represent the extreme ends of a spectrum of strategies, and different species of intertidal fish vary greatly in terms of the types of strategy utilized. Whatever the strategy utilized, fishes found in the high intertidal on rocky shores, and fishes that live in salt marshes are typically extremely resistant to environmental hypoxia.

One particularly interesting adaptation to low oxygen that is observed in intertidal fish is that of air breathing or aerial emergence. Intertidal fish exhibit a variety of strategies for air breathing and emergence. Some fish remain submerged, but skim oxygenated water from just below the surface where oxygen has diffused into the water from the air. This strategy is known as aquatic surface respiration (ASR) (**see also Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia). A fish performing ASR still ventilates its gills with water, but takes advantage of a more oxygenated microenvironment within a generally hypoxic zone. Many species of intertidal and estuarine fish exhibit increases in ASR behavior as oxygen tensions in the water decline. Some intertidal fishes are capable of efficiently extracting oxygen from air, and can even maintain metabolic rates in air as high as those seen when the fish are in the water. Unlike many freshwater air-breathing fish, however, most intertidal air-breathing fish do not have specialized air-breathing organs (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). Instead, most intertidal species rely on gas exchange across the gills or skin when respiring in air, and may also use the buccal or opercular cavity, or possibly the stomach.

Air-breathing intertidal fish utilize one of the two main behavioral strategies associated with air breathing. The so-called remainers do not actively emerge into the air. Instead, these species simply allow themselves to be stranded in air as the tide recedes. At low tide, these fishes can be found in moist areas under rocks or seaweed, where they are protected from desiccation. In contrast, active emergers intentionally crawl out of the water when it becomes hypoxic or otherwise inhospitable. The champions of this strategy are the mudskippers (family Gobiidae) and the rockskippers (family Blennidae). These species spend a substantial amount of time out of the water (**see also Air-Breathing Fishes**: The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction). For example, mudskippers feed and even engage in territorial displays when out of water. Mudskippers have a number of behavioral, anatomical, and physiological adaptations that allow them to function well in air. They have muscular fins that allow them to crawl or leap along the ground and unusually thick gills, which are stabilized to prevent the fine secondary lamellae from collapsing when the fish is in air. These thick gills, however, decrease the fish's ability to extract oxygen from the water, and may decrease their ability to survive if trapped below the surface in hypoxic water. As a result, the adaptations that allow these fish to function well in air also tend to cause them to emerge from the water sooner than do other fish species when water becomes progressively hypoxic. Some species of mudskipper will trap bubbles of air in their enlarged opercular cavities to allow them to remain submerged in hypoxic water (**see also Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes).

At high tide, many species of mudskipper retreat into burrows in the mud, and the water in these burrows can be quite hypoxic. Mudskippers maintain one or more air chambers within their burrows, which they supply with air from the surface (carried down in their large opercular chambers). Mudskippers place their eggs in these air chambers, allowing the eggs to develop in a high oxygen environment. When the eggs are ready to hatch, the male mudskipper (which guards the burrow) floods the air chamber with water. This submergence induces the eggs to hatch. Aerial incubation of the eggs is a common feature in intertidal fishes. For example, the salt marsh-dwelling common killifish (*Fundulus heteroclitus*) lays its eggs in mussel shells or at the base of seagrasses at the extreme high tide of the tidal cycle (i.e., the spring tide). The subsequent tides of the tidal cycle are not as high, so the eggs incubate in air. At the next spring tide, the eggs are submerged, and the drop in oxygen associated with submergence acts as a cue to cause the eggs to hatch.

Adaptations to Changing Temperature

In most intertidal zones, seawater temperatures are relatively stable throughout the year and from day to night compared with the potentially highly variable temperatures of freshwater and aerially exposed habitats. Thus, fishes living high in the rocky intertidal or in the freshwater/seawater mixing zone of estuaries can be exposed to extremely large temperature changes during a tidal cycle. **Figure 5** shows the temperature changes in tidepools at various heights in the rocky intertidal on Canada's Pacific Coast. Change in temperature can have profound effects on the physiology of ectotherms such as fish (**see also Temperature**: Effects of Temperature: An Introduction), in the absence of physiological specializations to cope with these fluctuations. As a result of the variable nature of their habitat, intertidal fish are remarkably eurythermal (able to cope with a wide range of temperatures). For example, the common killifish, *F. heteroclitus*, which is found in salt marshes along the east coast of North America, can be acclimated to temperatures ranging from ~2 °C to over 30 °C, and can tolerate abrupt changes in temperatures (e.g., from 5 °C to 33 °C, or even up to 37 °C, if the temperature ramps up gradually over the course of an hour or so). The role of heat shock proteins in protection from high temperatures (**see also Temperature**: Heat Shock Proteins and Temperature) has been intensively studied in several species of intertidal fish. Some species of intertidal fish appear to maintain unusually high levels of the constitutively expressed heat shock protein hsc70, which may serve to protect cellular functions against acute heat stress.

Other Environmental Factors

In addition to changes in salinity, oxygenation, and temperature, intertidal habitats also undergo changes in CO_2 and pH through the tidal cycle (**Figure 5**). In the confined environment of a tidepool, or within the burrow of a mudflat fish, respiration by animals, plants, and algae can cause large increases in the partial pressure of carbon dioxide. In contrast, during the day, photosynthesizing plants, algae and bacteria consume carbon dioxide. As a result of these processes, the partial pressure of carbon dioxide in an intertidal pool can vary more than 1000-fold. The partial pressure of carbon dioxide also affects the pH of water, because as CO_2 dissolves in water it forms carbonic acid (H_2CO_3), then bicarbonate (HCO_3^-) and a proton (H^+). Seawater is well buffered, but despite this high buffering capacity, the pH of an intertidal pool can vary between a pH of 6.9 and 10.3 across the tidal cycle because of the large changes in the partial pressure of CO_2 resulting from the combination of photosynthesis (removing CO_2) and respiration (adding CO_2). Changes in pH can cause damage to skin and to the surface of the gills, and are an important sublethal stressor for fish. As with other stressors, intertidal fish are typically fairly resistant to the effects of changes in pH and can tolerate a wide range of pH conditions.

See also: **Air-Breathing Fishes**: Circulatory Adaptations for Air-Breathing Fishes; The Biology, Diversity, and Natural History of Air-Breathing Fishes: An Introduction. **Behavioral Responses to the Environment**: Behavioral Responses to Hypoxia. **Chondrichthyes**: Physiology of Sharks, Skates, and Rays. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts; Mechanisms of Ion Transport in Freshwater Fishes. **Temperature**: Effects of Temperature: An Introduction; Heat Shock Proteins and Temperature.

Further Reading

Ballantyne JS and Robinson JW (2010) Freshwater elasmobranchs: A review of their physiology and biochemistry. *Journal of Comparative Physiology B* 180: 475–493.

Denny MW and Gaines SD (2007) *Encyclopedia of Tidepools and Rocky Shores*. Berkeley, CA: University of California Press.

Elliot M and Hemmingway KL (2002) *Fishes in Estuaries.* London. UK: Blackwell Science.

Horn MH, Martin KL, and Chotkowski MA (1999) *Intertidal Fishes: Life in Two Worlds*. San Diego, CA: Academic Press.

Ip YK, Kuah SSL, and Chew SF (2004) Strategies adopted by the mudskipper *Boleophthalmus boddaerti* to survive sulfide exposure in normoxia and hypoxia. *Physiological and Biochemical Zoology* 77(5): 824–837.

Ishimatsu A, Yoshida Y, Itoki N, *et al.* (2007) Mudskippers brood their eggs in air but submerge them for hatching. *Journal of Experimental Biology* 210: 3946–3954.

Little C (2000) *The Biology of Soft Shores and Estuaries.* Oxford, UK: Oxford University Press.

Little C, Williams G, and Trowbridge C (2009) *The Biology of Rocky Shores*, 2nd edn. Oxford, UK: Oxford University Press.

Martin KLM (1995) Time and tide wait for no fish: Intertidal fishes out of water. *Environmental Biology of Fishes* 44: 165–181.

McMahon BR (1988) Physiological responses to oxygen depletion in intertidal animals. *American Zoologist* 28: 39–53.

APPLIED ASPECTS OF FISH PHYSIOLOGY

Cellular, Molecular, Genomics, and Biomedical Approaches

Contents

Culture of Fish Cell Lines
Pigment Genes and Cancer Genes
Evolution of Fish Genomes
Fish as Model Organisms for Medical Research
Transgenesis and Chromosome Manipulation in Fish
Physiology of Triploid Fish
Growth Hormone Overexpression in Transgenic Fish
Molecular and Chromosomal Aspects of Sex Determination
Molecular Fish Pathology
Germ Cell Migration and Trans Sex
Global Gene Expression Profiling in Fish

Culture of Fish Cell Lines

NC Bols and A Kawano, University of Waterloo, Waterloo, ON, Canada

LEJ Lee, Wilfrid Laurier University, Waterloo, ON, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Sources
Equipment
Culture Vessels
Growth Media
Growth Temperature
Passaging
Cryopreservation
Applications
Further Reading

Glossary

Basal media A solution of buffering agents and of nutrients, which include a hexose, bulk ions, trace elements, amino acids, and vitamins.

Cell line Begins with the successful passaging of a primary culture.

CO_2 incubator A chamber that maintains temperatures a few degrees above ambient to approximately 40 °C and allows the injection of one or more gases, usually CO_2.

Cryopreservation Storing cells, usually in 10% dimethyl sulfoxide, at temperatures below −80 °C.

Culture vessels Commonly plastic flasks and also glass or plastic petri dishes.

Ecotoxicology An interdisciplinary science dealing with toxicants in the environment.

Epithelial-like cell line Majority of the cells attach and spread on the growth surface to have shapes like irregular patio blocks.

Fibroblast-like cell line Majority of the cells attach and spread on the growth surface to have long bipolar shapes.

Finite cell line Cells can undergo a limited number of population doublings.

Heat inactivation of serum Incubation at 56 °C for 30 min.

Immortal cell line Cells can be propagated indefinitely and are as same as a continuous cell line.

Laminar flow hood A cabinet in which sterile air is created by passing the air through a high-efficiency particle air filter in smooth or laminar flow and blown across a work area to create a sterile environment in which cultures can be manipulated.

Line Same as cell line.

Macrophage-like cell line	Majority of the cells express some macrophage properties, such as being highly phagocytic and having major histocompatibility class II proteins on the cell surface; often, proliferate loosely attached to the growth surface.
Passaging	Transfer of cells from one culture vessel to another. This is synonymous with subculturing, subcultivating, or splitting.
Primary cultures	A culture initiated directly from the cells, tissues, or organs of an animal.
Proliferation	An increase in cell number through cell division.
Senescence	Loss by cell lines of the ability to be propagated.
Serum	The fluid fraction after blood has been allowed to coagulate and trap blood cells with clotting factors into a solid clot.
Spheroid	An aggregate of cells in suspension within a culture.
Spontaneous immortalization	Cells acquire the ability to be grown indefinitely through only the routine maintenance of the cells in culture.
Trypsin	A protease from the pancreas that is used to passage cells.
Xenobiotic metabolism	Changes to foreign compounds brought about by cells or organisms.

Introduction

Cell lines are cultures of animal cells that can be propagated repeatedly and sometimes indefinitely. They arise from primary cell cultures. Primary cultures are initiated directly from the cells, tissues, or organs of animals and are typically used in experiments within a few days. By convention, the passaging or subcultivation of a primary culture begins a cell line. However, not all primary cultures yield cell lines. Instead, the cells of some subcultivated cultures die off slowly. Why the cells of other cultures keep on growing and allow these cultures to be continuously subcultivated is a complex and incompletely understood subject. The subject is intensively studied with mammalian cells because of the relevance of this to the mechanisms of cellular senescence and the origins of cancer cells. In the case of mammalian cell lines, some are finite. They can only be subcultivated a limited number of times before they senesce. Yet other mammalian cell lines are continuous or immortal and can be grown indefinitely. Continuing with the case of mammals, some primary cultures or finite cell lines can be experimentally immortalized to become continuous cell lines.

By contrast, with fish, little is known about cellular senescence and immortalization. Fortunately and surprisingly, many fish cell cultures have been subcultivated indefinitely. These cell lines are said to have become spontaneously immortalized. This is how nearly all fish cell lines have arisen. Most of these have been derived from bony fish, but they are called simply fish or piscine cell lines. The development of fish cell lines is described elsewhere (see the 'Further reading' section). Here, the sources and types of fish cell lines are noted and what is needed to maintain these cell lines is described, ending with a brief overview of how the cell lines are being used.

Sources

Like mammalian lines, fish cell lines can be stored frozen, aiding their distribution to researchers around the world. How piscine cell lines are cryopreserved is discussed later in the 'Cryopreservation' section. A recent list of repositories with fish cell lines has been presented (see the 'Further reading' section). Relative to the demand for human and rodent cell lines, the request for piscine cell lines is limited and so the number of fish lines in these cell banks is small. Despite the restricted choice, repositories are the best cell line sources. They assure quality and reliable delivery, but of course at a price.

Cell lines have been developed from most but not all tissues and organs of bony fish, with only a few from tumors, usually liver tumors. Most piscine cell lines are yet to be analyzed for differentiated properties and are described simply as being either epithelial-like or fibroblast-like. Exceptions are some lymphocyte and macrophage cell lines. **Figure 1** illustrates the morphologies in cultures of salmonid epithelial-, fibroblast-, and macrophage-like cell lines. Other properties commonly examined are the optimal growth conditions and the karyotype. Although diploid fish

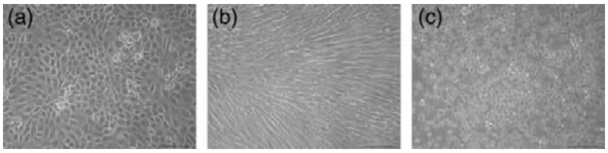


Figure 1 Phase-contrast microscopy appearance of cultures of an epithelial-like (a), a fibroblast-like (b), and a macrophage-like (c) cell line. The lines are RTgutGC from the epithelial layer of the rainbow trout intestine (a), AS20imf from the subepithelial layer of the Atlantic salmon intestine (b), and RTS11 from the rainbow trout spleen (c). Scale = 100 μm.

cell lines do exist, most cultures are heteroploid: they contain cells with chromosome numbers that are not an exact multiple of the haploid number.

Approximately 10 times as many fish cell lines have been described in the literature as have been placed in repositories. Lists of these have been prepared at several points over the last 30 years. Whereas ~29 000 species of bony fish in over 500 families have been classified, cell lines have been prepared from only about 75 species in 35 families. In the past, methods of confirming that a cell line was indeed from the stated starting species were problematic, but now species confirmation can be done reliably by DNA barcoding. This has revealed at least one surprise: epithelioma papulosum cyprini (EPC), which was thought to be a carp (*Cyprinus carpio*) cell line, is fathead minnow (*Pimephales promelas*). Over 10 different cell lines have been developed from each of several species, including catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), and zebrafish (*Danio rerio*). The total number of fish cell lines in the literature is now reaching close to 200, but many of these have likely been lost. Yet others are still available from the community of scientists developing and using piscine cell lines.

Whether from a repository or a colleague, the lines can be obtained as either a vial of frozen cells or as a flask of living cells. Sending flasks of cells is often easier because vials need to be embedded in dry ice, which imposes shipping restrictions and increases the shipping cost. Sending flasks of cell lines from coldwater fish in the summer might require placing the flask on an ice pack, but this poses no shipping problems. Getting living cultures is also easier. The cultures can often be subcultivated shortly after being received, and the problem of thawing the cells, the success of which can be variable, is avoided.

Equipment

At least three pieces of equipment are required to routinely maintain piscine cell lines. An inverted phase microscope is needed in order to visually inspect cultures. For passaging, a sterile environment is needed in order to open culture vessels and to remove or add cells without contaminating cultures with microbes. This is achieved in the working area of a laminar flow hood, which can be either vertical or horizontal, with the latter being less expensive. A vertical hood provides a current of sterile air between the worker and working area, protecting the worker from being exposed to any human pathogen that might inadvertently be associated with the cells. As there is no record of fish cell cultures containing human pathogens, a horizontal hood is often used. The third piece of equipment is a low-speed centrifuge to collect cells either for routine subcultivation or to set up experimental cultures. As discussed below, an incubator may or may not be required.

Culture Vessels

A vast array of plastic tissue culture ware has been developed for mammalian cells and nearly all these items work well with fish cell lines. Some of these items, such as flasks and petri dishes, are used for routinely growing cells, whereas others are used to monitor a particular endpoint. For the routine growth of piscine cell lines, nonvented flasks are favored because the loss of medium through evaporation is less. For RTS11, the most consistent growth is achieved with 12.5 cm^2 flasks, whereas for most other salmonid cell lines, 75 cm^2 flasks are convenient. In these, the cells grow to completely cover the flask surface and from a single flask, several multiwell plates can be set up for experimental purposes. With flasks >75 cm^2, RTL-W1 and RTgill-W1, and rainbow trout cell lines, are slow to cover the growth surface and often leave unfilled patches in the center.

Most plastic vessels are treated to promote animal cell attachment and proliferation, but some have hydrophobic surfaces to keep the cells in suspension. All these work for fish cell lines. In vessels with hydrophobic surfaces, suspended epithelial- and fibroblast-like cell lines aggregate over a few hours to form spheroids (**Figure 2**). Fish cells also attach to glass petri dishes. However, salmonid cell lines have been found to spread poorly in Lab Tek Chamber Slides (Nunc) with a glass surface, but do spread well when these slides have a plastic Permanox surface.

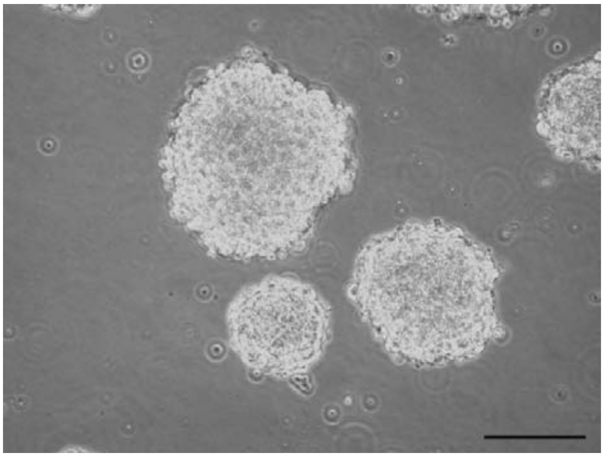


Figure 2 Phase-contrast microscopy appearance of spheroids formed in Nunc low-bind tissue culture dishes (coating of phospholipid polymer, 2-methacryloxyloxyethyl phosphorylchloline). The spheroids were initiated by adding 2×10^6 cells of the rainbow trout spleen stromal cell line (RTS34st) into 60 mm low-bind dishes. The medium was L-15 with 10% FBS, and after 24 h at room temperature, the culture was photographed. Scale = 100 μm.

Growth Media

The complete growth medium for the routine propagation of piscine cell lines has two essential parts, a basal medium and a supplement, with antibiotics being optional.

The basal medium constitutes an aqueous solution of buffering agents and of nutrients, which include a hexose, bulk ions, trace elements, amino acids, and vitamins. Basal media can be purchased, already made sterile in tissue-culture-grade water, or with all the constituents packaged as powders that need only be mixed with water. Some basal media are sold without glutamine because of its instability, and these are completed just before use by adding aliquots of a sterile concentrated glutamine solution (200 mM). Many different basal media are available commercially, and all were originally developed and optimized for mammalian cells. Fortunately, cells from other vertebrates, including both freshwater and marine fish, do well in them.

The choice of basal media for fish cells usually revolves around the availability of a CO_2 incubator, which is necessary to support the most common type of buffering system in basal media, sodium bicarbonate ($NaHCO_3$). For $NaHCO_3$ to act as buffer, a CO_2-rich atmosphere, usually 5% CO_2 and 95% air, is required and is provided for by a CO_2 incubator. Eagle's minimum essential medium (MEM) in Earle's salts is a basal medium that requires a CO_2 atmosphere and has been used frequently in the past to successfully grow a variety of fish cell lines. However, the use of CO_2 incubators for temperatures below room temperature is problematic for several reasons. As purchased, a CO_2 incubator can only raise the temperature, so a means of cooling has to be found for lower temperatures. The solubility of CO_2 increases at low temperatures, causing media to become acidic.

Two groups of basal media maintain their buffering capacity under normal atmospheric conditions (~0.03% CO_2) and are used to grow piscine cell lines. One group was developed for CO_2 incubators with slight modification. For example, MEM is made up in Hank's salts, which maintains the pH under atmospheric conditions. Another modification is the addition of organic buffers, such as HEPES. Many commercial basal media include either 15 or 25 mM HEPES plus the original concentration of sodium bicarbonate. Although buffering is usually optimal in a 5% CO_2 atmosphere, fish cells grow under atmospheric conditions. Another basal media group was formulated specifically for use in free gas exchange with air. The oldest is Leibovitz's L-15, which maintains physiological pH through a combination of salts, high basic amino acid concentrations, and galactose in place of glucose. A more recent addition is CO_2-independent medium (Gibco or Invitrogen). Unlike L-15, it contains sodium bicarbonate. Most fish cell lines are grown in L-15, but a few fish cell lines maintained in CO_2-independent medium grew well. These basal media are used without a CO_2 incubator.

The most common supplement is serum. Sterile sera from a few farm animals and sometimes fish sera are available commercially. Serum contains hundreds of proteins and is incompletely characterized, so it is described as an undefined supplement. No defined media have been reported yet that consistently support the proliferation of a piscine cell line through many passages.

Serum has to be added to basal media to improve fish-cell proliferation. For example, Atlantic salmon (*Salmo salar*) cell numbers slowly decline in L-15 over days to weeks (**Figure 3**). Yet, when L-15 is supplemented with 10% fetal bovine serum (FBS), cell number increases approximately eightfold over 2 weeks (**Figure 3**). Indeed, FBS with or without heat inactivation is the most common serum used for routine fish-cell growth. The usual FBS concentration is 10%, but ranges from 5% to 30% depending on the cell line. Sera from calves and horses are less expensive and infrequently used because of slower growth. Surprisingly, fish sera are rarely used, perhaps because FBS is cheaper and fish sera were found to be sometimes cytotoxic to fish cells.

For the routine maintenance of cell lines, antibiotics are added to media. Usually, these are penicillin at 100 IU with streptomycin at $100\,\mathrm{ug\,ml^{-1}}$ (penicillin/streptomycin). An alternative is to use gentamycin at $50\,\mathrm{ug\,ml^{-1}}$. Less frequently, an antifungal agent, amphotericin (fungizone), is added at variable concentrations. The use of antibiotics is often discouraged because consistent antibiotic use can lead to the development of resistant microbes and hide poor aseptic techniques.

Growth Temperature

The temperature range over which fish cells divide in culture is referred to as the proliferation zone and is wide. They tolerate an even wider temperature range,

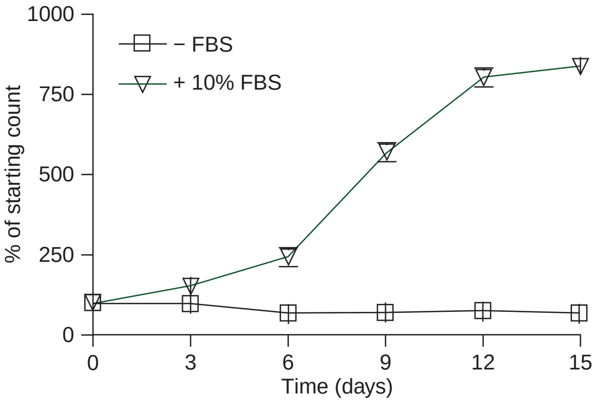


Figure 3 Change in cell number over time at room temperature for cultures of AS20imf in Leibovitz's L-15 either without a supplement (a) or with a supplement of 10% FBS (b). For each time point, cell number was counted with a Coulter counter in three replicate wells of a 12-well plate. The results are expressed as a percentage of the starting cell number, which was ~5.0×10^4 cells.

which has been defined as the endurance zone. The temperature chosen for a cell line depends on the purpose of the cultures, the species, and the availability of incubators. To maximize cell production, an optimal growth temperature should be selected. For cold-water fish, like salmon, this is in the 20–23 °C range; and for warm-water fish, like zebrafish, this is 26–30 °C. If the purpose is to just maintain the cultures for later use, lower temperatures allow the cells to slowly proliferate, reducing culture maintenance.

Nearly all fish cell lines grow at room temperature, which alleviates the need for an incubator or controlled rooms at other temperatures. The incubator type depends on the basal media. With L-15 or CO_2-independent media, a regular incubator can be used. However, if the temperatures are high and the incubator is not humidified, medium can rapidly evaporate from petri dish cultures, increasing the osmolarity and slowing cell growth. Therefore, in this situation and also for room temperature without an incubator, unvented flasks with the caps tightly closed are recommended. When buffering of the basal media is done only with sodium bicarbonate, a CO_2 incubator must be used.

Passaging

Passaging, which is also referred to as subculturing, subcultivating, or splitting, is the transfer of cells from one culture vessel in which the cells have been growing for days or weeks to two or more new culture vessels in which they will again be grown for days or weeks. This is routine maintenance and expands cell number. It is usually done in flasks.

Some fish white-blood cell lines, such as RTS11, grow in suspension or loosely attached to the growth surface and are the easiest to passage. A sterile cell scraper gently dislodges attached cells into the medium with the cells already in suspension. A pipette then removes aliquots of cell suspension into fresh medium.

In contrast to the lymphoid cell lines, most piscine cell lines are anchorage dependent and cells are removed from the flask growth surface before passage with or without enzymes as per commercially available step-by-step protocols. With fish cell lines, these steps are done at room temperature or lower. Trypsin is the most common enzymatic method, but the monolayer of cells must be rinsed with ethylenediaminetetraacetic acid to remove divalent cations prior to 0.01% trypsin being added. Trypsin usually causes the cells to round up and start detaching within 5 min, at which point medium with serum is added to stop the trypsin. Sometimes with older cultures, cells take longer to detach and sometimes cells clump on the addition of serum. Alternative methods include use of TryplE Select or Express (Invitrogen), trypsin-like enzymes produced by microbes. These are more convenient to use than trypsin and among their advantages is that serum need not be added to stop their actions. Nonenzymatic methods of splitting cultures act by chelating divalent cations. A commercial solution for this is cell dissociation solution nonenzymatic (Sigma), which is used to successfully passage several fish cell lines. Regardless of the dissociation method, a split ratio of one old confluent flask to two new flasks is common for slow-growing adherent cell lines from cold-water fish. With salmonid cell lines at room temperature, cultures can be commonly split one to two every 7–10 days. Higher split ratios can be used for more vigorously growing cell lines, such as EPC.

Cryopreservation

Cryopreservation of cell lines provides a supply of cells for some later date and involves the same methods used for mammalian cell lines. The usual cryoprotectants are dimethyl sulfoxide and glycerol at 5–10% (v/v) in the complete growth medium. Indefinite storage is done in Dewars of liquid N_2 at $-196\,^{\circ}\mathrm{C}$, but cells store for approximately a year in electrical freezers at $-80\,^{\circ}\mathrm{C}$. Cells are usually frozen slowly in several ways. One uses programmable coolers set at a cooling rate of often $-1\,^{\circ}\mathrm{C\,min^{-1}}$, but these are not usually available. Alternatively, vials in a polystyrene box, to ensure slow freezing, are placed in a -70 to $-90\,^{\circ}\mathrm{C}$ freezer for at least 2 h before being transferred to liquid N_2. A third strategy is to place vials in the atmosphere above the liquid N_2 in the Dewar and then after 2–24 h lower them into the liquid N_2. Rapid thawing and speedy cryoprotectant removal are key to getting high proportions (70–90%) of living cells, although even low viability (~10%) can lead to proliferating cultures of some fish cell lines.

Applications

The uses to which fish cell lines are being put keeps increasing. The first and most common application is to study viral diseases. The fastest growing area of use is in toxicology and ecotoxicology. Other research disciplines making use of fish cell lines include biomedicine, immunology, and animal physiology. Some applications defy easy classification, such as the use of a goldfish fibroblast cell line to explore the production of fish filets for long voyages in space.

See also: **Aquaculture**: Physiology of Fish in Culture Environments. **Toxicology**: The Toxicology of Organics in Fishes.

Further Reading

Babich H and Borenfreund E (1991) Cytotoxicity and genotoxicity assays with cultured fish cells: A review. *Toxicology In Vitro* 5: 91–100.

Benjaminson MA, Gilchriest JA, and Lorenz M (2002) *In vitro* edible muscle protein production system (MPPS): Stage 1, fish. *Acta Astronautica* 51: 879–889.

Bols NC, Dayeh VR, Lee LEJ, and Schirmer K (2005) Use of fish cell lines in toxicology of fish. In: Moon TW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes – Environmental Toxicology*, vol. 6, pp. 43–84. Amsterdam: Elsevier.

Bols NC, DeWitte-Orr SJ, Brubacher JL, Dixon B, and Ganassin RC (2003) Cell culture approaches in aquatic immunotoxicology. In: Mothersill C and Austin B (eds.) *In Vitro Methods in Aquatic Toxicology*, pp. 399–420. London: Springer.

Bols NC and Lee LEJ (1991) Technology and uses of cell cultures from the tissues and organs of bony fish. *Cytotechnology* 6: 163–187.

Bols NC and Lee LEJ (1994) Cell lines: Availability, propagation and isolation. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes*, vol. 3, pp. 145–159. Amsterdam: Elsevier.

Bols NC, Mosser DD, and Steels GB (1992) Temperature studies and recent advances with fish cells *in vitro*. *Comparative Biochemistry and Physiology* 103A: 1–14.

Clem LW, Bly JE, Wilson M, *et al.* (1996) Fish immunology, the utility of immortalized lymphoid cells – a mini review. *Veterinary Immunology Immunopathology* 54: 137–144.

Cooper JK, Sykes G, King S, *et al.* (2007) Species identification in cell culture: A two-pronged molecular approach. *In Vitro Cellular and Developmental Biology* 43: 344–351.

Dayeh VR, Schirmer K, Lee LEJ, and Bols NC (2003) The use of fish-derived cell lines for investigation of environmental contaminants. *Current Protocols in Toxicology* 1.5.1–1.5.17.

Fan L, Crodian J, and Collodi P (2004) Culture of embryonic stem cell lines from zebrafish. *Methods in Cell Biology* 76: 151–160.

Fent K (2001) Fish cell lines as versatile tools in ecotoxicology: Assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicology In Vitro* 15: 477–488.

Fryer JL and Lannan CN (1994) Three decades of fish cell culture: A current listing of cell lines derived from fishes. *Journal of Tissue Culture Methods* 16: 87–94.

Hightower LE and Renfro JL (1988) Recent applications of fish cell culture to biomedical research. *Journal of Experimental Zoology* 248: 290–302.

Lannan CN (1994) Fish cell culture: A protocol for quality control. *Journal of Tissue Culture Methods* 16: 99–108.

Lee LEJ, Dayeh VR, Schirmer K, and Bols NC (2009) Application and potential uses of fish gill cell lines: Examples with RTgill-W1. *In Vitro Cellular and Developmental Biology* 45: 127–134.

Nicholson BL (1985) Techniques in fish cell culture. In: Kurstak E (ed.) *Techniques in the Life Sciences*, vol. C1, pp. C015/1–16. Limmerick: Elsevier.

Villena AJ (2003) Applications and needs of fish and shellfish cell culture for disease control in aquaculture. *Reviews in Fish Biology and Fisheries* 13: 111–140.

Wolf K and Mann JA (1980) Poikilotherm vertebrate cell lines and viruses: A current listing for fishes. *In Vitro* 16: 168–179.

Wolf K and Quimby MC (1969) Fish cell and tissue culture. In: Hoar WS and Randall DJ (eds.) *Fish Physiology*, pp. 253–306. New York: Academic Press.

Pigment Genes and Cancer Genes

I Braasch, University of Oregon, Eugene, OR, USA

D Liedtke, University of Würzburg, Würzburg, Germany

© 2011 Elsevier Inc. All rights reserved.

Introduction
Pigment Cells in Fish
Genetic Control of Pigment Cell Development
Pigment Synthesis Genes
Color Change
Pigment Polymorphisms in the Wild
Pigment Cell Tumors
Further Reading

Glossary

Gene knockdown Inhibition of gene expression by the presence of a specific blocking agent such as antisense morpholinos.

Malignant tumor Severe tumor type that is able to metastasize.

Metastasis Spread of a tumor from the primary place of origin to another part of the body.

Mutagenesis screen Forward genetic method to find novel genes of interest by introducing gene mutations into the genome of a model organism.

Oncogene Gene driving tumor formation when mutated or expressed at abnormal levels.

Promoter The upstream sequence of DNA which regulates the expression of the downstream coding sequence of the gene. The promoter may sometimes be as big as or bigger than the gene itself. A so-called 'minimal promoter' is the length of the promoter required to achieve expression of the gene, but without additional positive or negative elements.

Receptor tyrosine kinase Cell-surface receptor that activates intracellular signaling cascades in response to binding of specific extracellular ligand molecules.

Transcription factor DNA-binding protein that influences the expression of genes by binding to their promoter region.

Tumor modifier Genetic factor that influences the characteristics of a specific tumor type.

Tumor suppressor Gene that prevents tumor formation.

Introduction

Pigmentation and color patterning of body and fins serve multiple functions in fish, including camouflage, communication, mate choice, or species recognition. Fish pigmentation is highly polymorphic and often sex-specific, particularly during the spawning season. Furthermore, many fish species change their pigmentation during their life cycle. Prominent examples for colorful fishes are coral reef fishes, poeciliids from Middle and South America (guppy, *Poecilia reticulata*; platyfish, *Xiphophorus maculatus*; swordtail, *Xiphophorus hellerii*) or the cichlids of the East African Great Lakes. (**see also Vision**: Color vision and Color Communication in Reef Fish)

Coloration of teleost fishes is based on six different major types of pigment cells. Other fish lineages and tetrapods, in contrast, have a smaller repertoire of such chromatophores. Studying the genetic basis of fish coloration has a long tradition, particularly in East Asia, where color morphs of carp and goldfish have been cultured for more than 1500 years. In the beginning of the last century, poeciliids became popular among geneticists to study the inheritance of color patterns and also of pigment cell-derived tumors.

The present article gives an overview of the types of pigment cells found in fish, the genetic control of their development and differentiation, and the genes that underlie color polymorphisms between natural fish populations. Subsequently, we discuss the longstanding interest in fish as model systems for the study of pigment cell-derived types of skin cancer.

Pigment Cells in Fish

Development of Pigment Cells

There are two major types of pigmented cells in the vertebrate body. The black cells of the retinal pigment epithelium, the dark cell layer in the eye, are of neuroectodermal origin. In contrast, the pigment cells of the skin (chromatophores), which are the focus of the present article, derive from the neural crest.

The neural crest is a transient cell population during early embryonic development that arises in the dorsal ectoderm between the neuroectoderm and the prospective epidermis. From this layer, neural crest cells migrate along different routes throughout the embryo to their

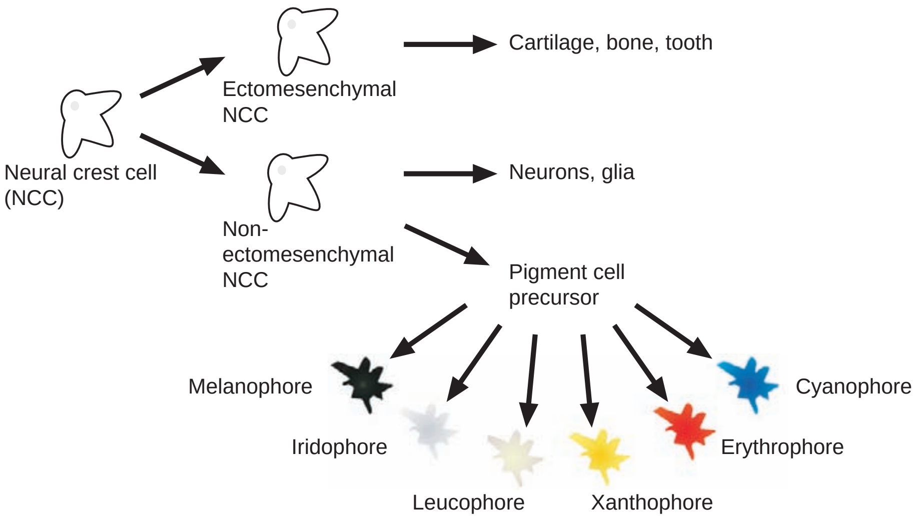


Figure 1 Specification of pigment cells from the neural crest.

final destinations. Neural crest cells give rise to around 50 different cell types including cartilage, neurons, and chromatophores. They are all initially multipotent, but they become gradually restricted to a particular cell fate during development. A first distinction can be made between ectomesenchymal and non-ectomesenchymal neural crest cells. The former give rise to cartilage, bone, and tooth tissues, while the latter develop into neurons, glia, and pigment cells. All pigment cell types develop from a common precursor cell (**Figure 1**).

Pigment Cell Types in Fish

In teleost fishes, six major pigment cell types can be recognized, each displaying characteristic pigments in specialized pigmentary organelles (**Figure 2**).

Melanophores synthesize the black to brown eumelanin in their melanosomes (**Figure 2**). In contrast to mammals and birds, teleosts do not synthesize the lighter pheomelanin.

The metallic-reflective iridophores store crystalline purines, particularly guanine, in so-called reflecting platelets (**Figure 2**(**a**)). The creamy-whitish leucophores also contain purines in their leucosomes (**Figure 2**(**b**)). Irido- and leucophores are closely related and generate structural colors by the reflection of light.

Yellow to red chromatophores are categorized according to their overall color either as xanthophores (yellow; **Figure 2**(**a**)) or as erythrophores (red). Both pigment cell types synthesize yellow to red pteridine pigments in their pigmentary organelles (pterinosomes), which are called xanthosomes or erythrosomes, respectively. In addition, xantho- and erythrophores may store yellow to red carotenoid pigments obtained from the food in carotenoid vesicles.

The blue cyanophores have so far only been found in two species of the family Callionymidae (dragonets), the mandarinfish *Synchiropus splendidus* and the picturesque dragonet *S. picturatus*. The pigmentary organelles of these cells, termed cyanosomes, contain a true blue pigment. In other fishes, blue is usually displayed as a structural color generated by iridophores.

The distribution of chromatophores, often combining stacked layers of different pigment cell types, generates

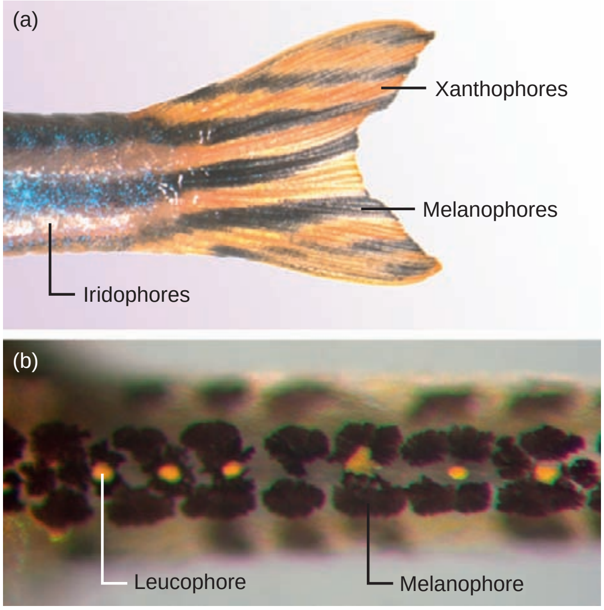


Figure 2 Teleost pigment cell types. (a) Tail and tailfin of an adult zebrafish showing the characteristic stripe pattern based on dark melanophores, yellow xanthophores and reflective iridophores. (b) Dorsal view on the trunk of a juvenile medaka. Cream-colored leucophores are surrounded by dark melanophores.

the endless diversity of stripes, spots, and patches of all types of colors in teleosts.

To the best of our knowledge, melanophores and iridophores are the only pigment cell types that have been described so far for agnathans (lamprey and hagfish), cartilaginous fishes (sharks and rays) and lobe-fin fishes (coelacanth and lungfish). (**see also The Skin**: Coloration and Chromatophores in Fishes).

Genetic Control of Pigment Cell Development

The identification of pigment genes, that is, genes that are involved in pigment cell development, pattern formation, pigment synthesis, or color change, has been spurred by the systematic analysis of pigment mutant collections of zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). An important resource for naturally occurring medaka pigmentation mutants is the Tomita collection. For zebrafish, a large collection of pigmentation mutants has been isolated from different large-scale mutagenesis screens. **Table 1** and **Figure 3** give some examples of pigmentation mutants for which the affected pigment gene has been found. In addition, some pigment genes have been functionally investigated by gene knockdown approaches. Several of the major pigment genes in fish will be introduced in the following.

Specification of Pigment Cells

The specification of the non-ectomesenchymal neural crest is regulated by the transcription factor Sox10. In the zebrafish *colorless* mutant, which is defective in the *sox10* gene, all types of pigment cells are severely reduced and defects in other non-ectomesenchymal derivatives are also observed.

All chromatophore types develop from a common precursor cell, but their relationships and the progression of fate restrictions to a particular type of chromatophore remain poorly understood. The analysis of zebrafish pigmentation mutants, however, has revealed that during development each type of chromatophore requires the expression of a characteristic transcription factor as well as of a transmembrane receptor from the family of receptor tyrosine kinases.

Melanophores

The zebrafish mutant *nacre* lacks almost all melanophores (**Figure 3**(**a**)). These fish are mutated in the *mitfa* gene. The Mitf transcription factor is the master regulator of melanophore development in vertebrates, and expression of *mitfa* is sufficient to specify precursor cells for the melanophore fate. Mitf binds to the promoters of melanin synthesis enzyme genes and upregulates their expression. The expression of *mitfa* itself is regulated by Sox10. Because of its specific activity, DNA constructs

Table 1 Examples of teleost pigment genes

Developmental/cellular function	*Gene*	*Teleost mutant*[a]	*Molecular function*
Neural crest specification	*sox10*	*colorless* (Dre)	Transcription factor
Melanophore specification	*mitfa*	*nacre* (Dre)	Transcription factor
Melanophore migration/survival	*kita*	*sparse* (Dre)	Receptor tyrosine kinase
Melanophore development	*tfap2a*	*lockjaw* (Dre)	Transcription factor
	hdac1	*colgate* (Dre)	Histone deacetylase
	trpm7	*touchtone* (Dre)	Cation channel
	erbb3b	*picasso* (Dre)	Receptor tyrosine kinase
Xanthophore specification	*pax3a*	KD (Dre)	Transcription factor
Xanthophore migration/survival	*csf1ra*	*panther* (Dre)	Receptor tyrosine kinase
Iridophore specification	*foxd3*	*mother superior* (Dre)	Transcription factor
Iridophore migration/survival	*ltk*	*shady* (Dre)	Receptor tyrosine kinase
Pattern formation	*cx41.8*	*leopard* (Dre)	Gap junction protien
	kcnj13	*obelix/jagular* (Dre)	Potassium channel
	fbxw4	*hagoromo* (Dre)	Protein degradation
Melanosome dispersal	*mc1r*	KD (Dre)	Melanocortin receptor
	mlpha	*j120* (Dre)	Protein transport
Melanin synthesis	*tyra*	*sandy* (Dre), *i* (Ola)	Enzyme
	tyrp1a/tyrp1b	KD (Dre)	Enzyme
	silva	*fading vision* (Dre)	Structural protien/enzyme
	oca2	*i-3* (Ola)	Melanosomal transporter
	slc45a2	*b* (Ola)	Melanosomal transporter
	slc24a5	*golden* (Dre)	Melanosomal transporter
Pteridine synthesis regulation	*mycbp2*	*esrom* (Dre)	?

[a] Names of mutant lines from zebrafish (Dre) or medaka (Ola).
Some genes have been studied by gene knockdown (KD).

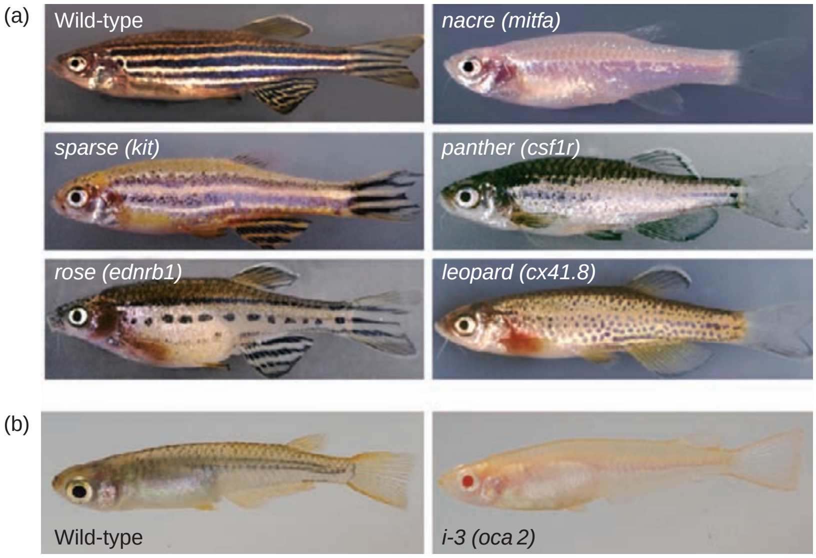


Figure 3 Examples of teleost pigmentation mutants. (a) Zebrafish mutants. Name and affected gene (in brackets) are given. (b) Wild-type medaka compared to the albino mutant *i-3*. (a) Adapted from figure 5 in Parichy DM (2006) Evolution of danio pigment pattern development. *Heredity* 92: 200–210.

containing the *mitfa* promoter can be used to artificially drive the expression of other genes into melanophores of transgenic fish. Such *mitfa* promoter constructs are essential tools for pigment cell and skin cancer research in fish (see below).

In teleosts, a second *mitf* gene, *mitfb*, regulates the expression of melanin synthesis enzymes in retinal pigment epithelium cells. The two *mitf* duplicates were generated in a teleost-specific whole genome duplication (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Evolution of Fish Genomes). Many pigment genes are present in two copies in teleosts due to this event (recognizable by the suffix ‘a’ or ‘b’ after the gene name).

The receptor tyrosine kinase Kita is required for the migration and survival of melanophores. The *sparse* mutant lacks Kita function and is characterized by loss of embryonic as well as early stripe melanophores. Late stripe melanophores, in contrast, are present in *sparse* mutants (**Figure 3**(**a**)). The Kita receptor is activated and sends intracellular signals upon binding to Kit ligand a (Kitla). Knockdown of *kitla* in zebrafish decreases and *kitla* overexpression increases the number of melanophores.

Xanthophores

The specification of xanthophores in zebrafish requires the function of the Pax3 transcription factor. Pax3 is known to be important for melanocyte specification in mammals. Knockdown of *pax3a* genes in zebrafish, however, leads to a decrease of xanthophores, while melanophores increase in number. Pax3 presumably regulates the expression of genes required for xanthophore differentiation like the pteridine synthesis enzymes.

Very similar to the role of Kita for melanophores, the related receptor tyrosine kinase Csf1ra is required for xanthophore migration and survival. The corresponding *panther* mutant is devoid of most xanthophores and this absence of xanthophores seems to be responsible for the loss of the adult melanophore stripe pattern (**Figure 3**(**a**)).

Iridophores and Leucophores

The presence of the Foxd3 transcription factor is essential for the differentiation of iridophores. Its function appears to be the downregulation of *mitfa* expression in those precursor cells that will give rise to iridophores.

Iridophores also need a specific receptor tyrosine kinase for their development. The *shady* mutant, which shows reduced numbers of iridophores, was shown to be defective in the *leucocyte tyrosine kinase* (*ltk*) gene.

Although there are leucophore mutants in medaka (e.g., *leucophore free*), so far no genes has been identified that is involved in leucophore development.

Color Pattern Formation

Studies on color pattern formation have been focused mostly on the adult stripe pattern in zebrafish (**Figure 1**(**a**)). Mathematical models predict a reaction–diffusion mechanism generating this pattern and developmental studies have shown that interactions among melanophores and xanthophores are essential for stripe formation.

In the *leopard* mutant (**Figure 3**(**a**)), the adult stripe pattern is disrupted into a series of spots. It is mutated in the gap junction gene *connexin 41.8 (cx41.8)*. Gap junctions mediate tight cell–cell contacts, which is in line with the cell–cell interaction theory of stripe pattern formation.

Several genes are involved in the development of more than one type of pigment cell. One example is the *ednrb1a* gene, which is expressed in melano-, irido-, and xanthophores. Loss of this gene leads to defects in stripe patterning in the zebrafish *rose* mutant (**Figure 3**(**a**)). The loss of the growth hormone somatolactin in the medaka mutant *color interfere* results in an increase in leucophores and a simultaneous decrease in xanthophores. Many more genes involved in chromatophore development have been identified (some further examples are given in **Table 1**), but their discussion is beyond the scope of this article.

Pigment Synthesis Genes

Many teleost pigmentation mutants are characterized by lighter pigmentation (hypopigmentation) of chromatophores because of defects in pigment synthesis. Some of the responsible genes have been identified.

Melanin Synthesis

In melanophores and the retinal pigment epithelium, black eumelanin is synthesized from tyrosine by enzymes of the tyrosinase family: tyrosinase (Tyr), dopachrome tautomerase (Dct), and tyrosinase-related protein 1 (Tyrp1) (**Figure 4** (**a**)). Loss of eumelanin formation leads to different types of albinism. Mutant lines of the *i* (medaka) and *sandy* (zebrafish) type are complete albinos devoid of melanin pigments. Both mutants have a defective *tyra* gene. Knockdown of *tyrp1* genes in the zebrafish leads to a brownish type of albinism.

Several transporter proteins that reside in the melanosome membrane (**Figure 4**(**a**)) also have an influence on melanin synthesis, although their exact roles for melanin synthesis remain unclear. The albinistic medaka mutants *i-3* (**Figure 3**(**b**)) and *b* have defects in the *oca2* and *slc45a2* (also known as *aim1*) gene, respectively. The zebrafish *golden* mutant possesses hypopigmented melanophores. It was found to be affected in the *slc24a5* transporter gene.

Pteridine Synthesis

The synthesis of yellow to red pteridine pigments is highly complex and less well understood than melanin synthesis. The pteridine synthesis pathway has different branches, one of them leading to the formation of sepiapterin as a precursor for different types of pteridine pigments. Another branch leads to the formation of tetrahydrobiopterin (**Figure 4**(**b**)). Tetrahydrobiopterin is an important cofactor of the tyrosinase enzyme in melanophores, which directly links melanin and pteridine synthesis.

Although several mutants with altered pteridine pigments have been found in zebrafish, only one of them has been cloned so far. A mutation in the *mycbp2* gene in the

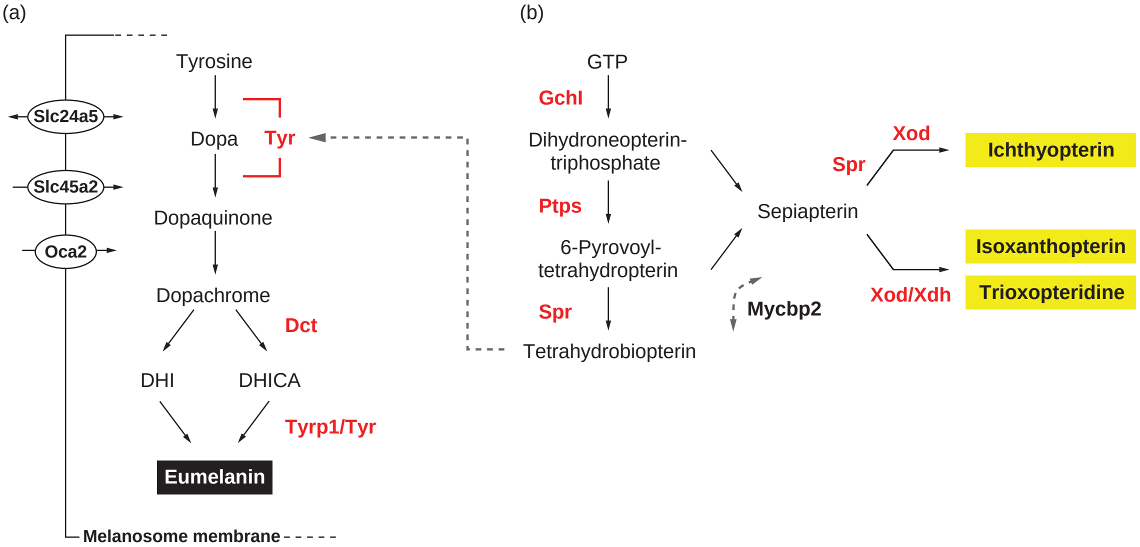


Figure 4 Pigment synthesis pathways. (a) Black eumelanin is synthesized from tyrosine by enzymes of the tyrosinase family. Tyr, tyrosinase; Dct, dopachrome tautomerase; Tyrp1, tyrosinase-related protein. Three melanosomal transporters (Slc24a5, Slc45a2, and Oca2) also play essential roles for melanin synthesis. (b) The pteridine synthesis pathway has two major branches: *de novo* synthesis of tetrahydrobiopterin (top to bottom) and formation of pteridine pigments via sepiapterin in xanthosomes (left to right). The switch between pathways is regulated by Mycbp2. In melanophores, Tetrahydrobiopterin is a cofactor of Tyr. GchI, GTP cyclohydrolase I; Ptps, 6-pyruvoyl tetrahydropterin synthase; Spr, sepiapterin reductase; Xod/Xdh, xanthine oxidase/dehydrogenase. Note that some intermediate pathway steps are not shown.

esrom mutant results in the reduction of yellow pigments. Mycbp2 may regulate the switching between different branches of pteridine synthesis (**Figure 4**(**b**)).

Color Change

Teleosts are famous for their ability to change colors (**see also Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals). Morphological color changes are based on slow changes of pigment amounts or the distribution of chromatophores. Physiological color changes, in contrast, are accomplished by rapid movements of pigmentary organelles within chromatophores. In response to the endocrine and nervous systems, pigmentary organelles are moved along microtubules to the pigment cell center (lightening) or the periphery (darkening) (**see also The Skin**: Coloration and Chromatophores in Fishes).

A large body of literature exists on the regulatory role of hormones such as the melanophore-stimulating hormone (MSH) and the melanin-concentrating hormone (MCH) for physiological color change. Knockdown of the melanocortin-1-receptor (Mc1r), the receptor of MSH, impairs the dispersal of melanosomes. Melanosome transport mutants have been isolated in zebrafish. One of them has a defect in the *melanophilin a* (*mlpha*) gene, which encodes a structural protein coordinating the movement of melanosomes along microtubules.

Pigment Polymorphisms in the Wild

Geneticists, ecologists, and evolutionary biologists have extensively studied color differences among fish populations and species in the wild. For example, classic genetic studies have identified numerous pigmentation loci responsible for color differences among guppy and platyfish populations, many of them residing on the sex chromosomes. There are, however, only few studies in which the particular gene underlying a naturally occurring color polymorphism in fish has been identified.

Blind Cavefish

The Mexican tetra (*Astyanax mexicanus*) is a dramatic example for naturally occurring pigmentation divergence. While surface populations have a silvery-grayish appearance, blind cave populations independently lost the ability for proper melanin synthesis. It was shown that some of these albino forms of *A. mexicanus* are caused by loss-of-function mutation in the *oca2* gene. Brownish cave populations, in contrast, show changes in the melanocortin 1 receptor (Mc1r) compared to the surface populations. Modulation of Mc1r function is well known for being responsible for pigmentation polymorphisms and melanism in mammals, birds, and reptiles (**see also Bony Fishes**: Blind Cavefish).

Danio Species

Parichy and colleagues used an elegant method to pinpoint the developmental pathways responsible for stripe pattern differences between zebrafish and other species of the genus *Danio*. Several *Danio* species were crossed with pigmentation mutants of the zebrafish. These studies showed that stripe pattern differences between zebrafish and the stripe-less pearl danio (*D. albolineatus*) are due to modulations of the aforementioned Csf1r and Kit receptor tyrosine kinase signaling pathways.

Stickleback

The three-spined stickleback (*Gasterosteus aculeatus*) is well known for its pigmentary diversity around the globe. A recent study determined the gene responsible for differences in melanophore distribution between freshwater populations with light gills and bellies and ancestral marine populations with dark gills and bellies. These differences in melanophore patterning were traced back to differences in the expression of the *kit ligand a* (*kitla*) gene causing changes in melanophore distribution.

Pigment Cell Tumors

Under abnormal conditions, the broad spectrum of pigment cell types in fish can give rise to different types of skin tumors. Teleosts are therefore suitable models for the study of skin cancers, such as malignant melanoma, which is one of the most aggressive cancers with high rates of metastasis formation in humans. Naturally occurring spontaneous tumor growth from pigment cells in fish has been studied since the 1920s. Scientific reports on abnormalities in the skin of fish include erythrophoroma, melanoma, and iridophoroma, but only the first two types have been studied in more detail.

Goldfish Erythrophoroma

Large malformations in the skin of the goldfish (*Carassius auratus*) that are caused by uncontrolled growth of red pigment cells have been described. Further studies identified these lesions as erythrophoroma, that is, tumors arising from erythrophores. In subsequent studies, erythrophoroma cells were cultured and the erythrophoroma cell lines have since then been used for investigations of general pigment cell and tumor properties. However, the genetic alterations that may have caused the erythrophoroma formation in goldfish remain elusive.

Xiphophorus Melanoma

An extensively studied example of fish skin tumors is the appearance of black tumor lesions (melanoma) in hybrid species of the genus *Xiphophorus*. By crossing, for example, platyfish (*X. maculatus*) and swordtail (*X. hellerii*), hybrids can be generated that have spots with high numbers of melanophores (hyperpigmentation) and also invasive melanoma in the following generations.

The spontaneous tumor formation in *Xiphophorus* hybrids can be explained by the interplay of a tumor locus (*Tu*) that is under the control of a repressor locus (regulator locus *R*). Different chromosomal locations of *Tu* and *R* enable the stepwise separation of these two loci by repeated crosses to swordtail.

As shown in the crossing scheme in **Figure 5**, the platyfish genome contains, as well as the *Tu* locus, also the *R* locus, which represses the development of melanoma. After crossing a platyfish to a swordtail, which possesses neither *Tu* nor *R*, hybrids are born which have one *Tu*- and one *R*-bearing chromosome. This first hybrid generation is characterized by hyperpigmentation. A second crossing of these hybrids to swordtail results in four different genotypes. Half of the individuals from this generation will not show a pigmentary phenotype, because their genome is free of the *Tu* locus. One-quarter of fish will be strongly hyperpigmented due to the presence of one *Tu* and one *R* locus allele. One-quarter of individuals will develop malignant melanoma due to the presence of the *Tu* locus and the absence of an *R* locus in their genome.

Positional cloning of the *Tu* locus identified the oncogene *xmrk* (*Xiphophorus* melanoma receptor kinase) as the genetic factor driving the transformation of a melanophore into a malignant melanoma cell. Xmrk is an epidermal growth factor receptor (Egfr) that belongs to the group of receptor tyrosine kinases. The *xmrk* gene originated by a local gene duplication of the *egfrb* gene, which is located like *xmrk* on the platyfish sex chromosome.

The *xmrk* gene is highly expressed in melanoma tissues. Two mutations keep the Xmrk receptor in a constantly active form, thereby allowing ligand-independent signaling. Xmrk turns on different signaling pathways such as the phosphatidylinositol 3-kinase, the Ras–Raf–Mapk, or the Fyn/focal adhesion kinase pathways. These downstream signaling cascades increase cell proliferation, cell survival, and cell motility of the transformed pigment cell. Such changes in cell characteristics as well as activation of the same signaling pathways are also observed in human melanoma. For example, aberrant signaling through the Raf–Ras–Mapk pathway is well described for human melanomas, and mutated receptor tyrosine kinases are known to be involved in human cancer formation and progression.

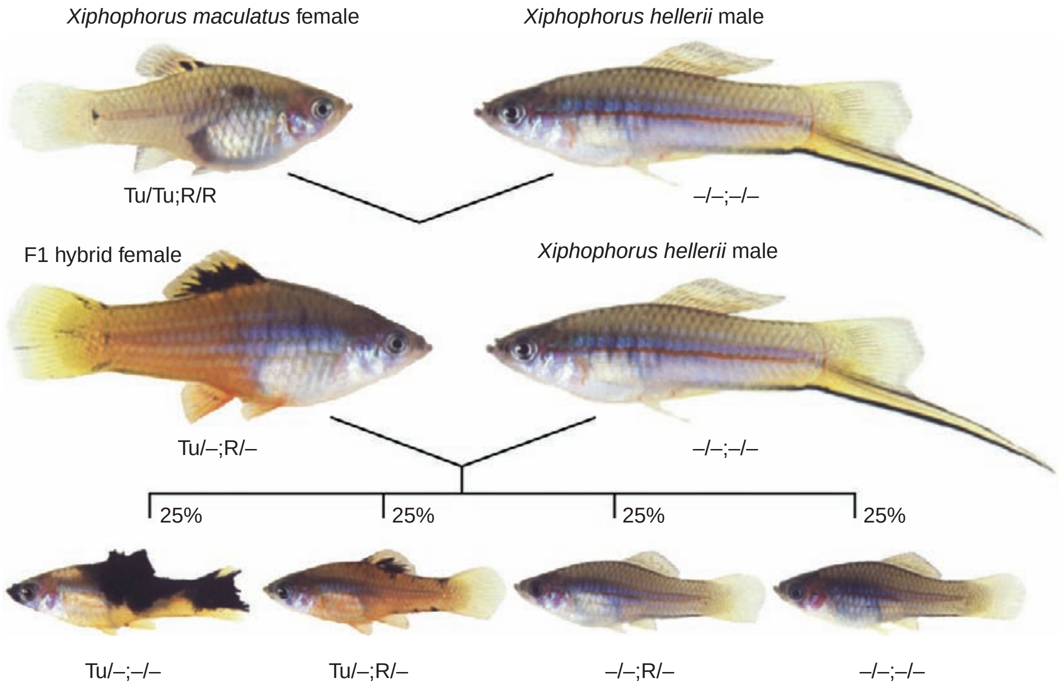


Figure 5 Crossing scheme of spontaneous melanoma formation in *Xiphophorus* (Gordon–Kosswig–Anders cross). Mating of a *X. maculatus* female (carrying *Tu* and the repressor *R*) to a *X. hellerii* male (lacking *Tu* and *R*) gives rise to a hyperpigmented hybrid generation (heterozygous for *Tu* and *R*). Further crossing of these hybrids to a *X. hellerii* male results in (from right to left) 50% fish with normal pigmentation (lacking *Tu*), 25% hyperpigmented fish (one copy of *Tu* and *R*), and 25% fish that develop malignant melanoma (carrying *Tu* but lacking *R*). Reproduced from figure 1 in Meierjohann S and Schartl M (2006) From Mendelian to molecular genetics: The *Xiphophorus* melanoma model. *Trends in Genetics* 22: 654–661.

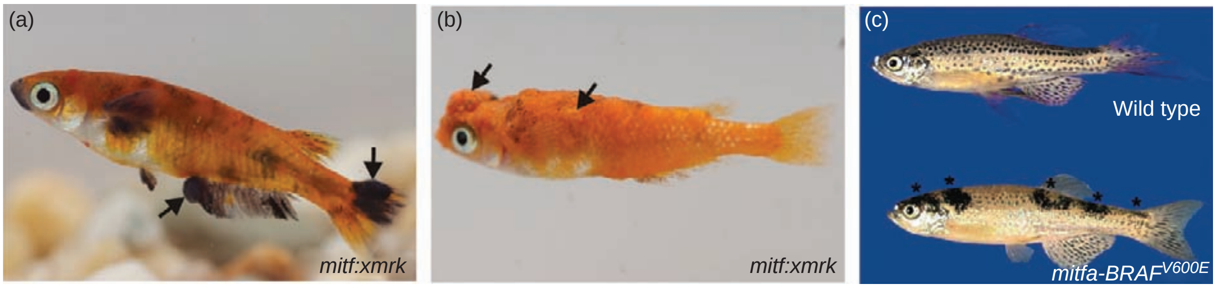


Figure 6 Different skin tumor phenotypes in transgenic medaka and zebrafish. Transgenic Medaka expressing *xmrk* in pigment cells under the control of the *mitfa* promoter develop (a) black melanoma and (b) red xantho-/erythrophoroma (arrows). (c) Zebrafish of the *leopard* strain expressing human *BRAF*^{V600E} in pigment cells (bottom) show growth of nevi (asterisks). Reproduced from figure 1B in Patton EE, Widlund HR, Kutok JL, *et al.* (2005) BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Current Biology* 15: 249–254.

The *Xiphophorus* crossings indicate that besides the presence of *Tu*, the absence of the tumor suppressor *R* is necessary for tumor formation (**Figure 5**). In the platyfish, *R* counteracts *xmrk* and impedes the formation of tumors. The corresponding gene has not been identified yet, but based on genetic studies, one major candidate gene has been identified, *cyclin-dependent kinase inhibitor 2* (*cdkn2*), which maps to the chromosomal region of the *R* locus. Cdkn2 proteins act as cell-cycle and cell-proliferation regulators and have been intensively studied in human melanoma. *CDKN2* genes are often mutated in human melanomas. Conclusive evidence that *cdkn2* in *Xiphophorus* is indeed the *R* locus-encoded gene, however, is still elusive.

Transgenic Skin Tumor Models

Besides the appearance of natural spontaneous pigment cell tumors, several new approaches have been developed to study melanoma formation in fish. Recently, progress in molecular biology opened up the possibility to design genetic models of skin tumors in laboratory fish species such as zebrafish and medaka (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Fish as Model Organisms for Medical Research). These skin tumor models show melanoma formation after chemical treatment, radiation, or the expression of an oncogene under a pigment cell-specific promoter.

One example for such a transgenic tumor model is the *mitf:xmrk* medaka, in which the *xmrk* gene from *Xiphophorus* is artificially expressed in medaka pigment cells under the control of the *mitfa* promoter. The transgenic fish develop, after a relatively short time, red xantho-/erythrophoroma and black melanoma (**Figure 6**). Both tumor cell types show specific characteristics of their own, such as different gene expression profiles, different tumor growth properties, and differences in invasiveness. Interestingly, appearance of the different tumor types depends on the genetic background of the fish, indicating the presence of different tumor modifiers genes.

Another example of newly established melanoma models is the *BRAF*^{V600E} zebrafish. Braf is a protein kinase of the Ras–Raf–Mapk pathway. In humans, mutations in this gene, for example, the common *BRAF*^{V600E} mutation, are associated with various cancers including malignant melanoma. In the transgenic zebrafish, the expression of the human *BRAF*^{V600E} gene under the control of the *mitfa* promoter drives the appearance of nevi (**Figure 6**(**c**)). Nevi, commonly called moles, are nonmalignant proliferations of melanophores. Further depletion of the tumor suppressor gene *p53* is necessary for the *BRAF*^{V600E} zebrafish to develop malignant melanoma. In a second zebrafish model, artificial expression of oncogenic human *RAS* in melanophores is sufficient to induce the formation of malignant melanoma.

Besides the transgenic models described here, other known melanoma oncogenes remain largely uninvestigated in fish. However, there are many promising candidate genes from human melanoma research including some of the pigment genes discussed in the previous sections (e.g., *mitf* and *kit*). Currently, fish models for other known oncogenes or other types of cancer are generated and make fish a valuable model system for cancer research.

See also: **Bony Fishes**: Blind Cavefish. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Evolution of Fish Genomes; Fish as Model Organisms for Medical Research. **Sensory Systems, Perception, and Learning**: Communication Behavior: Visual Signals. **The Skin**: Coloration and Chromatophores in Fishes.

Further Reading

Anders F (1991) Contributions of the Gordon–Kosswig melanoma system to the present concept of neoplasia. *Pigment Cell Research* 4: 7–29.

Bagnara JT and Matsumoto J (2006) Comparative anatomy and physiology of pigment cells in nonmammalian tissues. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, and Ortonne JP (eds.) *The Pigmentary System: Physiology and Pathophysiology*, 2nd edn., pp. 11–59. New York: Oxford University Press.

Braasch I, Volff JN, and Schartl M (2008) The evolution of teleost pigmentation and the fish-specific genome duplication. *Journal of Fish Biology* 73: 1891–1918.

Ceol CJ, Houvras Y, White RM, and Zon LI (2008) Melanoma biology and the promise of zebrafish. *Zebrafish* 5: 247–255.

Fujii R (1993) Coloration and chromatophores. In: Evans DH (ed.) *The Physiology of Fishes*, 1st edn., pp. 535–562. Boca Raton, FL: CRC Press.

Hirata M, Nakamura K, and Kondo S (2005) Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern. *Developmental Dynamics* 234: 293–300.

Ishikawa T, Masahito P, Matsumoto J, and Takayama S (1978) Morphologic and biochemical characterization of erythrophoromas in goldfish (*Carassius auratus*). *Journal of the National Cancer Institute* 61: 1461–1470.

Kelsh RN (2004) Genetics and evolution of pigment patterns in fish. *Pigment Cell Research* 17: 326–336.

Kelsh RN, Harris ML, Colanesi S, and Erickson CA (2009) Stripes and belly-spots: a review of pigment cell morphogenesis in vertebrates. *Seminars in Cell and Developmental Biology* 20: 90–104.

Kelsh RN, Inoue C, Momoi A, *et al.* (2004) The Tomita collection of medaka pigmentation mutants as a resource for understanding neural crest cell development. *Mechanisms of Development* 121: 841–859.

Meierjohann S and Schartl M (2006) From Mendelian to molecular genetics: The *Xiphophorus* melanoma model. *Trends in Genetics* 22: 654–661.

Mellgren EM and Johnson SL (2002) The evolution of morphological complexity in zebrafish stripes. *Trends in Genetics* 18: 128–134.

Michailidou C, Jones M, Walker P, *et al.* (2009) Dissecting the roles of Raf- and PI3K-signalling pathways in melanoma formation and progression in a zebrafish model. *Disease Models and Mechanisms* 2: 399–411.

Parichy DM (2006) Evolution of danio pigment pattern development. *Heredity* 92: 200–210.

Parichy DM, Reedy MV, and Erickson CA (2006) Regulation of melanoblasts migration and differentiation. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, and Ortonne JP (eds.) *The Pigmentary System: Physiology and Pathophysiology*, 2nd edn., pp. 108–139. New York: Oxford University Press.

Patton EE, Widlund HR, Kutok JL, *et al.* (2005) BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Current Biology* 15: 249–254.

Rawls JF, Mellgren EM, and Johnson SL (2001) How the zebrafish gets its stripes. *Developmental Biology* 240: 301–314.

Schartl M (1995) Platyfish and swordtails: A genetic system for the analysis of molecular mechanisms in tumor formation. *Trends in Genetics* 11: 185–189.

Schartl M, Wilde B, Laisney AG, *et al.* (2010) A mutated EGFR is sufficient to induce malignant melanoma with genetic background-dependent histopathologies. *Journal of Investigative Dermatology* 130: 249–258.

Ziegler I (2003) The pteridine pathway in zebrafish: Regulation and specification during the determination of neural crest cell-fate. *Pigment Cell Research* 16: 172–182.

Relevant Websites

http://www.espcr.org – European Society for Pigment Cell Research (ESPCR): Color Genes.

http://www.shigen.nig.ac.jp/medaka – Shared Information on GENetic Resources (SHIGEN): NBRP Medaka.

http://zfin.org – The Zebrafish Model Organism Database (ZFIN).

Evolution of Fish Genomes

J-N Volff, F Brunet, A Böhne, and D Galiana-Arnoux, Université de Lyon, Lyon, France

© 2011 Elsevier Inc. All rights reserved.

Genome Size and Chromosome Number in Fish
Ancestral Teleost-Specific Genome Duplication and Biodiversity
Diversity of Transposable Elements
Rapid Evolutionary Turnover of Sex Chromosomes
Conclusion
Further Reading

Glossary

Heterogamety Production of different types of gametes with different sex chromosome content in one sex of a species. For example, in male heterogamety (XY males and XX females), males produce gametes with either an X or a Y chromosome, whereas females produce only gametes with X chromosome.
Neofunctionalization Acquisition of a novel function by a gene.
Paralogons Large, duplicated regions in a genome.
Polyploidization Increase of the chromosome set number (ploidy), for example, through the fusion of unreduced gametes (with somatic ploidy).
Rediploidization Karyotype rearrangement restoring a diploid karyotype (2*n*) from a tetraploid karyotype (4*n*).
Retrotransposon Transposable element transposing through retrotransposition, that is, reverse transcription of messenger RNA (mRNA) and integration of the complementary DNA copy into a new DNA target site. Retrotransposition leads to the duplication of the element.
Reverse transcriptase RNA-dependent DNA polymerase that catalyzes reverse transcription of mRNA into complementary DNA during retrotransposition.
Speciation Evolutionary process leading to the formation of a new species.
Subfunctionalization Complementary distribution of the functions of an ancestral single-copy gene between duplicates after duplication.
Transposable element DNA sequence able to integrate autonomously or nonautonomously into a genome.

In vertebrates, fish are subdivided into jawless fish (hagfish and lampreys), cartilaginous fish (sharks and rays), and bony fish (coelacanth, lungfish, and ray-finned fish including teleost). Fish, particularly teleosts, display a remarkable level of diversity affecting their reproduction, development, morphology, physiology, ecology, behavior, and many other facets of their biology. Many biological processes such as sex determination, which are well conserved in the mammalian lineage, are particularly variable in fish, with major differences even between populations from the same species. This important biodiversity is also reflected by the number of species: with over 25 000 species, fish represent the most diverse group of vertebrates (about 50% of extant vertebrate species). Particularly, ray-finned fish represent over 95% of all living fish species, with about 99% of them belonging to teleosts. Altogether, there is a mass of observations indicating that fish can evolve very rapidly, urging on the search of genes and regions affected by adaptive changes in their genomes.

Besides cytogenetical analyses of chromosome number and DNA content measurements, genome projects and other sequencing programs have been performed for several key species. The genome of the pufferfish (*Takifugu rubripes*; Fugu) was the second vertebrate genome to be sequenced after the human genome, followed by that of *Tetraodon nigroviridis*, another pufferfish. Both species have been chosen for the compactness of their genome. Genome sequences are also available for the threespine stickleback (*Gasterosteus aculeatus*), the medaka (*Oryzias latipes*), and the zebrafish (*Danio rerio*). The genome will be sequenced soon for other species including cichlids, salmonids, and catfish, and survey sequencing has been already performed for the elephant shark (*Callorhinchus milii*; cartilaginous fish). Comparative analyses have illuminated our understanding of structure and evolution of genomes in fish and other vertebrates including human.

Among others, comparison of tetrapod and fish genomes allowed the reconstruction of the karyotype of their last common ancestor that lived approximately 450 million years ago.

Genome Size and Chromosome Number in Fish

Even prior to the advent of genome sequencing, the level of diversity affecting fish at the genomic level was already demonstrated by the measurement of DNA content in haploid cells (the so-called haploid C-value in picograms, with 1 pg roughly corresponding to 1000 megabases of DNA; in tetraploids the C-value may represent two genomes within a same nucleus). Haploid DNA content in ray-finned fish varies from 0.35 pg in the pufferfish (*T. nigroviridis*) to 9.32 pg in the shortnose sturgeon (*Acipenser brevirostrum*; a polyploid species), with a range of variation several fold greater than most other major vertebrate lineages. Some families have particularly compact genomes, for instance, the Tetraodontidae including *T. nigroviridis* and *T. rubripes*, or large genomes, for example, Acipenseridae (sturgeons), Salmonidae, and Polypetridae (bichirs). Outside of ray-finned fish, coelacanth, hagfish, and cartilaginous fish have large genomes (e.g., 16.41 pg in the Australian angel shark (*Squatina australis*)). However, the most impressive genome sizes are found in lungfish (132.83 pg in the marbled lungfish (*Protopterus aethiopicus*)).

Chromosome number, as assessed in normal somatic cells, is also variable, from 22 (in some mudminnows of the *Umbra* genus) to 250 (in the Russian sturgeon (*Acipenser gueldenstaedtii*)). As reported in amphibians and reptiles, but not in mammals and birds, early and recent episodes of polyploidization have been observed recurrently during ray-finned fish evolution. Examples include sturgeons, salmonids, catostomids (suckers), and cyprinids, as well as non-ray-finned fish (e.g., lampreys). However, over 50% of ray-finned fish species analyzed so far have either 48 or 50 chromosomes, reflecting probably their ancestral karyotype.

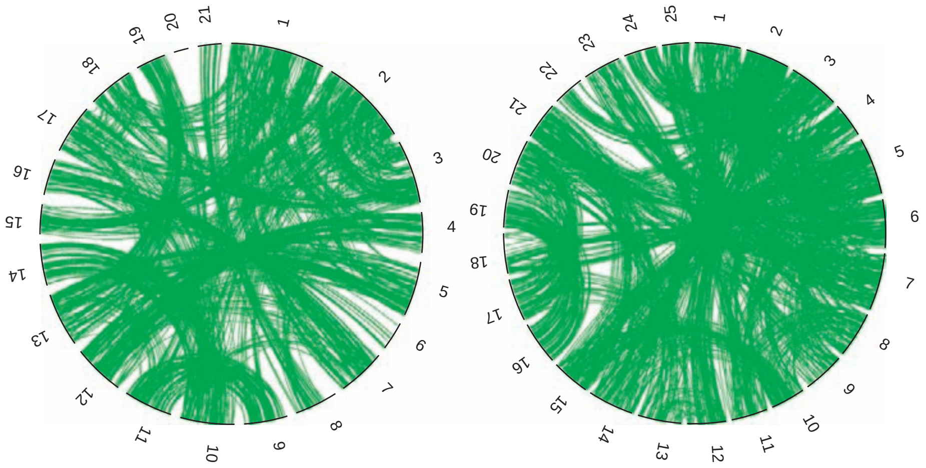
In many cases, higher DNA content results from polyploidization. However, 5–10-fold genome size differences are observed even between teleost species with the same chromosome number, suggesting variable degrees of genome compaction.

Ancestral Teleost-Specific Genome Duplication and Biodiversity

Many authors have reported the existence of genes present as single copies in tetrapods but duplicated in divergent teleost species. The classical example is provided by *hox* genes. This group of related genes, generally arranged in clusters, encodes transcription factors determining the positional specification of the anterior–posterior axis during early embryonic development in animals. The presence of four *hox* clusters in tetrapods compared to one cluster in invertebrates was one of the first hints suggesting that two rounds of genome duplications had taken place during early vertebrate evolution. Strikingly, teleost fish have even more *hox* clusters: at least seven clusters have been identified in zebrafish, pufferfishes, medaka, cichlids, and salmonids. This suggested the occurrence of an additional round of genome duplication during early evolution of the lineage, leading to teleosts after divergence from tetrapods. This teleost-specific genome duplication (TSGD) was confirmed through identification of thousands of protein-coding duplicated genes in teleost genomes that are single copy in tetrapods. Most of them have been formed within a same time window, about 320–350 million years ago. As expected from a whole genome duplication event, large syntenic duplicated regions (paralogons) have been identified in all teleost species studied so far (**Figure 1**). Altogether, convergent observations provide strong evidence for an ancestral genome duplication event having occurred after the divergence of bowfin, paddlefish, sturgeon, and bichir from the stem lineage leading to teleosts. This event was followed by rediploidization of the genome, since the great majority of teleost species has a diploid genome. After rediploidization, several thousands of pairs of gene duplicates generated by the TSGD have been conserved over several hundreds of million years in teleost genomes. Many of them are key developmental genes working as transcription factors or signaling molecules. Particular biological pathways (e.g., pigmentation) display a high rate of retention of TSGD duplicates.

According to Susumu Ohno and others, genome duplication has been proposed to generate a framework allowing genetic innovation and major evolutionary transitions. Hence, teleost fish represent an excellent model to understand the consequences of the massive duplication of genetic information on organismal complexity and biodiversity. Particularly, the availability of two divergent model species, the zebrafish (*D. rerio*) and the medaka (*O. latipes*), allows to establish not only the similarities but also the differences in evolutionary trajectories for a same pair of duplicates in two lineages separated by at least 150 million years of evolution.

Persistence of duplicated genes can be explained by different mutually nonexclusive mechanisms. Besides possible advantages of increased gene expression associated with the presence of a second functionally redundant copy, duplicated genes can experience functional divergence. This can be manifested by mutations changing the quantitative, spatial, or temporal transcriptional context or the coding potential of the copies. If one



Tetraodon nigroviridis

Danio rerio

Figure 1 Rose-window analysis of large duplicated regions (paralogons) in the genomes of the zebrafish *Danio rerio* and the pufferfish *Tetraodon nigroviridis*. Chromosomes are in black, individual duplicated genes are linked by green lines. The presence of large paralogons is diagnostic of an event of genome duplication.

duplicate acquires a novel, positively selected function different from the ancestral one, the process is called ‘neofunctionalization’. Alternatively, original functions performed by the ancestral gene might be distributed between both the duplicates through reciprocal mutations (subfunction partitioning/subfunctionalization). For example, from an ancestral single-copy gene expressed in testis and brain, after duplication one copy might be expressed only in brain (regulatory sequences for expression in testis have been lost) and the second copy only in testis (brain-specific regulatory sequences have been lost). Subfunctionalization can also occur through mutations in coding regions, for example, in different functional domains of a protein. Combinations of both models are of course possible: subfunctionalization of duplicated genes might correspond to a transition state to neofunctionalization. Subfunctionalization might allow innovation in one ancestral function without affecting the other (e.g., optimization of the function in brain without negative consequences on the function in testis). Many examples of TSGD duplicates having undergone subfunctionalization have been reported, including genes for the microphthalmia-associated transcription factor (MITF), the hormone precursor proopiomelanocortin, the Engrailed 2 homeoprotein and many others. Neofunctionalization has also been proposed to explain the maintenance of certain gene duplicates in fish, for example, through the acquisition of novel regulatory sequences or protein domains.

It has been proposed that the ancestral TSGD has provided the framework for the high level of biodiversity and speciation observed in teleosts. One possible mechanism is called ‘divergent resolution’ or ‘reciprocal gene loss’, a process in which each of the two populations loses a different copy from the same pair of duplicated genes during rediploidization. If divergent resolution occurs at multiple loci, this theory predicts genomic incompatibility associated with reduced fertility and/or viability of hybrids between the two populations (many genes will be completely absent or haplo-insufficient in hybrids), and therefore speciation. Accordingly, divergent resolution has been observed for some *hox* gene clusters in different teleost species and it has been estimated that as many as 1700 TSGD duplicate pairs (i.e., about 7% of original TSGD pairs) have lost different copies in *Tetraodon* and zebrafish. Alternatively, differential subfunctionalization of duplicate pairs in different populations would have similar effects as divergent resolution.

Taken together, there is compelling evidence that thousands of TSGD-derived genes have experienced lineage-specific loss or evolution (differential sub-/neofunctionalization), and that such a differential evolution has strongly contributed to biodiversity and, probably, speciation in teleost fish. Analysis of more recent events of tetraploidization in fish will allow determination of the initial steps of genome duplications. Of particular interest will be comparison of the process of rediploidization in two salmonid species, Atlantic salmon and rainbow trout, for which genome draft sequences should be available soon.

As observed in other organisms, more local events of gene duplication also contribute to organismal evolution and diversity in fish. For example, duplication and neofunctionalization of vertebrate vitellogenin genes might be associated with preadaptation of eggs to marine environment and might represent a key event in the evolution and success of teleosts to the marine environment.

Diversity of Transposable Elements

Transposable elements are sequences able to integrate into genomes. They are found in all genomes where they can induce various types of mutations. Through insertion they can disrupt genes and generate mutant phenotypes. In addition, many transposable elements have literally infested genomes and contribute very significantly to their size. For example, as much as 40–50% of the human genome is constituted by transposable elements. This is particularly true for retrotransposable elements, which generate new copies of themselves through messenger (mRNA) reverse transcription and complementary DNA (cDNA) integration, a mechanism of duplication called 'retrotransposition'. In contrast, most DNA transposons transpose through a cut-and-paste mechanism. Interspersed copies of the same transposable element integrated at different locations can recombine, generally by homologous recombination, and induce chromosomal rearrangements such as deletions, duplications, inversions, and translocations. Insertion of transposable elements in the vicinity of resident genes can modify their transcriptional context. Transposition itself can also produce different types of rearrangements at insertion sites. Parts of transposable elements inserted into introns can be recognized as exons by the host splicing machinery, thereby disrupting the open reading frame of a gene at the mRNA level (exonization). Taken together, the genomic effects of transposable elements are similar to those of any other form of genetic variability: some induced mutations can be deleterious to the host, but can also be beneficial when positively selected.

In addition, evidence has emerged that transposable elements serve as a genomic reservoir of regulatory and coding sequences, which can be recruited by resident genes during evolution. Many important genes in plants and animals have been formed directly from transposable elements (molecular domestication). Hence, transposable elements can be considered as major drivers of gene and genome evolution.

The presence of both DNA transposons and retrotransposable elements has been demonstrated in fish, but with a much lower global copy number than in mammals. Most of the 10 superfamilies of cut-and-paste DNA transposons recognized in living organisms have been identified, including Tc1/mariner, hAT, PIF/Harbinger, piggyBAC, and Merlin and P elements. Fish genomes also host Maverick/Polinton sequences, a group of self-synthesizing DNA transposons with long terminal-inverted repeats encoding a protein-primed DNA polymerase B and a retroviral-like integrase. Mavericks/Polintons encode a conserved set of proteins with homology to replication and packaging proteins of bacteriophages and eukaryotic double-stranded DNA viruses. Finally, fish genomes also contain two lineages of a particular superfamily of DNA transposons called 'Helitrons', which probably amplify through a rolling-circle mechanism and are able to capture other genomic sequences. Miniature inverted transposable elements (MITEs), nonautonomous sequences which do not encode any protein, are also found in fish. MITEs parasite the enzymatic machinery of coding autonomous elements to catalyze their own transposition.

All major types of reverse transcriptase-encoding retrotransposable elements have been identified in fish genomes. All groups of long terminal repeat (LTR) retrotransposons are represented, including Ty3/Gypsy, BEL-like, and Ty1/Copia retrotransposons. These elements are flanked by LTRs in direct orientation and encode among other a retroviral-like integrase. Endogenous vertebrate retroviruses, which are integrated remnants of past infections, are also found in fish genomes. Vertebrate retroviruses are structurally and phylogenetically related to Ty3/Gypsy, with an additional envelope gene necessary for infection. DIRS1-like tyrosine recombinase-encoding LTR retrotransposons, with more complex LTRs, are widespread in fish. Fish genomes contain retrotransposons without LTRs (called non-LTR retrotransposons of long interspersed nuclear elements (LINEs)). Some of these elements encode an apurinic–apyrimidinic endonuclease to cut their target integration site. Other fish non-LTR retrotransposons related to invertebrate elements have another type of endonuclease, possibly related to restriction enzymes. Finally, short interspersed nuclear elements (SINEs), which are noncoding retrotransposable elements using the non-LTR retrotransposition machinery of non-LTR retrotransposons, are present in fish genomes too.

Strikingly, fish genomes contain more types of transposable elements than mammalian genomes. Active Ty3/Gypsy, BEL-like, and Ty1/Copia, and DIRS1-like retrotransposons are absent in mammals. This is also the case for certain types of non-LTR retrotransposons and DNA transposons, for example, Helitrons. If we consider ancient phylogenetic families within a given type of element, the difference of transposon diversity between fish and mammals is even more impressive. For instance, within Ty3/Gypsy retrotransposons, at least nine ancient phylogenetic groups are present in fish and invertebrates. None of them is present as an active element in mammals. Taken together, from 26 ancient families of ancestral nonretroviral retrotransposable elements detected in at least one fish lineage and in invertebrates, only three are still present in human and mouse genomes, and most of them are not detected in chicken. Even the very compact genomes of pufferfishes with their low repeat content show a much higher diversity of endogenous retrotransposons than mammalian genomes. These observations suggest that a massive reduction of mobile DNA diversity has occurred during the evolution of the tetrapod lineage after its split from the ray-finned fish lineage.

There is no doubt that transposable elements contribute to genome and phenotype diversity in fish. Comparative analyses of sequenced fish genomes have demonstrated that different families can have very different copy numbers and levels of activity in different fish species. Some elements are very active in some species and have been completely eliminated in others. Even for widespread retrotransposons such as the Rex3 or Rex1 non-LTR retrotransposons, phylogenetic survey of different species revealed many independent bursts of retrotransposition potentially associated with speciation events. Many endogenous transposable elements are still active, as demonstrated by the identification of mutant phenotypes generated though gene disruption (e.g., the Tol2 DNA transposon in medaka or the TX1 retrotransposon in platyfish).

Rapid Evolutionary Turnover of Sex Chromosomes

Sex chromosomes are specialized chromosomes carrying genes that determine the sex of individuals (genetic sex determination). Males and females possess different sets of sex chromosomes. In mammals, males have an X and a Y chromosome (male heterogamety), while females have two X chromosomes. Birds have a genetic system with female heterogamety, with ZW females and ZZ males. Sex determination is controlled by the same XY system in the immense majority of mammals, and by the same ZW mechanism in most bird species. Both mammalian and bird sex-determination systems are ancient and have been formed at least 100 million years ago.

The situation is completely different in fish, where almost all possible forms of genetic sex determination have been observed (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular and Chromosomal Aspects of Sex Determination). Of course, not only simple systems with male or female heterogamety, but also more complicated systems with several or several pairs of sex chromosomes or without sex chromosomes are found, though with the involvement of several genomic loci (polyfactorial sex determination). Male or female heterogameties are frequently overridden by autosomal loci (autosomal influence) or by external parameters such as temperature, water pH, or social factors. In addition, different forms of hermaphroditism have been described in fish, as well as gynogenetic species reproducing as all-female clonal progenies. Importantly, plotting sex-determination mechanisms on a fish molecular phylogeny indicates a very frequent switch between sex-determination mechanisms in fish during evolution. Different systems of genetic sex determination can be found in a same fish genus and even in a same species (e.g., in the platyfish).

Frequent change of genetic sex-determination systems is associated with an astonishing turnover of sex chromosomes in fish. Comparative mapping experiments in different fish lineages have revealed that related species can have sex chromosomes from independent origins, even if they possess a same type of genetic sex determination. For example, comparative mapping of the sex-determining locus in different salmonid species with XY sex-determination mechanisms strongly suggests that they have different sex chromosomes.

In the best-studied model for sex determination, the medaka (*O. latipes*), the male-inducing sex-determining gene has been identified on the Y chromosome by two different research groups. This gene, alternatively called *DMY* and *dmrt1bY*, is the first master sex-determining gene to be identified in a nonmammalian vertebrate species. This master gene is rather recent (10 million years old) and has been formed after duplication and mutation of an autosomal gene called *Dmrt1*. When *dmrt1bY* is inactivated, XY genotypes are females. Transgenic expression of *dmrt1bY* induces testis differentiation and male development in XX genotypes. Hence, *dmrt1bY* is the bona fide master sex-determining gene of the medaka.

The Y-specific region is very small, and only contains *dmrt1bY* as a functional gene. There is no evidence of degeneration on the Y of neighbor genes also present on the X, as it is the case, for example, in mammals. Hence, medaka sex chromosomes might be of rather recent evolutionary origin. Accordingly, the age of *dmrt1bY* and of medaka Y chromosome has been estimated at only about 10 million years. The same master sex-determining gene is found in the related species *Oryzias curvinotus* but not in a more divergent *Oryzias* species, which has a different pair of sex chromosomes. This indicates that species from the same genus can have different sex chromosomes. Surprisingly, *O. luzonensis*, a sister species of *O. curvinotus* also with an XY system, has already lost *dmrt1bY* and possesses a new Y chromosome. Studies on other major models of genetic sex determination, such as the threespine stickleback (*G. aculeatus*), cichlids, and poeciliids, also support a very high turnover of master sex-determining genes and sex chromosomes in fish in association with the important level of diversity affecting sex determination.

Loci involved in sexual selection and hybrid sterility are found on various fish sex chromosomes. In the threespine stickleback (*G. aculeatus*), a newly evolved sex chromosome was recently shown to carry genes for male courtship display, that is, traits that contribute to behavioral isolation and therefore to speciation. Hence, sex chromosome turnover might play a role in reproductive isolation and speciation in fish.

Conclusion

Teleost fish genomes display several remarkable features suggesting a link between genome plasticity and phenotype diversity. Many duplicated genes have been formed through an ancient event of genome duplication. Divergent evolution of these duplicates has been observed in different fish lineages and might correspond to a major mechanism driving biodiversity and speciation in fish. Fish genomes contain many families of active transposable elements that can have very different activities in different fish lineages, thereby generating genomic and phenotypical diversity. In addition, sex chromosome turnover is very rapid in fish. Since sex chromosomes often carry genes involved in reproductive isolation, the rapid evolution of sex chromosomes might also significantly contribute to biodiversity and species formation. Finally, other features of fish genomes might favor diversity, such as a high rate of gene-linkage disrupting rearrangements as well as fast evolution of protein-coding and conserved noncoding, potentially regulatory sequences.

It is important to emphasize that most of these observations are based on key genome draft sequences from only five out of 25 000 fish species, with two of them being closely related (Fugu and *Tetraodon*). All of them are teleost species, making it difficult to assess the ancestral state within the ray-finned lineage (the situation before teleost radiation) or even within bony vertebrates (the situation before the split of ray-finned fish and tetrapod). Partial sequencing of the genome of the elephant shark (*C. milii*), a cartilaginous fish, has already provided important insights into this last aspect.

Clearly, much more work and many more fish genome sequences are required to gain a more complete picture of the structure and evolution of fish genomes. This will help to decipher the evolutionary mechanisms having shaped genomes and organisms between different fish lineages. Fish population genomics provides important information on the genome-wide patterns of sequence variation between the individuals within species. Rapid progress in genome-sequencing technologies, with a huge reduction of sequencing costs and time, has already opened a new era of fish genomics. We will soon have a very panoramic picture of the link between genome plasticity, biodiversity, and speciation in fish, the much diverse group of extant vertebrates.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular and Chromosomal Aspects of Sex Determination.

Further Reading

Aparicio S, Chapman J, Stupka E, *et al.* (2002) Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes. Science* 297: 1301–1310.

Böhne A, Brunet F, Galiana-Arnoux D, Schultheis C, and Volff JN (2008) Transposable elements as drivers of genomic and biological diversity in vertebrates. *Chromosome Research* 16: 203–215.

Jaillon O, Aury JM, Brunet F, *et al.* (2004) Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype *Nature* 431: 946–957.

Kasahara M, Naruse K, Sasaki S, *et al.* (2007) The medaka draft genome and insights into vertebrate genome evolution. *Nature* 447: 714–719.

Kitano J, Ross JA, Mori S, *et al.* (2009) A role for a neo-sex chromosome in stickleback speciation. *Nature* 461: 1079–1083.

Mank JE and Avise JC (2006) Phylogenetic conservation of chromosome numbers in Actinopterygiian fishes. *Genetica* 127: 321–327.

Mank JE and Avise JC (2009) Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sexual Development* 3: 60–67.

Matsuda M, Nagahama Y, Shinomiya A, *et al.* (2002) *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417: 559–563.

Nanda I, Kondo M, Hornung U, *et al.* (2002) A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes. Proceedings of the National Academy of Sciences of the United States of America* 99: 11778–11783.

Postlethwait J, Amores A, Cresko W, Singer A and Yan YL (2004) Subfunction partitioning, the teleost radiation and the annotation of the human genome. *Trends in Genetics* 20: 481–490.

Ravi V and Venkatesh B (2008) Rapidly evolving fish genomes and teleost diversity. *Current Opinion in Genetics and Development* 18: 544–550.

Roest Crollius H and Weissenbach J (2005) Fish genomics and biology. *Genome Research* 15: 1675–1682.

Schartl M (2004) A comparative view on sex determination in medaka. *Mechanisms of Development* 121: 639–645.

Taylor JS, Braasch I, Frickey T, Meyer A, and Van de Peer Y (2003) Genome duplication, a trait shared by 22000 species of ray-finned fish. *Genome Research* 13: 382–390.

Venkatesh B (2003) Evolution and diversity of fish genomes. *Current Opinion in Genetics and Development* 13: 588–592.

Volff JN (2005) Genome evolution and biodiversity in teleost fish. *Heredity* 94: 280–294.

Volff JN, Nanda I, Schmid M, and Schartl M (2007) Governing sex determination in fish: Regulatory putsches and ephemeral dictators. *Sexual Development* 1: 85–99.

Relevant Websites

http://www.genomesize.com – Animal Genome Size Database.

http://blast.ncbi.nlm.nih.gov – BLAST: Basic Local Alignment Search Tool.

http://www.ensembl.org – e!Ensembl.

Fish as Model Organisms for Medical Research

JN Cech and LI Zon, Howard Hughes Medical Institute, Boston, MA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Forward Genetic Screens
Reverse Genetics
Morpholino Gene Knockdown
Transgenesis
Transplantation
Chemical Biology
In Vivo Imaging
Blood
Heart
Kidney
Neuroscience
Muscle
Skin
Behavior
Future
Further Reading

Glossary

Development The process of an individual organism growing organically; a purely biological unfolding of events involved in an organism changing gradually from a simple to a more complex level.

DNA promoter The site on deoxyribonucleic acid to which ribonucleic acid polymerase binds preparatory to initiating transcription of a gene or an operon.

Genetics The branch of biology that deals with heredity, especially the mechanisms of hereditary transmission and the variation of inherited characteristics among similar or related organisms.

Germline Sequence of germ cells that have genetic material that may be passed to a child.

Hematopoiesis The process by which the cellular elements of the blood are formed.

Invertebrates Animal without a backbone.

In vivo Biological processes or experiments occurring or carried out in the living organism.

Model organism A species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the organism model will provide insight into the workings of other organisms.

Mutagenesis Any event, usually intentional, that changes genetic structure; any alteration in the inherited nucleic acid sequence of the genotype of an organism.

Neurodegenerative Characterized by the gradual and progressive loss of nerve cells.

Oncogene A normal gene that may be activated by radiation or a chemical carcinogen to cause cancer.

Phenotype Observable characteristics of an organism including different traits. Phenotype results from the expression of an organism's genes and the influence of environmental factors.

Physiology The biological study of the functions of living organisms and their parts.

Positional cloning The identification of a gene and its isolation as a cloned deoxyribonucleic acid fragment, starting from knowledge of its position on a genetic map or chromosome.

Vertebrates Animal with a backbone.

Xenotransplantation The surgical transfer of cells, tissues, or especially whole organs from one species to another.

Introduction

For the past 100 years, the study of human biology and disease has relied on the use of model organisms. An understanding of the underlying genetic and cellular components of disease in a model organism can often be translated to the human system. Ideally, animal models should be similar to humans in terms of development, physiology, and genetics. Furthermore, an ideal model organism should be easy to maintain in large numbers, because this allows for more varied experimental maneuvers to confirm findings. However, working with so-called higher species is fraught with economic, ethical, and practical concerns. For example, housing and working with monkeys or other large mammals is not very cost effective. Even the laboratory mouse, which has become the most widely used vertebrate model organism, is limited by the high cost of housing, the large amount of space occupied by relatively few individuals, and the relatively slow generation time. By contrast, nematode worms and fruit flies are easy to maintain in large numbers for a very

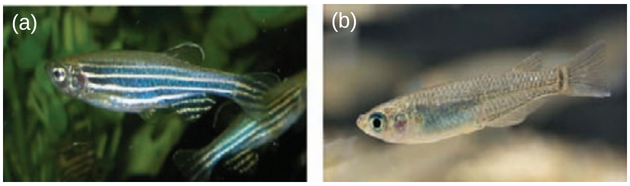


Figure 1 Fish model organisms. (a) Adult zebrafish. Photo by (http://commons.wikimedia.org/wiki/File:Zebrafisch.jpg). (b) Adult medaka, *Oryzias latipes* (Hamamatsu, Shizuoka, Japan). Photo by Azul. Photo by Seotaro (http://commons.wikimedia.org/wiki/File:Oryzias_latipes (Hamamatsu,Shizuoka,Japan,2007)-1.jpg).

low cost; however, as invertebrates they are less similar to humans. The goal is to find a model organism that is not only more closely related to humans, but also inexpensive to house and can be manipulated in large numbers. In the past decade, fish have emerged as promising model organisms that fit many of these criteria.

Fish, including zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) (**Figure 1**), are an advantageous model for many reasons. Like humans, fish are vertebrates, which often indicate that a given gene will function in a somewhat analogous manner in both species, especially as compared with nonvertebrate models. Their small size means that they can be housed in large numbers within small facilities. They reach sexual maturity in 3 months, providing a short duration for many experiments that require adults. Females can lay hundreds of eggs in 1 day and can mate multiple times a month. In addition, because the embryos develop outside the womb, one does not have to sacrifice the mother in order to access newly fertilized embryos. Because the embryos develop externally and are transparent, fish have become a premier model for *in vivo* visualization and manipulation of early embryonic development. Large-scale genetic screens, and the injection of morpholinos or transgenic constructs into embryos (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish) at the one- to two-cell stage have allowed researchers to manipulate even the earliest processes in embryonic development (**see also Bony Fishes**: Zebrafish).

In this article, we will first describe some key techniques used to study disease processes in fish. Then we will review some of the more important discoveries about disease in different organ systems. Throughout the discussion, the term 'fish' will imply both zebrafish and medaka. Although the two are not interchangeable, for the purpose of this article, we will focus on fish in general as the model organism of interest.

Forward Genetic Screens

One major utility of the fish is the capacity to perform forward genetic screens which rely on the principle of working from a phenotype to gene discovery. There are three ways to create mutations in which permanent genomic changes occur in the fish germ stem cells, including mutagenesis by exposure to a chemical (ENU, *N*-ethyl-*N*-nitrosourea), X-ray, or an insertional virus. These three methods create random germline mutations, which allow the altered DNA to become inherited in subsequent generations. The embryonic offspring of these animals are then screened to look for ones that have a phenotype of interest. This can be anything from a lack of blood cells, to an enlargement of the brain cells. Once an interesting mutant is found, the gene affected in that mutant is identified through techniques such as positional cloning. Most commonly, these screens are designed to identify genes or pathways that are required for various aspects of embryonic development, but more recently are being applied to adult phenotypes. Forward genetic screens have been used to identify fish mutants with abnormalities resembling human diseases such as anemia, congenital heart malformations, arrhythmic heartbeats, and muscular dystrophy. Subsequent cloning of the mutants has identified the genes specific to each disease and has provided insight into the mechanisms of the human disease. The identified gene products may also provide potential therapeutic targets.

Reverse Genetics

Reverse genetics involves perturbing a gene of interest and looking for the phenotypic consequences. A common reverse genetic system is gene knock-out in which sections of DNA are replaced, thus producing nonfunctioning gene products. This is accomplished by the manipulation of embryonic stem cells lines in culture that are then re-introduced into newly fertilized mouse embryos. Embryonic stem cells in the fish have been difficult to successfully culture and manipulate (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines), making this technique more challenging. Several new technologies have been developed that allow scientists to attempt reverse genetics in the fish model. TILLING, or targeting induced local lesions in genomes, was one of the first techniques used to create a targeted mutant for a human disease gene of interest. Adults are exposed to chemical mutagens, and the DNA from their germ cells is directly sequenced to identify mutations in the gene of interest. A new technology utilizing zinc-finger nucleases also gets around the caveat of needing embryonic stem cells to create mutants. Zinc finger proteins are designed to recognize a specific DNA sequence. Once bound, a nuclease attached to the zinc finger induces a break in the DNA, forcing attempted repair of the DNA. This repair often is incorrect and leads to mutagenic insertions or deletions at the site of the double stranded break. This is an exciting new technology that will greatly advance the study of human disease in fish. Reverse genetics has been used to mimic many human disorders, including hematopoietic diseases and neurodegenerative disorders, including Huntington's disease. Such disease models have exciting potential for medical research, for example, in the identification of good drug targets and in screening for small molecules that ameliorate disease progression or severity.

Morpholino Gene Knockdown

If a gene involved in a human disorder is already known, understanding its role in embryonic development can often give insight into that gene's function. One method of doing this is to knock down the gene's expression during embryonic development using morpholinos, antisense oligonucleotides that block gene expression by interfering with the translation of the gene product either by preventing initiation or by altering RNA splicing. Morpholinos are useful to study the function of a gene in embryo development by injecting the compounds into a one–two-cell stage embryo and comparing the growth and differentiation to that of untreated embryos. Some labs have even begun doing morpholino screens to better define certain signaling pathways. Morpholino gene knockdown has been useful in better understanding the genes involved in many human neurodegenerative diseases. Genes implicated in Alzheimer's disease and Parkinson's disease (PD) have been studied using this method, which has led to a better understanding of the functional role these genes play in both normal and diseased patients.

Transgenesis

In some cases, a gene is known to be associated with a disease but little is known of its function. One useful technique in the fish is transgenesis, in which expression of a gene of interest can be forced in a specific tissue of interest, allowing for detailed studies of that gene's function *in vivo*. This technique requires two major components: (1) the altered gene itself – in cancer studies, these are often oncogenes, which are mutated forms of genes that are responsible for proliferation, a prerequisite for most human cancer and (2) a DNA promoter, which allows for expression of that gene in only the tissue of interest. Transgenics are used to model diseases *in vivo* and watch their progression in real time. Many human cancers including leukemia and melanoma have been modeled in fish, allowing for a better understanding of disease initiation, metastasis, and treatment. Transgenics can also be used to tag specific cell types with a fluorescent protein (**Figure 2**). Not only can red blood cells, lymphocytes, neurons, and even entire organs be labeled, but fluorescently labeled cancer models have also been created. This technique has improved imaging of individual cells in adult fish, enhancing our ability to track cell proliferation, honing, and migration *in vivo*.

Transplantation

Transplantation is another technique often used in the fish model to study cancer tumorigenicity and bone marrow/hematopoietic stem cell (HSC) biology. Transplantation of cancer cells allows us to track individual cell migration and proliferation and to test the effectiveness of drugs on preventing these cancerous processes. In addition, the zebrafish has emerged as a powerful tool to find ways to improve HSC transplants, often used as a treatment for diseases such as leukemia. Transplantation allows us to test different variables affecting bone marrow engraftment after such treatments.

Researchers have also begun using the commonly farmed tilapia (*Oreochromis niloticus*) fish to study type I diabetes. Scientists have created transgenic tilapia carrying a humanized insulin transgene that, when grown to maturity, produce human insulin. The hope is that these transgenic fish can be used for xenotraplantation of insulin across species.

Chemical Biology

Most recently, the utility of fish for large-scale chemical screens has been realized. Thousands of embryos can be exposed to different chemicals from small molecule libraries and screened for interesting phenotypes. The fish is an ideal organism for this type of screen because water-soluble compounds can be directly added to the fishwater. In addition, female fish lay hundreds of embryos a day, permitting high-throughput *in vivo* screening, which is largely cost and space prohibitive in the murine system. In a single day, thousands of embryos can be exposed to hundreds of chemicals for effects on a variety of development pathways. Most often, screens are

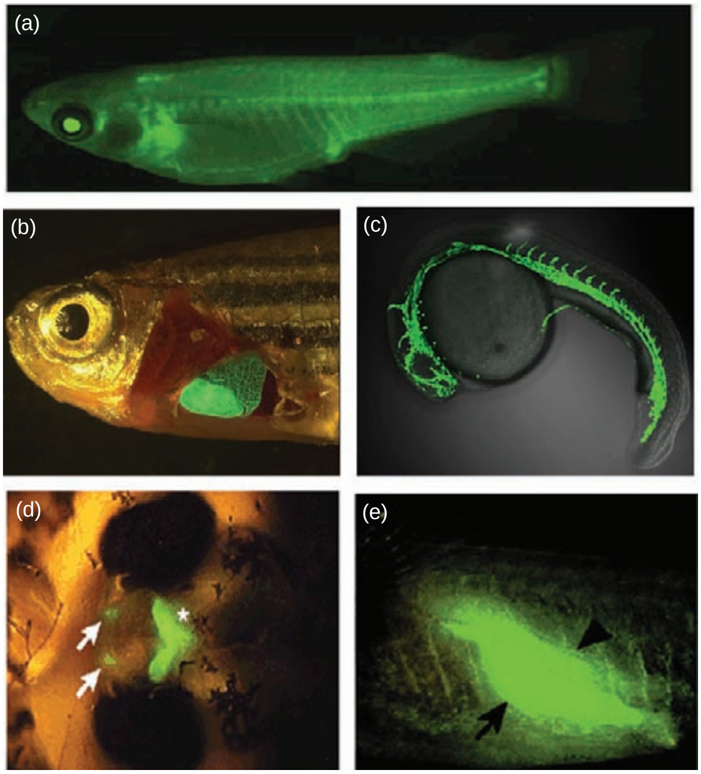


Figure 2 Transgenic fish. (a) Adult medaka with GFP expression driven by the cytoskeletal actin promoter. (b) Zebrafish transgenic with two heart chambers expressing GFP under control of the zebrafish cardiac myosin-light chain-2 (cmlc2) promoter. (c) fli1:GFP transgenic zebrafish embryo with vasculature expressing GFP under the Fli1 promoter. (d) Enhancer-trap lines expressing GFP specifically in the dorsal diencephalon (habenulae, shown by asterisk) and in nasal epithelium (arrows) in adult medaka. (e) GFP expression from the olvas–GFP transgene in the adult testis (arrows). (a,d) Reproduced from Wittbrodt J, Shima A, and Schartl M (2002) Medaka – a model organism from the far east. *Nature Reviews Genetics* 3: 53–64. (b) Rottbaucer *et al*. (2006) Cardiac myosin light chain-2. A novel essential component of thick-myofilament assembly and contractility of the heart. *Circulation Research* 99(3): 323. (c) Photo courtesy of Marina Mione, The Fire Institute of Molecular Oncology. (e) Reproduced from Wakamatsu *et al*. (2001) The see-through medaka: A fish model that is transparent throughout life. *National Academy of Sciences* 98(18): 10046–10050.

used to better characterize embryonic phenotypes or for the purposes of drug discovery. Using chemical screens in combination with transplantation studies has allowed researchers to look for compounds that increase the success rate of bone marrow transplants or that can specifically inhibit growth of certain types of cancer cells. Chemical screening in zebrafish has also expanded to include *in vivo* toxicological screening of pharmacological compounds prior to preclinical human studies.

In Vivo Imaging

The ability to look at individual cells and organs *in vivo* during development is extremely beneficial for disease modeling. There is no other model organism besides the fish in which one can watch the development of vertebrate tissues such as blood, spinal cord, and bones in real time. Fish embryos are also useful for studying the initiating events in cancer formation. For many cancers, we do not know when cells first become cancerous, or what they do at the earliest stages. Where and when do they become diseased and begin migrating, and does this metastasis occur in specific locations? Using transgenics, cancer cells can be tagged with green fluorescent protein (GFP) allowing even the most primitive cancer cells to be visually tracked in a living animal.

In contrast, evaluation of adult processes in zebrafish has been limited due to the opacity of the mature animal. Inspired by studies of blood flow and vascularization in the naturally occurring glass catfish (*Kryptopterus bicirrhis*), researchers have recently created a nearly transparent adult zebrafish termed *casper* (**Figure 3**), which will allow for the power of the zebrafish system to be utilized for a broad array of adult studies. Named *casper* for its ghost-like clarity, scientists have been able to use this mutant fish to visualize internal tumors, and document cancer cell migration in adult fish cancer models. These transparent fish have also been used to study the engraftment of blood stem cell, a clinically important process to understand to improve bone marrow transplants in humans.

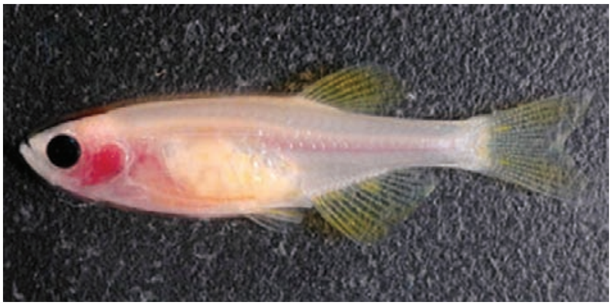


Figure 3 *Casper* zebrafish mutant. Photo courtesy of Richard White, Dana-Farber Cancer Institute, Boston MA.

Research in the past decade has proved that, in conjunction with the described experimental techniques, fish are an excellent model organism to understand many aspects of human biology. While fish research has only been underway for several decades, it has already contributed to the understanding of a number of human diseases. Next we will look at some of the more important discoveries regarding disease of organ systems, including blood, heart, kidney, brain, muscle, and skin.

Blood

Hematopoiesis, the formation of blood, involves differentiation of blood stem cells into different mature blood cells utilized for oxygen exchange, immune response, and clotting. Disruption of any of these processes is at the root of many human blood diseases and disorders. The fish is a powerful model to study hematopoiesis, as the genetic regulation and blood cells types are highly conserved from fish to humans. Over 50 hematopoietic mutants have been characterized with phenotypes that can be divided into five classes: bloodless, blocked progenitor proliferation, blocked progenitor differentiation, hypochromia, and photosensitive blood (**see also Blood**: Cellular Composition of the Blood and Erythropoiesis in Fishes).

Anemia

To date, most insight has been gained into red cell function, such as iron metabolism, heme synthesis, and globin expression. This is significant because one of the most common human hematopoietic disorders is anemia, a deficiency of red blood cells. By modeling different anemias in the fish, a much more detailed understanding of the causes of the multiple forms of human anemia can be found, and the feasibility of potential therapies can be evaluated.

One specific example is the human disease, sideroblastic anemia. Using forward genetics, scientists identified a mutant fish, sauternes (sau), which presented with immature circulating erythroid cells, a characteristic of the human condition. Using positional cloning, a mutation of the gene ALAS2 (Delta-aminolevulinate synthase 2) was found on chromosome 9. Likewise, it is known that sex-linked congenital sideroblastic anemia in humans is due to a mutation in the ALAS2 gene. This mutant fish model for human sideroblastic anemia can be used to study the enzyme's function and its role in anemia, with the hope of finding more effective treatments.

Hematopoietic Stem Cells

HSCs are another area of intense scientific study. For several decades, transplantation of human HSCs into patients with defective hematopoiesis has been used as a therapy to repopulate the patient's hematopoietic system. This is most commonly used for patients with cancers of the blood system. However, HSC transplant is still a highly toxic procedure, in part because it takes several weeks for the transplant to engraft in the recipient and there is a limited amount of HSCs available to patients. Researchers hypothesized that a better understanding of how to increase the total number of HSCs in fish could lead to possible improvements in bone-marrow transplants in humans. A team of investigators followed up this hypothesis and used the fish to perform a chemical screen looking for chemicals that either increased or decreased HSCs in the fish embryo. In the screen, they found that prostaglandin E2 increased the number of HSCs. After pretreating mouse HSCs with prostaglandin prior to transplantation, they found an expansion of functional HSCs, which led the researchers to hypothesize that prostaglandin E2 could be used as a therapy for patients needing bone-marrow transplants. The clinical trials for prostaglandin are currently underway. This example shows how using a zebrafish screen led to a discovery of a novel role for a chemical, which ultimately has led to a Food and Drug Administration (FDA)-approved clinical trial on human patients.

Leukemia

Cancer of blood, or leukemia, is a broad class of diseases, which are incompletely understood. Leukemia is marked by abnormalities of the bone marrow in which there is overproduction of cells called blasts, which are immature red, white, or platelet blood cells (**Figure 4**). While there are some treatments available to leukemia patients, little is known about why the disease initiates and how it progresses. Fish allow us to model leukemia to better understand what happens in the earliest stages even before diagnosis is possible. Researchers have been able to model types of human leukemias in fish using the transgenic approach, in which various oncogenes known to be important in human leukemia can be studied. Some examples using this approach include: (1) the rag2 promoter driving either the myc oncogene, or the

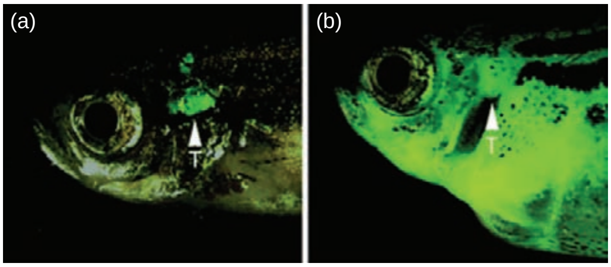


Figure 4 Leukemia transgenic adult *rag2-EGFP-mMyc* zebrafish. (a). Normal GFP expression in the thymus of the control rag2-GFP fish. (b). GFP-labeled leukemic lymphoblasts in the leukemia fish model (rag2-EGFP-mMyc). Arrowheads mark location of the thymus (T). Reproduced from Langenau *et al.* (2005) 102(17): 6068.

Notch1/bcl2 oncogenes, results in T-cell acute lymphoblastic leukemia (T-ALL), (2) the beta actin promoter (expressed in most cells) driving the runx1 oncogene leads to early B-lineage acute lymphoblastic leukemia, and (3) the beta actin promoter driving the KRAS oncogene leads to a myeloproliferative disorder replicating human chronic myelomonocytic leukemia. Other oncogenes are formed when two separate genes translocate and become expressed jointly. The BCR/ABL oncogene has been used in fish to model human chronic myeloid leukemia, and the TEL-AML1 oncogene is used to model acute lymphoblastic leukemia.

Heart

Over 40 cardiovascular mutants have been defined in fish, and are divided into two groups: defective cardiac formation or cardiac function.

Cardiac Formation

Malformations of the heart have devastating effects in human patients. While surgeons can repair holes, replace valves, and even transplant new hearts, what causes the original deformations is unknown. Using a forward genetic mutagenesis screen, a zebrafish mutant gridlock was found that has a heart defect resembling the human condition of coarctation of the aorta, a congenital malformation of the heart, for which, in many cases, the cause is unknown. The fish was found to have malformation of the aorta, leading to decreased blood flow to the tail. This is functionally similar to human patients, in which malformations of the aorta restrict blood flow to the lower parts of the body. Because the early development of the human disease is difficult to study, the fish model is useful because embryos known to carry the gridlock defect can be studied from the moment they are fertilized to allow for more insight into the initiating steps of the disease. In addition, a chemical screen was performed on this model of coarctation of the heart revealing two chemicals, GS4012 and GS4898, which were able to completely rescue the mutant without causing developmental defects. In addition to the coarctation model, other mutants of clinically relevant cardiac malformations of heart fusion and size have been created. The mutants miles apart (mil) and bonnie and clyde (bon) have disrupted heart fusion, causing differentiation and development of two hearts, a phenotype resembling the human condition of cardia bifida. Mutants of other cardiac malformations include cloche (no endocardium), valentine (large heart), and heart and soul (small dense heart).

Cardiac Function

Diseases of heart malfunction are critical to understand. If the heart beats too fast, it can lead to heart failure; if the heart beats too slowly, blood cannot supply sufficient oxygen needed by all organs. Scientists have also been able to use the fish as a model to study cardiac function. In an example using transgenesis, a cardiovascular research team developed a unique assay to quantify the embryonic heart rate in fish. The researchers created a transgenic fish in which green fluorescent protein (GFP) was expressed exclusively in the myocardium. They then developed a computer program that could analyze and calculate the heart rate in living fish. Use of this technique is exciting for groups studying a variety of heart-rate disorders. Atrial fibrillation is the most common human cardiac arrhythmia, characterized by the heart beating too quickly. In some cases, this disorder is familial; however, little is known about its cause. Another disorder is supraventricular tachycardia, which is characterized by a sudden, almost instantaneous increase in heart rate. In order to gain insight into these conditions, forward genetic screens have been performed to isolate mutants with abnormalities in heart rate. The fish mutant breakdance (bre) has an arrhythmia in which the ventricle beats only with every second atrial contraction, resembling the human Long QT syndrome. The human short-QT syndrome has been modeled in the fish mutant reggae (reg) that shows cardiac fibrillation due to accelerated cardiac repolarization. Different mutations in the same gene, Zerg, are responsible for both phenotypes. Other cardiac function mutants include: slow mo (slow heart rate/sinus bradycardia), dead beat (weak ventricle contractility), beach bum (weak contractility of both chambers), tremblor (fibrillation), and ping pong and yoyo (retrograde flow and regurgitation of blood).

Kidney

There are many diseases of kidney tissues, some of which involve the formation of fluid-filled cysts in the kidney tubules. Autosomal dominant polycystic kidney disease (ADPKD) is one such common genetic human disease that often leads to renal failure. While extensive studies have been done to elucidate the mutations of the PKD1 gene that are involved in the human disease, it is still unclear how and why these fluid-filled cysts develop. Recently, a mutagenesis screen in zebrafish identified three genes that encode components of intraflagellar transport particles needed in cilia formation. It has been proposed that the primary cilia act as flow-sensing organelles in the kidney cells. Once flow in the tubules is optimized, the cilia can signal the kidney cells to stop replicating. In mutated forms, however, the cilia do not signal properly, leading to outgrowths of the kidney that develop into cysts (**Figure 5**). The results of these experiments suggest that mutations in these ciliary genes are one major cause of PKD. There are multiple kidney diseases involving cyst formation that likely may also be linked to these ciliary genes. In addition, because the embryos are transparent, researchers are able to visualize the early signs of cyst formation in vivo well before they are visible in other model systems.

Neuroscience

Neurodegenerative Disease

Huntington's disease is an inherited neurodegenerative condition characterized by cognitive defects, dementia, and psychiatric dysfunction. While extensive research has identified a mutation in the human protein, huntingtin, the precise function of this gene remains poorly understood. Researchers have recently identified a zebrafish homolog to the Huntington's disease gene, called zhd, which encodes the protein huntingtin. Identification of this gene will allow researchers to understand its function in normal development, using morpholino knockdown. In addition, a knockout of this gene (using TILLING or zinc fingers) can help to identify mutant fish, which exhibit characteristics of patients with Huntington's disease.

Alzheimer's is a neurodegenerative disease caused by fragments of a protein, amyloid precursor protein (APP). A fragment of APP called amyloid beta peptide can aggregate and form amyloid plaques, which accumulate in brain neurons. Pen-2 is an enzyme required for the formation of the amyloid B protein plaques. Researchers used the zebrafish to better understand the role of Pen-2 in Alzheimer's development by designing a morpholino to block transcription of the Pen-2 gene. Knockdown of Pen-2 had a negative effect on cell survival indicated by an increase in apoptosis. Because apoptosis in the adult nervous system is often an initiating step in many neurodegenerative diseases, the fish provides a useful tool for deciphering this complex signaling pathway in the origins of Alzheimer's disease. While it is still unclear how Pen-2 affects the formation of plaques in adult neurons, research focused on better understanding its normal function will enhance our knowledge of brain development, neuronal apoptosis, and the signaling mechanisms involved in plaque formation leading to Alzheimer's.

PD is another neurodegenerative disease affecting 1 in 800 people. Patients suffer from physical rigidity and tremors in addition to cognitive psychological abnormalities. Previous studies have shown that PD is, in part, due to loss of dopaminergic cells; however, we still do not know exactly why the neurons die. Aspects of PD can be induced by mutations in the A2A adenosine receptor (Adr) genes, or due to the administration of a neurotoxic drug called 1-methyl-4-phenyl-1,2,4,5-tetrahydropyridine (MPTP) that causes degeneration of dopaminergic cells. In the past 2 years, zebrafish homologs to the Adr genes have been identified. The function of these genes in early development was studied using morpholino technology, which induced loss of dopaminergic neurons

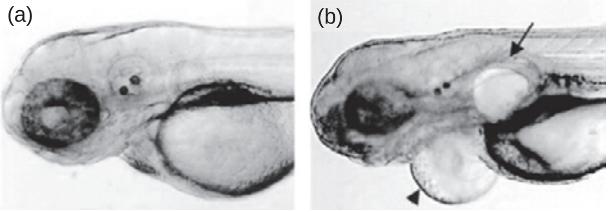


Figure 5 Kidney cysts in zebrafish embryos. (a) Control embryo. (b) The arrow in (b) shows a kidney cyst, and the small arrowhead shows pericardial edema. Reproduced from Sun and Hopkins (2001).

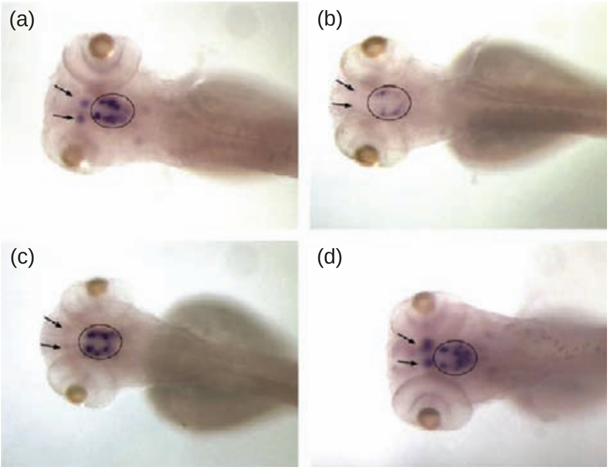


Figure 6 Caffeine protects dopaminergic neurons from MPTP-induced neurotoxicity. 5 dpf embryos hybridized with *dat* probe, staining ventral diencephalon neurons. (a) Untreated control embryo. (b) Drug-treated embryo with 40 μM MPTP shows decreased neuronal staining. (c) Embryo treated with 10 μM caffeine and also 40 μM MPTP shows increase in staining compared to MPTP treatment in (b). (d) Embryo treated with 10 μM caffeine shows normal staining. Black arrows indicate bilateral pretectal cluster of neurons. Reprinted from Boehmle *et al.* (2009) Identification of zebrafish A2 adenosine receptors and expression in developing embryos. *Gene Expression Patterns* 9(3): 144.

similar to that effected by MPTP. Because caffeine is a known A2A AdR antagonist, researchers hypothesized that caffeine would be neuroprotective against MPTP, and this was found to be selectively true – the results showed the caffeine protected ventral diencephalic but not pretectal neurons (**Figure 6**). The zebrafish Parkinson's model is the first description of following the disease state from its earliest stages. It also further implicates AdR antagonists as promising therapeutic treatments.

Muscle

Muscular Dystrophy

Muscular dystrophy is an inherited disease of skeletal muscle in which abnormalities of several muscle proteins, such as dystrophin and dystroglycan, contribute to the disease. A better understanding of how these proteins cause muscular degeneration would provide therapeutic opportunities. Recently, a zebrafish mutagenesis screen was performed to isolate mutants with defects in muscle formation. One mutant, referred to as sapje (sap), was confirmed to resemble many aspects of the disease. Positional cloning of the affected gene revealed a mutation in the dystrophin (DMD) gene, one of the proteins known to be affected in human patients. The sap mutant was then studied during early embryonic development to gain insight into the origins of muscular dystrophy. The muscles of human adult patients suffering from DMD show tearing and scaring along the entire muscle region. However, the sap mutant showed that dystrophin is specifically lost from the ends of the muscle fibers in developing embryos. Because we cannot assess where damage is occurring in developing humans, the zebrafish model suggests that muscle attachment failure to the cytoskeleton is also a major contributor to DMD. Other dystrophic mutants reflecting various aspects of MD have been developed, and include softy, unresolved, and candyfloss (a model of congenital muscular dystrophy (MDC1A).

In addition to studying mutants, researchers also used morpholino technology to further examine the function of the genes involved in muscular dystrophy. Dystroglycan is another component of the dystrophin-associated protein complex (DAPC), which is found at the membrane of skeletal muscle fibers and provides a structural link between the cytoskeleton and the extracellular matrix. Downregulating the dystroglycan gene during embryonic development demonstrated that the protein is essential for normal differentiation of muscle fibers. While loss of dystroglycan does not cause muscular dystrophy, it does result in the inability of the muscle fibers to form the normal organization of proteins required for proper muscle contraction. It is likely that defects in dystroglycan can be linked to more rare forms of the disease such as Fukuyama-type muscular dystrophy.

Muscle Tumors

Little is known about the origins, progression, or treatment of muscle cancer. The zebrafish has recently emerged as a tool in understanding a type of muscle tumor called rhabdomyosarcoma. Activation of the RAS oncogene pathway is known to occur in some human muscle tumors. Studies using a transgenic fish ubiquitously expressing the kRAS oncogene under a beta-actin promoter resulted in tumor formation resembling human rhabdomyosarcoma. The childhood form of this disease is especially devastating; at the time of a metastatic diagnosis, less than 25% of patients surviving past 5 years. While the rag2 promoter described in leukemia is known to be specific to T cells, it was recently discovered that it also is expressed in embryonic zebrafish muscle cells. Surprisingly, linking the kRAS oncogene to the Rag2 promoter created a fish model of childhood rhabdomyosarcoma. The reasons for this remain unclear. Using this model as a basis for research, further evaluation of human rhabdomyosarcoma reported that the majority of human rhabdomyosarcoma tumors also shows an activated RAS pathway. Researchers hope to use the zebrafish model to further explore the specifics of the RAS pathway responsible for tumor progression.

Skin

Pigmentation

An everlasting puzzle in human biology surrounds the question of the genetic origin of skin color: why do people of different races have different skin pigmentation, and why do people within those races show varying degrees of coloration? It is known that variations of skin pigmentation are due to differences in the number, density, and size of melanosomes, the pigmented organelles within melanocytes. Previous to studies using zebrafish, it was largely unknown what genes cause these variations between populations. A zebrafish mutant named golden shows delayed and reduced production of the melanin pigment (**Figure 7**). This mutant resembles light-skinned humans. Positional cloning revealed the golden mutant was caused by a mutation in the nckx5 gene, a homolog to the mammalian slc24a5 gene. Functional conservation of these genes was supported by injecting human slc24a5 messenger RNA (mRNA) into the golden mutant, resulting in rescued melanin pigmentation. Analysis of the human golden gene showed that, at the 111-amino-acid position, there could be either a threonine or an alanine variant, called a single nucleotide polymorphism (SNP). Comparing this to human population studies, they reported that nearly 99% of European Americans expressed the Thr allele, and close to 95% of African, indigenous American, and East Asian populations expressed the Ala allele. These data revealed that this single gene accounts for nearly 40% of human skin pigment differences. The fish model provided an exciting example of how a mutagenesis screen can be used to first find a phenotype of interest in the fish and then use the fish gene to determine the human homolog and its function.

Fish provide a unique tool for studying disorders of skin pigmentation. Fish have three distinct pigment cell types: melanophores (the melanin-containing cells called melanocytes in humans), xanothophores (give the fish a slight yellowish hue), and the iridophores (contain the reflective platelets on fish skin). However, the most studied of these pigment types in human disease are the melanophores. Albinism in humans has many forms due to two major types of melanocyte defects. In one set, patients are entirely missing the cell type (melanocytes) that produce melanin, and, in the other, the melanocytes are present but are defective and unable to produce the melanin pigment. A fish model of the former was created in a fish mutant termed nacre (**Figure 8**). Nacre is a homolog to the human MitF, a gene known to be required for eye and pigment cells in the mouse and human. The

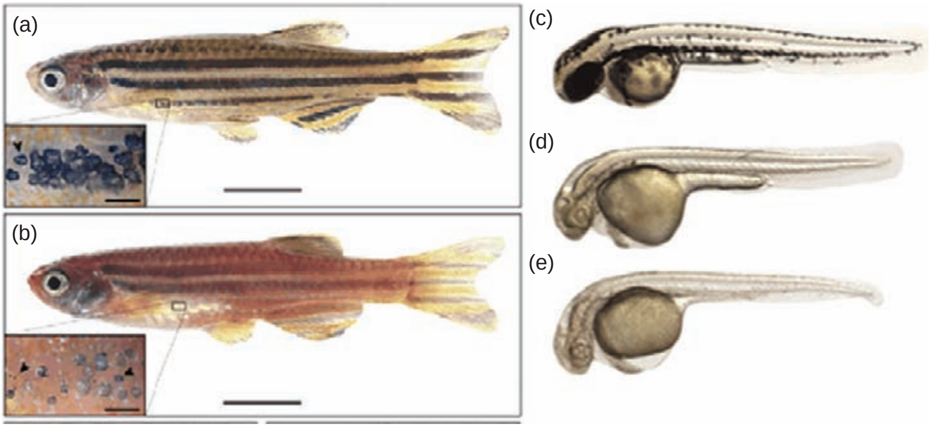


Figure 7 Phenotype of *golden* adult and embryonic zebrafish. (a) Adult wild-type zebrafish and (b) adult zebrafish *golden* mutant. Insets show melanophores (arrowheads). Adult *golden* mutants have melanophores than smaller, more pale, and transparent. (c) Wild-type zebrafish embryp 55-hpf. (d) *Golden* zebrafish mutant embryo 55-hpf. (e) Morpholino to the translational start site of *slc24a5* phenocopies the *golden* embryonic mutation. From Lamason *et al.* (2005) SLC24A5, a putative cation exchanger affects pigmentation in zebrafish and humans. *Science* 310: 1782.

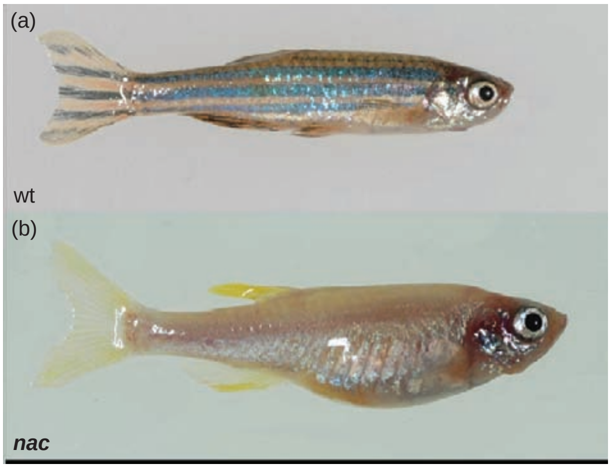


Figure 8 Phenotype of (a) adult WT zebrafish and (b) adult nacre mutant zebrafish.

nacre mutant shows an absence of melanocytes throughout development. Other types of albinism result from defective melanocytes that cannot produce pigment. This disorder is phenocopied in a mutant fish named *sandy*, which has defects in the melanin-producing enzyme tyrosinase. Modeling human pigmentation disorders in the fish will allow scientists to gather more information about embryonic development and possible therapies for more detrimental disorders of this class (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Pigment Genes and Cancer Genes and **The Skin**: Coloration and Chromatophores in Fishes).

Melanoma

Melanoma is a particularly devastating cancer, which arises from melanocytes in human skin. With little known therapy, once metastatic disease is diagnosed, there are nearly no effective available therapies. Understanding the beginning stages of melanoma are crucial to finding methods to prevent cancer cells from forming, or delaying the metastasis of the disease. To gain to insight into the origins of this disease, researchers have studied the two hits necessary to produce most cancers. One hit is a mutation of a gene that causes excess cell proliferation, a hallmark of most cancers. In human melanoma, this is usually due to mutations in the BRAF oncogene. The second hit is something that prevents cells from undergoing cell death (apoptosis). In melanoma, this second hit often comes from loss of the p53 tumor suppressor gene. The normal function of p53 is to signal cells to apoptose when cell programming goes awry. The p53 null mutant is unable to send this apoptosis signal, thus allowing the abnormal oncogenic cells to multiply. Using this information, scientists created a melanoma model in fish by expressing the BRAF oncogene under the melanocyte-specific MitF promoter in the background of a p53 null fish. Nearly 100% of these transgenic fish eventually form melanoma tumors that histologically resemble human tumors (**Figure 9**). These fish mutants are being studied extensively with the hope of finding therapies for the growth and metastasis of melanoma cells (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Pigment Genes and Cancer Genes).

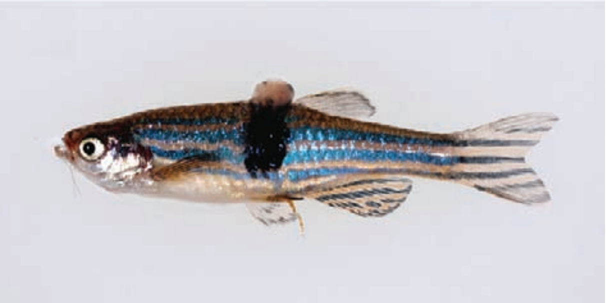


Figure 9 Adult zebrafish melanoma model. Photo courtesy of Richard White, Dana-Farber Cancer Institute, Boston, MA.

Behavior

Addiction

While a relatively new field, studying psychological or behavioral traits in the zebrafish is beginning to emerge as a useful adjunct to mammalian studies. Researchers studying diseases of addiction, most notably to alcohol and nicotine, have begun using the fish as a model organism. Scientists have been able to accurately mimic the enhanced dopamine levels found in human patients repeatedly exposed to addictive drugs. A forward genetic screen produced a fish mutant, too few, that cannot produce sufficient dopaminergic and serotonergic neurons in the forebrain. After multiple pre-exposure to morphine, when given the choice between normal or morphine-containing water, normal fish show a preference for the drug water. The too few morphant, however, does not show this same preference as their wild-type siblings, implicating that choice behavior and addiction is partially mediated through the dopaminergic and serotonergic neurons in the central nervous system.

Sleep

Sleep disorders such as narcolepsy are in the beginning phases of being modeled in fish. The neuropeptide hypocretin is a key regulator of sleep in humans. A fish mutant was created in which there is a mutation for the gene encoding the receptor of this neuropeptide. These fish display a contrasting phenotype to narcolepsy. Rather than excessive sleepiness or cataplexy, the fish suffer from short and fragmented period of sleep in the dark. Understanding what genetically separates these abnormal patterns of fish sleep from narcolepsy will better our understanding of both disorders, and possibly lead to novel therapies. Current research is focused on delineating the normal role of sleep, its cycle, and modeling other sleep-associated disorders in fish.

Mood

Researchers are also interested in studying different mood behaviors in fish. Most recently, a novel aggressive mutant, speigel, was identified. It appears that serta, the serotonin transport gene, plays a role in the speigel mutant. Current experiments are testing whether selective serotonin reuptake inhibitors affect the aggressive behavior in speigel. Future studies hope to evaluate if other behavioral traits can also be linked to specific genetic mutations.

Future

The field of fish research has provided us with many discoveries about human disease over the past decades and will undoubtedly continue to grow and expand in the future. It is likely that significant areas of research will further involve functional genomics, chemical screening, and cancer genomics. In the post-genomic era, where the genomes of many species have now been fully sequenced, a major challenge is to try and understand the function of these gene sequences. The rapidity of transgenesis and increasing tools in reverse genetics will allow for high-throughput analysis of understanding gene functions that are conserved across vertebrate species. In addition, until this point, the capacity for chemical screens in the fish has been restricted to relatively limited small molecule libraries. However, with increasing automation and novel assay design, truely high-throughput screens (i.e., 50000–500 000 compounds) will be feasible in the near future. This will allow for a comprehensive examination of in vivo phenotypes induced by structurally diverse small molecules. Lastly, 'deep' resequencing of cancer genomes is rapidly accelerating, but the importance of these newly discovered gene mutations is largely unknown. Utilizing reverse genetics and transgenesis, the zebrafish is well poised to allow for detailed modeling of novel cancer genes in a high-throughput, relevant model organism.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines; Growth Hormone Overexpression in Transgenic Fish.

Further Reading

Amacher SL (2008) Emerging gene knockout technology in zebrafish: Zinc-finger nucleases. *Briefings in Functional Genomics* 7(6): 460–464.

Amsterdam A and Hopkins N (2006) Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. *Trends in Genetics* 22(9): 0168–9525.

Baker K, Warren KS, Yellen G, and Fishman MC (1997) Defective"pacemaker" current (Ih) in a zebrafish mutant with a slow heart rate. *Proceedings of the National Academy of Sciences of the United States of America* 94(9): 4554–4559.

Boehmlera W, Petkoa J, Wolla M, *et al.* (2009) Identification of zebrafish A2 adenosine receptors and expression in developing embryos. *Gene Expression Patterns* 9(3): 144–151.

Bretaud S, Li Q, Lockwood BL, *et al.* (2007) A choice behavior for morphine reveals experience-dependent drug preference and underlying neural substrates in developing larval zebrafish. *Neuroscience* 146(3): 1109–1116.

Campbell WA, Yang H, Zetterberg H, *et al.* (2006) Zebrafish lacking Alzheimer presenilin enhancer 2 (Pen-2) demonstrate excessive p53-dependent apoptosis and neuronal loss. *Journal of Neurochemistry* 96(5): 1423–1440.

Craig J, Ceol I, Houvras Y, *et al.* (2008) Pigmentation biology: Melanoma biology and the promise of zebrafish. *Zebrafish* 5(4): 247–255.

Currie PD and Lieschke GJ (2007) Animal models of human disease: zebrafish swim into view. *Nature Reviews Genetics* 8(May): 353–367.

Dahme T, Katus HA, and Rottbauer R (2009) Fishing for the genetic basis of cardiovascular disease. *Disease Models and Mechanisms* 2: 18–22.

David I, Bassett DI, and Currie PD (2003) The zebrafish as a model for muscular dystrophy and congenital myopathy. *Human Molecular Genetics* 12(2): R265–R270.

David Hassel MS, Edward P, Scholz MD, *et al.* (2008) Deficient zebrafish ether-à-go-go_related gene channel gating causes short-QT syndrome in zebrafish reggae mutants. *Circulation* 117: 866–875.

Guo S (2004) Linking genes to brain, behavior and neurological diseases: What can we learn from zebrafish? *Genes Brain, and Behavior* 3(2): 63–74.

Hall TE, Bryson-Richardson RJ, Berger S, *et al.* (2007) The zebrafish candyfloss mutant implicates extracellular matrix adhesion failure in laminin? 2-deficient congenital muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America* 104(17): 7092–7097.

Hostetter CL, Sullivan-Brown JL, and Burdine RD (2003) Zebrafish pronephros: A model for understanding cystic kidney disease. *Developmental Dynamics* 228(3): 514–522.

Ingham PW (2009) The power of the zebrafish for disease analysis. *Human Molecular Genetics* 18(Review Issue 1): R107–R112.

Karlovich CA, John RM, Ramirez L, Stainier DY, and Myers RM (1998) Characterization of the Huntington's disease (HD) gene homologue in the zebrafish *Danio rerio*. *Gene* 217(1–2): 117–125.

Kim TAE, Sellin LK, Benzing T, *et al.* (1999) The polycystic kidney disease 1 gene product modulates Wnt signaling. *Journal of Biological Chemistry* 274(8): 4947–4953.

Kopp R, Schwerte T, and Pelster B (2005) Cardiac performance in the zebrafish breakdance mutant. *The Journal of Experimental Biology* 209(11): 2123–2134.

Lamason RL, *et al.* (2005) SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 310(Dec): 1782–1786.

Langenau DM, Keefe MD, Storer NY, *et al.* (2007) Effects of RAS on the genesis of embryonal rhabdomyosarcoma. *Genes and Development* 21(11): 1382–1395.

Lister JA, Robertson CP, and Lepage T (1999) Nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development* 126(17): 3757–3767.

MacRae CA and Peterson RT (2003) Zebrafish-based small molecule discovery. *Chemistry and Biology* 10(Oct): 901–908.

North TE, Goessling W, Walkley CR, *et al.* (2007) Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* 447(7147): 1007–1011.

Norton WHJ, Webb K, Harris M, *et al.* (2008) Approaches to analyse mood disorders in zebrafish. *Proceedings of Measuring Behavior*. The Netherlands: Maastricht.

Schiffer NW, Broadley S, Hirschberger T, *et al.* (2007) Identification of anti-prion compounds as efficient inhibitors of polyglutamine protein aggregation in a zebrafish model. *Journal of Biological Chemistry* 282(12): 9195–9203.

Stainier DY, Fouquet B, Chen JN, *et al.* (1996) Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. *Development* 123(1): 285–292.

Wheeler GN and Brändli AW (2009) Simple vertebrate models for chemical genetics and drug discovery screens: Lessons from zebrafish and *Xenopus*. *Developmental Dynamics* 238(6): 1287–1308.

Wittbrodt J, Shima A, and Schartl M (2002) Medaka – a model organism from the far east. *Nature Reviews Genetics* 3: 53–64.

Yelon D (2001) Cardiac patterning and morphogenesis in zebrafish. *Developmental Dynamics* 222(4): 552–563.

Yokogawa T, Marin W, Faraco J, *et al.* (2007) Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biology* 5(10): 2379–2397.

Transgenesis and Chromosome Manipulation in Fish

N Maclean, University of Southampton, Southampton, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Fish Transgenesis
Further Reading

Glossary

Antifreeze proteins Small proteins secreted into the blood of some species of Arctic fish to prevent their blood freezing in the Arctic winter seas. Some Antarctic fish also produce antifreeze but they are quite different in action from the antifreezes of Arctic fish and have clearly evolved separately. Antifreeze coding genes have been isolated from some fish species such as ocean pout and winter flounder and used transgenically in attempts to make Atlantic salmon resistant to freezing.

Diploid Having two complete chromosome sets.

Exon A part of the DNA sequence of a gene which is expressed in the protein. Following initial transcription, intron sequences are spliced out and the exon sequences spliced together to produce the relevant messenger RNA.

Gene A hereditary unit consisting of a single strand of DNA occupying a specific locus within a chromosome, and in many cases carrying the genetic code for a protein.

Gene expression The production of a phenotypic trait by a gene. This involves the transcription of the gene into RNA and subsequent translation of the RNA into protein.

Gene integration The incorporation of a DNA gene sequence into the chromosomal DNA of the cell nuclei following the gene's introduction into the cell.

Gene manipulation Any artificial work with chromosomes or genes, including gene sequence isolation, modification, cloning, and subsequent use in transgenesis. See transgenesis.

Genetic engineering Essentially synonymous with gene manipulation (see transgenesis).

Genome The entire set of genes and noncoding DNA present in the haploid cell of a particular organism, but not including the separate mitochondrial genome.

Genotype The genetic constitution of an organism. Only part of the genotype is expressed in the phenotype, since recessive alleles present in a heterozygous state are not expressed in the phenotype.

Germ line transmission Passage of a gene through the eggs and sperm from parent to offering. This will normally only occur if the gene in question is present in the chromosomal DNA of the gonads of the parents.

Green fluorescent protein (GFP) A widely used reporter gene used in transgenesis. The original was derived from the genome of a jellyfish, and the protein product shows green fluorescence without the need for activation, substrate provision, or the death of the animal.

Haploid A genetic state in which a cell or organism possesses only one copy of each gene and chromosome. Sperm and egg cells are haploid, and fertilization of the egg by the sperm restores diploidy.

Intron A part of the DNA sequence of a gene which is not expressed in the protein but spliced out between transcription and translation.

lac Z A gene from the bacterium *E. coli* which is often used as a reporter gene in work with fish. When an appropriate development procedure is used, the lac Z provides a blue color. Only of use with dead material. lac Z means the Z gene from the lac operon, a small cluster of genes in the bacterium which act as a unit.

Luciferase A reporter gene derived from a species of firefly. For luminescence to show, the substrate luciferin is required.

Meganuclease A particular category of restriction endonuclease enzyme with a very long DNA target sequence.

Morpholino A molecule used to knock down gene translation. This is achieved by blocking access of ribosomal initiation complex to an mRNA with modified antisense oligomers, and thus preventing from making a targeted protein.

Mosaic expression Nonuniform expression of a transgene in an organism, implying that only some tissues carry copies of the transgene, or that the expression is only activated in certain tissues. The latter situation is more commonly referred to as tissue-specific expression.

Phenotype Observable characteristics of an organism including different traits. Phenotype results from the expression of an organism's genes and the influence of environmental factors.

Polar body The tiny cell released by the oocyte during reduction division. Although technically a cell, the polar body has almost no cytoplasm, but contains a nucleus. Triploid induction may involve retention of one of the polar bodies, induced by pressure or temperature shock.

Primary oocyte A stage in vertebrate egg production prior to the two rounds of division which produce the egg. The primary oocyte has four copies of each chromosome, the secondary oocyte following the first reduction division only two, and the egg following the second reduction division only one. These reduction divisions each involve the release of a polar body.

Prion A misfolded protein particle capable of causing diseases such as mad cow disease (BSE). The misfolded prion protein is capable of inducing similar misfolding to the relevant proteins of the host, thus causing disease. The genes coding for the prion protein are present in the genome of the affected organism.

Promoter The upstream sequence of DNA which regulates the expression of the downstream coding sequence of the gene. The promoter may sometimes be as big or bigger than the gene itself. A so-called minimal promoter is the length of the promoter required to achieve expression of the gene, but without additional positive or negative elements.

Red fluorescent protein (RFP) An alternative to the more commonly used reporter gene (GFP). Like the latter, RFP is derived from a jellyfish and needs neither substrate nor activation for fluorescence.

Reporter gene A gene sequence used to provide a signal, usually visible, that a gene sequence is present in a cell or organism after transgenesis. Common examples are lac Z, GFP and RFP genes, and the luciferase gene.

Restriction enzyme Endonuclease enzymes which are highly specific and cut DNA only within a particular target sequence.

Sleeping Beauty transposon A transposable element developed within the laboratory of Prof. Perry Hackett in the USA. The original inactive element was recovered from a salmon genome, and then returned to full active transposable activity by a series of targeted mutations. It has come to be widely used to facilitate gene integration during transgenesis.

Tetraploidy The genetic state of a cell or organism in which four copies of each chromosome are present, usually two being of paternal origin and two of maternal origin. Tetraploidy can sometimes occur naturally as a result of a failure of a nucleus to divide after DNA replication and mitosis. Tetraploid organisms may be sterile, but are not invariably so.

Transgenesis Transfer of new genetic material to an organism or cell, or between organisms. The resulting organisms are then said to have been genetically manipulated (GM).

Transposon A DNA element, often a few kilobases in size, which can insert itself within a genome or move from one site to another within a genome.

Triploid Having three complete chromosome sets.

Introduction

Transgenesis is the introduction of a novel gene into the genome of an organism. Such organisms are then referred to as genetically manipulated or GM. When it became possible in the 1980s to isolate and clone genes from a variety of organisms, the possibility of transgenesis arose, and this was initially achieved in mice and *Drosophila.* Clearly, the target of any transgenesis program is to have the novel gene of interest isolated, introduced into the cells of the recipient organism, integrated into the chromosomal DNA, and eventually transmitted to the progeny of the organism as part of the normal genetic endowment. Finally, it is expected that the relevant gene will be expressed in the cells and tissues of the recipient organism and its progeny. Thus, there are various steps in the progression between gene isolation and having a batch of transgenic animals, and these are usefully referred to as introduction, incorporation, transmission, and expression.

Following success with all of these stages in mice using a rat growth hormone gene, leading to the production of rapidly growing enlarged GM mice, work started in a few laboratories to achieve the same end point with fish. This initial ambition was realized with fish of various species such as common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*).

Chromosome manipulation refers to the artificial alteration of the ploidy of an organism, that is to change the chromosome number from the normal state to haploidy, triploidy, or tetraploidy. It is chiefly of interest in fish as a means of producing triploid fish, which are most frequently sterile and sometimes also grow faster than their diploid siblings.

Fish Transgenesis

Gene Isolation

It has been known since Mendel's time that hereditary factors exist within the nuclei of all living cells, and with the discovery of DNA as the genetic material and its structure as a double helix of nucleotide pairs, it became clear that a gene was a length of DNA, usually of a few thousand bases. In order to appreciate the problems of isolating genes, it is necessary to step back and consider for a moment the structure of a gene. As shown in **Figure 1**, it consists of a linear DNA sequence within a DNA double helix. The gene is only one strand of the double helix, referred to as the sense strand, while the other strand to which it is hydrogen-bonded is referred to as the anti-sense strand. The strand has polarity and runs from a $3'$ end to a $5'$ end, referred to in genetic jargon as the upstream end and the downstream end, respectively. The first part of the upstream sequence is the promoter or regulatory sequence, and each gene has its own individual promoter (sometimes the word 'gene' includes the promoter, and in other terminology it does not). The promoter may be as long as or longer than the actual gene coding sequence. The latter sequence is also frequently interrupted by introns, which do not contribute to the final protein product when the gene is read. The bits that do contribute are called exons, so a gene is most frequently an interrupted DNA sequence.

In order to isolate genes, DNA is purified by a process of preferential solution, enzyme digestion of unwanted material, and centrifugation. Pulling out the desired sequence and separating it from the rest of the chromosomal DNA depends on preferential hybridization, often using analogous sequences from other organisms. In order to undertake transgenesis, genes are required in large numbers of copies (many millions) and this scale-up is known as cloning. Most commonly, genes are cloned by being placed inside the plasmids of a bacterium, the plasmids reintroduced into living bacteria, followed by growth of large numbers of the new gene-transformed bacteria. Finally, after growth of a large culture of such bacteria, the cells are harvested, the plasmids isolated, and the gene of interest recovered in bulk by preferential digestion with the so-called restriction enzymes. The end product of this process is a small volume of, say, 10 ml of dilute saline in which are dissolved many millions of copies of the desired DNA sequence.

Gene Introduction

Usually, the novel genes are introduced into the fertilized eggs of the chosen fish species, and one of the attractions of fish as a model system for transgenesis is that they lay large number of eggs. Often the unfertilized eggs and milt (sperm) are artificially stripped from captive fish and then mixed in a dish to allow fertilization and provide a batch of newly fertilized eggs for the gene introduction. Sometimes unfertilized eggs or young embryos have been employed for attempted transgenesis, but much more frequently fertilized eggs are used.

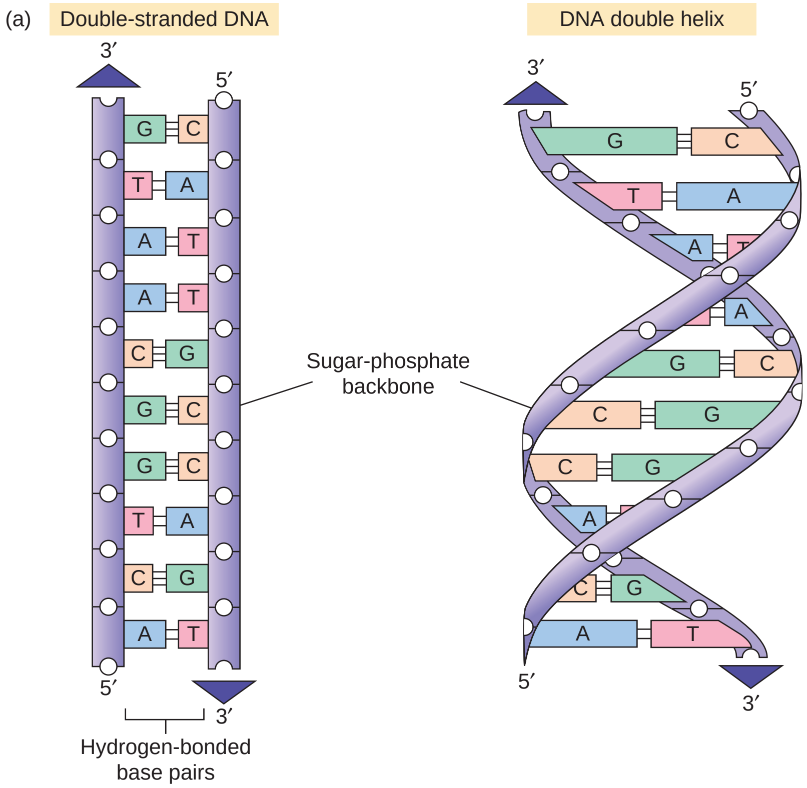


Figure 1 (Continued)

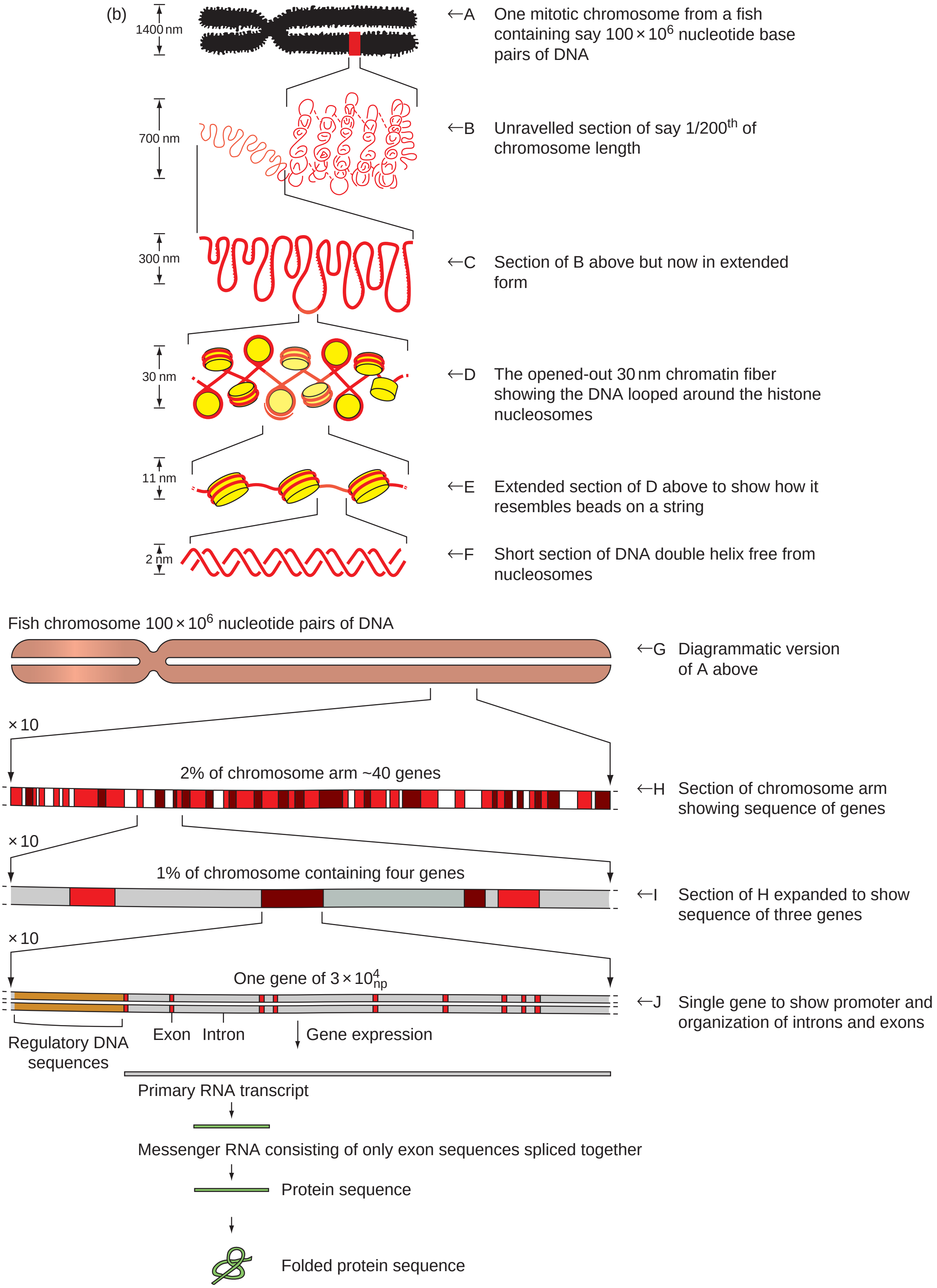

(b)
1400 nm
←A One mitotic chromosome from a fish containing say 100 × 10^6 nucleotide base pairs of DNA
700 nm
←B Unravelled section of say 1/200th of chromosome length
300 nm
←C Section of B above but now in extended form
30 nm
←D The opened-out 30 nm chromatin fiber showing the DNA looped around the histone nucleosomes
11 nm
←E Extended section of D above to show how it resembles beads on a string
2 nm
←F Short section of DNA double helix free from nucleosomes
Fish chromosome 100 × 10^6 nucleotide pairs of DNA
←G Diagrammatic version of A above
× 10
2% of chromosome arm ~40 genes
←H Section of chromosome arm showing sequence of genes
× 10
1% of chromosome containing four genes
←I Section of H expanded to show sequence of three genes
× 10
One gene of 3 × 10^4 np
←J Single gene to show promoter and organization of introns and exons
Regulatory DNA sequences
Exon
Intron
Gene expression
Primary RNA transcript
Messenger RNA consisting of only exon sequences spliced together
Protein sequence
Folded protein sequence

Figure 1 (a) Gene and its structure within a DNA double helix. (b) The linear structure of a gene and its promoter, showing the intron and exon sequences. The whole sequence is initially transcribed into the high-molecular-weight RNA transcript, but this molecule is then modified by the splicing out of the introns, so that the resultant messenger RNA contains only the exon sequences spliced together. The message is therefore, in almost all cases, much shorter than the gene. Partly redrawn from figures by Alberts B, *et al*. (2002) *Molecular Biology of the Cell*. New York: Garland Science.

In the case of the pioneering work with mice, the novel gene copies were introduced by injection into the egg cell nucleus. However, in fish eggs the nucleus is too small and invisible even when using a dissecting microscope, so the gene copies are introduced into the cytoplasm, in the hope that some will find their way to the chromosomes, perhaps during mitosis, and then be incorporated. Since this is a very low frequency event, very large numbers of gene copies, often in excess of 1 million per egg, have to be introduced to give any chance of successful integration.

Sometimes the eggs are placed in a dilute saline solution along with the gene copies and a powerful electric current used to drive some of the copies into the eggs. This is called electroporation. More commonly, the genes in solution are injected into the eggs using fine glass needles and a dissecting microscope. A mechanical machine known as a gene pulser can be used to ensure the appropriate volume and pressure. In some eggs, such as those of carp and zebrafish, the egg chorion is sufficiently soft to allow easy penetration by a fine glass needle, but with eggs of fish species such as salmon, trout, and tilapia, the chorion is very tough and the needle can only be inserted into the egg via the micropyle, which is the narrow tube in the chorion that permits sperm entry. Large numbers of eggs must be injected to allow recovery of some in which chromosome integration has followed on from gene injection.

Gene Incorporation

Since gene translocation (movement of a length of coding DNA from one chromosomal site to another) is a known natural phenomenon in genetics, it is evident that at least theoretically, once inside a cell nucleus, DNA can integrate into a chromosome and so come to permanently occupy a new locus. Following the introduction of many thousands of copies of the transgene, there is the low but significant possibility that one or more of these copies will be integrated, and indeed this is the finding in all transgenic fish programs. A range of methods have been tried to increase the probability of incorporation, and perhaps the most successful of these has proved to be the co-injection, along with the transgene, of molecules of meganuclease. Meganucleases are a kind of restriction endonuclease enzyme which has a long recognition sequence. The trick here is to flank the transgene with two meganuclease recognition sites and co-inject together with molecules of meganucleases. This has led to highly efficient transgenesis in zebrafish with improved integration frequencies and germ line transmission. Although in the fish transgenesis so far practiced, the transgene integration sites are randomly distributed throughout the chromosomes, it could be that the future site-specific integration could be achieved with the clever manipulation of meganucleases.

It is worth stressing that the use of the meganuclease technique, with its increased frequency of transgene integration, also goes some way to offset another problem with fish transgenesis, namely mosaic expression following integration of the transgene in the first-generation fish (and, as a result, frequent failure of such fish to pass the transgene on to progeny because the gonads are not one of the tissues carrying the transgene construct). Such mosaic expression clearly results from late incorporation of a transgene copy after some rounds of embryonic cell division, so that only one cell out of say 8 or 16 cells actually comes to be involved in an integration event.

Another technique now important in achieving good integration frequencies with fish transgenesis is the use of transposable elements. A number of such elements have been tried, and one notable example is the Sleeping Beauty transposon, so called because it was reactivated by targeted mutagenesis of an ancient inactive transposable element recovered from salmon. Here the procedure is to place the transgene construct within the transposon sequence prior to injection. High rates of transgene integration in fish have been achieved in this way. It is worth emphasizing that damage to host genes by transgene integration (so-called insertional mutagenesis) into chromosomes is rare in fish. This is probably explained by the large amount of essentially redundant DNA found in vertebrate genomes, so most often transgenes are integrated in nonfunctional noncoding DNA sequences.

Gene expression

When transgenesis was first envisaged as an experimental technique, there were substantial fears that transgenes would fail to express due to selective DNA methylation or some other gene-silencing mechanism. Fortunately, this has proved to be a rare phenomenon. Gene expression is of course mainly determined by the promoter driving the gene of interest within the construct, and often in the early stages of an experimental program in transgenesis, ubiquitously expressing promoters are employed to bring about gene expression at all times in all tissues. Once expression has been achieved, further work with tissue-specific promoter sequences can be attempted.

It should not be assumed that transgene expression is always indicative of chromosomal integration of the construct. Indeed, the so-called transient expression of unintegrated copies is frequently observed, but this will not survive long once embryonic formation is under way. A device that is often used in a transgenesis program is the employment of reporter sequences. These are genes such as green or red fluorescent protein (GFP or RFP) genes recovered from jellyfish, or the luciferase gene recovered from a firefly, or the beta

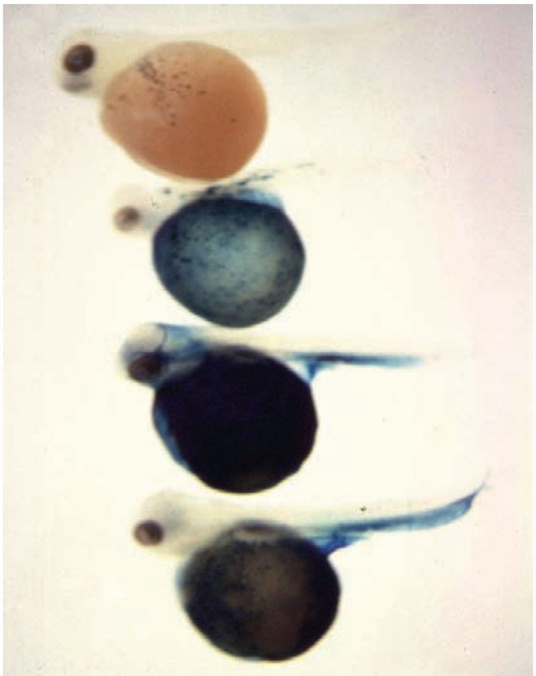


Figure 2 A series of newly hatched tilapia embryos following egg injection with a lac Z reporter gene and subsequent treatment to reveal the color. The uppermost embryo is nontransgenic and the lower three show different degrees of expression in the skeletal muscle. The yolk sacs are also stained. This is not in the yolk itself but in the thin layer of cells covering the yolk. Figure from Maclean laboratory.

galactosidase enzyme gene derived from the bacterium *Escherichia coli.* All of these genes, when coupled with an appropriate promoter, provide a visible color within the cells of the recipient organism (see **Figure 2**). The color may be directly visible, or made visible by the addition of the substrate luciferin in the case of luciferin, or of a blue color from beta galactosidase when X gal staining is carried out. It is understandable, therefore, that constructs made up of chosen promoters, driving both reporter genes and other genes of interest in tandem, are often employed in establishing the early stages of a transgenesis program with fish.

There are few experiences as exciting and rewarding as visualizing the tissue-specific expression of a reporter gene within the cells of a young fish following the demanding and labor-intensive process of injecting large numbers of fish eggs.

As mentioned earlier, expression of a gene is substantially a function of the promoter with which it is teamed, and promoters can be quite complex sequences, containing negative as well as positive transcription enhancers. Thus, designing a promoter to give the desired level and tissue specificity of expression is a highly important aspect of achieving a satisfactory expression profile within the transgenic fish.

Gene Transmission

Most frequently, the Holy Grail of any transgenesis program is to achieve germ line transmission of the transgene construct to progeny fish. Often this will have been previously attempted using a reporter gene construct to ensure that it is indeed feasible. Almost invariably, since transgene integration is a rare event, integration will have occurred into only one of a pair of chromosomes in the set, and so the original transgenic fish will be heterozygous for the transgenic sequence. Since transmission is mendelian, when the transgenic fish is crossed with a wild type, about half of the progeny will carry the transgene and they too will be heterozygous. Only by further crossing of two of the positive progenies will homozygous transgenics be obtained, and these will be approximately 25% of the resulting progeny, with another 25% wild type and 50% heterozygous transgenics.

Occasionally, odd numbers of positive progeny will be found, resulting from two or more separate transgene integrations in the same chromosome set. Heterozygous progeny should of course be nonmosaic, so that every cell and tissue should carry the same single incorporated transgene in one chromosome of the set.

If the transgene is driven by a ubiquitous promoter, then expression should be evident in all tissues of the progeny fish, or, if the promoter is specific in its expression to say, liver, then only liver cells will express in the progeny. Both situations are illustrated in **Figure 3**.

There is often interest in the long-term persistence of the transgene construct. There are examples cited in the literature of transgenes being lost from the chromosomes at some time after its initial germ line transmission. This presumably may result from some failure at mitosis or meiosis, but this seems to be very rare. More commonly, the explanation for subsequent transgene loss is that the transgene was never integrated in the first place but was transmitted and expressed as a nonintegrated nuclear episome. Examples of such entities certainly exist in the literature.

The Usefulness of Transgenic Fish

There is a wide spectrum of different applications through which transgenic fish have already proved to be useful and also offer great promise for the future. They include studies on gene regulation and exploitation of transgenes as signals of gene expression in model fish systems. Second, strains of GM fish can be produced which promise to offer aquaculture strains that improve on those currently used. Third, transgenic fish can be designed to allow environmental monitoring of pollutants. Lastly, there is promise that transgenic fish can be used as biofactory systems for the production of valuable

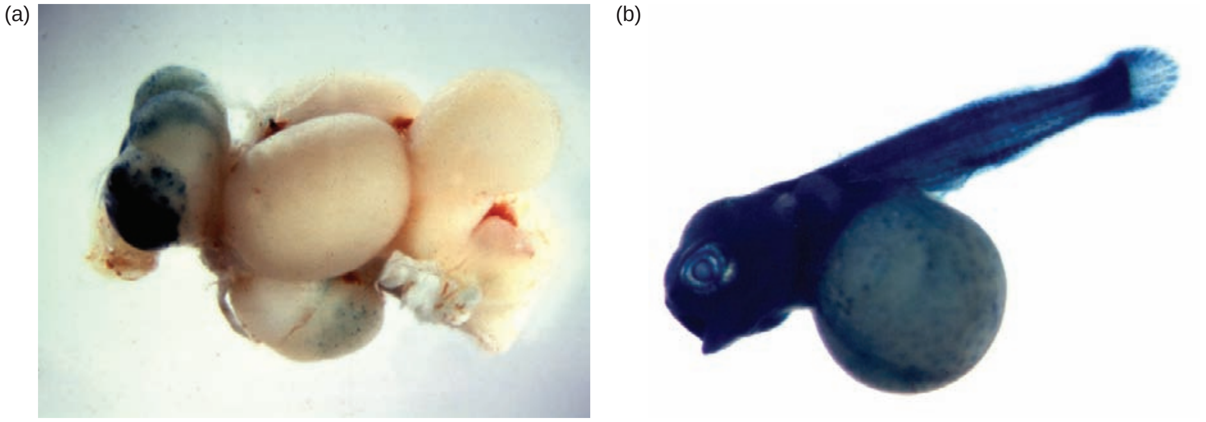


Figure 3 (a) The brain of an adult transgenic tilapia fish dissected out and treated to show localized tissue-specific expression of the lac Z reporter gene in the optic lobes. (b) A tilapia embryo transgenic for carp beta actin promoter spliced to the lac Z gene, showing all over-expression of this ubiquitously expressing promoter. Treated to show lac Z expression. (a, b) Figure from Maclean laboratory.

pharmaceuticals or of cells or tissues which offer benefits to human medicine.

Studies on gene regulation with transgenic fish

These studies have mainly been carried out with small model fish species such as zebrafish (*D. rerio*) or medaka (*O. latipes*), and sometimes with larger species such as goldfish (*Carassius aureus*) or tilapia (*Oreochromis niloticus*) as well. Transgenesis is an attractive methodology because reporter genes can be used to indicate levels of gene expression in particular tissues, and, when combined with the comparative transparency of the embryos of these fish species, to demonstrate in which cells and tissues particular genes are active. They can even be used to track cell movement *in vivo*.

A good example is provided by the splicing of a reporter gene for fluorescent protein to a promoter for a gene called vasa, which is only ever expressed in the early germ cells. The germ cells then light up in the positive fish, allowing them to be counted and their path of travel from the site of origin to the embryonic gonad displayed (see **Figure 4**). Labeling of specific cells within transgenic embryos has also allowed other specific cell migrations to be charted.

The cellular specificities of particular promoters can also be determined in this way, as well as the fine-scale analysis of the structure of particular promoter sequences, so that positive upregulators or downregulators (suppressors) of specific gene expression can be discovered.

Much of the attraction of GM mice for researchers follows from the ability to knock out expression of a single gene and then study the phenotype of the transgenic mice carrying the knock-out. In this way, for example, it has been shown that mice in which the PrP gene that codes for prion protein (as in mad cow disease) is knocked out are apparently normal. So here is a gene which appears to have no function in normal humans, but when its protein product is misfolded it can cause serious disease. Although this gene knock-out technology is not effective in GM fish, an alternative methodology involving the use of specific oligonucleotide morpholinos permits short-term gene knock-out and therefore studies on early expression of important developmental genes.

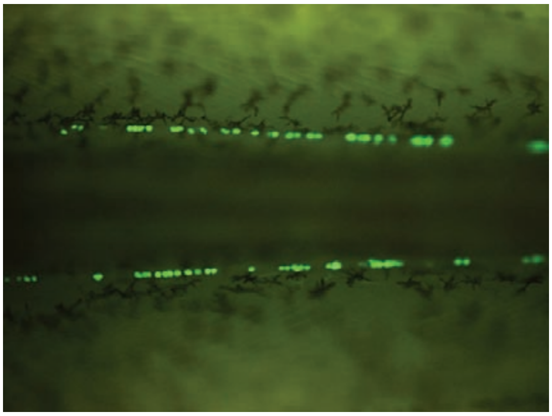


Figure 4 Primordial germ cells of rainbow trout labeled with a green fluorescent reporter gene driven by the vasa promoter from the same species. Vasa expression is specific to these cells. Photo kindly provided by Prof. Yoshizaki. From Takeuchi Y, Yoshizaki G, Kobayashi T, and Takeuchi T (2002) Mass isolation of primordial germ cells from transgenic rainbow trout carrying the green fluorescent protein gene driven by the *vasa* gene promoter. *Biology of Reproduction* 67: 1087–1092.

A further way in which transgenic model fish have contributed to our knowledge of gene regulation follows from the exploitation of particular hybrid crosses between swordtail species (*Xiphophorus*) fish. Some of these hybrids suffer from a form of pigmented melanoma. The transgenic regulation of tumor suppressor genes in these fish which grow melanomas has revealed valuable information about cancer activation and suppression in humans (see **Figure 5**).

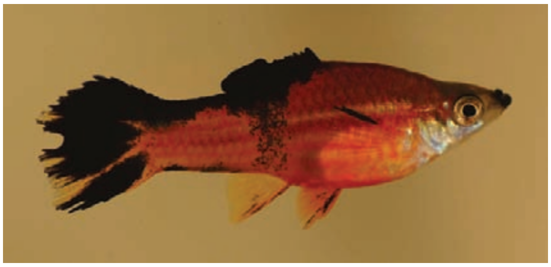


Figure 5 Spontaneous melanoma formation in a crossbred *Xiphophorus* fish. The expression and suppression of tumors in these fish have been manipulated transgenically in Prof. Manfred Schartl's lab in Germany to reveal the genes involved in this cancerous tumor formation. This has proved informative for the understanding of tumor formation in humans. Photo kindly provided by Prof. Manfred Schartl. From Meierjohann S and Schartl M (2006) From Mendelian to molecular genetics: The *Xiphophorus* melanoma model. *Trends in Genetics* 22(12): 654–661.

Development of GM fish offering potential benefits to aquaculture

With the decline of ocean fish stocks as a result of extensive over-fishing, aquaculture has an ever-increasing role in the production of animal protein for human food. Although aquaculture has long been critically important in the economies of countries such as Thailand and China, in recent years the aquaculture of rainbow trout (*O. mykiss*), various salmon species, and of Atlantic cod (*Gadus morhua*) has become very important in countries such as Chile, Norway, and North America.

The parameters which have been modified to date in appropriate species of GM fish include growth enhancement, freeze resistance and cold tolerance, disease resistance, and sterility.

Growth-enhanced transgenic mice were famously produced in the USA in 1982, and since then it became an ambition of fish biologists to produce similar strains of growth enhanced GM fish. As shown in **Figure 6**, fast-growing strains of various salmon species, tilapia, carp, and mud loach have been successfully produced. Some of these strains appear to be commercially attractive, especially since no other undesirable traits are apparent in such fish. These fish can also be made sterile, sometimes reversibly. An additional transgenic modification with some of these fish is to produce them autotransgenically, meaning that the entire transgene sequence of promoter, structural gene, and downstream DNA sequence has been recovered from the same species as is now made transgenic, so such fish carry no novel DNA sequences, but have simply undergone a minor rearrangement of genes in their own genome. If autotransgenesis is coupled with reversible sterility in the future, it would surely offer fish which would be widely acceptable in aquaculture.

Another parameter of interest in fish for aquaculture is resistance to cold or freezing. The latter involves resistance to freezing in water which is below the freezing temperature of freshwater. Many species of Arctic fish such as winter flounder (*Pseudopleuronectes americanus*) carry specific genes which code for antifreeze protein. These small proteins are released into the blood and tissues of Arctic fishes, and prevent freezing of the fish. Genes for antifreeze protein have been recovered from such fish species and transferred and expressed in species such as Atlantic salmon (*Salmo salar*) to allow their aquaculture production in the Arctic. However, to date the synthesis of antifreeze protein by such transgenic fish is insufficient to render them truly freeze-resistant.

Cold tolerance to temperatures below 10 °C would be a considerable benefit to carp culture in China or tilapia culture in Israel, and there is certainly some possibility that cold-tolerant transgenic strains of such fish could be developed.

Development of transgenic fish which are resistant to common fish diseases such as furunculosis in salmon could also have great potential. While there is evidence

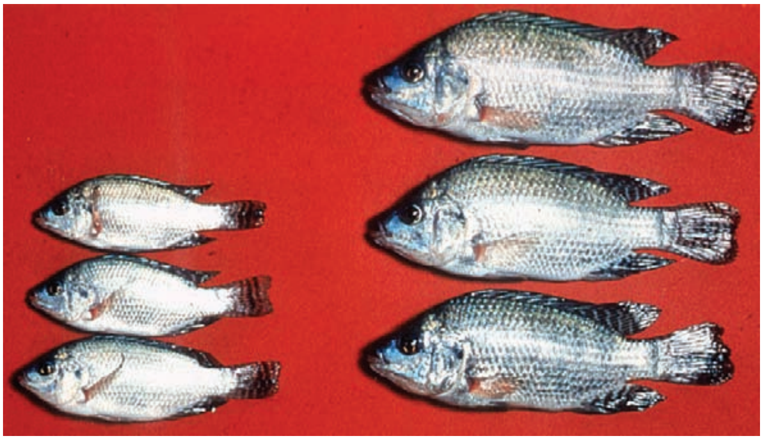


Figure 6 Growth-enhanced transgenic tilapia expressing a growth hormone transgene from a different fish species. Three control fish are on the left and three transgenically growth-enhanced fish are on the right. Figure from Maclean laboratory.

in the literature of the production of GM fish strains with improved levels of disease resistance, such work is still at a very early stage of development.

The last parameter to be considered as a potential benefit to aquaculture is sterility. Such a feature would prevent the spread of transgenes into wild fish stocks should transgenic fish escape. Nontransgenic sterility can be achieved in fish by triploid induction. In addition, the antagonization of some of the sex hormones such as gonadotropin-releasing hormone (GnRH) allows reversibly sterile strains of GM fish to be produced. Such fish would remain sterile unless the production of the GnRH antisense is negated by injection of GnRH at an early stage of development. There is some experimental evidence in tilapia that this research goal is indeed achievable.

Fish as Environmental Indicators

It is at least theoretically possible to design GM fish to act as indicators of environmental pollution. The rationale is to combine a promoter for a gene such as metallothionein, which is activated by heavy metals such as cadmium or copper, to a reporter gene, for example, GFP. The fish would then glow green if the water contained a high but nonlethal level of a heavy metal. This has indeed been attempted but the evidence for success is still somewhat incomplete.

GM Fish as Biofactories

Since fish are comparatively cheap to produce and easy to keep, it is clear that they may make attractive systems for the production of pharmaceuticals. Rival methodologies exist for yeast cells, GM plants, GM chickens (for production of egg protein), and GM mammals (for production of milk).

The system which has been tried in fish in Southampton is to produce transgenic tilapia (*O. niloticus*) capable of expressing a human blood clotting factor, factor VII. This factor is not important in hemophilia but is important in the control of internal bleeding such as occurs following gunshot injury, or from rupture of spleen or liver following car accidents. Currently, human factor VII is produced in human tissue culture cells and is very expensive. The strategy employed with tilapia was to render the fish transgenic for a construct consisting of a liver specific promoter (vitellogenin) spliced to a copy of the gene from human factor VII. The tilapia fish did indeed synthesize human factor VII capable of clotting human cells, but the research effort stopped before the amount of factor VII produced by the fish was of truly commercial significance.

Another application with medical potential has been followed up in Toronto, Canada. This is to humanize the tilapia copy of the insulin gene so that tilapia produce human insulin, with a view to making tilapia liver tissue from such fish available for transplant to humans suffering from advanced insulin-dependent diabetes.

Colorful Fish for the Pet Trade

A further use for GM fish has been found with glowfish, transgenic zebrafish which express GFP or RFP (from jellyfish) in their muscle. This has no undesirable effect on the fish but endows them with a novel color property. Such fish have been authorized for sale in the pet trade in the USA (see **Figure 7**).

Problems with Transgenic Fish

GM animals and plants have aroused considerable public anxiety, especially where they might be used for food production. Such anxiety is currently maximal in

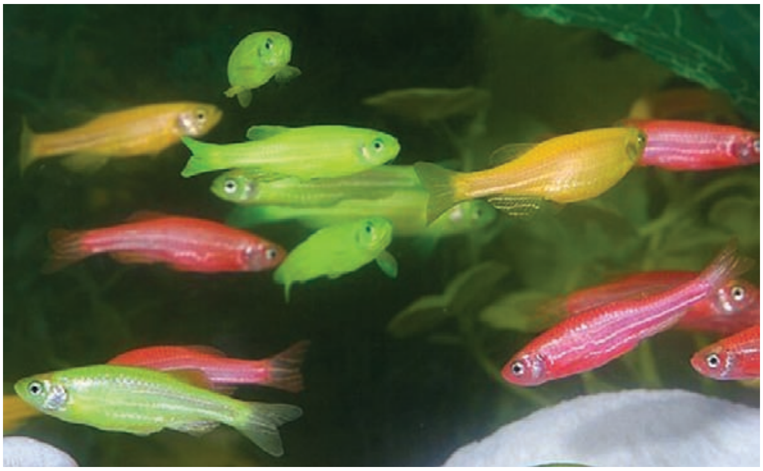


Figure 7 Glowfish which are a line of transgenic zebrafish offered for sale as pets in the USA. Reproduced from www.glofish.com.

Europe and fairly minimal in the USA. GM fish present some particular problems because of their mobility and the comparative difficulty of ensuring physical containment at all times. There is no doubt that GM organisms could be produced by accident or intention which would prove hazardous to health if eaten, just as some non-GM organisms are poisonous. However, provided the design of transgenic organisms is wisely planned, no significant health hazards need arise. Certainly, there is no particular property acquired by a promoter or coding sequence when used as a transgene which would render it more hazardous than the normal sequence in the nontransgenic organism.

The containment of transgenic fish can be achieved either physically, for example, by their culture in a land-locked lake or secure aquaculture facility, or by ensuring that they are sterile and so unable to mate with wild individuals should they escape. Where it has been proposed to exploit transgenic fish in commercial aquaculture, as for example with growth-enhanced GM Atlantic salmon in North America, it has been proposed to use all-female triploid fish which are indeed sterile. It is an interesting question to determine how dangerous an accidental escape of GM fish might be if they were, for example, growth enhanced. Present genetic evidence favors the view that such fish are significantly less fit than normal wild fish, and would eventually die out through disease or predation. However, at the present time, it is difficult to be 100% sure that extinction would always follow from accidental escape, and it is clearly better to ensure containment at all times.

Concerns have also been raised about the hazards of transgenesis induction, in terms of unexpected side effects of the transgenesis process. As with normal plant and animal breeding, new transgenic strains should obviously be rigorously tested before being used for commercial production and especially if destined for medical use or for food. Theoretical possibilities exist for nontarget genes being accidentally inactivated through transgene insertion, or through transgenes affecting the expression of adjacent gene sequences following integration. There is even a theoretical possibility of the accidental activation of viral or proto-oncogene sequences following transgenesis. Such possibilities seem to be more theoretical than real, and so far such problems have not been encountered in practice.

Chromosome manipulation in fish

Chromosome manipulation is usually taken to mean ploidy manipulation, most frequently triploidy. Triploid fish can offer certain advantages. They are normally sterile, especially when female, and the lack of gonadal development may lead to increased growth as compared to wild-type diploids when the fish would normally be sexually mature. However, most frequently triploids are produced to ensure sterility when such fish are used for stocking. To understand how triploid fish are produced, it is necessary to know something about normal gametogenesis in fish. The fish egg results from two rounds of division of a primary oocyte which carries four copies of each chromosome, that is, it is tetraploid. In two rounds of division the primary oocyte produces first a diploid cell plus a tiny polar body carrying the other chromosome set, and then in a further division a haploid egg cell is produced and a second tiny polar body is expelled. If the oocytes are recovered from a fish and treated to either heat-shock or pressure-shock, the elimination of the second polar body may be prevented, leading to a diploid egg. On fertilization with normal haploid sperm, such an egg develops into a triploid embryo and organism.

Although both male and female triploid fish are commonly sterile, occasionally some triploid males may secrete small amounts of viable sperm. For this reason all-female triploid fish are favored.

The production of all-female triploids is ensured by the use of sperm from female fish which have been emasculated by injection with male sex hormone. Such females go on to develop testes and secrete sperm, but it is all genetically female-determining sperm, since the male is the heterogametic sex. Thus, all fish resulting from the fertilization of diploid eggs with female-determining sperm are all-female triploid fish, and these can be guaranteed to be sterile.

The physiology of triploid fish is described in **Cellular, Molecular, Genomics, and Biomedical Approaches**: Physiology of Triploid Fish.

Applications of triploidy

Triploid fish are now widely favored in the management of wild trout rivers for stocking purposes. For example, the province of British Columbia, Canada, has a very large, province-wide stocking program for its lakes that had been depleted of wild stocks by overfishing. These fish do not divert energy into sexual development or secondary sexual characteristics. Furthermore, the techniques of transgenesis and triploid induction are sometimes combined in fish to produce lines of sterile GM fish which would not pose an environmental risk to interbreeding with any wild stock. Sterile triploids are also of considerable interest as mentioned previously, when GM fish are likely to be used in commercial situations where accidental escape could not always be avoided.

Although semantically transgenesis can be viewed as a chromosome manipulation, in most countries the two procedures are viewed as distinct, the term gene manipulation being reserved for cases where the novel genes are artificially manipulated. However, it is well to be aware that in some countries such as Germany chromosome manipulation as in triploid induction is categorized as GM and is covered by the same legislation.

Gynogenesis and androgenesis

Triploidy is not the only interesting aspect of chromosome manipulation in fish. Both gynogenesis (all maternal inheritance) and androgenesis (all paternal inheritance) can be used to produce inbred clonal lines of fish.

Diploid gynogenetic fish can be generated by blocking first cleavage during oocyte maturation, or by blocking the extrusion of the second polar body. Often such eggs are fertilized with irradiated sperm to ensure further embryo development without any male genetic contribution.

Diploid androgenic fish can be produced by irradiating eggs then fertilizing with normal sperm, and then allowing doubling of the paternal genome by blocking the first cleavage, resulting in a doubling of the sperm donated genome.

Although such clonal lines of fish are normally less attractive for aquaculture and more prone to disease and reduced growth, they are of considerable interest to scientists studying fish immunology, since clonal fish may accept tissue grafts such as scales from one another without rejection. The Laboratory of Fisheries at Wageningen Agricultural University in the Netherlands has used such gynogenetic and androgenetic homozygous clones of fish for studies on fish tissue transplantation.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Physiology of Triploid Fish.

Further Reading

Benfey TJ (1999) The physiology and behaviour of triploid fishes. *Reviews in Fisheries Science* 7: 39–67.

Bill BR, Petzold AM, Clark KJ, Schimmenti LA, and Ekker SC (2009) A primer for morpholino use in zebrafish. *Zebrafish* 6(1): 69–77.

Bongers AB, Sukkel M, Gort G, Komen J, and Richter CJ (1998) Development and use of genetically uniform strains of common carp in experimental animal research. *Lab Animal* 32(4): 349–363.

Devlin RH, Yesaki TY, Biagi CA, *et al.* (1994) Extraordinary salmon growth. *Nature* 371: 209–210.

Dunham RA, Warr G, Nichols A, *et al.* (2002) Enhanced bacterial disease resistance of transgenic channel catfish, *Ictalurus punctatus*, possessing cecropin genes. *Marine Biotechnology* 4: 338–344.

Fletcher GL, Davies PL, and Hew CL (1992) Genetic engineering of freeze-resistant Atlantic salmon. In: Hew CL and Fletcher GL (eds.) *Transgenic Fish*, pp. 190–208. London: World Scientific.

Hew C, Poon R, Xiong F, *et al.* (1999) Liver-specific and seasonal expression of transgenic Atlantic salmon harbouring the winter flounder antifreeze protein gene. *Transgenic Research* 8: 405–419.

Hwang G, Müller F, Rahman MA, *et al.* (2004) Fish as bioreactors: Transgene expression of human coagulation factor VII in fish embryos. *Marine Biotechnology* 6: 485–492.

Kapuscinski AR, Hayes KR, Li S, and Dana G (eds.) (2007) *Environmental Risk Assessment of Genetically Modified Organisms, Volume 3, Methodologies for Transgenic Fish*. Wallingford: CAB International.

Maclean N (1994) *Animals with Novel Genes* (reprinted 2009). Cambridge: Cambridge University Press.

Maclean N (2003) Genetically modified fish and their effects on food quality and human health and nutrition. *Trends in Food Science and Technology* 14: 242–252.

Meierjohann S and Schartl M (2006) From Mendelian to molecular genetics: The *Xiphophorus* melanoma model. *Trends in Genetics* 22(12): 654–661.

Nam YK, Maclean N, Hwang G, and Kim DS (2008) Autotransgenic and allotransgenic manipulation of growth traits in fish for aquaculture: A review. *Journal of Fish Biology* 72: 1–26.

Nam YK, Noh JK, Cho YS, *et al.* (2001b) Dramatically accelerated growth and extraordinary gigantism of transgenic mud loach *Misgurnus mizolepis*. *Transgenic Research* 10: 353–362.

Palmiter RD, Brinster RL, Hammer RE, *et al.* (1982) Dramatic growth of mice that develop from eggs micro-injected with metallothionein-growth hormone fusion gene. *Nature* 300: 611–615.

Pohajdak B, Mansour M, Hrytsenko O, *et al.* (2004) Production of transgenic tilapia with Brockmann bodies secreting (desThrB30) human insulin. *Transgenic Research* 13: 313–323.

Rahman MA and Maclean N (1999) Growth performance of transgenic tilapia containing an exogenous piscine growth hormone gene. *Aquaculture* 173: 333–346.

Schartl M, Wilde B, Laisney JA, *et al.* (2010) A mutated EGFR is sufficient to induce malignant melanoma with genetic background-dependent histopathologies. *Journal of Investigative Dermatology* 130(1): 249–258.

Takeuchi Y, Yoshizaki G, Kobayashi T, and Takeuchi T (2002) Mass isolation of primordial germ cells from transgenic rainbow trout carrying the green fluorescent protein gene driven by the *vasa* gene promoter. *Biology of Reproduction* 67: 1087–1092.

Thermes V, Grabher C, Ristoratore F, *et al.* (2002) I-SceI meganuclease mediates highly efficient transgenesis in fish. *Mechanisms of Development* 118: 91–98.

Thorgaard GH (1986) Ploidy manipulation and performance. *Aquaculture* 57: 57–64.

Van Muiswinkel WB, Tigchelaar AJ, Harmsen EG, and Rijnders PM (1986) The use of artificial gynogenesis in studies on the immune system of carp (*Cyprinus carpio* L.). *Veterinary Immunology and Immunopathology* 12(1–4): 1–6.

Wang R, Zhang P, Gong Z, and Hew C-L (1995) Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. *Molecular Marine Biology and Biotechnology* 4: 20–26.

Relevant Websites

http://www.glofish.com – GloFisH.

Physiology of Triploid Fish

TJ Benfey, University of New Brunswick, Fredericton, NB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Reproductive Physiology
Hematology, Gas Transport, and Cardiovascular Physiology
Respiration and Metabolism
Stress Response and Environmental Tolerances
Immunology and Disease Resistance
Nutrition and Growth
Conclusions
Further Reading

Glossary

Aneuploid Having any number of chromosomes other than an exact multiple of one complete chromosome set.
Chromosome set A single complete complement of chromosomes.
Dioecious Having separate sexes.
Diploid Having two complete chromosome sets.
Gene dosage The number of copies of a given gene.
Heterozygous Having variability in alleles for a given gene or genes.
Hypotonic Having a lower salt concentration.
Mean erythrocyte hemoglobin concentration The mean concentration of hemoglobin per erythrocyte.
Parthenogenesis Development in the absence of a paternal genome.
Polyploid Having more than two complete chromosome sets.
Sterile Unable to produce viable offspring.
Tetraploid Having four complete chromosome sets.
Triploid Having three complete chromosome sets.

Introduction

Most fish are diploids, with their genomes comprised of two sets of homologous chromosomes. Polyploid fish have a greater number of complete chromosome sets, with the most common being triploids (three sets) and tetraploids (four sets). Although polyploidy is more common in plants than in animals, tetraploidy has played an important role in vertebrate evolution as a mechanism for providing redundant genes that can mutate with less likelihood of being lethal. However, additional sets of chromosomes require a larger nucleus to house them, and this often results in a larger cell size as well. Adaptive benefits of polyploidy could potentially be counterbalanced by physiological constraints arising at the organismal level from this increased cell size. The fact that numerous anscestral polyploid lineages have survived their diploid progenitors indicates that the genetic benefits of polyploidy can outweigh any physiological consequences.

There are many examples of both naturally occurring and artificially induced triploidy in fishes. Naturally occurring triploids fall into two categories: occasional spontaneous triploid individuals within dioecious diploid species and self-sustaining triploid populations with atypical modes of reproduction. Fish of the former category have been reported in a diversity of species, from sharks to teleosts. Fish of the latter category occur as female hybrids between two closely related species and typically reproduce by parthenogenesis. Triploidy has been induced in many species of fish, generally with the aim of producing sterile populations for aquaculture or fisheries management. This is most commonly achieved through thermal or hydrostatic pressure treatment of eggs (a shock) shortly after fertilization, thereby interfering with the completion of meiotic reduction of the maternal genome within the oocyte (**see also Cellular, Molecular, Genomics, and Biomedical Approaches:** Transgenesis and Chromosome Manipulation in Fish).

Triploids can potentially differ from conspecific diploids in a number of fundamental ways. Those that arise spontaneously or are induced in species that normally exist as dioecious diploids are more heterozygous, have unbalanced gene dosage, and are sterile. All triploids, independent of their origin, also have larger cell nuclei to accommodate their larger genomes, resulting in increased cell volume in many cell types. Increased cell volume in triploids is balanced by reduced cell numbers (to maintain normal organ and body size), reduced cellular and nuclear surface area per unit volume, and increased intracellular diffusion distances.

The genetic consequences of triploidy (heterozygosity and gene dosage) are not considered in this article. Rather, the focus is on the physiological effects of triploidy on fish biology, which are ultimately linked to sterility and increased cell volume.

Reproductive Physiology

Although there are a few examples of self-sustaining triploid populations of fish that have arisen through hybridization events, these are rare and exist as female clones that require males from one of the progenitor species to activate development of their eggs. Aside from these exceptions, triploids are reproductively sterile. This is a direct consequence of having an odd number of chromosome sets. The unpaired chromosome set leads to a failure of the normal pairing and crossing-over of homologous chromosomes that occurs during meiosis I.

At the time when diploid females are sexually mature, triploid ovaries are largely devoid of vitellogenic oocytes (**Figure 1**). The small numbers of oocytes that do occasionally become vitellogenic in triploids are rarely – if ever – ovulated. Steroid biosynthesis is greatly diminished or absent in triploid ovaries due to their small number of developed follicles. As a result, triploid females retain juvenile endocrine profiles throughout their lives, whether at the level of the ovary (steroids), liver (vitellogenin), pituitary (gonadotropins) or hypothalamus (gonadotropin releasing hormone), and they do not develop secondary sexual characteristics (**Figure 2**). All of these endocrine systems are fully competent and respond appropriately to exogenous steroid treatment to replace the absent endogenous steroids (**see also Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction).

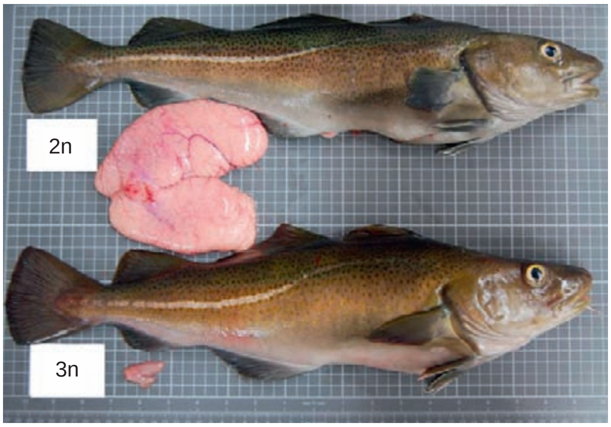


Figure 1 Ovarian development in sibling diploid (2n) and triploid (3n) Atlantic cod, *Gadus morhua*. Photo: Nathaniel Feindel, University of New Brunswick.

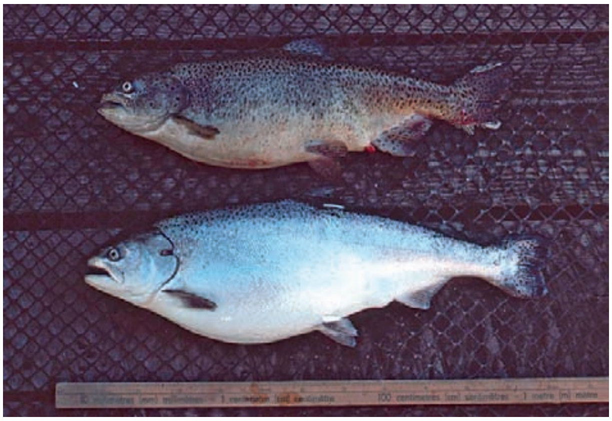


Figure 2 Secondary sexual characteristics in a sexually mature diploid female chinook salmon, *Oncorhynchus tshawytscha* (top), compared to a sibling triploid female that has retained the juvenile appearance (bottom). Note the darker coloration and protruding vent in the diploid. Photo: Tillmann Benfey, University of New Brunswick.

The situation for triploid males is quite different: they often produce functional, post-meiotic spermatozoa, although in much smaller numbers than diploids. These cells are larger than the haploid spermatozoa produced by diploid males, and flow-cytometric measurement of DNA content shows them to be aneuploid with a distribution of genomic DNA content that peaks midway between haploid and diploid. Triploid males appear outwardly to mature normally. They develop relatively large testes (**Figure 3**) through the pre-meiotic proliferation of spermatogonia, and their testes are fully invested with functional steroidogenic cells. As a result, triploid males have reproductive endocrine profiles identical to those of

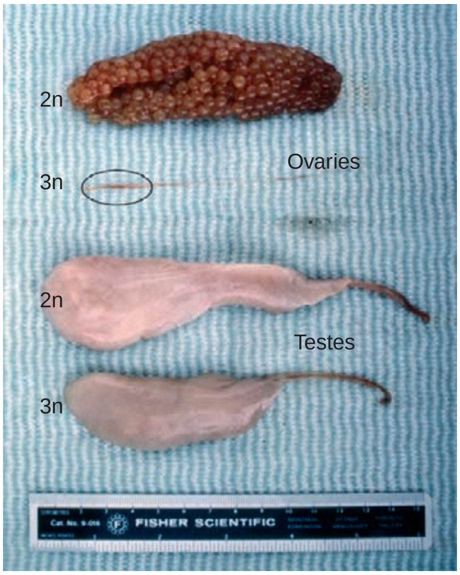


Figure 3 Gonadal development in sibling diploid (2n) and triploid (3n) pink salmon, *O. gorbuscha*. Photo: Tillmann Benfey, University of New Brunswick.

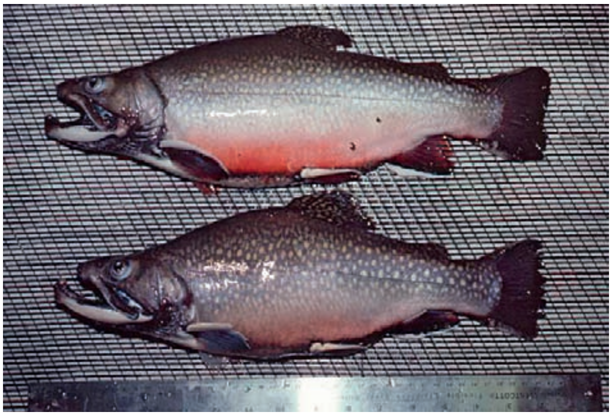


Figure 4 Secondary sexual characteristics in a sexually mature diploid male brook charr, *Salvelinus fontinalis* (top) compared to a sibling triploid male (bottom). Note the similar characteristic darker coloration, reddish abdomen, white leading edges to the ventral fins, humped back and hooked lower jaw. Photo: Tillmann Benfey, University of New Brunswick.

diploids, in spite of producing greatly reduced numbers of spermatozoa. They also develop typical secondary sexual characteristics (**Figure 4**), and they have normal rates of precocious maturation and spawning behavior. Their spermatozoa are able to fertilize eggs, but the resultant offspring are aneuploid and die early in development. Some studies have found sperm motility to be the same for triploid and diploid males, but others have found the former to have reduced sperm motility (**see also The Reproductive Organs and Processes:** Anatomy and Histology of Fish Testis and Vitellogenesis in Fishes).

Hematology, Gas Transport, and Cardiovascular Physiology

The effect of triploidy on cell size is clearly demonstrated for the nucleated erythrocytes of fish, which are approximately 40% larger in volume than those of diploids (**Figure 5**). This increase in cell size is so extensively documented that it is often the sole criterion used to confirm ploidy level in fish. There tends to be a proportionately greater increase in cell length than in cell width or height, with the result that triploid erythrocytes not only appear larger but also more elongated than diploid erythrocytes when viewed in blood smears. Although not well quantified, any such increase in cell size necessarily reduces the cellular surface area to volume ratio.

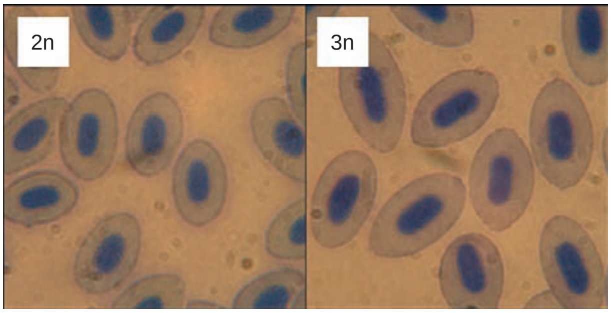


Figure 5 Diploid (2n) and triploid (3n) Atlantic salmon, *Salmo salar*, erythrocytes at identical magnification. Photo: Tillmann Benfey, University of New Brunswick.

Increased erythrocyte volume is balanced by reduced cell numbers, such that there is no effect of triploidy on the proportion of the blood volume occupied by blood cells (i.e., hematocrit). Most studies have also shown equivalent blood hemoglobin concentrations in diploids and triploids. Mean erythrocyte hemoglobin concentration (**see also Blood:** Cellular Composition of the Blood) is similar in diploids and triploids, but the absolute amount of hemoglobin per cell is higher in triploids because of their larger cell volume.

Based solely on hemoglobin concentrations, the oxygen-carrying capacity of individual erythrocytes should be higher for triploids than for diploids, while blood oxygen-carrying capacity should be the same. However, the percentage oxygen saturation of hemoglobin in triploid chinook salmon is about 75% of diploid values over a range of swimming speeds, that is, their hemoglobin is less fully saturated (**see also Transport and Exchange of Respiratory Gases in the Blood:** Hemoglobin). Consequently, they characteristically have lower arterial blood oxygen content (about 70% of diploid values). The cause of this is unclear; the limited data available for chinook and Atlantic salmon indicate that it is not likely due to any significant effect of triploidy on the principal factors controlling hemoglobin's binding affinity for oxygen, such as blood pH or erythrocyte organic phosphate concentration. What has not been adequately considered (or tested) is whether the reduced surface area of erythrocytes (relative to their volume) limits the rate at which oxygen enters and exits the cells. However, surface area does not appear to limit carbon dioxide exchange across the erythrocyte cell membrane in triploids. Another possibility is that gill vascular dimensions and anatomy (**see also Design and Physiology of Arteries and Veins:** Anatomical Pathways and Patterns and Branchial Anatomy) are altered in triploids.

Capillary diameter must be carefully regulated to minimize the distance for gas exchange between erythrocytes (**see also Design and Physiology of Capillaries and Secondary Circulation:** Capillaries, Capillarity, and Angiogenesis) and the surrounding tissues without causing excessive resistance to blood flow. This is achieved through erythrocyte deformation as they pass through capillaries that typically are narrower than erythrocyte diameter. It is not known whether capillary diameter is increased in triploids to accommodate their larger cells or whether triploid erythrocytes undergo greater deformation as they pass through the capillaries. Greater deformation and/or increased pressure on the cells as they pass through the microvasculature could affect their structural integrity. A single *in vitro* study of blood viscosity in triploids concluded that increased erythrocyte size does not increase vascular resistance. Several studies have shown that triploid erythrocytes are more resistant than diploid cells to rupture (hemolysis) when exposed to hypotonic solutions (**Figure 6**), suggesting that they differ in cell membrane composition and/or cytoskeletal structure. This would be an interesting compensatory mechanism to protect erythrocytes from size-related damage resulting from their passage through the microvasculature, and warrants further study. Perhaps unrelated, but also needing further research, is the origin and physiological implications of characteristic erythrocyte nuclear and cellular abnormalities routinely observed in triploid fish (**Figure 7**).

Several studies have shown triploids to be less able to survive chronic exposure to what would be considered high, but sublethal, temperatures for diploids. Triploid trout reared at elevated temperature have exceptionally high heart rates, and it has been suggested that mortalities at such temperatures may be due to myocardial infarctions or aneurysms. However, *in vivo* and *in vitro* studies of cardiac performance have concluded that it is not remarkably affected by triploidy. These studies have not included diploid controls, and further research is needed on the effects of triploidy on cardiac function and output. A single study, using brook charr at embryo-larval stages, found no effect of triploidy on heart rate or cardiac output.

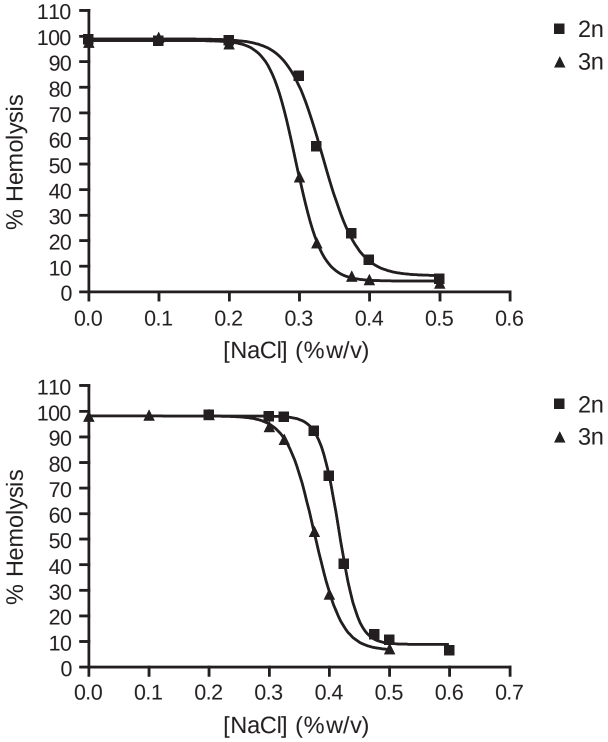


Figure 6 Osmotic fragility curves of diploid (2n) and triploid (3n) brook charr (top) and Atlantic salmon (bottom) blood. Each point represents the mean of six values. From Tam A and Benfey TJ, University of New Brunswick, unpublished.

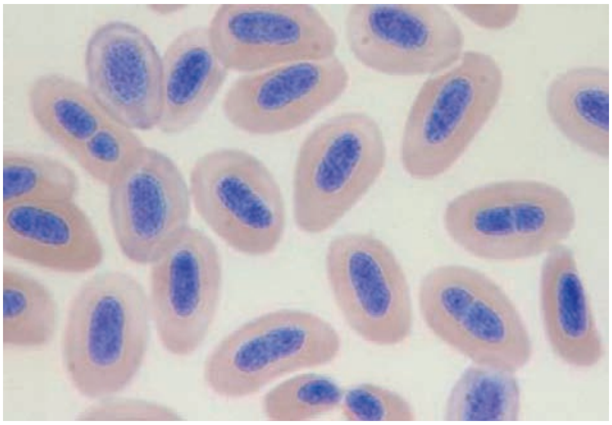


Figure 7 Triploid brook charr erythrocytes showing characteristic abnormalities such as bisected nuclei and pinched cells. Photo: Michelle Atkins, University of New Brunswick.

Respiration and Metabolism

Effective respiration in fish requires an efficient mechanism for moving water across the gills. Triploid salmonids and Atlantic cod occasionally have lower jaw deformities that prevent full closure of the mouth (**Figure 8**). Triploids also sometimes have a higher incidence of shortened opercula that are missing the skin flap used to seal the exits from the opercular cavities. The efficiency of the buccal-opercular pumping system used for gill irrigation depends on the ability to seal all compartments within the system, suggesting that under some

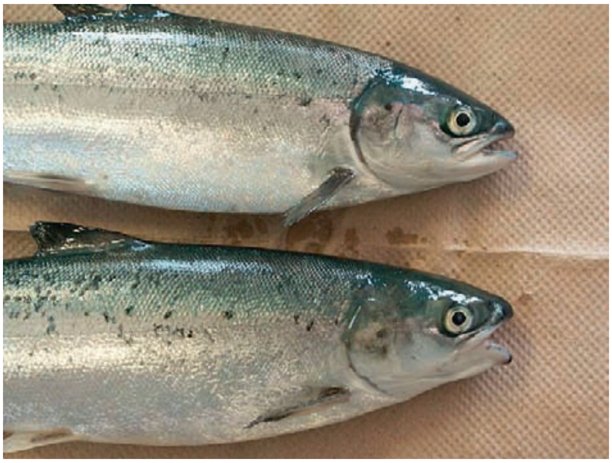


Figure 8 Diploid (top) and triploid (bottom) Atlantic salmon post-smolts showing characteristic lower jaw deformity more frequently observed in the triploid. Photo: Saranyan Pillai, University of New Brunswick.

circumstances (lower jaw deformity and/or shortened opercula), triploids may have reduced respiratory efficiency. Although there are no direct data on the efficiency of triploid respiratory systems, triploid Atlantic salmon with lower jaw deformities do less well in aerobic swimming tests than triploids and diploids lacking this deformity. Triploid brook charr have also been shown to have lower oxygen consumption rates than diploids at equivalent gill irrigation frequencies, but this was independent of their opercular condition and did not consider any differences in irrigation volume.

Gill anatomy (**see also Design and Physiology of Arteries and Veins:** Branchial Anatomy) can also be affected by triploidy. For instance, zones of missing filaments have been observed on the gill arches of triploid Atlantic salmon and they also have a lower total macroscopic gill surface area (**see also Ventilation and Animal Respiration:** Gill Respiratory Morphometrics) compared with diploids. Although there is no evidence of gill filament deformities in triploid tench (*Tinca tinca*), their gill secondary lamellae are longer and thinner than those of diploids, and the lamellar diffusion distance for respiratory gas exchange is approximately 30% shorter. This closer proximity of water and blood flow through the gills of triploids suggests some level of anatomical compensation, although there have been no studies on lamellar blood perfusion in triploids of any species.

Oxygen consumption rates are largely unaffected in triploids under a variety of test conditions. Numerous studies have shown that triploidy does not affect critical swimming speed (U_{crit}, a common proxy for aerobic capacity). However, triploids may accrue a larger oxygen debt during such swim tests, as suggested by the lower arterial blood oxygen content and hemoglobin–oxygen saturation noted above as well as higher post-exercise oxygen consumption rates noted in some (but not all) studies. Compared to diploids, triploid Atlantic salmon have reduced endurance when forced to swim at speeds above their maximum sustainable swimming speed (**see also Swimming and Other Activities:** Applied Aspects of Fish Swimming Performance), suggesting reduced anaerobic capacity. However, the data on blood and muscle levels of anaerobic metabolites after anaerobic exercise are equivocal as to whether there is a ploidy effect.

Stress Response and Environmental Tolerances

Triploid salmonids exhibit a typical physiological stress response when exposed to common acute stressors encountered in aquaculture, such as chasing, confinement, transportation, or seawater transfer. The peak levels and time course of recovery of plasma cortisol, glucose and lactate following exposure to such stressors are the same for both ploidies. However, triploids often do not survive as well as diploids under conditions of chronic stress brought about by typical aquaculture conditions. The two environmental stressors that have been examined in some detail in terms of physiological responses by triploids are temperature and salinity.

The critical thermal maximum (**see also Temperature:** Measures of Thermal Tolerance), which is the temperature at which fish lose their ability to maintain equilibrium and turn belly up when exposed to rapid temperature increases, is the same for triploids and diploids. The chronic lethal maximum, which is estimated using slower rates of temperature increase to provide some opportunity for thermal acclimation, is also unaffected by ploidy. However, triploids have markedly higher mortality rates than diploids when first acclimated to sublethally high temperatures and then exposed to stressors such as increased salinity or exhaustive exercise. The specific cause of mortality under such conditions is not known, but triploids have been shown to have a lower thermal optimum than diploids (based on the relationship between acclimation temperature and routine metabolic rate).

Physiological adaptation to the marine environment is an energetically demanding phase of the natural life history of salmonid fishes that can be associated with high mortality rates in aquaculture. Independent studies with a number of salmonid species have shown that triploids do not differ from diploids in their abilities to osmoregulate after transfer to seawater (**see also Osmotic, Ionic and Nitrogenous-Waste Balance:** Osmoregulation in Fishes: An Introduction), as evidenced by their similar plasma osmolalities and ion concentrations and gill Na^+/K^+-ATPase activities post-transfer. Interestingly, triploid chinook salmon smolts have been shown to have, on average, lower gill Na^+/K^+-ATPase activity prior to saltwater transfer (during the pre-adaptation stage), but this was not the case for non-smolting rainbow trout (*O. mykiss*). Plasma IGF-I levels, which are associated with osmoregulatory ability, do not differ between triploids and diploids.

Immunology and Disease Resistance

Just as for erythrocytes, triploid leukocyte size is increased and numbers reduced compared to diploids. Differential cell counts, used to estimate relative abundances of the different types of leukocytes, are generally the same for triploids and diploids. Respiratory burst and phagocytic activities are higher for triploid leukocytes due to their larger size, but when expressed per unit blood volume, there is no effect of triploidy. The balance between cell size and numbers also results in there being no tissue-level effect of triploidy on complement (blood), bactericidal (blood), and lyzozyme (blood and muscle) activities. However, a study with Atlantic salmon showed that complement activity took slightly longer to respond to, and recover from, a bacterial infection mimic (intraperitoneal injection with lipopolysaccharide) in triploids compared with diploids. Triploidy has been shown to have no effect on vaccine efficacy at the cellular and whole-animal level (**see also Hormonal Responses to Stress:** Impact of Stress in Health and Reproduction).

It is not clear whether triploidy affects disease resistance in fish under aquaculture conditions where it is impossible to control exposure levels and fish condition. However, under controlled experimental condition, triploids appear to have reduced disease resistance. For instance, although the effect of bacterial (*Vibrio anguillarum*) concentration delivered through intramuscular injection on mortality rates was found to be the same for unvaccinated triploid and diploid ayu (*Plecoglossus altivelis*), the average time to death was shorter in triploids. In unvaccinated coho salmon (*O. kisutch*) exposed to this same pathogen by immersion, the overall mortality was higher and average time to death shorter for triploids compared with diploids.

Nutrition and Growth

The earliest studies with triploid fish and amphibians assumed that they would grow larger than diploids as a result of their larger cells, as is often the case with gigantism in polyploid plants. This is clearly not the case for triploid vertebrates, including fish. A concomitant reduction in cell numbers balances any increase in cell size. Although it is unclear what the underlying homeostatic control mechanism is for balancing cell size and number, the end effect is that with the exception of the ovaries, organ size and body size are generally the same in juvenile diploids and triploids of the same age.

The physiology of nutrition and growth has received surprisingly little attention in triploids considering the focus of triploidy as a management tool in aquaculture. The limited data available are inconclusive as to whether triploidy affects food conversion efficiency, with some studies finding no effect and others finding both improved and reduced efficiency in triploids. At juvenile stages, there may be minor effects of triploidy on proximate composition and dietary digestibility coefficients, but again these are inconsistent among studies. The relationship between circulating IGF-I levels and growth rate is the same for triploids as for diploids. As juveniles, triploids generally grow no faster than diploids, with some studies finding equivalent growth rates and others finding triploid growth to be inferior. However, in species where diploids have low or no growth during the spawning period, triploid females tend to show continued growth. For this reason, long-term growth studies have often found triploid females to grow to be the largest fish (**see also Hormonal Responses to Stress:** Stress Effect on Growth and Metabolism and **Cellular, Molecular, Genomics, and Biomedical Approaches:** Growth Hormone Overexpression in Transgenic Fish).

Conclusions

The physiology of triploids has received far less research attention than that of diploids because triploidy is, for the most part, not a natural state in the life history of fishes. The fact that triploidy induction leads to sterility is of relevance to aquaculture, and much of the research on triploids has focused on economically important culture traits in a small number of commercially relevant species. However, the effects of triploidy on cell size and number make these fishes interesting models for basic research on how cell size and surface area to volume relationships affect physiological processes. Triploids tend to be similar to diploids in whole-animal tests of physiological performance; determining what sorts of compensatory mechanisms are involved in accommodating differences at the cell and tissue level should be a fruitful area of future research.

See also: **Blood:** Cellular Composition of the Blood. **Cellular, Molecular, Genomics, and Biomedical Approaches:** Growth Hormone Overexpression in Transgenic Fish; Transgenesis and Chromosome Manipulation in Fish. **Design and Physiology of Arteries and Veins:** Anatomical Pathways and Patterns; Branchial Anatomy. **Design and Physiology of Capillaries and Secondary Circulation:** Capillaries, Capillarity, and Angiogenesis. **Hormonal Control of Reproduction and Growth:** Endocrine Regulation of Fish Reproduction. **Hormonal Responses to Stress:** Impact of Stress in Health and Reproduction; Stress Effect on Growth and Metabolism. **Osmotic, Ionic and Nitrogenous-Waste Balance:** Osmoregulation in Fishes: An Introduction. **Swimming and Other Activities:** Applied Aspects of Fish Swimming Performance. **Temperature:** Measures of Thermal Tolerance. **The Reproductive Organs and Processes:** Anatomy and Histology of Fish Testis. **Transport and Exchange of Respiratory Gases in the Blood:** Hemoglobin. **Ventilation and Animal Respiration:** Gill Respiratory Morphometrics. Vitellogenesis in Fishes.

Further Reading

Atkins ME and Benfey TJ (2008) Effect of acclimation temperature on routine metabolic rate in triploid salmonids. *Comparative Biochemistry and Physiology* 149A: 157–161.

Benfey TJ (1999) The physiology and behaviour of triploid fishes. *Reviews in Fisheries Science* 7: 39–67.

Benfey TJ (2009) Producing sterile and single-sex populations of fish for aquaculture. In: Burnell G and Allan G (eds.) *New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management*, pp. 143–164. Cambridge: Woodhead Publishing Ltd.

Benfey TJ and Bennett LE (2009) Effect of temperature on heart rate in diploid and triploid brook charr, *Salvelinus fontinalis*, embryos and larvae. *Comparative Biochemistry and Physiology* 152A: 203–206.

Bernier NJ, Brauner CJ, Heath JW, and Randall DJ (2004) Oxygen and carbon dioxide transport during sustained exercise in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 61: 1797–1805.

Budiño B, Cal RM, Piazzon MC, and Lamas J (2006) The activity of several components of the innate immune system in diploid and triploid turbot. *Comparative Biochemistry and Physiology* 145A: 108–113.

Flajšhans M and Piačková V (2006) Difference in blood and water diffusion distance in gill lamellae of diploid and triploid tench *Tinca tinca* (L.). *Journal of Fish Biology* 69: 1870–1873.

Galbreath PF, Adams ND, Sherill LW, and Martin TH (2006) Thermal tolerance of diploid versus triploid rainbow trout and brook trout assessed by time to chronic lethal maximum. *Environmental Biology of Fishes* 75: 183–193.

Leggatt RA and Iwama GK (2003) Occurrence of polyploidy in the fishes. *Reviews in Fish Biology and Fisheries* 13: 237–246.

Lijalad M and Powell MD (2009) Effects of lower jaw deformity on swimming performance and recovery from exhaustive exercise in triploid and diploid Atlantic salmon *Salmo salar* L. *Aquaculture* 290: 145–154.

Maxime V (2008) The physiology of triploid fish: Current knowledge and comparisons with diploid fish. *Fish and Fisheries* 9: 67–78.

Piferrer F, Beaumont A, Falguière J-C, *et al.* (2009) Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293: 125–156.

Sadler J, Pankhurst PM, and King HR (2001) High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* 198: 369–386.

Sadler J, Wells RMG, Pankhurst PM, and Pankhurst NW (2000) Blood oxygen transport, rheology and haematological responses to confinement stress in diploid and triploid Atlantic salmon, *Salmo salar*. *Aquaculture* 184: 349–361.

Schafhauser-Smith D and Benfey TJ (2001) The reproductive physiology of three age classes of adult female diploid and triploid brook trout (*Salvelinus fontinalis*). *Fish Physiology and Biochemistry* 25: 319–333.

Schafhauser-Smith D and Benfey TJ (2003) *In vitro* steroid production by triploid ovarian follicles. *General and Comparative Endocrinology* 133: 279–286.

Shrimpton JM, Sentlinger AMC, Heath JW, Devlin RH, and Heath DD (2007) Biochemical and molecular differences in diploid and triploid ocean-type chinook salmon (*Oncorhynchus tshawytscha*) smolts. *Fish Physiology and Biochemistry* 33: 259–268.

Taylor JF, Needham MP, North BP, *et al.* (2007) The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. *General and Comparative Endocrinology* 152: 314–325.

Growth Hormone Overexpression in Transgenic Fish

RH Devlin, Fisheries and Oceans Canada, West Vancouver, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Brief History of Genetic Engineering in Fish
Physiology of GH Is Well Known
How GH Transgenic Fish Are Made
Growth Effects of GH Transgenes in Fish
Expression of Transgenes and Endocrine Effects
Phenotypic Effects – Morphology
General Viability and Immunology
Nutritional Requirements and Energy Use
Oxygen Uptake, Exercise, and Energy Use
Behavior
Application in Aquaculture, and Environmental Risk Assessments
Further Reading

Glossary

Acromegaly Deformation of cranial structures typified by excess cartilage and bone growth.

Compensatory growth A period of accelerated growth following a period of stress such as food deprivation.

Gene construct A synthetic DNA molecule often containing regulatory and coding regions from different genes.

Orexigenic Stimulating appetite.

Pleiotropic Affecting multiple systems.

Promoter The upstream sequence of DNA which regulates the expression of the downstream coding sequence of the gene. The promoter may sometimes be as big or bigger than the gene itself. A so-called minimal promoter is the length of the promoter required to achieve expression of the gene, but without additional positive or negative elements.

Transgenic An organism possessing foreign DNA introduced by genetic engineering.

Brief History of Genetic Engineering in Fish

Genetic engineering, or transgenesis, involves the introduction of novel DNA into an organism by processes that do not normally occur in nature. Studies in the early 1980s showed that transgenic mice overexpressing growth hormone (GH) displayed a remarkable doubling of body size compared to nontransgenic littermates. These findings powerfully displayed the potential of genetic engineering to modify traits in vertebrates for use in basic science and for applied purposes.

Such growth enhancement was also recognized for its potential to enhance human food production in agriculture, and hence numerous reports soon appeared describing genetic engineering of commercially important livestock, including pigs, sheep, and cattle. Growth responses in these species were much more limited than those seen in mice, perhaps because these other species had long histories of genetic selection for enhanced growth under domestication (see below).

Genetic engineering of fish similarly began in the early 1980s (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Transgenesis and Chromosome Manipulation in Fish). Currently, more than 35 species of fish have been genetically engineered with gene constructs designed to alter many traits, including growth, reproduction, disease resistance, and flesh quality. The first report of GH transgenesis in fish was from Dr. Zuoyan Zhu in Wuhan, China in 1986, working with the weather loach *Misgurnus anguillicaudatus*. In these studies, overexpression of GH genes increased growth in nontransgenic counterparts, and even beyond that seen in the transgenic mouse.

These early experiments kindled a large number of similar studies with fish, primarily using mammalian GH gene constructs, with the objective of generating strains with enhanced growth rate for potential use in aquaculture. Subsequently, the use of gene constructs comprised of fish sequences were developed, which in general functioned more effectively than nonpiscine gene constructs.

GH transgenesis remains a focus of activity for fish genetic engineering, generating strains for basic science investigations of growth physiology, behavior, ecology, and evolution. Some strains are also being actively pursued for application in aquaculture. However, globally, all transgenic fish are currently reared in specialized confinement facilities to prevent fish escape, and none are known to have entered natural environments.

Physiology of GH Is Well Known

Fish GHs, either purified from fish pituitaries or synthesized as a recombinant protein using bacterial expression systems, are active in stimulating growth in fish. Indeed, many fish possess a broad capacity to respond to GH family proteins (GH, prolactin (PRL), placental lactogen (PL), and somatolactin (SL)) from piscine and mammalian sources. Confirming an adequate response of a fish species or strain to GH is a critical research step that should be undertaken before embarking on a lengthy and expensive program to generate GH transgenic fish strains overexpressing this hormone.

Wild-type fish strains express normal GH genes at high levels in the pituitary gland and at very low levels in several other tissues. GH protein is stored in the pituitary. Its release is tightly regulated by neural controls and a suite of neuropeptides (growth hormone-releasing hormone (GHRH), somatostatin, gonadotropin-releasing hormone (GnRH), etc.) in response to internal metabolic (e.g., energy status and reproductive status) and external environmental (e.g., temperature, photoperiod, and food supply) cues that adjust growth rates to match seasonal and short-term conditions (**see also Hormonal Control of Reproduction and Growth**: Endocrinology of Fish Growth). GH is controlled through a negative feedback loop such that high circulating GH is capable of repressing its own synthesis in, and release from, the pituitary. This is an important feature of GH gene regulation, which becomes critical in the design of appropriate GH gene constructs for transgenesis (see below). GH functions by binding to the GH receptor which activates the janus kinases/signal transducers and activators of transcription (JAK/STAT) intracellular signaling systems to stimulate effector genes such as insulin-like growth factor-I (IGF-I). It is IGF-I that plays a primary role in stimulating growth, although some direct action of GH is also thought to occur.

How GH Transgenic Fish Are Made

To overexpress GH in transgenic fish, it is necessary to overcome the negative feedback controls that normally operate to limit GH production. In fact, simply adding additional copies of GH genes, with their native regulatory sequences, has not resulted in enhanced growth in transgenic fish. GH gene constructs therefore replace the normal regulatory sequences (promoters) of GH genes with those from other genes that are not sensitive to GH feedback regulation (see **Figure 1**). Promoters that have been used to drive GH sequences are often strong constitutive promoters – that is, those that are active at most times and in many cell types – and include those from the ocean pout antifreeze, metallothionein-B, β-actin, and cytomegalovirus-immediate early (CMV-IE) genes.

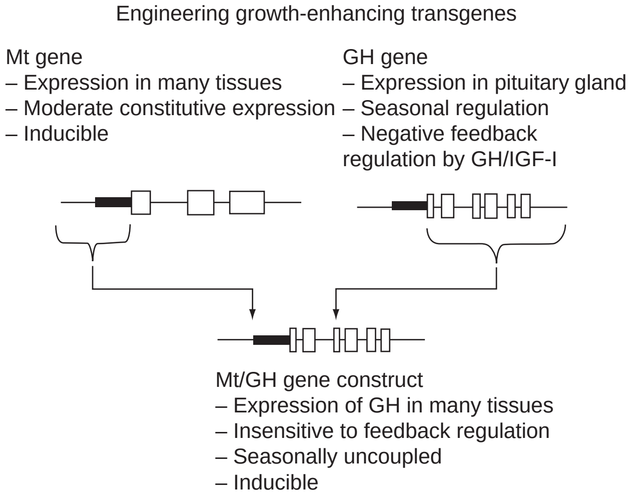

Engineering growth-enhancing transgenes
Mt gene
– Expression in many tissues
– Moderate constitutive expression
– Inducible
GH gene
– Expression in pituitary gland
– Seasonal regulation
– Negative feedback regulation by GH/IGF-I
Mt/GH gene construct
– Expression of GH in many tissues
– Insensitive to feedback regulation
– Seasonally uncoupled
– Inducible

Figure 1 Example method of generating a gene construct for overexpression of GH. GH, growth hormone; Mt, metallothionein.

GH gene constructs are introduced into transgenic fish by mechanical (microinjection and particle bombardment), electrical (electroporation), or biological (pantropic viral) approaches. Microinjection has been primarily used, allowing introduction of small volumes (2–5 nl) of DNA through fine glass needles (tip sizes approximately 2–5 μm) into the cytoplasm of newly fertilized eggs where it later enters the nucleus and becomes integrated into host chromosomes. In most cases, transgenes appear to be very stable once integrated, and are faithfully transmitted to subsequent generations in a Mendelian fashion.

Growth Effects of GH Transgenes in Fish

The first laboratories to show strong growth acceleration in fish were those of Zuoyan Zhu with loach, and Garth Fletcher and Choy Hew with Atlantic salmon (*Salmo salar*). Growth effects of GH in transgenic fish actually can vary widely by strain and species, ranging from very little or no stimulation to as much as 34-fold (loach) or 37-fold (coho salmon, *Oncorhynchus kisutch*) increases in body weight (**Figure 2**). A general observation is that fish species that naturally grow fast (e.g., carps, catfish (*Ictalurus punctatus*), tilapia (*Oreochromis* sp.), and zebrafish (*Danio rerio*)) are more difficult to growth stimulate, probably because their metabolic machinery naturally operates at near full capacity. Cold-water species such as salmon and rainbow trout (*Oncorhynchus mykiss*) show very significant growth acceleration, primarily because control nontransgenic individuals are generally very slow growing.

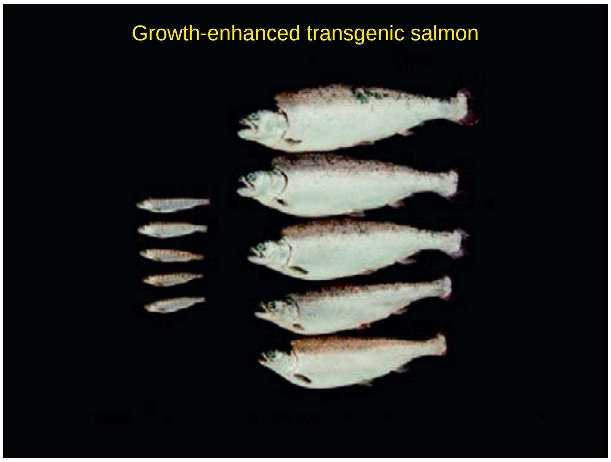


Figure 2 Normal (left) and GH transgenic (right) coho salmon at 1 year of age.

In coho salmon, GH transgenesis begins its action early, resulting in faster embryonic development and subsequent stimulation of growth through freshwater and marine growth phases. Thus, GH transgenesis can cause an overall compression of the life history and cause animals to undergo developmental stage changes at the wrong time of the year. Some GH transgenic fish (Pacific salmon) mature sexually at a younger age but with approximately normal body size, whereas others (rainbow trout) mature at a normal age, but with significantly larger bodies. What drives these differences is unknown at this time.

Expression of Transgenes and Endocrine Effects

GH transgenes drive the expression of GH mRNA in engineered fish, which in turn elevates production of GH protein. In many cases, the promoters that are used cause overexpression of GH mRNA in all tissues. However, this is not always the case as some (e.g., the ocean pout antifreeze and sockeye salmon (*Oncorhynchus nerka*) metallothionein-B promoters) express transgene mRNA at very different levels among tissues. Furthermore, species-specific differences in transgene function and effect exist. For example, an all-salmon DNA GH transgene driven by the metallothionein-B promoter stimulates growth strongly in salmon and charr (*Salvelinus alpinus*), but has no effect in tilapia. The reason for this variability is being actively investigated.

In part due to the difficulty in developing assays for GH hormone for various fish species, few have attempted to examine for elevated production of GH protein in the large number of GH transgenic fish species. Where examined, circulating GH is indeed elevated, but the increase relative to wild type can be slight (e.g., ~1.5-fold) or as much as 40-fold (with certain constructs in coho salmon). The large variance in apparent induction level of GH, particularly in salmonids, arises in part from the strong natural fluctuations in levels of this hormone seasonally. GH is found at very low levels in winter in nontransgenic fish, but remains constitutively expressed in GH transgenic fish. Expression of GH in the pituitary of salmon is strongly repressed in older GH transgenic fish (due to negative feedback regulation – see above), resulting in diminished pituitary size and dampening of brain/pituitary regulation of growth.

IGF-I levels can also change in GH transgenic fish. In salmon with GH expression driven by the metallothionein-B promoter, IGF-I mRNA is elevated in liver and other tissues, and circulating levels of hormone are increased. By contrast, IGF-I levels may be higher or lower than wild type in salmon when GH expression is driven by the ocean pout promoter. In GH transgenic tilapia, IGF-I mRNA and protein increase in several tissues, but circulating serum IGF-I levels are lower, perhaps because higher IGF-I-binding protein levels are also found. Tissues removed for culture (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines) from GH transgenic salmon can continue to show enhanced growth (in the form of synthesis of cartilage) *in vitro*, revealing that local (paracrine) effects are functioning. GH receptor gene expression is also induced in GH transgenic salmon, which may facilitate signal transduction and stimulation of IGF-I gene expression. Nutritional modulation of IGF-I expression also occurs such that GH transgenic fish fed a wild-type ration level show normal levels of this hormone. Thus, the available data indicate that there are construct and species-specific effects, but in general, elevated GH arising from extrapituitary expression of the transgene stimulates IGF-I gene expression and protein production in liver and other tissues, which in turn act in paracrine and/or endocrine fashions to stimulate growth, provided sufficient intake of energy occurs.

Other critical hormones are involved as well. For example, triiodothyronine (T3), a thyroid hormone that has strong positive effects on growth, is elevated in GH transgenic salmon due to an inhibition of T3 degradation rates. T3 levels in GH transgenic fish are unaffected by ration level suggesting a more direct action of elevated GH, as opposed to being mediated by secondary signals such as IGF-I.

In addition to effects on specific genes in the GH/IGF-I axis, microarray analysis (a technique that allows examination of the mRNA levels of thousands of genes simultaneously) has shown that GH transgenesis in fish affects the expression of many other genes (**Figure 3**), including those for lipid metabolism, iron homeostasis,

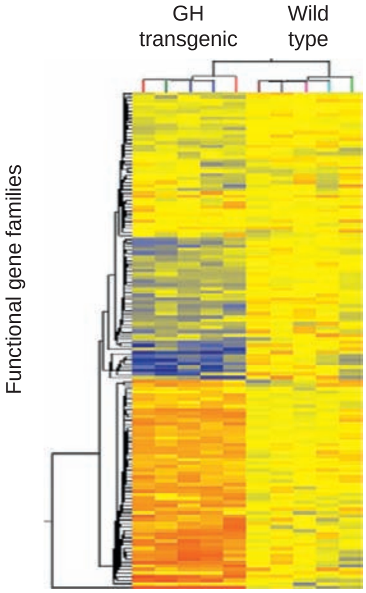


Figure 3 Heat map and hierarchical clustering of liver gene expression differences between wild-type (left five lanes) and GH transgenic salmon (right five lanes). Red bars indicate increased, and blue decreased, gene expression relative to wild type.

mitochondrial function, carbohydrate metabolism, cellular proliferation, and innate immunity. By comparing gene expression patterns among wild-type, fully fed GH transgenic (fast growing), and ration-restricted GH transgenic (growing at wild-type rates) salmon, it is apparent that some genes are directly affected by GH overexpression and some are indirectly influenced by growth rate. Thus, at the cellular level, GH expression has very broad pleiotropic effects on many pathways that are expected to influence physiological, morphological, and behavioral phenotypes of the organism, as discussed below.

Phenotypic Effects – Morphology

In addition to changes in body size with age, GH transgenesis can cause disproportionate development of organs and tissues in fish. In many vertebrates, overproduction of GH leads to an acromegaly syndrome. A striking example of this occurs in some GH transgenic salmon strains that show excessive overgrowth of cartilage in the cranium and operculum (**Figure 4**). Some deformation of head shape exists in GH transgenic tilapia and catfish. Many strains do not show these phenotypes, or are only mildly affected, suggesting that an appropriate level of GH expression is necessary in GH transgenic fish to maximize growth without inducing abnormalities (**Figure 5**).

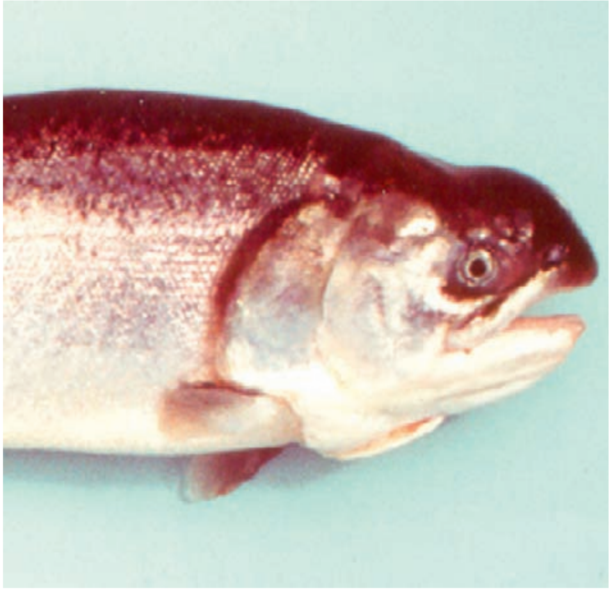


Figure 4 Phenotype of a severely acromegalic GH transgenic coho salmon. Reproduced from Devlin RH, Yesaki TY, Donaldson EM, and Hew CL (1995) Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon (*Oncorhynchus Kisutch*). *Aquaculture* 137: 161–169, with permission from Elsevier.

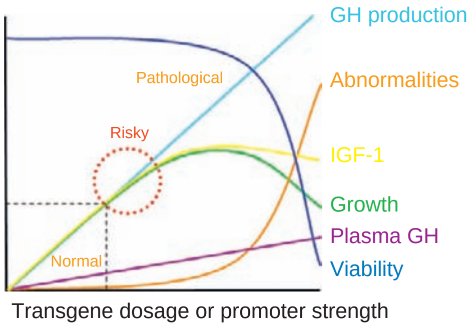


Figure 5 Relationship between GH expression, growth, viability, and abnormalities. Boxed area indicates zone where stimulation can occur without inducing pathologies.

In salmon, treatments of wild-type fish with T3 can lead to a similar acromegaly phenotype. In GH transgenic fish, the severity of such deformations can be reduced through treatment with anti-thyroid compounds (e.g., 6-n-propyl-2-thiouracil), suggesting that the acromegaly is mediated at least in part by T3.

Changes in the morphologies of specific organs or tissues have also been noted in GH transgenic fish. Smaller average cell size has been observed in GH transgenic salmon muscle, indicating that growth is occurring primarily by hyperplasia, rather than by hypertrophy, as it occurs in mammals. In GH transgenic Atlantic but not coho salmon gill surface area is reduced relative to wild fish, whereas gut surface area is increased in both species. The enhancement of gut functional size in GH transgenic coho salmon arises from a direct effect of the GH transgene, but also is affected indirectly by their enhanced food intake (food intake is known to influence gut development in many other vertebrates). Enlarged hearts have also been noted in GH transgenic Atlantic salmon resulting in enhanced cardiac output, and reduced eye and brain size occurs in GH transgenic Pacific salmon. Thus, it is clear that some allometric development of organ systems arises from GH transgenesis in fish, presumably due to differential responsiveness to, or nonuniform expression and paracrine effect of, GH among tissues.

General Viability and Immunology

The general viability (under culture conditions) of GH strains can vary widely, and depends on the level of expression from the transgene (**Figure 5**). Strong promoter constructs and certain strains with weak promoters can produce fish that are grossly deformed (see above) and have poor viability. The viability of strains with a more normal morphologically can approach or equal that of wild-type fish. Viabilities of GH transgenic fish are influenced by environmental conditions, for example, seminatural conditions with predators or low food supplies can reduce survival of these animals (see below).

GH overexpression can also affect disease processes in GH transgenic fish. Treatments of nontransgenic fish and fish cells with GH protein have been reported to enhance disease resistance and/or immune function. In GH transgenic carp, lysozyme and serum bactericidal activity are elevated, suggesting that disease resistance may be enhanced. By contrast, GH coho salmon do not show elevated lysozyme and other immune parameters. When challenged with the bacterial pathogen *Vibrio anguillarum*, survival is reduced, indicating a reduced innate disease resistance. However, GH transgenic fish surviving an initial bacterial infection show high resistance to the same pathogen, indicating that their acquired immune response still functions effectively. It is apparent that different species and strains respond distinctly to GH with respect to disease resistance.

Nutritional Requirements and Energy Use

Enhanced growth rate of GH transgenic fish is fueled by elevated energy intake and utilization. In GH transgenic salmon, feed intake (adjusted for body size) is as much as threefold greater per day than that of nontransgenic fish, depending on development stage and strain. Feed utilization (conversion efficiency) is enhanced by between 10% and 20%. Other species (such as tilapia, carp, and loach) also increase feed intake. GH transgenic rohu and one strain of GH transgenic tilapia, however, reduce feed intake. Instead, in both of these cases, feed conversion in GH transgenic fish was strongly enhanced (as much as fourfold for the tilapia), a physiological transformation that supported their enhanced growth with reduced feed intake.

In GH transgenic carp and salmon, feed intake and effects on conversion efficiency are strongly dependent on the composition of the feed. When protein levels in the feed are low, GH transgenic carp primarily enhance growth through higher food intake, whereas feed conversion efficiency is enhanced when protein levels are high. GH transgenic salmon appear to possess a new set point for energy intake, and grow at similar rates by increasing feed intake to meet energy demands when provided with low-energy feeds. However, eventually a threshold is reached where intake of very low-energy feeds is physically constrained by gut capacity, and maximal growth cannot be sustained. By contrast, feed conversion efficiency is enhanced with provision of high-energy feeds. In both GH transgenic and control carp, temporary feed restriction can lead to a compensatory growth response. By contrast, growth compensation is not observed in a very fast growing strain of GH transgenic coho salmon. Such differences between carp and salmon may depend on the levels of growth stimulation between the species and strains.

Starvation trials, and feeding trials using diets containing different levels of lipid, protein, and carbohydrate, have shown that GH transgenic salmonids, tilapia, and carp preferentially utilize lipid as an energy source, sparing protein for growth. When available energy intake is restricted, GH transgenic salmon reduce levels of body lipid stores, consistent with the lipolytic action of GH, whereas when feed availability is not limited, body lipids are much higher than in wild-type salmon. Excess deposition of fat arises from the behavioural stimulation of appetite by GH (see below), which results in greater feed intake than is required to support somatic growth.

Oxygen Uptake, Exercise, and Energy Use

A basic question surrounding GH transgenic fish energetics is whether their growth rate is driven by an obligate enhancement of metabolic processes, or do they possess increased scope that enables opportunistic use of increased energy when available? GH transgenic coho salmon show elevated oxygen uptake during exercise and during food absorption, but not during routine activity, suggesting they are not forced into a high metabolism at all times. By contrast, GH transgenic Atlantic salmon show elevated oxygen uptake during routine culture, during exercise, or following 24 h of starvation, indicating that they maintain high metabolic rates for more extended periods. GH transgenic tilapia also show elevated oxygen uptake during routine activity or during exercise.

The interaction between growth, feed intake, and energy stores can complicate investigation of metabolic rates. For example, GH transgenic fish consume more food and deposit more body fat prior to experiments, and thus remain in a positive energy balance for an extended period compared to control animals. Following prolonged starvation of GH Atlantic salmon, oxygen uptake eventually drops to normal levels, similar to the response seen in coho salmon. In GH Atlantic salmon, no difference in tolerance to low oxygen has been detected despite having a higher critical oxygen uptake level. The situation is more complex in GH transgenic carp, where family differences in tolerance to low oxygen exist, suggesting only a modest effect of the transgene relative to normal variation associated with this trait. During embryonic development (where available energy is fixed), GH transgenic coho salmon eggs are less able to survive periods of hypoxia, suggesting their demand or transport ability for oxygen differs from wild type.

Exercise capabilities of GH transgenic fish have been measured for tilapia, salmonids, and carp. In general, GH transgenic fish reduce maximum prolonged swimming speed (U_{crit}) and increase oxygen requirement during exercise. However, rearing conditions and age of fish can strongly influence these effects (e.g., U_{crit} has been found to be unaffected or reduced for the same strain of GH Atlantic salmon in different studies). Structural (e.g., muscle fiber size and body morphology) and metabolic changes in muscle discussed above are likely responsible in part for reduced swimming capacity.

The significant changes in growth and energy use in GH transgenic fish are supported by changes in levels of metabolic enzymes. While some inconsistencies in the available data are apparent (perhaps arising from differences in species, strain, ration level, fish age), in general changes reflect an enhanced ability to utilize available energy. For example, in juvenile GH transgenic tilapia and salmon, enzymes involved in amino acid catabolism (for energy production) increase both in liver and muscle, and in salmon, enzymes involved in carbohydrate use increase. Equally, however, many enzymes are unaffected by GH transgenesis, indicating that their normal levels allow sufficient flux through metabolic pathways to support the enhanced growth rates seen in these animals.

GH strongly influences osmoregulatory ability in fish, particularly in species such as salmon, which undergo a parr-to-smolt transformation (a physiological adaptation to allow ion regulation in marine conditions) (**see also Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts). Smolt development is influenced by body size and hence growth, and thus GH transgenic salmon can achieve smolt status much sooner than wild-type strains. Wild-type coho and Atlantic salmon normally smolt in their second spring at approximately 18 months postfertilization, whereas GH transgenic strains can smolt when 6 months old during their first summer. This precocious transformation is accompanied by typical body silvering (**Figure 2**), and enhanced osmoregulatory ability (as determined by plasma sodium levels and gill Na^+-K^+-ATPase activity levels) that facilitate survival following transfer to seawater. Detailed studies in GH transgenic Atlantic salmon show that smolt development occurs under a wide range of environmental conditions, in contrast to wild-type salmon which require relatively specific temperatures and photoperiods to initiate this change in physiology. In a euryhaline tilapia species, GH transgenesis also facilitates osmoregulatory tolerance to seawater conditions.

Behavior

Strong enhancement of feeding motivation and appetite (see **Video Clip 1**) support the metabolic and growth effects of GH overexpression. Many GH transgenic fish strains consume more food than nontransgenic fish of the same size (see above for exceptions), and for salmon and carp, this translates into higher feeding motivation such that GH transgenic fish can effectively outcompete wild-type fish for food in their environment. GH transgenic fish can eventually become satiated, but they return to active feeding more rapidly than nontransgenic fish, and do so even when their guts are filled, suggesting dominance of brain-driven regulation of short-term feeding activity (as opposed to peripheral controls such as stomach stretch receptor or anorexigenic peptide signals). A striking feature of GH transgenic coho salmon is they continue to feed at very high levels throughout all seasons, whereas nontransgenic salmon strongly reduce appetite and feeding motivation during the fall and winter in response to environmental conditions, indicating that GH transgenesis strongly alters central regulation of feeding.

The regulation of feeding behavior involves many orexigenic and anorexigenic neuropeptides and neurotransmitters. In nontransgenic fish, neuropeptide Y (NPY) and cholecystokinin (CCK) positively and negatively influence appetite, respectively, and NPY mRNA levels increase in the preoptic areas of the brain in starved salmon. However, the expression of genes for these two neuropeptides is unaffected in GH transgenic salmon brain subregions, suggesting more complex control (e.g., perhaps at the level of specific brain nuclei). Gut CCK mRNA levels differ between winter and summer more strongly in nontransgenic than GH transgenic fish, and suppression of appetite with the CCK inhibitor devazepide is less effective in GH transgenic fish, suggesting that seasonal regulation of feed intake is dampened (see discussion above regarding alteration of brain/pituitary-level control of growth).

GH mRNA expression is enhanced in multiple brain regions in GH transgenic Pacific salmon. However in

Atlantic salmon, intracerebroventricular (ICV) injection of GH protein does not stimulate feeding behavior, suggesting a more indirect mode of action for GH-mediated appetite stimulation. As indicated above, IGF-I levels (both circulating hormone and tissue mRNA) are elevated in fully fed GH transgenic fish but only when they are fed satiating rations. GH transgenic salmon fed wild-type ration levels have normal levels of IGF-I, but still retain the strongly enhanced feeding behavior, indicating that circulating IGF-I is not directly regulating feeding behavior. ICV injection of GH however does influence swimming behavior (by modulating dopaminergic stimulation in the brain), a response similar to that seen in GH transgenic fish, which show more rapid movement in response to feeding, and greater tendency to disperse in naturalized environments.

In nature, foraging activity increases contact with predators, and consequently, in order to maximize fitness, many organisms balance the need to acquire food resources (to allow growth and reproduction) with the risk of dying from predation. A consequence of enhanced feeding motivation in GH transgenic fish is that they spend more time foraging than do wild-type fish, and are more willing to expose themselves to predation risk (**Figure 6**).

In naturalized conditions, GH transgenic fish suffer greater mortality in the presence of predators, indicating a shift away from the evolved foraging/predation risk balance found in wild-type fish. This effect is exacerbated by low food supplies (which requires increased foraging), or by early emergence from the safety of natal redds (which exposes GH transgenic fish to predators first). Under highly competitive conditions with low food supplies, GH transgenic fish engage in cannibalism, causing crashes of small experimental populations.

Reproduction can also be influenced by GH transgenesis, both with respect to spawning ability and gamete characteristics. In medaka (*Oryzias latipes*), GH transgenic males grow larger and have a breeding advantage over wild type, whereas in salmon, GH males and females have impaired spawning ability. Sperm quality and quantity is reduced in GH transgenic males, while in GH transgenic females egg size is reduced and fecundity increased. Rearing conditions can strongly affect the development of reproductive capabilities, and thus knowing the effect of GH transgenesis alone is difficult, particularly for larger species kept in captivity.

Application in Aquaculture, and Environmental Risk Assessments

GH transgenic fish have considerable potential to enhance production efficiency in aquaculture by shortening production times and enhancing feed conversion efficiency. For many species of fish, genetic engineering can generate strong improvements in strain performance much faster (two to three generations) than can selective breeding or domestication. However, for some species (e.g. rainbow trout) that have been long domesticated and are already growing very rapidly, adding GH transgenes has a much reduced effect compared to the response seen in slow-growing wild-type strains (**Figure 7**). Analyses of gene

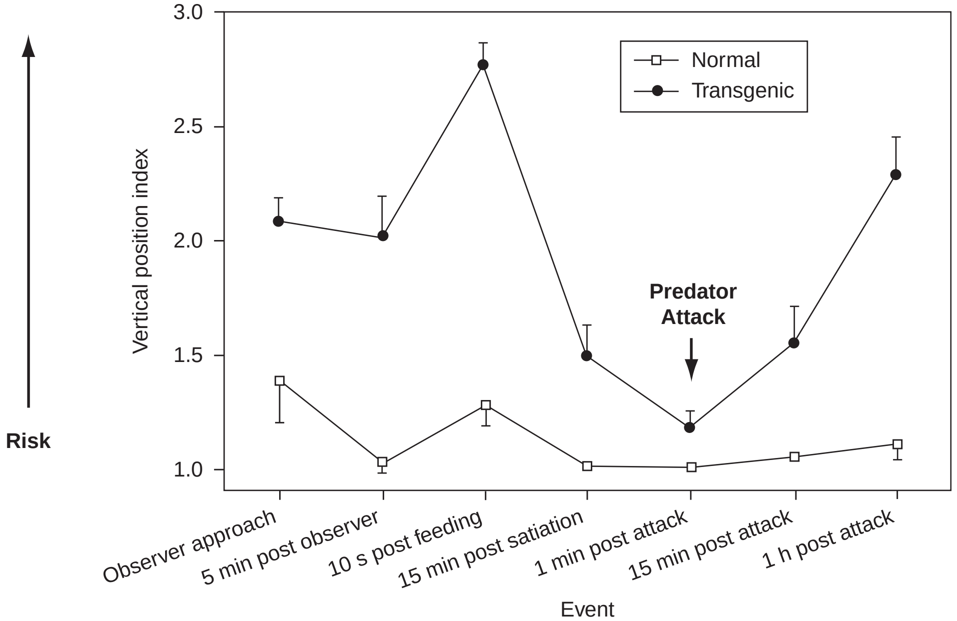


Figure 6 Risk-taking behavior of GH transgenic and wild-type salmon. Redrawn from Sundström LF, Devlin RH, Johnsson JI, and Biagi CA (2003) Vertical position reflects increased feeding motivation in growth-transgenic coho salmon (*Oncorhynchus Kisutch*). *Ethology* 109: 701–712.

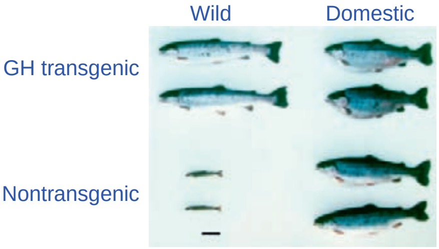


Figure 7 Effect of GH transgenesis in slow-growing wild-type and fast-growing domesticated strains of rainbow trout. Scale = 5 cm. Reproduced from Devlin RH, Biagi CA, Yesaki TY, Smailus DE, and Byatt JC (2001) Growth of domesticated transgenic fish. *Nature* 409: 781–782.

expression (using microarrays; **see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Global Gene Expression Profiling in Fish) and growth-modulating hormones (GH, IGF-I, T3) show that domestication and GH transgenesis actually modify physiological pathways in similar ways; thus, growth stimulation of domesticated strains by additional overexpression of GH from a transgene may be anticipated to be dampened.

A major issue associated with GH transgenic fish for use in aquaculture is the potential environmental risks they may pose should they inadvertently escape from research or production facilities and enter nature. Concern has centered mainly on the potential ability for GH transgenic fish with novel phenotypes (such as heightened feeding motivation) to outcompete other organisms in ecosystems and alter community structure and/or biodiversity. Under some conditions, GH transgenic fish have been hypothesized to be able to cause extinctions of populations (known as a Trojan Gene effect) through the interaction of pleiotropic phenotypes (e.g., high mating success driving the transgene into populations

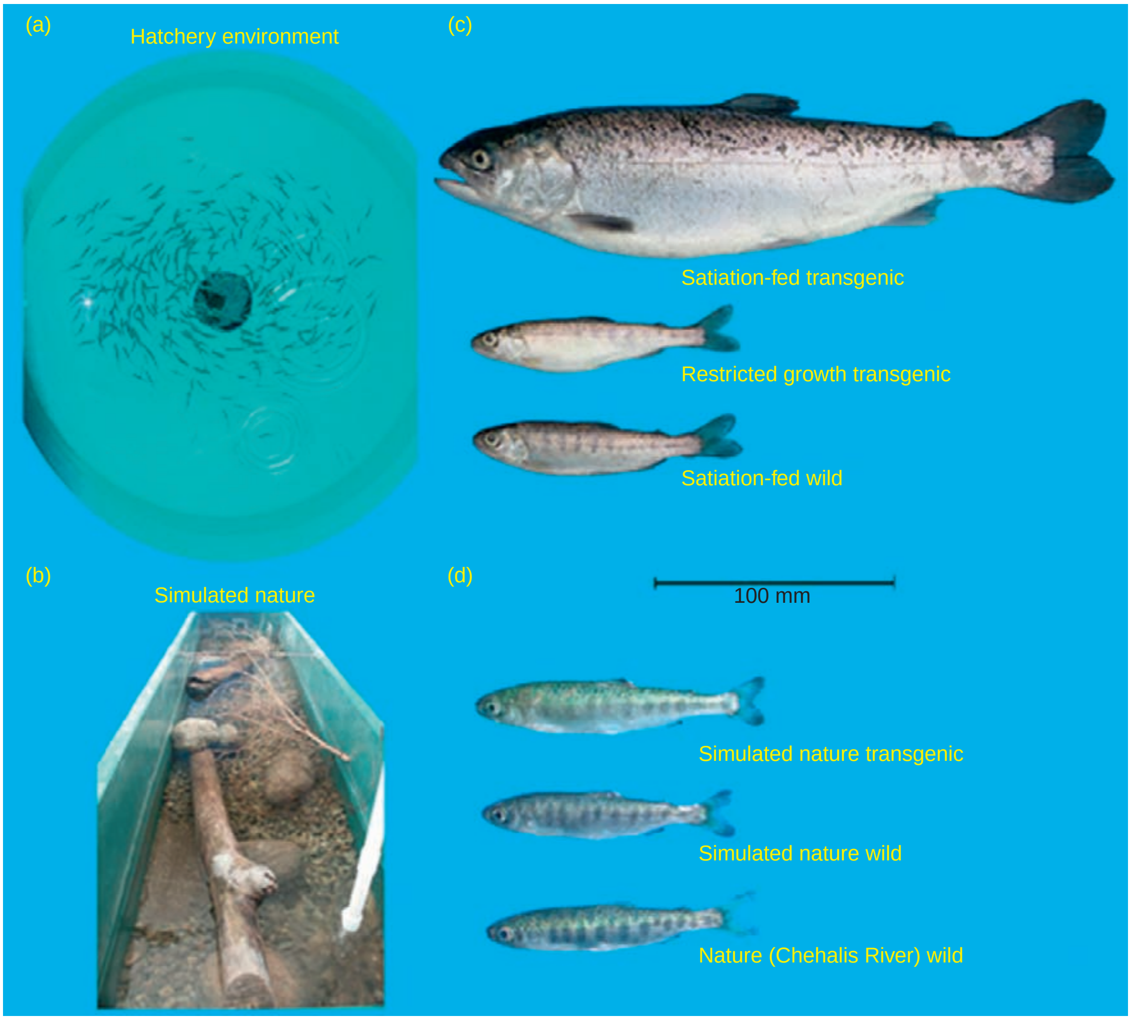


Figure 8 Influence of rearing environment on growth in GH transgenic coho salmon. Note GH transgenic fish show reduced growth under naturalized conditions. Reproduced From Sundström LF, Lohmus M, Tymchuk WE, and Devlin RH (2007) Gene–environment interactions influence ecological consequences of transgenic animals. *Proceedings of the National Academy of Sciences of the United States of America* 104: 3889–3894.

combined with poor viability which simultaneously reduces survival).

The effects of GH transgenic fish are expected to be highly dependent on their fitness in nature (i.e., ability to survive and reproduce) as well as their interaction with various ecosystem components (e.g., prey, predators, and pathogens). At this time, predicting the effects of GH transgenic fish in nature based on data generated in laboratories is difficult, since many of the traits affecting fitness and consequences are also affected by rearing and experimental environmental conditions (**Figure 8**). Due to such uncertainties, containment methods using physical approaches (e.g., land-based culture systems) or biological methods (e.g., sterilization by triploidy or transgenic methods) are being explored to minimize exposure of GH transgenic fish to an ecosystem, and potentially allow safe application of this technology outside of laboratory facilities.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Culture of Fish Cell Lines; Global Gene Expression Profiling in Fish; Transgenesis and Chromosome Manipulation in Fish. **Hormonal Control of Reproduction and Growth**: Endocrinology of Fish Growth. **Osmotic, Ionic and Nitrogenous-Waste Balance**: Mechanisms of Gill Salt Secretion in Marine Teleosts.

Further Reading

Abrahams MV and Sutterlin A (1999) The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. *Animal Behaviour* 58: 933–942.

Devlin RH, Biagi CA, Yesaki TY, Smailus DE, and Byatt JC (2001) Growth of domesticated transgenic fish. *Nature* 409: 781–782.

Devlin RH, Sakhrani D, Tymchuk WE, Rise ML, and Goh B (2009) Domestication and growth hormone transgenesis cause similar changes in gene expression in coho salmon (*Oncorhynchus kisutch*). *Proceedings of the National Academy of Sciences of the United States of America* 106: 3047–3052.

Devlin RH, Sundstrom LF, and Muir WM (2006) Interface of biotechnology and ecology for environmental risk assessments of transgenic fish. *Trends in Biotechnology* 24: 89–97.

Devlin RH, Yesaki TY, Biagi CA, *et al.* (1994) Extraordinary salmon growth. *Nature* 371: 209–210.

Donaldson EM, Fagerlund UHM, Higgs DA, and McBride JR (1979) Hormonal enhancement of growth. In: Hoar WS, Randall DJ, and Brett JR (eds.) *Fish Physiology: Bioenergetics and Growth*, pp. 455–597. New York: Academic Press.

Du SJ, Gong Z, Fletcher GL, *et al.* (1992) Growth enhancement in transgenic Atlantic salmon by use of an "all fish" chimeric growth hormone gene construct. *Biotechnology* 10: 176–181.

Dunham RA (2004) *Aquaculture and Fisheries Biotechnology: Genetic Approaches*. Wallingford: CABI Publishing.

Hew CL and Fletcher GL (1992) *Transgenic Fish*. Singapore: World Scientific Press.

Kapuscinski AR, Hayes KR, Li S, and Dana G (2007) *Environmental Risk Assessment of Genetically Modified Organisms. Vol. 3. Methodologies for Transgenic Fish*. Wallingford: CABI Publishing.

Martinez R, Estrada MP, Berlanga J, *et al.* (1996) Growth enhancement in transgenic tilapia by ectopic expression of tilapia growth hormone. *Molecular Marine Biology and Biotechnology* 5: 62–70.

Muir WM and Howard RD (1999) Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* 96: 13853–13856.

Nam YK, Cho HJ, Cho YS, *et al.* (2001) Accelerated growth, gigantism and likely sterility in autotransgenic triploid mud loach *Misgurnus mizolepis*. *Journal of the World Aquaculture Society* 32: 353–363.

Nam YK, Maclean N, Hwang G, and Kim DS (2008) Autotransgenic and allotransgenic manipulation of growth traits in fish for aquaculture: A review. *Journal of Fish Biology* 72: 1–26.

Nicholl DST (2008) *An Introduction to Genetic Engineering*. Cambridge, NY: Cambridge University Press.

Rahman MA, Mak R, Ayad H, Smith A, and Maclean N (1998) Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia (*Oreochromis niloticus*). *Transgenic Research* 7: 357–369.

Sundström LF, Lohmus M, Tymchuk WE, and Devlin RH (2007) Gene–environment interactions influence ecological consequences of transgenic animals. *Proceedings of the National Academy of Sciences of the United States of America* 104: 3889–3894.

Venugopal T, Anathy V, Kirankumar S, and Pandian TJ (2004) Growth enhancement and food conversion efficiency of transgenic fish *Labeo rohita*. *Journal of Experimental Zoology* 301: 477–490.

Wong AC and van Eenennaam AL (2007) Transgenic containment of genetically engineered fish. *Transgenic Research* 16: 862.

Zhu Z (1992) Generation of fast growing transgenic fish: Methods and mechanisms. In: Hew CL and Fletcher GL (eds.) *Transgenic Fish*, pp. 92–119. Singapore: World Scientific.

Relevant Websites

http://www.aquabounty.com – AquaBounty Technologies.

For supplementary material please also visit the companion site: http://www.elsevierdirect.com/companions/9780123745453

Molecular and Chromosomal Aspects of Sex Determination

KR Siegfried, Massachusetts General Hospital, Boston, MA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Genetic Sex Determination
Molecular Control of Sex Differentiation
Further Reading

Glossary

Androgenesis Formation of offspring possessing only paternal genetic information.

Gynogenesis Formation of offspring possessing only maternal genetic information; a type of parthenogenesis where embryogenesis is triggered by the presence of sperm, although the sperm does not contribute any genetic material.

Heteromorphic chromosomes A chromosome pair with visible size differences.

Homomorphic chromosomes A chromosome pair with no visible size differences.

Sex chromosome A chromosome whose presence or absence correlates with the sexual identity of the organism.

Sex determination Genetic or environmental process whereby the gender (male or female) is established in an individual. In most species, sex determination depends on the genotype (genotypic sex determination, GSD), but in some it depends on the value of some environmental variables during a specific developmental stage (environmental sex determination, ESD), with temperature being the most common one (temperature-dependent sex determination, TSD). Even in species with ESD, there is always a genetic component controlling sex determination.

Sex differentiation Process whereby a sexually undifferentiated gonad transforms into a testis or an ovary, according to genetic information, the influence of the environment or both. Sex differentiation results in the gonadal or phenotypic sex.

Introduction

The study of sex chromosomes and sex-linked traits in the fruit fly *Drosophila melanogaster* by Calvin Bridges around 100 years ago provided the ultimate proof that genes were associated with chromosomes. In the following years, the genes that determine sex in *D. melanogaster*, the nematode *Caenorhabditis elegans*, and mammals were elucidated. Despite the conservation in many developmental mechanisms observed in these animals, the genes controlling sex determination surprisingly were not well conserved in evolution. As an example, the mammalian sex determination gene *SRY* (sex-determining region on the Y chromosome*),* which acts as a dominant male-determining gene on the Y chromosome, is present only within therian mammals (all mammals except monotremes). Thus, all other animals determine sex through different means. Some animals harbor a different sex determination gene, while the others use environmental stimuli to determine sex, to name two examples.

The primary sex determination signal sets up a cascade of events triggering sex differentiation, that is, the development of sexually dimorphic structures or morphologies (**see also Hormonal Control of Reproduction and Growth**: Endocrine Regulation of Fish Reproduction; **Reproduction**: The Diversity of Fish Reproduction: An Introduction; **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis; Regulation of Spermatogenesis). The initiation of development of the testis or ovary from an undetermined gonadal primordium is typically the first hallmark of sex differentiation. Comparative analysis of gene expression in many vertebrate species has led to the discovery that many genes functioning in sex differentiation are expressed in the developing gonads, oftentimes exhibiting sexually dimorphic expression. Therefore, it is likely that the genes regulating sex differentiation of the vertebrate gonad have conserved roles in this process despite the differences in the primary sex determination cue.

In fish, the methods by which sex is determined vary widely across species. Sex can be determined by genomic inputs, environmental inputs (such as temperature or social cues), or by a combination of these. Certainly, the endocrine system plays a major role in sex determination (**see also Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish). However, how the endocrine system and genetic or environmental signals (**see also Social and**

Reproductive Behaviors: Socially Controlled Sex Change in Fishes) integrate to give the final outcome of male or female sexual fate is not well understood. This article discusses the genetic control of sex determination and gonadal sex differentiation.

Genetic Sex Determination

The genome oftentimes provides a significant contribution to the determination of sexual fate in fish. Chromosomal sex determination, where one gene commonly acts as the primary sex determination cue, is prevalent. Another mechanism through which sex can be genetically determined is polygenic sex determination, where the combination of multiple genes tips the fate choice toward either male or female. The genetic control of sex determination can be elucidated by karyotype analysis, identification of sex-linked traits, or through genetic experiments.

Chromosomal Sex Determination

Many fish species utilize chromosome-based sex determination mechanisms. In chromosomal sex determination, a single gene commonly regulates sex determination. The chromosome harboring this gene is dubbed the 'sex chromosome' as its inheritance correlates with sex due to coinheritance with the sex determination gene. Chromosomal sex determination generally comes in two varieties: XX/XY and ZZ/ZW. In the former case, males are heterogametic (XY) and females are homogametic (XX). In the latter, females are heterogametic (ZW) and males are homogametic (ZZ). It is also possible for one sex to be lacking a chromosome altogether, for example, some animals have XX/X0 or ZZ/Z0 sex determination. Many examples of chromosomal sex determination exist in fish; some are given in **Table 1**.

Chromosomal sex determination may be identified by cytogenetic techniques if one sex chromosome appears visibly different from the other. When the sex chromosomes are heteromorphic, large size differences are apparent through staining of chromosomes. When the differences are very small between the sex chromosomes, synaptonemal complex analysis can identify unpaired regions that could indicate unique sequences on the chromosome in only one sex. Fluorescent *in situ* hybridization (FISH) can also be used to visualize sequences that are sex specific. Using this method, sex-specific loci or repetitive elements mark the sex chromosomes. In addition to cytogenetic techniques, or in cases where no visible differences can be detected by the above techniques, analysis of sex-linked traits or genetic experiments has revealed the sex determination mechanisms of many fish (see below).

An indication of chromosomal sex determination is the coinheritance of a trait or character with sex, a phenomenon dubbed 'sex linkage'. When a trait or character is coinherited with the Y chromosome, it segregates from fathers to sons. Conversely, when the trait or character is linked to a W chromosome, it segregates from mothers to daughters. Thus, sex linkage can reveal the chromosomal nature of sex determination. These traits or characters can be visible traits (e.g., pigmentation or ornamentations), protein signatures, or DNA sequences. One must take care when using visible traits or protein signatures to assess sex linkage as these can also be influenced by sex-specific hormonal regulation. Therefore, it is essential to experimentally determine if the trait is sex specific as a result of coinheritance with the sex determination locus or due to the sex-specific hormone regulation.

Genetic experiments have been successfully used to discover sex determination mechanisms. Fish that consistently yield 1:1 sex ratios are likely to have chromosomal sex determination; those that deviate from 1:1 sex ratios probably have polygenic or environmental sex determination. Alternatively, they may have chromosomal sex determination, but have additional genetic or

Table 1 Examples of genetic sex determination systems

Common name	*Species*	*XY*	*ZW*	*Polygenic*
Carp	*Cyprinus carpio*	•		
European sea Bass	*Dicentrarhus labrax*			•
Guppy	*Poecilia reticulata*	•		
Japanese eel	*Anguilla japonica*		•	
Medaka	*Oryzias latipes*	•		
Rainbow trout	*Oncorhynchus mykiss*	•		
Threespine stickleback	*Gasterosteus aculeatus*	•		
Tiger pufferfish	*Takifugu rubripes*	•		
Tilapia	*Oreochromis niloticus*	•		
	Oreochromis aureus		•	
Zebrafish	*Danio rerio*			•

environmental factors that can override the predominant sex determination gene. For those animals with suspected chromosomal sex determination, whether an XY or ZW system is functioning can be elucidated by performing experiments using hormone-induced sex-reversed fish or by inducing gynogenesis or androgenesis. In the former experiment, matings of sex-reversed fish with normal fish give predictable sex ratios that reveal the chromosomal mechanism (see **Figure 1** for detailed description). In the latter experiments, gynogenetic or androgenetic fish are produced resulting in fish with all genetic material derived from a female or male fish, respectively. Again, predictable sex ratios arise depending on the chromosomal mechanism of sex determination (**Figure 2**).

Multifactoral Sex Determination

Polygenic sex determination

Oftentimes sex ratios are not 1:1 or the experiments outlined in **Figures 1** and **2** yield unpredicted outcomes. This may indicate a polygenic sex determination system where the combined effect of multiple genes determines sex. The presence of polygenic sex determination is difficult to prove as unpredictable sex ratios might also arise due to environmental influences. A good indication of polygenic sex determination is through pair-wise matings or through analyses of androgenic or gynogenic fish. Successive matings of individual pairs of fish can reveal whether or not sex is genetically determined. Whenever possible, the progeny should be reared under constant conditions to eliminate the possibility of environmental influences. If successive matings between individual pairs generally give rise to clutches with consistent sex ratios, then there are probably genetic factors determining sex. To confirm this result, two experiments can be significant. First, fish resulting form gynogenesis or androgenesis will give unpredictable sex ratios. For example, in zebrafish (*Danio rerio*), androgenesis has been reported to give rise to nearly all male populations, nearly all female populations, or mixed-sex populations. This discrepancy might be due to the genetic makeup of the parent fish being used. A key experiment for proving that there is a genetic contribution to sex is by conducting selection experiments. One selects for populations with higher male-to-female sex ratios, for example, over successive generations. If the high male-to-female sex ratio increases

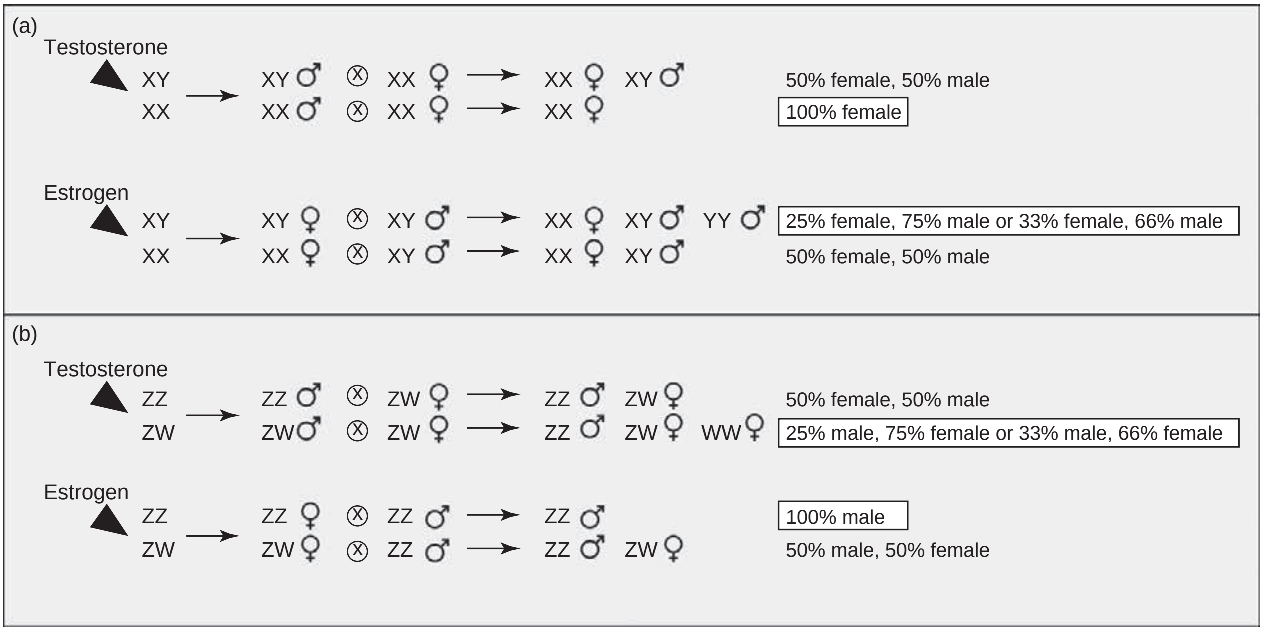


Figure 1 Hormone treatment and crossing scheme to decipher the mechanism of sex chromosome determination. Fish are treated with hormones prior to sex differentiation. At the time of treatment, the sex of the fish is not discernable; thus, the population is mixed XX and XY or ZZ and ZW. The fish treated with testosterone all develop into males, whereas those treated with estrogens all develop as females regardless of the sex chromosome content. Hormone-treated fish are individually crossed to normal males or females. In all experiments, half of the resulting clutches will give rise to 50/50 sex ratios. These are derived from the non-sex-reversed individuals in the treatment (e.g., XY males in the testosterone-treated group) and are not informative. However, those clutches giving rise to sex ratios deviating from 50/50 can reveal whether the sex chromosome system is XY or ZW. These informative clutches are highlighted. (a) In organisms with an XY sex determination system, sex reversal with testosterone treatment followed by crossing to natural females gives rise to some clutches that are 100% female. Treatment with estrogen followed by crossing to natural males leads to clutches with sex ratios of either 25/75% female to male when YY individuals are viable or 33/66% female to male when the YY chromosomal content is lethal. (b) In organisms with a ZW sex determination system, the resulting sex ratios from hormone-treated sex-reversed fish followed by crossing to natural males and females is counter to those observed from organisms with XY sex determination.

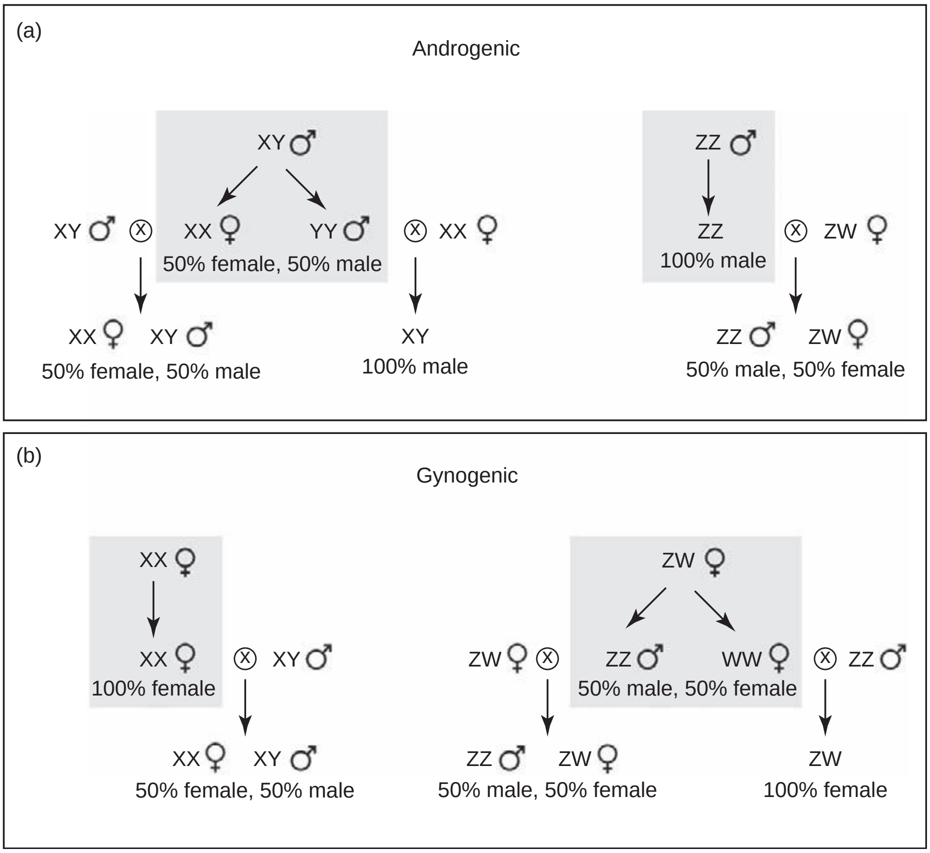


Figure 2 Use of androgenesis and gynogenesis to determine the sex chromosome system. Production of fish from diploid gametes derived from male or female individuals' results in predictable sex ratios depending on the sex chromosome mechanism (shown in the shaded boxes). Crossing of the resulting fish to natural males or females can confirm the sex chromosome contents through production of predictable sex ratios, shown outside of the shaded boxes. (a) Diploid gametes from XY male fish give rise to populations with 50/50 sex ratios of XX females to YY males offspring, assuming viability of YY individuals. By contrast, those resulting from ZZ males give 100% ZZ males. (b) Gynogenesis results in 100% females when the parent females have XX sex chromosomes or 50/50 sex ratios when parent females have ZW sex chromosomes, assuming viability of WW females.

over each generation, then it is likely that the researcher has enriched for male-determining loci or alleles. This is a clear indication that multiple genetic factors determine sex.

Modifiers

It is not uncommon to find exceptions to the above sex determination mechanisms. For example, in fish with chromosomal sex determination, a small percentage of XX males may be present in the population. When different strains are available, the percentage of these exceptional males is often strain dependent, indicating genetic modifiers. For example, in medaka (*Oryzias latipes*), some strains produce more than 10% XX males, whereas others exhibit no XX males. This strain dependency indicates an autosomal gene or genes that can control sexual fate in the absence of the predominant sex determination gene. As yet, the genes that act as modifiers to sex determination have not been identified. In addition to genetic modifiers, environmental stimuli can also override the genetic sex determination signal, leading to exceptional males or females.

Sex Determination in Medaka

The medaka fish have been studied for decades with respect to various aspects of fish biology including sex determination. Medaka have an XY sex determination system with a dominant male-determining gene on the Y chromosome determining male sexual fate. The Y chromosome is homomorphic, but can be visualized by FISH using Y-specific probes. Currently, it is the only fish for which the sex determination gene has been identified.

The medaka sex determination gene is called *DMY* (also known as *dmrt1bY*). *DMY* is a homolog of the doublesex and mab-3 related transcription factor 1 (*DMRT1*) gene. *DMY* and *DMRT1* encode transcription factors containing a conserved zinc finger DNA-binding domain, dubbed the 'DM domain'. The vertebrate *DMRT1* gene name is derived from its relation to the doublesex gene in the fruitfly *D. melanogaster*, which is essential for both male and female sex determination, and the male abnormal-3 (*mab-3*) gene in the nematode *C. elegans*, which is important for specification of male-specific cell fates. In vertebrates, *DMRT1* is known to function as, or to be associated with, sex-specific development of many animals including chicken, alligator, mouse, and humans. Medaka possess two *DMRT1* paralogs, *DMRT1* and *DMY* (also known as *DMRT1a* and *DMRT1bY*, respectively). *DMY* is expressed in the male gonad during the time window of sex determination and through adulthood. The autosomal *DMRT1* gene is expressed in the same gonadal cell types as *DMY* but at a later time point, after sex is determined.

The medaka Y chromosome arose between 10 and 18 million years ago through duplication of the *DMRT1*-containing region and insertion onto another chromosome. The duplicated *DMRT1* gene subsequently evolved to become the *DMY* sex-determining gene, resulting in a Y chromosome with a small Y-specific region. This region contains 258 kb of sequence not present on the X chromosome with *DMY* being the only functional gene. *DMY* is a recently acquired sex determination gene and is not common among fish species. It is present and functions as the male sex determination gene only in medaka and its sister species *Oryzias curvinotus*, and is thus a recently acquired gene. The *DMRT1* gene has been identified and mapped within the genome of several fish species, but so far is not located at same position as the sex-determining region in any fish other then medaka.

Molecular Control of Sex Differentiation

The first signs of sexual dimorphism are typically seen in the gonads. The gonads consist of somatically derived tissues and the germ line. The somatic tissues and germ line are derived from different embryological origins and come together to form the gonad during embryogenesis, typically through migration of the germ cells to the site of the gonad anlage (primordium). Gametogenesis requires the association of the germ line with somatically derived support cells. In the testis, these support cells are the Sertoli cells and in the ovary they are the granulosa cells. The steroid-producing cells of the gonad are also somatically derived. These are the Leydig cells and thecal cells in the testis and ovary, respectively (**see also The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis).

Sex Differentiation of the Gonad

Sex differentiation in teleost fish is typically divided into two stages. The first being germ line sex differentiation which is characterized by a dichotomy in germ line proliferation and oftentimes the onset of meiosis between the sexes. Somatic sex differentiation follows, which is characterized by seminiferous tubule morphogenesis and efferent duct formation in the testis and formation of follicles and the ovarian cavity in the ovary.

The first sexually dimorphic feature in teleost fish is typically a dichotomy in germ cell number, with females having more germ cells than males. How this dichotomy is established molecularly is not known. In medaka, the sex determination gene, *DMY*, is expressed specifically in male somatic gonadal cells, thought to be Sertoli cell precursors. This expression commences prior to the sex-specific differences in germ cell number. It has been postulated that one of the early roles of *DMY* in testis development is to limit germ cell divisions in males. However, because *DMY* is a transcription factor, it probably regulates gene expression within the Sertoli cells and their precursors. One possibility is that *DMY* regulates the expression of a gene encoding a molecule that signals the germ line to inhibit mitosis.

Somatic sex differentiation is characterized by morphogenesis of somatic gonadal structures. In the testis, somatic sex differentiation consists of formation of the seminiferous tubules and efferent ducts. In the ovaries, it consists of follicle and ovarian cavity formation. During testis sex differentiation, the Sertoli cells enclose the germ line to form the seminiferous tubules. A basement membrane is produced between the Sertoli cells and another somatic cell type called the peritubular myoid cells. Thus, only germ line and Sertoli cells reside within the seminiferous tubules. In the ovary, the granulosa cells surround individual oogonia to form follicles. There is reciprocal dependency on the oocyte and granulosa cells for follicle development. The granulosa cells and oocyte remain intimately associated through cytoplasmic processes that pass through the developing zona pellucida (future eggshell), which forms between the oocyte and granulosa cell layer.

The ovarian granulosa cells and testicular Sertoli cells are thought to be derived from a common precursor cell lineage in the gonadal pirmordium. The common lineage of these two cell types has been demonstrated in mice and medaka. Thus, specification of these two cell types from a common precursor cell population is a critical step in somatic sex differentiation of the gonad.

Genes Involved in Gonadal Sex Differentiation

The molecular mechanisms underlying somatic sex differentiation in fish are as yet not understood. Somatic sex differentiation has been well studied in mammals, leading to the discovery of many genes that function in this process. In recent years, investigators have begun assaying the potential role of genes known to be involved in mammalian sex differentiation in fish, primarily through gene expression analysis. Genes that have been investigated in fish with potential roles in testis differentiation are *SOX9* (Sry related HMG box gene 9), *AMH* (anti-Müllerian hormone/Müllerian inhibiting substance), *DMRT1, SF1* (steroidogenic factor 1, also known as Nr5a1), and *WT1* (Wilms tumor 1). Of these, *SOX9*, *AMH*, and *DMRT1* exhibit sexually dimorphic expression during sex differentiation in at least some fish species suggesting a possible role in this process. In the ovary, two genes have been investigated that have potential roles in ovary sex differentiation: *CYP19A1* (cytochrome P450), which encodes aromatase, and *FOXL2* (**Table 2**).

The *SOX9* gene has been suggested to function in sex determination or gonad sex differentiation in many vertebrates. In mammals, the mammalian sex determination gene *SRY* directly activates *SOX9*. *SOX9* is both necessary and sufficient for male sex determination in both mice and humans. It is thought to drive Sertoli versus granulosa cell fate, thereby controlling testis fate and leading to morphogenesis of the seminiferous tubules. In fish, sexually dimorphic *SOX9* expression does not typically correlate with onset of germ line sex differentiation. However, the sex-specific expression of *SOX9* correlates with the onset of seminiferous tubule formation in several fish species, suggesting that *SOX9* may have a conserved role in this process.

The *AMH* gene often exhibits sex-specific upregulation during sex differentiation of male fish. In the developing testis, *AMH* is expressed in the pre-Sertoli cells and Sertoli cells and remains expressed through adulthood. It is also expressed in ovarian follicle cells of previtellogenic and vitellogenic ovaries. AMH is a secreted glycoprotein of the transforming growth factor beta family (TGFβ) of signaling molecules. In medaka, *AMH* has a clear role in germ cell proliferation and sex determination. Fish with a mutation in the *amh-receptor 2* (*AMHR2*) gene, which encodes for the AMH receptor, exhibit non-sex-specific overproliferation of the germ line, defects in oogenesis, and XY sex reversal (i.e., development of ovaries in a genetically male individual). Thus, in medaka, AMH signaling functions in several processes in gonad development, including testis sex differentiation.

The *DMRT1* gene has been implicated in male-specific development in many animal species. Its role as the medaka sex determination gene has been described previously in this article. Although *DMRT1* has not been implicated as the master sex determination gene in other fish, its expression in the testis oftentimes correlates with the onset of sex differentiation. In some fish, such as the Nile tilapia (*Oreochromis niloticus*), male-specific expression of gonadal *DMRT1* precedes any morphological signs of sex differentiation, including germ line sex differentiation. However, in other species, such as *Oryzias luzonensis*, testicular *DMRT1* expression correlates with the onset of somatic sex differentiation of the testis, but is not expressed earlier during germ line sex differentiation. Thus, *DMRT1* may have a conserved role in sex differentiation of somatic structures and, in some species, a possible additional role in influencing germ line sex differentiation.

Few genes have been investigated during female sex differentiation in fish. Estrogen clearly plays an important role in female sex differentiation (**see also Hormonal**

Table 2 Genes expressed during gonadal sex differentiation

Gene name	*Protein*	*Tissue expressed*	*Expression during sex differentiation*[a]	*Function*
SOX9	HMG transcription factor	Sertoli cells	In developing testes during formation of Seminiferous tubules	Not tested
AMH	Secreted glycoprotein	Sertoli cells	Dimorphic expression prior to somatic sex differentiation in some species Medaka: no dimorphicexpression	Medaka: regulation of germ line proliferation, oogenesis, male sex determination
DMRT1	Zinc finger transcription factor	Sertoli cells some species in germ cells	In developing testis during somatic sex differentiation In some species prior to germ line sex differentiation	Medaka: DMY paralog is the master male sex determination gene
CYP19A1	Aromatase	Granulosa cells Thecal cells	Prior to or at the onset of sex differentiation	Estrogen production
FOXL2	Forkhead transcription factor	Granulosa cells Thecal cells	Prior to or at the onset of sex differentiation	Activates *CYP19A1* expression Tilapia: sex differentiation

[a] The precise expression of the genes listed in this table is variable depending on the fish species.

Control of Reproduction and Growth: Endocrine Control of Sex Differentiation in Fish). The granulosa cells produce the enzyme aromatase, which produces estrogen. Aromatase is encoded by the *CYP19A1* gene. In the ovary, *CYP19A1* expression is often dimorphically expressed during early stages of gonadal sex differentiation suggesting a role of estrogen signaling in ovary sex differentiation. Furthermore, estrogen treatment during the time when sex is determined is typically sufficient to feminize fish, resulting in fully functional females.

The forkhead transcription factor gene, *FOXL2*, has been shown to be an important regulator of ovarian cell fates in mammals. It also exhibits early dimorphic expression and is expressed in the ovarian granulosa cells in several fish species. In fish, it binds to the *CYP19A1* promoter and activates transcription in the ovary. In the Nile tilapia, the function of *FOXL2* has been investigated using transgenic fish expressing a dominant negative form of FOXL2 protein. In these fish, a mutated version of the *FOXL2* gene was introduced that produces a protein that interferes with the function of the wild-type protein, thereby resulting in overall loss or reduced function of the FOXL2 protein. These fish display masculinized gonads in XX fish indicating an important function of *FOXL2* in gonadal sex differentiation in females.

See also: **Hormonal Control of Reproduction and Growth**: Endocrine Control of Sex Differentiation in Fish; Endocrine Regulation of Fish Reproduction. **Reproduction**: The Diversity of Fish Reproduction: An Introduction. **Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes. **The Reproductive Organs and Processes**: Anatomy and Histology of Fish Testis; Regulation of Spermatogenesis.

Further Reading

Baroiller JF, D'Cotta H, Bezault E, Wessels S, and Hoerstgen-Schwark G (2009) Tilapia sex determination: Where temperature and genetics meet. *Comparative Biochemistry and Physiology. Part A. Molecular and Integrative Physiology* 153: 30–38.

DeFalco T and Capel B (2009) Gonad morphogenesis in vertebrates: Divergent means to a convergent end. *Annual Review of Cell and Developmental Biology* 25: 19.1–19.26.

Devlin RH and Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191–364.

Kondo M, Nanda I, Schmid M, and Schartl M (2009) Sex determination and sex chromosome evolution: Insights from medaka. *Sexual Development* 3: 88–98.

Matsuda M (2005) Sex determination in the teleost medaka, *Oryzias latipes. Annual Review of Genetics* 39: 293–307.

Matsuda M, Nagahama Y, Shinomiya A, *et al.* (2002) DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417: 559–563.

Nanda I, Kondo M, Hornung U, *et al.* (2002) A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes. Proceedings of the National Academy of Sciences of the United States of America* 99: 11778–11783.

Orban L, Sreenivasan R, and Olsson PE (2009) Long and winding roads: Testis differentiation in zebrafish. *Molecular and Cellular Endocrinology* 312: 35–41 (doi:10.1016/j.mce.2009.04.014).

Siegfried KR (2010) In search of determinants: Gene expression during gonadal sex differentiation. *Journal of Fish Biology* 76: 1879–1902.

Volff JN, Nanda I, Schmid M, and Schartl M (2007) Governing sex determination in fish: Regulatory putsches and ephemeral dictators. *Sexual Development* 1: 85–99.

Yamamoto T (1969) Sex differentiation. In: Hoar HS and Randall DJ (eds.) *Fish Physiology: Reproduction and Growth Bioluminescence, Pigments, and Poisons,* vol. 3, pp. 127–131. New York: Academic Press.

Molecular Fish Pathology

CJW Scott and PC Morris, Heriot-Watt University, Edinburgh, UK

B Austin, University of Stirling, Stirling, UK

© 2011 Elsevier Inc. All rights reserved.

Introduction
Bacterial Fish Pathogens
Extracellular Products
Excretion Systems
Infection
Fish Viruses
Methods of Control
Conclusions
Further Reading

Glossary

Asymptomatic A case in which an animal carries a disease-causing agent but does not show any symptoms associated with the disease.

Bioinformatics The use of information technology in studying molecular biology.

Cytopathic effect An effect where the infecting virus results in a change in the host cell morphology.

Endemic A state in which the pathogen remains within the animal population but only results in disease occasionally.

Exophthalmia The swelling and protrusion of one or both eyes.

Genome All the genetic information for one living organism or virus.

Glycoprotein A molecule which consists of both protein and carbohydrate.

Immunocompromised A state in which the immune system of the animal is unable to fight an infection.

LD_{50} An abbreviation of lethal dose, 50%. This is the amount of a substance (e.g., number of bacteria) required to kill 50% of a given population.

Molecular biology The study of biological molecules including DNA, RNA, proteins, and lipids.

Necrosis Death of living cells and body tissue.

Neutralizing epitope A molecule which, when recognized by the immune system, can effectively neutralize the pathogen.

Pathogen An infectious agent that can cause disease.

Pathogenesis A stage of events that eventually leads to the development of disease caused by a pathogen.

Probiotics Microorganisms which, when applied orally to fish, have a tangible health benefit.

Proteases Enzymes which break down protein.

Proteomics The broad study of proteins and their structures.

Pro-toxins Proteins or compounds, which only become toxins after further modifications (e.g., proteolytic cleavage).

Quorum sensing A form of bacterial cell-to-cell communication, which allows bacteria to control gene expression, particularly with respect to the bacterial population.

Reverse transcriptase An enzyme which transcribes single-stranded RNA to double-stranded DNA.

RNA polymerase An enzyme which produces RNA.

Systemic viremia A condition where the virus has spread throughout the body.

Virion A completely formed virus or viral particle in the infective form.

Virulence A broad term used to describe the ability of an organism to cause disease.

Virulence factor A molecule (e.g., protein) which is involved in establishing disease or maintaining the diseased state.

Virus A small infectious agent which cannot replicate outside the host cell.

Zoonosis Any infectious disease that can be passed from animals to humans.

Introduction

Fish may be exposed to bacterial and viral pathogens, both in the environment and within aquaculture sites. Many of these pathogens can cause significant losses to a wide range of aquatic species and can remain persistent or endemic within populations. Depending on the etiological agent and host, disease symptoms may vary dramatically from overt (see examples of bacterial infections in **Figure 1**) to asymptomatic. Moreover,

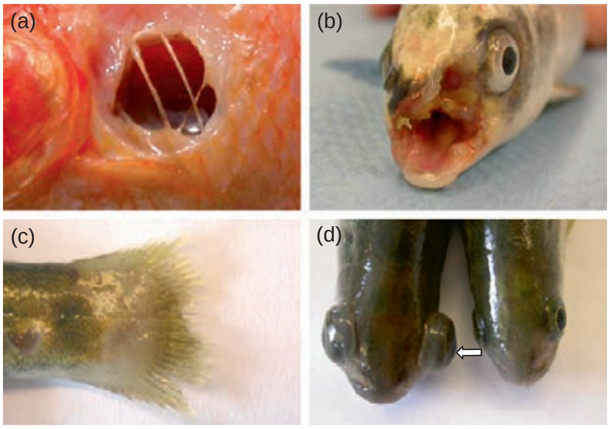


Figure 1 Common symptoms associated with bacterial infections. (a) *Aeromonas salmonicida* infection causing ulceration across the body wall in goldfish. (b) Erosion of ghost carp mouth caused by *Aeromonas bestiarum* infection. (c) Tail rot of a rainbow trout caused by *Aeromonas hydrophila*. (d) Rainbow trout showing signs of a bilateral exophthalmia (indicated by arrow) whereas the fish on the right is a healthy specimen. The etiological agent was identified as *Renibacterium salmoninarum*. Reproduced from Austin B and Austin D (2007) *Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish*, 4th edn. Weilheim: Springer-Praxis Publishing Ltd.

disease symptoms may not be specific for any one pathogen, making diagnosis difficult. Stressed or overcrowded livestock, suboptimal diets, and poor rearing conditions can also contribute to the development of disease. In addition, some fish pathogens (particularly bacterial) can be zoonotic and cause disease in immunocompromised humans. Despite rigorous control measures and immunization strategies, however, there are still significant outbreaks of disease. Nevertheless, advancements in molecular biology and bioinformatics have significantly increased our understanding of fish diseases, pathogenicity, and virulence. This article first gives a general introduction to bacterial fish pathogens and common virulence factors associated with microbes. Attention then turns to viruses, their structures, and life cycles, before covering some topical viruses currently problematic in aquaculture. Finally, current control and preventative measures are discussed, followed by a brief conclusion on the future of molecular fish pathology.

Bacterial Fish Pathogens

Many bacterial species are known to cause disease in fish (some are illustrated in **Table 1**) and often share key features or similarities in the molecular machinery used in establishing an infection (e.g., attachment, invasion, spread, and immune invasion). For example, many bacterial pathogens problematic to aquaculture rely heavily on extracellular products in establishing disease.

Extracellular Products

Extracellular products (ECPs), also known as extracellular enzymes, are secreted by a wide range of bacteria and include amylases, proteases, hemolysins, lipases, and phospholipases. Many ECPs act synergistically to aid colonization, evade host immune systems, kill host cells, and utilize nutrients via the breakdown of macromolecules.

Proteases

Proteases are among the most common components of ECPs. They often aid colonization and provide peptides and amino acids from the breakdown of host proteins. These enzymes have been grouped into four functional classes, that is, the metallo-, serine-, cysteine-, and aspartate proteases. Proteases are produced by many fish pathogens, including *Aeromonas hydrophila*, *A. salmonicida*, *A. jandaei*, *Edwardsiella tarda*, *Yersinia ruckeri*, and *Vibrio vulnificus*. In particular, the metalloproteases and serine proteases contribute significantly to virulence.

Metalloproteases

Y. ruckeri, which causes enteric redmouth disease (ERM) in salmonids, produces a well-characterized metalloprotease. This enzyme is a 47-kDa metallo-endopeptidase belonging to the serralysin family which is secreted via a type I secretion system and is encoded by the *yrp1* (*Yersinia* related protease 1) gene. *Y. ruckeri* strains possessing a mutation in the *yrp1* gene are less virulent *in vivo* compared to their wild-type counterparts. This protease has the ability to break down many host protein substrates including fibrinogen, actin, gelatin, laminin, and myosin. Although this ECP is important for pathogenesis, a number of *Y. ruckeri* strains do not produce it, and are classified as Azo$^+$ or Azo$^-$ depending on their ability to break down azocasein.

Serine proteases

When the serine protease of *A. salmonicida* is purified and injected into fish, it causes liquefaction of muscle tissue, a common pathological sign of furunculosis. This protease is encoded by the *aspA* gene and has a predicted molecular weight of 70 kDa. The enzyme possesses a conserved serine protease binding site, is sensitive to the protease inhibitor phenylmethanesulfonylfluoride (PMSF) and cation chelator ethylenediaminetetraacetic acid (EDTA), and performs optimally in alkaline conditions (pH 9.5) The enzyme may exert an indirect role in pathogenesis by proteolytically cleaving (and thereby activating) excreted pro-toxins.

Table 1 A list of currently topical bacterial fish pathogens

Pathogen	*Disease*	*Host*	*Source*
Gram-positive			
Lactococcus garvieae (formerly *Enterococcus seriolicida*)	Streptococcosis	Many fish species	Australia, Europe, Saudi Arabia, Red Sea, South Africa, Taiwan, and USA
Mycobacterium marinum	Mycobacteriosis (form of fish tuberculosis)	Many fish species	Worldwide
Streptococcus iniae (formerly *S. shiloi*)	Streptococcosis	Freshwater and costal fish	Saudia Arabia, Red Sea, South Africa, Taiwan, and the USA
Streptococcus parauberis	Streptococcosis	Turbot	Spain
Gram-negative			
Aeromonas hydrophila (formerly *A. liquefaciens* and *A. punctata*)	Hemorrhagic septicemia, red sore disease and fin rot.	Many freshwater species	Worldwide
Aeromonas salmonicida subsp. *salmonicida* (formerly *Hemophilus piscium*)	Furunculosis, ulcer disease and carp erythrodermatitis.	Salmonids, cyprinids, and marine species	Worldwide
Edwardsiella ictaluri	Enteric septicemia of catfish	Catfish	Indonesia, USA, and Vietnam
Edwardsiella tarda	Edwardsiellosis and redpest	Freshwater species	Japan, Spain, and USA
Flavobacterium psychrophilum (formerly *Cytophaga psychrophila*)	Cold water disease, necrotic myositis, and rainbow trout fry syndrome	Salmonids and sea lamprey	Australia, Europe, Japan, and North America
Hafnia alvei	Hemorrhagic septicemia	Cherry salmon and rainbow trout	Bulgaria, England, and Spain
Pasteurella skyensis	Pasteurellosis	Atlantic salmon	Scotland
Photobacterium damselae subsp. *piscicida* (formerly *Pasteurella piscicida*)	Vibriosis	Damsel fish, sharks, redbanded sea bream, turbot and yellowtail	Europe and USA
Piscirickettsia salmonis	Coho salmon syndrome and salmonid rickettsial syndrome	Salmon and sea bass	Canada, Chile, Greece, Norway and Scotland
Pseudomonas anguilliseptica	Red spot and winter disease	Rainbow trout, cod, eels, black spot sea bream and gilthead	Finland, France, Japan, Portugal, Scotland and Spain
Serratia liquefaciens	Septicemia	Atlantic salmon, turbot, and charr	France, Scotland and USA
Tenacibacterium maritimum (formerly *Flexibacter maritimus*)	Bacterial stomatitis, gill disease, and necrotic myositis	Many fish species	Europe, Japan, and North America
Vibrio anguillarum	Vibriosis	Most marine fish	Worldwide
Vibrio cholerae (non-O1)	Septicemia	Ayu, goldfish	Australia and Japan
Vibrio harveyi (formerly *V. carchariae* and *V. trachuri*)	Eye disease, necrotizing enteritis and vasculitis	Many fish species	Europe, Japan, Taiwan, and USA
*Vibrio (*now *Aliivibrio) ordalii*	Vibriosis	Most marine fish species	Worldwide
Vibrio salmonicida	Vibriosis (cold water), Hitra disease	Atlantic salmon	Canada, Norway, and Scotland
Vibrio vulnificus	Septicemia and ovate pompano (*Trachinotus ovatus*)	Eel	Europe, Japan, P.R. of China,. and USA
Yersinia ruckeri	Enteric redmouth (ERM) and salmonid blood spot	Salmonids	Australia, Europe, North and South America

Hemolysins/Cytolysins

Iron is an essential factor for pathogenesis and is required by bacteria to grow and proliferate during infection. Although iron is found in abundance within fish, it is often present in protein complexes (=hemoproteins) within host cells. For example, hemoglobin is found within erythrocytes but is not readily accessible for bacteria. Hemolysins are therefore required to lyse cells and release cellular contents, making them available for the pathogen. These enzymes may also lyse other cell types including polymorphonuclear cells, mast cells, and neutrophils. This aids colonization and contributes to virulence and tissue damage. When lysing cells other than erythrocytes, the hemolysins are more commonly known as cytolysins. Although many pathogens produce hemolysins, *Vibrio* spp. and *Aeromonas* spp. produce hemolysins that exert a role in fish and human pathogenesis. *Vibrio* spp. are associated with four different families of hemolysins including the HlyA family, the thermostable direct hemolysin (TDH) family, and the thermostable hemolysin (δ-VPH) family. For some *Aeromonas* spp., however, the hemolysin/cytolysin termed aerolysin plays an important role in virulence and infection (**see also Blood**: Cellular Composition of the Blood and Erythropoiesis in Fishes).

Aerolysin

The conformation of the aerolysin protein, its secretion, and mode of action on host cells have been well characterized. The toxin is initially transported out of the cell as an inactivated protoxin known as proaerolysin. Following secretion, the enzyme is activated by proteolysis of the C-terminal, either by host proteases or by those secreted by the pathogen. Once activated, the protein binds to the eukaryotic host receptor glycophorin where it oligomerizes to form a ring-like structure that creates a pore in the host plasma membrane, eventually leading to permeabilization and cell death.

Lipases

Host cells may be destroyed via the hydrolysis of cytoplasmic membrane phospholipids. In particular, the glycerophospholipid:cholesterol acyltransferase (GCAT), which is produced by *Vibrio* spp. and *Aeromonas* spp., is a highly potent acyltransferase that hydrolyzes many different types of lipids. This lipase is secreted as a pro-toxin and is again activated via proteolytic cleavage. Studies have shown that virulent strains of *A. salmonicida* with a mutation in the serine protease are unable to activate the GCAT pro-toxin *in vivo*. Evidence also indicates that GCAT can contribute to membrane degradation and hemolytic activity alongside aerolysin. The 35-kDa lipase is produced predominately during the stationary phase of growth and acts in conjunction with lipopolysaccharides LPS (GCAT–LPS) to break down phospholipids, including glycerophospholipids and diacylglycerols. GCAT may associate with LPS to prevent proteolytic clearing of the acyltransferase by the host immune system. Injection of the GCAT monomer causes symptoms associated with tissue liquefaction and the formation of furuncles (**Figure 2**). In addition, injecting both the serine protease and GCAT can increase muscle liquefaction. Nevertheless, virulent *A. salmonicida* strains possessing a mutation in the GCAT gene showed no reduction in LD_{50}, suggesting that this lipase/phospholipase may only contribute slightly to virulence.

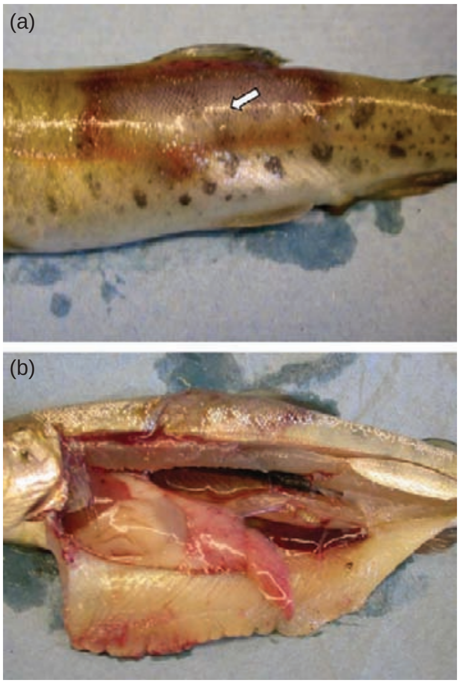


Figure 2 Rainbow trout showing signs associated with a typical *A. hydrophila* infection. (a) Furuncles (indicated by arrow) are swollen areas on the surface of fish and arise from underlying muscle and tissue liquefaction. (b) Exposed internal tissue shows extensive tissue degradation and liquefaction. Photographs: Courtesy of Springer Praxis Ltd and Dr A. Newaj-Fyzul.

Excretion Systems

Before the ECPs can be effective in virulence, they must first be transported across the bacterial outer membrane to the environment or into the fish cell. This is accomplished using large, membrane-bound transport systems, which are often composed of protein complexes. There have been six different types of transport systems identified to date (termed I–VI), although not all are associated with the transport of virulence factors in every organism.

Type I secretion systems (T1SSs) span the inner and outer membrane and are associated with Gram-negative bacteria. This system is important for virulence, in the export of both ECPs and molecules destined for the outer membrane. For example, *P. fluorescens*, which causes a general septicemia for fish, uses the T1SS to transport the adhesion factor LapA to the outer membrane. Type II secretion systems (T2SSs) can also transport ECPs to the environment or host cell cytosol in a two-stage process. Many bacterial fish pathogens use T2SSs for secreting ECPs including some *Aeromonas* spp. and *Vibrio* spp. For example, *A. hydrophila* is able to secrete a wide array of ECPs via T2SSs including a phospholipase, a DNase, a protease, and the well-characterized aerolysin.

Type III secretion systems (T3SSs) deliver proteins directly into the cytosol of host cells and consist of two large protein rings that span the inner and outer membrane. The OMP ring is connected with the large, needle-like structure that extends from the cell's surface to puncture host cells and allow the delivery of effector molecules. T3SSs are thought to aid adherence, invasion, and colonization, and may cause tissue damage, by inducing either apoptosis or necrosis. Mutational studies have shown that *E. tarda*, which causes edwardsiellosis in fish, uses a T3SS in establishing disease.

Type IV secretion systems (T4SSs) span the membrane envelope of Gram-negative bacteria which often possess pili and are involved in conjugation and horizontal gene transfer. This system may therefore enable mobile genetic elements, such as plasmids encoding virulence genes and genes associated with antibiotic resistance, to be transferred between pathogens. A number of fish pathogens use a type VI secretion system (T6SS) to translocate effectors into the host cell including some *Edwardsiella* spp., *A. hydrophila*, and *V. cholerae.* In addition to a T3SS, *E. tarda* contains a gene cluster called *evp*, which is associated with a T6SS. Mutations in these genes attenuated virulence in a gourami fish model, and some proteins encoded by this group of genes have since been studied in detail.

Bacterial Surface Molecules

Lipopolysaccharides

Although ECPs are secreted from the cell, many bacterial fish pathogens can possess toxins which are bound to the outer membrane known as endotoxins. A common class of endotoxin, which is found in *A. salmonicida*, *A. hydrophila*, *V. anguillarum*, *V. vulnificus*, and *E. ictaluri*, is LPS. This endotoxin is found in a number of animal pathogens, including those of higher and lower vertebrates. Although some fish pathogens may possess LPS, they are not endotoxigenic for fish and are merely a component of the outer membrane. LPS has a conserved chemical structure (**Figure 3**) consisting of three regions: a membrane-bound lipid A region, a variable polysaccharide core, and an outer polysaccharide region known as the 'O' antigen. In terms of pathogenicity, the lipid A region is immunogenic and is primarily responsible for elucidating a number of responses in fish. Although a toll-like receptor (TLR)-4 in mammals recognizes the conserved lipid A feature of LPS, the methods of recognition in fish have not been fully established. Nevertheless, recognition of LPS in fish can result in the production of pro-inflammatory cytokines, acute phase proteins (APPs), and antiviral type I interferons. This often leads to the stimulation of other immunological factors such as neutrophils, monocytes, and macrophages. In severe cases, the immune response can be overstimulated by LPS, eventually leading to septic (=endotoxic) shock. However, unlike higher vertebrates, fish are believed to be relatively resistant to this type of shock.

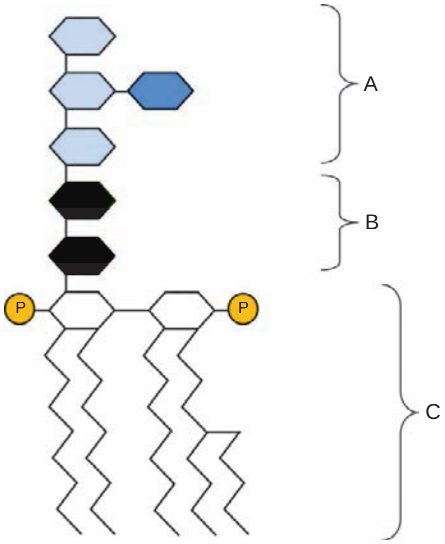


Figure 3 The three conserved structures of LPS. These features include the outer 'O' polysaccharide region (a), a variable polysaccharide core (b), and long-chain fatty acids linked to diglucosamine units which make up the conserved lipid A region(P = phosphate groups) (c). Adapted from Swain P, Nayak SK, Nanda PK, and Dash S (2008) Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: A review. *Fish and Shellfish Immunology* 25: 191–201.

Although in extreme cases endotoxic shock can lead to death, it is more likely to be associated with some physiological effects (e.g., increased respiratory rates, hypotension, and anorexia). However, this may only be an initial response to the LPS toxin (=hyper-responsiveness), with the second exposure stimulating a negligible response. Although a potential virulence factor, administering LPS in low quantities to fish has given cross-protection and enhanced immunity, suggesting that LPS may be a successful immunostimulant in aquaculture. Additionally, LPS may also contribute to bacterial adhesion to host tissue.

S-Layers

The surface layer (S-layer or A-layer) is an important structure for pathogenicity. This flat, paracrystalline surface protein covers the entire outer membrane surface of the pathogen, forming a sheet of tetragonal protein structures. In *A. salmonicida*, the S-layer is composed of large VapA subunits, whereas in *A. hydrophila* the S-layer is composed of a similar protein monomer called AhsA. The *VapA* gene from *A. salmonicida* encodes an ~51-kDa protein with a 21-amino-acid N-terminal sequence which is cleaved once the protein is transported out of the cytoplasmic membrane. This protein sheet is thought to protect the pathogen from multiple factors including phagocytic killing, serum borne factors, bacteriophage, and proteases. It also increases hydrophobicity and association with macrophages. *A. salmonicida* strains, which are unable to produce this S-layer, are subsequently avirulent, whereas VapA^- *A. hydrophila* are only slightly reduced in virulence, suggesting the involvement of other factors. S-layers also aid binding to host cell material (e.g., laminin and fibronectin) which helps maintain the carrier state of the organism.

Attachment and Colonization

There are a number of outer-membrane-associated molecules that aid binding and colonization. In addition to LPS and S-layers, polysaccharide capsules and slime layers (found in both Gram-negative and Gram-positive bacteria) can also facilitate adherence and invasion of host tissue. For example, *Lactococcus garvieae*, which causes a generalized septicemic condition termed 'lactococcosis' in marine and freshwater fish, produces capsules. Capsule-producing strains (termed KG–) were resistant to phagocytosis and more virulent *in vivo* compared to noncapsulated strains (termed KG+). Hence, the presence of a capsule aids attachment and prevents phagocytosis and serum killing.

Similarly, *Streptococcus iniae*, which causes meningoencephalitis in salmonids, possesses a capsule that prevents phagocytosis and attack from factors of the fish innate and humoral immune system. More virulent serotype II isolates of *S. iniae* produce a larger capsule which covers more bacterial surface antigens, thereby providing further protection from phagocytic attack. Serotype II isolates are able to survive within phagocytes, thus providing transport to the central nervous system (CNS) or can cross the blood–brain barrier as free bacteria in the bloodstream. Cells within phagocytes can also induce apoptosis which is thought to facilitate escape from the host cell and prevent inflammation. Although the capsule is important in immune evasion, surface proteins are able to bind trout immunoglobulins via the crystallizable fragment known as the Fc region.

Slime layers may aid *E. tarda* and *Flavobacterium psychrophilum* attachment to host cells. In *F. psychrophilum*, which causes coldwater disease and rainbow trout fry syndrome, slime layers may aid attachment and colonization as pili, fimbriae, or flagellin has not been observed. Pili, such as IV pili, are also used by *V. vulnificus* and *A. salmonicida* to achieve attachment. With regard to *V. vulnificus*, a mutation in the gene encoding a PilD peptidase (*vvpD*) resulted in the loss of pili and extracellular toxin secretion via a T2SS. Although this mutation caused an overall reduction in pathogenicity, it could not be determined if this was due to an absence of pili or loss of ECP excretion. Adhesins, like pili, are also used for adhesion and colonization. *A. hydrophila*, for example, possesses a 43-kDa adhesin termed AHA1 which selectively binds to L-fructose and D-mannose residues present on glycosylated OMPs of host cells. Adhesion mediated via AHA1 is thought to aid epithelial cell invasion.

Flagellin

In addition to being a PAMP, flagellin plays a significant role in motility, adhesion/colonization, and biofilm formation. For instance, *V. vulnificus* mutants possessing mutations in flagellum genes (*flgC* and *flg E*) have reduced motility, cellular adhesion, and cytotoxicity and increased LD_{50} values. Workers have suggested that a reduction in cytotoxicity is primarily due to reduced adhesion to host cells, thereby preventing the delivery of cytotoxins. As flagella can be associated with T3SS, a mutation in flagella genes could inhibit the secretion of toxins associated with this type of transport system, which has been the case for *Yersinia enterocolitica.* Given the rise in nonmotile pathogens (e.g., nonmotile *E. tarda*) causing disease in vaccinated fish, flagellin may be a topical research subject in the coming years.

Regulation of Virulence Factors

The expression of many bacterial virulence factors is regulated by a number of environmental cues, including temperature, pH, and osmolarity. Iron availability, however, is thought to regulate a number of virulence genes for many fish pathogens.

Iron Acquisition

As iron is not readily available for pathogens, measures must be taken to scavenge and sequester iron. For many pathogens, this is usually accomplished using both cellular and extracellular components which aid iron solubilization and transportation across the microbial membrane envelope. For example, *V. anguillarum*, which causes a hemorrhagic septicemia termed 'vibriosis', is able to sequester iron via a number of routes. *V. anguillarum* produces hemolysins/cytolysins which release hemoglobin from the host cell. These molecules can then be recognized on the surface of the pathogen through specific, iron-regulated outer membrane proteins which transports the heme component across the outer membrane and into the cytosol. The receptor protein and transport system is known as the TonB system and is primarily responsible for the utilization of heme from hemoglobin and hemin. In *V. anguillarum*, as with other *Vibrio* spp., two TonB systems (TonB1 and TonB2) function in transporting iron across the cytoplasmic membrane. Although both TonB1 and TonB2 systems transport heme and the siderophore ferrichrome, only TonB2 is involved in transporting the siderophores vanchrobactin, enterobactin, and anguibactin in some *V. anguillarum* strains.

Siderophores are small-molecular-weight proteins which are secreted by the bacterium and have a high affinity for iron. Essentially, these molecules compete with the host for iron and play an important role in establishing disease. For a number of *Vibrio* spp., including *V. anguillarum*, a number of siderophores can be produced. For example, *V. anguillarum* serovar O1 strains possessing a 65-kbp plasmid (pJM1) were 10^7 times more virulent than those in which the plasmid had been lost. Subsequent studies showed that this plasmid encoded genes involved in the transport and biosynthesis of the siderophore anguibactin. Although serovar O2 strains of *V. anguillarum* lack pJM1, and therefore the transport and biosynthesis of anguibactin, they are able to transport the siderophore vanchrobactin. Whereas mutations in either one of the two TonB systems results in reduced virulence, particularly the TonB2 system, double mutants (TonB1$^-$ and TonB2$^-$) are completely avirulent. Given the total lack of virulence when both systems are disrupted, it is clear that the ability to acquire iron is an important feature for pathogenicity.

Quorum Sensing

Although nutrient availability can regulate the expression of genes associated with virulence, bacteria must also control gene expression in response to the bacterial population or cell density. For this to be possible, bacteria must achieve cell-to-cell communication, a feature which is accomplished via quorum sensing. Quorum sensing involves the recognition of small signal molecules which are secreted by the bacterial cell. In particular, Gram-negative bacteria produce acylated homoserine lactones (AHLs) or autoinducers (e.g., AI-2) which are continuously produced by the organism and recognized on the surface of the outer membrane via specific, two-component receptors. Depending on the concentration of AHL or autoinducer, different genes (including those associated with virulence) will be expressed or repressed downstream.

A. salmonicida, *A. hydrophila*, *Vibrio* spp., and *Y. ruckeri* produce AHLs, suggesting a potential role for quorum sensing in regulating pathogenicity. However, not all fish pathogens produce these molecules. Some workers have suggested that gene expression with regard to quorum sensing is related to cell density, whereas others suggest that it is used to determine how quickly degradative proteins would diffuse away from the bacterium. *V. harveyi*, which causes necrotizing enteritis, eye disease, and luminous vibriosis, possesses a well-characterized quorum-sensing system. This organism uses three channels to regulate quorum-sensing-associated gene expression. The first channel is regulated by an acylated homoserine lactone (AHL), called harveyi autoinducer 1 (HAI-1), whereas the second and third channels are regulated by a furanosyl borate diester (AI-2) and cholerae autoinducer 1 (CAI-1), respectively.

For *V. harveyi* a number of virulence-related genes are regulated by this sensing mechanism including an extracellular toxin, a metalloprotease, siderophore production, and proteins associated with a type III secretion system. Studies indicate that the A1-2 and CAI-1 channels are required for virulence in brine shrimp (*Artemia franciscana*), whereas the HAI-1 is not. Therefore, given the importance of quorum sensing for *V. harveyi* and other fish pathogens, studies are now suggesting that this may be a novel target to prevent outbreaks of disease.

Virus Structures

Like bacteria, many different types of viruses are known to infect fish. Unlike bacteria, however, viruses are very small infectious particles (10–400 nm in diameter) which show a great deal of biological diversity. During their extracellular phase, viruses are unable to undergo division or replicate outside the host cell and possess no or few active enzymes. They also lack crucial genetic information required to generate metabolic energy or construct viral components (e.g., proteins). Therefore, viruses are dependent upon host metabolism and are often regarded as 'obligate intracellular parasites'.

Although virion structures are highly variable, they are generally composed of two to three parts including a viral genome (nucleic acid or NA) and a protein coat (capsid or capsule). In addition, some viruses may also have a lipid envelope which contains viral proteins.

Nucleic Acid

The function of viral NA is principally to encode viral enzymes and structural proteins. Unlike the genomes of cells, the composition and size of viral genomes are highly variable and are unique in that they are composed of DNA or RNA. The NA can also be double stranded (dsDNA or dsRNA) or single stranded (ssDNA or ssRNA). The genome may also be split (=segmented) and linear or circularized. For viruses with an ssDNA or RNA genome, strands are said to have a positive ((+) ssDNA or (+) ssRNA) or negative ((–) ssDNA or (–) ssRNA) polarity.

Protein Coat

The protein coat (or capsid) is in close association with viral NA. Both the NA and protein coat, when referred together, are known as the nucleocapsid. The main role of the coat is to maintain stability and protect fragile NA from chemical, physical, or enzymatic damage. The coat can also ensure that NA is effectively administered to the host for replication and may also aid attachment for nonenveloped viruses. Capsids are composed of multiple protein subunits (protomers) which are arranged under two major morphological types: helical and icosahedral.

Helical

This type of symmetry is associated with rod- or filamentous-like virus particles which have a single protomer type stacked around a central axis to form a helical-shaped cylinder. For viruses possessing a helical capsid, the genome is usually (−) ssRNA or (−) ssDNA and is bound to the inner coat via interactions with the positive charges on proteins. The total length of the helical capsid is dependent upon the length of the viral genome. Whereas a number of bacterial and plant viruses (notably the tobacco mosaic virus) lack an envelope, all vertebrate viruses with a helical coat possess a membrane. In addition, all animal viruses have a (−) ssRNA genome and exhibit a high level of similarity in their molecular structure.

Icosahedral

An icosahedron (isomeric) capsid is a regular polyhedron with 20 triangular faces, each one being made up of three protein subunits. Therefore, the smallest icosahedron is made up of 60 subunits (known as the triangulation number or $T = 1$). For the simplest icosahedron of 60 units, all triangular subunits have an identical shape with symmetry around a two-, three- and fivefold axis. Viruses with large genomes, however, require large icosahedral coats which are made up of multiples of 60 units ($T > 1$) and can form many complex shapes and structures.

Envelope

Some viruses, possessing a helical or icosahedral capsid, may have a lipid envelope which is sourced from host membranes. Envelopes can also have viral proteins which span the entire membrane. In this case, the extracellular domain is glycosylated and can have a number of functions including the recognition of host receptors, fusing with the host cytoplasmic membrane or aiding attachment to other virus particles.

Infection

As viruses are unable to replicate during the extracellular phase of the life cycle, the virus must be able to penetrate target host cells, begin viral synthesis, and escape the cell as fully functional virus particles. In general, this complex process can be considered in stages, although the process is often continuous.

Attachment

The initial attachment of a virus to the host cell usually involves the recognition of specific macromolecules (=receptors) present on the surface of the host cytoplasmic membranes. The presence or absence of these specific molecules determines if the target host cell is appropriate for replication of the virus, a feature often referred to as viral host tropism. Host cell receptors can include both proteins, particularly glycoprotein, and polysaccharides present on glycoproteins or glycolipids. Whereas glycoproteins are believed to be relatively specific receptors, carbohydrates are thought to be less specific as many different glycoproteins may share carbohydrate side chains with similar configurations. In some cases, multiple receptors must be recognized by the virus before attachment to the membrane can be achieved. Although many different types of receptors exist, they can be broadly grouped into different classes including membrane-associated receptors, transmembrane transporters/channels, and immunoglobulin-like superfamily molecules.

Penetration and Uncoating

Once bound to the membrane, the virus or viral NA must be transported across the membrane and into the host cytosol before replication can occur. Generally, although some viruses may directly inject viral NA, viral transportation across the membrane can be achieved using three different processes: translocation, endocytosis, and membrane fusion.

Unlike other methods of viral transportation across the membrane, translocation requires energy and involves the transportation of the entire virus across the cytoplasmic membrane and into the cytosol. In this case, the receptor on the cytoplasmic membrane is a transporter which recognizes and binds to the capsid protein of nonenveloped viruses, resulting in the active uptake of the entire virion. Unlike translocation, however, receptor-mediated endocytosis is a common form of viral uptake. This process involves the attachment of viruses on coated membrane pits which then pinch off to form coated vesicles filled with viruses. These vesicles can then fuse with lysosomes after the removal of clathrin, although virion or NA can escape before or after this process depending on the viral type. Both membrane and naked viruses may be taken up by receptor-mediated endocytosis, but again the method of escape from the endosome may vary depending on the virus. Nevertheless, the method of virus uptake by viral fusion is only associated with enveloped viruses. This involves the recognition of viral proteins by host receptors, eventually leading to the fusion of viral envelopes with the host cytoplasmic membrane and the release of the nucleocapsid into the host cytosol.

Regardless of the methods used for penetration, the protein coat must be completely or partially removed before the viral NA is exposed. This process is poorly understood and is thought to vary depending on the virus and nucleocapsid structure, although it may involve the action of viral or host proteases.

Viral Replication

Following the administration of genetic material, the next stage includes the hijacking of cellular processes and the production of functional messenger RNA (mRNA), which is essential for both protein production and genomic replication, with the exception of (+) ssRNA viruses which can be directly translated without further modifications. As viruses differ in the nature of their genetic material, a number of steps must be taken to convert different types of NA to mRNA. Moreover, viruses can be classified depending on mRNA production, a feature known as the Baltimore classification system.

This classification system places viruses under one of several groups (I–VI) depending on the features of mRNA synthesis and the genome. This includes whether the NA is DNA or RNA, single or double stranded, the intracellular location of NA replication, the involvement of a reverse transcriptase, and the polarity (+ or −) of ssRNA. Nevertheless, viruses lack the ability to produce proteins or replicate NA and therefore make use of host amino acids, transfer RNA (tRNA) and ribosomes.

Assembly

Following the replication of NA and production of viral protein, the virus must be assembled before it can be released. The site of viral assembly is dependent upon both the method of NA replication and the method of release from the host cell. Both the structure and assembly of the virus are predetermined, and are usually independent of the formation of covalent bonds. Other methods of interactions, such as protein–protein, protein–NA, and protein–lipid interactions, are important for virus assembly. Once assembled, however, the virus must go through maturation. This is the process by which the virus becomes infectious and usually involves the activity of viral proteases or cellular enzymes, which result in major conformational changes in capsid proteins. Following maturation, the virus may use one of two ways to escape the cell. If the virus is a nonenveloped particle, then it can leave the cell through lysing the cytoplasmic membrane. However, if the virus possesses a lipid envelope then it may escape through budding (**Figure 4**), a process by which the virus effectively obtains the outer membrane as it is released. It is also important to note that other intracellular membranes may be the source of the envelope, including the nucleus, endoplasmic reticulum, and Golgi apparatus.

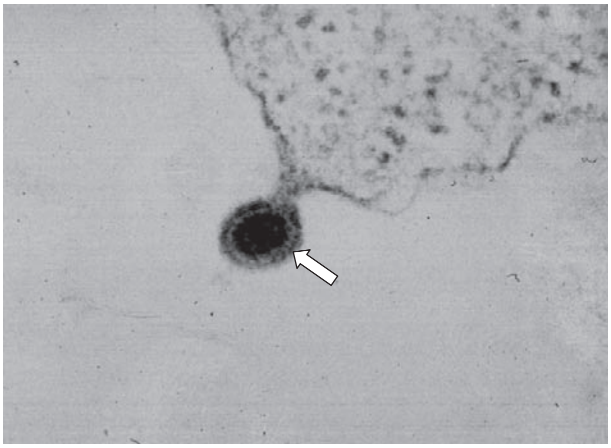


Figure 4 A retrovirus budding from a host plasma membrane. Viral proteins present on the surface of the virion coat are also visible (indicated) (magnification ×98 000). Reproduced with kind permission of Springer Science and Business Media.

Fish Viruses

Like bacteria, many fish viruses are known to affect fish in aquaculture and many aspects of virulence have been studied at the molecular level for many different viruses from various families. Some topical viruses, which are currently problematic for aquaculture, are discussed in the following.

Birnaviridae

Infectious pancreatic necrosis virus

Infectious pancreatic necrosis virus (IPNV) has been extensively studied at the molecular level and has been isolated from numerous fish species including salmonids (e.g., Atlantic salmon) and nonsalmonids (e.g., eels, yellowtail, turbot, halibut, and striped bass). Depending on the viral dosage and the age of host, the disease can cause high mortalities and give varying symptoms. In general, the virus causes a disease which is characterized by lesions in the pancreas (particularly for salmonids) and can also include infected kidney macrophage, gut epithelial cells, and liver. An example of the cytopathic effect (CPE) caused by IPNV on infected Chinook salmon embryo cells (CHSE-14) is illustrated in **Figure 5**.

IPNV belongs to the genus *Aquabirnavirus* and is biochemically similar to the *Drosophila* X virus (DXV) and infectious bursal virus (IBDV) of chickens. IPNV is a nonenveloped, spherical icosahedron (60 nm in diameter) (**Figure 6**) which possesses an RNase-resistant dsRNA genome ranging from 2.3 to 3 kb. As the NA is dsRNA,

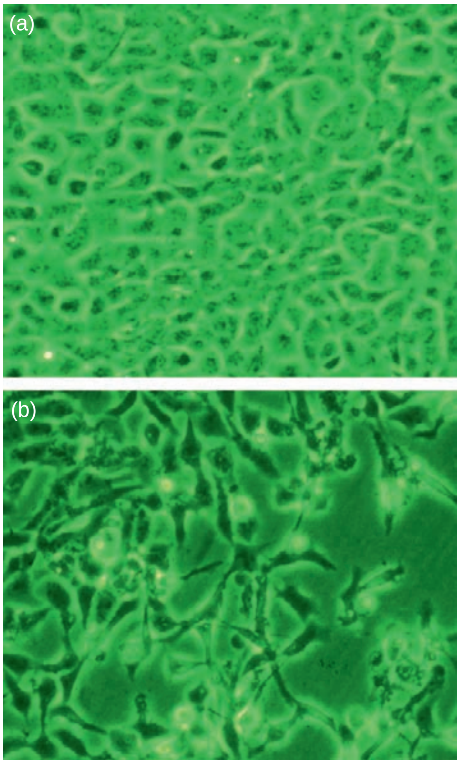


Figure 5 An example of the cytopathic effect (CPE) on cells infected with IPNV. (a) Growth of normal epithelioma papillosum cyprini (EPC cell line) (magnification ×10). (b) CHSE-14 cell line infected with IPNV showing significant changes in cell morphology (magnification ×10).

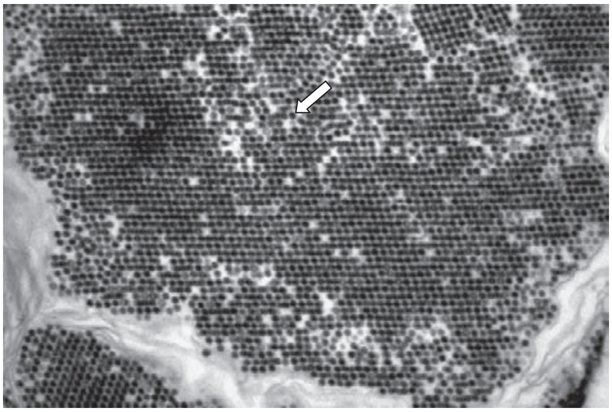


Figure 6 A rotifer cell showing an infection with IPNV. Each spherical object (indicated) represents a single icosahedral IPN virion which forms large virus arrays within the host cell (magnification ×98 000).

members of the Birnaviridae are classified as type III viruses in the Baltimore classification system. The genome is composed of two segments: segment A and segment B, with different strains showing a similar genomic arrangement. Segment A possesses a large open reading frame (ORF) encoding a polyprotein of 106 kDa which is cleaved by viral proteases to produce preVP2 and VP3. VP3 is thought to be an internal capsid protein, although studies suggest that at least part of the molecule is exposed at the surface. The preVP2 undergoes further proteolytic cleavage later in the viral process to give VP2. A second, smaller ORF also exists in segment A, and encodes a small (17 kDa) arginine-rich protein, the function of which remains unknown. Segment B, however, encodes the 94.4-kDa viral RNA-dependent RNA polymerase (RdRp) termed VP1. This protein can exist within two forms; either as a free polypeptide or as a genome-linked protein (Vpg) which is covalently attached to the 5′ regions of RNA.

The infection process is relatively well understood and has been studied both *in vivo* and *in vitro*. Replication occurs primarily at low temperatures and ceases at higher temperatures (>28 °C) when replicated in fathead minnow cells. Following entry into fish via oral or anal routes, the virus attaches to a 100–200-kDa protein receptor present on fish cell membranes via the VP2 capsid protein, resulting in internalization of the virus. However, it is unknown if the virus undergoes uncoating of the capsid as the viral RNA polymerase is still fully functional when the virus is encapsulated. Nevertheless, the genome can be synthesized *in vitro* via VP1-primed, semi-conservative, strand displacement transcription. In this case, VP1 has a dual functioning role, acting both as a primer and as an RNA polymerase to produce plus (+) RNA replicative intermediates. Gene expression, protein synthesis, and the roles of viral proteases (particularly with respect to polyprotein processing) have also been studied.

Orthomyxoviridae

Infectious salmon anemia virus

Infectious salmon anemia virus (ISAV) causes infectious salmon anemia (ISA), a disease which is characterized by exophthalmia, intestinal hemorrhaging, and tubular and liver necrosis, although fish may remain asymptomatic. Infection and transmission occur primarily in seawater, suggesting that the virus infects salmonids during the marine phase of the lifecycle. Horizontal transmission of virus between fish is believed to be primarily through urine and feces, with lice having been implicated as carriers.

ISAV belongs to the genus *Isavirus* and is the only fish virus belonging to the Orthomyxoviridae family. This virus also shares a number of biochemical similarities with the influenza A virus. Although ISAV proteins exhibit relatively low amino acid sequence identity to other members of the Orthomyxoviridae, genomic hybridization with conserved probes confirmed that this virus was correctly placed within this family. Viral particles are enveloped (90–140 nm) and possess a (–) ssRNA genome that is ~14.3 kb in size and composed of eight ssRNA segments. ISAV is classified as a type V virus in the Baltimore classification system.

The virus can survive in seawater for at least 20 h (4 days at 6 °C in blood and kidney tissue) and is sensitive to lipid solvents and low pH. All eight genomic ssRNA sequences have been sequenced and found to encode a putative PB2 and hemagglutinin, whereas expression studies have indicated at least nine structural proteins and one nonstructural protein, which are encoded within the genome.

A well-studied feature of ISAV is the dual-functioning hemagglutinin esterase (HE). This enzyme is a hemagglutinin-binding receptor which aids attachment to host cell material present on the surface of cells, primarily sialic acids (Sias). During attachment, virus particles may incorrectly bind to Sias which are not associated with cells, or with Sias associated with previously infected cells, resulting in a reduction in infection rates. Should this occur then the receptor can act as an esterase (siliate-O-acetyltransferase), otherwise referred to as a receptor-destroying enzyme (RDE), and detach from the bound molecule by acetylating the Sias. Once the virus attaches to the correct Sias, the virus is internalized. Following internalization, the virus envelope fuses with the host endosome membrane and is thought to replicate within host cells in a manner that is not dissimilar to the closely related influenza virus.

Togaviridae

Salmon alphaviruses

Salmon alphaviruses SAVs are known to be pathogenic for salmonids including Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). A number of virus subtypes (termed SAV 1, 2, and 3) have been associated with a variety of diseases. SAV 1 (salmon pancreas disease virus (SPDV)) and SAV 3 (which is specific to Norway) are associated with salmon pancreatic disease (PD), whereas SAV 2 (sleeping disease virus (SDV)) is linked with sleeping disease (SD).

Based on physical characteristics and genome sequences, the viral agent was placed within the genus *Alphavirus* and Togaviridae family. This spherical, enveloped virus has a (+) ssRNA genome which is 11–12 kb in length and shows relatively high levels of identity between SAV 1 and SAV 2 subtypes. SAVs belong to the type IV class of viruses within the Baltimore system. The genome is composed of two ORFs and encodes five structural and four nonstructural proteins. Structural proteins are encoded within the 3′ region of the genome and include the outer membrane glycoproteins (E2, E3, and E1) and capsid, whereas the 5′ region encodes proteins associated with viral replication (e.g., the viral RNA polymerase). Amino acid identities between SAV 1 and SAV 3 are high for both structural (95%) and nonstructural proteins (93.6%), but relatively low between other SAV strains (30% and 40%). Differences in overall protein structures and posttranslation modifications have also been documented between SAV 1 and SAV 2. Once inside the host cell, the virus can replicate within the cytoplasm and is released via budding through the plasma membrane. This budding process forms the viral envelope that possesses the E1 and E2 glycoproteins which form heterodimers on the membrane surface.

Rhabdoviridae

A number of viruses pathogenic for fish are grouped within the Rhabdoviridae family, including infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), and spring viremia of carp virus (SVCV).

IHNV infects Atlantic salmon in aquaculture, whereas rainbow trout are known to be susceptible in the environment. The disease is characterized by necrosis of the anterior kidney, including necrosis of the hematopoietic kidney tissue and pancreas. However, this disease can be distinguished from VHSV infection in that fish with IHNV show necrosis of the eosinophilic granular cells of the intestinal wall. Infection has been shown to occur through the gills and esophagus epithelium, resulting in a systemic viremia where they spread to the vital organs, causing a necrosis which can often result in death. VHSV (or Egtved virus) is an important viral disease in aquaculture, particularly for salmonids. This virus causes a viral hemorrhagic septicemia (VHS) and is characterized by hemorrhaging in the muscles, eyes, skin, and meninges; necrosis of internal organs (particularly the kidney and liver); exophthalmia; lethargy; and darkening of the skin. SVCV (=*Rhabovirus carpio*) also poses as a threat to carp (particularly *Cyprinus carpio*) and causes numerous symptoms including exophthalmia, skin hemorrhaging, hemorrhagic enteritis, and ascites.

All three members of the Rhabdoviridae discussed earlier are membrane-bound, bullet-shaped viruses (**Figure 7**) which have a nucleocapsid containing a (–) ssRNA genome that is ~11 kb in length. Members of the Rhabdoviridae belong to the type V class of viruses in the Baltimore classification system. The genome is linear and encodes six proteins for IHNV and VHSV, but five proteins for SVCV. Interestingly, the genome composition is somewhat similar for VHSV and IHNV, whereas the SVC genomes vary slightly. As a result, VHSV and IHNV have been re-classified within the genus *Novirhabdoviridiae*, whereas SVC is classified within the genus *Vesiculovirus*. All three genomes, however, encode similar proteins including an outer G protein, an RdRp, a nucleoprotein, a membrane protein which maintains viral shape, and a protein which aids transcription. The G protein, which initiates endocytosis during infection, has been studied

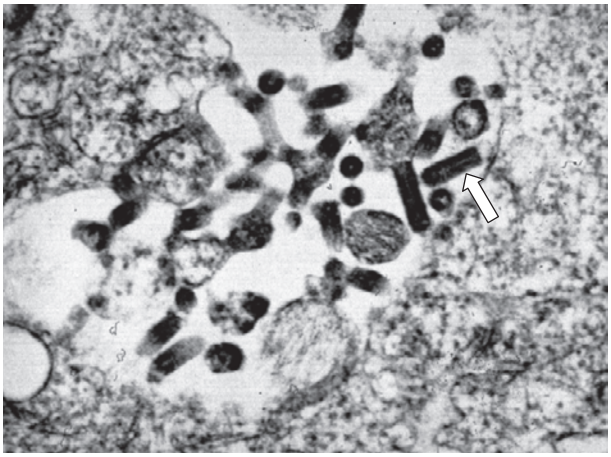


Figure 7 Kidney tissue from Atlantic salmon infected with VHSV. A fully formed, bullet-shaped VHS virion (180 nm in length) is clearly visible (indicated) (magnification ×59 000).

with regard to recombinant vaccine development. Viral replication has also been studied *in vitro*, as has the conditions and mode of spread required for infection.

Orthoretroviridae

Walleye dermal sarcoma virus (WDSV) and walleye epidermal hyperplasia virus 1 and 2 (WEHV1 and WEHV2) are of particular interest not only with regard to fish pathology, but also in understanding oncogenesis and cell proliferation. WDSV causes benign skin lesion in vascularized tumors, whereas WEHV causes a hyperproliferative skin disease which forms plaques of thickened epidermis. Belonging to the retroviruses, these closely related species cause seasonal proliferative skin lesions. For example, during the summer, autumn, and winter seasons, tumors and lesions continue to develop, whereas the level of spliced and unspliced retrovirus RNA remains relatively low. However, during the spring, these lesions regress, but virus replication and infectivity dramatically increase. Studies to date suggest that walleyes do not contract the condition in subsequent years, nor have there been any cases where WDS or WEH has caused mortality.

The genomes of WDSV and WEHV (1 and 2) have been studied at the molecular level. They possess a number of ORFs, including Orf C, B, and A. The WDSV Orf-C protein has been shown to induce apoptosis in cell culture, a factor which may be related to regressing tumors. Both Orf A and Orf B are somewhat similar, with Orf A encoding a retroviral cyclin (rv-cyclin) which shows some identity to cellular C-type and D-type cyclins. Extensive work has been undertaken to try and fully understand the role of rv-cyclin in the viral cycle. Furthermore, WDSV and WEHVs cyclins in walleyes infections may act as a model to fully understand oncogenic properties of cyclins in human cell replication and oncogenesis.

Methods of Control

General control methods for fish pathogens can include movement restrictions (with or without a culling policy), disinfection of ponds and utensils, nonspecific immunostimulants (e.g. β-1,3 glucans), dietary supplements such as vitamins, and the use of genetically disease-resistant stock. In addition, more specific methods (e.g., vaccines) have been developed for both bacterial and viral pathogens.

Bacterial Control

Although antimicrobial compounds (e.g., oxytetracycline) have been used for many years to prevent bacterial disease outbreaks, this is a contentious issue as it allows the development and spread of antibiotic resistance genes. Molecular approaches have been used with some vaccine development, although the bulk of the commercial fish vaccines uses traditional approaches, notably the formalin inactivation of broth cultures. These can be administered by intraperitoneal injection, immersion (the fish are bathed in a diluted preparation), or orally on feed. More modern approaches include the development of DNA vaccines and live, attenuated genetically engineered vaccines (e.g., the aromatic-dependent (*aro*) cells of *A. salmonicida*). However, there is debate over the value of live vaccines because of concerns about reversion to virulence and possible issues with diagnosis, that is, there could be confusion between live vaccine cells and virulent pathogen.

Viral Control

In a similar fashion to bacteria, the development of virus vaccines has been done using attenuated and recombinant vaccines. Subunit vaccines for IPNV produced by recombinant DNA techniques have involved the major capsid protein, VP2, with the gene being cloned and expressed in *Escherichia coli.* Vaccination by intraperitoneal injection and immersion led to the development of antibody. As a component in a multivalent vaccine, VP2 led to a suppression of viral replication in Atlantic salmon following challenge. IHNV has also been another target for recombinant vaccine development. For example, fragments of the G (glycoprotein) genes of IHNV have been cloned into *E. coli.* Plasmid-encoded viral epitopes were then mobilized into an avirulent isolate of *A. salmonicida* A440 for use as a shuttle system for the expression of glycoprotein fragments. This resulted in rainbow trout developing immunity against IHNV. Plasmid vectors encoding viral nucleoprotein or glycoprotein genes under the control of a cytomegalovirus promoter have been used to vaccinate rainbow trout, which received the glycoprotein with or without the nucleoprotein encoding plasmid, produced IHNV-neutralizing antibodies, and were protected against challenge. Protective immunity was transferred from hen fish to fry, which survived challenge. A third example involves VHSV. For this virus, a 2-propiolactone-inactivated preparation and attenuated vaccine were effective in providing protection against this virus in rainbow trout. When applied by intraperitoneal injection, a recombinant baculovirus vaccine was immunogenic and protective, albeit expensive. DNA-based vaccination using VHSV G (glycoprotein) and N (nucleocapsid) proteins, which were cloned individually into an expression plasmid, has been successful. Following injection of G and luciferase genes into rainbow trout muscle, there was a rapid decrease in luciferase activity, indicating that the fish developed a cellular immune response to the antigen. The transfected fish cells were killed, thereby stopping further expression of the G protein and luciferase. Juvenile rainbow trout, which were injected intramuscularly with the G construct, were protected against challenge after 52 days and exhibited viral neutralizing activity within serum.

Conclusions

Aquaculture continues to be hindered by disease despite attempts to understand the molecular basis of fish pathogenicity. However, this field has seen some dramatic breakthroughs within the last 20 years, although some questions still remain unanswered. For example, the methods used by some bacteria (e.g., *Y. ruckeri*) to evade serum killing and survive host defenses are still unclear. Equally, questions still exist over the role of some bacterial macromolecules, such as ECPs (e.g., the metalloproteases of *V. vulnificus*) in causing disease. Furthermore, some processes of viral replication (including uncoating of the viral coat) are still relatively unknown, as are some neutralizing epitopes (e.g., IPNV) which could be used in recombinant vaccine development. Nevertheless, future advancements in NA sequencing, protein sequencing, and *in vivo* analysis will undoubtedly expanded the field of molecular fish pathology.

Acknowledgment

Many thanks to Fiona Muir, Institute of Aquaculture, Stirling University for kindly providing the figures.

See also: **Blood**: Cellular Composition of the Blood; Erythropoiesis in Fishes. **Gut Anatomy and Morphology**: Pancreas.

Further Reading

Agnew W and Barnes AC (2007) *Streptococcus iniae*: An aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology* 122: 1–15.

Ahne W, Bjorklund HV, Essbauer S, *et al.* (2002) Spring viremia of carp (SVC). *Diseases of Aquatic Organisms* 52: 261–272.

Austin B, Altwegg M, Gosling PJ, and Joseph S (eds.) (1996) *The Genus Aeromonas*. Chicester: John Wiley and Sons Ltd.

Austin B and Austin D (2007) *Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish*, 4th edn. Weilheim: Springer-Praxis Publishing Ltd.

Balcázar JL, de Blas I, Ruiz-Zarzuela I, *et al.* (2006) The role of probiotics in aquaculture. *Veterinary Microbiology* 114: 173–186.

Brémont M (2005) Reverse genetics on fish rhabdoviruses: Tools to study the pathogenesis of fish rhabdoviruses. *Current Topics in Microbiology and Immunology* 292: 119–141.

Cann AJ (2005) *Principles of Molecular Virology*, 4th edn. San Diego, CA: Academic Press.

Chinchar VG (2002) Ranaviruses (family Iridoviridae): Emerging coldblooded killers. *Archives of Virology* 147(3): 447–470.

Chinchar VG, Hyatt A, Miyazaki T, and Williams T (2009) Family Iridoviridae: Poor viral relations no longer. *Current Topics in Microbiology and Immunology* 328: 123–170.

Defoirdt T, Boon N, Sorgeloos P, Verstraete W, and Bossier P (2008) Quorum sensing and quorum quenching in *Vibrio harveyi*: Lessons learned from *in vivo* work. *International Society for Microbial Ecology* 2: 19–26.

Dobos P (1995) The molecular biology of infectious pancreatic necrosis virus (IPNV). *Annual Review of Fish Diseases* 5: 25–54.

Feranández L, Méndez J, and Guijarro JA (2007) Molecular virulence mechanisms of the fish pathogen *Yersinia ruckeri*. *Veterinary Microbiology* 125: 1–10.

Fivaz M, Abrami L, Tsitrin Y, and Gisou van der Goot F (2001) Not as simple as punching a hole. *Toxicon* 39: 1637–1645.

Gulig PA, Bourdage KL, and Starks AM (2005) Molecular pathogenesis of *Vibrio vulnificus*. *The Journal of Microbiology* 43: 118–131.

Hoffmann B, Beer M, Schu-tze H, and Mettenleiter TC (2005) Fish rhabdoviruses: Molecular epidemiology and evolution. *Current Topics in Microbiology and Immunology* 292: 81–117.

Jacobs JM, Stine CB, Baya AM, and Kent ML (2009) A review of mycobacteriosis in marine fish. *Journal of Fish Diseases* 32: 119–130.

Jones MK and Oliver JD (2009) *Vibrio vulnificus*: Disease and pathogenesis. *Infection and Immunity* 77(5): 1723–1733.

Kibenge FSB, Munir K, Kibenge MJT, Joseph T, and Moneke E (2004) Infectious salmon anemia virus: Causative agent, pathogenesis and immunity. *Annual Health Research Reviews* 5(1): 65–78.

Lemos ML and Osorio CR (2007) Heme, an iron supply for *vibrios* pathogenic for fish. *Biometals* 20: 615–626.

Lliev DB, Roach JC, Mackenzie S, Planas JV, and Goetz FW (2005) Endotoxin recognition: In fish or not in fish? *FEBS Letters* 579(29): 6519–6528.

López CS and Crosa JH (2007) Characterization of ferric-anguibactin transport in *Vibrio anguillarum*. *Biometals* 20: 393–403.

Lorenzen N, Lorenzen E, Einer-Jensen K, and LaPatra SE (2002) DNA vaccines as a tool for analysing the protective immune response against rhabdoviruses in rainbow trout. *Fish and Shellfish Immunology* 12(5): 439–453.

Mohanty BR and Sahoo PK (2007) Edwardsiellosis in fish: A brief review. *Journal of Biosciences* 32(7): 1331–1344.

McLoughlin MF and Graham DA (2007) Alphavirus infections in salmonids – a review. *Journal of Fish Diseases* 30: 511–531.

Nematollahi A, Decostere A, Pasmans F, and Haesebrouck F (2003) *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases* 26: 563–574.

Noonan B and Trust TJ (1995) The molecular biology of *Aeromonas salmonicida*. *Annual Review of Fish Diseases* 5: 95–111.

Roberts RJ (ed). (2009) *Fish Pathology*, 3rd edn, London: Harcourt Publishers Ltd.

Skall HF, Olesen NJ, and Mellergaard S (2005) Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. *Journal of Fish Diseases* 28: 509–529.

Swain P, Nayak SK, Nanda PK, and Dash S (2008) Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: A review. *Fish and Shellfish Immunology* 25: 191–201.

Toranzo AE, Magariños B, and Romalde JL (2005) A review of the main bacterial fish diseases in mariculture systems. *Aquaculture* 246: 37–61.

Vendrell D, Balcázar JL, Ruiz-Zarzuela I, *et al.* (2006) *Lactococcus garvieae* in fish: A review. *Comparative Immunology, Microbiology and Infectious Diseases* 29: 177–198.

Whittington J, Becker JA, and Dennis MM (2010) Iridovirus infections in finfish – critical review with emphasis on ranaviruses. *Journal of Fish Diseases* 33(2): 95–122.

Zhang X-H and Austin B (2005) Haemolysins in *Vibrio* species. *Journal of Applied Microbiology* 98: 1011–1019.

Germ Cell Migration and Trans Sex

M Kinoshita, Kyoto University, Kyoto, Japan

© 2011 Elsevier Inc. All rights reserved.

What Is a Germ Cell?
How Specified Are PGCs?
Specific Markers for PGCs
When Is a PGC Specified?
PGC Migration
Why Do PGCs Migrate?
High Plasticity in Sex Determination of Fish
What Is a Trans Sex Phenomenon?
When Do Fish Change Sex?
Plasma Steroids Play a Critical Role in Sex Change
Further Reading

Glossary

Aromatase Enzyme belonging to the cytochrome P450 family of proteins and plays a key role in the biosynthesis of estrogens through metabolism of androgens such as testosterone.

Green fluorescent protein (GFP) This protein is originally cloned from jellyfish. When it is excited with blue light it exhibits green fluorescence. GFP is one of the most popular reporter proteins in the bio-imaging field because it can be detected without sacrificing cells and organisms.

Hermaphroditism Sexual pattern characterized by individuals that function both as males and females either simultaneously or sequentially.

Medaka A small freshwater teleost fish that lives in temperate zones and, along with zebrafish, is an excellent vertebrate model organism. Its genomic DNA has been fully sequenced.

Nuage An electron-dense cytoplasmic inclusion that is usually observed in association with mitochondria in germ cells. Nuage has been recognized as a marker of a germ-cell line since the early days of germ-cell study. It consists of an aggregation of RNA and proteins that are thought to be essential for the specification of a germ cell. Nuage has been referred to as a germinal-dense body, a nucleolus-like body, and a chromatoid body. Functionally, nuage resembles the pole plasm of an insect and the germ plasm of frogs.

Pluripotent epiblast cell A type of cell derived from an inner cell mass that can differentiate into any cell type except for that comprising extra embryonic tissue. Conversely, a totipotent cell can differentiate into all cell types, including extra embryonic tissue.

Primordial germ cell (PGC) The precursor of a germ cell, which will develop as a gamete; sperm or oocyte. Morphologically, a PGC is distinguished from a somatic cell by its large round shape, large nuclei, and presence of a nuage.

Totipotency The ability to differentiate into all types of cells in an organism.

Trans sex The phenomenon where an individual changes its sex during its lifetime.

Untranslated region (UTR) Messenger RNA (mRNA) contains a coding region, which is translated into protein, and regions that are not translated. If it locates in the 5′ region and 3′ region, it is termed 5′-UTR and 3′-UTR, respectively. Sometimes, 3′-UTR contains regulatory regions, where proteins and micro-RNA bind that can affect mRNA's stability, location, and translation efficiency.

Zebrafish A small freshwater teleost fish that originated from the tropical zone. Zebrafish, as well as medaka, is an excellent model organism for vertebrate development, due to the many kinds of artificial mutants and application of molecular biology techniques.

What Is a Germ Cell?

Among the many kinds of cell lineages, a germ-cell line is the only one that can provide the genetic link between generations and transmit genetic information to the next generation. In this sense, germ cells are an immortal cell line beyond generations, in contrast to a somatic cell lineage, which is destined to die after a fixed number of mitotic divisions, or disappear with the death of the organism.

A germ-cell line contains both male and female gametes, and their precursor cells. In the early stage of development, certain cells are specified as precursors of a germ cell (primordial germ cell (PGC)) and will develop as germ cells. After colonization in a gonad, PGCs differentiate into gametes (sperms or oocytes) by their interaction with the surrounding cells and/or environmental factors.

In most organisms, the specification of germ cells occurs in a specific position distinct from the final position where gonads will form. Thus, during the developmental process, germ cells (or PGCs) migrate to the gonadal anlage.

How Specified Are PGCs?

Germ cells are currently specified in two distinct ways. In many invertebrate and nonmammalian vertebrate species, including fish, cells that inherit asymmetrically localized, maternally provided determinants are specified as a PGC. So far, the determinants of a germ cell are not precisely identified in fish. In zebrafish, however, some maternally supplied materials, including *vasa* and *nanos* transcript, localize at cleavage furrows at the two- and four-cell stages. These materials are thought to be determinants of a germ cell because their removal results in a severe reduction in the number of germ cells.

In mammals, on the other hand, cytoplasmic determinants for germ cells have not been found, but induction signals from neighboring somatic cells before or during early gastrulation are thought to specify PGCs from pluripotent epiblast cells. BMP4 and BMP8b from extra-embryonic ectoderm are thought to be necessary in this process.

Specific Markers for PGCs

In early studies on PGCs in fish, PGCs were identified only by their morphological features because they have features that are distinct from somatic cells. PGCs are round and larger than other cells with large nuclei, and possess prominent nucleoli and a distinct nuclear membrane. Additionally, PGCs contain characteristic inclusions, nuage (also referred to as germinal-dense, nucleolus-like, or chromatoid bodies), which are electron-dense particles that can be observed in cytoplasm with an electron microscope (**Figure 1**). Nuage is often observed in association with mitochondria and is thought to be an aggregate of RNA and proteins. Because nuage is observed in many kinds of germ cells, such as oogonia, oocytes, spermatogonia, and spermatocytes, it is thought that nuage has essential roles in the development and/or maintenance of germline cells.

In *Drosophila*, a special cytoplasm, called pole plasm, is located in the posterior portion of the egg. During embryonic development, the pole plasm is required to establish anterior–posterior patterning and the specification of germ cells. RNA and proteins accumulate at the pole plasm and assemble in polar granules. Thus, nuage in fish shares resembling morphological features and is thought to share the same roles as pole plasm (or polar granules). The RNA molecules *vasa*, encoding an RNA helicase, and *nanos*, encoding zinc finger protein, are the components of polar granules. So far, their homologs have been found in several teleost fish, including zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), goldfish (*Carassius auratus*), rainbow trout (*Oncorhynchus mykiss*), and loach (*Misgurnus anguillicaudatus*). These homologs are found in germ cells in fish. Recently, it has been confirmed that the *vasa* homolog is located in nuage of zebrafish. Thus, *vasa* and *nanos* messenger RNA (mRNA) have been used as germ-cell-specific markers to elucidate the origin and migration route of germ cells in fish.

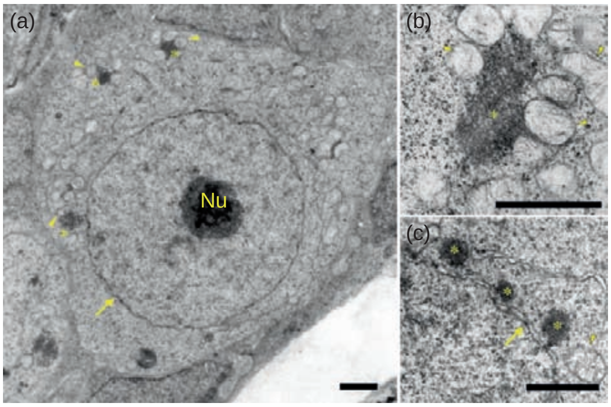


Figure 1 Nuage in medaka PGCs. Nuage is a characteristic inclusion in PGCs. Nuage is an electron-dense particle and is often observed with mitochondria. The asterisk represents nuage, arrowheads indicate mitochondria, arrows indicate nuclear membrane, and Nu indicates nucleolus. Bars represent 1 μm. These figures were kindly provided by Dr. Hamaguchi of Niigata University, Japan.

When Is a PGC Specified?

The utilization of germ-cell-specific molecules, such as *vasa* mRNA and *nanos* mRNA, and recent advances in molecular biology techniques reveal more precise information on the specification of PGCs.

Current knowledge shows that the precise period of PGC specification seems to vary in fish species, and that generally PGCs seem to be specified early in development. In zebrafish, a clump of *vasa* mRNA, a PGC marker, localizes at a cleavage furrow, not in the cytoplasm, until the eight-cell stage. It then localizes in the cytoplasm of four cells at the 32-cell stage, suggesting that at this stage PGCs are specified in zebrafish. The expression pattern of *vasa* in goldfish is identical to that of its confamilial zebrafish (Cyprinidae). In loach (*Misgurnus anguillicaudatus*), the specification pattern resembles that of zebrafish: *vasa* transcript remains at the cleavage furrow until the 32-cell stage and after the 64-cell stage each aggregate of the *vasa* transcript settles into a single blastomere. In contrast to the regional localization of *vasa* transcript in the zebrafish embryo, the transcript in medaka distributes uniformly until late gastrulation. However, using the 3′-untranslated region (3′-UTR) of *nanos*, another PGC-marker, green fluorescent protein (GFP) technology (see below) reveals that GFP-expressing cells scatter around the animal pole at the onset of gastrulation, which is the earliest time point recorded for the detection of PGCs in medaka to date.

PGC Migration

Several methods have been applied to trace PGC migration in a fish embryo. Early morphological observations used light and/or electron microscopy. Once PGC-specific molecular markers, such as *vasa* and *nanos* transcripts, were identified, whole mount *in situ* hybridization used these molecules as probes, making the tracking of the migration route of PGCs easier than morphological analysis. As GFP technology advanced, transgenic fish (**see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Transgenesis and Chromosome Manipulation in Fish and Growth Hormone Overexpression in Transgenic Fish) became subjected to this study. Usually, an artificial DNA construct, in which the GFP gene is placed between the promoter region and the 3′-UTR of the *vasa* gene, is introduced into fertilized eggs by microinjection. In this transgenic fish strain, germ cells, including PGCs, express the GFP gene. Such transgenic strains exist for medaka, rainbow trout, and zebrafish. Another GFP technology that is useful for tracking the migration route of PGCs is the RNA injection technique. The 3′-UTR of *vasa* and *nanos* mRNA involves signals that stabilize RNA and promote translation specifically in PGCs. The introduction of fusion RNA, containing a GFP gene followed by *vasa* or *nanos* 3′-UTR, into fertilized eggs by microinjection leads to the production of GFP, specifically in PGCs (**Figure 2**). With both GFP technologies, PGCs are labeled with green fluorescence and the most advantageous of these technologies is that the movement of PGCs can be traced in living organs in real time using fluorescent microscopy and without sacrificing embryos.

Although the details in the mode of migration of PGCs vary among fish species, a common theme exists for fish investigated to date. After PGCs are specified and segregated from somatic cells, they move to the marginal zone (epiboly stage). Then, PGCs move toward the dorsal midline and align along the lateral mesoderm (gastrula and neurula stage). Finally, the cluster of PGCs moves posteriorly along the body axis and reaches the gonadal primordium, where gonads will form and PGCs give rise to sperms and oocytes. The migration pathway of PGCs

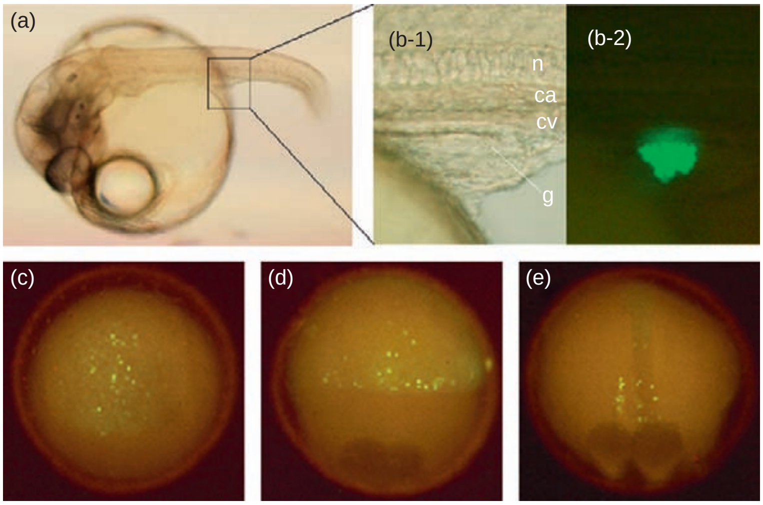


Figure 2 Imaging PGCs with GFP technology. There are two ways to visualize PGCs with GFP technology. One is to establish a transgenic strain that contains a GFP gene regulated by PGC-specific regulatory sequences. The other is the injection of mRNA containing a GFP-coding sequence and PGC-specific UTR. Transgenic medaka harboring an olvas-GFP gene, which contains the GFP gene and a regulatory region of medaka *vasa* gene (a, b). GFP-*nanos* 3′-UTR mRNA-injected embryos (c–e). Usually, mRNA injection enables the identification of PGCs in an earlier stage of embryogenesis. (a, b-1) Normal light images; (b-2, c–e) fluorescent images. (a) Stage 31; (c) animal pole of early gastrula stage corresponding to **Figure 5**(**a**); (d) dorsal view of mid-gastrula stage corresponding to **Figure 5**(**b**); (e) dorsal view of late gastrula stage corresponding to **Figure 5**(**c**). ca, caudal artery; cv, caudal vein; n, notchord; g, gut. (c–e) Modified from Kurokawa H, Aoki Y, Nakamura S, *et al*. (2006) Time-laps analysis reveals different modes of primordial germ cell migration in the medaka *Oryzias latipes. Development Growth Diferentiation* 48: 209–221.

has been relatively well investigated in zebrafish and medaka. In both species, there are two modes of PGC migration. One is passive movement according to somatic cell movement, such as extension and conversion; the other is active migration, as indicated by the following. PGCs migrate in a different direction to the surrounding somatic cells. Although PGCs locate randomly in each embryo, they finally settle together in the gonads. When PGCs migrate, pseudopodia are observed (**Figure 3**). In addition, a guide molecule, stromal cell-derived factor 1 (SDF1), has been found. SDF1 is expressed in somatic cells and indicates the direction where PGCs should migrate. PGCs express CXC chemokine receptor 4 (CXCR4), a receptor of SDF1, and respond to this chemoatractant, SDF1. This signal and receptor (SDF1/CXCR4) system is thought to be a master system of active migration of PGCs, as indicated by the following experiments: the disruption of either SDF1 or CXCR4 disturbs normal PGC migration, and ectopic expression of SDF1 leads PGC to a new SDF1 source. In the following section, recent knowledge obtained from zebrafish and medaka is described.

Kunwar and collaborators have summarized the zebrafish migration pathway and mechanism of PGCs. Once PGCs are specified at the 32-cell stage, mitosis is suppressed and the number of PGCs remains at 4 until the blastula stage (1000-cell stage). PGCs then divide several times and, at the dome stage, four clusters of PGCs randomly locate with respect to the body axes of the embryo. Two of the four clusters locate relatively close to the embryonic body and the other two locate some distance from the embryonic body. The clusters migrate along the embryonic margin toward the dorsal side (**Figure 4**(**a**)). At around 60% of the epibody stage, the cluster that reaches close to the midline then starts to move away from the midline (**Figure 4**(**b**)). Subsequent migration toward the gonadal primordium is active, with movement depending on the SDF1/CXCR4 chemokine system. As shown in **Figure 4**(**c**), the clusters that come close to the midline align at the border between the head and trunk mesoderm, and the other clusters, which come from the ventral part and locate some distance from midline, align at the lateral border of the mesoderm. They then move to an intermediate position, around the first somite of the embryo (**Figure 4**(**d**)). The more anteriorly located clusters migrate posteriorly, whereas posterior clusters migrate anteriorly along the mesoderm (**Figure 4**(**e**)). By 24 h postfertilization, most PGCs are assembled into a single cluster on each side and have reached the region corresponding to the eighth to tenth somites to form two gonads (**Figure 4**(**f**)). At this time, PGCs can be identified morphologically. In addition to SDF1, hydroxymethylglutaryl coenzyme A reductase (HMGCoAR) and Staufen-related proteins (an RNA-binding protein) are thought to influence PGC migration and/or survival.

Both Kurokawa and co-workers and later Saito have summarized the PGC migration and the formation of gonadal primordia for medaka. At the onset of gastrulation (stage 12), about 10 PGCs locate around the animal pole as scattered motile cells (**Figure 5**(**a**)). The active migration of these cells depends on the SDF1/CXCR4 system toward the marginal zone where SDF1 is expressed (stage 15: mid-gastrula stage; **Figure 5**(**b**)). During gastrulation, PGCs extend filopodia and

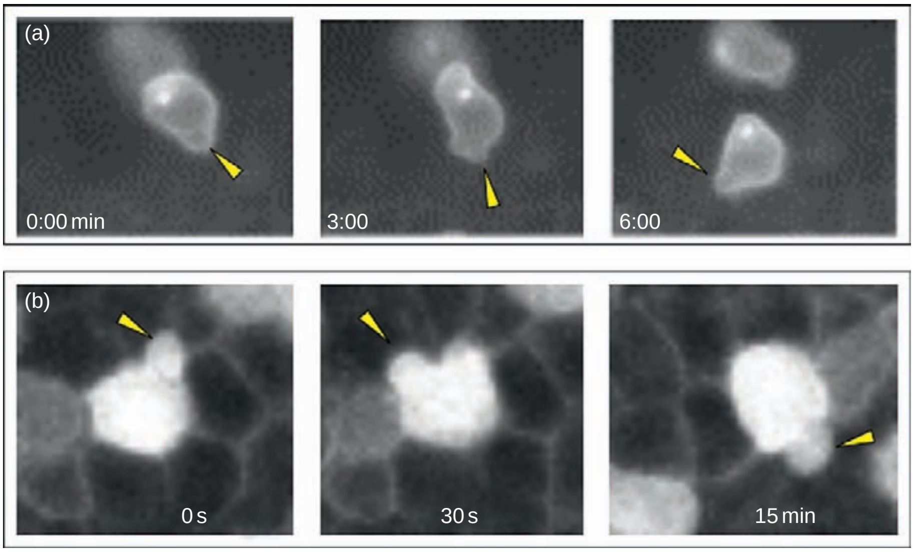


Figure 3 Pseudopodia in PGCs. Time-lapse images of moving PGCs of zebrafish (a) and medaka (b). Arrowheads indicate pseudopodia. (a) Modified from Raz E (2004) Guidance of primordial germ cell migration. *Current Opinion in Cell Biology* 16: 169–173 (review). (b) Modified from Kurokawa H, Aoki Y, Nakamura S, *et al*. (2006) Time-lapse analysis reveals different modes of primordial germ cell migration in the medaka *Oryzias latipes. Development Growth Diferentiation* 48: 209–221.

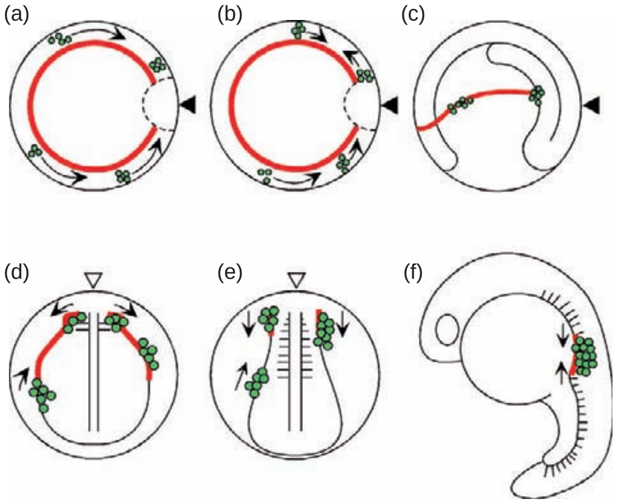


Figure 4 Scheme of PGC migration in zebrafish. Green particles represent PGCs; red line shows the expression of SDF1 (consult text for details). (a) Animal pole at dome stage; (b) animal pole at 60% epiboly stage; (c) lateral view at 80% epiboly; (d) frontal view of two-somite stage; (e) frontal view of eight-somite stage; (f) lateral view at 24-h postfertilization. Closed and open triangles represent dorsal and anterior, respectively. Reproduced from Weidinger G, Wolke U, Koprunner M, Klinger M, and Raz E (1999) Identification of tissues and patterning events required for distinct steps in early migration of zebrafish primordial germ cells. *Development* 126: 5295–5307.

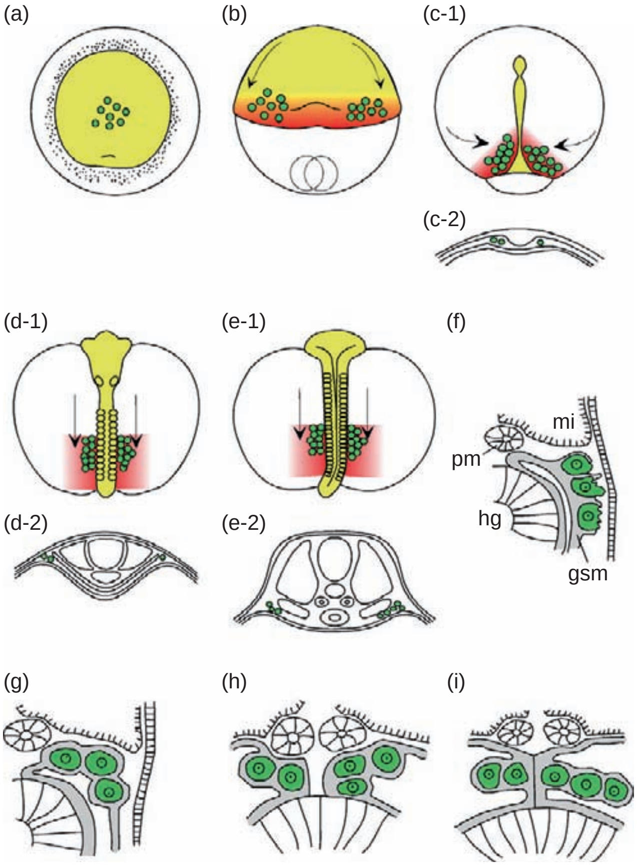


Figure 5 Schematic of PGC migration in medaka. Green particles represent PGCs; red parts represent the expression of SDF1 (consult text for details). (a) Animal pole at early gastrula stage (stage 12); (b) frontal view at mid-gastrula stage (stage 15); (c) frontal view and cross section at late gastrula stage (stage 17); (d) frontal view and cross section at 12-somite stage (stage 23); (e) frontal view and cross section at 23-somite stage (stage 26); (f–i) cross section at stage 30–35. hg, hindgut; gsm, gonadal somatic mesoderm; mi, miotome; pn, pronephros. Modified from Saito D (2009) Gonads. In: Kinoshita M, Murata K, Naruse K, and Tanaka M (eds.) *Medaka: Biology, Management, and Experimental Protocols*, pp. 247–252. Ames, IA: Wiley-Blackwell; and Hamaguchi S and Shinomiya A (2000) The establishment of germ line and their sex differentiation in the teleost. *Protein Nucleic acid Enzyme* 45: 2690–2698 (in Japanese).

lamellipodia and move faster than surrounding somatic cells. After staying in the marginal zone for few hours, PGCs migrate toward the midline and align along the lateral plate mesoderm (stage 17: early neurula stage; **Figure 5**(**c**)). In this stage, the PGCs seem to migrate coupled with neighboring somatic cells, and so this mode of movement is thought to be passive and caused by undergoing convergent movement. During migration from the animal pole to the lateral plate mesoderm (from early gastrula to early neurula stage), PGCs proliferate and increase in number to 30–40. As somitogenesis proceeds, PGCs actively migrate posteriorly along the body axis to catch up with the SDF1-expressing domain, depending on the SDF1/CXCR4 system, and arrive at the 11th–13th somite position where gonadal precursors are located (stage 26: 23-somite stage; **Figures 5**(**d**) and **5**(**e**)). Similar to zebrafish, HMGCoAR also seems to be involved in this migration process. In the gonadal primordium-forming process, PGCs approach the gonadal primordium and each PGC is segregated and enveloped by gonadal somatic mesoderm (**Figures 5**(**f**) and **5**(**g**)). PGCs and gonadal somatic cells come from the right and left side of the hindgut, come across at the dorsal side of the hindgut, and form a single gonadal primordium (**Figures 5**(**h**) and **5**(**i**)). Later, this single gonadal primordium divides into the right and left lobes.

Why Do PGCs Migrate?

There is currently no clear explanation as to why PGCs migrate, but it is possible to speculate as follows. During the developmental process, many kinds of cell types specialize and differentiate. This means that many kinds of differentiation-inducing factors are produced and secreted. In order to retain a state of nondifferentiation and totipotency, PGCs should avoid the effects of such factors, and so the cells might be waiting for the differentiation of the somatic gonadal premordium, where they finally settle, and so keep moving until this occurs.

High Plasticity in Sex Determination of Fish

PGCs differentiate into sperms or oocytes once they settle in the gonadal ridge, which develops into the testes or ovaries.

In vertebrates, there are two modes for the determination of sex: genotypic sex determination (GSD) and environmental sex determination (ESD). In GSD, individual sex is determined by whether or not the sex chromosome is inherited. In ESD, the sex of each individual is determined by the local environment, such as temperature.

Mammals and birds follow GSD. In mammals, the sex-determining gene, *sry*, has been identified and the individuals inheriting this gene become male.

In fish, the mode of sex determination depends on the species. Medaka follows GSD and its sex-determining gene, *dmY*/*dmrt1Y*, has been identified. Individuals that inherit *dmY*/*dmrt1Y* become male. In addition to medaka, some fish species have a sex chromosome and follow GSD. However, it is well known that sex differentiation can be altered by the treatment of high or low temperature and by steroid hormones in fish such as medaka, tilapia, salmonid, and flounder. Thus, these fish possess high plasticity in sex determination and differentiation, in contrast to the rigid sex-chromosome determination in mammals and birds. (As for sex determination, **see also Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular and Chromosomal Aspects of Sex Determination).

What Is a Trans Sex Phenomenon?

In this article, trans sex is defined as the phenomenon where an individual changes its sex during its lifetime. Trans sex has been scarcely observed in terrestrial vertebrates, but in fish it is known to occur in some teleosts, mostly coral fish, but not in the elasmobranchii (**see also Social and Reproductive Behaviors**: Socially Controlled Sex Change in Fishes).

Fish that can change sex are recognized as hermaphrodites. The trans sex phenomenon is caused by switching the functional gonad, from testis to ovary or vice versa. Trans sex in fish is recognized as sequential hermaphroditism and is divided into three types:

1. protogyny, fish that function as a female and later as a male;
2. protandry, fish that function as a male and later as a female; and
3. reciprocal sex change, fish that can change their sex reversibly.

In addition, there is simultaneous hermaphroditism, in which an individual can function as both male and female simultaneously. Fish species belonging to each type are listed in **Table 1**.

Sequential Hermaphroditism

Protogynous fish

This sex change type is common in fish and includes many species. Red porgy (*Pagrus pagrus*) has a bisexual gonad, in which a dorsal ovarian area and a ventral testicular area are separated by connective tissue (delimited type of gonad: **Figure 6**(**a**)). In the female phase, the ovarian part is dominant. As sex change proceeds, mature oocytes and

Table 1 List of sex change types in fish

Type of sex change		*Common name*	*Scientific name*
Sequential hermaphroditism	Protogyny	Harlequin sandsmelt	*Parapercis snyderi*
		Orange sea-perch	*Pseudanthias squamipinnis*
		Red hind	*Epinephelus guttatus*
		Honeycomb grouper	*Epinephelus merra*
		Red grouper	*Epinephelus morio*
		Sevenband grouper	*Epinephelus septemfasciatus*
		Saddleback wrasse	*Thalassoma duperrey*
		Threespot wrasse	*Halichoeres trimaculatus*
		Blue-headed wrasse	*Thalassoma bifasciatum*
		Multicolor fin rainbow fish	*Halichoeres poecilopterus*
		Stoplight parrotfish	*Sparisoma viride*
		Parrotfish	*Scarus psittacus*
		Yellowback sea bream	*Dentex tumifrons*
		Swamp eel, ricefield eel	*Monopterus albus*
		Red porgy	*Pagrus pagrus*
	Protandry	Black porgy	*Acanthopagrus schlegeli*
		Gilthead sea bream	*Sparus aurata*
		Striped sea bream	*Lithognathus mormyrus*
		Anemonefish	*Amphiprion* (genus)
		Common snook	*Centropomus undecimalis*
		Giant perch, barramundi	*Lates calcarifer*
		Flathead	*Platycephalus* sp.
		Ribbon eel, ribbon moray	*Rhinomuraena quaesita*
	Reciprocal sex change	Bluestreak cleaner wrasse	*Labroides dimidiatus*
		Red-head goby	*Paragobiodon echinocephalus*
		Okinawa rubble goby	*Trimma okinawae*
		Star-bambooleaf wrasse	*Pseudolabrus sieboldi*
		Rusty angelfish	*Centropyge ferrugata*
		Yellow hawkfish	*Cirrhitichthys aureus*
Simultaneous hermaphroditism		Hamlet	*Hypoplectrus* (genus)
		Mangrove killfish	*Rivulus marmoratus*
		Longnose lancetfish	*Alepisaurus ferox*
		Greeneyes	*Chlorophthalmus albatrossis*

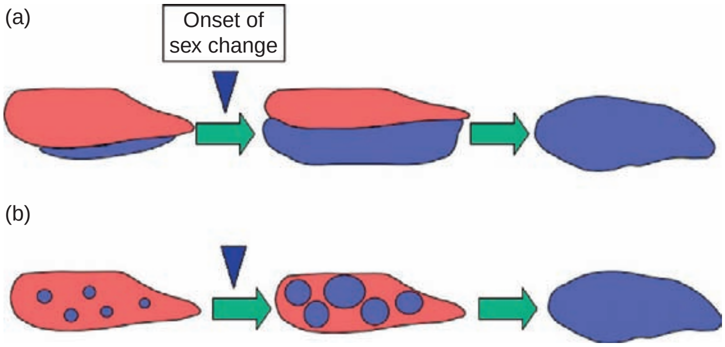


Figure 6 Schematic of two types of gonads observed in sequential hermaphroditism. (a) Delimited type. The ovary and testis are separated by connective tissue. In the first sex phase, a corresponding type of gonad (ovarian or testicular part) is dominant and functional (pink). As sex change proceeds, the dominant gonad part (pink) shrinks and the other gonad part (blue) expands. Finally (second sex phase), the other gonad (blue) becomes dominant and functional. (b) Undelimited type. In the first sex phase, the corresponding type of gonad is dominant and functional. The precursor cells of the other gonad type scatter in the gonad of the first phase. As sex change proceeds, the scattered cells grow and finally (second sex phase) the gonadal tissue has been replaced.

other ovarian cells shrink, and testicular cells, Leydig cells, and spermatogonia expand. Conversely, in wrasse, testicular precursor cells are scattered within the ovary in the female phase (undelimited type of gonad: **Figure 6**(**b**)). After changing to a male, the testicular cells occupy the gonad. However, most protogynous species contain absolutely no testicular tissue in the ovarian tissue in the female sex phase. In the case of Saddleback wrasse, *Thalassoma duperrey*, it takes 3–4 weeks for the gonads to fully change from an ovary to a testis.

Protandrous fish

In black porgy (*Acanthopagrus schlegeli*), gilthead sea bream (*Sparus aurata*), and striped sea bream (*Lithognathus mormyrus*), gonads first differentiate as immature ovaries, followed by the development of bisexual gonads where a testis and immature ovary are separated by connective tissue (delimited-type gonad). Over time, testicular tissue becomes dominant and functional, resulting in an initial male sex phase. In anemonefish, the gonads first differentiate into immature ovaries, and then testicular tissue develops and functions within the immature ovaries without the connective tissue boundary between the two tissue types (undelimited-type gonads). On the other hand, common snook (*Centropomus undecimails*) and giant perch (*Lates calcarifer*) have only testicular tissue in their gonads during the male phase.

Reciprocal sex-change fish

The fish classified in this type usually function as a male or a female, but they have a testis and ovary simultaneously. In the male phase, the testis is mature and functional but the ovary is immature. In the female phase, the ovary is mature and functional but the testis is immature. However, unlike protogynous and protandrous fish, the immature gonadal tissue does not disappear but remains in the gonad. This system enables rapid sex change compared to protogynous and protandrous fish. Actually, in the case of the Okinawa rubble goby, *Trimma okinawae*, it takes only 5 and 10 days to switch the gonad from ovary to testis and vice versa, respectively. Some fish classified in this type are currently first noticed as protogynous or protandrous fish. Therefore, further studies in this area may change the classification of some fish.

Simultaneous Hermaphroditism

Fish in this type have both testicular and ovarian functional gonadal tissues at the same time, meaning that they can release sperm and spawn the eggs any time after maturation. At spawning, a couple trades off between the sperm and the eggs; that is, in the first round of spawning behavior one acts as a male and releases sperm and the other acts as a female and releases the eggs. In the next turn, the previous male acts as a female and vice versa.

When Do Fish Change Sex?

In protogynous and protandrous fish, their sex changes as the fish grow. A model termed the 'size-advantage model' is proposed to explain the advantage of changing sex according to body size. According to this model, changing sex is advantageous at certain body sizes when the rate of reproductive success differs between males and females according to body size (**Figure 7**).

Additionally, some social factors, such as the disappearance of males or females, also induce sex change (**see also Social and Reproductive Behaviors**: Sexual Behavior in Fish). In the case of reciprocal sex-change fish, sex change occurs when two same-sex fish have to live together, such as if the timing that each fish loses its spouse means that an individual of the other sex is not available. In such a case, finding an individual of the opposite sex that is a long way away can be more energetically expensive and can entail a higher survival risk than staying and changing sex.

In any event, changing sex depends on the balance of the cost and benefit, that is, the energy expenditure and overall success of reproduction. In this sense, the trans sex phenomenon has not been observed in elasmobranchii, possibly because their totally differentiated sexual dimorphism with external genitalia for internal fertilization requires too high a cost/energy to rearrange them.

Plasma Steroids Play a Critical Role in Sex Change

In the case of protogynous fish, there is an induced decrease of estrogen (17-β-estradiol (E2)) and/or subsequent increase of androgen (11-ketotestosterone (11-KT))

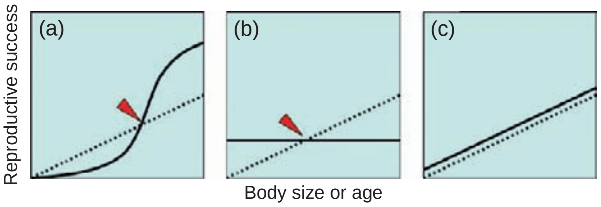


Figure 7 Size-advantage model. Reproductive success generally depends on body size in female fish. Conversely, that of male fish is affected by body size, social system, mating system, and so on. In species where large males monopolize a harem of females, protogyny is advantageous for producing more progeny. Namely, an individual functions as a female with a small body size, then changes its sex to male when it grows to a certain body size (a). Protandry is advantageous in species where small and large males have equal opportunities for mating because small fish can produce more sperms than oocytes (b). In species where males and females are equal in body size, sex change is not advantageous (c). Solid line represents male, and dotted line represents female. Arrowhead represents the time of sex change.

in serum to rearrange an ovary into a testis. Blocking estrogen synthesis with an aromatase inhibitor (AI), such as fadrozole, induces sex change to a male. In the case of protandrous fish, the increase of E2 in plasma induces sex change to female. Additionally, AI or 11-KT treatment suppresses this type of sex change.

The sex change process can be summarized as follows. First, the recognition of the change in body size or circumstance in the nervous system triggers this process and changes the endocrine system. This is followed by an increase in sex steroids that leads to a rearrangement of gonads into the opposite sex.

See also: **Cellular, Molecular, Genomics, and Biomedical Approaches**: Growth Hormone Overexpression in Transgenic Fish; Molecular and Chromosomal Aspects of Sex Determination; Transgenesis and Chromosome Manipulation in Fish. **Social and Reproductive Behaviors**: Sexual Behavior in Fish; Socially Controlled Sex Change in Fishes.

Further Reading

Blaser H, Eisenbeiss S, Neumann M, *et al.* (2005) Transition from non-motile behaviour to directed migration during early PGC development in zebrafish. *Journal of Cell Science* 118: 4027–4038.

Boldajipour B, Mahabaleshwar H, Kardash E, *et al.* (2008) Control of chemokine-guided cell migration by ligand sequestration. *Cell* 132: 463–473.

Braat AK, Speksnijder JE, and Zivkovic D (1999) Germ line development in fishes. *International Journal of Developmental Biology* 43: 745–760 (review).

Devlin RH and Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191–364.

Dumstrei K, Mennecke R, and Raz E (2004) Signaling pathways controlling primordial germ cell migration in zebrafish. *Journal of Cell Science* 117: 4787–4795.

Fujimoto T, Kataoka T, Sakao S, *et al.* (2006) Developmental stages and germ cell lineage of the loach (*Misgurnus anguillicaudatus)*. *Zoological Science* 23: 977–989.

Kokokiris L, Fostier A, Athanassopoulou F, Petridis D, and Kentouri M. (2006) Gonadal changes and blood sex steroids levels during natural sex inversion in the protogynous Mediterranean red porgy, *Pagrus pagrus* (Teleostei: Sparidae). *General and Comparative Endocrinology* 149: 42–48.

Kunwar PS, Siekhaus DE, and Lehmann R (2006) *In vivo* migration: A germ cell perspective. *Annual Review of Cell and Developmental Biology* 22: 237–265 (review).

Kurokawa H, Aoki Y, Nakamura S, *et al.* (2006) Time-lapse analysis reveals different modes of primordial germ cell migration in the medaka *Oryzias latipes*. *Development Growth Differentiation* 48: 209–221.

Nakamura M, Hourigan TF, Yamauchi K, Nagahama Y, and Grau EG (1989) Histological and ultrastructural evidence for the role of gonadal steroid hormone in sex change in the protogynous wrasse *Thalassoma duperrey*. *Environmental Biology of Fishes* 24: 117–136.

Okutsu T, Suzuki K, Takeuchi Y, Takeuchi T, and Yoshizaki G (2006) Testicular germ cells can colonize sexually undifferentiated embryonic gonad and produce functional eggs in fish. *Proceedings of the National Academy of Sciences of the United States of America* 103: 2725–2729.

Otani S, Kitauchi T, Saito T, *et al.* (2005) The formation of primordial germ cells from germline cells in spherical embryos derived from the blastodisc of 2-cell embryos in goldfish, *Carassius auratus*. *International Journal of Developmental Biology* 49: 843–850.

Raz E (2004) Guidance of primordial germ cell migration. *Current Opinion in Cell Biology* 16: 169–173 (review).

Raz E and Reichman-Fried M (2006) Attraction rules: Germ cell migration in zebrafish. *Current Opinion in Genetics and Development* 16: 355–359.

Saito D (2009) Gonads. In: Kinoshita M, Murata K, Naruse K, and Tanaka M (eds.) *Medaka: Biology, Management, and Experimental Protocols*, pp. 247–252. Ames, IA: Wiley-Blackwell.

Wu GC, Du JL, Lee YH, Lee MF, and Chang CF (2005) Current status of genetic and endocrine factors in the sex change of protandrous black porgy, *Acanthopagrus schlegeli* (Teleostean). *Annals of the New York Academy of Sciences* 1040: 206–214.

Yoshizaki G, Takeuchi Y, Sakatani S, and Takeuchi T (2000) Germ cell-specific expression of green fluorescent protein in transgenic rainbow trout under control of the rainbow trout vasa-like gene promoter. *International Journal of Developmental Biology* 44: 323–326.

Global Gene Expression Profiling in Fish

AY Gracey, University of Southern California, Los Angeles, CA, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Why Is Global Gene Expression Measured?
How Is Global Gene Expression Measured?
Sources of Variation in Gene Expression Data
Physiological Insights Arising from Global Gene Expression Data
Further Reading

Glossary

Expressed sequence tag An EST is a short DNA sequence derived from a cloned mRNA representing portions of a gene transcript. The sequence is generally several hundred base pairs from one or both ends of the cloned mRNA.

Hybridization The process of annealing two complementary single-stranded nucleic acids into a single double-stranded molecule.

Hypoxia Low partial pressures of oxygen in external or internal environments.

mRNA An RNA molecule that carries the protein-coding sequence of a gene (CDS) and which is preceded and flanked by a 5′- and a 3′-untranslated region, respectively (5′-UTR and 3′-UTR), which do not contain protein-coding information but which may contain regulatory sequences.

Oligonucleotide A short synthetic nucleotide polymer.

Transcriptome The full set of mRNA molecules (transcripts) produced by the biological system under observation.

Introduction

The physiology of an organism is determined by the combined activities of thousands of genes that are coordinated both temporally and spatially. A central dogma of biology is that genomic DNA is transcribed into messenger RNA (mRNA), which is then translated into protein. This dogma is the foundation behind the study of genomics, transcriptomics, and proteomics, the so-called core 'omics' approaches. Genomics is the study of the genomes of organisms, while transcriptomics and proteomics refer to the study of the complement of mRNA and protein molecules in the organism, respectively. Of these three levels of biological regulation, the study of the regulation of global gene expression at the level of the entire transcriptome has received particular attention.

Why Is Global Gene Expression Measured?

The regulation of mRNA abundance is just one of several levels at which biological regulation occurs and phenotype results from integration at all levels of control. The quantitative relationship between expression of an mRNA molecule and that of its encoded protein is sometimes questioned, since transcript amounts are not always tightly correlated with expression of its encoded protein, and because protein function can be affected through complex post-translational modifications and molecular interactions. The induction of an mRNA transcript is often short term and transient, lasting only long enough for the protein it encodes to be synthesized. Thus, correlations between changes in transcript and ensuing protein levels are likely to be more evident by measurements made during responses to a changed environment or to physiological stimuli rather than in the ensuing steady state. Nevertheless, almost all changes in cell state are accompanied by changes in gene expression, and the regulation of mRNA abundance is an important and perhaps principal means of physiological regulation.

There are two major types of application for which global gene expression data are used. The first aims at identifying individual genes or sets of genes that are differentially expressed across specific conditions or samples. This type of experimental approach is sometimes described as discovery driven or hypothesis generating, which contrasts with more conventional hypothesis-driven approaches in which the expression of specific candidate genes is measured between samples. Examples of this discovery-driven approach would include comparing expression data between groups of fish sampled under different environmental conditions, comparing the transcriptional response of a tolerant versus sensitive fish species, comparing different tissues within the same individual, or comparing transcriptional differences between separate fish species. The overall goal of these applications is to screen a large number of genes so as to identify a subset whose expression is regulated in relation to differences in the phenotypes or genotypes of the samples. Detailed analysis of the functional roles of these differentially expressed genes can provide new insights into the molecular background of the physiological state of the fish.

The second type of application seeks to classify samples according to the gene expression signature they exhibit. This approach works on the assumption that animals of similar physiological state will share similar expressed signatures and that expression data can serve as a surrogate for the physiological state of the fish. In essence, this type of application treats each gene's expression as a phenotypic trait and pattern analysis of hundreds to thousands of these traits allows individuals or samples to be grouped together or separated according to the expression traits they exhibit. An example of this approach would be comparing the transcriptional data of individuals under a range of specific conditions and then grouping the conditions according to the gene expression responses they elicit. This approach makes little or no use of the identity or function of the differentially expressed genes and instead the gene expression signatures serve as a classifier of the physiological state of the individuals under the different conditions.

How Is Global Gene Expression Measured?

There are two main methods through which global gene expression data can be collected. The first method is hybridization based, in which the RNA molecules in a sample are first labeled with a detectable marker such as a fluorescent dye, and then hybridized to large collection of DNA probes that are bound to a solid phase (**Figures 1(a) and 1(b)**). This arrayed collection of DNA probes is typically called a DNA microarray or a DNA chip. Each probe is specific to a particular gene and the amount of labeled RNA that hybridizes to each probe is a measure of the mRNA abundance of each gene in the sample. While arrays are available for a growing number of fish taxa, it seems unlikely that arrays will become available for every species of fish. To address this shortfall, an approach termed heterologous hybridization can be employed in which RNA from one species is hybridized to an array constructed from probes designed from a different species. The utility of this approach depends on the degree to which nucleotide sequence differences between the species affect the kinetics of the nucleic acid hybridization at each arrayed probe. This approach is likely to be most successful between phylogenetically related species, but nevertheless differences in the rate of gene sequence evolution may mean that certain genes will hybridize to the array better than others leading to false assumptions regarding gene abundance.

The second expression profiling method uses high-throughput sequencing to directly sequence the population of RNA molecules within a sample, with mRNA abundance measured by the frequency with which the sequence of a specific gene is detected within the sequencing data (**Figure 1(c)**). This approach is sometimes referred to as whole transcriptome, shotgun sequencing, RNA sequencing, or RNA-Seq. Until recently, hybridization-based approaches were the main method by which gene expression data were collected, but recent innovations in sequencing technology and reductions in cost may mean that sequencing-based methods will dominate in the future. Either way, both methods can measure the mRNA abundance for almost every gene in the transcriptome, offering a global perspective on the role of the gene expression in the physiology of an animal.

The implementation of either method requires that pre-existing sequence data are available in the form of either genomic or gene transcript sequence. Transcript sequences can be produced by sequencing mRNAs that are cloned as cDNA copies. The resulting sequences are often referred to as an expressed sequence tags (ESTs), and represent single sequence reads from one end of cloned mRNAs. Microarrays are fabricated using either polymerase chain reaction (PCR) products or oligonucleotides as probes, and are termed cDNA or amplicon arrays, or oligonucleotide or oligo arrays, respectively. Probes derived from PCR products are generated by targeted amplification of specific genes based on their DNA sequence, or by amplification of cloned mRNAs. When oligonucleotides are used as probes, the sequences of the arrayed oligonucleotides are designed to match specific parts of the gene transcript. The need for pre-existing sequence data is reduced with RNA sequencing approaches because the sequencing effort itself is capable of producing complete gene sequences. Nevertheless, a reference set of gene sequences is required so that the resulting RNA sequences can be mapped to specific genes, the gene expression being a function of the number of sequences from that gene discovered in the transcriptome, relative to the length of the gene.

Sources of Variation in Gene Expression Data

The major sources of variation in gene expression screens are attributed to environmental and biological factors. Transcript levels are responsive to a wide variety of

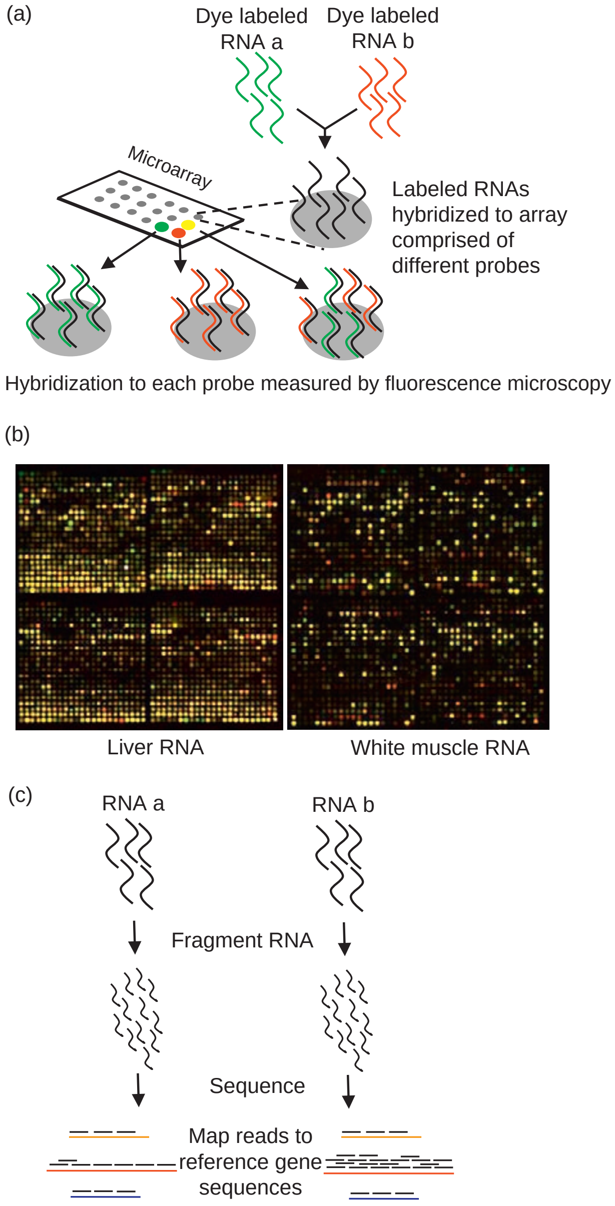

(a)
Dye labeled RNA a
Dye labeled RNA b
Microarray
Labeled RNAs hybridized to array comprised of different probes
Hybridization to each probe measured by fluorescence microscopy
(b)
Liver RNA
White muscle RNA
(c)
RNA a
RNA b
Fragment RNA
Sequence
Map reads to reference gene sequences

Figure 1 Schematic depiction of the two major global gene expression profiling methods. (a) A microarray of arrayed DNA probes is hybridized with either one of two target RNA samples that are labeled with different dyes. The amount of target RNA that hybridizes to each DNA probe is determined using a fluorescence scanner and is a measure of the abundance of that mRNA in the sample. (b) Examples of fluorescent images of a carp microarray hybridized with RNA isolated from liver or white muscle tissue. Note that the fluorescent intensity for each spot is different between the two tissues and is a measure of differences in mRNA abundance between the tissues. (c) In RNA sequencing the sample RNA is fragmented and the fragments sequenced to great depth. The resulting sequences are aligned with and mapped to reference gene sequences. Relative abundance of a transcript is a function of how many sequence fragments derived from the gene were discovered relative to the length of the gene. The red gene is relatively more abundant in RNA b.

environmental variables and so variation due to these factors can be reduced by careful experimental design that excludes unwanted sources of variation. Particularly important variables are temperature, oxygen, salinity, photoperiod, and diet. Experiments are best conducted with fish that have been housed for a period of time under common-gardened conditions where all these variables are carefully controlled and standardized across experimental treatments. Gene expression studies in mammals indicate that the circadian rhythm has a potent effect on global gene expression levels and so the time of day at which samples are taken should be standardized. Efforts to interpret transcriptional differences between individuals sampled in their native habitat are always confounded due to the myriad of environmental differences that might exist between any two habitats. Variation at the measurement level does exist but can be controlled to an extent by technical replication and statistical methods.

A major source of biological variation is related to genetic differences between individuals. With the exception of highly inbred lines of fish, most fishes will differ to some extent at the genetic level. There is an established association between sequence variation and gene expression variation. Indeed, variation in transcript abundance between individuals is a heritable trait and provides the principal means by which genetic differences have their phenotypic effect. In the Atlantic killifish, *Fundulus heterclitus*, analysis of inter-individual variation in metabolic gene expression in the brain, heart, and liver reports that >50% of genes exhibit statistically significant differences in transcript abundance across individuals. This variation appears to be meaningful and differences in cardiac metabolism in killifish are correlated with changes in cardiac gene expression. Individuals with similar cardiac metabolism are also similar with respect to the relative abundance of metabolic genes that they are expressing. While correlation is different from causality, it suggests that patterns of gene expression can explain metabolic variation and in turn can be used to classify individuals according to physiological state.

Another important source of biological variation is behavioral. In Nile tilapia (*Oreochromis niloticus*) populations, males can be socially dominant or subordinate, or reproductively active or suppressed, and different brain gene expression patterns are observed in fish displaying each of these behavioral types. Behavioral differences such as these are observed in many fish species, and because these behaviors are reversible, brain gene expression patterns may be extremely plastic changing in accordance with the behavior currently displayed by the individual. Gene expression differences also play an important role in future behavioral traits. Sedentary versus migratory populations of brown trout, *Salmo trutta*, exhibit profound differences in liver gene expression long before the process of migration is initiated. Fish that are destined to migrate out of streams display a particular pattern of gene expression, which indicates that this behavior is preprogrammed, and, furthermore, this expression pattern is detected in all populations that migrate. Thus, behavior patterns displayed in either the present or future are manifest as gene expression differences.

Physiological Insights Arising from Global Gene Expression Data

Insights into Environmental Acclimation and Adaptation

Fish are a diversified vertebrate family and are found in almost every aquatic habitat on Earth. Their occurrence in a wide range of environmental habitats is a testament to the diversity of their physiologies and their capacity to adapt to different environmental conditions. The ability of an organism to change its phenotype in response to changes in the environment is termed phenotypic plasticity. Phenotypic plasticity is achieved through a process termed acclimatization or acclimation, in which an organism adjusts its physiology to suit the prevailing environmental conditions. Acclimatization refers to this process in field settings where many environmental variables are changed, while acclimation refers to the process when it is studied in the laboratory and the changing variables are carefully controlled. In both instances, this process occurs over a short time and within the lifetime of the organism. This has made fish favored subjects for analysis of the mechanisms underlying phenotypic plasticity. In contrast, evolutionary adaptation is brought about by the accumulation of genetic changes that enhance fitness of the species in the context of its environment. The resulting adaptations are traits upon which natural selection has acted to improve or maintain their function adequate to the particular environment. Most adaptive changes are accomplished either by changes in the temporal, spatial, or quantitative expression of a gene, or alternatively by changes in the function of the encoded protein. These genetic modifications occur through either sequence changes in the regulatory or coding regions of a gene, respectively.

A phenotype results from an interaction between genes and the environment, and so monitoring global gene expression changes has become a standard approach for understanding the molecular basis for alterations in physiological state and phenotype. These gene expression screens have the potential to identify specific genes, pathways, or classes of genes whose expression is actively regulated during environmental change. The addition of temporal (when they are expressed) and spatial information (in which tissues they are expressed) regarding the expression of these genes provides insights into the coordination of phenotypic plasticity and the role of the different organ systems in bringing about physiological change at the level of the whole organism. The two environmental variables that affect all fish regardless of habitat are temperature and oxygen availability.

Temperature

The majority of fish are poikilothermic and their body temperature matches that of their environment. This makes temperature one of, if not the most important, environmental variables with which fish must contend. This is especially true of temperate fishes that typically experience annual temperature ranges from 2 °C to greater than 30 °C. Most temperate fish exhibit phenotypic plasticity over this temperature range by adopting compensatory strategies that serve to maintain physiological performance at relatively constant levels despite the acute rate effects of temperature on all biochemical processes. These compensatory responses are associated with profound changes in global gene expression in fishes.

A survey of the transcriptional changes that occur in seven tissues of common carp, *Cyprinus carpio*, as function of time, and three different extents of cooling, reveals that >50% of genes in the transcriptome are differentially expressed during cold acclimation (**Figure 2**). All tissues respond to cold with a common transcriptional response that comprises genes involved in preserving energy charge, protein turnover, nucleic acid binding, and cell stress. The vast majority (>95%) of the genes that make up this common response increases in expression upon cooling, reflecting a basic paradigm of cold acclimation that organisms frequently compensate for the rate-depressing effects of cold by synthesizing more enzymes to increase biochemical performance. Generally, it appears that gene induction is a graded function of the thermal perturbation, and is transient with transcript levels falling after several weeks at the respective colder acclimation temperatures. The brain is the most active tissue with respect to gene expression changes, particularly with respect to genes linked to cholesterol, fatty acid, and sterol metabolism, consistent with the importance of lipids for proper function of the central nervous system. In most carp tissues, genes involved in the tricarboxylic acid cycle are consistent with other studies that have shown that mitochondrial enzyme activities are elevated in cold-adapted fish populations. Similarly, genes involved in protein translation initiation and protein folding are upregulated consistent with evidence from prokaryotes that suggests that cold compromises the translational apparatus.

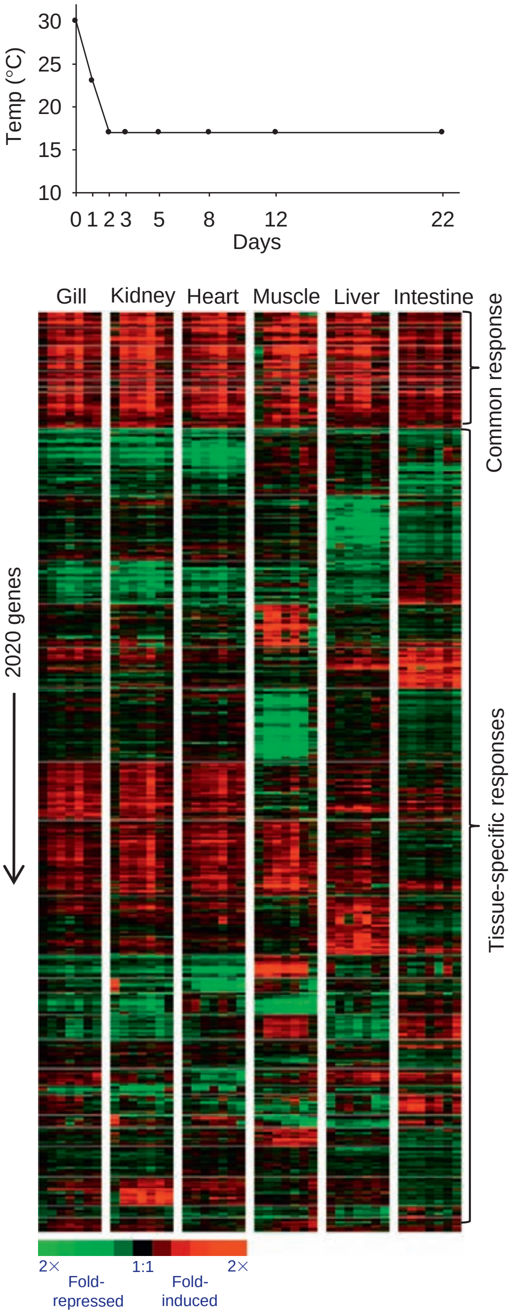


Figure 2 Global gene expression profile of 2020 genes in tissues of cooled carp. Each row represents a different gene and each column represents the expression of the corresponding transcript in carp tissues at 1-, 2-, 3-, 5-, 8-, 12-, and 22-day time points during cold acclimation from 30 to 17 °C. The expression of each gene is presented as the ratio of transcript abundance in each cooled sample relative to its mean abundance in the control 30 °C acclimated fish. Red color indicates a relative increase in transcript abundance with cooling, and green represents a decrease.

Over evolutionary timescales, species can offset the rate effects of temperature by evolving enzymes that are tailored to perform optimally at the typical habitat temperature of the species. While this appears to be a universal strategy, comparison of transcriptomes across taxa offers insights into the role of differential gene expression in the process of evolutionary adaptation. Relative to temperate species, the transcriptome of a cold-adapted Antarctic notothenioid, *Dissostichus mawsoni,* exhibits a preponderance of elevated genes. The functional roles of these elevated genes are very similar to those of the classes of genes that are induced in cold-acclimating carp, such processes as protein degradation, lipid transport, molecular chaperones, and reactive oxygen species scavengers. The concordance between gene expression patterns over both evolutionary and acclimatory timescales indicates the central role that elevating gene expression plays as a mechanism to offset the rate-depressing effects of cold. It also suggests that studying the mechanisms that underpin environmental acclimation provides insights into the processes that underpin evolutionary adaptation to a particular environmental parameter.

Environmental evolutionary adaptation also occurs at a local level when barriers exist that restrict mating and gene flow between populations. Local adaptation is the evolution of traits that confer enhanced fitness to a particular population in a local environment. When comparing the transcriptomes of genetically distinct populations of Atlantic killifish whose range is distributed along a latitudinal thermal gradient of 8–19 °C, the expression of

18% of metabolic genes correlates with local habitat temperature. Interpreting these data within the context of the genetic relatedness of the populations suggests that the expression level of 22% of the habitat temperature-correlated genes is explained by natural selection. The majority of genes whose expression is affected by natural selection are orthologs of genes that are induced in cold-acclimating carp providing further evidence that similar physiological strategies regulated by differential gene expression are employed to deal with cold over both immediate and evolutionary timescales.

Oxygen

Dissolved oxygen levels can fluctuate in aquatic habitats due to a variety of factors. Limitation in availability of oxygen (hypoxia) is a debilitating stress and leads to rapid adaptive changes in ventilator physiology coupled to changes in metabolic organization. Differential gene expression is central to this metabolic response and is mediated in part by the transcription factor, hypoxia-inducible factor 1α, which increases the downstream expression of a suite of genes that enhance anaerobic metabolism and delivery of oxygen to tissues. While much is known about the hypoxia molecular response in mammals because of its relevance in biomedicine, relatively little is known about the response in fish, which in contrast to mammals exhibit a far greater tolerance to protracted periods of hypoxia. When challenged with hypoxia, the long-jaw mudsucker goby, *Gillichthys mirabilis*, alters gene expression so as to reduce the major energy-demanding processes such as contractile protein synthesis and cell division in muscle, while in the liver genes associated with anaerobic glycolysis and gluconeogenesis are elevated. Notably, very few genes exhibit differential expression in brain. During hypoxic stress, the brain is likely to receive a preferential supply of oxygen and glucose, so alterations in gene expression may be minimal relative to tissues such as skeletal muscle that undergo substantial decreases in activity. Similar expression patterns are observed in long-term hypoxia-acclimated zebrafish, *Danio rerio*. In zebrafish gill, genes associated with oxidative phosphorylation and protein synthesis are reduced, while glycolytic genes responsible for generating adenosine triphosphate (ATP) in the absence of oxygen are elevated. The apparent reduction in energy-demanding processes in these two divergent taxa suggests that this might be a common response to hypoxia in fish and may provide a common mechanism that allows diverse fish taxa to acclimate successfully to a reduced level of oxygen.

In common carp, a gene expression screen for hypoxia-regulated genes across multiple tissues reveals that myoglobin, an intra-cellular oxygen-binding protein, is expressed in tissues other than skeletal and cardiac muscle. Specifically, hypoxia exposure is associated with elevated expression of myoglobin in liver, gill, and brain tissue. The detection of myoglobin in nonmuscle tissues is an unprecedented finding, and suggests that myoglobin may play a previously undocumented role in the hypoxic response of fish. This discovery exemplifies the power of global gene expression screens in revealing unexpected physiological responses. It is also a reminder that assumptions regarding the functional role of specific genes may need to be revised when they are investigated within the context of the environmental responses of fish.

See also: **Buoyancy, Locomotion, and Movement in Fishes**: Buoyancy in Fishes. **Cellular, Molecular, Genomics, and Biomedical Approaches**: Molecular Fish Pathology. **Sensory Systems, Perception, and Learning**: Circadian Rhythms in Fish.

Further Reading

Brown PO and Botstein D (1999) Exploring the new world of the genome with DNA microarrays. *Nature Genetics* 21: 33–37.

Buckley BA (2007) Comparative environmental genomics in non-model species: Using heterologous hybridization to DNA-based microarrays. *Journal of Experimental Biology* 210: 1602–1606.

Cossins AR and Crawford DL (2005) Fish as models for environmental genomics. *Nature Reviews Genetics* 6: 324–333.

Cossins AR, Fraser EJ, Hughes M, and Gracey AY (2006) Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *Journal of Experimental Biology* 209: 2328–2336.

Crawford DL and Oleksiak MF (2007) The biological importance of measuring individual variation. *Journal of Experimental Biology* 210: 1613–1621.

Gibson G and Weir B (2005) The quantitative genetics of transcription. *Trends in Genetics* 21: 616–623.

Gracey AY and Cossins AR (2003) Application of microarray technology in environmental and comparative physiology. *Annual Review of Physiology* 65: 231–259.

Gracey AY, Fraser EJ, Li W, *et al.* (2004) Coping with cold: An integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences of the United States of America* 101: 16970–16975.

Miller KM and Maclean N (eds.) (2008) Fish microarrays. *Special Issue: Journal of Fish Biology* 72: 2039–2434.

Mortazavi A, Williams BA, McCue K, Schaeffer L, and Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-seq. *Nature Methods* 5: 621–628.

Podrabsky JE and Somero GN (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus. Journal of Experimental Biology* 207: 2237–2254.

Quackenbush J (2007) Extracting biology from high-dimensional biological data. *Journal of Experimental Biology* 210: 1507–1517.

APPLIED ASPECTS OF FISH PHYSIOLOGY

Toxicology

Contents

The Toxicology of Metals in Fishes
The Toxicology of Organics in Fishes
The Effects of Toxicants on Olfaction in Fishes

The Toxicology of Metals in Fishes

CJ Kennedy, Simon Fraser University, Burnaby, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

How Do We Classify Metals?
Environmental Fate and Bioavailability of Metals
Biological Fate and Toxicokinetics
Biological Effects and Toxicodynamics
Further Reading

Glossary

Bioaccumulation	The process by which substances accumulate in the tissues of living organisms from all sources in the environment.
Bioconcentration	The accumulation of chemicals or metals in living organisms to higher concentrations than in environment from water.
Biomagnification	Increase in concentration of chemicals or metals through the food chain to highest levels in highest trophic level.
Contaminant	Any substance that enters a system where it is not normally found.
Dose	Amount of chemical absorbed, usually per unit weight.
Dose–response	The relationship between the amount of chemical absorbed and the effects observed.
Essential metal	A metal that has a defined biological role.
Heavy metal	A metal of relatively high density (specific gravity greater than about 5) or of high relative atomic weight.
Metallochaperone	Any of a family of proteins that move metal ions to specific sites within a cell.
Metallothionein	A small protein that a group of cysteine-rich proteins with an ability to bind heavy metals.
Nonessential metal	A metal which does not have a known biological role.
Pollutant	Contaminant that adversely alters properties of the environment.
Toxicodynamics	The process of interaction of chemical substances with target sites leading to adverse effects.
Toxicokinetics	Processes of the uptake of potentially toxic substances, biotransformation, distribution, and elimination.
Xenobiotic	Chemicals that are foreign to a biological system.

How Do We Classify Metals?

In the periodic table (**Figure 1**), metalloids occur along the diagonal line through the p block from boron to astatine. Elements in the upper right of this line display increasing nonmetallic behavior; elements in the lower left display increasing metallic behavior.

Metals can be categorized as biologically essential and nonessential; nonessential metals (e.g., tin (Sn), aluminum (Al), cadmium (Cd), mercury (Hg), and lead (Pb)) have no demonstrated biological function (also called xenobiotics or foreign elements). The toxicity of nonessential metals rises with increasing concentration (**Figure 2**). Essential metals (e.g., copper (Cu), zinc (Zn), and iron (Fe)) are

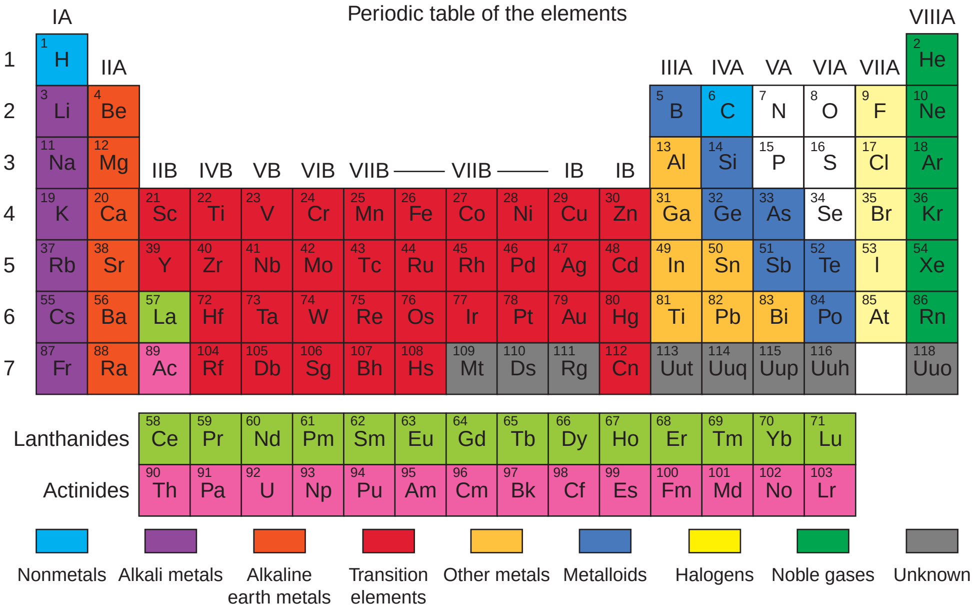

Periodic table of the elements
IA
IIA
IIIA IVA VA VIA VIIA
VIIIA
IIB IVB VB VIB VIIB —— VIIB —— IB IB
1 1 H 2 He
2 3 Li 4 Be 5 B 6 C 7 N 8 O 9 F 10 Ne
3 11 Na 12 Mg 13 Al 14 Si 15 P 16 S 17 Cl 18 Ar
4 19 K 20 Ca 21 Sc 22 Ti 23 V 24 Cr 25 Mn 26 Fe 27 Co 28 Ni 29 Cu 30 Zn 31 Ga 32 Ge 33 As 34 Se 35 Br 36 Kr
5 37 Rb 38 Sr 39 Y 40 Zr 41 Nb 42 Mo 43 Tc 44 Ru 45 Rh 46 Pd 47 Ag 48 Cd 49 In 50 Sn 51 Sb 52 Te 53 I 54 Xe
6 55 Cs 56 Ba 57 La 72 Hf 73 Ta 74 W 75 Re 76 Os 77 Ir 78 Pt 79 Au 80 Hg 81 Ti 82 Pb 83 Bi 84 Po 85 At 86 Rn
7 87 Fr 88 Ra 89 Ac 104 Rf 105 Db 106 Sg 107 Bh 108 Hs 109 Mt 110 Ds 111 Rg 112 Cn 113 Uut 114 Uuq 115 Uup 116 Uuh 118 Uuo
Lanthanides 58 Ce 59 Pr 60 Nd 61 Pm 62 Sm 63 Eu 64 Gd 65 Tb 66 Dy 67 Ho 68 Er 69 Tm 70 Yb 71 Lu
Actinides 90 Th 91 Pa 92 U 93 Np 94 Pu 95 Am 96 Cm 97 Bk 98 Cf 99 Es 100 Fm 101 Md 102 No 103 Lr
Nonmetals
Alkali metals
Alkaline earth metals
Transition elements
Other metals
Metalloids
Halogens
Noble gases
Unknown

Figure 1 Periodic table of the elements showing various metals and elements with metallic properties (metalloids).

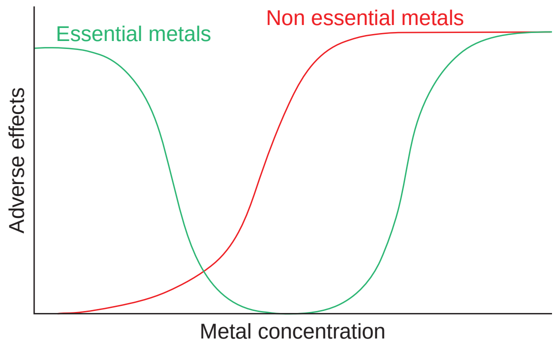


Figure 2 The concentration-adverse-response curves for essential and nonessential metals. Essential metal deficiency leads to adverse health effects as does high concentrations. Nonessential metals are toxic at low concentrations, and exhibit a typical toxicological concentration-response curve.

those with a functional biologic role. Thus, a deficiency of an essential metal causes an adverse health effect, whereas high concentrations can result in toxic effects that are equivalent to or worse than those caused by nonessential metals (**Figure 2**).

Metals are also commonly categorized as either light or heavy (typically greater than 50-Da molecular weight) metals (HMs). A further classification scheme of HMs considers both essentiality and toxicity: Class A HMs include Fe, which is essential in relatively high concentrations. Class B HMs have no established biological role and, at low concentrations, exhibit little or no toxicity (e.g., lanthanum and strontium). Class C HMs include essential metals, but are very toxic at higher concentrations (e.g., Zn, Cu, nickel (Ni), cobalt (Co), molybdenum (Mo), and chromium (Cr)). Class D HMs are toxic at even very low concentrations, and have no clear biological function (e.g., Hg, Pb, Cd, Sn, and uranium (U)).

Environmental Fate and Bioavailability of Metals

Most released metals enter the aquatic environment as a result of direct input, atmospheric deposition, and erosion due to precipitation. Distribution in water is determined by the physico-chemical properties of the metal and is influenced by complex and diverse environmental parameters including dissolved organics, pH, salinity, hardness, and sedimentary load.

Soluble metals include simple dissolved metal ions, metal ion complexes with organic ligands and with inorganic anions. For dissolved metals, there is considerable evidence that the dissolved ionic form is the major bioavailable form and correlates best with metal toxicity. Bioavailability refers to the proportion of metal that can be absorbed by the organism in relation to the total amount of metal in the environment. The bioavailable fraction is accessible to the epithelial surfaces of the animal and can either diffuse or be transported into the cells. Metals can also be found in organic forms as bioavailable organometallic compounds (e.g., organomercurials and organotins (**Figure 3**)). Metals sorbed onto suspended mineral and organic particles are in particulate forms, and the relative importance of these complexes to metal toxicity is still unclear.

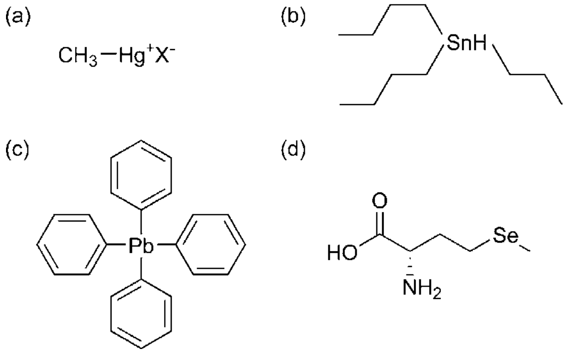


Figure 3 Several examples of organometals: (a) methyl mercury, (b) tributyl tin, (c) tetraethyl lead, and (d) selenomethionine.

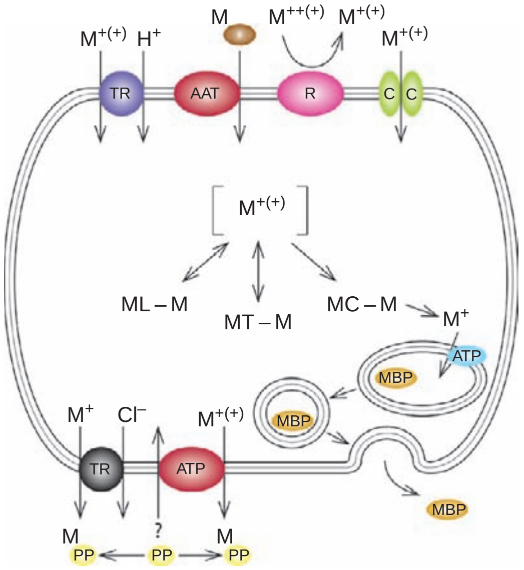


Figure 4 Summary of metal (M in various valence states) transformation (R: metal reductase), transport (TR, transporter (red in apical membrane and black for basolateral membrane); AAT, amino acid transporter; CC, channel; ATP, active transporter (red to extracellular matrix and blue into Golgi apparatus), and vesicular transport), and metal binding to metallothionein (MT), metallochaperones (MCs), nonspecific ligands (MLs), plasma proteins (PPs) and metal-binding protein (MBP).

Biological Fate and Toxicokinetics

Metal toxicity is dependent on its absorption, distribution, metabolism, and excretion, collectively termed ‘toxicokinetics’. HMs accumulate in the tissues of aquatic animals and become toxic when concentrations reach toxicity thresholds, values that vary considerably between metals, metal species, and taxonomic species and organism life stage.

Absorption

The major sites of metal uptake in fish are the gills, digestive system, and, to a lesser extent, the integument. Metal bioavailability is influenced by chemical speciation and complexation in the environmental matrix. The soluble ionic form is believed to be the bioavailable fraction, that is, the form that is absorbed; therefore, the free metal ion concentration rather than the total metal concentration in the environmental matrix is the major determinant of metal toxicity.

There are two main processes for metal uptake: membrane protein transporters/channels (i.e., carrier mediated, not active transport) and passive diffusion. Metals can move into cells simultaneously via several separate and parallel transport mechanisms. For example, Cu uptake in gills can occur through both Na-sensitive (via epithelial Na channels (ENaC)) and Na-insensitive (via putative high-affinity Cu transporter (Ctr) family of proteins) pathways.

The valence of metal ions (free metals) and their propensity to bind to organic material limits their ability to traverse cell membranes without interacting with membrane protein constituents. For class A–C HMs, these mechanisms include both transporter proteins (e.g., Fe^{2+}/H^+ symporter (divalent metal transporter 1) or the zinc transporter-1) and channels (e.g., the epithelial calcium channel and the ENaC) (**Figure 4**). No such mechanisms have evolved for class D HMs (e.g., Cd); therefore, they are assumed to take advantage of these essential transport mechanisms. Metal uptake can be enhanced by the maintenance of metal concentration gradients through binding to intracellular ligands (e.g., metallochaperones (MCs), metallothioneins (MTs), or metal-binding proteins), as well as their efflux (e.g., via ATPases or exocytosis) across basolateral membranes (**Figure 4**).

Lipophilic molecules can passively diffuse through cell membranes, and several lipophilic metal compounds move into fish via this route. Metals can form neutrally charged chloro-complexes (e.g., $HgCl_2$ and $CdCl_2$), and complexation with various hydrophobic ligands, such as xanthates, diethyldithiocarbamates, and dithiophosphates, can increase their lipophilic characteristics. Some metals can exist as lipophilic organometallic compounds (e.g., organomercurials, organotins, and organoselenium) as well.

Distribution, Storage, and Sequestration

Metals such as copper do not exist as free ions in the intracellular milieu (intracellular free Cu concentrations are believed to be 10^{-18} M) due to binding to various chelation sites. For example, MCs are soluble, cytosolic intracellular trafficking proteins that deliver metal ions to specific target proteins (e.g., Atx1 in a Cu secretory pathway). These trafficking proteins are not detoxification proteins; they simply guide and protect the metal ion while facilitating appropriate partnerships (**Figure 4**).

MTs are ubiquitous low-molecular-weight proteins rich in cysteines with a high affinity for specific metals. They function in metal homeostasis by binding essential metals and in defense by sequestering nonessential metals. MTs have been identified in most tissues, including the blood, and can be induced in tissues that are active in metal uptake, storage, and excretion (**Figure 4**). The induction of MTs may not only increase the tolerance of fish to metals, but also decrease metal excretion rates due to sequestration; ecologically, this may increase the potential for bioconcentration within a fish and transfer through a trophic food chain.

Extracellular transport of metals occurs via the circulatory system by diverse plasma proteins. Protein-mediated transport can be either specific (e.g., Fe by transferrin) or nonspecific (e.g., Ni by serum albumin) or both (**Figure 4**).

Biotransformation

Biotransformation is strictly defined as the enzymatic conversion of xenobiotics into more water-soluble metabolites in order to enhance excretion. This definition does not apply to metal ions; however, enzymes can alter their redox state (e.g., membrane-bound cupric reductases; **Figure 4**) and metal toxicokinetics. Organometals (e.g., organic forms of Sn, Hg, and Pb) pose unique challenges to organisms due to their lipophilic nature or their incorporation into biological molecules (e.g., Se incorporation into methionine). The biotransformation rates of organometals are molecule specific, and use the same enzymes responsible for the metabolism of other organic xenobiotics (e.g., tributyl tin (TBT) is oxidized by cytochrome P450).

Excretion

There are several major and minor sites of metal elimination in fish including the hepatobiliary system, kidneys, gills, alimentary system, eggs, embryos, and skin and scales. Compared to mammals, fish have a limited capacity to excrete metals. Excretion is metal and species specific; for example, there is a threefold difference between the excretion rates of inorganic Hg (faster) versus methyl Hg (slower) that is partly responsible for the predominance of methyl Hg in fish tissues. Toxicokinetic modeling suggests that most metal elimination can be described by a two-compartment model where metal is first accumulated into a rapidly exchanging compartment from which metals are easily eliminated (e.g., complex to low-affinity intra- and extracellular ligands, i.e., Hg half-life ($t_{1/2}$ = days to weeks), and a slowly exchanging compartment in which metals are tightly bound (e.g., MTs, Hg $t_{1/2}$ = months to years).

Hepatobiliary excretion is a major route of metal excretion. The liver is highly perfused, has a high binding affinity for many metals (e.g., high-MT content), and has a biotransformation system for organometal metabolism and high titers of transporting ATPases (e.g., Cu-ATPase and a canalicular multiorganic anion transporter). Although believed to be an important route for metal excretion (e.g., Pb), specific details on renal metal excretory mechanisms are generally lacking for fish.

Bioaccumulation, Bioconcentration, and Biomagnification

Most metals are taken up by fish, that is, they bioaccumulate, and many have the potential to bioconcentrate (accumulation from the water to higher concentrations in the fish). Biomagnification is the accumulation from food resulting in higher concentrations in the fish. Most metals are not expected to biomagnify to any significant degree; however, lipophilic organic metals that are not easily biodegraded (e.g., triphenyltin and organoselenium) do so. For example, methyl Hg biomagnifies; it has high uptake and low elimination rates and attains its highest concentration at the top of the aquatic food chain in large predatory species such as the tuna, swordfish, and sharks.

Biological Effects and Toxicodynamics

The complexities of metal speciation and form on toxicokinetics are also reflected in diverse toxicodynamics (metal–receptor interactions, i.e., effects). Metals can be classified by their physiological mechanism of action into three broad categories, two that are based on action and one on location: monovalent metals (e.g., Ag(I) and Cu(I)) that affect Na transport; divalent metals (e.g., Cd and Zn) that disrupt Ca metabolism; and metals that act centrally (e.g., Pb and Hg).

Nonspecific protein binding is a hallmark of HM toxicity. Metals such as Hg and Cd bind nonspecifically to many proteins, particularly at sulfhydryl (–SH) groups, which are common functional groups on many proteins. This binding degrades the functionality of multiple proteins, which is why metal exposure can result in varied effects on many systems, and can result in multiple higher-level effects.

A discussion on metal toxicity would not be complete without mention of methods used to predict metal toxicity. Currently, there is much interest in the biotic ligand model (BLM) that is a chemical equilibrium-based model used as a tool to predict acute metal toxicity in aquatic organisms. Water-quality constituents can influence the extent of metal binding to the biotic ligand (e.g., Na or Ca channel proteins in gills) by competing with the metal for binding sites. The BLM is used to predict the degree of metal binding, accumulation, and toxicological response.

Specific metal toxicities in fish are highlighted below. They have been chosen by several criteria: relative toxicity, environmental relevance, and availability of toxicokinetic and toxicodynamic information.

Aluminum (Al)

The bioavailability and toxicity of Al varies with its chemical speciation. Bioconcentration is believed to be minimal and biomagnification insignificant. Acute (24–96 h) lethal concentration to 50% (LC_{50}) values in fish range from 0.095 to 235 mg l^{-1}.

In freshwater (FW) fish, Al exposure can cause significant ion disturbances and reduced plasma osmolarity. Failed ion regulation is arguably the primary cause for Al-related mortality (although several authors cite respiratory disturbance as the main mechanism). Some of these disturbances are due to change in gill Na^+/K^+-ATPase activity. In marine fish, low saltwater (SW) tolerance in Al-exposed fish is related to a decline in Na^+/K^+-ATPase activity.

At high Al concentrations, as with other metals, the other major physiological system affected is the respiratory system. Effects include hypertrophy and lifting of the outer epithelium, congestion of the secondary lamellae, and degeneration of chloride and epithelial cells. Reductions in apparent lamellar surface area and significant increases in gill mucus density further contribute to respiratory dysfunction, leading to increased coughing, hyperventilation, and decreased oxygen uptake.

Behavioral effects of Al include effects on predatory ability, swimming performance, and feeding activity, which may be caused in part by demyelination of nerves in the brain. Other neurotoxic effects on the olfactory organs of fish are also seen (**see also** **Toxicology**: The Effects of Toxicants on Olfaction in Fishes).

Cadmium (Cd)

The bioconcentration factors (BCFs) for Cd range from 152 to 756. The evidence as to whether or not Cd biomagnifies in aquatic foodwebs is inconclusive, but several studies indicate that biomagnification is insignificant.

The acute toxicity of Cd is highest at neutral pH, with LC_{50} values of 0.8–48 μg l^{-1} for FW fish. Marine species appear to be more tolerant, likely due to the effects of higher pH and other ions (e.g., Ca) on Cd bioavailability. For marine species, acute LC_{50} values range between 200 and 700 μg l^{-1}. Comparisons of toxicities between studies with most metals are difficult and can be improved by converting to common hardness values ($CaCO_3$ equivalents).

The main toxic effects of Cd in fish occur in gills and kidneys resulting in osmoregulatory disturbances. Specifically, its main effects occur through the obstruction of Ca uptake via Ca channels resulting in hypocalcaemia. This, however, appears to cause only moderate alterations in overall osmotic regulation: in FW, Cd causes modest declines in plasma Na and Cl; and in SW, the protective effects of other ions increase the concentrations necessary to elicit these effects.

Cd is an endocrine pathway modulator in teleosts. The hypothalamus–pituitary–gonadal (controlling sexual differentiation and reproduction) and the hypothalamus–pituitary–interrenal (controlling stress) axes are prime targets of Cd. Developmental effects, such as reduced growth and effects on reproduction, are effects likely mediated by this mechanism.

Cd also affects hematological parameters resulting in anemia. This occurs through several identified mechanisms: interference with hematopoiesis, potentially through a reduction in the absorption of Fe for hemoglobin, a slowing of erythrocyte maturation, and malformed and fragile circulating erythrocytes.

Cd exposure causes oxidative stress resulting in DNA fragmentation by stimulating the production of reactive oxygen species (ROS) and by inhibiting antioxidant enzymes that scavenge ROS; this may lead to apoptosis and pathological effects in several tissues. Cd is also immunomodulatory, inhibiting and stimulating different immune system components.

Chromium (Cr)

Cr does not significantly bioconcentrate or biomagnify in aquatic organisms. Cr exists as both trivalent (III) and hexavalent (VI) Cr, the latter being more toxic. Acute LC_{50} values of Cr(VI) in FW range from 0.1 to 930 mg l^{-1}. In SW, acute toxicity ranges from 16.3 to 200 mg l^{-1}. The acute toxicity of Cr(III) in FW ranges from 3.3 to 77.5 mg l^{-1}. Adverse effects in chronic studies include reduced growth, chromosomal aberrations, reduced disease resistance, and morphological changes. Cr is also a mutagen, carcinogen, and teratogen in vertebrates.

Copper (Cu)

There is a moderate potential for bioconcentration, but not biomagnification of Cu in fish. The acute LC_{50} value of Cu to FW fish can be as low as 2.8 μg l^{-1} in soft water and is inversely correlated with hardness. Toxicity in SW is much lower, with LC_{50} values in the range of 0.011–3 mg l^{-1}.

In FW, Cu exerts inhibitory effects on branchial ionoregulation during acute exposures. With metals that affect Na transport (e.g., via inhibition of Na^+/K^+-ATPase), the following sequelae are believed to occur; the inhibition of sodium uptake results in a redistribution of ions and water between internal compartments, which in turn triggers a sequence of events that potentially lead to cardiovascular collapse.

Chronic effects of Cu include effects on reproduction, growth, behavior, olfaction (**see also Toxicology**: The Effects of Toxicants on Olfaction in Fishes), development of embryos, and disease resistance.

Lead (Pb)

Pb can be bioconcentrated from water, but it does not biomagnify and concentrations tend to decrease with increasing trophic level. Although organic Pb compounds have the potential to biomagnify, evidence for such is contradictory.

As with other metals, the acute toxicity of Pb varies considerably with water hardness. In soft water, LC_{50} values range from 1 to 400 mg l^{-1}. In SW, LC_{50} values range from 0.3 to 3 mg l^{-1}. Organic Pb can be more acutely toxic; values as low as 3 μg l^{-1} are lethal to rainbow trout (*Oncorhynchus mykiss*).

Pb exerts toxic effects in fish through disruption of Ca balance and the interference with Na and Cl regulation, which places its toxic mechanism midway between metals such as Cd and Cu. Lead-induced ionoregulatory toxicity is not only exclusively a branchial phenomenon, but also involves the disruption of ionoregulatory mechanisms at the kidney and digestive tract.

Pb causes cancer, adversely affects liver and thyroid function, and decreases disease resistance. Other effects include muscular, spinal, and neurological degeneration, growth inhibition, reproductive problems, and hematologic effects such as disruption in hemoglobin synthesis.

Mercury (Hg)

There is a high potential for Hg bioconcentration and biomagnification (in organic form), with BCFs up to 100 000. Organic Hg compounds are more readily absorbed and poorly excreted in comparison with inorganic forms.

Acute LC_{50} values for inorganic Hg range from 150 to 900 μg l^{-1} in both FW and SW. Acute toxicity concentrations for methyl Hg range from 24 to 125 μg l^{-1}. Unlike most other toxic metals, water hardness has a negligible effect on Hg toxicity.

Hg causes altered osmoregulation in both FW and SW fish, although unlike other metals, the effects are not diminished in SW. Methyl Hg appears to produce osmoregulatory disturbances at concentrations approximately 10–20 times lower than inorganic mercury in FW fish.

The primary targets of Hg are the central nervous system (CNS) and kidneys in fish. At concentrations below 1 μg l^{-1}, Hg can cause neurological effects including loss of appetite, brain lesions, cataracts, abnormal motor coordination, and behavioral changes. Significant anemia and reduced oxygen uptake occur in fish exposed to inorganic Hg, possibly through accumulation in the kidney and inhibition of uroporphyrinogen I synthetase (a heme biosynthetic enzyme) as well as through reductions in the deformability of erythrocytes and premature destruction. Other sublethal adverse effects that are commonly reported include impaired growth, reproduction, and development. Reproductive effects are partly mediated by suppressed levels of plasma sex steroids, inhibition of gonadal development, altered reproductive behavior, reduced egg production, and reduced spawning.

Nickel (Ni)

Little bioconcentration of Ni occurs, with BCFs in the range of 2–52. There is also little evidence of significant biomagnification of Ni. Ionic Ni is very toxic to fish embryos (LC_{50} 11–113 μg l^{-1}), but considerably less toxic in larger fish (7–140 mg l^{-1} depending on water hardness).

Unlike many metals which cause respiratory distress only at higher concentrations, the main effect of Ni is on respiration (arguably as with Al) rather than on ionoregulation (e.g., Cu, Ag, Cd, and Zn), most likely causing sufficient gill damage to critically impair branchial gas exchange, leading to eventual suffocation. Other effects include altered immunological parameters, reduced growth, and there is some evidence that it is a carcinogen and weak mutagen as well.

Selenium (Se)

Se can significantly bioconcentrate and biomagnify. The bioaccumulation of Se through the diet is believed to be greater than the direct uptake from water. Organoselenium forms (e.g., selenoamino acids (selenomethionine, selenocysteine, and selenocysteine)) are much more bioavailable than inorganic forms and play the most important roles in Se ecotoxic effects. These forms can be transferred through various aquatic consumers into the top-level piscivorous fish.

Se functions as both an essential nutrient and environmental toxicant, with a narrow margin between essential and toxic effects. Acute LC_{50} values for Se range from 0.62 to 96.8 mg l^{-1} for FW fish, and range from 0.6 to 86 mg l^{-1} in marine species. Data suggest that selenite-Se is more toxic than selenate-Se.

Adverse effects observed in fish exposed to Se include loss of equilibrium and other neurological disorders, liver damage, reproductive failure, reduced growth, reduced locomotion, chromosomal aberrations, reduced hemoglobin and increased white blood cell count, and necrosis of the ovaries. Research to date indicates that the most ecologically significant Se toxicity in fish arises from maternally transferred Se that accumulates in egg yolks during vitellogenesis, likely as selenoamino acids. Deformities and edema occur when developing larvae utilize the yolk sac resulting in larvae with gross abnormalities.

Silver (Ag)

Ag is one of the most toxic metals to fish. The LC_{50} value for Ag (as nitrate) in FW is usually in the range of 5–70 $\mu g\ l^{-1}$. Ag chloride is 300 times less toxic, Ag sulfide is 15 000 times less toxic, and Ag thiosulfate is 17 500 times less toxic. Water hardness is an important factor affecting the toxicity of most metals, but appears to be less important for Ag. The toxicity of Ag is considerably lower in SW (acute LC_{50} range: 330–2700 $\mu g\ l^{-1}$).

The primary mechanism of toxicity in FW is the inhibition of active Na and Cl uptake at the gills, whereas the intestine has been indicated as the site of toxicity in SW fish. As described for Cu, this effect can lead to critically low blood volume, high viscosity of the blood, and high blood pressure leading to mortality. Ag affects gill structure and function at high concentrations leading to asphyxiation, convulsions, and abnormal surfacing behaviors. At environmentally realistic concentrations, toxicity is not via suffocation, but via osmoregulatory impedence. Chronically elevated concentrations of Ag can also cause developmental abnormalities and reduced growth.

Thallium (Tl)

Fish exposed simultaneously to Tl sulfate (Tl_2SO_4) and Tl had BCF values of 34, and Tl is not expected to biomagnify. Tl can be as toxic as Cu on a weight basis with acute LC_{50} values ranging from 0.86 to 132 $mg\ l^{-1}$ in FW fish. Tl can also cause reductions in larval fish growth and reduced embryo hatchability.

Tin (Sn)

The most environmentally important Sn compounds are organotins. The bioconcentration potential of organotins is directly related to the lipophilicity of the specific organotin. For example, BCFs for TBT have been found to range from 200 to 4580 compared to lower values for water-soluble inorganic Sn.

Acute toxicity of organotin compounds is strongly influenced by the length of the alkyl chains attached to the tin. TBT is generally less toxic than trimethyl tins (TMTs)- and triethyl tins (TETs). LC_{50} values for TBT range from 2.6 to 12.7 $\mu g\ l^{-1}$. Of the tri-substituted organotins that exert the highest toxicity, TMT and TET are primarily neurotoxic, while TBT has a broader range of toxic effects. A multitude of cellular effects has been seen in fish exposed to organotins and includes disturbance of Ca^{2+} homeostasis and the induction of apoptosis, DNA fragmentation, inhibition of oxidative phosphorylation and ATP-synthesis, and inhibition of ion pumps. Although TBT exposure leads to endocrine disruption, and developmental and reproductive abnormalities, its proximal mechanism(s) of action involves acting as a Ca ionophore causing general membrane perturbation leading to apoptosis.

TBT exposure can also adversely affect egg hatchability, larval viability, and survival and growth in fish. These effects are often associated with overall retardation of development including striking morphological effects and severe malformations of the body axis of larvae including CNS and spinal cord malformalities.

Behavioral effects of organotins include alterations to swimming behavior that were believed to be associated with neurotoxicological manifestations in the brain. Interestingly, TBT accumulates in brain regions via axonal transport from water-exposed sensory organs.

The immunotoxicity of organotin compounds is only partially understood in fish; however, it is believed that TBT oxide and dibutyl tin (metabolites of TBT) induce thymic atrophy, and hyperplasia of the hematopoietic interstitial tissue of kidneys resulting in reduced immune defense parameters.

Zinc (Zn)

Zn can bioconcentrate, with typical BCFs around 150. Biomagnification is not a significant process in zinc toxicity, and concentrations can decrease with increasing trophic level. In FW, acute LC_{50} values range between 63 and 170 $mg\ l^{-1}$. Zn interferes with Ca uptake at the gill that, like Cd, causes hypocalcaemia or calcium deficiency. To a lesser extent, Zn disturbs Na and Cl fluxes, resulting in a net branchial ion loss in FW. Irreversible destruction of the gill epithelium occurs at high Zn concentrations causing reductions in oxygen uptake, tissue hypoxia, osmoregulatory failure, and acidosis. Long-term Zn toxicity affects growth, egg fragility and mortality, and reproduction.

See also: **Toxicology:** The Effects of Toxicants on Olfaction in Fishes.

Further Reading

Atchison GJ, Henry MG, and Sandheinrich MB (1987) Effects of metals on fish behavior: A review. *Environmental Biology of Fishes* 18: 11–25.

Bury NR, Walker PA, and Glover CN (2003) Nutritive metal uptake in teleost fish. *Journal of Experimental Biology* 206: 11–23.

Clearwater SJ, Farag AM, and Meyer JS (2003) Bioavailability and toxicity of dietborne copper and zinc to fish. *Comparative Biochemistry and Physiology Part C* 132: 269–313.
Craig PM, Hogstrand C, Wood CM, and McClelland GB (2009) Gene expression endpoints following chronic waterborne copper exposure in a genomic model organism, the zebrafish, *Danio rerio. Physiological Genomics* 40: 23–33.
Foulkes EC (2000) Transport of toxic heavy metals across cell membranes. *Proceedings of the Society for Experimental Biology and Medicine* 223: 234–240.
Gray JS (2002) Biomagnification in marine systems: The perspective of an ecologist. *Marine Pollution Bulletin* 45: 46–52.
Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment* 326: 1–31.
Heath AG (1995) *Water Pollution and Fish Physiology*. Boca Raton, FL: CRC Press.
Jezierska B, Lugowska K, and Witeska M (2009) The effects of heavy metals on embryonic development of fish (a review). *Fish Physiology and Biochemistry* 35: 625–640.
Malins DC and Ostrander GK (eds.) *Aquatic Toxicology Molecular, Biochemical, and Cellular Perspectives*. Boca Raton, FL: CRC Press.
Mirza RS, Green WW, Connor S, *et al.* (2009) Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. *Ecotoxicology and Environmental Safety* 72: 677–683.
Pane EF, Richards JG, and Wood CM (2003) Acute waterborne nickel toxicity in the rainbow trout *(Oncorhynchus mykiss)* occurs by a respiratory rather than ionoregulatory mechanism. *Aquatic Toxicology* 63: 65–82.
Paquin PR, Gorsuch JW, and Apte S (2002) The biotic ligand model: A historical overview. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 133: 3–35.
Power M, Klein GM, Kuiguer KRRA, and Kwan MKH (2002) Mercury accumulation in the fish community in a sub-arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology* 39: 819–830.
Roesijadi G and Robinson WE (1994) Metal regulation in aquatic animals: Mechanisms of uptake, accumulation and release. In: Malins DC Ostrander GK (eds.) *Aquatic Toxicology Molecular, Biochemical, and Cellular Perspectives*, pp. 387–420. Boca Raton, FL: CRC Press.
Sorensen EMB (1991) *Metal Poisoning in Fish*. Boca Raton, FL: CRC Press.
Wang WX and Rainbow PS (2008) Comparative approaches to understand metal bioaccumulation in aquatic animals. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 148: 315–323.
Weber DN and Spieler RE (1994) Behavioural mechanisms of metal toxicity in fish. In: Malins DC Ostrander GK (eds.) *Aquatic Toxicology Molecular, Biochemical, and Cellular Perspectives*, pp. 421–467. Boca Raton, FL: CRC Press.
Wood CM (2001) Toxic responses of the gill. In: Schlenk D and Benson WH (eds.) *Target Organ Toxicity in Marine and Freshwater Teleosts,* vol. 1, pp.1–89. London: Taylor and Francis.

Relevant Websites

http://www.env.gov.bc.ca – British Columbia; Water Quality.
http://ceqg-rcqe.ccme.ca – CCME.
http://www.epa.gov – EPA: United Stated Environmental Protection Agency; Ecsological Toxicity Information.

The Toxicology of Organics in Fishes

CJ Kennedy, Simon Fraser University, Burnaby, BC, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Biological Fate
Specific Organic Contaminants
Chemicals of Emerging Concern
Further Reading

Glossary

Bioaccumulation The process by which substances accumulate in the tissues of living organisms from all sources in the environment.

Bioconcentration The accumulation of chemicals or metals in living organisms to higher concentrations than in environment from water.

Biomagnification Increase in concentration of chemicals or metals through the food chain to highest levels in highest trophic level.

Contaminant Any substance that enters a system where it is not normally found.

Dose Amount of chemical absorbed, usually in mass per unit weight.

Dose–response The relationship between the amount of chemical absorbed and the effects observed.

Endocrine disruptors An exogenous substance (xenobiotic) that interferes with endocrine function, the synthesis, transport, degradation, or actions of hormones.

Hydrophilicity (water linking in Greek) A physical property of a molecule that can transiently bond with water (H_2O) (dissolve in it) through hydrogen bonding.

Hydrophobicity (water linking in Greek) The physical property of a molecule that is repelled from a mass of water.

Lipophilicity (fat liking in Greek) The ability of a chemical compound to dissolve in fats, oils, lipids, and nonpolar solvents.

Octanol–water partition coefficient The octanol–water partition coefficient or K_{ow} is the ratio of the concentrations of a chemical in an octanol and water system.

Organic compound A compound that contains one or more C–H bonds.

Organochlorine An organochlorine is an organic compound containing at least one covalently bonded chlorine atom.

Personal-care products Consumer products used for beautification and in personal hygiene.

Pharmaceutical A pharmaceutical drug can be loosely defined as any chemical substance intended for use in the medical diagnosis, cure, treatment, or prevention of disease.

Pollutant Contaminant that adversely alters properties of the environment.

Polybrominated diphenyl ethers (PBDEs) Organobromine compounds that are used as flame retardants, and are polyhalogenated compounds, consisting of two halogenated aromatic rings.

Polychlorinated biphenyls (PCBs) A class of organic compounds with 1–10 chlorine atoms attached to biphenyl, a molecule composed of two benzene rings.

Polycyclic aromatic hydrocarbon Polycyclic aromatic hydrocarbons (PAHs) are also known as polyaromatic hydrocarbons or polynuclear aromatic hydrocarbons, and are chemical compounds that consist of two or more fused aromatic rings.

Toxicodynamics The process of interaction of chemical substances with target sites leading to adverse effects.

Toxicokinetics Processes of the uptake of potentially toxic substances, biotransformation, distribution, and elimination.

Xenobiotic Chemicals that are foreign to a biological system.

Introduction

In the sixteenth century, Paracelcus, a German-Swiss alchemist and physician, highlighted one of the most fundamental aspects of toxicology in his quote: “All things are poison and nothing is without poison, only the dose permits something not to be poisonous.” Today, concern about environmental contamination and pollution is usually limited to those compounds that have relatively high toxicity. Nature produces such chemicals; for example, the potent bacterially derived neurotoxin tetrodotoxin is used as a defensive biotoxin and predatory venom by a number of fish species including puffer and angelfishes. However, it has been the relatively recent phenomena of industrial pollution and the release of novel synthetic compounds into the biosphere that has heightened interest and concern, and focused research toward understanding the fate and effects of harmful substances to humans and wildlife, including fish.

The Stockholm Convention (2001) was an international convention that brought into force an agreement that essentially moves to outlaw chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment. This applies particularly to persistent pollutants including the so-called dirty dozen chemicals (aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polychlorinated dibenzo-*p*-dioxins (PCDDs; dioxins), polychlorinated dibenzofurans (PCDFs; furans), mirex, and toxaphene).

Several definitions exist for organic compounds (with many exceptions) and include compounds containing one or more C–H bonds, compounds that include C–C bonds, or molecules that simply contain carbon. Many of the molecules classified as organic today have no connection whatsoever, material or structural, to any substance found in living organisms (the historical definition of the word). In toxicology, organic compounds are classified as natural or synthetic. Natural organics refer to toxins that are produced by plants or animals. Compounds that are prepared by reaction of compounds (natural or not) are referred to as synthetic. This article focuses on anthropogenic synthetic chemicals (foreign chemicals are often called xenobiotics) of environmental significance.

Understanding the toxicity of environmental contaminants to fish requires knowledge regarding their environmental fate (to understand contact between fish and chemical, i.e., exposure), toxicokinetics (absorption, distribution, biotransformation, and excretion of the chemical), and toxicodynamics (effects of the toxicant) according to the toxic action paradigm (**Figure 1**).

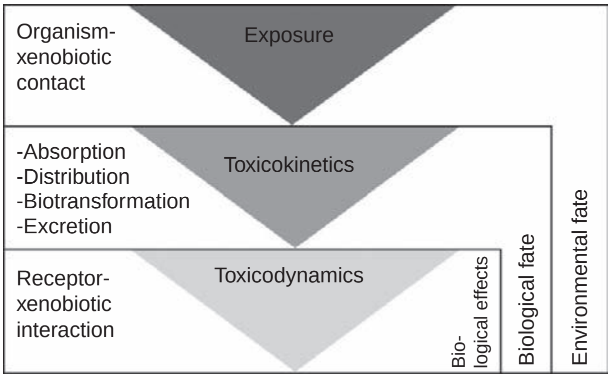


Figure 1 The toxic action paradigm. Knowledge regarding environmental fate, toxicokinetics, and toxicodynamics is fundamental to understanding how organic chemicals impact fish and other organisms.

Biological Fate

The distribution of a chemical in the aquatic environment is determined by a complex interplay between the physico-chemical properties of the compound and a diverse environment. For the purpose of understanding the partitioning of such chemicals, the aquatic environment can be visualized as several units: the atmosphere, water, sediments, and biota. Two key chemical characteristics, vapor pressure and water solubility, determine the partitioning and final concentrations of organic chemicals in the environmental units.

Vapor pressure is a measure of the tendency of molecules and atoms to escape from a liquid or a solid into the gas phase. The higher the vapor pressure of a compound, the higher is its partitioning into the atmosphere. Toxic chemicals such as persistent organic pollutants (POPs; even with low vapor pressures) can volatilize and be transported globally, affecting ecosystems far from the original pollution sources. Equally interesting, volatilization from contaminated water bodies can aid in their decontamination. For example, the Great Lakes decreased their combined levels of PCBs by 10 tons from 1992 to 1996 by chemical movement into the atmosphere.

Water solubilities of organic chemicals can range from very water soluble (hydrophilic, e.g., ionized or polar molecules) to those such as dioxins and polybrominated diphenyl ethers (PBDEs), which are hydrophobic (water fearing) and have very limited water solubility. Hydrophilic molecules will dissolve in the water easily, whereas hydrophobic chemicals tend to bind to sediment or the lipid of organisms. K_{ow} is a value that describes the partitioning of a chemical between octanol (an organic solvent and a surrogate for lipids in biota and for organic carbon) and water. K_{ow} is arguably the most important measure of a compound's hydrophobicity, associated environmental partitioning, and uptake potential in organisms. Chemicals with log K_{ow} values between 4 and 8 are hydrophobic, while lower values are indicative of more water-soluble compounds.

Bioaccumulation of organic chemicals is usually defined as the uptake of chemical from any matrix in the environment by an organism. Bioconcentration specifies the accumulation of chemical from the abiotic environment (e.g., from water to gills or through skin) into an organism, resulting in concentrations higher than in the environment. Biomagnification specifies the accumulation of chemical from its diet to higher concentrations than are found in its prey. Biomagnification, therefore, accounts for increases in the concentrations of some chemicals through successive trophic levels due to food alone. A high log K_{ow} value and a low biodegradation rate are the two factors primarily responsible for bioconcentration and biomagnification. For example, in Lake Michigan, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE: a high log K_{ow} organochlorine (OC) pesticide) was a strongly biomagnified compound, increasing 28.7 times in average concentration from plankton to fish. PCBs increased 12.9 times in average concentration from plankton to fish, while toxaphene increased by an average factor of 4.7. A simple way of viewing this tendency is that the greater the K_{ow} of a compound, the less it likes being dissolved in the water surrounding the fish and the more it likes being dissolved in the lipids of the fish.

Chemical Uptake

The three major sites of uptake for organic chemicals in fish are the integument, gills, and digestive system. Integuments can act, in part, as barriers to the outside environment and limit the absorption of xenobiotics (even with the secondary skin circulation in fish), partially due to the presence of scales in fish. Conversely, respiratory systems and digestive systems are designed for transfer between the organism and the environment. For example, extraction efficiencies of organic chemicals from the aqueous environment can be typically >80% at the gills (e.g., pentachlorphenol). Log K_{ow} correlates fairly well with the extent of uptake in fish, with compounds having higher log K_{ow} values tending to accumulate more rapidly. For chemical concentration ratios that might be expected in nature, gill uptake can account for the majority of a fish's body burden for chemicals of log K_{ow} values of <5, but as the K_{ow} increases, the contribution from dietary uptake rapidly rises largely because there is less of the chemical dissolved in the water and more in the food. Thus, K_{ow} is primarily responsible for altering the bioavailability of the chemical to either the gill or gut; the ease with which a chemical can cross the gill or gut membrane also increases with K_{ow}.

Several mechanisms exist by which organic chemicals can move into cells. Simple passive diffusion is the primary mechanism by which lipophilic/hydrophobic toxicants move into cells. The lipid bilayer of the cell membrane is a barrier to most nonionized hydrophilic molecules and ions, although small molecules of approximately molecular weight (MW) <100 can traverse this barrier by diffusing through aqueous channels. In addition to this, larger water-soluble molecules can still gain entry into cells through active transport and facilitated diffusion. In these cases, membrane-associated carrier proteins provide transport and are linked either to energy-producing enzymes or to the co-transport of other molecules.

Chemical Biotransformation

Biotransformation is the enzymatically catalyzed transformation of a parent (original) xenobiotic into metabolic products called 'metabolites'. The excretory mechanisms of fish are essentially water based; excretion of foreign chemicals is into water via the gills, urine via the kidneys, or feces via the hepatobiliary route. Any absorbed hydrophobic/lipophilic contaminant, such as PCBs, PBDEs, or OC pesticides, cannot utilize these excretory pathways unless the parent chemical is transformed into more water-soluble products by biotransformation. Thus, once high K_{ow} chemicals are absorbed, it is very difficult to excrete them back to the aquatic environment.

Biotransformation results in water-soluble metabolites that are more easily excreted. Biotransformation reactions in fish are categorized into phase I (asynthetic) and phase II (conjugation or synthetic) reactions (**Figure 2**). In phase I reactions, a chemical can undergo oxidation, reduction, or hydrolysis reactions (the three most common in fish), which either reveals or adds on a polar functional group (e.g., –OH and –COOH), usually rendering the molecule more water soluble than the original. The cytochrome P450-dependent monooxygenases comprise a group of heme-containing enzymes involved in the metabolism of

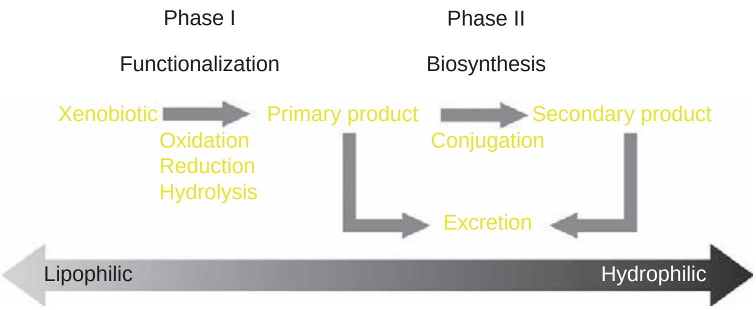


Figure 2 Biotransformation processes in fish. Phase I reactions add or unmask polar functional groups to a xenobiotic, while phase II reactions conjugate xenobiotics or their phase I metabolites to endogenous molecules. Generally, both types of reactions result in more water-soluble metabolites.

thousands of endogenous and xenobiotic compounds. The various P450 forms have been classified into families and subfamilies based on their amino acid sequences and are well studied in fish.

Phase I reactions prepare xenobiotics for phase II reactions if they are not rendered water soluble enough to be excreted. In phase II reactions, an endogenous molecule (e.g., glutathione, glucuronic acid, sulfate, and amino acids (e.g., taurine)) is covalently bound to a polar functional group on either the original chemical (e.g., pentachlorophenol has an –OH group), or one of its metabolites (e.g., the phase I metabolite of the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene, 3-OH B[a]P) producing a phase II metabolite which is very water soluble and easily excreted. Phase II metabolites are usually less toxic than the parent molecule. Even so, the biotransformation of many chemicals via phase I reactions can result in more toxic metabolites, a process called 'bioactivation'. For example, the PAH B[a]P is metabolized to B[a]P 7,8-dihydrodiol-9,10-epoxide which is the ultimate carcinogenic form of this PAH and responsible for increased neoplasia found in flatfish associated with PAH-contaminated sediments (**Figure 3**). The bioconcentration and biomagnification of high K_{ow} contaminants in fish (e.g., methylmercury, OC pesticides, and PCBs) are due in part to their recalcitrance to biodegradation by this process.

Biotransformation capability can be enhanced by prior exposure to some contaminants in a process called 'induction'. For example, P450 isoforms in the 1A (CYP1A) subfamily are inducible by planar PAH and halogenated aromatic hydrocarbon (HAH), including chlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans. Induction of cytochrome CYP1A is controlled by the Ah receptor (AhR), a soluble protein with a high binding affinity for planar PAH and HAH. The aryl hydrocarbon receptor nuclear translocator (ARNT) and the AhR together form a transcription factor complex through which altered gene expression and toxicity occur. Their dimerization is dependent upon high-affinity binding of planar PAH and HAH to the AhR. The AhR:ARNT complex then binds specific, *cis*-acting DNA elements, altering the transcription of target genes, including phase I and phase II detoxifying enzymes, growth factors and their receptors, and numerous unidentified genes. In addition to the AhR, other factors such as temperature, hormonal status, stress, and the presence of inhibitors may influence the expression and function of fish biotransformation enzymes.

Chemical Excretion

The main excretory routes in fish for organic xenobiotics mentioned above include the branchial, urinary, and fecal routes. High K_{ow} compounds are not easily excreted via any of these routes because they are water based. However, some ionizable organics (e.g., chlorinated

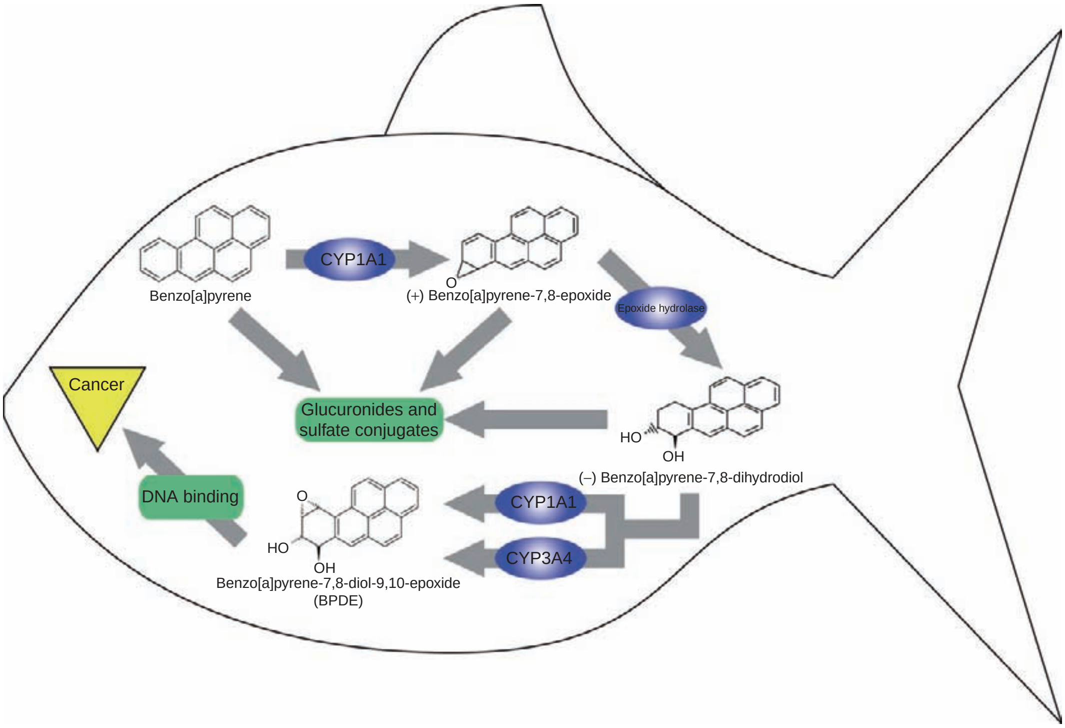

Benzo[a]pyrene
CYP1A1
O
(+) Benzo[a]pyrene-7,8-epoxide
Epoxide hydrolase
Cancer
Glucuronides and sulfate conjugates
HO
OH
(–) Benzo[a]pyrene-7,8-dihydrodiol
DNA binding
O
HO
OH
Benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE)
CYP1A1
CYP3A4

Figure 3 Biotransformation can bioactivate compounds such as benzo[a]pyrene to reactive metabolites. B[a]P metabolites can bind to DNA and result in neoplasm formation in fish.

phenols) may be excreted via the gills and kidney. As undissociated molecules, they may pass through epithelial membranes, becoming ionized (and hence water soluble) in the external microenvironment of the gill or in urine, depending on pH of the aqueous medium. Few organic chemicals or their metabolites are excreted via the gills other than by this mechanism.

Most elimination of organic compounds or their biotransformed derivatives occurs via the kidneys and hepatobiliary route. In teleost kidney tubules and in hepatocytes, as well as other tissues, a number of xenobiotic (or metabolite) export pumps exist, which result in efficient vectorial transport of their substrates from the basolateral (facing extracellular fluid) to the luminal side of cells (facing canaliculi or lumen for excretion into bile or urine). These pumps include members of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of proteins (**Figure 4**). The ABC family of membrane proteins accepts substrates with an unusually wide range of structural features. *p*-Glycoprotein accepts primarily cationic compounds. Multidrug resistance proteins (Mrps) accept anionic compounds, and both accept uncharged molecules and some polypeptides. The expression of xenobiotic efflux pumps in kidney and liver can be upregulated several fold over a period of days to weeks in response to a number of signals, including, substrates, toxicants, and heat stress. At the basolateral membrane are members of the solute carrier transport superfamily of proteins, the organic anion transporters (Oat1 and -3) and organic cation transporters (Oct2), as well as efflux pumps from the Mrp subfamily (Mrp1). Oat1 exhibits a broad substrate specificity and is responsible for the cellular uptake of most amphiphilic organic anions. High levels of expression of Mrp2, multidrug resistance (MDR) or *p*-glycoprotein, and organic cation/carnitine transporter (OCTN1) are seen in barrier and excretory tissues, for example, intestine, blood–brain barrier, liver and renal proximal tubule, and are targeted to the luminal membrane, which puts them in the right location to limit xenobiotic entry into the body and brain and to drive excretion into bile and urine.

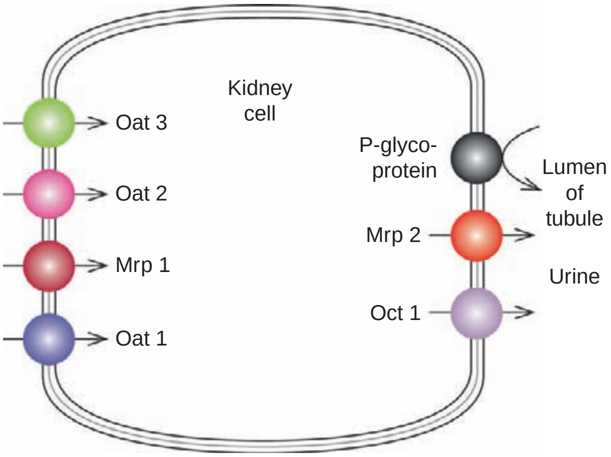


Figure 4 Various transport proteins are involved in excretory tissues or tissues in contact with the environment. Here, several known transporters in fish are shown on the basolateral side of the cell facing the extracellular fluid or blood (organic anion transporters (Oat) and multidrug resistance protein (Mrp)) and on the luminal side of the cell facing the environment (in this case, the lumen of the kidney tubule containing urine; organic cation transporter (Oct), Mrp, and P-glycoprotein).

Multixenobiotic resistance (MXR) is a phenomenon that increases the potential for survival of fish in polluted environments through enhanced expression of one or more MDR-like transport mechanisms that restrict xenobiotic uptake. Protein analysis has indicated the presence of proteins immunologically related to *p*-glycoprotein in tissues from marine and freshwater fish. Such transport systems are present at some basal level in animals maintained in unpolluted environments and they appear to be upregulated on exposure to pollutants.

Specific Organic Contaminants

Pesticides

Pesticides can be broadly classified as insecticides, herbicides, and fungicides. Insecticides fall into three major groups: the OCs, organophosphates (OPs), and carbamates (**Figure 5**). Legacy OCs include DDT, chlordane, dieldrin, aldrin, endrin, heptachlor, lindane, methoxychlor, and toxaphene (**Figure 5**). OC insecticide action is through disruption of the sodium channels of neurons, resulting in continuous firing of nerves and paralysis. Their lethal concentrations (LCs) vary greatly, and they have been phased out because of their high toxicities (teleost LC_{50} values in low $\mu g\ l^{-1}$), persistence, and potential to bioaccumulate. OC pesticides, such as DDT, are extremely persistent and biomagnify; DDT and its metabolite DDE can act as endocrine disruptors, interfering with hormonal function of estrogen, testosterone, and other steroid hormones, in fish and vertebrate predators.

OPs and carbamates have largely replaced OC pesticides due to their lowered persistence. Their mechanism of action is to inhibit acetylcholinesterase, allowing the neurotransmitter acetylcholine in cholinergic nerves to continue stimulating postsynaptic nerves indefinitely, causing muscle weakness or paralysis. OPs are less toxic to fish (LC_{50} values in fish in the mg l^{-1} range) than OCs, but have in some cases been replaced by less toxic carbamates.

All of the above insecticides, including others in the botanical (e.g., pyrethroids) class, as well as herbicides and fungicides, have molecule-specific effects. The effects can have a variety of target sites and toxicities; for example, hematological, gas exchange, olfactory (**see also Hearing and Lateral Line**: Lateral Line Structure), ionoregulatory and osmoregulatory, carbohydrate metabolism, the immune system, as well as neurological effects

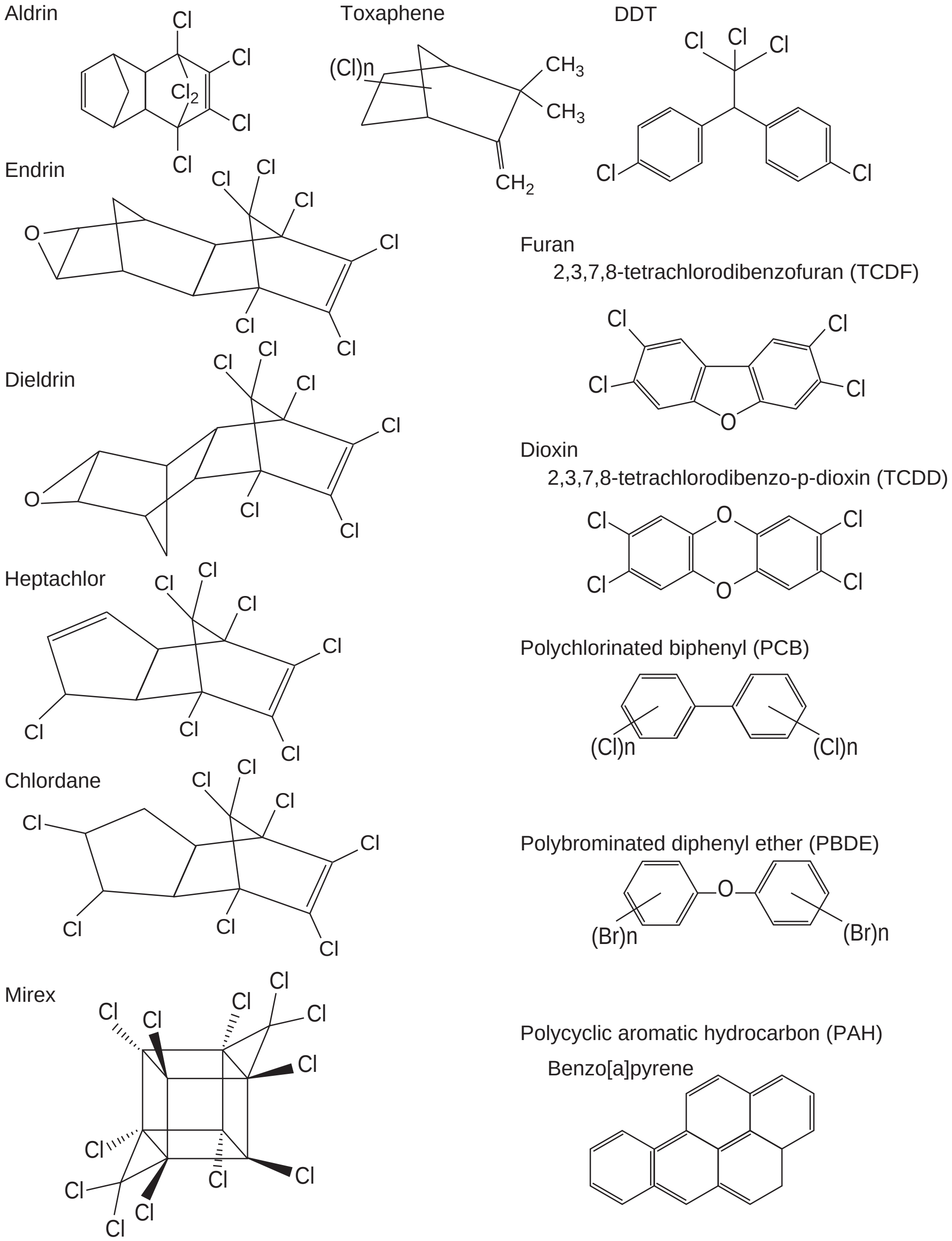

Aldrin
Toxaphene
(Cl)n
CH3
CH3
CH2
DDT
Endrin
Furan
2,3,7,8-tetrachlorodibenzofuran (TCDF)
Dieldrin
Dioxin
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
Heptachlor
Polychlorinated biphenyl (PCB)
(Cl)n
(Cl)n
Chlordane
Polybrominated diphenyl ether (PBDE)
(Br)n
(Br)n
Mirex
Polycyclic aromatic hydrocarbon (PAH)
Benzo[a]pyrene

Figure 5 Structures of some important (present and historical) organic compounds including the pesticides aldrin, endrin, dieldrin, heptachlor, chlordane, mirex, toxaphene, and DDT, as well as other persistent organic pollutants, furans, dioxin, PCB, and PBDE. A representative PAH, benzo[a]pyrene is also shown.

including impaired learning behaviors, slowed reflexes, and reductions in reproductive success in fish.

Regulatory attention has resulted in bans against many legacy pesticides and restricted the use of many OPs and carbamates, and has led to the development of new pesticides with less persistence and more target specificity. This group of newer compounds is called current-use pesticides (CUPs). Concerns with CUPs include high usage, the prevalence of these chemicals in the environment at low levels, unknown effects on nontargets, and concerns of synergism between compounds. To date, several significant sublethal effects have been demonstrated with CUPs in fish, including moderate immunomodulation, endocrine disruption, energetic costs, and effects on olfaction.

Polycyclic Aromatic Hydrocarbons

One of the most ubiquitous and important classes of environmental contaminants are the PAHs, which consist of two or more fused benzene rings which may have alkyl side groups containing nitrogen, sulfur, and oxygen (**Figure 5**). These contaminants are produced mainly as a result of incomplete combustion of fossil fuels or biomass, as well as being constituents of petroleum. PAH environmental distribution and toxicity are structurally dependent and although there is a wide range of water solubilities of PAHs, they are generally considered to be a hydrophobic class. The degree to which fish accumulate various PAHs from water, sediment, and food depends upon the physical and chemical properties of the PAH (e.g., size and log K_{ow}), environmental conditions (e.g., dissolved oxygen content (DOC), and biological factors (PAH metabolism and depuration rates, feeding characteristics of organisms, fat content of tissue, life stage, etc.)) PAHs generally do not bioconcentrate or biomagnify in fish due to extensive metabolism.

In general and with some exceptions, fish appear to be the most sensitive of aquatic organisms to PAHs. The acute toxicity of PAHs varies greatly with the molecule, but generally short-term effects are due to low MW PAHs (LPAHs), with toxicity increasing with increasing substitution on the molecule. The minimum LC_{50} for LPAHs is $30\,\mu g\ l^{-1}$ for phenanthrene in rainbow trout (*Oncorhynchus mykiss*). LPAHs are relatively more soluble in water than high MW PAH (HPAH) and, therefore, LC_{50} values for HPAHs may not reflect reality because of their low solubility. Alkyl homologs of PAHs are generally more toxic to fish than the parent compound. Several PAHs, accumulated by aquatic organisms during exposure, have been shown to be severely toxic when the contaminated organisms were exposed to sunlight or ultraviolet radiation. The phototoxicity of a PAH is a function of the efficiency of conversion of ground-state molecules to the excited triplet state and the probability of the excited intermediate reacting with a target molecule.

PAHs have common and chemical-specific chronic mechanisms of action (e.g., activation of the AhR pathways) underlying their toxicity. The toxicological significance of environmental contamination of PAHs depends in large part on the metabolic fate of the compounds inasmuch as metabolism can lead either to the formation of toxic and carcinogenic intermediates or to detoxification. A wide variety of chronic effects have been reported for PAH exposure in fish. LPAHs are known irritants, and can stimulate the ascending neural pathways of the hypothalamus–pituitary–interrenal (HPI) axis and cause a stress response. In addition, these LPAHs can cause gill hyperplasia and other morphological effects. PAHs can also affect the functioning of gills by causing ionoregulatory disturbances. Effects of PAHs in larval and juvenile fish include morphological (e.g., necrosis of brain and spine), histopathological, and genetic damage, as well as affecting development (e.g., arrested development and reduced hatching success). PAHs in the $\mu g\ l^{-1}$ range can affect immune system components and pathogen resistance. PAHs have been shown to reduce the swimming ability of fish and potentially reduce marine survival in salmonids. Some PAHs may act directly as endocrine disruptors, targeting multiple sites in the HPI axis including the perception of stimuli, the pituitary or adrenocortical tissues, the synthesis and secretion stress hormones (e.g., cortisol), and target tissue responsiveness to glucocorticoid stimulation.

Some HPAHs are mutagenic, carcinogenic, and inducers of tumors in fish. During biotransformation, some PAHs are converted to arene oxide intermediates, which can be carcinogenic and/or mutagenic. Fish collected from PAH-contaminated marine areas show a high incidence of hepatic lesions including neoplasm.

Dioxins

Dioxins are organic chemicals that contain 210 structurally related individual PCDDs and PCDFs. They are produced in the incineration of chlorine-containing substances, chemical and pesticide manufacturing, and in the bleaching of paper (**Figure 5**). These chemically related compounds vary in their physical and chemical properties and toxicity; however, generally, higher chlorination leads to lower degradation rates, higher partitioning to sediments and biota, and increased toxicity (the most toxic is 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD)). Dioxins are highly persistent in the environment with reported half-lives in sediment ranging from months to years. Bioaccumulation factors vary among the congeners and generally increase with chlorine content up through the tetra congeners and then generally decrease with higher chlorine content. For example, bioconcentration factors (BCFs) for TCDD in fish ranges from 37 900 to 128 000, whereas BCFs for the octo-chlorinated dibenzo-*p*-dioxin (OCDD) congeners range from 34 to 2226.

Fish, as oviparous vertebrates, are particularly sensitive to the developmental effects of dioxin. This fact has led to reduced and, in some cases, failed recruitment of young fish into breeding populations.

The mechanism of action for dioxin and dioxin-like compounds is mainly through activation of AhR and subsequent transcriptional upregulation of the AhR gene battery that includes CYP1A. However, the role of these genes in dioxin-induced toxicity is still not well understood. Blue sac disease is a syndrome in fish embryos and larvae that includes induction of CYP1A enzymes, yolk sac and pericardium edema, hemorrhaging in the head and tail regions, craniofacial deformities, a wasting syndrome, and early larval mortality, which are seen in TCDD and other chlorinated, planar aromatic compound exposures. This toxicity may begin by AhR-mediated CYP1A induction and its increased activity, resulting in an increase in reactive oxygen species (ROS) through an inefficient coupling of the P450 complex with nicotinamide adenine dinucleotide phosphate (NADPH)-P450 oxidoreductase or cytochrome b_5. Enhanced localized oxidative stress in the cardiovascular system, and in particular the vascular endothelium of the developing embryo, leads to an increase in membrane permeability, ischemia, and circulatory failure. This mechanism has been identified as a primary target of dioxin-induced toxicity. Events such as apoptosis in the digestive tract, gills, and neural tissues and cells lead to a myriad of secondary effects in various tissues affected by dioxins which, in turn, may lead toward eventual toxicity and death.

Polychlorinated Biphenyls

PCBs (commonly called Aroclors) are organic compounds with 1–10 chlorine atoms attached to biphenyl (209 distinct congeners, **Figure 5**). They were widely used as dielectric fluids in transformers, capacitors, and coolants. PCBs are still prevalent environmental contaminants worldwide, even though they were banned in most countries in the 1970s and prescribed by the Stockholm Convention in 2001.

PCB persistence, biomagnification potential, and toxicity are all congener specific. PCBs bioconcentrate and biomagnify to a high degree (maximum BCF was 975 000 when expressed on whole fish basis). Aroclors containing 42–54% chlorine appear to be the most acutely toxic formulations of PCBs with values ranging from 100 to 3000 $\mu g\ l^{-1}$. Data suggest that PCBs are as toxic to marine organisms as they are to freshwater organisms.

Coplanar PCBs can mimic the effects of dioxin as agonists of the AHR (those halogenated in meta- or para-positions are among the most toxic PCBs), and the similarity of certain congeners to biological ligands of steroid nuclear receptors can result in endocrine system disruption. Select PCB congeners can produce (although less than dioxins) fish early-life-stage mortality associated with yolk sac edema and hemorrhaging (blue sac disease). Because only a small portion of the total mass of PCB mixtures contains coplanar congeners that elicit AhR-mediated dioxin-like effects, it is possible that non-dioxin-like effects may also play a role in PCB toxicity through various mechanisms. While environmental levels of PCBs do not produce overt lethality in adult fish, however, their presence in combination with other chlorinated dioxins and furans poses an increased risk to early-life-stage survival of many fish species. Reproductive abnormalities induced by PCB exposure include altered sex ratios, testicular abnormalities such as disorganization of spermatogenic elements and inhibition of spermatogenesis, altered steroid biosynthetic patterns, reduced fertilization, reduced egg production and limited development of eggs, and complete reproductive failure. Other effects in fish include formation of neoplasms, as well as biochemical and cellular perturbations such as anemia, hyperglycemia, and altered cholesterol metabolism.

Chemicals of Emerging Concern

Polybrominated Diphenyl Ethers

PBDEs consist of two halogenated aromatic rings, and are classified according to the average number of bromine atoms in the molecule (**Figure 5**; 209 possible congeners). They are used as flame retardants in a wide array of products including plastics, textiles, polyurethane foam, and other materials. PBDEs have emerged as a major environmental pollutant. They are similar in structure to PCBs and resist degradation in the environment, and have been detected in fish that feed in offshore waters. This indicates that they have become incorporated into pelagic and deep-sea food webs. The toxicology of PBDEs is not well understood, but PBDEs have been associated with tumors, neurodevelopmental toxicity, and thyroid hormone imbalance. The toxicology of PBDEs to fish is not well understood. However, PBDEs are believed to bind to the same cellular receptors and to have toxic effects that are similar to those reported for the structurally related PCBs. PBDEs possess endocrine-disrupting properties; hydroxy-PBDE congeners have structural similarities with the thyroid hormones and short-term exposure to less-brominated PBDE congeners may interfere with thyroid function and disrupt hormonal balance. Embryonic exposure can result in the development of morphological, cardiac, and neural deficits that impair survivorship for fish larvae. Behavioral effects include irritation, twitching, abnormal swimming behavior, and labored respiration. Some species show reduced survival of young at hatch and reduced growth and condition, as well as reproductive effects including lower sperm production. However, the concentrations of PBDE required to cause most of the above effects in fish were considerably higher than recorded tissue or environmental concentrations. Therefore, the risk of PBDE contamination to fish while currently unknown may be low.

Pharmaceuticals and Personal Care Products

Pharmaceuticals and personal care products (PPCPs) are bioactive chemicals that include prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, cosmetics, sun-screen products, and diagnostic agents and nutraceuticals (e.g., vitamins) from a variety of sources including human activity, residues from pharmaceutical manufacturing, residues from hospitals, illicit drugs, veterinary drug use especially antibiotics and steroids, and agribusiness. PPCPs can enter aquatic environments through human and animal excretion as well as disposal of unwanted medications, some of which are not amenable to wastewater treatment processes. Natural and synthetic estrogens from the contraceptive pill are an example of a PPCP that has effects on fish populations at concentrations found in the environment, including increased feminization of males, intersex, hermaphroditic fish, female-skewed sex ratios, increased vitellogenin synthesis, arrested reproductive development in males, reduced fecundity, and induction of developmental abnormalities.

Perfluorooctane Sulfonate

Perfluorooctane sulfonate (PFOS; **Figure 5**) has been manufactured for over 50 years and has been used for several industrial and commercial purposes including fire retardants and stain-resistant treatments. Fluorinated organic compounds such as PFOS have not received due toxicological attention because their measurement is difficult. Recently, it has been reported that PFOS is widely distributed on a global scale and can be persistent and bioaccumulative and can be found worldwide in wildlife. Related perfluorinated compounds have been shown to affect cell–cell communication, membrane transport and process of energy generation, and peroxisome proliferation. In fish, PFOS can cause inflammation-independent leakage of liver cells and disturbance of DNA metabolism homeostasis in liver tissue, as well as an increase in hepatic fatty acyl-coenzyme A (CoA) oxidase activity, and oxidative damage. PFOS may also be an endocrine disruptor, and has been shown to alter steroid hormone and vitellogenin concentrations in plasma, and may be responsible for reduced fecundity and effects on ovaries.

See also: **Hearing and Lateral Line**: Lateral Line Structure.

Further Reading

Arnot JA and Gobas FAPC (2004) A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environmental Toxicology and Chemistry* 2: 2343–2355.

Bard SM (2000) Multixenobiotic resistance as a cellular defense mechanism in aquatic organism. *Aquatic Toxicology* 48: 357–389.

Cantrell SM, Lutz LH, Tillitt DE, and Hannink M (1998) Correlation of 2,3,7,8-tetrachorodibenzo-p-dioxin-induced apoptotic cell death in the embryonic vasculature with embryotoxicity. *Toxicology and Applied Pharmacology* 148: 24–34.

Gray JS (2002) Biomagnification in marine systems: The perspective of an ecologist. *Marine Pollution Bulletin* 45: 46–52.

Heath AG (1995) *Water Pollution and Fish Physiology*. Boca Raton, FL: CRC Press.

Hodgson E and Levi PE (1994) *Introduction to Biochemical Toxicology*. Norwalk, CT: Appleton and Lange.

Kannan K and Geisy JP (1998) Dioxin-like and non-dioxin-like effects of polychlorinated biphenyls (PCBs): Implications for risk assessment. *Critical Reviews in Toxicology* 28: 511–569.

Kennedy CJ and Farrell AP (2006) Effects of exposure to the water-soluble fraction of crude oil on the swimming performance and the metabolic and ionic recovery post-exercise in Pacific herring. *Environmental Toxicology and Chemistry* 25: 2715–2724.

Klaasen CD, Amdur MO, and Doull J (2001) *Casarett & Doull'sToxicology: The Basic Science of Poisons*. New York, NY: Macmillan.

Kurelec B (1992) The multixenobiotic resistance mechanism in aquatic organisms. *Critical Reviews in Toxicology* 22: 23–43.

Mackay D (1995) *Multimedia Environmental Models: The Fugacity Approach*, 2nd edn. Boca Raton, FL: CRC Press.

Malins DC and Ostrander GK (eds.) (1994) *In Aquatic Toxicology Molecular, Biochemical, and Cellular Perspectives*. Boca Raton, FL: CRC Press.

Varanasi U (1989) *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*. Boca Raton, FL: CRC Press.

Relevant Websites

http://ceqg-rcqe.ccme.ca – Canadian Council of Ministers of the Environment (CCME).http://www.env.gov.bc.ca – Environmental Protection Division; Water Quality Guidelines (Criteria) Reports.http://www.epa.gov – US EPA; Region 5 Superfund; Ecological Toxicity Information.

The Effects of Toxicants on Olfaction in Fishes

KB Tierney, University of Alberta, Edmonton, AB, Canada

© 2011 Elsevier Inc. All rights reserved.

Introduction
Odorants, Contaminants, and Their Relevance to Physiological and Behavioral Response
Olfactory–Toxicity Interactions
Future Studies
Further Reading

Glossary

Ecological death Death attributed to natural processes such as predation; a result that contaminant exposure may increase the probability of.

Electro-olfactogram (EOG) The summed electrical response recorded from a group of receptor cells in response to an odorant stimulus.

Generator potential Local membrane depolarization in neurons, including OSNs, that may lead to an action potential.

Local effect Describes an effect that is limited to the site of application.

Pesticide A catchall term for agents/compounds that are intended to remove undesirable organisms; subcategories include herbicides, insecticides, and fungicides.

Pharmaceuticals A catchall term for agents/compounds that are intended to be of human medical use, such as fluoxetine.

Systemic effect Describes an effect that occurs spatially removed from the application site.

Introduction

At the headwaters of world renowned salmon runs in British Columbia, Canada, local fishermen offer testimonials of capturing returning adult fish that were completely blind. Without question we know that Pacific salmon rely on their sense of smell, that is, olfaction, to navigate much of their return river migration. However, salmon are not alone in their olfactory dependence; since discovering this remarkable ecophysiological phenomenon nearly 50 years ago, olfaction in fishes is now known to be necessary for migration of other fish species, for detecting predators, for recognizing related and unrelated fishes, for locating food, and for synchronizing the release of egg and sperm (**see also Smell, Taste, and Chemical Sensing**: Chemosensory Behavior). All of these indispensible behaviors are predicated on the functionality of the relatively unprotected olfactory sensory neurons (OSNs) of the nose. What a growing body of research demonstrates is that these neurons are not only exquisitely sensitive to dissolved odorants, but that they are also highly susceptible to impairment by exposure to the ever-increasing number of aquatic contaminants introduced into the aquatic ecosystem by humans. With unknown numbers of ecologically, culturally, and commercially invaluable fish stocks currently at risk for extinction, the need to understand the impact that contaminants make on olfactory-mediated behaviors has never been greater.

Understanding the ways that contaminants alter olfaction is challenging. The olfactory system is very complex and inextricably integrated into other suborganismal systems (**see also Smell, Taste, and Chemical Sensing**: Chemoreception (Smell and Taste): An Introduction). For this reason, isolating olfactory effects from others or determining the proportion of olfactory effects contributing to effects observed at the organismal level may not always be possible. Confounding all of this is that the mechanisms by which the majority of contaminants alter or interact with OSNs remains unknown. Nevertheless, an increasing number of techniques exist to investigate the effects and their mechanisms at organizational levels from the molecular to the behavioral. A growing field of research endeavor is the mechanistic linking of impaired enzyme expression/activity with behaviors that are relevant to survival. This article provides a succinct review of the relevant mechanics of olfaction, followed by a discussion on the known routes and examples by which contaminants affect olfaction and downstream responses, and finally directions are provided for future research.

Odorants, Contaminants, and Their Relevance to Physiological and Behavioral Response

Fishes live in a mosaic of dissolved compounds. The process of olfaction of a dissolved compound, that is, an odorant, begins with its binding to protein receptors at the apical surface of OSNs. The number of odorants is near limitless; the way in which they are perceived is not. Olfaction of chemical signals occurs through just 100–150 different distinct receptors, one type per OSN. There are three morphologically distinct OSN classes: ciliated, microvillar, and crypt. Each has specific types of receptor proteins. Ciliated OSNs express odorant receptor and vomero-nasal receptors, which bind amino acids and bile salts, respectively. Microvillus OSNs express vomero-nasal receptors and bind amino acids and nucleotides. Crypt OSNs express vomero-nasal receptors and may bind pheromones and/or amino acids. All OSNs transduce odorants using G-protein-coupled receptors (GPCRs), although the second messenger systems differ. In ciliated OSNs, G-proteins stimulate adenylate cyclase (AC) to produce cAMP, whereas in microvillar and crypt OSNs G-proteins stimulate phospholipase C to produce IP_3. In all OSNs, the second messengers act to open cation channels in the cell membrane, which then cause the membrane potential to depolarize. If the depolarization is sufficient (a generator potential), an action potential will be generated in an olfactory nerve and will be conducted back to the olfactory bulb (OB) for the first level of signal integration.

Second-order neurons in the OB may then conduct the signal to the higher brain (telencephalon, TE) for further processing and output, which may consist of physiological responses, such as hormone release or swimming behavior. (For more detailed information on fish olfaction, **see also Smell, Taste, and Chemical Sensing**: Morphology of the Olfactory (Smell) System in Fishes and Neurophysiology of Olfaction.)

The dissolved mosaic of compounds now includes many of human origin. As with all other life, fishes are adapted to the historic environment and its natural odorants and toxins. Humans have rapidly introduced pesticides, pharmaceuticals, and high concentrations of metals, to name just a few of the many types of contaminants. Our contributions are numerous and global, with contaminants reaching even the most remote and formerly pristine waterways. Just south of Alaska, for example, six classes of pesticides were found in a watershed virtually free of development; their presence can only be attributed to either aerial deposition or import courtesy of migratory fishes. In streams proximal to human activities, complex contaminant mixtures can consist of more than 50 compounds. This means that there is probably not a fish on the planet that will not be exposed to a synthetic contaminant that its olfactory machinery has been designed for, nor as of yet has had time to adapt to. Ideally, fishes that sense that they are in polluted water should move to other areas, and this has been shown in some cases for metals. However, migration may not be an option for some fishes (such as those that live in lakes), and there is no reason to expect that all contaminants will be perceived as noxious. In fact, some contaminants elicit behavioral attraction responses. Alarmingly, some fishes show an attraction response to toxicants. In other words, given the choice, fish may elect to put themselves in situations that will have negative fitness consequences.

The effects of the vast majority of aquatic contaminants on fish olfaction are unknown. Even when known for a specific compound, the effects of exposure to mixtures of compounds, as that they typically exist in, remain unknown. Furthermore, within a class of structurally similar compounds (e.g., carbamate insecticides), toxicities can differ considerably (e.g., >100-fold). Nevertheless, there are essentially four ways by which introduced agents can affect olfaction and its derived responses: (1) acting as odorants; (2) affecting the perception/reception of odorants; (3) indirectly altering neural circuitry associated with the processing of olfactory signals; and (4) impairing the functionality of processes necessary to an olfaction-based response, such as swimming ability (**Figure 1**). An ultimate goal of studies on olfaction and contaminants is to determine the relevance of the olfactory impairment to the survival of fishes in the environment.

Contaminants as Odorants

Generator potential responses of OSNs to natural odorants such as amino acids can be recorded with the help of small, sensitive electrodes placed immediately above the surface of the olfactory epithelium of a living fish (see **Figure 2**). The generator potentials, recorded as electro-olfactograms (EOGs), resemble action potentials, but are in fact the summed electrical responses of several neurons.

Contaminants can also generate EOGs, which means they would ultimately be perceived by the fish as odors. However, contaminants can generate EOGs through two routes: one analogous to odorants – by binding to odorant sites on GPCRs – and another not – by activating proteins associated with signal transduction (e.g., AC). In the former route, many contaminants have molecular components structurally similar to natural odorants, and so may bind existing GPCR-binding sites. One of the most widely used herbicides, Roundup®, contains an active ingredient (glyphosate) that evokes EOGs similar in magnitude to those of the amino acid L-serine (on a mass to mass basis). An OSN response to this active ingredient would not be unexpected since glyphosate is based on the amino acid glycine. As mentioned above, amino acids can activate both ciliated and microvillar OSNs. In contrast, contaminants may have no clear resemblance to amino acids, such as bile salts and pheromones, but can generate EOGs all the same, such as the insecticide carbaryl. Whether any given contaminant acts through GPCR binding, generator potential responses

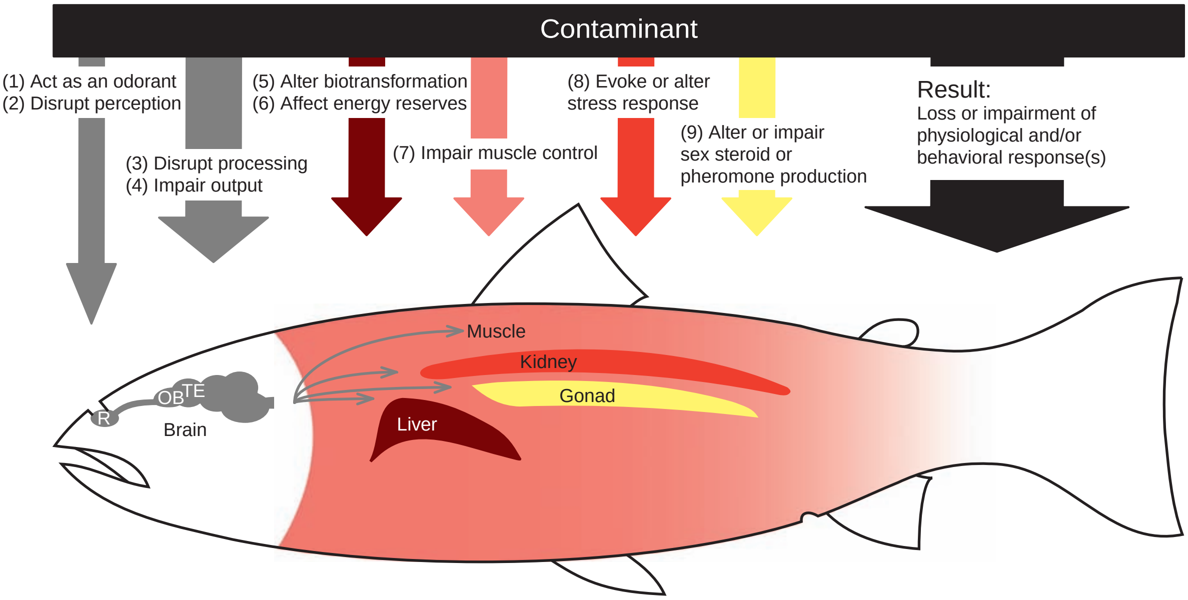


Figure 1 There are numerous routes by which contaminants can affect olfaction and downstream responses in fishes. The effects span from acting and competing with natural odorants, to altering the production of sex pheromones, to impairing behaviors critically important to survival, such as predator avoidance. OB, olfactory bulb; R, olfactory rosette; TE, telencephalon.

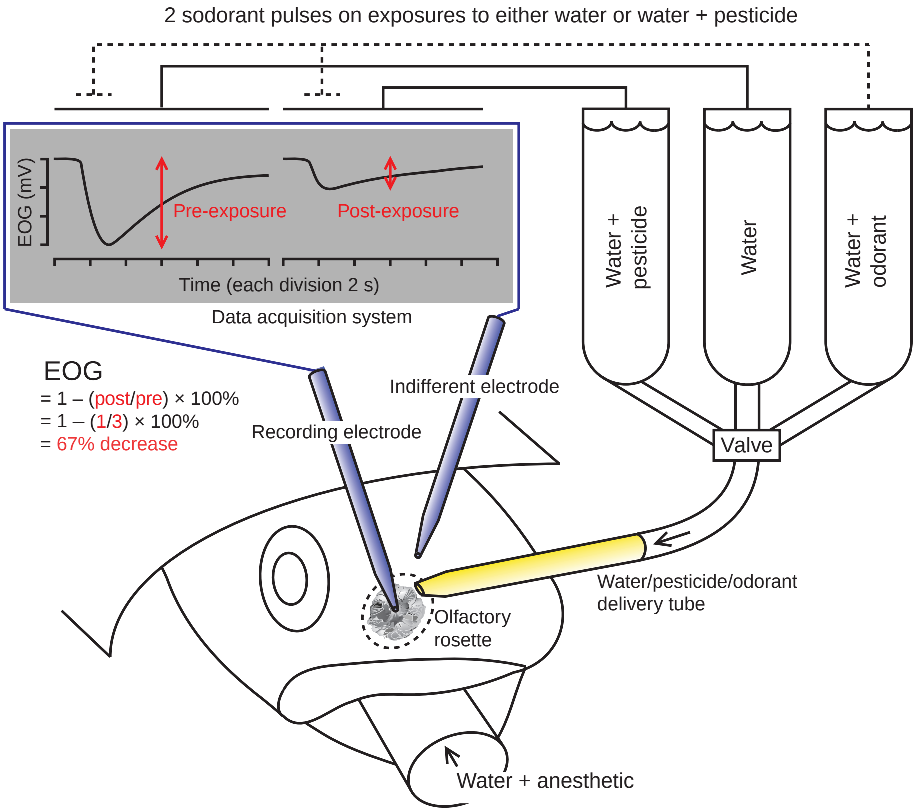


Figure 2 One of the best ways to determine whether contaminants affect olfactory neurons is to expose the olfactory tissue of anesthetized fish to contaminants and then pulse a known odorant of survival relevance over the tissue and measure responses. Decreased responses indicate olfactory impairment.

may be conducted back to the brain, and evoke physiological and/or behavioral responses. An issue is that contaminants that act through binding the odorant (i.e., ligand) site of a GPCR may also have added effects. Many contaminants have reactive, functional groups that may bind sites on the GPCRs or associated transduction machinery, and thereby inhibit/alter their functionality.

Contaminants That Impair OSNs

Being as exposed as they are, OSNs are open to a direct physical assault from waterborne compounds. Many contaminants affect OSNs structurally, even at the molecular level. These effects can occur very rapidly (<30 s) and persist (>20 min). Typically, impairments of this nature alter OSN responses to natural odorants such as amino acids (which have food, predator, and home stream olfactory bouquet (HSOB) relevance) and bile salts (which have social relevance). Therefore, impacts can be investigated by exposing either the isolated olfactory tissue (*in situ* preparations) or the whole animal (in tanks), and then recording the responses of OSNs to natural odorants (**Figure 2**). Exposure to altered pH, metals, pesticides, and other contaminants may all reduce OSN responses to amino acids.

Potent OSN toxicants include copper and certain pesticides, with impairment thresholds in the range of parts per billion (ppb) and lower. Copper was one of the very first contaminants investigated in this regard, and investigation continues owing to its release through mining activities and brake dust. Just a 30 min exposure to 5 ppm copper can reduce EOG response to L-serine (a predator scent) by as much as 50%. Synthetic compounds can have lower toxicity thresholds; OP and fungicide concentrations of ≤ 1 ppb can decrease L-serine EOGs to the same degree. Certain OSN toxic contaminants such as OPs have reactive functional groups, in this case phosphate groups (PO_4), that are known to bind with and inhibit enzymes, such as acetylcholinesterase (AChE). Although AChE may not be directly involved with olfaction, its functionality may be important to OSN function.

As may be expected, not all OSN types are equally sensitive to contaminants. The herbicide linuron, for example, reduces the L-serine EOGs, but not those for a bile salt (taurocholic acid, TChA). Differential impact on OSNs means that some behaviors may be selectively affected by contaminants. In this example, fish passing through a linuron-contaminated environment might not be as adept at avoiding predators, but would have no problem identifying kin. Overall, these types of effects are alarming since concentrations of these contaminants have been found repeatedly in fish bearing waterways.

OSNs can defend against contaminant insults. Chemical detoxification systems in OSNs and in the supporting tissues involve phase I and II detoxification enzymes that act to break up and/or solubilize compounds reaching and/or penetrating the olfactory epithelium. The expression of phase I and II enzymes can be induced by contaminant exposure; however, there is no guarantee that expression will be sufficient to stave off injury. At least one recent publication found that activity increases in a class of enzymes that solubilize compounds in order to facilitate their removal (glutathione S-transferase, GST) were insufficient to prevent OSN functionality impairments from realistic contaminant concentrations.

Contaminants That Affect Abilities Necessary to Olfaction-Based Responses

There are numerous routes by which contaminants can alter olfactory-based responses in addition to impairing OSNs. Many contaminants are readily miscible in water and are taken up by fishes (by gills, gut, and other routes). With uptake, contaminants may cause systemic effects through distribution beyond a local effect on OSNs.

Neurotoxic contaminants are prime candidates for causing systemic effects. The uptake of neurotoxins may impact the ability of the brain to process olfactory cues, affecting its ability to integrate olfactory responses with other external and internal signals of survival relevance as well as impairing the fish's ability to control muscle (**Figure 1**). Esfenvalerate (a pyrethroid) and chlorpyrifos (an OP) are two example neurotoxic contaminants that cause systemic effects. The pyrethroids impair the closing of the voltage-gated sodium channels that are critical for the generation of neuronal action potentials. As mentioned above, the OPs inhibit AChE, slowing the degradation of the neurotransmitter acetylcholine (ACh), which is involved in neuronal synapses in the brain as well as the neuromuscular synapses of skeletal, smooth, and cardiac muscles.

With the impairment of responses known to involve olfaction, a critical question is whether the downstream, systemic effect is more or less sensitive than the OSN local effect. Consider either impaired OSN responses or a change in desire/ability could contribute to a decrease in food odor response. Chlorpyrifos, for example, decreases OSN responses to TChA and L-serine following exposure to ≥ 1 ppb and impairs white muscle performance at concentrations ≥ 10 ppb. These data clearly suggest that the OSN impairment and not other effects would be of more behavioral relevance. The opposite is true for the herbicide atrazine, a common groundwater contaminant. Typically, exposure to female urine will evoke a priming response in male salmon and other species triggering an increase in plasma testosterone. Atrazine exposures in the parts per trillion (ppt) range can impair this response in male salmon, whereas OSN responses are not altered until the ppb range. Clearly varying sensitivities of orders of magnitude make predicting olfactory-based exposure outcomes very challenging.

One of the most intriguing olfactory paradoxes exists for migratory salmonids. As juveniles, they imprint to HSOB, which is used later to assist adult river navigation. Many of the streams that juveniles live in are filled with contaminants, including the two identified above as being odorants. As they mature, they will move out to the potentially or relatively cleaner ocean water. However, at maturity, they will return to freshwater and seek out that imprinted HSOB. Unfortunately, some of the contaminants they may have imprinted to over months of residence years earlier may now impair their OSNs because with relatively rapid transition to the freshwater environment, they will not have the detoxification machinery ready for coping with the contaminants. Without correct olfactory information, their ability to make accurate behavioral decisions (i.e., choose the right home stream) may be impaired.

Olfactory–Toxicity Interactions

The responses that olfactory cues evoke depend on the state of the animal. Broadly speaking, the signal is defined by the receiver. Therefore, contaminants that change the state of the animal can change the nature of an olfactory response. Well-known examples are contaminants that disrupt the endocrine system. Consider a male fish exposed to feminizing contaminants such as 4-nonylphenol (4-NP) for extended periods may respond as female to male pheromones. Although this type of effect is not directly an olfactory effect, it is through olfaction that the effect may be behaviorally evident.

Even in the short run, contaminants can act through the endocrine system to affect odorant responses. In fishes and other vertebrates, stress hormones can be upregulated following exposures to toxic compounds such as pesticides. Sensory cues perceived under a stressful situation may have altered meaning, or lose meaning altogether. For example, when fish are exposed to alarm pheromone (extract from conspecific skin), they typically exhibit an alarm response consisting of freezing. With pesticide exposure, these behaviors can be lost or worse: alarm pheromones can make fish more active. Such aberrant behavior may not result in outright death, but it may increase the probability of ecological death, such as through predation. Given many contaminants have multiple mechanisms of toxicity, interactions between sensory impairment and other physiological effects are likely common.

Even compounds that do not have overt toxicity, such as some polycyclic aromatic hydrocarbons, may have ramifications all the same. As discussed earlier, for a fish to remove (biotransform) compounds from the olfactory epithelium, proteins such as GSTs need to be made and so energy and nutrients are required. Coping with contaminant insult may take energy away from various olfactory-related processes, such as pheromone production and gonadal maturation.

Future Studies

Olfaction is an external to internal signal relay system that spans organizational levels, each interfaced by thresholds. Contaminants can act on the exposed OSNs, on the brain, on physiological systems in diverse ways, and through multiple pathways, all with potential to abolish or cause aberrant behaviors. The state-of-the-art research in olfactory toxicity research is two pronged, and consists of in-depth investigations at relatively low and high biological organizational levels. In the former, to elucidate the mechanisms of toxicity, molecular tools such as gene set analysis are being used. With knowledge of which genes undergo expression changes following contaminant exposure, we will be able to target candidate protein receptors and cellular systems. In the latter, behavioral endpoints using high-throughput screening methods are now available, in part, due to the wholesale adoption of zebrafish models. With behavioral endpoints of survival relevance, we will be able to predict how subtle changes in organism performance may alter their survivorship in the environment. With an increasing number of contaminants, with unpredictable changes in environmental condition (such as temperature), and with invasive species, the challenge is to determine how olfaction will function to ensure the success of fishes in a modern ecology.

See also: **Smell, Taste, and Chemical Sensing**: Chemosensory Behavior; Morphology of the Olfactory (Smell) System in Fishes; Neurophysiology of Olfaction.

Further Reading

Baldwin DH and Scholz NL (2005) The electro-olfactogram: An *in vivo* measure of peripheral olfactory function and sublethal neurotoxicity in fish. In: Ostrander GK (ed.) *Techniques in Aquatic Toxicology,* vol. 2, pp. 257–276. Boca Raton, FL: CRC Press.

Evans RE and Hara TJ (1985) The characteristics of the electro-olfactogram (EOG): Its loss and recovery following olfactory nerve section in rainbow trout (*Salmo gairdneri*). *Brain Research* 330: 65–75.

Gilliom RJ, Barbash JE, Crawford CG, *et al.* (2006) *The Quality of Our Nation's Waters, Pesticides in the Nation's Streams and Ground Water, 1992–2001*, vol. 1291, 184pp. Reston, VA: USGS.

Hamdani EH, Lastein S, Gregersen F, and Døving KB (2008) Seasonal variations in olfactory sensory neurons fish sensitivity to sex pheromones explained? *Chemical Senses* 33: 119–123 (doi:10.1093/chemse/bjm072).

Hansen A and Zielinski B (2005) Diversity in the olfactory epithelium of bony fishes: Development, lamellar arrangement, sensory neuron cell types and transduction components. *Journal of Neurocytology* 34: 183–208.

Hara TJ (2005) Olfactory responses to amino acids in rainbow trout: Revisited. In: Reutter K and Kapoor BG (eds.) *Fish Chemosenses*, pp. 31–64. Enfield, CT: Science Publishers.

Hara TJ, Ueda K, and Gorbman A (1965) Electroencephalographic studies of homing salmon. *Science* 149: 884–885.

Harris KA, Dangerfield N, Woudneh M, *et al.* (2008) Partitioning of current-use and legacy pesticides in salmon habitat in British Columbia, Canada. *Environmental Toxicology and Chemistry* 27: 2253–2262.

Hino H, Miles NG, Bandoh H, and Ueda H (2009) Molecular biological research on olfactory chemoreception in fishes. *Journal of Fish Biology* 75: 945–959.

Robinson PD (2009) Behavioural toxicity of organic chemical contaminants in fish: Application to ecological risk assessments (ERAs). *Canadian Journal of Fisheries and Aquatic Sciences* 66: 1179–1188.

Sloman KA and Wilson RW (2006) Anthropogenic impacts upon behaviour and physiology. In: Sloman KA, Wilson RW, and Balshine S (eds.) *Behaviour and Physiology of Fish*, *Fish Physiology,* vol. 24, pp. 413–461. San Diego, CA: Elsevier.

Tierney KB and Kennedy CJ (2008) Background toxicology. In: Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele H, and Gerwick WH (eds.) *Oceans and Human Health: Risks and Remedies from the Seas,* vol. 1, ch. 6, pp. 101–120. New York, NY: Elsevier.

Tierney KB, Sampson JL, Ross PS, Sekela MA, and Kennedy CJ (2008) Salmon olfaction is impaired by an environmentally realistic pesticide mixture. *Environmental Science and Technology* 42: 4996–5001.

Tierney KT, Baldwin DH, Hara TJ, *et al.* (2010) Review: Olfactory toxicity in fishes. *Aquatic Toxicology* 96: 2–26 (doi:10.1016/j.aquatox.2009.09.019).

Tilton F, Tilton SC, Bammler TK, *et al.* (2008) Transcriptional biomarkers and mechanisms of copper-induced olfactory injury in zebrafish. *Environmental Science and Technology* 42: 9404–9411.

Vielma A, Ardiles A, Delgado L, and Schmachtenberg O (2008) The elusive crypt olfactory receptor neuron: Evidence for its stimulation by amino acids and cAMP pathway agonists. *Journal of Experimental Biology* 211: 2417–2422.

Zarzo M (2007) The sense of smell: Molecular basis of odorant recognition. *Biological Reviews* 82: 455–479.

Zielinski BS and Hara TJ (2007) Olfaction. In: Zielinski BS and Hara TJ (eds.) *Sensory Systems Neuroscience, Fish Physiology,* vol. 25,pp. 1–43. New York, NY: Elsevier.

Relevant Websites

http://www.ec.gc.ca – Environment Canada.

http://www.pesticideinfo.org – Pesticide Action Network (PAN) Pesticide Database.

http://extoxnet.orst.edu – The EXtension TOXicology NETwork (EXTOX NET).

http://www.epa.gov – United States Environmental Protection Agency (US EPA).

APPLIED ASPECTS OF FISH PHYSIOLOGY

Aquaculture

Physiology of Fish in Culture Environments

CA Myrick, Colorado State University, Fort Collins, CO, USA

© 2011 Elsevier Inc. All rights reserved.

Introduction
Bioenergetics
Temperature
Oxygen and Oxygen Demand
Carbon Dioxide and Acid–Base Balance
Osmoregulation, Salinity Tolerance, and Smoltification in Cultured Fishes
Exercise and Cultured Fishes
Further Reading

Glossary

Aquaculture The rearing of aquatic organisms under controlled or semi-controlled conditions; sometimes referred to as underwater agriculture.

Bioenergetics Quantitative analysis of the energetic processes of living organisms.

Smoltification Series of physiological and morphological changes that a fish may undergo when migrating from freshwater to seawater.

Specific dynamic action (**SDA**) The additional amount of oxygen or energy consumed above standard metabolic rate (SMR) as a result of feeding, measured during the period after feeding until metabolic rate returns to SMR; SDA represents the energetic cost of digestion, assimilation, and protein turnover.

Introduction

Aquaculture, the rearing of aquatic organisms under controlled or semi-controlled conditions, is practiced for a variety of reasons. These include production of (1) food fish, (2) fish for stock enhancement (e.g., stocking Pacific salmon, *Oncorhynchus* spp., in the Pacific Northwest region of North America), and (3) aquarium fish, as well as captive rearing of rare or endangered fishes, either for reintroduction programs or to establish refuge populations. Regardless of the final disposition of the fish reared in aquaculture, the success of the culture operation largely depends on the culturist's ability to understand and manage the physiological responses of the cultured fish to their environment.

Maximizing fish production (kilogram of fish produced per unit time or kilogram of fish produced per unit volume of water), while simultaneously maintaining acceptable fish health and quality, can be achieved by providing optimal physiological conditions for growth and performance. Although this sounds superficially simple, the conflicting demands of maximizing profit wherein more fish need to be raised in a fixed amount of time and fixed amount of water versus the ability of the culturist to provide physiologically optimal conditions in a culture system with a high loading density make profitable aquaculture a challenging endeavor. Achieving a profitable equilibrium between culture conditions and production becomes especially difficult when fish are being raised under intensive conditions where the loading or stocking density of fish (kilogram of fish per unit volume water or unit flow of water) is high enough to exceed the natural carrying capacity of the culture vessel. In the most extreme cases, fishes that are reared in recirculating aquaculture systems can be held at very high densities ($>100\,000\ \mathrm{kg\,ha^{-1}}$).

Just about any aspect of fish physiology could have an effect on the success of an aquaculture operation; however, there are six key areas that generally apply in most situations and for most fishes: bioenergetics, the effects of temperature, oxygen and oxygen consumption rates, carbon dioxide and acid–base balance, osmoregulation and salinity tolerance, and swimming performance. Although seemingly distinct aspects of the physiology of fish, these components can be incorporated into the generalized bioenergetic framework proposed by Warren and Davis in 1967.

Bioenergetics

Food (energy) consumed by a fish is used to meet the metabolic demands of existing tissues, is lost to the environment as wastes, or, if it is in surplus, can be synthesized into new tissue. This accounting process is commonly referred to as the fish's energy budget, or bioenergetics. The general equation used to illustrate the partitioning of energy of fish in aquaculture (or other settings), as proposed by Warren and Davis in 1967, is given as

$$C = (M_r + M_a + \mathrm{SDA}) + (F + U) + (G_s + G_r)$$

where C is the energy consumption rate, M_r the standard metabolic rate, M_a the active metabolic rate, SDA the specific dynamic action, F the rate of energy loss in fecal matter, U the rate of energy loss in urine, G_s the rate of energy allocation to the growth of somatic tissues (proteins, lipids, and structural elements), and G_r the rate of energy allocation to the growth and maturation of reproductive tissues (**see also Temperature**: Measures of Thermal Tolerance).

In aquaculture, the general goal is to maximize the rate of G_s (sometimes also G_r), while minimizing the allocation of energy to the other compartments. Achieving this balance requires the culturist to maximize the surplus of energy available, while simultaneously providing physiologically optimum conditions for growth and minimizing the necessity for the fish to allocate energy to nongrowth compartments such as M_r or M_A. Reducing fish activity to levels that maximize the production of somatic tissue, and thus reducing M_a, is fairly straightforward and can be achieved through the careful design of the culture systems (e.g., raceways or tanks). Reducing the energy spent on various aspects of M_r, such as osmoregulation, oxygen uptake, and acid–base balance, requires greater effort because the physicochemical environment influences these aspects of a fish's physiology. Fortunately, our understanding and ability to manipulate environmental factors such as dissolved oxygen concentration, salinity, and pH are sufficiently advanced such that techniques and equipment are available to do so.

For further discussion on this topic within the encyclopedia, **see also Energetic Models**: Bioenergetics in Aquaculture Settings.

Temperature

Water temperature is the environmental variable that exerts the greatest influence on an aquaculture system. It not only directly affects the physiology of fish contained in the culture system, but also affects other physical and chemical variables, such as water viscosity, dissolved oxygen and carbon dioxide concentrations, and ammonia toxicity levels. The responses of various aspects of a fish's physiology to a change in temperature generally follow one of three patterns (**Figure 1**).

The first pattern (**Figure 1(a)**) shows a physiological function that is temperature independent. This is generally not observed in fish, except for a few eurythermal species or over ranges for which the Q10 (**see also Temperature**: Effects of Temperature: An Introduction) is small.

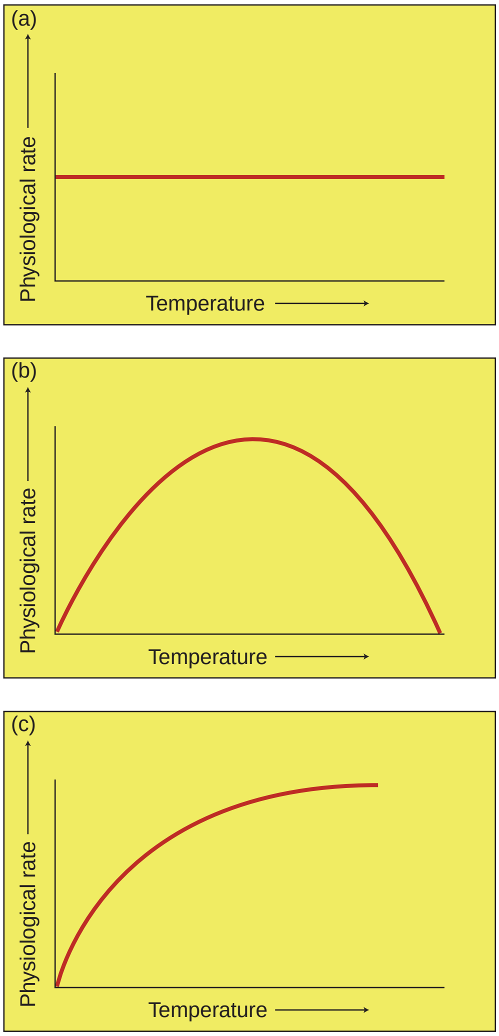


Figure 1 General responses of fish physiological processes to temperature showing possible temperature-independent (a) and temperature-dependent (b, c) patterns.

The second pattern (**Figure 1**(**b**)) is that observed for many physiological functions (e.g., growth rate) that increase with temperature to some maximum, often referred to as the optimal temperature for that function, and then decline as the temperature continues to increase.

The third pattern (**Figure 1**(**c**)) is where the rate of the physiological function (e.g., oxygen consumption rate) increases as temperature increases.

At the most fundamental level, a fish culturist should be cognizant of the temperatures that are tolerated by the species of interest at the acute and chronic levels, as well as the suboptimal and optimal temperatures for that species. **Figure 2** provides an example of such information for the rainbow trout (*Oncorhynchus mykiss*).

At the extremes of thermal tolerance are the acute upper and lower thermal limits, often referred to as the critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min}). These are temperatures that the fish can survive for a short period of time (seconds to minutes) and still recover. For rainbow trout, the CT_{min} approaches 0 °C, while the CT_{max} ranges between 27 and 30 °C or more, depending on the acclimation temperature. Temperatures that are slightly more moderate than the critical thermal limits are often referred to as the incipient lethal limits, with the incipient upper lethal limit (IULT) and the incipient lower lethal limit (ILLT). These are temperatures that fish can tolerate for prolonged periods (hours to days) but that will ultimately prove lethal. For rainbow trout, the ILLT is close to 0 °C, while the IULT ranges from 25 to 27 °C, depending on the strain of trout and its acclimation temperature. Culturists do not typically rear fish under conditions approaching either incipient lethal or critical limits, except during unusual weather events or system failures. However, fish from aquaculture facilities that are released into the environment could encounter temperatures of this nature, adding value to this information.

Within the range bracketed by incipient lethal temperatures is a thermal region that contains both the optimal and the suboptimal, but not the lethal, temperatures. At suboptimal temperatures, a fish may not be able to fulfill its life cycle, but the individual fish can survive. For example, rainbow trout can survive and grow very well at 19 °C, but they spawn most successfully at temperatures below 10–12 °C. The optimal temperature range is the one where the rate of the physiological function is maximized. For rainbow trout, depending on the strain, the optimal range for growth lies between 15 and 19 °C.

Fish culturists try to rear fish at temperatures that approach the optimum for growth. It may be possible to provide fish with water of a constant temperature when spring-fed water supplies or recirculating culture systems are being used. If the primary water supply is subject to seasonal temperature fluctuations (e.g., from a local river or lake), then the culturist must understand the influences of temperature on metabolic processes and adjust their management accordingly (for further discussion on this topic, **see also Temperature**: Membranes and Temperature: Homeoviscous Adaptation).

When evaluating thermal tolerance data, one must consider the temperature to which the fish were acclimated (e.g., held for >2 weeks) prior to testing. Fishes have the ability to become thermally acclimated, thus slightly raising or lowering their ability to tolerate temperature extremes. There are limits to this acclimation, however, and it is generally considered impractical to try to acclimate coldwater species such as rainbow trout to temperatures more suitable for a warm-water species such as a Nile tilapia (*Tilapia niloticus*; optimal temperature 28–30 °C).

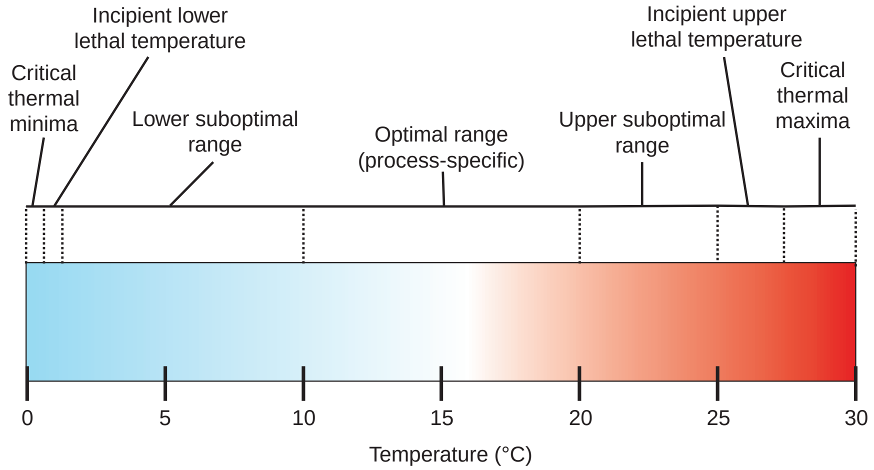


Figure 2 Thermal profile for rainbow trout (*Oncorhynchus mykiss*) showing critical thermal maxima and minima, incipient lethal temperatures, and suboptimal and optimal temperature ranges.

Oxygen and Oxygen Demand

After temperature, oxygen is the most influential of environmental factors when considering the physiology of fishes in aquaculture. The challenge in aquaculture, particularly in systems with a high fish-loading rate, is delivering sufficient oxygen to meet the metabolic demands of the fish to such a degree that no depression of growth is observed.

Effects of Mild Environmental Hypoxia

Under normal culture conditions, severe environmental hypoxia (low partial pressure of oxygen, P_{O_2}, in the water) is rare, and anoxia (zero oxygen) generally is not encountered. Severe environmental hypoxia is defined as P_{O_2} levels approaching the threshold P_{O_2} where there is insufficient oxygen for the organism to sustain a completely aerobic existence (**see also Hypoxia**: The Expanding Hypoxic Environment). The exceptions to this are during failures of aeration equipment, or when high densities of fish are confined in a small volume of water, such as in a fish-transport tank. When hypoxic conditions do occur, even at relatively mild levels (e.g., dissolved oxygen at 60% of air-saturated levels), long-term effects on fish growth and performance can be observed. The growth rate of fish, including rainbow trout and white sturgeon (*Acipenser transmontanus*), declines as the P_{O_2} decreases from normoxic (i.e., dissolved oxygen near air saturation) to hypoxic levels above the threshold P_{O_2}.

To avoid problems associated with mild levels of hypoxia, fish culturists use a variety of techniques to introduce atmospheric air, or for greater transfer efficiency, compressed and liquid oxygen into their culture systems to counter the fish's high oxygen demand. These aeration systems allow the rearing of fish at high stocking densities, and can also lead to complications related to carbon dioxide excretion and acid–base balance.

Sources of Increased Oxygen Demand in Cultured Fishes

How oxygen demand (or oxygen consumption rates) is affected by factors such as fish size, water temperature, activity, and SDA is discussed elsewhere in the encyclopedia (**see also Energetics**: General Energy Metabolism, **Temperature**: Effects of Temperature: An Introduction, and **Food Acquisition and Digestion**: Cost of Digestion and Assimilation). With regard to fish size, large fish use more oxygen than small fish, but small fish use more oxygen than large fish on a gram oxygen per gram fish basis (a higher mass-specific metabolic rate; **see also Energetics**: General Energy Metabolism). This is one of the reasons why aquaculture facilities that raise salmonids (members of family Salmonidae, including salmon, trout, and char) in raceways keep their smallest fish in the top raceways (receiving first-use water), while larger fish are held in the lower raceways (receiving second-, third-, or even fourth-use water). The raceways positioned further downstream contain less oxygen due to the respiration of the fish in the more upstream raceways.

Because fish oxygen demand also increases with water temperature, fish culturists need to monitor dissolved oxygen concentrations in their culture systems as daily and seasonal temperature fluctuations change the metabolic rates of the fish contained therein. The degree to which temperature affects metabolic rate and oxygen demand can be determined using the Q10 approach (for more details on this topic within the encyclopedia, **see also Temperature**: Effects of Temperature: An Introduction).

The specific dynamic action associated with the processing and assimilation of ingested food during daily culture operations can easily double oxygen demand for many hours. Recent studies on common carp (*Cyprinus carpio*), southern catfish (*Silurus meridionalis*), and other species demonstrated that the post-feeding oxygen demands can be 2–4 times as high as the pre-feeding oxygen demand. This increased oxygen demand generally peaks within 2–6 h after feeding and remains elevated for periods exceeding 24 h, depending on the water temperature. Clearly, this is something that a fish culturist needs to account for, along with increases in microbial oxygen consumption rates within the system that may accompany SDA increases as the flux of nitrogenous wastes stimulates higher levels of bacterial activity. The timing, magnitude, and duration of the SDA effect are affected by feed composition (in particular, the source and proportion of protein to other ingredients). In addition, fish size, meal size, and feeding frequency can all affect the timing, magnitude, and duration of the SDA effect (for more details on this topic within the encyclopedia, (**see also Food Acquisition and Digestion**: Cost of Digestion and **Integrated Response of the Circulatory System**: Integrated Responses of the Circulatory System to Digestion).

Carbon Dioxide and Acid–Base Balance

When fish in a culture system use oxygen to meet their aerobic metabolic demands, they produce carbon dioxide (CO_2) that is transported from the tissues by the blood to the gills, where it is excreted. Because of the nature of the carbonate system, the addition of aqueous carbon dioxide to the environment (culture system) will decrease the pH, leading to problems with environmental acidosis; these conditions can have negative impacts on fish growth and on the performance of culture system components such as biofiltration units.

If the carbon dioxide is allowed to build up in the culture system, the water becomes hypercarbic (synonymous with hypercapnic, which is a term often used in the literature). Studies on white sturgeon showed that severe hypercapnia (45–75 $\mathrm{mg\,L^{-1}}$ CO_2) reduces growth by 38% compared with fish held under normocarbic (synonymous with normocapnic) conditions. Similar effects have been seen in juvenile Atlantic salmon (*Salmo salar*) and juvenile spotted wolffish (*Anarhichas minor*). The juvenile wolffish also showed signs of nephrocalcinosis, but this may have resulted from a combination of factors, including high environmental carbon dioxide levels and dietary sources such as magnesium deficiencies or an unbalanced feed mineral composition.

Hypercarbic conditions during fish-transport operations can impact the post-transport survival of fish. Walleye (*Sander vitreus*) transported in tanks where the P_{CO_2} was allowed to increase had higher blood P_{CO_2}, bicarbonate ion concentrations ($[HCO_3^-]$), and stress indicators (plasma cortisol and blood glucose) than fish transported in tanks fitted with ram-air scoops that prevented the increase in water P_{CO_2}. Such measures, along with vigorous aeration of water in static culture systems, particularly those using oxygen injection systems, can prevent problems with hypercarbic conditions that would otherwise lead to a disruption of the ability of the fish to maintain an adequate internal acid–base balance (for more details on fish acid–base balance and hypercapnia effects, **see also Transport and Exchange of Respiratory Gases in the Blood**: Carbon Dioxide Transport and Excretion).

Osmoregulation, Salinity Tolerance, and Smoltification in Cultured Fishes

A detailed discussion of osmoregulation, salinity tolerance, and smoltification in cultured fishes is beyond the scope of this article. However, some portions of these topics are relevant to aquaculture, primarily those regarding the stress response and stress mitigation, and in relation to the parr–smolt transformation of juvenile anadromous salmonids.

Osmoregulation – Implications for Aquaculture

When fish encounter stressful conditions, their responses include primary, secondary, and tertiary stages. One phase of the secondary response is an increase in gill perfusion through lamellar recruitment and an increase in gill permeability. While such actions are adaptive in that they allow for greater oxygen uptake, they also allow for greater water and solute fluxes, and thus can disrupt ionic and osmoregulatory stability if the stressor persists – termed the 'osmo-respiratory compromise'. Fish in aquaculture become stressed during many standard culture activities including cleaning of culture vessels, handling, transportation, and crowding. Evidence from careful monitoring of stress indicators, including plasma cortisol concentrations and plasma glucose levels, suggests that the most stressful periods are those involving handling and air exposure. Mortality resulting from an osmoregulatory disturbance may be delayed by 24 h or more. Fish chronically exposed to sublethal levels of stress will show reduced growth rates and have higher rates of disease incidence (**see also Role of the Gills**: The Osmorespiratory Compromise).

In addition to adopting culture practices that minimize the rough handling of fishes, careful management of water quality, and, in particular, water temperature and salinity, can help reduce the osmotic gradients between the fish and their environment, thus reducing the osmotic and ionic gradients that are present. A general approach is to add sodium chloride (NaCl) at concentrations of 1–5 $\mathrm{g\,L^{-1}}$ to the system water and to reduce the water temperature by up to 5 °C from the source tank. Studies on largemouth bass (*Micropterus salmoides*) hauled in water that had NaCl added and that was cooled demonstrated that posthaul survival could be doubled, particularly when the posthaul fish were held in water that also had salt added to it. In addition to simple NaCl solutions, there are a number of commercially available solutions designed to mitigate the effects of stress on fish. Some of the solutions are designed to reduce the toxicity of ammonia; a similar effect can be achieved by using granular zeolite (synonymous with clinoptilolite). While these commercial formulations have been shown to be effective, most have not been approved for use on food fish; therefore, fish culturists should consult the manufacturers and regional regulatory agencies before employing them (**see also Nitrogenous-Waste Balance**: Excretion of Ammonia).

Salinity Tolerance

Managing the salinity tolerance of fish in aquaculture is primarily a concern of culturists raising anadromous salmonids such as Atlantic salmon and Arctic char (*Salvelinus alpinus*) that combine a freshwater rearing phase of fry and juveniles with a longer saltwater grow-out period. The attraction of the saltwater grow-out period includes the greater availability of space and water, a potential reduction in the number of diseases, and the potential for higher growth rates in saltwater, as many wild strains of salmonids grow faster in seawater than in freshwater.

Recognizing the transformation of the freshwater-adapted juveniles into seawater-adapted juveniles and timing the subsequent transfer of the fish into saltwater culture systems is critical – moving juvenile fish that are not capable of osmoregulating in seawater will generally cause high levels of mortality. The timing of such movements is well established for Atlantic and Pacific salmon, but is still being determined for emerging species such as Arctic char. In the case of some strains of Arctic char, growth was highest in salinities of 0–10 ppt, and fish transferred to salinities of 20–30 ppt had higher mortality rates and lower growth rates.

For more information on the physiological aspects of osmoregulation and salinity tolerance within the encyclopedia, **see Osmotic, Ionic and Nitrogenous-Waste Balance**: Osmoregulation in Fishes: An Introduction.

Exercise and Cultured Fishes

Cultured fishes are generally held in tanks, raceways, or net pens that provide a heterogeneous velocity field. While this velocity heterogeneity may provide fish with areas where they can rest and reduce energy expenditures (reducing M_a), it does not always provide the greatest benefit in terms of growth potential. Studies on the effects of mild exercise (forced swimming at 1–2 body lengths per second) on striped bass (*Morone saxatilis*) and rainbow trout have demonstrated that fish that are forced to exercise can demonstrate higher growth rates than those that only engage in volitional swimming. The trade-off is that the higher growth rates are associated with higher food consumption rates; hence, fish culturists should conduct a cost–benefit analysis to determine if the gain in production is offset by the increased production costs. There may be other benefits of the exercise, such as flesh quality.

Another area where subjecting cultured fish to exercise may be beneficial is when the fish are being raised for stock enhancement. Studies have shown that exercised fish have swimming performances that are closer to those of wild fish than cultured fish that do not experience an exercise regime. This increased swimming performance can help prevent the higher rates of downstream displacement, predation mortality, and release stress that cultured fish often experience.

See also: **Energetics**: General Energy Metabolism. **Energetic Models**: Bioenergetics in Aquaculture Settings. **Food Acquisition and Digestion**: Cost of Digestion and Assimilation. **Hypoxia**: The Expanding Hypoxic Environment. **Osmotic, Ionic and Nitrogenous-Waste Balance:** Osmoregulation in Fishes: An Introduction. **Temperature**: Effects of Temperature: An Introduction; Membranes and Temperature: Homeoviscous Adaptation. **Transport and Exchange of Respiratory Gases in the Blood:** Carbon Dioxide Transport and Excretion.

Further Reading

Adams SM and Breck JE (1990) Bioenergetics. In: Schreck CB and Moyle PB (eds.) *Methods for Fish Biology*, pp. 389–415. Bethesda, MD: American Fisheries Society.

Becker CD and Genoway RG (1979) Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes* 4: 245–256.

Carmichael GJ and Tomasso JR (1988) Survey of fish transportation equipment and techniques. *Progressive Fish Culturist* 50: 155–159.

Carmichael GJ and Tomasso JR, Jr. (2001) Fish transportation In: Wedemeyer GA (ed.) *Fish Hatchery Management*, 2nd edn., pp. 641–660. Bethesda, MD: American Fisheries Society.

Carmichael GJ, Tomasso JR, Simco BA, and Davis KB (1984) Characterization and alleviation of stress associated with hauling largemouth bass. *Transactions of the American Fisheries Society* 113: 778–785.

Lawson TB (1995) *Fundamentals of Aquacultural Engineering*. New York, NY: Chapman and Hall.

Lutterschmidt WI and Hutchinson VH (1997) The critical thermal maximum: History and critique. *Canadian Journal of Zoology* 75: 1561–1574.

Moyes CD and West TG (1995) Exercise metabolism of fish. In: Hochachka PW and Mommsen TP (eds.) *Biochemistry and Molecular Biology of Fishes,* vol. 4, pp. 367–392. Amsterdam: Elsevier.

Warren CE and Davis GE (1967) Laboratory studies on the feeding, bioenergetics, and growth of fish. In: Gerkin SD (ed.) *The Biological Basis of Freshwater Fish Production*, pp. 175–214. Oxford: Blackwell Scientific Publications.

Wedemeyer GA (1996) *Physiology of Fish in Intensive Culture Systems*. New York, NY: Chapman and Hall.

INDEX

NOTES:

Cross-reference terms in *italics* are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references – not all of these cross-references have been included in the index cross-references.

The index is arranged in set-out style with a maximum of three levels of heading. Major discussion of a subject is indicated by **bold** page numbers. Page numbers suffixed by *t* and *f* refer to Tables and Figures respectively. *vs.* indicates a comparison.

This index is in letter-by-letter order, whereby hyphens and spaces within index headings are ignored in the alphabetization. Prefixes and terms in parentheses are excluded from the initial alphabetization.

ABBREVIATIONS:

Ca^{2+} – calcium
CO_2 – carbon dioxide
DNA – deoxyribonucleic acid
ELL – electrosensory lateral line lobe
EOD – electric organ discharge
GABA – γ-aminobutyric acid
GM – genetically modified
H_2S – hydrogen sulfide
HPI axis – hypothalamic – pituitary – interrenal axis
IGF – insulin-like growth factor
MCHC – mean cell hemoglobin concentration
MRC – mitochondrion-rich cell
NaCl – sodium chloride
NKA – Na^+/K^+-ATPase
NKCC – Na^+, K^+, $2Cl^-$ cotransporter
OSN – olfactory sensory neuron
PAHs – polycyclic aromatic hydrocarbons
PBDEs – polybrominated diphenyl ethers
PCBs – polychlorinated biphenyls
RBC – red blood cell
SDA – specific dynamic action
TNF – tumor necrosis factor

A

A-band
  cardiomyocyte myofibril, 1008–1009, 1009*f*
  definition, 1006
Abductor muscle
  definition, 1095
  gill arch, 1096–1097
Abiotic, definition, 1629, 1695
Abiotic factors
  definition, 1629, 1664, 1688, 1959
  growth effects *see under* Growth, environmental effects
  influence on energy partitioning/production, 1666*f*, 1668
  see also Environmental influences; *specific biotic factors*
ABR (auditory brainstem response) measurement *see* Audiograms
Absorption
  definition, 1664
  gut *see* Intestinal absorption
  metals, 2062*f*, 2063
Acceleration reaction *see* Added mass (acceleration reaction)
Accessory hearts, **1073–1076**
  branchial *see* Branchial hearts
  caudal *see* Caudal hearts
  hagfish *see under* Hagfish
  portal, 1074, 1775
Accessory olfactory bulb, 204
Acclimation
  definition, 1006, 1389
  salinity *see* Salinity acclimation
  soft water, osmorespiratory compromise, 1392, 1392*f*
  thermal *see* Thermal acclimation/adaptation
Acclimatize (acclimate), definition, 1197, 1221
Accommodation, lens, 106
Acellular bone
  cartilaginous fish, 429
  definition, 428
Acetazolamide
  definition, 899
  inhibition of carbonic anhydrase, 866–867, 868*f*, 901*f*, 905
4-Acetoamido-4′-isothiocyano-2,2′-disulfonic stilbene (SITS), 911
  definition, 909
Acetylcholine (ACh), 786, 1069
  cardiac action potential effects, 1042
  catecholamine release role, 1528, 1528*f*
    studies using *in vitro* saline-perfused posterior cardinal vein preparation, 1529, 1529*f*
  definition, 783
  enteric nervous system, 1335
    gut motility role, 1297, 1299*f*
  see also Cholinergic system
Acetylcholinesterase, 786
  definition, 783
Acid secretion, gastric *see* Gastric acid secretion
Acid–base balance
  carbonic anhydrase role, 900, 900*f*, 904, 905, 907*f*
  cultured fishes, 2087
  definition, 899, 1411
  elasmobranchs, 1817
  gut role, 1427
    see also Gut ion/acid–base regulation
  hagfish, 1776
  lampreys, 1792*f*, 1797
  renal role, 1415, 1416*f*, 1417*f*
  role in conservation of protein structure/function, 1684*f*, 1685
  sturgeons, 1804
  see also pH; Red blood cells (RBCs), pH regulation
Acid–base equivalents, definition, 899
Acidosis
  definition, 865
  ventilatory response *see* Ventilatory response to CO_2/H^+
Acipenseriformes, 1801
  ampullary organs, 352
  see also Paddlefish; Sturgeons, physiology
Acorn worms
  CNS development, molecular basis, 9
  PNS/motor neurons, evolution/developmental transformation, 9, 10
Acoustic behavior *see* Sound production
Acoustic Doppler velocimetry (ADV), 582
Acoustic monopole/dipole, 271
Acoustic particle motion, 276, 279*f*, 280
  definition, 262, 298
  sound pressure *vs.*, 298–299

Acoustic particle velocity, definition, 270
Acoustic power
  transmission loss, 272
  see also Sound
Acoustic pressure *see* Sound (acoustic) pressure
Acoustic stimuli and behavior
  biological relevance, 265–266, 267–268
  conditioning, for hearing threshold measurement, 267, 267*f*, 277*f*
    classical (Pavlovian), 277
    instrumental avoidance, 277
  sharks, attraction to sounds, 265
    interpretation of field experiments, 268
  startle response, 266
  see also Bioacoustics; Hearing; Sound production; *specific behaviors*
Acoustic tag, 1930
  definition, 1928
Acromegaly
  definition, 2016
  growth hormone transgenic fish, 2018*f*, 2019
ACTH *see* Adrenocorticotropin hormone (ACTH)
Actin, deep-sea fishes, 1957
Actin filament *see* Thin filaments
Actinistia *see* Coelacanths
Actinopterygians *see* Ray-finned fish
Action potential (AP)
  cardiac *see* Cardiac action potential
  conduction velocity, 1717, 1721
    temperature effects, 1721
  definition, 398, 973, 1030, 1038, 1717
  duration, 1722
    temperature effects, 1722
  generation, 401, 401*f*, 1717*f*, 1720
  muscle *see* Muscle action potential (MAP)
  see also Excitable membranes; Ion channels
Activation, definition, 1717
Activation energy, definition, 1688
Active electrolocation, **375–386**, 348–349
  behavioral performance during object detection/analysis, 377, 378*f*, 379*f*
  central processes *see* Active electrolocation, central processes
  definition, 347, 375, 387
  discovery/historical aspects, 348
  electroreceptors, 379
    ampullary *see* Ampullary electroreceptors
    tuberous electroreceptors *see* Tuberous electroreceptors
  EOD modulation/electrical imaging, 375, 376*f*, 377*f*
  foveation, 378, 380*f*
  peripheral encoding, 380
    lateral coding of EOD amplitude and electrical imaging, 380, 381*f*
    waveform encoding, 380, 382*f*
  prereceptor mechanisms, 376
  see also Electric organ(s) (EOs); Electric organ discharge (EOD); Electrocommunication
Active electrolocation, central processes, 380
  information processing in electrosensory lateral line lobe, 380
    circuitry, 380, 382*f*
  latency code to rate code transformation in granule cells, 381
  spatial coding, 383, 383*f*, 384*f*
    plasticity, 384, 385*f*
Active ion transport *see* Ion transport
Active metabolic rate (AMR), definition, 812
Active metabolism
  definition, 846, 847
  measurement *see* Swimming respirometers
  see also Maximal metabolic rate (MMR)
Active site
  carbonic anhydrase, 901, 902*t*
  definition, 899
Active tension
  definition, 1060
  see also Sarcomere length (SL)–tension relationship
Activins, 1501–1502, 1503*f*, 1507
Activity patterns
  during compensatory growth, 754
  hypoxia response, 765, 1768
  see also Swimming
Acute hypoxia, circulatory system responses *see under* Hypoxia, circulatory system responses
Acute thermal change
  mitochondrial effects, 1711*f*, 1712
  see also Temperature effects
Acyl chain, definition, 1709
Acyl group, definition, 1725
Ad libitum food consumption
  definition, 1629
  growth rate and, 1632*f*
    temperature effects, 1629*f*, 1630–1631
Adaptation
  air-breathing *see* Air-breathing fish
  biochemical *see* Biochemical adaptations, general principles
  definition, 777
  environmental *see* Environmental acclimation/adaptation visual *see* Visual adaptations
  see also specific adaptations
Adaptive radiation, air-breathing fish, 1858
Added mass (acceleration reaction), 538
  definition, 535, 581
  momentum effects, 543, 544*f*
Added mass coefficient, 543
  definition, 535
Addiction (human), fish models, 1995
Additive color mixing, 154*f*, 155, 155*f*
Adductor muscle
  definition, 1095
  gill arch, 1096–1097
Adelphophagy, 679
  definition, 678
Adenohypophysis, 1458
  definition, 419, 1457
  histology, 1458, 1458*f*, 1462
    elasmobranchs, 1458–1459, 1460*f*
    hagfish, 1459, 1461*f*
    hormone immunocytochemistry, 1460, 1461*f*, 1462*f*
    lamprey, 1459, 1460*f*
    neurohypophysis relations, 1458, 1460*f*
    pars distalis *see* Pars distalis
    pars intermedia *see* Pars intermedia
  hormones, 1458*f*, 1460
    see also specific hormones
  see also Pituitary gland
Adenosine
  definition, 1824
  metabolic depression role in anoxic crucian carp, 1829
Adenosine triphosphate *see* ATP (adenosine triphosphate)
Adherens junction, cardiomyocytes, 1012*f*, 1012–1013
Adrenaline (epinephrine)
  biosynthesis, 1524, 1525*f*
  cardiac action potential effects, 1042
  definition, 1389
  gut expression, 1336
  gut motility role, 1299, 1299*f*
  release, 1525–1526
    see also Catecholamine release
  stress response activity, 1516*f*, 1516–1517, 1517*f*, 1518, 1519
    exercise-associated osmorespiratory compromise, 1389–1390
    hypoxia, 1521, 1522*f*
    proactive *vs.* reactive coping styles, 1521*f*
  see also Catecholamine(s); Stress response, hormonal
Adrenergic, definition, 80, 1104, 1524
Adrenergic receptors, 1531*f*, 1532
  definition, 1221
  see also Catecholamine(s)
Adrenergic response, red blood cells *see under* Sodium/proton exchange, RBC
Adrenergic tone, GI vascular beds, 1136
Adrenoceptor, definition, 1104
Adrenocorticotropin hormone (ACTH)
  definition, 1450, 1466
  embryonic expression, 1454
  osmoregulation role
    freshwater, 1469, 1470
    seawater, 1468
  stress response role, 1475–1476, 1476*f*, 1516*f*, 1517
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Adrenomedullins, vascular resistance control, 1106
Advertisement calls *see* Spawning/advertisement calls
Aerobic, definition, 1758, 1887
Aerobic metabolism
  definition, 829, 838
  pathways, 1757*f*, 1758
    environmental influences *see under* Metabolism, environmental influences
  see also Mitochondrial respiration; Oxidative phosphorylation
Aerobic scope, 847, 1639
  definition, 846, 1588, 1636, 1738, 1764, 1887, 1903
  environmental influences *see* Fry paradigm
  feeding rate and, 1589
  multiple activity use, 812
    in tunas, 1906*f*, 1908
  ontogenetic changes, 1576*f*, 1577*f*, 1578, 1580
  pelagic fish, 1893–1894
  for survival *see* Standard metabolic rate (SMR)
  swimming performance and, 1658–1659
  temperature dependence, 1740*f*, 1741–1742, 1742*f*, 1744
Aerobic swimming
  circulatory system responses, 1216
    blood flow redistribution, 1217
    improved oxygen loading at gills, mechanism, 1217
    improved oxygen unloading at tissues, mechanism, 1218
    increased tissue oxygen delivery, mechanisms, 1216
      increased cardiac output, 1216
      increased cardiac stroke volume, 1216
      increased heart rate, 1216
    increased tissue oxygen extraction, mechanism, 1216
  energetic cost, 1638, 1638*f*
    measurement, 1638*f*, 1639
  see also Swimming
Aerolysin, pathogenesis role, 2035
Aeromonas salmonicida, translocation across intestinal epithelium, 1328, 1329*f*
Afferent, definition, 1, 854
Afferent branchial arteries (ABAs), 1096*f*, 1097*f*, 1101, 1101*f*
Afferent filament arteries (AFAs), 1097*f*, 1098, 1101, 1101*f*
Afferent lamellar arterioles (ALAs), 1097*f*, 1101*f*, 1102
African lungfish, heart, outflow tract, 1016–1018, 1019*f*
Agastric, definition, 1311
Aggression
  compensatory growth-associated, 754
  electric signaling role, 703, 703*f*, 704*f*
  fight responses, periaqueductal gray role, 63
  foraging activity and, 1591
Aggressive sounds, 314, 314*f*
Agility
  definition, 575, 575
  effectors, 577
    fish size, 578
  measurement, 576
  see also Maneuverability
Aging, heat shock response and, 1736

Aglomerular kidneys, 1411
  see also Kidney(s)
Agnathan(s)
  brains *see* Agnathan brain, functional morphology
  definition, 16, 110, 236
  endogenous opioid peptides, proopiomelanocortins, 93*t*
  feeding methods, 598
  lateral line system, 344, 345*f*
  life cycles, 16
  physical features, 16, 17*f*
  taxonomy, 16
  see also Hagfish; Lampreys
Agnathan brain, functional morphology, **16–25**, **1–5**
  forebrain, 21
    diencephalon, 21, 22*f*
      thalamus *see under* Thalamus
    pineal organ *see under* Pineal organ
    secondary prosencephalon, 23, 24*f*
  hindbrain, 17, 18*f*
  midbrain, 20, 20*f*
  research, 17
  see also Brain(s)
Agonist, definition, 656
Agonistic, definition, 684
Agonistic sound signaling, 685*t*, 688*f*, 689
Air, water *vs. see under* Water
Air-breathing fish, 767, 768*f*, **1850**
  adaptive radiations, 1858
  ancillary functions of air-breathing, 1857*f*, 1858
  carbon dioxide excretion, 914
  cardiac anatomy, 1004
  cardiorespiratory interactions, central control, 1187, 1188*f*
  circulation *see* Air-breathing fish, circulatory adaptations
  cycle of *Hoplosternum littorale*, 768–769, 769*f*
  diversity, 1851*f*, 1852
  evolution, 1855*f*, 1856
  gill respiratory morphometrics, 808*f*, 809
  hypercarbic ventilatory response, 866, 867*f*, 868
  hypoxic hyperventilatory response, 1752*f*, 1753
  intertidal and estuarine, adaptive strategies, 1963
  natural history, 1858
  organs *see* Air-breathing organs (ABOs)
  parental care, 1852
  phylogeny, 1851*f*, 1852
    air-breathing organ and, 1851*f*, 1853, 1853*f*
  respiratory adaptations, **1861**
    gills, 1871*f*, 1872
    skin respiration, 1872*f*, 1873
  see also Air-breathing organs (ABOs)
  respiratory rhythm generation, 862, 863*f*
  types, 1854
    amphibious *see* Amphibious air breathing
    aquatic air breathers, 1854
    obligate *see* Obligatory air breathing
  see also Hypoxia, behavioral responses
Air-breathing fish, circulatory adaptations, **1875**
  coronary circulation, 1078*f*, 1084, 1885
  diversity, 1876*f*, 1877–1879
  early studies, 1879
  lungs/respiratory gas bladders, 1881
    gars and primitive teleosts, 1876*f*, 1881*f*, 1882
    lungfish, 1876*f*, 1878*t*, 1879*f*, 1881
    Osteoglossiforms, 1876*f*, 1881*f*, 1882
    polypterids and *Amia*, 1876*f*, 1877*f*, 1880*f*, 1881*f*, 1882
    teleost species, other, 1876*f*, 1883
  mouth breathers, 1876*f*, 1883
  problems, 1877*f*, 1879
    solutions/structural modifications, 1878*t*, 1880
  stomach/intestinal air breathers, 1876*f*, 1884
  suprabranchial chambers, 1876*f*, 1883*f*, 1884
Air-breathing organs (ABOs), 768, **1861**
  ancillary functions, 1858–1859
  blood flow patterns *see* Air-breathing fish, circulatory adaptations
  definition, 1850, 1861
  digestive tube, 1870
    esophagus, 1870
    intestine, 1871
      see also Intestinal air breathing
    stomach, 1869*f*, 1870
  evolution, 1851*f*, 1853, 1853*f*, 1861*f*, 1862
  function, 1863
  head region, 1868
    modified chambers, 1867*f*, 1868
    suprabranchial chambers *see* Suprabranchial chambers (SBCs)
  lungs *see* Lung, fish
  natural history, 1862
  novel, 1868
  respiratory gas bladders *see* Respiratory gas bladder
  type I cells, 1865–1866
    definition, 1862
  type II cells, 1865–1866
    definition, 1862
  types, 1862
  ventilation, 1862
  see also Air-breathing fish
Alar plate, 2–3, 3*f*
  definition, 1
Alarm calls, 314, 315*f*, 689–690
Alarm substance, 231, 783–784
  definition, 783
  effect of cadmium on response, 784*f*, 784–785
  evolution, 233
  fright reaction, 231–232, 232
  olfactory detection, 232
  putative, 232
  surfacing postponement and, 768
Alarm substance cells, 232, 232*f*
ALAS2 gene, mutation, 1990
Albinism
  cavefish, 1845, 1848
  definition, 1843
  fish models/studies using, 1995
Albumin, capillary permeability, 1159
Alcohol dehydrogenase (ADH), 1825*f*, 1827–1828
Aldosterone, 1474
  synthesis, 1475, 1475*f*
  see also Corticosteroid(s)
Alevins, Pacific salmon, migration
  feeding ground, 1947
  first, 1947
Alkaline lakes, fish ionic regulation, 1386
Alkaline tide, 1427
  definition, 1429
  rectal gland impact, 1435
Alkaloids, definition, 1664
Allocentric cues, 721
  definition, 720
  learning, 722
Allometric, definition, 1573
Allometric exponent, definition, 1566
Allosteric binding, definition, 921, 935
Allosteric regulation, definition, 887
Allostery, hemoglobin *see under* Hemoglobin(s), fish
Alosa sapidissima (American shad), hearing, auxiliary anatomy, 274
Alphastat regulation, 1684*f*, 1685
  cardiac contractility and, 1056*f*, 1058
  definition, 1054, 1681
Alternating current (AC) fields, 359–360
  definition, 359
Alternative reproductive tactics, 614
Aluminium, biological effects and toxicodynamics, 2065
  respiratory, 787
Alzheimer's disease, fish models in, 1993
Amacrine cells, 124*f*, 125
Amargosa River, 1831*f*, 1833
  pupfish *see Cyprinodon nevadensis amargosae*
Ambient noise, definition, 304
Ambient pressure, effect on deep-sea fish *see* Deep-sea fishes, pressure effects
American eel (*Anguilla rostrata)*
  larval migration, 1939–1940
  specific dynamic action, 1609*t*, 1612
  wake, 541, 541*f*
American shad, hearing, auxiliary anatomy, 274
AMH gene, 2029*t*, 2030
Amia, circulatory patterns, 1876*f*, 1881*f*, 1882
Amines
  as gut neurotransmitters, 1335, 1335*f*
  see also specific amines
Amino acids, 209
  absorption, 1314, 1314*f*
  ATP production role, 952*f*, 953, 955–956
  composition, protein modification, 1706
  definition, 208, 218, 1664
  dietary requirements of cultured fish *see under* Cultured fish, dietary requirements
  dispensable *see* Dispensable amino acid (DAA)
  functions, 1618
  gustatory responses *see under* Gustation, neurophysiology
  as gut neurotransmitters, 1339
  indispensable *see* Indispensable amino acids (IDAAs)
  olfactory responses, 209, 210*f*, 211*f*, 228–230
  stimulation of feeding behavior, 227–228, 229*f*, 230*f*
  substitutions in enzymic temperature adaptation, 1706
Ammocoete(s), 1779*f*, 1781
  anatomy, 1780*f*, 1789*f*
  definition, 16, 1395, 1780
  environmental challenges, 1791
  feeding, apparatus, 1780*f*, 1784
    see also Oral hood, larval lamprey
  kidney histology, 1397*f*, 1397*f*
    opisthonephros, 1404–1406, 1409*f*
    pronephros, 1399, 1399*f*, 1400*f*, 1401*f*
  metamorphosis, 1779*f*, 1781, 1781*f*, 1782*f*, 1783
  osmoregulation, 1796
  respiration, 1789*f*, 1791
  see also Lampreys
Ammocoete mitochondrion-rich (AMR) cells, 1794*f*, 1796
Ammonia/ammonium (NH_3/NH_4+), **1437–1443**
  ammonia gas (NH_3)–ammonium ion (NH_4+) reaction, 1438
  blood–brain barrier permeability, 1441
  excretion *see* Ammonia excretion
  feeding and exercise, 1442
  gill ventilation and, 1439
  growth effects, 1632
  sources/sinks, 1438*f*
  toxicity *see* Ammonia toxicity
  transfer across cell membranes, 835, 1439
  ureogenesis, 1441
  volatilization (ammonia), 1440
  see also Ammonia gas (NH_3); Ammonium ions (NH_4+)
Ammonia excretion, **1437–1443**, 835, 836*f*, 1444
  fish embryos, 835, 1447, 1448*f*
  gill ventilation and, 1439
  lampreys, 1797
  pH and, 1438, 1438*f*
  rates, cultured fish, 1673
  renal role, 1416–1417
  skin role, 474, 835
  ureogenesis, 1441
  see also Nitrogen excretion
Ammonia gas (NH_3)
  ammonium ion (NH_4+) reaction, 1438
  gill ventilation and, 1439
  transfer across cell membranes, 1439
  volatilization, 1440
  see also Ammonia/ammonium (NH_3/NH_4+)
Ammonia-tolerant fish, 1441

Ammonia toxicity, 1440
  exercise and, 1442, 1442*t*
  feeding and, 1442
  reduction strategies, 1440
    detoxification to glutamine, 1440, 1440*f*
    ureogenesis, 1441
Ammonium ions (NH_4+)
  ammonia gas reaction, 1438
  gill ventilation and, 1439–1440
  postprandial elevation, 1442
  transfer across cell membranes, 1439, 1439
  see also Ammonia/ammonium (NH_3/NH_4+)
Ammonotelic, definition, 829, 1437, 1444
Ammonotelic fish, 1444, 1445
  definition, 1664
  see also Ammonia excretion
Amniotes
  brains, 26–27, 34–35
  definition, 26, 1395
Amoore, J.E., San Francisco Bay Bridge Analogy, 185, 185*f*
Amphibians
  caecilian, ampullary organs, 354
  urodele, ampullary organs, 354
Amphibious air breathing, 770, 1856
  definition, 1850
Amphibious fishes, 770
  adaptations, 1858
    air breathing *see* Amphibious air breathing
    visual, 108, 108*f*
  see also specific fish
Amphibious vision, 108, 108*f*
Amphidromy, 1922
  definition, 1921
Amphioxus, PNS/motor neurons, evolution/developmental transformation, 10
Amphipathic, definition, 1311, 1725
Amphipod, definition, 1675
Amphiprion (clownfish), sound production, 325
Amplitude, definition, 311
Amplitude coding, 367
  pulse-type fish, 367, 367*f*, 369*t*
  wave-type fish, 367*f*, 368, 369*t*
    physiology of ELL neurons receiving electroreceptor input, 367*f*, 368
  see also Electric organ discharge (EOD), central control
Amplitude modulation
  definition, 366
  electric organ discharge, 366–367
    see also Amplitude coding
Ampullae of Lorenzini, 350, 351, 359, 409
  cartilaginous fish, 351, 351*f*, 352*f*
  definition, 350
  development, 410
  morphology, 361, 361*f*
  see also Ampullary electroreceptors; Ampullary electrosensory system, physiology
Ampullary electroreceptors, 351, 379
  Acipenseriformes, 352, 353*f*
  ampullae of Lorenzini *see* Ampullae of Lorenzini
  caecilian/urodele amphibians, 354
  cartilaginous fish, 351, 351*f*, 352*f*
  coelacanths, 353
  definition, 350, 409
  development
    derived ampullary organ, 410
    primitive ampullary organ, 410
  lungfishes, 353, 353*f*
  magnetic field detection and, 729
    see also Magnetic sense
  morphology, 351, 361, 361*f*, 410*f*
  physiology, 363
    see also Ampullary electrosensory system, physiology
  Polypteriniformes, 352
  teleosts, 354, 354*f*, 355*f*
    development, 410
    gymnotids, 356, 356*f*
    Osteoglossiformes, 354
      Gymnarchus, 355, 356*f*
      mormyrids, 355, 356*f*
      notopterids *see under* Notopterids
    silurids, 356, 356*f*, 357*f*
  tuberous organs *vs.*, 356–357
Ampullary electrosensory system, physiology, **359–365**
  electrosensory-guided behavior, 364
  higher electrosensory areas, 363
  information processing in hindbrain, 363
  morphology and, 361, 361*f*
    electric field measurement, 361, 362*f*
  receptors, 363
Amygdala
  definition, 707
  learning role, 709–710
Amylase, 1281*t*, 1282
  isoforms, 1304–1305
Amylin, appetite suppression, 1485*f*, 1485–1486, 1512*t*, 1513
Amyloid beta peptide, 1993
Amyloid precursor protein (APP), 1993
Anabantoids
  hearing, auxiliary anatomy, 274
  suprabranchial chambers, 1868*f*, 1870
Anabas testudineus, suprabranchial chambers, 1868*f*, 1870
Anabolism, definition, 1206, 1623, 1629
Anadromy/anadromous fish, 1922, 1946
  definition, 1466, 1921, 1946
  ion regulation, 1950–1951
    hormonal control, 1471, 1472*f*, 1948–1949
    see also Freshwater fish, gill ion regulation; Mitochondrion-rich cells (MRCs), seawater fish
  see also Migration; Smoltification
Anaerobic, definition, 1758, 1887, 1903
Anaerobic metabolism, **1757**
  definition, 829, 1759
  pathways, 1759
    creatine phosphate hydrolysis, 1759
    environmental influences *see under* Metabolism, environmental influences
    glycolysis *see* Glycolysis
  reasons for using, 1761
    exhaustive exercise, 1760*f*, 1761, 1762
    hypoxia exposure, 1761, 1762
Anaerobic processes, definition, 1771
Anaerobic swimming
  circulatory system responses, 1218
  energetic cost, 1640
Anaerobiosis, hagfish, 1773
Anal fins, skeletal supports, bony fish, 443–445, 445*f*
Analgesia
  definition, 713
  effects, 714, 718
  opioid, 95
  stress-induced *see* Stress-induced analgesia
Analgesics, 714, 716
  definition, 713
Anamniotes, definition, 26
Anastomose, definition, 1085, 1095
Anatomy, functional *see* Functional anatomy, fish
Anchovy (*Engraulis enerasicolus*), muscle fiber anatomy and microvasculature, 1149, 1149*f*, 1150
Androgen(s)
  definition, 656, 670, 1553
  effect on electrocommunication systems, 705
  parental care role, 670, 676
    defense, 675
  sex change role, 665–666, 666*f*
  sex differentiation role, 1494
  sexual behavior role, 659–660, 660
  spermatogenesis role, 631, 631*f*, 633*f*
  see also 11-Ketotestosterone; Testosterone
Androgen receptors (ARs), sex differentiation role, 1496
Androgenesis, 2027*f*, 2028
  chromosome manipulation, 2008
  definition, 2025
Androgenic endocrine disruptors, 787
Androstenedione (AD), as pheromone
  female preovulatory, 1556–1557
  male-derived, 1560–1561
Anemia
  definition, 984
  effects, 986–987
  fish models for studying, 1990
  infectious salmon anemia, 2038
  sideroblastic, 1990
Aneuploid, definition, 2009
Angelfish (*Pygoplytes diacanthus*)
  additive color mixing, 154*f*, 155
  body colors, close range, 151*f*, 153*f*, 154–155
Angiogenesis, 1147
  definition, 1142
  ontogenetic, response to muscle growth, 1146, 1148, 1148*f*
  seasonal cold acclimation response, 1147
Angiotensin, catecholamine release role, 1530
Angiotensin II (ANG II)
  catecholamine release role, 1530
  vascular resistance control, 1105–1106
Angle of attack, 570, 572
  definition, 564
Anglerfish, bioluminescence, 500, 500*f*
Anguilla anguilla see European eel (*Anguilla anguilla*)
Anguilla japonica (Japanese eel), spawning area, 1940
Anguilla rostrata see American eel (*Anguilla rostrata)*
Anguilliform swimming, 548, 549*f*
Anguilliformes *see* Eel(s)
Angular acceleration, 246, 246*f*
Animal(s)
  behavior
    definition, 647
    see also Behavior, fish
  Bohr effect occurrence, 923
    see also Bohr effect
  evolution, 7, 7*f*
  see also Mammals
Anion effects, oxygen-binding properties of hemoglobin, 891, 891*f*
Anion exchanger
  band-III protein *see* Band-III protein anion exchanger
  definition, 879
  erythrocytes, 880, 881*f*, 883*f*, 911
  see also Carbon dioxide (CO_2) transport/excretion
Anlage
  definition, 194
  olfactory, 194
Anodal hemoglobins, 949
Anorexigenic, definition, 1341, 1509
Anorexigenic complex, 1484, 1485*f*
  definition, 1483
  orexigenic complex interactions, 1485, 1485*f*
  see also Appetite suppression
Anosmia, effect on feeding behavior, 230
Anosmic, definition, 227
Anoxia
  avoidance, crucian carp, 1829
  definition, 765, 829, 1221, 1746, 1764, 1824, 1887
  tolerance, 1825
    crucian carp *see under* Crucian carp
    goldfish *see under* Goldfish
  see also Hypoxia
Anoxic energy deficiency, avoidance in crucian carp, 1827
Antagonist, definition, 656
Antarctic fish
  cardiovascular adaptations to temperature, 1203, 1204*f*
  environmental conditions, 996
  nervous system, temperature compensation, 1723
  vascular system

    dual artery supply to muscle, 1143
    total peripheral resistance (TPR), 1151
  see also Cold acclimation/adaptation; Icefish
Anterio-ventral post-gill arteries, 1091
Anterior pituitary *see* Adenohypophysis
Anterior tuber (AT), auditory/lateral line connections, 284f, 285f, 291
Anthropogenic influences/disruptions, **783–789**
  future studies, 788
  hypoxic environments, 1748
  migration *see under* Migration
  neurotoxins/interference of brain function, 786
  reproductive impairment, 787
  respiratory toxicants, 787
  sensory information
    mechanoreception, 785, 785f
    olfaction, 783, 784f
      see also Olfactory toxicity
    sound *see* Human-generated sounds
    taste, 786
    vision, 786
  see also Pollution/pollutants; *specific anthropogenic disruptions*
Anti-Müllerian hormone (AMH)
  sex differentiation role, 1497
  spermatogenesis role, 631, 631f
Antibiotics, pollution, dangers to fish, 2076
Antibody
  definition, 1807
  elasmobranchs, 1816
Antidromic, definition, 66
Antidromic stimulation
  definition, 1178
  Mauthner cell, 68, 69f
Antifreeze proteins, 1701
  definition, 1998
  integument, 474
  transgenic fish, 2006
Antigen, definition, 1457
Antihistamine, sleep induction, 775
Antimicrobial properties, vitellogenins, 645
Antinutritional factor (ANF), 1671–1672
  definition, 1664
Antiporter, definition, 1444
Antisense genetic research approaches, 1822
  see also Morpholinos
Antiserum, definition, 1457
Apelin, appetite stimulation, 1512t, 1513
Aphakic aperture, 105, 105f
  definition, 102
Aphotic, definition, 1887
Apical, definition, 1311, 1322
Apical membrane
  definition, 1229, 1429
  rectal gland, 1431f, 1432, 1434
Apoptosis
  definition, 116, 796, 1466, 1709, 1843
  prolactin-associated, 1469
  role in interlamellar cell mass regression in hypoxia, 797
Aposematic (warning) coloration, 151, 151f
  definition, 150
Apparent digestive efficiency, definition, 1597, 1598
Appendicular skeleton
  bony fish, 445, 446f, 447f
  cartilaginous fish, 424
    batoids, 426
    chimaeroids, 426
    sharks, 425
Appetite
  assessment, 1511
  regulation *see* Appetite regulation
  see also Food consumption/intake
Appetite regulation, 1484, **1509–1514**, 1485f
  environmental factors, 2020
  gender effects, 2020
  genetic effects, 2020
  growth hormone transgenic fish, 2020, 2022–2023
  hormonal, 1512, 1512t
    brain/pituitary hormones, 1512, 1512t
    feeding status/circulating metabolite level effects, 2020
    peripheral hormones, 1512, 1512t
      see also specific hormones
  mechanisms, 1509, 1510f
    fish as study model, 1510
  reproductive status and, 2020
  stimulation *see* Appetite stimulation
  study methods and approaches, 1511
  suppression *see* Appetite suppression
  see also Foraging decisions (energetics), when to forage? (motivation to feed)
Appetite stimulation, 1484, 1485f, 1512t
  apelin, 1512t, 1513
  galanin, 1484, 1485f, 1512, 1512t
  ghrelin, 1345, 1346f, 1484, 1485f, 1512t, 1513
  neuropeptide Y, 1484, 1512, 1512t
  orexins, 1484, 1485f, 1512, 1512t
Appetite suppression, 1485, 1485f, 1488, 1512t
  amylin, 1485f, 1485–1486, 1512t, 1513
  cholecystokinin, 1344, 1485, 1485f, 1512, 1512t
  cocaine- and amphetamine-regulated transcript, 1485, 1485f, 1512, 1512t
  corticotropin-releasing hormone, 1485, 1512, 1512t
  cortisol, 1485–1486, 1519
  gastrin-releasing peptide, 1512t, 1513
  insulin, 1485f, 1485–1486, 1512t
  leptin, 1346, 1485–1486, 1512t, 1513
  melanocyte-stimulating hormone, 1485, 1485f
Appositional growth, definition, 428
Aquaculture, physiological responses, **2084–2089**
  bioenergetics, 2085
  carbon dioxide and acid–base balance, 2087
  exercise, 2089
  osmoregulation, 2088
  oxygen/oxygen demand, 2086
    effects of mild environmental hypoxia, 2086
    sources of increased demand, 2087
  salinity tolerance, 2088
  temperature, 2084f, 2085f, 2086
Aquaculture/aquaculture settings
  bioenergetics *see under* Bioenergetics
  definition, 2084
  dominance hierarchies, 650
  fish physiology *see* Aquaculture, physiological responses
  genetically modified fish
    applications, 2004f, 2005
      see also under Growth hormone transgenesis
  induced triploidy, 2010, 2014
    see also Triploid fish, physiology
  infection and disease, 2013–2014
  reasons for, 2084
  stress factors, 2013
  swimbladder malfunction, causes, 532
  use of eddies, 586
  see also Cultured fish
Aquaporins, 1367–1368
  definition, 1419, 1444
  discovery, 1367–1368
  gut, marine teleosts, 1421f, 1422
  as osmosensors, 1376
  paracellular cellular water transport, 1368, 1368f
  transcellular water transport, 1367–1368, 1368f
  see also Water homeostasis
Aquatic air breathers, 1854
  see also Air-breathing fish; Continuous air breathing; Facultative air breathing
Aquatic environment
  terrestrial environment *vs.*, 183
    chemoreception, 183–184
  see also Freshwater; Seawater
Aquatic surface respiration (ASR), 766, 1753, 1855f, 1856, 1963
  definition, 765, 1751, 1850
  intertidal species, 766, 767f
  morphological adaptations, 766, 767f
  parental care and, 769
  see also Hypoxia, behavioral responses
Aquatic toxicants *see* Toxicants
Aqueous solutions, respiratory gas properties, 830
Arachidonic acid
  dietary requirement of cultured fish, 1621
  metabolites, vascular resistance control, 1106
Arapaima gigas, gill remodeling, 802, 1393f, 1393–1394
Archerfish, water shooting behavior, 145f, 146, 147, 148f
Archinephric ducts, 1396f, 1397–1398, 1398f, 1398f, 1405f
Archinephros, 1396f, 1397
Archival tag, 1933
  definition, 1928
Arctic char, olfactory responses
  amino acids, 209–210
  prostaglandins, 213, 213f
Area composita
  definition, 1006
  mammalian cardiomyocytes, 1012f, 1012–1013
Area octavolateralis
  definition, 46
  Latimeria, 53f, 54f
  lungfishes, 48f, 50
Areolar cartilage
  cartilaginous fish, 430f, 431
  definition, 428
Arginase (ARG), urea cycle, 1445, 1445f
Arginine vasotocin (AVT), 675–676, 1462
  definition, 670
  inactivation, 1194
  sexual behavior role
    females, 661
    males, 660
  sexual change role, 668, 669f
  vascular resistance control, 1106
Argininosuccinate lyase (ASL), urea cycle, 1445, 1445f
Argininosuccinate synthase (ASS), urea cycle, 1445, 1445f
ARGOS satellite tracking systems, 1930, 1931, 1933
Aromatase
  definition, 662, 2046
  sex change role, 667
Arrhenius breakpoint temperature (ABP), 1692f, 1693
  definition, 1688, 1709
  mitochondria, tropical fish *vs.* Antarctic fish, 1713
Arrhenius equation, 1692
  definition, 1688
Arrhenius plot, 1692f, 1693
  definition, 1688
Arterial, definition, 909, 916, 1067, 1119, 1887, 1903
Arterial blood pressure, 1105
  central, 1035t, 1036
  hypoxia effects, 1223
  vascular resistance relationship, 1105
    see also under Arterial blood pressure regulation
  see also Blood pressure
Arterial blood pressure regulation, 1172, 1172f
  cardiac output, 1172, 1172f
  local control, 1173
  post-hemorrhage, baroreceptor-mediated negative feedback mechanism, 1172f, 1173
  remote control, 1174
  total peripheral resistance, 1172f, 1173
    local control *see* Systemic vascular resistance, local control
    remote control *see* Systemic vascular resistance, remote control
Arterial compliance, 981, 981f
  definition, 929
Arterial outflow vessels *see* Outflow tract, heart
Arterial system, 1086, 1104
  post-gill arteries *see* Post-gill (systemic) arteries
  pre-gill arteries, 1086, 1086f
  resistance vessels *see* Resistance vessels
  see also specific arteries

Arterioarterial anastomosis
  definition, 1161
  prelamellar, 1102
  secondary, 1162, 1164*f*
Arterioles
  definition, 1104
  resistance, 1104–1105
   see also Systemic vascular resistance
Arteriosus
  definition, 1067
  see also Outflow tract, heart
Artery
  anatomy, 1104–1105
  definition, 973
  resistance *see* Systemic vascular resistance
  see also specific arteries
Ascending octaval (AO) nuclei, 284*f*, 286–287
Aschoff's rule, 738
Ash, definition, 1664
Ash Meadows, 1831*f*, 1833
  pupfish, 1833*f*, 1834
    Cyprinodon nevadensis mionectes see Cyprinodon nevadensis mionectes
    Devils Hole *see Cyprinodon diabolis*
Aspect ratios, 571
  definition, 564
Assembly, viral, 2032*f*, 2036
Assimilation markers, 1606
Associative learning, 708
  see also Classical conditioning
Astaxanthin, 694
  definition, 692
Astroscopidae, electric organ development, 415
Astyanax mexicanus, 1843*f*, 1844
  distribution, 1843*f*, 1844
  orientation mechanisms, 722–724, 724*f*
  troglomorphism *see under* Blind cavefish
Asymptomatic, definition, 2032
Athletic fishes, hematocrit, 986, 987*f*
Atlantic bluefin tuna (*Thunnus thynnus*)
  breeding migration route, 1933*f*
  diving behavior, 1934*f*
  long-term tracking, 1933*f*, 1935
  tag attachment, 1932, 1932*f*
  visceral rete, 1122, 1123*f*
Atlantic cod *see* Cod, Atlantic (*Gadus morhua*)
Atlantic killifish, 2058
Atlantic salmon (*Salmo salar*)
  diploid and triploid
    jaw deformities, 2011*f*, 2012
    physiological differences, 2010*f*, 2012
  ear, 253*f*, 254
  olfactory sensitivity to testosterone, 212
Atlantic sharpnose shark, conus arteriosus, 1026–1027, 1028*f*
ATP (adenosine triphosphate)
  consumption, 952, 952*f*
    hypoxia effects, 1769
  definition, 887, 1566, 1645
  erythrocytic concentration, control, 883, 885*f*
ATP (adenosine triphosphate) production, 951, 952, 952*f*, 1710, 1758
  fuels, 952*f*, 952–953, 955–956
    selection, 1760*f*, 1761–1762
  hypoxia/anoxia effects, 1765*f*, 1766, 1827
    see also Anaerobic metabolism
  pathways, 1758
    aerobic *see* Aerobic metabolism
    anaerobic *see* Anaerobic metabolism
  see also Cellular respiration; Mitochondrial respiration; Oxidative phosphorylation
ATPase
  Ca^{2+} *see* Ca^{2+}-ATPase
  definition, 1045, 1903
Atractoscion nobilis (white seabass), spawning/acoustic behavior, 315–316, 317*f*, 318*f*
Atrazine contamination, olfactory effects, 2081–2082
Atresia, definition, 1541
Atrial action potential, 1041, 1041*f*
Atrial fibrillation, transgenesis and fish models, 1991–1992
Atrial natriuretic peptide (ANP), capacitance curve and cardiac output, effect, 1115
Atrial pressure, 1032, 1032
Atrio-ventricular canal, 1032
  action potential, 1041, 1041*f*
Atrium, 1030–1031
  anatomy, 999, 999*f*, 1000, 1001*f*, 1002, 1068
    shape/size variations, 1003, 1003*f*
  definition, 998, 1030, 1903
  filling, pressure and, 1032
  see also Cardiac cycle; Heart
Attachment
  bacterial *see under* Bacterial pathogenicity
  viral, 2035
Attenuation, definition, 270
Audiograms
  auditory evoked potentials (AEP/ABR), 267, 267*f*, 278
  behavioral, 276, 277*f*
  definition, 276, 304
  elasmobranch hearing, 266, 267*f*
Auditory afferents
  coding of sounds, 293
    frequency coding, 294, 294*f*
    intensity coding, 293, 293*f*
    temporal coding, 294, 295*f*
  mixed synaptic transmission, Mauthner cell as model, 70*f*, 71
  octavolateral efferents *vs.*, 296–297
  phase locking *see* Phase locking
  tuning curve, 294, 294*f*
Auditory/lateral line CNS, anatomy, **283–291**
  central anatomy, 284*f*, 286
    forebrain connections, 290
      anterior tuber of hypothalamus, 291
      central posterior nucleus, 291
      preglomerular complex, 284*f*, 290
    midbrain torus semicircularis/lateral mesencephalic complex, 284*f*, 289, 290*f*
    nuclei projecting into midbrain torus semicircularis/lateral mesencephalic complex, 284*f*, 285*f*, 286, 286*f*
      higher-order nuclei, 289, 289*f*
      lateral line nuclei *see* Lateral line nuclei
      octaval nuclei *see* Octaval nuclei
  cross-talk between systems, 288
  evolution, 283
    brain circuits, 283, 284*f*
    sensory organs, 283
  organization, 284–285, 285*f*
Auditory plasticity, 295
  definition, 292
Auditory scene analysis (ASA), 298, 302
  definition, 276, 298
  source segregation, 281
Auditory system, 241, 292, 293*f*
  definition, 252
  see also Auditory/lateral line CNS, anatomy; Ears, fish; Hearing mechanisms
Auditory system structure, **252–261**
  sharks and rays, 262, 263*f*
    endolymphatic pores/ducts, 262–265, 264*f*
    fenestra ovalis, 262, 264, 264*f*
    macula neglecta, 262, 264, 264*f*, 265*f*
    otoconia (otoliths), 262, 263–264
    parietal fossa, 262, 264, 264*f*, 265
  see also Lagena; Otophysic connections; Saccule; Utricle; *specific structures*
Auditory threshold, definition, 304
Auditory transduction, 293
  definition, 292
  see also Hearing
Autapomorphy
  air-breathing fish, 1852
  definition, 1850
Autocrine factors
  definition, 1429
  rectal gland secretion control, 1434
Autocrine signaling, 1191
  definition, 1190, 1515
Autonomic nerves
  cranial *see* Cranial autonomic nerves
  spinal *see* Spinal autonomic nerves
Autonomic nervous system (ANS), **80–88**
  chromaffin tissue and *see under* Chromaffin cells
  definition, 80, 1067, 1229, 1332
  of different fish groups, 81
    cyclostomes *see under* Cyclostomes
    dipnoans *see under* Lungfishes
    elasmobranchs *see under* Elasmobranchs
    teleosts *see under* Teleosts
  functional aspects, 84
    cardiac control, 84, 85*f*, 1068
    control of iris, 86, 86*f*, 87*f*
    gastrointestinal blood flow regulation, 1211
  gut *see* Gut innervation
  neurotransmitters *see* Autonomic neurotransmitters
  pathways, 1068
    see also Parasympathetic nervous system; Sympathetic nervous system
Autonomic neurons, extrinsic innervation/chemical codes, 1068
  gut *see* Gut innervation
  heart, 1068
    see also Intracardiac neurons/neurotransmitters
Autonomic neurotransmitters, 1069
  cardiac *see* Intracardiac neurons/neurotransmitters
  definition, 1067
  see also Neurotransmitters; *specific neurotransmitters*
Autosomal dominant polycystic kidney disease (ADPKD), 1992
Autostyly, 423
  definition, 419
Avascular, definition, 929, 1119
Avoidance behaviors
  anti-predator *see* Predator avoidance
  dangerous temperatures, 762, 762*f*, 763*f*
  noxious/unpalatable substances, 230, 230*f*
  optic tectum role, 141
Axial skeleton
  bony fish *see under* Bony fish skeleton
  cartilaginous fish, 421, 422*f*, 423*f*
    batoids, 422*f*, 425
    chimaeroids, 426
    sharks, 424
Axon(s)
  definition, 123, 1457
  myelinated *see* Myelinated axons
  retinal ganglion cells, 131

B

Background noise, definition, 304
Backward swimming, 577
  see also Maneuverability
Bacteria
  commensal *see* Commensal bacteria
  pathogenic, 2033*t*
    see also Bacterial pathogenicity
  translocation across intestinal epithelium, 1328, 1328*f*, 1329*f*
  see also specific bacteria
Bacterial diseases, 2033*t*
Bacterial pathogenicity, 2033*t*
  attachment and colonization, 2037
    flagellin role, 2037
  bacterial surface molecules, 2036
    lipopolysaccharides, 2036
    S-layers, 2037
  control methods, 2038
  extracellular products *see* Extracellular products (ECPs), disease role

  - quorum sensing, 2038
  - regulation of virulence factors, 2037
    - iron acquisition, 2037
- Bacterial surface molecules *see under* Bacterial pathogenicity
- *Bairdiella chrysoura see* Silver perch (*Bairdiella chrysoura*)
- Balance
  - elasmobranch, 1809
  - *see also* Vestibular system
- Balistiform swimming, 571
  - definition, 564
- Band-III protein anion exchanger, 911, 911*f*
  - definition, 909
- Bandwidth, definition, 270
- Barbels, 487–488
  - gustatory role, 218–219, 224
  - sensory nerve and brain connections, 192
  - taste buds, 187, 189–190
- Baroreceptors, 978
  - definition, 977
  - *see also* Arterial blood pressure regulation
- Barreleye fish, bioluminescence, 501
- Barriers
  - anthropogenic, 1653*f*, 1654, 1925*f*, 1926
    - *see also* Dams
  - blood–brain *see* Blood–brain barrier (BBB)
  - gut *see under* Gut
  - immunological *see* Immunological barrier
- Basal, definition, 1322
- Basal ganglia, 63, 63*f*
  - definition, 56
- Basal labyrinth, definition, 1381
- Basal lamina, definition, 1395
- Basal media
  - definition, 1965
  - fish cell culture, 1968
- Basal metabolic rate (BMR)
  - definition, 951
  - measurement, 1567
  - tissue contributions, fish *vs.* mammals, 951–952, 952*t*
  - *see also* Metabolic rate (MR); Standard metabolic rate (SMR)
- Basal plate, 2–3, 3*f*
  - definition, 1
- Basement membrane, definition, 102, 1284
- Basic helix-loop-helix (bHLH) genes, 8, 10, 13
- Basic helix-loop-helix (bHLH) proteins, definition, 6
- Basic prey model (BPM), 1593
- Basking shark (*Cetorhinus maximus*)
  - satellite tracking, 1931
  - swimming speed, 1581
- Basolateral, definition, 1311, 1807
- Basolateral membrane
  - definition, 1229, 1429
  - rectal gland, 1431*f*, 1432, 1434
- Basolateral tubular network, definition, 1366
- Basophils, 989*f*, 990
- Bathypelagic, definition, 497
- Batoids
  - cranial muscles, 455, 457*t*
    - actions, 457*t*
    - branchiomeric, 455, 456*f*, 458*f*
    - insertions, 455, 456*f*, 457*t*
    - phylogenetic relationships, 455
  - definition, 1807
  - skeletal anatomy, 421*f*, 425
    - appendicular skeleton, 426
    - axial skeleton, 422*f*, 425
    - visceral arches, 425
      - *see also* Cartilaginous fish; Elasmobranchs; Rays; Skates
- BCR/ABL oncogene, 1991
- Beacon, definition, 720
- Behavior, fish
  - anthropogenic influences *see* Anthropogenic influences/disruptions
  - avoidance *see* Avoidance behaviors
  - chemosensory *see* Chemosensory behavior
  - compensatory growth effects *see* Compensatory growth, behavioral effects
  - feeding *see* Feeding behavior
  - growth hormone transgenic fish, 2021*f*, 2022
  - hagfish, deployment of slime
    - defense and competition avoidance function, 513–515, 515*f*
    - escape struggles and seawater mixing, 510, 511*f*
  - human influences *see* Anthropogenic influences/disruptions
  - metabolic rate suppression strategies, 1767*f*, 1768
  - physiology and *see* Behavior–physiology interrelations
  - response to hypoxia *see* Hypoxia, behavioral responses
  - response to parasites *see* Host manipulation; Parasite(s)
  - response to temperature *see* Temperature effects; Temperature preferences
  - sexual *see* Sexual behavior
  - *see also individual species*; *specific behaviors*
- Behavioral disorders (human), fish models, 1995
- Behavioral studies
  - fish responses to human-generated sounds, 307
  - magnetic sense *see under* Magnetic sense
  - visual capabilities *see* Visual capabilities, behavioral studies
- Behavioral thermoregulation, 1696–1697, 1911*f*, 1912
  - brook and rainbow trout, 762, 762*f*
  - definition, 758
  - striped bass, 762, 763*f*
  - *see also* Temperature preferences
- Behavior–physiology interrelations, 783, **647–648**
  - environmental interactions, 648
  - sensory systems, 647
  - social/reproductive behaviors, 648
- Benthic, definition, 98, 166, 744, 1887
- Benthic fish
  - definition, 1954
  - *see also* Deep-sea fishes
- Benthic operations, robotic fish use, 607, 608*f*, 609*f*, 610*f*, 611*f*
- Benthopelagic, definition, 520
- Benthopelagic fish, buoyancy, 521, 523, 525
- Benzolamide
  - definition, 899
  - inhibition of carbonic anhydrase, 901*f*, 905, 906*f*
- Bicarbonate
  - blood, 909–910
    - *see also* Carbon dioxide (CO$_2$) transport/excretion
  - intestinal *see* Intestinal bicarbonate
  - renal reabsorption, 1416, 1416*f*
- Bichirs
  - ampullary organs, 352
  - intracardiac neurons/neurotransmitters, 1069, 1070*f*
- Bifurcate, definition, 1085, 1095
- Bigeye tuna (*Thunnus obesus*), bulbus arteriosus, anatomy, 1016*f*
- Bilayer, definition, 1709
- Bile, 1303
  - gall bladder storage, 1303, 1304*f*
- Bile acids, 211, 1303, 1303*f*
  - definition, 208, 218
  - digestion role, 1304
  - gustatory responses, 219*f*, 221, 223*f*
  - olfactory responses, 211, 211*f*
- Bile salt-dependent lipase, 1281–1282
- Billfishes, endothermy, 1914–1915, 1918*f*
  - heater organ, 1916*f*, 1916–1917
- Bimodal respiration, 1852, 1854
  - clariids, 1858
  - definition, 1850, 1861, 1876
  - *see also* Air-breathing fish
- Bio-logging
  - definition, 1636
  - estimation of exercise-related energy expenditure, 1642–1643
- Bioaccumulation, 2069, 2071, 2074–2075
  - bioenergetics model applications, 1679
  - definition, 2062
  - metals, 2064
  - *see also* Toxicants
- Bioacoustic parameter, definition, 684
- Bioacoustics, **236–243**
  - current perspectives, 241
  - hearing *see* Hearing
  - historical perspectives, 238, 238*f*, 239*f*, 240*f*, 241*f*
    - Tavolga's work, 240–241
  - sound production *see* Sound production
- Bioactive substance, definition, 471
- Bioavailability
  - definition, 1664
  - nutrient, assessment, 1671
- Biochemical adaptations, general principles, **1681**
  - conservation of gene regulatory ability, 1686
  - conservation of membrane function, 1685
    - homeophasic adaptation *see* Homeophasic adaptation
    - homeoviscous adaptation *see* Homeoviscous adaptation
  - conservation of optimal values, 1681*f*, 1682
  - conservation of protein structure/function *see* Protein structure/function, conservation
- Biochemical strategies, metabolic rate suppression, 1767*f*, 1768
- Bioconcentration, 2069, 2071, 2072
  - definition, 2062
  - factors (BCF), 2075, 2076
  - metals, 2064
- Biodiversity
  - coral reefs *see under* Coral reefs
  - pelagic ocean, 1929
  - teleost-specific genome duplication (TSGD) and, 1981, 1981*f*
- Bioenergetics, **1563**
  - animal, 1666*f*, 1667
  - cultured fish *see* Bioenergetics, in aquaculture
  - definition, 1623, 1668, 2084
  - in ecosystems, modeling *see* Bioenergetics models
  - energy partitioning, 1666*f*, 1668
    - environmental influences, 1666*f*, 1668
    - simple model, 1666*f*, 1667*f*, 1668
  - feeding and digestion *see* specific dynamic action (SDA)
  - flowchart for fish, 1563*f*, 1564
  - growth and reproduction costs, 1909
  - models *see* Bioenergetics models
  - swimming efficiency *see* Swimming efficiency, bioenergetics
  - thermal limits for physiological processes, 1742*f*, 1743
  - *see also* Energetics; Energy budget; Metabolic rate; Thermodynamics
- Bioenergetics, in aquaculture, 2085, **1664**
  - chemical analyses and energy determination, 1669
  - energy expenditure, 1672, 1672*f*
  - feeds/feedstuff
    - biological evaluation (growth trials), 1668*t*, 1669*t*, 1670, 1673
    - chemical analyses, 1669
    - effect on metabolic rate, 1672*f*, 1672–1673
    - growth and nutrient retention efficiency, 1673
    - intake assessment, 1670*f*, 1671
    - nutrient bioavailability assessment, 1671
  - future perspectives, 1673
  - nitrogenous excretion rates, 1673
  - units of measurement, 1669
- Bioenergetics models, **1675**
  - applications, 1675–1676

- Bioenergetics models (*continued*)
    - assessment of environmental effects on fish growth and consumption, 1678
    - contaminant accumulation in fish, 1679
    - estimation of fish role in nutrient cycling, 1679
    - estimation of strength of predator–prey trophic link, 1678
    - fisheries management, 1677
    - limitations, 1679
  - growth *see* Growth, bioenergetic models
  - overview, 1675
  - Wisconsin models *see* Wisconsin bioenergetics models
- Biofactories, transgenic fish as, 2006
- Biogenesis, mitochondrial *see* Mitochondrial biogenesis
- Bioinformatics, definition, 2032
- Bioinspiration, 604
  - definition, 603
- Biological clocks, 736
  - circadian *see* Circadian clock
  - circannual, 743
  - circatidal, 742
  - lunar, 742
  - semilunar, 742
- Biological variation, sources, 2058
- Bioluminescence, 418, 497
  - definition, 98, 166, 416, 497
- Bioluminescent fish, 167, **497–503**, 168*f*
  - counterillumination *see* Counterillumination
  - flash kinetics, 498
  - future perspectives, 502
  - habitat, 498
  - light environment and, 498
  - light organs, 497
  - observation, 498
- Bioluminescent fish species, 499
  - anglerfish, 500, 500*f*
  - barreleye fish, 501
  - cardinal fish, 501
  - dragonfish, 502, 502*f*, 503*f*
  - flashlight fish, 499
  - hatchetfish, 499, 499*f*
  - lanternfish, 501, 501*f*
  - midshipman, 499, 500*f*
  - ponyfish, 501
  - tubeshoulders, 501
- Bioluminescent signaling, 693
- Biomagnification, 2069, 2071, 2074, 2076
  - definition, 2062
  - metals, 2064
- Biomechanics, inner ear of fishes *see* Inner ear
- Biomimetics
  - definition, 516, 603, 604
  - fish robotics approach, 604
- Biotelemetry
  - definition, 1636
  - estimation of exercise-related energy expenditure, 1642–1643
- Biotic, definition, 1695
- Biotic factors
  - definition, 1629, 1664
  - growth effects *see under* Growth, environmental effects
  - influence on energy partitioning/production, 1666*f*, 1668
  - *see also specific biotic factors*
- Biotransformation, 2070*f*, 2072
  - induction, 2072–2073
  - metals, 2064
  - PAHs, metabolic fates, 2075
    - activation (increased toxicity), 2071*f*, 2072
- Biparental care, 672, 673*f*
  - *see also* Parental care
- Bipolar cells, 124*f*, 125
- Birds
  - *Diplostomum spathcaeum* hosts, 778–779, 779*f*
  - *Schistocephalus solidus* hosts, 780*f*, 780–781
- Birnaviridae *see under* Viral diseases
- Birth, nutrient provision *see under* Parental nutrient provision
- Bisazir, sea lamprey population control, 1786
- Biting, 469, 598
  - associated muscles, 464*f*, 465*t*, 470
- Black marlin (*Makaira indica*), swimming speed, 1581
- Black Sea, hypoxic area, 1748, 1749
- Bladder, urinary, water homeostasis role, 1372
- Blaska-type respirometer, 851, 851*f*
- Blastoderm, 1285
  - definition, 1284
- Blazka-type respirometer, 1655*f*, 1658
- Blind cavefish, **1843**
  - troglomorphism, 1843*f*, 1844
    - color polymorphism, 1976
    - constructive traits, 1845
      - feeding apparatus amplification, 1845–1846, 1847
    - evolution, 1848
    - inheritance of troglomorphic traits, 1848
    - regressive traits, 1844
      - eye degeneration *see* Eye degeneration, blind cavefish
  - *see also Astyanax mexicanus*
- Blood
  - carbon dioxide transport *see* Carbon dioxide (CO_2) transport/excretion
  - cells/cellular composition, **984–991**
    - red blood cells *see* Red blood cells
    - separation, 985, 985*f*
    - white blood cells *see* Leukocytes
  - definition, 984
  - formation *see* Erythropoiesis; Hematopoiesis
  - hagfish, 1773
  - hemoglobin *see* Hemoglobin(s)
  - non-Newtonian properties, 980, 980*f*
  - nutrients, appetite/food intake effects, 1486, 1513
  - oxygen affinity *see* Oxygen binding, fish hemoglobins
  - oxygen binding *see* Oxygen binding, fish hemoglobins
  - pH *see under* pH
  - plasma *see* Plasma
- Blood diseases, fish models, 1990
  - anemia, 1990
  - hematopoietic stem cells, 1990
  - leukemia, 1991
- Blood flow
  - coronary *see* Coronary blood flow
  - distribution, 817
  - gastrointestinal *see* Gastrointestinal blood flow (GBF)
  - redistribution, gastrointestinal tract, 1209
  - vascular resistance and, 1105
  - *see also* Circulation
- Blood gas-equilibrium curve
  - nonlinear nature, effect on gas exchange efficiency, 826, 827*f*
  - oxygen *see* Oxygen equilibrium curves (OECs)
- Blood pressure, 1143–1144
  - balance with oncotic pressure, 1144–1145
  - definitions, 979, 1073
  - maintenance during exercise, 817
  - regulation *see* Arterial blood pressure regulation
  - secondary circulation, 1167
  - *see also* Arterial blood pressure
- Blood vessels
  - functional definitions, 982
  - gill *see* Gill vessels
  - resistance *see* Resistance vessels
  - *see also* Cardiovascular system; Venous system
- Blood viscosity
  - adaptations in Antarctic species, 1203
  - hematocrit and, 980, 980*f*, 987
  - *see also* Fahraeus–Lindqvist effect
- Blood volume
  - hagfish, 1773–1774
  - mean circulatory filling pressure and *see under* Mean circulatory filling pressure
  - stressed *see* Stressed blood volume
  - total, determinants, 982
  - unstressed *see* Unstressed blood volume
- Blood–brain barrier (BBB), 1144, 1145
  - ammonia permeability, 1441
  - elasmobranchs, 1816
- Blood–oxygen binding *see* Oxygen binding, fish hemoglobins
- Blue marlin (*Makaira nigricans*)
  - bulbus arteriosus, 1016*f*, 1018, 1020*f*
  - satellite tracking, 1932
- Blue sac disease, 2075–2076
- Blue-striped grunt (*Haemulon sciurus*), hearing audiogram, 277*f*, 277–278
- Bluefin tuna, Atlantic *see* Atlantic bluefin tuna (*Thunnus thynnus*)
- Bluegill sunfish (*Lepomis macrochirus*), wake, 541, 541*f*, 542*f*
- *Bmal* genes, 741
- Body coloration *see* Coloration
- Body design, fast start performance and, 592*t*, 594
- Body-fin form, maneuverability and, 579
- Body mass
  - cardiovascular variables, 1035*t*
  - considerations, respiratory physiology techniques, 848
  - scaling *see* Scaling, body mass
- Body orientation
  - neural control, 61, 61*f*
    - dorsal light response, 61
  - *see also* Vestibular system
- Body shape
  - fast start performance and, 594
  - maneuverability effects, 579
  - pelagic fish, 1890*f*, 1891
  - *see also individual species*
- Body size
  - fast start performance and, 592*t*, 594
  - lens focal length and, 107
  - maneuverability effects, 578
  - SDA impact, 1615
  - standard metabolic rate and, 1570
- Body temperature
  - effect on gut anatomy, 1274
  - SDA impact, 1613*f*, 1615
  - *see also entries beginning temperature*
- Body–caudal fin (BCF) swimmers, 603
  - robotic fish, 603
- Bohr effect, 748, **921–928**, 1903, 1911, 1912
  - benefits, 926
  - definition, 520, 526, 791, 887, 909, 916, 917, 921, 929, 935, 944, 1752, 1903
  - discovery, 921, 922*f*
  - evolution, 926, 927*f*
    - jawed vertebrates, 926*f*
  - fish hemoglobins *see* Bohr effect, fish hemoglobins
  - Haldane effect and *see* Bohr–Haldane effect
  - modulating factors, 922
  - molecular mechanisms in vertebrate hemoglobins, 923, 924*f*, 925*f*
    - mammals, 924, 925*f*
  - occurrence in animals, 307*f*, 923
  - Root effect *vs.*, 921, 922, 923*f*, 929, 930*f*, 935–936, 936*f*
- Bohr effect, fish hemoglobins, 522, 888, 888*f*, 890, 922*f*, 947
  - evolution, 926, 926*f*
    - hemoglobin buffer properties and, 927*f*, 928
  - lampreys *see under* Lampreys
  - modulating factors, 922
  - molecular mechanisms, 924*f*, 925, 925*f*, 1531*f*, 1532
  - occurrence, 923
  - Root effect *vs.*, 921, 923*f*, 935–936, 936*f*
  - teleosts *see under* Teleosts
  - *see also* Bohr effect

- Bohr–Haldane coefficient, 917
  - optimal, 917, 918f
- Bohr–Haldane effect, 890, 917
  - nonlinear, within OEC, 916–917, 918
  - theoretical coefficient optimal for oxygen delivery, 917, 918f
- Bone
  - acellular *see* Acellular bone
  - cartilage *vs.*, 429
  - cellular, definition, 428
  - chondral, definition, 434
  - compound, definition, 434
  - perichondral *see* Perichondral bone
  - *see also* Bony fish skeleton
- Bone marrow transplantation, fish model, 1989, 1990–1991
- Bone morphogenetic protein (BMP), 8
  - definition, 6
  - pathway, 9
- Bony fish
  - cranial muscles *see* Bony fish cranial muscles
  - definition, 236, 1457
  - endogenous opioid peptides
    - prodynorphins, 92t
    - proenkephalins, 90t
    - proopiomelanocortins, 93t
  - lateral line system
    - development, 339, 342f, 343f
    - structural variation, 338, 341f, 342f
  - lobe-finned *see* Sarcopterygians
  - ray-finned *see* Ray-finned fish
  - skeleton *see* Bony fish skeleton
  - *see also individual species*
- Bony fish cranial muscles, **463–470**
  - functional anatomy, 465t
    - biting/tearing, 464f, 465t, 469
    - buccal pumping, 463, 464f, 465t, 467f, 468f
    - pharyngeal jaw processing, 465t, 468f, 470
    - premaxillary protrusion, 464f, 469
    - suction feeding, 464f, 465t, 467, 467f
- Bony fish skeleton, **434–448**
  - appendicular skeleton, 445, 446f, 447f
  - cranial axial skeleton, 435
    - dorsal branchial arches, 440–442, 441f
    - lower jaw, 439, 439f
    - skull, 435, 436f, 437f
    - suspensorium, 439–440, 440f
    - upper jaw, 435, 438f, 438–439
    - ventral hyoid/branchial arches, 440, 441f
  - functional anatomy, current knowledge, 417
  - hierarchical study approach, 434
  - postcranial axial skeleton, 442, 443f, 444f, 445f
    - caudal, 442–443, 443f, 444f
    - median fin supports, 443–445, 445f
    - scale jacket, 442
    - vertebral column, 442, 443f, 444f
- Bottlenose dolphin, whistles, 315, 315f
- Boundary layer
  - definition, 581
  - diffusive *see* Diffusive boundary layer (DBL)
  - eddies and, 586
- Bower, 657–658
  - definition, 656
- Bowman's capsule *see* Glomerular capsule
- *Brachyhypopomus*, electrocytes, 391, 392f, 393f
- Bradycardia
  - definition, 1221, 1876
  - hypoxia-induced, 1222, 1223f
    - crucian carp, 1226–1228, 1227f
- Bradykinins, vascular resistance control, 1106
- BRAF oncogene, 1995
- Brain(s), **1–5**
  - development, 2, 3f
  - function, anthropogenic disruptions, 786
  - functional morphology, **1–5**
    - agnathan *see* Agnathan brain, functional morphology
    - cartilaginous fish *see* Cartilaginous fish, brains
    - organization, 2, 3f, 4f
    - ray-finned fish *see* Ray-finned fish, brains
    - sarcopterygians *see under* Sarcopterygians
  - gustatory and olfactory areas, 190
    - neural organization, 192f
      - *see also* Telencephalon
  - heat exchangers serving, 1125, 1126t
  - historical perspective, 3
  - learning and memory areas, 708, 708f
  - mass, body size scaling, 1579f, 1581
  - medullary lobes, 190, 190f
  - organization, 2, 3f
  - sound source localization mechanisms, 302, 302f
  - structural variations across taxa, 4
  - *see also specific areas/structures*
- Brain hormones, appetite regulation, 1512, 1512t
- Brain slices
  - crucian carp, anoxia effects, 1828, 1829
  - definition, 1824
- Brain temperature
  - telemetry, 1918f, 1919
  - *see also* Cranial endothermy
- Braincase, bony fish, 435, 437f
- Brain–hypophyseal–gonadal (BPG) axis, 627, 628f, 1500, 1501f
- Brain–pituitary relations, 1460, 1461f
  - *see also* Hypothalamic–pituitary–interrenal (HPI) axis
- Brainstem, definition, 26, 56, 854
- Brain–sympathetic–chromaffin cell (BSC) axis, stress response, 1516, 1516f, 1517f
- Braking, 571, 578
  - effect of fish size, 578
  - hydrodynamics, 573
- Branchial, definition, 1444
- Branchial anatomy *see under* Gill(s)
- Branchial chemoreceptors
  - carbon dioxide, 866, 867f
  - oxygen *see* Oxygen chemoreceptors, branchial
- Branchial circulation, 1086, 1086f
  - pre-gill arteries, 1086, 1086f
  - *see also* Post-gill arteries
- Branchial hearts, 1074, 1775
  - definition, 1073
- Branchial ionocytes *see* Gill ionocytes; Ionocytes
- Branchial nerves, 1097f, 1098, 1102
- Branchial remodeling *see* Gill remodeling
- Branchial respiration
  - definition, 838
  - development, 843, 843f, 844f
  - *see also* Gill(s); Respiration
- Branchial vascular resistance, systemic vascular resistance and, 1132, 1133f
- Branchial veins, 1101f, 1101–1102
- Branchiomeric, definition, 449
- Branchiomeric muscles
  - batoids, 455, 456f, 458f
  - holocephalans, 459, 459f, 460f
  - sharks, 450, 450f, 453f, 454f
- Branchiopores, lamprey, 1789f, 1791
  - external, 1790f
    - definition, 1788
  - internal, 1790f
    - definition, 1788
- Breeding behavior *see* Sexual behavior
- Breeding coloration
  - compensatory growth effects, 756
  - *see also* Coloration
- Breeding tubercles, 488
- Brett-type respirometer, 851, 851f, 1655f, 1658
- *Brevoortia patronus* (gulf menhaden), hearing, auxiliary anatomy, 274
- Brockmann body
  - definition, 1276, 1483
  - insulin/amylin production, 1485–1486
  - morphology, 1277, 1278f
- Brooding, physiological mechanisms, 675
- Brook trout (*Salvelinus fontinalis*)
  - behavioral thermoregulation, 762, 762f
  - gill remodeling, 801
  - triploid fish characteristics, 2010f, 2011f, 2012
- Brown eye phenotype, cavefish, 1848
- Brown trout (*Salmo trutta*), olfactory responses
  - electroencephalography, 214f, 215
  - olfactory bulb, 215–216, 216f
  - prostaglandins, 213, 213f
- Brush border
  - definition, 1395
  - renal tubules, 1402, 1406f
- Buccal cavity, 1095–1096, 1269
  - definition, 597, 765, 1095
- Buccal pumping, 463
  - associated muscles, 464, 464f, 465t, 467f, 468f
  - definition, 463
- Buffers, definition, 1045
- Bulbospinal pathway, electrogenesis, 391
- Bulboventricular vale, 1086–1087
- Bulbus arteriosus, 1018, 1086–1087
  - anatomy/form, 999f, 1000, 1001f, 1003, 1015, 1016f, 1017f, 1018
    - bulbus type, 1018, 1019f
    - endocardial cells, 1019–1020, 1021f
    - internal elements, 1018, 1020f
    - layers, 1018, 1020f
    - tubular type, 1018
  - Antarctic species, 1018–1019, 1021f, 1203, 1204f
  - blue marlin, 1016f, 1018, 1020f
  - definition, 1015
  - function, 1021, 1021f, 1022f, 1023f
  - rainbow trout, 1018, 1022, 1024f
  - tuna, 1023–1024
    - bigeye, 1016f
    - yellowfin *see under* Yellowfin tuna
- Buoyancy, **520–525**, **516–519**
  - adaptations, 521, 523
    - changing depth, 522
    - deep-sea fishes *see under* Deep-sea fishes, pressure effects
    - dense fishes, 521
    - keeping afloat, 523, 524f
    - life on the bottom, 523
    - lifestyle and, 521
  - center of *see* Center of buoyancy
  - control
    - air-breathing organ and, 1858–1859
    - elasmobranchs, 1810
    - swimbladder role *see* Swimbladder
  - current knowledge, 518
  - definition, 516, 1887, 1903
  - energy cost, 523–525
  - fish density and, 520–521
  - historical perspective, 517, 517f
  - negative *see* Negative buoyancy
  - neutral *see* Neutral buoyancy
  - pelagic fish, 1892
  - positive, definition, 159, 516, 520, 1887
- Burst-and-coast swimming, 1659–1660
- Burst firing, regulation, electrosensory lateral line lobe, 371, 371f
- Burst swimming, 1908
  - definition, 1887
  - pelagic fish, 1894, 1897
  - recovery period, 812, 1908

C

- C-start, 587–588, 588f
  - definition, 73–74, 587
  - double-bend response *see* Double-bend response
  - hydrodynamics, 590, 591f
  - Mauthner cell role, 74f, 74–75
    - *see also* Mauthner cells
  - muscle activity, 589
  - neural control, 588
  - single-bend response *see* Single-bend response
  - *see also* Fast start

C-type natriuretic peptide (CNP), rectal gland activation role, 1433*f*, 1434
Ca^{2+}-ATPase
  definition, 1045
  plasma membrane *see* Plasma membrane Ca^{2+}-ATPase (PMCA)
  sarcoplasmic reticulum *see* Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA)
$[Ca^{2+}]_i$, definition, 1060
Cadaverine, olfactory responses, goldfish, 214
Cadmium (Cd), biological effects and toxicodynamics, 2065
  effect on alarm substance response, 784*f*, 784–785
Caecilian amphibians, ampullary organs, 354
Calcification
  'continuous cartilage', 433
  types, 431–433
Calcified cartilage
  cap (prismatic) *vs.* body (globular), 428
  cartilaginous fish, 431
  see also Areolar cartilage
Calcitonin gene-related peptide (CGRP)
  amino acid sequence, 1336*f*
  enteric nervous system, 1336
Calcium (Ca^{2+})
  osmosensory signal transduction role, 1374
  pCa_{50}, 1064
    definition, 1060
Calcium-activated potassium channels, definition, 56
Calcium activation
  cardiomyocyte contraction, 1055, 1055*f*
  definition, 1054
Calcium affinity
  cardiac troponin C *see under* Cardiac troponin C (cTnC)
  definition, 1054
Calcium flux
  cardiac action potential, 1039, 1040, 1041
  routes in cardiac excitation–contraction coupling *see* Calcium transient
  see also Cellular calcium flux; Ryanodine receptors
Calcium-induced-calcium release (CICR), 1049, 1049, 1049*f*
  definition, 1045
Calcium pump
  definition, 1045
  PMCA *see* Plasma membrane Ca^{2+}-ATPase (PMCA)
  SERCA *see* Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA)
Calcium-sensing receptor (CaSR), rectal gland, 1435
Calcium sensitivity
  cardiac myofilament, 1056, 1056*f*, 1060–1061, 1064, 1064*f*
    see also Cardiomyocyte contraction
  definition, 1054
Calcium transient, 1046, 1046*f*, 1060
  definition, 1045, 1060
  falling phase, 1046*f*, 1051
    Ca^{2+} storage in sarcoplasmic reticulum, 1051
    extracellular Ca^{2+} efflux, 1051
    temperature effects, 1051, 1052*f*
      see also Plasma membrane Ca^{2+}-ATPase (PMCA); Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA)
  rising phase, 1046*f*, 1047
    Ca^{2+}-induced Ca^{2+}-release (CICR), 1049, 1049*f*
    extracellular Ca^{2+} influx, 1047, 1048
      L-type Ca^{2+} channel *see* L-type calcium channel (LTCC)
      sodium–calcium exchanger (NCX), 1048
    intracellular Ca^{2+} influx, sarcoplasmic reticulum, 1049
    temperature effects, 1050, 1050*f*
Calcium uptake, freshwater fish gill ionocytes, 1361*f*, 1363
Calls
  alarm, 689–690
  amplitude, 688, 688*f*
  characteristics, 687, 687*f*
  constraints, 688
  courtship/display, 687, 687*f*, 688*f*, 689
  definition, 684
  duration, 687, 688*f*
  female, 689
  female preference for male call, 689
  frequency, 688
  male, 689, 689, 690
  multipulsed, 687*f*, 688
  simple *vs.* complex, 687, 687*f*
  tonal sounds, 688
  see also Sound production; Vocal fishes
Calorie, definition, 1206
Calorimetry, respiratory metabolism measurement, 846–847, 1566
  see also Respirometers
Camouflage, 490
  disruptive (high-contrast), 151*f*, 152, 154*f*
  protective behavior, reef fish, 152–153, 153*f*
    see also Coral reefs
  see also Color changes; Coloration
Canal neuromasts, 337, 339*f*
  distribution, 337, 340*f*
  function, 331, 332*f*
  hair cell orientation, 337, 340*f*, 342*f*
  innervation, 337–338, 340*f*
  morphological variation, 337, 340*f*
Cancer
  genes, **1971**
    see also Pigment cell tumors
  human, fish models, 1989
    skin cancer, 1977, 1977*f*, 1978, 1989, 2004*f*, 2005
    in vivo imaging, 1989, 1989*f*
  see also entries beginning tumor
Candidate gene, definition, 1843
Cannibalism, growth hormone transgenic fish, 2023
Cannula, definition, 1104
Capacitance
  definition, 1111
  venous *see* Venous capacitance
Capacitance coefficient
  definition, 820, 893
  respiratory gases
    air *vs.* water, 820, 821, 821*t*, 822, 822*f*
    oxygen, blood, 823, 823*f*, 895*t*, 897
    oxygen, water, 895*t*, 897
Capacitance vessels, **1111–1118**
  definition, 982–983
  see also Venous capacitance
Capillaries, **1142–1153**, 1146*t*
  definition, 973
  fluid movement across *see* Transcapillary fluid movement
  function, 1144
    anisotropic/isotropic capillary beds, 1142, 1145, 1147*f*, 1148*t*
    oxygen diffusion and capillary density, 1145
    regulation, red blood cell role, 884
    wall permeability, 1144–1145
  microvasculature in fish circulatory system, 1143
    angiogenesis, 1147
    related to oxygen demand, 1148
    research coverage, 1146–1147, 1150, 1151
    triploid fish, erythrocyte integrity, 2012
  protein permeability, 1159
  skeletal muscle supply, 1149
    variation between species, 1150, 1911
  structure, 1143
    compared with mammals, 1143–1144, 1145*f*
  thermal environment, adaptations, 1150
    cold acclimation, 1147, 1151
    cold-adapted species, 1151
  vascular origins (ontogeny), 1146
    adaptive scaling to muscle growth, 1146–1147, 1148*f*
  see also Retia
Capsaicin
  definition, 1178
  effect on cardiorespiratory interactions, 1181–1183
Capsule-producing bacteria, 2037
Captive fish
  appetite, environmental control, 1514
  see also Cultured fish
Carangiform, definition, 387
Carangiform fish, electric organ location, 396
Carangiform swimming, 548, 549*f*, 552
Carassius auratus see Goldfish
Carassius carassius see Crucian carp
Carbamino compounds
  definition, 909
  formation, 910
Carbamoyl phosphate synthetase (CPS), urea cycle, 1445, 1445*f*
Carbohydrases, 1281*t*, 1282
Carbohydrate(s)
  absorption, 1317, 1317*f*
  ATP production role, 952*f*, 953
  definition, 1664
  digestion, 1306*f*, 1307
  digestive efficiency, 1600*t*, 1601
  functions, 1618
  metabolism, elasmobranchs, 1813
Carbon dioxide (CO_2)
  capacitance coefficient, air *vs.* water, 821*t*, 822
  chemoreceptors *see* Carbon dioxide (CO_2) chemoreceptors
  diffusion across membranes, 834
    adult fish, 834, 835*f*
    developing fish, 834
    see also Carbon dioxide (CO_2) transport/excretion
  exchange *see* Carbon dioxide (CO_2) transport/excretion
  gustatory responses, 222, 224*f*, 225*t*
  partial pressure, effect on blood–oxygen binding, 921, 922*f*
    see also Bohr effect
  physiological responses in cultured fishes, 2087
  role in gas secretion into swimbladder, 913–914
  ventilatory response *see* Ventilatory response to CO_2/H^+
Carbon dioxide (CO_2) chemoreceptors, 866
  branchial, 866, 867*f*
  central, 868
  extra-branchial, 868
  gustatory/olfactory, 868
  stimulus specificity, 867*f*, 869
Carbon dioxide (CO_2) incubator
  definition, 1965
  fish cell culture, 1968
Carbon dioxide (CO_2) transport/excretion, **909–915**, 834
  blood, 880, 883*f*, 910, 910*f*, 911*f*
    effect of temperature and variations in environmental CO_2 levels, 914
    pH regulation role, 912, 913*f*, 913*f*
  excretion patterns, 814, 816, 817, 834, 835*f*, 913
    across gills, 914
    air-breathing fish, 914
    developing fish, 834
    elasmobranchs, 913
    hagfish, 911–912
    lampreys, 911–912, 913, 1792*f*, 1794–1795
    teleosts, 911, 912, 913
  oxygen interaction *see* Oxygen–carbon dioxide interaction
  see also Carbonic anhydrase (CA)
Carbon monoxide (CO)
  catecholamine release role, 1531
  vascular resistance control, 1107, 1107
Carbonic anhydrase (CA), **899–908**
  active site, 901, 902*t*
  catalytic mechanism, 900
  catalytic reactions, 834, 899, 910–911, 911*f*
    dehydration, 793–794, 899, 900*f*, 905, 906*f*, 907*f*

    hydration, 899, 905, 905–907, 907*f*, 919, 920*f*
  definition, 791, 829, 899, 909, 916
  discovery, 900
  electrometric ΔpH assay, 904
  gills, 900*f*, 904, 905, 906*f*
  inhibition, effects, 866–867, 868*f*, 899, 901*f*, 905, 906*f*
  isoforms, 834, 901
    extracellular, 901
    intracellular, 901, 904
    membrane-associated, 901, 903–904
    methodological approaches for discerning function, 904
    phylogenetic relationships among mammals and fish, 901–903, 904*f*
    physiological functions, 905, 906*f*, 907*f*, 907*f*
  red blood cells, 899, 900*f*, 904, 905, 906*f*, 910–911, 911*f*, 911–912, 913
    see also Carbon dioxide (CO_2) transport/excretion
  roles, 900, 907
    acid–base balance, 900, 900*f*, 904, 905, 907*f*
    carbon dioxide excretion, 899, 900*f*, 905, 906*f*
      see also Carbon dioxide (CO_2) transport/excretion
    ion transport, 905–907, 907*f*
Carboxyl ester lipase (CEL), 1281–1282
Carcharodon carcharias see White shark
Cardiac, definition, 998
Cardiac action potential, **1038–1044**, 974–975, 1068
  definition, 1038
  ionic basis, 1040
  phases, 1039, 1039*f*
  shapes in different heart sites, 1040, 1041*f*
    atrial myocytes, 1041
    atrio-ventricular canal, 1041
    pacemaker cells, 1040
    ventricular myocytes, 1041
  species-specific differences, 1042, 1042*f*
    autonomic nervous transmitter effects, 1042
    oxygen shortage effects, 1042
    temperature effects, 1043, 1043*f*
  temperature effects, 1043, 1043*f*, 1721, 1722
  see also Cardiac excitation–contraction coupling
Cardiac anatomy, **998–1005**
  air-breathing fish, 1004
  arterial outflow vessels *see* Outflow tract, heart
  atrium *see under* Atrium
  comparative, 1003
    shapes and sizes, 1003, 1003*f*
  coronary capillaries, 1143, 1145–1146
  cyclostomes, 1004
  ductus Cuvier *see under* Ductus Cuvier
  elasmobranchs, 1004
    see also Conus arteriosus
  general, 999
    macroscopic, 999, 999*f*, 1068
    microscopic, 1000, 1000*f*, 1001*f*
  heart mass
    plasticity, 816
    tunas, 1905*f*, 1908*f*, 1909
  lungfish *see under* Lungfishes
  sinus venosus, 999*f*, 999–1000, 1001
  ventricle *see under* Ventricle
  see also Cardiomyocyte(s); Heart; *specific structures*
Cardiac arrhythmias, fish models, 1991–1992
Cardiac chambers, 1030–1031
  anatomy, 999, 999*f*
  filling/emptying *see* Cardiac cycle
  see also Atrium; Outflow tract, heart; Sinus venosus; Ventricle
Cardiac circulation
  coronary circulation *vs.*, 1082
  definition, 1077, 1078
  schematic representation, 1078*f*
  see also Circulation
Cardiac conditioning, responses to magnetic fields, 728, 729*f*
Cardiac conducting system, 1071
  see also Cardiac action potential
Cardiac control, autonomic nervous system, 84, 85*f*, 1068
  see also Intracardiac neurons/neurotransmitters
Cardiac cycle, 1031
  cardiac filling, 1031, 1031*f*, 1032
    vis-a fronte see Vis-a-fronte cardiac filling
    vis-a-tergo see Vis-a-tergo cardiac filling
  definition, 1030
  electrical events, 1032*f*, 1033
  flow events (cardiac chamber filling/emptying), 1031, 1031*f*
  pressure events, 1031, 1032*f*
  pumping assistance, 1034
    contractions by other cardiac chambers, 1034
    other mechanisms, 1034
Cardiac excitation–contraction coupling, 974–975, 1046, 1046*f*
  calcium and contractile element, **1054–1059**
    adaptation to low temperatures, 1056
    in changing temperatures, 1058
    temperature effect on Ca^{2+} binding, 1055
    see also Cardiomyocyte contraction
  fish myocyte, 1046, 1047*f*
  mammalian myocyte, 1046, 1046*f*
  routes of cellular calcium flux *see* Calcium transient
  temperature effects
    Ca^{2+} transient
      falling phase, 1051, 1052*f*
      rising phase, 1050, 1050*f*
    cardiomyocyte contraction, 1058
      Ca^{2+} binding by contractile elements, 1055, 1056*f*
    changing temperatures, 1058
Cardiac filling, 1031, 1031*f*, 1032
  vis-a fronte see Vis-a-fronte cardiac filling
  vis-a-tergo see Vis-a-tergo cardiac filling
Cardiac muscle length–tension relationship, **1060–1066**
  cellular level *see* Sarcomere length (SL)–tension relationship
  whole heart level *see* Frank–Starling relationship
Cardiac myofilament calcium sensitivity, 1056, 1056*f*, 1060–1061, 1064, 1064*f*
  see also Cardiomyocyte contraction
Cardiac output (V_b), 815*f*, 816, 1034, 1035*t*
  aerobic swimming-associated increase, 1216
    blood flow redistribution and, 1217
    maximal, 1216
    mechanism, 1216
  anaerobic swimming burst, 1219
  arterial blood pressure regulation, 1172, 1172*f*
  definition, 159, 812, 973, 1030, 1034, 1060, 1132, 1190, 1206, 1215, 1887
  hormone inactivation and, 1194*f*, 1196
  hypoxia response, 1115, 1222, 1223*f*
  importance of Frank–Starling relationship, 1061, 1061*f*
  role in increasing gastrointestinal blood flow post-prandially, 1207, 1209*f*
  temperature effects *see* Temperature effects, cardiovascular system
  venous system role, 1112–1113
    see also Venous capacitance
Cardiac oxygen demand, definition, 1077
Cardiac pacemaker, 1071
Cardiac pumping, physiology, **1030–1037**
  assistance mechanisms, 1034
    contractions by other cardiac chambers, 1034
  cardiac cycle *see* Cardiac cycle
  cardiac output *see* Cardiac output
  cardiac power output, 1035*t*, 1036
  cardiac stroke volume *see* Cardiac stroke volume
Cardiac scope
  definition, 1200
  temperature effects, 1200, 1201*f*
Cardiac shapes and sizes, variation, 1003, 1003*f*
Cardiac stroke volume (V_s), 1034, 1035*t*, 1036*f*
  aerobic swimming-associated increase, mechanism, 1216
  definition, 1030, 1215
  factors affecting sensitivity to filling pressure, 1035
Cardiac troponin C (cTnC), 1055, 1055*f*
  human
    amino acid sequences, 1056, 1057*f*
    Ca2^{+} affinity, 1056, 1057*f*
  phylogenetic analysis, 1057, 1058*f*
  trout
    amino acid sequences, 1056, 1057*f*
    Ca2^{+} affinity, 1056, 1057*f*
      pH effects, 1059, 1059*f*
      temperature effects, 1047, 1057*f*
    low temperature adaptation, 1056
  see also Cardiomyocyte contraction
Cardiac troponin complex, 1055, 1055*f*
  definition, 1054
  see also Cardiomyocyte contraction
Cardiac troponin I (cTnI), 1055
Cardiac troponin T (cTnT), 1055
Cardiac vagal preganglionic neurons (CVPNs), 1179–1180
  definition, 1178
  respiratory neuron interactions, 1187, 1189
    elasmobranchs, 1181–1183, 1182*f*
    teleosts, 1183–1184, 1187*f*, 1188*f*
Cardiac work, 1035*t*, 1036
Cardinal fish, bioluminescence, 501
Cardinal hearts, hagfish, 1074, 1774–1775
Cardiolipin, 1710
  definition, 1709
  warm acclimation and, 1715
Cardiomyocyte(s), 1007
  contraction *see* Cardiomyocyte contraction
  definition, 159, 1006, 1060
  excitability, 1719*f*, 1720–1721
    fish *vs.* mammals, 1723
    membrane ion channel expression, 1721, 1721
  length–tension relationship *see* Sarcomere length (SL)–tension relationship
  resting membrane potential, 1039, 1040, 1041
  ultrastructure *see* Cardiomyocyte ultrastructure
Cardiomyocyte contraction, 1038, 1054, 1055*f*
  adaptation of trout contractile element to low temperature, 1056, 1057*f*
  Ca^{2+} activation, 1055, 1055*f*
  Ca^{2+} transient and, 1046, 1046*f*, 1060
    see also Calcium transient
  environmental temperature/pH effects, 1058, 1059*f*
  future research directions, 1059
  temperature effects on Ca^{2+} binding, 1055, 1056*f*
  see also Cardiac action potential; Cardiac excitation–contraction coupling; Cardiac troponin C (cTnC); Sarcomere length (SL)–tension relationship
Cardiomyocyte ultrastructure, **1006–1014**
  cell–cell connections, 1012, 1012*f*
  dimensions, 1007, 1007*t*, 1008*f*
  fat and glycogen deposits, 1010
  membrane, 1008, 1008*f*
  mitochondria, 1010, 1010*f*, 1013, 1909–1911
  myofibrils, 1008, 1009*f*, 1013
    sarcomere, 1008–1009, 1009*f*
  nucleus, 1009*f*, 1010, 1013
  percentage volumes of cellular constituents, 1011, 1012*t*, 1013, 1013*f*
  sarcoplasmic reticulum, 1011, 1011*f*, 1013
Cardiorespiratory coupling (CRC), 1179
  definition, 1178
Cardiorespiratory interactions (CRIs), 1179, 1180*f*, 1180*f*
  central control, **1178–1189**
    air-breathing fish, 1187, 1188*f*
    elasmobranchs, 1180, 1181*f*, 1182*f*
    neural basis, 1187

Cardiorespiratory interactions (CRIs) (*continued*)
    teleosts, 1183, 1184*f*, 1185*f*, 1186*f*, 1187*f*, 1188*f*
  definition, 1178
Cardiorespiratory synchrony (CRS), 1179, 1180*f*
  definition, 1178
  generation, 1184
Cardiovascular oxygen transport, 816
  definition, 812
  see also Oxygen transport/exchange
Cardiovascular (circulatory) system, 1086, **977–983**, **973–976**, 1132
  blood flow *see* Blood flow
  blood pressure *see* Blood pressure
  cardiac output *see* Cardiac output (V_b)
  construction, 981, 981*f*
  control *see* Cardiovascular system control
  current knowledge, 974
  digestion, responses in *see* Gastrointestinal blood flow (GBF), regulation during digestion
  exercise responses *see under* Exercise
  extent and arrangement in fish, 1143
  features, 982, 982*f*
  function, 977
  historical perspective, 973
  hypoxia responses *see* Hypoxia, circulatory system responses
  integrated control *see* Cardiovascular system control
  integrated responses to
    digestion *see* Gastrointestinal blood flow (GBF)
    hypoxia *see* Hypoxia, circulatory system responses
    swimming *see* Aerobic swimming; Exercise
    temperature *see* Temperature effects, cardiovascular system
  operating principles, 978
  pelagic fish, 1893
  postprandial responses *see* Gastrointestinal blood flow (GBF), regulation during digestion
  resistance vessels *see* Resistance vessels
  respiratory system interaction *see* Cardiorespiratory interactions (CRIs)
  schematic representation, 1133*f*
  sustained swimming responses, 813*f*, 816
  triploid fish, 2011
  see also Arterial system; Capillaries; Circulation; Heart; Red blood cells; Vascular anatomy; Venous system
Cardiovascular system control, **1169–1177**, 975–976
  integrative analysis, 1172
    arterial blood pressure regulation *see* Arterial blood pressure regulation
    tissue perfusion regulation, 1174, 1175*f*
  integrative responses
    digestion *see* Gastrointestinal blood flow (GBF), regulation during digestion
    exercise *see* Exercise
    hypoxia *see* Hypoxia
    temperature *see* Temperature effects, cardiovascular system
  negative feedback mechanism, 1170, 1171*f*
    examples, 1174
      baroreceptor-mediated restoration of arterial blood pressure following hemorrhage, 1174, 1175*f*
      exercise-/digestion-related, 1176
      local regulation of blood flow in response to oxygen demand, 1176, 1176*f*
  rheostasis mechanism, 1171, 1171*f*
    examples, 1176
      programmed rheostasis, 1177
      reactive rheostasis, 1176
  systems, 1170, 1171*f*
    components, 1170, 1170*f*
    feedforward, 1171, 1171*f*
      examples, 1176
    integration, 1171
    negative feedback *see* Cardiovascular system control, negative feedback mechanism
    rheostasis *see* Cardiovascular system control, rheostasis mechanism
Care, parental *see* Parental care
Carnivore, definition, 1664
Carotenoid(s)
  digestion, 1309
  pigmentation, 490*t*, 491
  reflected-light signaling, 694
Carotid artery, 1086*f*, 1089
  internal, retia, 1125
Carp(s)
  common *see* Common carp (*Cyprinus carpio*)
  crucian *see* Crucian carp
  goldfish *see* Goldfish (*Carassius auratus*)
  growth hormone transgenesis, 2020
  hormonal pheromones *see under* Hormonal pheromones
  see also Cyprinids
Carrier-mediated transport, 977
CART (cocaine- and amphetamine-regulated transcript), appetite suppression, 1485, 1485*f*, 1512, 1512*t*
Cartilage
  bone *vs*., 429
  types in cartilaginous fish *see* Cartilaginous fish, skeletal tissues
Cartilaginous fish
  ampullary electroreceptors, 351, 351*f*, 352*f*
    development, 410
  brains *see* Cartilaginous fish, brains
  characteristics, 26
  cranial muscles *see* Cartilaginous fish, cranial muscles
  electric organs, 409–410
    development, 412
  endogenous opioid peptides
    prodynorphins, 92*t*
    proenkephalins, 90*t*
    proopiomelanocortins, 93*t*
  lateral line system, 341, 344*f*, 345*f*
  skeletal anatomy *see* Cartilaginous fish, skeletal anatomy
  skeletal tissues *see* Cartilaginous fish, skeletal tissues
  see also Elasmobranchs; *individual species*
Cartilaginous fish, brains, **26–36**
  development, 26–27
  forebrain, 27*f*, 32
    diencephalon, 32
    hypothalamus, 31*f*, 32
    preoptic area, 34
    telencephalon, 34, 34*f*
  hindbrain, 27, 27*f*, 28*f*, 30*f*
    cerebellum, 29, 30*f*
  midbrain, 27*f*, 31, 31*f*
  see also Brain(s)
Cartilaginous fish, cranial muscles, **449–462**
  batoids *see under* Batoids
  holocephalans *see under* Holocephalans
  sharks *see under* Sharks
  terminology, 451*t*
Cartilaginous fish, skeletal anatomy, **419–427**
  batoids *see under* Batoids
  chimaeroids *see under* Chimaeras
  functional, current knowledge, 417
  general, 420*f*, 421, 421*f*
    appendicular skeleton, 424
    axial skeleton, 421, 422*f*, 423*f*
    visceral arches, 420*f*, 422
  sharks *see under* Sharks
Cartilaginous fish, skeletal tissues, **428–433**
  cartilage types, 429, 430*f*
    areolar cartilage, 430*f*, 431
    fibrocartilage, 430*f*, 432
    neural arch cartilage, 430*f*, 432
    tessellated cartilage, 429–431, 430*f*
      reinforcements, 430*f*, 432–433
    vascular cartilage, 430*f*, 432
Catabolism, definition, 1623, 1629
Catadromy/catadromous life, 1922
  definition, 1921, 1937
  eels, 1938–1939, 1941
    see also Eel migration
Catalytic rate, 1703, 1705
Cataracts, *Diplostomun spathcaeum*-induced, 778–779, 779*f*
Catecholamine(s), **1524–1533**, 1534
  biosynthesis, 1524, 1525*f*
    site *see* Chromaffin cells
  blood levels, 1535
  circulating, effects and mechanism of action, 1531*f*, 1532
    vascular resistance control, 1105
  definition, 80, 1104, 1132, 1206, 1389, 1541
  degradation, 1532, 1532*f*
  as gut neurotransmitters, 1335
  receptors *see* Adrenergic receptors
  release into circulation *see* Catecholamine release
  storage site *see* Chromaffin cells
  stress response activity, 1516, 1516*f*, 1516–1517
    adrenaline *see under* Adrenaline
    dominance and, 652
    exercise-related osmorespiratory compromise, 1389–1390
    growth effects, 1537
    hypoxia response *see under* Hypoxia
    metabolic effects, 1536, 1536*f*
    noradrenaline *see* Noradrenaline
    termination, 1532, 1532*f*
    see also Stress response, hormonal
  see also specific catecholamines
Catecholamine release, 1525–1526, 1534
  control mechanisms *see* Catecholamine release, control mechanisms
  stimuli, 1526
    hypercapnia and exercise, 1527
    hypoxia, 1526, 1527*f*, 1529–1530, 1535*f*, 1535–1536, 1755
  threshold, definition, 1524
Catecholamine release, control mechanisms, 1528, 1528*f*, 1534
  cholinergic, 1528, 1528*f*
    studies using *in vitro* saline-perfused posterior cardinal vein preparation, 1529, 1529*f*
  noncholinergic, 1528*f*, 1530
    angiotensin, 1530
    gasotransmitters, 1531
    natriuretic peptides, 1530
    pituitary adenylate cyclase activating polypeptide, 1530
    vasoactive intestinal polypeptide, 1530
Catecholaminergic neurons
  agnathan, 19, 23
  cartilaginous fish, 29, 31*f*, 33–34
Catfish (Siluriformes)
  clariid *see* Clariids
  electrosense, 364, 365
  gustatory system (*Ictalurus* spp.)
    body surface taste buds, 187, 189–190
    brain, facial lobe, 190*f*, 191, 192
    relationship with tactile neurology, 192
  heat tolerance (*Ictalurus punctatus*), 1700
  Indian catfish (*Heteropneustes fossilis*), suprabranchial chambers, 1868*f*, 1870
  silurid *see* Silurids
  specific dynamic action, 1609*t*, 1611*f*, 1612
  testis morphology, 617–618, 620*f*
  see also individual species
Cathodal hemoglobins, 949
Caudal, definition, 1, 854
Caudal hearts, 1074, 1093
  eels, 1075
  hagfish, 1075, 1774–1775

  sharks, 1075
Caudal neurosecretory system (CNSS), development, 1454
Caudal peduncle, definition, 1085
Caudal skeleton, bony fish, 442–443, 443*f*, 444*f*
Caveolae
  cardiomyocytes, 1008, 1008*f*
  definition, 1006
Celiacomesenteric artery (CMA), 1086*f*, 1090, 1090–1093, 1133*f*, 1133–1134, 1134*f*, 1135*f*, 1206–1207
  see also Gastrointestinal blood flow (GBF)
Cell body, definition, 854
Cell culture, fish cell lines *see* Fish cell lines
Cell imaging techniques (microscopy)
  differential staining, 1383*f*, 1385, 1387
  fluorescent labeling, lectin dyes, 512*f*, 513, 514*f*
  freeze fracture electron microscopy, 1381, 1383–1384
  immunohistochemical (IHC) labeling, 1382*f*, 1386, 1387, 1387–1388
Cell lines, 1966
  definition, 1965
  fish *see* Fish cell lines
  mammalian, 1966
Cell membrane(s)
  deep-sea fishes, 1957
  excitable *see* Excitable membranes
  fluidity *see* Cell membrane fluidity
  functions, 1725
    conservation, 1685
      homeophasic adaptation *see* Homeophasic adaptation
      homeoviscous adaptation *see* Homeoviscous adaptation
  proteins *see* Membrane proteins
  respiratory gas diffusion across *see under* Diffusion
  structure, 1726
    lipid components, 1726*f*, 1727
      see also Lipid rafts; *specific lipids*
    lipid-dependent proteins, 1726
      see also Membrane proteins
    mechanisms for controlling composition, 1728
    regional specializations, 1727
  temperature effect
      see under Cell membrane fluidity; Temperature effects
  transport, 952*f*, 954
      see also specific membrane transporters
  see also entries beginning membrane
Cell membrane fluidity, 1728
  cholesterol role, 1728, 1731
  environmental effects, 1725–1726, 1729
    homeoviscous adaptation *see* Homeoviscous adaptation (HVA)
    mechanisms for sensing/responding to changes, 1731
Cell proliferation, intestinal epithelium, 1286
Cell-surface-area-to-volume ratio, definition, 1045
Cell–cell connections, cardiomyocytes, 1012, 1012*f*
Cellular bone, definition, 428
Cellular calcium flux
  cardiac action potential, 1039, 1040, 1041
  routes in cardiac excitation–contraction coupling *see* Calcium transient
  see also Calcium flux; Ryanodine receptors
Cellular homeostasis response (CHR), osmotic stress, 1376
Cellular metabolism, 951, 952*f*, 952*t*
  ATP production/consumption *see under* ATP (adenosine triphosphate)
  tissue contributions, fish *vs.* mammals, 951–952, 952*t*
  see also Cellular respiration; Metabolism
Cellular respiration, **951–958**
  ATP production *see under* ATP (adenosine triphosphate)
  cell membrane transport, 952*f*, 954
  cytosolic transport, 954
  environmental and energetic stress, effects, 956
  fish, 955
    novel species for study, 957
  fuel and oxygen delivery to tissues, 953, 957
  limits, 957
  mitochondrial transport, 952*f*, 955
    see also Mitochondrial respiration
Cellular stress response (CSR), osmotic stress, 1376
Celsius scale, 1690–1691
Census of Marine Life (COML), 1935
Center of buoyancy, 566*f*
  definition, 564
Center of mass, 566*f*
  definition, 564
Central arterial blood pressure, 1035*t*, 1036
Central auditory processing, 296
  see also Auditory/lateral line CNS, anatomy; Hearing mechanisms
Central chemoreceptors, CO$_2$/H$^+$, 868
Central circulation, hagfish, 1775
Central nervous system (CNS)
  definition, 6
  evolution
    acorn worm, molecular basis, 9
    centralization/condensation of subepidermal nerve net, 8, 10
    suppression of neurogenesis in ectoderm, 9
  see also Brain(s); *entries beginning neural*
Central neural mechanisms, cardiorespiratory interactions *see under* Cardiorespiratory interactions (CRIs)
Central pattern-generating (CPG) network, 57–58
  definition, 56, 398
  electric organ discharge, mormyriform pulse fish, 406–407, 407*f*
  location, 57*f*
  locomotor pattern generation, 58
  dorsal fins, 58
  escape swimming, 59
  pectoral fins, 59
  trunk movements, 57*f*, 58, 59*f*
  see also Motor control systems
Central posterior (CP) nucleus, auditory/lateral line connections, 284*f*, 291
Central projections
  auditory/lateral line system *see under* Auditory/lateral line CNS, anatomy
  olfactory tract, 205, 205*f*
Central venous system, 1088*f*, 1092
Centrifugal fibers, 124*f*, 126
Cephalochordates, amphioxus, PNS evolution/developmental transformation, 10
Ceramide, definition, 1725
Ceratobranchial bone, 1096, 1096*f*, 1097*f*
Ceratodus forsteri, ears, 256*f*
Ceratotrichia, 424, 425
  definition, 419
Cercariae, *Diplostomun spathcaeum*, 778–779, 779*f*
Cerebellar body, cartilaginous fish, 30, 30*f*
Cerebellum, 64
  agnathan, 17, 19
  cartilaginous fish, 29, 30*f*
  definition, 56, 707
  Latimeria, 47, 53*f*, 54, 54*f*
  learning role, 708–709, 709*f*
  lungfishes, 47, 50
  ray-finned fish, 3, 4*f*, 39, 40*f*
  see also Hindbrain
Cerebrospinal fluid-contacting (CSF-c) neurons
  agnathans, 23–25, 24*f*
  cartilaginous fish, 31*f*, 33–34
  definition, 26
Cestodes
  definition, 777
  Schistocephalus solidus see Schistocephalus solidus
Cetorhinus maximus see Basking shark
Changing depth, buoyancy adaptations, 522
Channa micropeltes, suprabranchial chambers, 1869*f*, 1870
Channels
  definition, 218
  ion *see* Ion channels
Channids, suprabranchial chambers, 1869*f*, 1870
Chaperones *see* Molecular chaperones
Character displacement, 1584
  definition, 1583
Chat tag, definition, 1928
Chemical codes/coding
  autonomic neurons *see under* Autonomic neurons
  definition, 1067
  gut neurotransmitters, 1339
Chemical screening, fish models, 1989
Chemical synapses
  definition, 66
  electrical synapses and, primary auditory afferents, Mauthner cell as model, 70*f*, 71
Chemoreception, **183–186**
  experimental approaches, 184
  terrestrial *vs.* aquatic environment, 183–184
  see also Gustation; Olfaction
Chemoreceptors
  carbon dioxide *see* Carbon dioxide (CO$_2$) chemoreceptors
  definition, 765, 854, 871, 1095
  oxygen
    branchial *see* Oxygen chemoreceptors, branchial
    extra-branchial, 1755
Chemosensory behavior, **227–235**
  alarm substance responses *see* Alarm substance
  feeding *see under* Feeding behavior
  predator–prey interactions, 233
  reproduction, 230, 231*f*
    see also Hormonal pheromones
Chemotrophic, definition, 744
Chilid, haplochromine, definition, 1675
Chimaeras, lateral line system, 336, 341, 343, 345*f*
Chimaeroids
  skeletal anatomy, 426
    appendicular skeleton, 426
    axial skeleton, 426
    visceral arches, 426
  see also Cartilaginous fish
Chinook salmon (*Oncorhynchus tshawytscha*)
  diploid and triploid
    physiological differences, 2012, 2013
    sexual development, 2009*f*, 2010–2011
  eggs, size and oxygen uptake, 1573*f*, 1574*f*, 1575–1576
  growth, scaling of respiratory surface area, 1578*f*, 1580
  hypothalamic–pituitary–interrenal (HPI) axis development, 1455
  see also Salmon migration
Chirps, 702–703, 703*f*, 704*f*, 705, 706
  definition, 699
  effect of androgens, 705
Chloride (Cl$^-$) uptake
  freshwater fish gill ionocytes, 1361*f*, 1362
  see also NKCC (Na$^+$, K$^{+,}$ 2Cl$^-$ cotransporter)
Chloride cells *see* Mitochondrion-rich cells (MRCs)
Chloride shift, 911
  definition, 909
Chlororus sordidus (parrotfish), color properties, 155, 155*f*
Choanoflagellates, 7, 8
Cholecystokinin (CCK), 1344
  appetite suppression role, 1344, 1485, 1485*f*, 1512, 1512*t*
  enteric nervous system, 1338–1339
  gallbladder contraction role, 1344, 1344*f*
  gastrointestinal blood flow regulation, 1212
  gut motility role, 1299, 1299*f*
Cholesterol
  cell membranes, 1726*f*, 1727, 1728
    homeoviscous adaptation, 1723, 1729*t*, 1731
    role, 1728
  definition, 1725

Cholinergic, definition, 66, 80, 1104, 1524
Cholinergic control, catecholamine release *see under* Catecholamine release, control mechanisms
Cholinergic system
  agnathan, 19
  cartilaginous fish, 29, 32
  definition, 1067
  effect of toxicants, 786
  ray-finned fishes, 41, 43
  see also Acetylcholine (ACh)
Cholinesterase (ChE), 786
  definition, 783
  effect of toxicants, 786
Chondral bone
  definition, 434
  see also Bony fish skeleton
Chondrichthyans *see* Cartilaginous fish
Chondrocranium, 421–422
  batoids, 421*f*, 422*f*, 425
  definition, 449
  sharks, 420*f*, 424
Chondrocytes, 429
  definition, 428, 1483
Chondrostei
  Acipenseriformes *see* Acipenseriformes
  brain structure, 38*f*
  Polypteridae *see* Polypteridae
Chordates, 7
  CNS induction/neurogenesis suppression in ectoderm, 9
  craniate *see* Craniates
Chorion, definition, 1444
Choroid, definition, 929, 935, 1119
Choroid rete mirabile, 931–932, 932*f*, 941, 1128
  association with high PO_2 values in eyes, 1129*t*, 1130
  evolution, 941, 942*f*
  structure, 1128–1130, 1129*f*
  swimbladder rete *vs.*, 1125–1127, 1127*f*
  vascular supply, 932, 932*f*
    see also Pseudobranch
Chorusing behavior, 685*t*, 688
Chromaffin cells, 1525, 1526*f*
  autonomic nervous system and, 81, 81*f*
    cyclostomes, 82, 85, 85*f*
    dipnoans, 83–84, 85*f*
    elasmobranchs, 82, 85, 85*f*
    teleosts, 82, 85, 85*f*
  definition, 1104, 1524
  see also Catecholamine(s)
Chromatic aberration, 103–104, 104*f*
Chromatophore(s), 418, 489, 490, 490*t*, 1972
  color change and *see* Color changes
  colors, 491
  communication role, 473
  definition, 416, 489, 490
  development *see* Chromatophore development
  genes *see* Pigment genes
  location, 490, 491*f*
  morphology, 477*f*, 478*f*, 479*f*, 481*f*, 484
  origin, 490
  tumors *see* Pigment cell tumors
  types, 1971*f*, 1973, 1995
    see also specific types
  see also Coloration
Chromatophore development, 1971*f*, 1972
  genetic control, 1973
    color pattern formation, 1972*t*, 1973*f*, 1975
    iridophores, 1975
    leukophores, 1975
    melanophores, 1974
    specification, 1973
    xanthophores, 1974
Chromatosomes
  color change and, 492, 492, 494
  definition, 489, 490–491
Chromium (Cr), biological effects and toxicodynamics, 2065
Chromophore, 111, 112*f*
  definition, 98, 110, 116
  exchange, 120, 121*f*
Chromosomal sex determination, 2025*t*, 2026*f*, 2027, 2027*f*
Chromosome(s)
  heteromorphic *see* Heteromorphic chromosomes
  homomorphic, definition, 2025
  number, fish, 1981
  Y, evolution, 2029
Chromosome manipulation
  definition, 2001
  fish, 2007
    androgenesis, 2008
    applications, 2007
    gynogenesis, 2008
    triploidy *see* Triploid fish, physiology
Chromosome set, definition, 2009
Chronic hypoxia, circulatory system responses *see under* Hypoxia, circulatory system responses
Chronotropy, definition, 1030
Chronotypes, 773
  definition, 772
Chyme, 1303
  definition, 1206, 1229, 1292, 1301
  see also Digestion
Chymotrypsin, 1281, 1281*t*, 1304–1305, 1305*f*, 1305*t*
Cichlids, parental care, 671*f*, 671–672
  brooding, 675–676
  costs/benefits, 674, 674*f*
  defense, 675
  evolution, 675
Ciliary marginal zone (CMZ), proliferation, blind cavefish, 1847
Ciliated olfactory neurons, 195, 200*f*, 201*f*, 214–215
Circadian, definition, 736, 772, 773
Circadian clock, 736, 773
  definition, 736
  genes, 741
  location, 741, 773–774
  sleep regulation
    fish, 774, 775
    mammals, 774
    see also Sleep
  see also Circadian rhythms
Circadian rhythms, **736–743**
  definition, 98, 159, 1483
  diurnal, 740, 773
  entrainment, 739
    definition, 736, 737
  expressions of, 737
    anticipation of dawn/dusk, 739
    demand feeding, 737–738
    food-anticipatory activity, 738–739
    freerunning rhythms, 737, 737*f*
    locomotion, 737, 737*f*
    sun–compass orientation, 739
  freerunning period, 737, 737*f*, 738
    effect of pinealectomy, 741, 741*f*, 742*f*
  masking, 739
    definition, 736
  nocturnal, 740, 773
  phase shifts, 739, 739*f*
    definition, 736
  pineal organ and, 165, 741
  transient cycles, 739, 739*f*
    definition, 736
  see also Circadian clock
Circannual clock, 743
Circatidal, definition, 736
Circatidal rhythms, 742
Circuits, vascular, 980, 980*f*, 1132, 1133*f*
Circulating catecholamines *see* Catecholamine(s)
Circulating hormones *see under* Hormone(s)
Circulating metabolite levels
  appetite/food intake effects, 1486, 1513
  effect on appetite, 1513
Circulation, 1086
  air-breathing fish *see* Air-breathing fish, circulatory adaptations
  cardiac *see* Cardiac circulation
  central, hagfish, 1775
  comparative, mammal *vs.* fish, 1877*f*, 1879
  coronary *see* Coronary circulation
  definition, 535, 581
  elasmobranchs, 1811
  gastrointestinal *see* Gastrointestinal circulation
  hormone inactivation, **1190–1196**
    in fish *see* Hormone inactivation, fish
    in mammals *see* Hormone inactivation, mammals
  lampreys, 1792*f*, 1793
  primary, secondary circulation *vs.*, 1162, 1163–1165, 1164*f*
  retia *see* Retia mirabilia
  secondary *see* Secondary circulation
  see also Cardiovascular (circulatory) system; Vascular anatomy
Circulation time
  definition, 1190
  hormones and, 1191, 1195
Circulatory fluid balance, **1154–1160**
  see also Transcapillary fluid movement
Circulatory shunting, air-breathing fish, 1878*t*, 1879*f*, 1880
  see also Air-breathing fish, circulatory adaptations
Circulatory shunts, definition, 1876
Circulatory system *see* Cardiovascular (circulatory) system
Circumventricular organs
  cartilaginous fish, 33–34
  definition, 26
Clade, definition, 1833
Cladistia
  brain structure, 38*f*, 42–44, 44*f*
  see also Polypteridae
Clariids
  bimodal respiration, 1858
  suprabranchial chambers, 1867*f*, 1870
  see also Catfish (Siluriformes)
Clark-type oxygen electrode, 846–848
Classical conditioning
  cerebellum role, 708
  definition, 713
  noxious stimuli avoidance, 714
Cleaner fish
  cleaner–client mutualism, 155
    guild flag coloration, 151*f*, 156
  deceptive mimicry, 156, 156*f*
Climate change
  definition, 1738
  frequency of extreme events, 1741
  impacts *see* Climate change impacts
Climate change impacts, **1738**
  bioclimatic-envelope modeling, 1744
  ecosystem, levels of impact, 1697, 1738
    population and community effects, 1701, 1740
  environmental hypoxia contribution, 1748*f*, 1749
  fish growth, assessment using bioenergetics modeling, 1678
  local extinction risks, 1701, 1744
  migration, 1927
  physiological basis, 1741
    performance patterns and energy budget, 1743
    thermal tolerance, 1740*f*, 1741
  projection, modeling, 1744
  temperature preferences, 763
Climate models *see* Global climate models (GCM)
Climbing fibers, definition, 56
Clock gene, 741, 741
Clocks *see* Biological clocks
Closed respirometers, 848–849
  static, 848–849, 849*f*, 1568
  swimming *see* Swimming respirometers

Closed-system temperature change
  definition, 1887
  pelagic fish and, 1901
Clownfish, sound production, 325
Club cells, 232, 232*f*
  definition, 476
  morphology, 482, 483*f*
Club endings, 70*f*, 71, 74*f*, 75–76, 78
Clupeids
  definition, 283
  octaval nuclei, 287, 287*f*, 288
Clutch size, compensatory growth effects, 755–756, 756–757
Co-transporter, definition, 1359
Coastal ecosystems, hypoxic areas, 1747*f*, 1748
  eutrophication effects, 1748–1749
Coastal migration, salmon, 1949
Coastal shelf, eel migration from, 1938*f*, 1940
Cocaine- and amphetamine-regulated transcript (CART), appetite suppression, 1485, 1485*f*, 1512, 1512*t*
Cod, Atlantic (*Gadus morhua*)
  environmental temperature effects
    distribution limits and range, 1740–1741
    population productivity, 1741
  hearing abilities, 278–279, 279*f*, 279*f*, 279–280
Coefficient of synchronization, 294–295
  definition, 292
Coelacanths, 46
  ampullary organs, 353
  outflow tract, heart, 1016–1018, 1019*f*
  rectal gland *see* Rectal gland, dogfish
  urea retention, 1446
  see also Latimeria
Coelom, definition, 1395
Coevolution, definition, 966
Cognition
  definition, 707
  see also Learning and memory
Cognitive abilities, fish, 45
  see also Learning and memory
Coho salmon (*Oncorhynchus kisutch*), growth hormone transgenesis
  behavioral effects, 2022–2023
  energetics, 2020
  growth effects, 2017*f*
    influence of rearing environment, 2022*f*
  immune effects, 2020
  phenotypic effects, 2018*f*, 2019–2020
Cold acclimation/adaptation
  antifreeze protein production *see* Antifreeze proteins
  capillary diameter, 1151, 1152*f*
  cardiovascular adaptations/responses, 1199*f*, 1200–1203
    Antarctic fishes, 1203, 1204*f*, 1204*f*
    cardiomyocytes, 1007–1008, 1010, 1011
    see also Temperature effects, cardiovascular system
  crucian carp *see under* Crucian carp (*Carassius carassius*)
  insights from global gene expression data, 2058*f*, 2059
  intracellular lipids, oxygen storage, 1151
  metabolic, 1580–1581
  mitochondrial membrane remodeling, 1715
  mitochondrial role, 970, 971*f*, 1647, 1713*f*, 1714*f*, 1714–1715
    cold-induced shifts to lipid metabolism, 1647, 1649
    genomic analysis, 1715, 1715*f*
  neuron excitability, 1723
  seasonal angiogenesis, 1147, 1151, 1151*f*
  see also Antarctic fish; Thermal acclimation/adaptation
Cold stress, heat shock protein induction, 1734
Cold tolerance
  cultured fish, transgenesis, 2006
  see also Cold acclimation/adaptation
Collagen, definition, 1015
Collimating, definition, 692
Colloid osmotic pressure *see* Oncotic pressure
Collothalamus, ray-finned fish, 37, 42, 44–45
Collothalmic, definition, 37
Colonization, bacterial *see under* Bacterial pathogenicity
Color
  complementary
    additive mixing, for camouflage, 154*f*, 155
    contrast, for display/communication, 154–155
  definition, 150, 150
  spectrally complex, 153*f*, 155, 155*f*
  structural, reflected-light signaling, 694, 695*f*, 696*f*
  see also Chromatophore(s); Coloration; Visual communication
Color changes, 418, 490, 1975
  mechanisms, 492, 492*f*
  morphological, 492, 493*f*
    definition, 489
  physiological, 492, 493*f*
    definition, 489
  regulation, 493
Color coding, ganglion cells, 127, 128*f*
Color communication, reef fish *see* Reef fish, color vision/communication
Color constancy
  behavioral studies, 146
  definition, 143
Color detection, behavioral studies *see under* Visual capabilities, behavioral studies
Color discrimination, behavioral studies, 145
Color opponency, 128
Color pattern formation, genetic control, 1972*t*, 1973*f*, 1975
Color processing, optic tectum, 135
Color vision
  adaptation for coral reefs, 152
  partial color blindness in reef fish, 151
  processing, optic tectum, 135
  reef fish *see* Reef fish, color vision/communication
  skin coloration and, 490
  see also Visual capabilities, behavioral studies
Coloration, **489–496**, 418, 1971–1972
  aposematic (warning), 150, 151, 151*f*
  changes *see* Color changes
  compensatory growth effects, 756
  dietary influences, 493
  effect of social status, 653
  fish color vision and, 490
  integumentary patterns, 494, 495*f*
  intrapattern scale autografts, 495
  iridescent *see* Iridescent coloration
  pelagic fish, 1890*f*, 1892
  pigmentary, 490, 1971–1972
    definition, 489
    reflected-light signaling, 694
    see also entries beginning pigment
  roles, 489
    communication, 473
    protective, 473
    see also Camouflage
  structural, 490
    definition, 489
  white, 694–695
  see also Chromatophore(s); Visual communication
Colorful fish
  genetically-modified for pet trade, 2005*f*, 2006
  see also Reef fish, color vision/communication
Command neuron, definition, 73
Command nucleus, electric fish, 399–402, 402*f*, 405*t*
  see also Electric organ discharge (EOD), central control
Command signals, definition, 56
Commensal bacteria
  definition, 1322
  gut, 1323
  see also Gut, microbiota
Common carp (*Cyprinus carpio*)
  gill remodeling, 799–800, 800*f*
  hypothalamic–pituitary–interrenal (HPI) axis development, 1455
  pain response, 715, 715*f*, 716*f*, 716–717, 717*f*, 718*t*
  specific dynamic action, 1609*t*, 1611*f*, 1612
Common killifish *see under* Killifish
Communication
  color and reef fish *see* Reef fish, color vision/communication
  definition, 684
  electrical signaling *see* Electrocommunication
  integument role, 472*f*, 473
  visual *see* Visual communication
  vocal *see* Vocal fishes
Communication modality
  definition, 684
  sound production *vs.* other modalities, 690
    see also Sound production
Compact myocardium
  cardiac shape and, 1003
  fish growth and, 1078, 1079*f*
  oxygen supply, 1078
    coronary vessels, 1080, 1081*f*
      see also Coronary artery; Coronary circulation
  proportion, inter-species variation, 1004
  structure, 1000*f*, 1002, 1078, 1079*f*
  see also Myocardium
Compass, 721
  definition, 720
  see also Spatial orientation
Compatible solutes
  definition, 1960
  see also Osmoregulation
Compensatory growth, 1489, 1634
  behavioral effects *see* Compensatory growth, behavioral effects
  definition, 752, 1483, 2016
  growth hormone transgenic fish, 2020
  mechanism, 752
    see also Hyperphagia
Compensatory growth, behavioral effects, **752–757**
  during compensation, 752, 753*f*, 753*f*
    activity patterns, 754
    aggressiveness/aggression risk, 754
      see also Dominance hierarchies
    risk-taking behavior, 753
    time–place learning, 755
  deferred/long-term, 755
    breeding behavior, 755
    breeding coloration/sexual attractiveness, 756
    clutch/offspring care, 756, 756*f*
    predator avoidance, 755
Competition, foraging, strategies for dealing with, 747*f*, 748
Competition theory, 1584
Competitive behavior, 649–650, 650*f*, 651
  see also Dominance hierarchies
Competitive exclusion, 1584
  definition, 1583–1584
Complement system, definition, 1541
Complementary colors *see under* Color
Complex I *see under* Electron transport system (ETS)
Complex II *see under* Electron transport system (ETS)
Complex III *see under* Electron transport system (ETS)
Complex IV *see* Cytochrome *c* oxidase (complex IV)
Complex V *see* F_1F_0 ATPase (complex V)
Compliance, 981
  arterial *see* Arterial compliance
  definition, 977, 1111
  venous *see* Venous compliance
Compliance vessels
  definition, 982–983
  see also Compliance
Compound bone
  definition, 434
  see also Bony fish skeleton

- Concentration gradient, definition, 1429
- Conditioning, classical, 277
  - acoustic stimuli *see* Acoustic stimuli and behavior
  - avoidance, acoustic stimuli, 277
  - avoidance of painful stimuli, 714
  - small field motion detection and, 147, 148f
- Conductance, definition, 829
- Conductance vessels, definition, 982–983
- Conducting system, heart, 1071
- Conduction velocity, definition, 1717
- Cones, 150
  - definition, 98, 150, 1807
  - mosaics, 114, 114f, 121
  - types, 113
  - visual pigments, 110–111
    - adaptations to photic environment *see under* Photic environment
    - double cones, 113–114, 114f
    - evolution, 111, 113f
    - single cones, 113–114
  - *see also* Photoreceptors
- Conformation, definition, 1703
- Connective tissue, definition, 1457
- Connexins, cardiomyocyte cell–cell connections, 1012
- Conservation
  - desert pupfish, 1841
  - fisheries, threats, 1653
    - *see also* Anthropogenic influences/disruptions; *specific threats*
- Conspecifics
  - definition, 1629, 1937
  - feeding/growth effects, 1634
- Construction behaviors, 657, 657f
  - *see also* Nest building
- Construction industries, noise generation, 305t, 305–306
- Constructive interference, 105
  - definition, 102
- Constructive traits
  - blind cavefish troglomorphism *see under* Blind cavefish
  - definition, 1843
- Consumption *see* Food consumption/intake
- Contaminant(s)
  - accumulation *see* Bioaccumulation
  - definition, 2062, 2069
  - *see also* Toxicants
- Continental shelf, eel migration and, 1938f, 1940
- Continuous air breathing, 1854–1855, 1855f
  - definition, 1850
  - *see also* Air-breathing fish
- Continuous swimming
  - definition, 1887
  - pelagic fish, 1893–1894
- Contractile element
  - cardiomyocytes *see* Cardiomyocyte contraction
  - definition, 1054
  - *see also specific contractile elements*
- Contractile reaction
  - cardiomyocytes, 1054–1055
    - *see also* Cardiomyocyte contraction
  - definition, 1054
- Contractility, definition, 1045
- Contralateral, definition, 564
- Conus arteriosus, 1023
  - anatomy/form, 1000, 1003, 1015, 1018f, 1023
  - Atlantic sharpnose shark, 1026–1027, 1028f
  - definition, 1015
  - development, 1015, 1017f
  - dogfish *see under* Dogfish
  - function, 1028
  - phylogeny, 1015
  - sturgeons *see under* Sturgeons, physiology
- Convection
  - cardiovascular system, 893, 977
  - definition, 820, 829, 893, 977, 1444
  - forced *see* Forced convection
  - gas transport during development, 836, 836f
  - natural *see* Natural convection
  - requirement, 821–822, 822f
- Convergence, definition, 1843
- Cooperation in fish *see* Parental care
- Cooperativity, fish hemoglobins, 945, 946f
  - *see also* Oxygen binding, fish hemoglobins
- Coping, definition, 1515
- Coping styles, stress, 1520, 1521f, 1522f
- Copper toxicity, 2066
  - effects of social status, 654
  - olfactory sensory neurons, 2081
- Coral reefs
  - biodiversity and visual environment, 150
    - evolution, 157
  - color vision/communication on, **150–158**
    - *see also* Reef fish, color vision/communication
  - as dense ecosystem, 150–151
- *Coregonus cluperformis* *see* Lake whitefish
- Cornea, 105, 105f
- Corollary discharge
  - definition, 366
  - electric organ *see* Electric organ corollary discharge (EOCD)
- Coronary artery, 1086f, 1091
  - anatomy, 1080, 1081f
  - histology, 1081, 1082f
  - origin and routing, 1080
- Coronary blood flow
  - definition, 1077
  - exercise effects, 1081–1082, 1082f
  - hypoxia effects, 1082f, 1082–1083
  - regulation, 1081
- Coronary circulation, **1077–1084**
  - air-breathing fish, 1078f, 1084, 1885
  - anatomy, 1078
    - in relation to ventricular architecture, 1078, 1079f
  - definition, 1067, 1077
  - evolution, 1078–1079
  - fishes that have, 1078
  - percentage of cardiac supply, 1080
  - physiology, 1081
    - blood flow regulation, 1081
    - limitations on myocardial oxygen supply, 1083
  - schematic representation, 1078f
  - *see also* Coronary artery
- Coronary vascular tone
  - definition, 1077
  - regulation, 1083
- Coronary vessels
  - definition, 1887
  - *see also* Coronary artery
- Coronet cell, cartilaginous fish, 33f, 33–34
- Cortex/cortices, definition, 1
- Corticosteroid(s), **1474–1482**
  - central regulation of release, 1475, 1476f
  - definition, 1474
  - glucocorticoids, 1474, 1537
    - corticosterone *see* Corticosterone
    - cortisol *see* Cortisol
    - cortisone *see* Cortisone
  - mineralocorticoids, 1474
    - aldosterone *see* Aldosterone
    - DOC *see* 11-Deoxycorticosterone (DOC)
  - plasma levels, 1476
  - signaling *see* Corticosteroid signaling system
  - synthesis, 1451f, 1452, 1475, 1475f
  - target tissue, 1479, 1479f
- Corticosteroid receptors
  - activation, 1478, 1478f
  - definition, 1474
  - evolution, 1480, 1481f
  - tissue expression (teleosts), 1479
  - transactivation studies, 1476–1477, 1477f
  - types, 1476
  - *see also* Corticosteroid signaling system; Glucocorticoid receptors (GRs); Mineralocorticoid receptors (MRs)
- Corticosteroid signaling system
  - evolution, 1480, 1481f
  - molecular mechanisms, 1452, 1478, 1478f
    - *see also* Corticosteroid receptors
  - regulation by stress exposure, 1479
    - *see also* Cortisol; Stress response, hormonal
  - *see also* Corticosteroid receptors
- Corticosterone, 1474
  - plasma levels, 1476
  - *see also* Corticosteroid(s)
- Corticotropin-releasing factor/hormone (CRF/CRH)
  - appetite suppression, 1485, 1512, 1512t
  - definition, 1450
  - signaling systems, embryonic, 1454, 1454f
  - stress response role, 1475–1476, 1476f, 1516f, 1517
  - *see also* Hypothalamic–pituitary–interrenal (HPI) axis
- Cortisol, 1450
  - biosynthesis, 1475, 1475f
    - embryonic, 1454
      - *see also* Hypothalamic–pituitary–interrenal (HPI) axis development
  - definition, 1466
  - dominance behavior role, 651, 652, 653, 653f
  - effect on intestinal epithelial barrier, 1329
  - ion regulation role, 1468
    - freshwater acclimation, 1469
    - growth hormone/IGF-I axis interaction, 1471
    - seawater acclimation, 1468, 1948–1949
  - lymphocyte number reduction, 989, 991
  - physiological actions, 1474–1475, 1516f
    - *see also stress response activity (below)*
  - plasma levels, 1476
  - release, 1475, 1476f, 1537
    - *see also* Hypothalamic–pituitary–interrenal (HPI) axis
  - reproductive hormone interactions, 1545f, 1547
    - *see also* Reproduction, stress effects
  - sex change role, 667
  - signaling/mechanisms of action, 1452, 1537, 1537f
    - *see also* Corticosteroid signaling system
  - stress response activity, 1516f, 1517f, 1518, 1519
    - appetite suppression, 1485–1486, 1519
    - associated receptors *see* Cortisol receptors
    - growth effects, 1519, 1539
    - hydromineral regulation effects, 1519, 1520f
    - hypoxia, 1521, 1522f
    - immune suppression, 1549f, 1550
    - metabolic effects, 1538
    - reproductive effects, 1519
  - stress response activity, modifying factors
    - coping styles, 1520–1521
    - maternal stress and offspring development, 1521–1522
  - target tissue, 1479, 1479f
  - *see also* Stress response, hormonal
- Cortisol receptors, 1451f, 1452, 1478, 1478f, 1518
  - *see also* Corticosteroid receptors; Glucocorticoid receptors (GRs); Mineralocorticoid receptors (MRs)
- Cortisone, 1474
  - plasma levels, 1476
  - *see also* Corticosteroid(s)
- Cost of digestion and assimilation *see* specific dynamic action (SDA)
- Cost of transport (COT)
  - definition, 1636, 1639–1640
  - swimming speed *vs.*, 1638f, 1639–1640
- Costamere
  - cardiomyocytes, 1012f, 1012–1013
  - definition, 1006
- Cottonball Marsh, 1832f, 1834
  - pupfish, 1832f, 1834
- Countercurrent, definition, 935, 1119, 1807

Countercurrent exchangers, 1120
  see also Retia mirabilia
Countercurrent flow
  definition, 1095
  lamellar blood–respiratory water, 820, 821*f*, 1097*f*, 1098
    efficiency *see* Gas exchange organs, efficiency
Countercurrent gas exchangers, 1120
Countercurrent heat exchangers, 1120, 1121
  see also Retia mirabilia
Countercurrent multiplication, 1127
  Root effect, 931, 932*f*
    see also Retia
  see also Choroid rete mirabile; Swimbladder rete
Countercurrent multipliers, for swimbladder and retina, 1125
Counterillumination, 498
  definition, 416, 497
Counterphase, definition, 123
Countershading, 490
  definition, 489
Courtship, definition, 656
Courtship behaviors, 658
  calls, 687, 687*f*, 688*f*, 689
  compensatory growth effects, 756
  electric signaling role, 703*f*, 704, 704*f*
  female readiness, 659
  hypoxia effects, 1768
  male readiness, 658
  visual recognition (sexual dimorphism), 658
  see also Sexual behavior
Cover, use in surfacing strategies, 768
Cranial autonomic nerves
  definition, 80, 1332
  dipnoans, 84
  elasmobranchs, 82
  evolution *see* Cranial nerve(s), evolution
  gut innervation, 1333, 1334*f*
  respiratory system innervation, 855, 855*f*, 856*f*, 857*f*, 858*f*
    see also Respiratory rhythm generation
  teleosts, 82
  see also Autonomic nervous system (ANS); Cranial nerve(s)
Cranial axial skeleton, bony fish *see under* Bony fish skeleton
Cranial endothermy, 1915, 1915, 1916
  advantages, 1919
  heater organ tissue, 1916, 1916*f*
    excitation–thermogenic coupling, 1917*f*, 1917–1918
    modifications/morphological features, 1917
  telemetry of brain temperature, 1918*f*, 1919
Cranial muscles
  bony fish *see* Bony fish cranial muscles
  cartilaginous fish *see* Cartilaginous fish, cranial muscles
  functional anatomy, current knowledge, 417
Cranial nerve(s)
  definition, 854
  eighth, 264, 266
    definition, 236
  evolution, **6–15**
    acorn worm CNS development, 9
    craniates, 11
      jawed, 13
    see also Central nervous system (CNS)
  gustatory system, 190, 190*f*, 191*f*
  tenth (vagus nerve)
    definition, 1178, 1509
    see also Cardiorespiratory interactions (CRIs); *entries beginning vagal*
  see also Cranial autonomic nerves
Craniates
  PNS/motor neuron, evolution/developmental transformation, 11
    jawed craniates, 13
Creatine phosphate, 959–960
  definition, 959, 966, 1645
  hydrolysis, 1759
Crepuscular, definition, 772, 1921
Crepuscular chronotype, definition, 773
Cristae
  definition, 1006
  see also Mitochondria
Critical masking ratios (CRs), sounds, 280, 280*f*
Critical oxygen tension (P_{crit})
  definition, 1389, 1764
  see also under Partial pressure of oxygen
Critical swimming speed (U_{crit}), 812
  definition, 812, 1389, 1645
  extension of swimming period/velocity beyond, 1649*f*, 1650
  protocol development (Brett), 1656*f*, 1658
Critical thermal maximum (CTMax), 1698, 1740*f*, 1742–1743
  definition, 1197, 1732
  desert pupfish, 1836*f*, 1837, 1837*f*
  endpoints, 1698
  laboratory determination, 1698, 1698
  used for environmental quality bioassay, 1700*t*, 1701
Critical thermal minimum (CTMin), 1698, 1740*f*, 1742–1743
  desert pupfish, 1837, 1837*f*
  endpoints, 1699
Croaking gouramis, sound production, 322, 322*f*, 325
Cross-bridge, definition, 1054
Cross-correlation
  definition, 123
  retina, 129
Cross-tolerance, heat shock protein role, 1736
Crossing over, definition, 616
Crucian carp (*Carassius carassius*), **1824**
  anoxia responses/tolerance, 799, 1714, 1825–1827, 1829
    avoidance of anoxic energy deficiency, 1827
    ethanol production, 1825*f*, 1827, 1829
    glycogen stores, 1762, 1825*f*, 1828
    heart activity/cardiac function, 1226, 1227*f*, 1826*f*, 1828, 1829
    metabolic depression, 1828
      GABA role, 1828*f*, 1829
      mechanisms, 1829
      NMDA receptor activity, 1829
      protein synthesis, 1827*f*, 1829
    neural depression, 1828
    tissue anoxia avoidance strategies, 1829
    see also Anaerobic metabolism
  cold acclimation
    capillary growth, 1147
    myocyte excitability, 1721–1722
  eurythermy, 1826–1827
  gill remodeling *see under* Gill remodeling
  habitat, 1825
  morphology, 1824*f*, 1825–1826
  occurrence, 1825
Crude fiber, definition, 1665
Crude protein, definition, 1665
Cryopreservation
  definition, 1965
  fish cell lines, 1969
Crypsis
  definition, 744
  visual, 747*f*, 747–748
    see also Camouflage
Crypt cells, olfactory receptor neurons, 195, 201*f*
Cryptochrome (Cry) genes, 741
Crystalline lens *see* Lens
Crystallins, lens, 106
Cues, 692
  definition, 692
  egocentric *see* Egocentric cues
  magnetic, navigation, 721, 723
  olfactory
    orienting, 1924
    see also Pheromone(s)
  solar, orienting, 1922*f*, 1923
Culture, cell, fish cell lines *see* Fish cell lines
Culture environments *see* Aquaculture/aquaculture settings
Culture vessels
  definition, 1965
  fish cell lines, 1966*f*, 1967
Cultured fish
  bioenergetics *see* Bioenergetics, in aquaculture
  food consumption/intake
    assessment, 1671
    factors influencing, 1670*f*, 1671
    see also Cultured fish, dietary requirements
  physiology *see* Aquaculture, physiological responses
  self-feeding, 711, 712*f*
  see also Aquaculture/aquaculture settings
Cultured fish, dietary requirements, **1617**
  amino acids/proteins, 1618
    balance and impact of other nutrients, 1618, 1621
    estimation methods, 1619, 1619*f*
    physiological basis, 1618
  fatty acids, 1621
  minerals and vitamins, 1621
Cupula, 330–331, 336–337
  definition, 236, 329
  structure, 330*f*, 338*f*
Current speed, growth effects, 1632
Currents, oceans, leptocephalus transportation and, 1939*f*, 1940
Cutaneous
  definition, 1444
  see also Integument
Cutaneous excretion, 474, 835
  nitrogen, early life stages, 1447
Cutaneous respiration, 472*f*, 473, 1872*f*, 1873
  branchial respiration *vs.*, 845
  during development, 841, 842*f*
    transition to branchial respiration, 844, 844*f*
  oxygen uptake, 814
Cutaneous veins, 1093
Cuticle, 480–481, 482*f*
  definition, 476
Cyanophores, 490*t*, 491, 1973
  development, 1971*f*
Cyclostomes
  autonomic nervous system, 82
    cardiac control, 85, 85*f*
    chromaffin tissue and, 82, 85, 85*f*
  cardiac anatomy, 1004
  definition, 16, 1085
  see also Agnathan(s); Hagfish; Lampreys
CYP1A1, pillar cells, 1192–1194
CYP19, sex differentiation role, 1494, 1496*f*
CYP19A1/*CYP19A1* gene, sex differentiation role, 1494, 1496*f*, 2029*t*, 2030
Cyprinids
  gill remodeling, 799–800, 800*f*
  number of species, 1820
  palatal organ and vagal lobe, 191–192, 193*f*
  Weberian ossicles *see* Weberian ossicles
  see also individual species
Cypriniformes
  specific dynamic action, 1609*t*, 1611*f*, 1612
  see also Cyprinids
Cyprinodon (desert pupfish), **1832**
  conservation, 1841
  habitats, 1831*f*, 1833
  hybrids, 1841
  physiological studies, 1837
    critical thermal maxima/minima, 1836*f*, 1837, 1837*f*
    energetics, 1840*f*, 1841
    metabolism, 1838*f*, 1839
    thermal limits to reproduction, 1839
  see also individual species

Cyprinodon diabolis
  conservation, 1841
  habitat, 1834f, 1834–1837
    temperature fluctuations, 1834–1837, 1835f
  physiological studies
    breeding ledge occurrence, 1839f, 1840f, 1841
    critical thermal maximum, 1836f
    energetics, 1841
    thermal limits to reproduction, 1839
  population estimates, 1834–1837, 1835f
Cyprinodon macularius
  conservation, 1841
  energetics, 1840f, 1841
  habitat, 1831f, 1834
Cyprinodon nevadensis amargosae
  habitat, 1834
  physiological studies
    critical thermal maximum, 1837, 1837f, 1837–1838
    energetics, 1841
    metabolism, 1839–1841
Cyprinodon nevadensis mionectes
  conservation, 1841
  critical thermal maximum, 1836f
  habitat, 1833f, 1834
  physiological studies
    critical thermal maximum, 1836f, 1837–1838
    metabolism, 1839–1841
Cyprinodon nevadensis nevadensis
  habitat, 1833f, 1834
  physiological studies
    critical thermal maximum, 1836f
    thermal limits to reproduction, 1839
Cyprinodon nevadensis pectoralis, habitat, 1834
Cyprinodon radiosus
  conservation, 1841
  habitat, 1831f, 1834
Cyprinodon salinus
  habitat, 1832f, 1834
  physiological studies
    critical thermal maximum, 1836f
    metabolism, 1838f, 1839
Cyprinodon salinus milleri, habitat, 1832f, 1834
Cyprinus carpio see Common carp (*Cyprinus carpio*)
Cystic fibrosis transmembrane conductance regulator (CFTR)
  definition, 1354, 1466
  mitochondrion-rich cell, 1467
    freshwater fish, 1364
    seawater fish, 1355–1356, 1357f
Cytochrome *c* oxidase (complex IV), 963, 963f, 1710
  definition, 959, 966
  effect of cold acclimation on activity, 1714f, 1715
  synthesis, 961–962, 962f
  see also Electron transport system (ETS)
Cytochromes P450 (CYPs)
  1A1, pillar cells, 1192–1194
  CYP19, sex differentiation role, 1494, 1496f
  CYP19A1/*CYP19A1* gene, sex differentiation role, 1494, 1496f, 2029t, 2030
Cytokines
  definition, 1373, 1541
  osmosensing role, 1377f, 1378
    TNF-α and interleukins, 1378
Cytolysins, pathogenesis role *see under* Extracellular products (ECPs), disease role
Cytopathic effect, definition, 2032
Cytoskeletal strain, as osmosensory signal, 1375
Cytoskeleton, definition, 1429
Cytosol, definition, 1045
Cytosolic transport, 954
Cytotoxicity, definition, 1541

D

Daily, definition, 736
Dampening, definition, 1169
Dams
  development, 1652f, 1653f, 1654
  fish passageways *see* Fishways
  impacts
    migration, 1653f, 1654, 1925f, 1926, 1951
    nonmigratory species, 1654–1655
    riverine ecosystems, 1654
    tropical species, 1658–1659
Damselfish
  behavior and color background, 153f
    Dascyllus spp., 154f
    Pomacentrus ambionensis, UV vision and colors, 156, 157f
Dangerous temperatures, avoidance, 762, 762f, 763f
Danio, color polymorphism, 1976
Danio rerio see Zebrafish
Daphnia see Water flea
Darwinian fitness *see* Fitness (Darwinian)
Davis and Warren growth model, 1623f, 1626
Dawn, anticipation of, 739
Deactivation, definition, 1717
Deacylation–reacylation, homeoviscous adaptation, 1729t, 1730
Dead zones *see under* Hypoxic environments
Deamination, definition, 1437, 1437–1438
Death Valley, 1831f, 1833
  pupfish
    Cyprinodon nevadensis nevadensis see Cyprinodon nevadensis nevadensis
    Cyprinodon salinus see Cyprinodon salinus
Decapentaplegic (Dpp)
  definition, 6
  pathway, 9
Decibel (dB), definition, 311
Deep-brain photosensitivity, 160, 160f
Deep-diving species, monofocal lenses, 108
Deep-scattering layer, 523
  definition, 520
Deep sea
  definition, 1953f, 1953f, 1954, 1954
  light environment, 167, 167f
    luminescence and, 167, 168f, 498
  water characteristics, 1954–1955
  zonation, 1953f
Deep-sea fishes, **1953**
  definition, 1954
  elasmobranchs, 1810
  feeding habits, 1955
    trophic guilds, 1956
      demersal, 1956
      pelagic, 1956
  Haldane and Huxley illustration, 1954f, 1955
  ocular anatomy *see under* Deep-sea fishes, ocular anatomy
  species diversity, 1955
  visual adaptations *see* Deep-sea fishes, visual adaptations
  see also individual species
Deep-sea fishes, ocular anatomy, 168
  eye shape, 169, 169f
    methods of visual field enlargement, 169f, 170f, 171f, 172, 172f
    tubular eyes, 169, 169f, 170f, 171f
  lenses, 170f, 173, 173f
  retinal structure, 173, 174f
    modifications to increase sensitivity, 173, 175f, 176f
    photoreceptor renewal, 174
    regional specializations, 175, 177f, 178f
    tapeta, 176, 178f, 179f
  teleosts, 168, 169f
Deep-sea fishes, pressure effects, 1956
  biochemical structures/reactions, 1957
    cell membranes, 1957
    metabolic rate, 1957
    proteins, 1957
    visual pigments, 180
  buoyancy adaptations, 1956
    lipid inclusions, 1957
    swimbladder, 1956
    watery fluids/gelatinous tissues, 1957
Deep-sea fishes, visual adaptations, **166–182**, 108
  to photon-limited environment, 118, 167, 1892
    ocular anatomy *see* Deep-sea fishes, ocular anatomy
    visual pigments *see* Visual pigments, deep-sea fish
Deep waters
  photoreceptor/visual pigment adaptations, 117, 118f
  see also Deep sea
Defense mechanisms
  hagfish, 1776
    see also Hagfish slime
  parental care, 675
Definitive host
  definition, 777
  Diplostomun spathcaeum, 778–779, 779f
  Schistocephalus solidus, 780f, 780–781
Dehydration reaction
  carbonic anhydrase, 899, 900f, 905, 906f, 907f
  definition, 899
Delayed rectifier
  cardiac action potential, 1040, 1041
  definition, 1038
Demand feeding, freerunning rhythms, 737–738
Demersal fish
  definition, 1954
  trophic guilds, 1956
  see also Deep-sea fishes
Denaturation, definition, 1703
Denatured proteins, as heat shock response trigger, 1735f, 1736
Dendritic chromatophores, 491, 491f
  color change and, 492
Dense fishes, buoyancy adaptations, 521
Density
  air *vs.* water, 820
  fish *see* Fish densities
  freshwater, 521
  seawater, 521
Deorphanized receptors, olfactory, 185
11-Deoxycorticosterone (DOC), 1474
  plasma levels, 1476
  synthesis, 1475, 1475f
  see also Corticosteroid(s)
Deoxyribonucleic acid (DNA), 2000f, 2001
Depolarization
  cardiac action potential phase, 1039, 1040
  definition, 871, 1038, 1045, 1717
  diastolic *see* Diastolic depolarization
Depth
  increasing, photoreceptor/visual pigment adaptations, 117, 118f
  preference, pelagic fish, 1894
Derived condition, definition, 1772
Dermal bone
  definition, 336, 434
  lateral line canals, 338, 341f
    morphological variation, 338, 341f
  see also Bony fish skeleton
Dermal rays *see* Fin rays
Dermis, 477, 477f, 484
  definition, 476
  specialized dermal elements, 484
    chromatophores *see* Chromatophore(s)
    fin rays *see* Fin rays
    scales *see* Scales
Dermotrichia, 487
  see also Fin rays
Descending octaval nuclei (DON), 284f, 286–287, 287f, 288f
  directional selectivity, 302
Desert pupfish *see Cyprinodon* (desert pupfish)
Desmosomes
  cardiomyocyte cell–cell connections, 1012, 1012f
  definition, 194, 1006
  olfactory epithelium, 201
Determinate growth, indeterminate growth *vs.*, 1486

Detoxification systems
  ammonia *see under* Ammonia toxicity
  olfactory sensory neurons, 2081
  see also specific systems
Detritus
  definition, 1597
  digestive efficiency, 1599f, 1599t, 1600t, 1602
Deuterostomes, 7, 8, 9
  CNS evolution, 8
  motor neurons *see* Motor neurons
Development
  definition, 1986
  embryonic *see* Embryogenesis/embryonic development
  gas exchange during *see* Gas exchange during development (respiratory transitions)
  gills *see under* Gill(s)
  gut motility, 1299
  see also Ontogeny; *specific anatomic structures*
Developmental plasticity, hypoxic hyperventilatory response, 1755
Devils Hole, 1834f, 1834–1837
  pupfish *see Cyprinodon diabolis*
Diacylglycerol, definition, 1725
Diadromy, 1921f, 1922
  definition, 1921
Diapause, definition, 829
Diastole, 982–983, 1031
  definition, 973, 977, 1030, 1197, 1221
  see also Cardiac cycle
Diastolic depolarization, 1040–1041
  acetylcholine effects, 1042
  definition, 1038
Diastolic pressure, definition, 977, 979, 1030
Diel, definition, 1887, 1921
Diel-cycling hypoxia, 1746–1748
Diencephalic locomotor region (DLR), 57–58, 58f, 59f
Diencephalon
  agnathan, 21, 22f
  cartilaginous fish, 32
  definition, 1, 56, 159, 1457
  photosensitivity, 160, 160f
  ray-finned fish, 40f, 41
  see also Forebrain
Diet model, foraging, 745
Diet shifts, ontogenic, 1594
Dietary effects
  digestive efficiency *see under* Digestive efficiency
  gut anatomy, 1273
  skin coloration, 493
Dietary quality, effect on digestive efficiency, 1599f, 1601
Dietary requirements, cultured fish *see* Cultured fish, dietary requirements
Dietary toxicants
  effects in dominant *vs.* subordinate individuals, 654
  see also Toxicants
Differentiation *see* Organ differentiation
Diffraction, 103
  definition, 102
Diffusion, 833, 977
  in aqueous media, 833, 833t
  definition, 791, 829, 1348, 1389
  through membranes, 834
    ammonia, 835, 1439
    carbon dioxide, 834, 835f
    oxygen, 834
  time consideration, 836
  see also Gas transport and exchange; Oxygen diffusion; Passive diffusion
Diffusion capacity, 804, 814–816
  definition, 803, 1876
  gills, measurement, 806
Diffusion coefficients, air *vs.* water, 820, 821
Diffusion conductance, 824, 827
Diffusion distances, gill morphometrics, 806
Diffusion limited, definition, 1437
Diffusive boundary layer (DBL)
  definition, 829, 838
  developing fish and, 832f, 832–833, 839, 840f
Digesta
  definition, 1597
  digestive efficiency measurement, 1597t, 1598–1599
Digestion, 1301, 1306
  carbohydrate, 1306f, 1307
  carotenoids, 1309
  circulatory system responses *see* Gastrointestinal blood flow (GBF), regulation during digestion
  definition, 1206, 1229, 1301, 1665
  elasmobranch, 1813
  lipid, 1306f, 1308, 1309f
    see also Lipases
  minerals, 1306f, 1309
  process, 1306, 1306f
  protein, 1305t, 1306f, 1307
  specific dynamic action *see* specific dynamic action (SDA)
  visceral endothermy and, 1919f, 1920
  vitamins, 1306f, 1309
Digestive efficiency, **1596**
  apparent, definition, 1597, 1598
  concepts, 1596f, 1597
  definition, 1597
  determination, 1596f, 1597–1598
    digestive strategy considerations, 1598f, 1600
    effects of collection methods, 1597t, 1598–1599
    energy losses to nitrogen excretion, 1596f, 1600
    leaching considerations, 1600
    nondietary fecal material, 1596f, 1598
  dietary effects, 1599t, 1601
    dietary quality, 1599f, 1601
    nutrient classes, 1600t, 1601
  fish digestive strategies, 1603
    assimilation markers, 1606
    digestive enzyme activities and nutrient transporters, 1604f, 1605
    gut length, 1602f, 1605
    gut morphology, 1604
    intake and transit time, 1598f, 1603
    mastication and trituration, 1605
  true, definition, 1597, 1598
Digestive enzymes, 1305t, 1604f, 1605
  elasmobranchs, 1813
  gastric, 1302, 1305t
  pancreatic *see* Pancreatic enzymes
Digestive system *see* Gut
Digital particle image velocimetry (DPIV), 599, 599f, 600
  definition, 597
  see also Particle image velocimetry (PIV)
Digitiform gland *see* Rectal gland, dogfish
17α, 20β–Dihydroxy-4-pregnene-4-one (DHP), 1500
  formation, 630f, 1502–1503, 1504f
  oogenesis role, 1500, 1501f, 1502
  spermatogenesis role, 630, 632, 1502, 1503f
Dijkgraaf, Sven, 299–300, 329, 334–335
Dilution, hormone inactivation mechanism in mammals, 1191
Diodon liturosus (porcupine fish), antipredator morphology, 750, 750f
Dioecious, definition, 2009
Dioxins, 2075
Dipeptides, absorption, 1314f, 1315
Diploblasts, 7, 8
Diploid, definition, 1998, 2009
Diploid androgenic fish, generation, 2008
Diploid gynogenetic fish, generation, 2008
Diplostomun spathcaeum
  fish phenotype manipulation, 778, 779f
    cataract formation, 778–779, 779f
  life cycle, 778–779, 779f
Dipnoi *see* Lungfishes
Direct calorimetry
  definition, 1566
  metabolic rate measurement, 846–847, 1566
Direct current (DC) fields, 359–360
  definition, 359
Directional hearing, **298–303**
  behavioral evidence, 299, 300f
  brain mechanism, 302, 302f
  definition, 73
  theories, 301
    vector detection and phase model, 301
  unresolved issues, 76, 302
Discharge activity, definition, 854
Discharge-related events, effect on migration, 1926
Disease
  bacterial, 2033t
    see also Bacterial pathogenicity
  effects on gastrointestinal tract
    anatomy, 1274
    barrier function, 1329, 1330f
  stress-mediated, 1550f, 1551
    see also Health, stress effects
  viral *see* Viral diseases
  see also specific diseases
Disease resistance
  cultured fish, transgenesis, 2006
  effects of triploidy, 2013
  elasmobranchs, 1816
  triploid fish, 2013
Disease susceptibility, effect of social status, 653–654
Disinhibition, definition, 56
Dispensable amino acid (DAA), 1618–1619
  definition, 1617
Display
  courtship *see* Courtship behaviors
  definition, 684
Display calls, 687, 687f, 688f, 689
  see also Sound production
Dissolved oxygen (DO)
  absence *see* Anoxia
  effect of temperature, 1631
  effect on energetic costs of swimming, 1641f, 1642
  growth relationship, 1630f, 1631
  importance of, 1746
  intertidal habitats, adaptations to changing oxygenation, 1962f, 1963
  low levels *see* Hypoxia
  measurement methods, 846–848
  see also entries beginning oxygen
Distal, definition, 123, 419
Distance–time performance, fast start performance and, 592t, 593–594, 594f
Distress, sound production and, 685t, 689–690
Distribution, biological, toxic metals, 2062f, 2064
Distribution, geographical
  effects of global warming, 1740
  thermal niche adaptations, 1701
    tuna, 1912, 1913
  see also Migration
Disturbance
  definition, 684
  sound production and, 685t, 689–690
Disulfide bonds, 1707
  protein stabilization, 1707
Diurnality, 740, 773
Divalent, definition, 1311
Diversification, air breathing and, 1858
Diverticulum, definition, 98, 166, 1457
Diving
  monofocal lenses in deep-diving species, 108
  tracking technology
    problems, 1930–1932, 1935
    successful long term tagging, 1928f, 1930, 1934f
  tuna *see under* Tuna
Dmrt1/*Dmrt1* gene, sex differentiation role, 1497, 2028–2029, 2029t, 2030
DMRT1 gene, 2028–2029

DMY/*DMY* gene, sex differentiation role, 1497, 2028–2030
DNA, structure, 2000f, 2001
DNA microarray *see* Microarray analysis
DNA promoter, definition, 1986
DNA transposons, 1983
DOC *see* 11-Deoxycorticosterone (DOC)
Docosahexaenoic acid (DHA), dietary requirement of cultured fish, 1621
Dogfish
  cardiorespiratory interactions (CRIs), central control, 1181f, 1181–1183, 1182f
  conus arteriosus, 1023–1024
    Scyliorhinus canicula, 1023–1024, 1025f, 1026f, 1026–1027, 1027f
    Scyliorhinus sellaris, 1024–1026, 1026f, 1027f
  rectal gland *see* Rectal gland, dogfish
  see also Sharks
Dolphin, bottlenose, whistles, 315, 315f
Dominance
  behaviors, **649–655**
    hierarchies *see* Dominance hierarchies
    social status *see* Social status
  causes/predictors, 651
  definition, 649, 752, 1629
  measurement, 650
  studies on, reasons, 654
  subordinance *vs. see under* Subordinate individuals
Dominance hierarchies, 649, 754
  agonistic sound signaling and, 685t, 689
  aquaculture conditions, 650
  assessment, 650–651
  concept, 649, 650f
  definition, 649
  juvenile salmonids, 650, 650f
  pain-related responses and, 717–718, 718f, 719
  reasons for studying, 654
  tropical fish, 650
  see also Social status
Dopamine
  gonadotropin inhibition, 1505, 1505f
  gut motility role, 1299f
  see also Catecholamine(s)
Doppler effect, definition, 581
DOR (δ opioid receptor), 96
Dorsal, definition, 419
Dorsal aorta, 1086f, 1088f, 1089–1090
  definition, 1119
  hagfish, 1093
Dorsal fins
  locomotion and, 58
  skeletal supports, bony fish, 443–445, 445f
Dorsal light reflex, 145, 145f
  definition, 143
Dorsal light response, 61
Dorsal motor nucleus of the vagus (DVN)
  definition, 1178
  respiration-related activity, 1181–1183
    elasmobranchs, 1182f, 1183
    teleosts, 1187
Dorsal octavolateral nucleus (DON)
  definition, 359
  information processing, ampullary electrosensory system, 363, 364f
Dorsolateral placodes, 410
  definition, 409
Dose, definition, 2062, 2069
Dose–response
  definition, 2062, 2069
  metals, 2061f, 2062–2063
Double-bend response, 589, 589f
  definition, 587
  see also C-start
Double cones, 113–114, 114f
Downstream migration
  eels, 1938f, 1941
  salmon, 1948
  see also Smoltification
Drag, definition, 1887
Drag coefficient, 524–525
Drag power, 1637
  definition, 1636
Dragonfish, bioluminescence, 502, 502f, 503f
Dredging, noise generation, 305t, 306
Drilling, noise generation, 305t, 305–306
Drugs, effect on venous capacitance/cardiac output, 1114
Drumming muscles, 322–323
  extrinsic, 323–324, 324f, 325f
    definition, 321
  intrinsic, 323–324, 324f
    definition, 321
  lacking direct connections to swimbladder, 324
    piranha, 324, 326f
  sexual dimorphisms, 324f, 325
Duchenne muscular dystrophy, 1993–1994
Ductus arteriosus
  air-breathing fish, 1876f, 1878t, 1879f, 1882
  definition, 1876
Ductus Cuvier, 1092, 1093, 1112
  anatomy, 999f, 999–1001, 1086f, 1088f
  cyclostomes, 1004
  definition, 998
Durophagy
  definition, 434
  jaw adaptations, 435, 438–440
Dusk, anticipation of, 739
Dye flow visualization, 542
Dynamic energy budget (DEB) model, 1627, 1627f
Dystroglycan, 1994
Dystrophin, 1993–1994

E

Ears, fish, 241, 254
  evolution, 237
  historical studies, 238, 239f
  inner ear *see* Inner ear
  inter-species variation, 254, 256f
  location, 254, 255f
  physics, 299, 299f
  structure *see* Auditory system structure
  see also Hearing
Ears, vertebrate, 252
  fish *see* Ears, fish
  inner ear anatomy, 252, 253f
  sensory cells, 252, 254f, 255f
Earth, magnetic field, 726–727, 727f
  navigation role, 732, 734f
Eavesdroppers, 689–690, 692, 697–698
  definition, 692
Echocardiography, definition, 1197
Echolocation, 317
Ecological death
  definition, 2078
  olfactory–toxicity interactions and, 2082
Ecomorphological paradigm, 1583f, 1584
  definition, 1584
  performance consequences, 1586
Ecomorphology
  definition, 597
  feeding, 600
Ecophysiological theory, thermal tolerance, 1701
Ecosystems, bioenergetics models *see* Bioenergetics models
Ecotoxicology
  definition, 1965
  use of fish cell lines, 1969
  see also Toxicants
Ectoderm, 1285
  definition, 1284
  suppression of neurogenesis, 8, 9
Ectodermal cell, definition, 1457
Ectothermic, definition, 1629
Ectotherms, 1914
  definition, 992, 1006, 1054, 1665, 1688, 1695, 1732, 1887–1888, 1914
  heat shock proteins, 1733
    see also Heat shock response, fish
  responses to environmental temperature, 1696–1697, 1700
    compensatory responses to chronic low temperature, 956
Eddies, 582, 582f, 583
  definition, 520, 581
  fish interactions *see* Eddy–fish interactions
Eddy–fish interactions, 584
  importance of scale, 583, 583f
  response latency, 583
  use of eddies, 586
  see also Turbulence
Edema, 1112, 1161
  definition, 796, 1111, 1161
Ediculae, definition, 1861
Eel(s)
  American *see* American eel (*Anguilla rostrata)*
  anguilliform swimming, 548, 549f
  caudal heart, 1075
  European *see* European eel (*Anguilla anguilla*)
  glass *see* Glass eels
  Japanese (*Anguilla japonica)*, spawning area, 1940
  otoliths, 1939f, 1941
  specific dynamic action, 1609t, 1611f, 1612
Eel migration, **1937**
  from coastal shelf, 1938f, 1940
  downstream (return to sea), 1938f, 1941
  freshwater life, 1938f, 1941
  larval, 1937f, 1938f, 1939, 1940
  predation risk reduction, 1940–1942
  spawning, 1938f, 1943
Effector, definition, 1169
Efferent, definition, 1, 854
Efferent branchial arteries (EBAs), 1096f, 1097f, 1101, 1101f
Efferent filamental arteries (EFAs), 1096f, 1098, 1101, 1101f, 1102
Efferent lamellar arterioles (ELAs), 1097f, 1101f, 1102
Efflux, definition, 1359
Egestion
  definition, 1675
  simple energy budget, 1675f, 1676
Egg(s)
  definition, 635
  size, limiting factors, 1575
    capsule surface area and thickness, 1575–1576
    comparison with amphibian eggs, 1575–1576
    effect of hatching, 1574f, 1576
  yolk *see* Yolk, egg
  see also Spawning
Egg capsule
  definition, 829, 838
  gas exchange and, 832, 840, 841f, 1575
  surface area and thickness, 1575–1576
Egg eating *see* Oophagy
Egocentric cues
  definition, 720
  learning, 721, 721, 724
Egocentric direction, definition, 244
Eicosapentaenoic acid (EPA), dietary requirement of cultured fish, 1621
Ejection fraction, definition, 1060
Elasmobranchs, **1807**
  acid–base regulation, 1817
  autonomic nervous system, 82, 83f
    cardiac control, 85, 85f
    chromaffin tissue and, 82, 85, 85f
    control of iris, 87f
    extrinsic innervation of gut, 1333, 1334f
  buoyancy regulation, 1810
  carbon dioxide excretion, 913
  cardiac anatomy, 1004
    see also Conus arteriosus
  cardiorespiratory interactions, central control, 1180, 1181f, 1182f

  circulation, 1811
    GI tract, 1133–1134
  deep-water species, 1810
  definition, 236, 262, 1085, 1888
  digestion, 1813
  electric organs, 1809
  endocrinology, 1814t, 1816
  endothermy, 1816
  evolution and diversity, 262
  excretion, 1815
  freshwater species, 1810
  immune system and disease resistance, 1816
  intermediary metabolism, 1813
    carbohydrate, 1813
    ketone body, 1815
    lipid, 1815
    nitrogen, 1812f, 1813
  kidneys, 1412
  muscle physiology and swimming, 1809
  neurophysiology, 1816
  osmo- and ionoregulation, 1810f, 1811f, 1812
    freshwater species, 1349t, 1352
    gill ionocytes (MRCs), 1384f, 1386–1387
    marine species, 1349t, 1351
    stenohaline and euryhaline species, 1963
    see also Rectal gland, dogfish
  pelagic *vs.* demersal
    hearing sensitivity, 267, 267f
    inner ear anatomy, 264–265, 265f, 266f
  pituitary gland structure, 1458–1459, 1460f
  rectal gland *see* Rectal gland, dogfish
  reproduction, 1812
  respiration, 1811
  sensory biology, 1808
    audition, 1808
    balance and motion detection, 262–263, 263f, 264, 266f, 1809
    electro- and magnetoreception, 365, 726, 1808
      see also Magnetic sense
    mechanoreception, 1809
    olfaction, 1808
    taste, 1808
    vision, 1809
  specific dynamic action, 1609t, 1611, 1611f
  urea retention, 1445, 1446f, 1815–1816
  vascular anatomy, 1093
  see also Cartilaginous fish; Rays; Sharks; Skates
Elasmoid scales
  definition, 476
  morphology, 481f, 483f, 485f, 486, 486f, 487
Elastase, pancreatic, 1281, 1281t
Elastic cartilage, definition, 428
Elastin, definition, 1015
Electric fields
  alternating current *see* Alternating current (AC) fields
  definition, 347
  direct current *see* Direct current (DC) fields
  measurement, ampullary organs, 361, 362f
  sources, 359, 360f
Electric fish, 398, 399f
  historical aspects, 347–348
  pulse-type *see* Pulse-type electric fish
  wave-type *see* Wave-type electric fish
  see also Electric organ discharge (EOD)
Electric image, 376, 377f
  definition, 375
  see also Active electrolocation
Electric lobes, electric rays, 27, 28f
Electric organ(s) (EOs), **387–397**, 410
  definition, 347
  development *see* Electric organ development
  discharge *see* Electric organ discharge (EOD)
  discovery/historical aspects, 348
  diversity, 387
  elasmobranch, 1809
  evolution, 387, 388f
  location, 398, 399f
  morphological organization and role in electrogenesis, 388
    bulbosinal and peripheral networks, 391, 394f, 395f, 396f
    electrocytes *see* Electrocytes
    post-effector mechanisms, 395, 396f
  myogenic *see* Myogenic electric organ
  neurogenic *see* Neurogenic electric organs
Electric organ corollary discharge (EOCD), 368–369, 369–370, 381–383, 382f, 384
  definition, 366, 375
Electric organ development, 412
  Chondrichthyes, 412
  Teleostei, 413
    Astroscopidae, 415
    Gymnotiformes, 414, 414f
    Osteoglossomorpha, 413, 413f
    Siluriformes, 414
Electric organ discharge (EOD), 366, **398–408**, 375, 409–410, 699, 700
  aggression role, 703, 703f, 704f
  central control *see* Electric organ discharge (EOD), central control
  continuous, 701
  corollary discharge *see* Electric organ corollary discharge (EOCD)
  courtship role, 703f, 704, 704f
    see also Chirps
  definition, 347, 375, 398, 699
  discovery/historical aspects, 348
  evolution, 387, 388f
  mechanisms, 399, 400, 400f
    generation of commands by CNS, 401, 401f, 402f
    synchronous firing of electrocytes, 389, 392–393, 401
      see also Electrocytes
  modulation as source of environmental information, 375, 376f, 377f
    see also Active electrolocation
  modulations, 400, 702, 703f
    amplitude, 366–367
      see also Amplitude coding
    frequency, 366–367, 400
      see also Time coding
    mechanisms, 403, 405t
      see also under Electric organ discharge (EOD), central control
  projections to higher-order neurons, 368
  pulse-type *see* Pulse-type electric organ discharge
  species/sexual identification role, 701
  wave-type *see* Wave-type electric organ discharge
  waveforms
    processing, torus semicircularis, 373
    representative, 399f
    variability, 391, 392f
  see also Electric organ(s) (EOs); Electrocommunication; Tuberous electroreceptors
Electric organ discharge (EOD), central control
  command generation (regular rhythm), 401, 401f, 402f
    see also Command nucleus, electric fish; Pacemaker nucleus
  time patterns, 403, 405t
    gymnotiform pulse fishes, 403, 403f, 404f, 405f
    gymnotiform wave fishes, 403, 406f
    mormyrid pulse fishes, 406, 407f
    mormyrid wave fish, 407, 407f
Electric ray, electric lobes, 27, 28f
Electric shock, avoidance, 709, 709f, 714–715, 715f
Electric signals
  detection *see* Electrosense
  generation *see* Electric organ discharge (EOD)
Electrical gradient, definition, 1354
Electrical inhibition, Mauthner cell, 68, 69f
Electrical resonance, definition, 73
Electrical synapses
  chemical synapses and, primary auditory afferents, Mauthner cell as model, 70f, 71
  definition, 66
Electro-olfactogram (EOG), 184, 2080
  definition, 183, 1553
  responses
    amino acids, 209, 210f, 211f
    bile acids, 211f, 211–212
    contaminants, 2079f, 2080–2081
    gustatory responses *vs.*, 218, 219f
    prostaglandins, 212, 213f
    steroid hormones, 212, 212f
Electrocardiogram (ECG), 1039
  cardiac cycle, 1032f, 1033
  definition, 1030, 1038
Electrochemical gradient, definition, 1311, 1354
Electrocommunication, **699–706**
  definition, 347, 387
  discovery, 700
  evolution, 705
  hormonal influences, 705
  as neuroethology model, 706
  signal detection *see* Electrosense
  signal generation, 700
    see also Electric organ discharge (EOD)
Electrocytes, 387–389, 700
  Brachyhypopomus, 391, 392f, 393f
  definition, 387, 699
  Gymnarchus niloticus, 391
  Gymnotus, 391, 392f, 393, 393f
  hormonal influences, 705
  myogenic, 389–391, 390f
  neural, 389, 389f
  Rhamphychthys, 391, 392f, 393, 393f
  synchronous firing, 389, 392–393, 401
  waveform generation, 389, 390f
    variety of waveforms, 391, 392f, 393f
  see also Electric organ(s) (EOs); Electric organ discharge (EOD)
Electroencephalogram (EEG), olfactory bulb responses, 213, 214f, 215
Electrogenesis *see* Electric organ discharge (EOD)
Electrogenic transporter, definition, 1359
Electrolocation, 723
  active *see* Active electrolocation
Electrolyte, definition, 1457
Electrometric ΔpH assay, definition, 899
Electrometric measurements, dissolved oxygen, 846–847, 847–848
Electromotor neuron, 389, 389f, 393–394, 394f, 395f
  definition, 387
Electromyogram, definition, 854
Electromyography (EMG)
  activity measurement in undulatory swimming, 551
    related to muscle length change, 551–552, 552f
    sequential timing along body axis, 551, 552f
  definition, 516, 547
  limitations, 552
Electron transport system (ETS), 959–960, 963f, 1710, 1757f, 1758
  complex I, 963, 963f
    definition, 959, 966
  complex II, 963, 963f
    definition, 959, 966
  complex III, 963, 963f
    definition, 959, 966
  complex IV *see* Cytochrome *c* oxidase (complex IV)
  complex V *see* F_1F_0 ATPase (complex V)
  definition, 959, 966, 1709
  oxidation, 963, 963f
  see also Oxidative phosphorylation (OXPHOS)
Electroneutral transporter, definition, 1359
Electroreception, 409
  definition, 387
  elasmobranch, 1808
  evolution, 359, 360f, 705
  sensory organs *see* Electroreceptor(s)
  see also Active electrolocation; Electric organ(s) (EOs); Electroreceptor(s)

- Electroreceptor(s), **350–358**, 379, 410*f*, 699, 700–703, 705
  - ampullary organs *see* Ampullary electroreceptors
  - comparative aspects, **409–415**
  - definition, 347
  - development *see* Electroreceptor development
  - lamprey end buds *see under* Lampreys
  - monotreme electroreceptive organs, 358
  - tuberous organs *see* Tuberous electroreceptors
  - *see also* Active electrolocation; Electric organ discharge (EOD)
- Electroreceptor development, 410
  - derived ampullary/tuberous electroreceptor organs, 410
    - Teleostei, 410
      - Gymnotiformes, 412
      - Osteoglossiformes, 410, 411*f*, 412*f*
      - Siluriformes, 411
  - lampreys, 410
  - primitive ampullary organ, 410
- Electroretinogram (ERG), definition, 98, 166
- Electrosense, 348–349, 364, 723
  - definition, 347, 366
  - discovery/historical aspects, 348
  - predator detection, 365
  - prey sensing, 364
- Electrosensory lateral line lobe (ELL), 366–367, 368–369, 379
  - circuitry, 380, 382*f*
  - granule cells, latency code to rate code transformation, 381
  - physiology of neurons receiving input from amplitude-sensitive electroreceptors, 369
    - amplitude-coding in wave-type gymnotiforms, 369, 370*f*
    - cancellation of self-generated stimuli, 369
    - regulation of burst firing, 371, 371*f*
    - wave-type gymnotiform *vs.* wave-type mormyriform weakly electric fish, 371
- Electrosensory systems
  - ampullary *see* Ampullary electrosensory system, physiology
  - tuberous *see* Tuberous electrosensory system, physiology
- Electrotonic, definition, 123
- Elephantfish *see* Mormyrid electric fish
- Elephantnose fish *see Gnathonemus petersii*
- Elvers
  - geographical distribution, 1937*f*
  - migration, 1938*f*, 1941
  - *see also* Eel(s)
- Embryo(s)
  - body mass scaling *see under* Scaling, body mass
  - definition, 336
  - development *see* Embryogenesis/embryonic development
  - gas exchange *see* Gas exchange during development
  - genetic manipulation with morpholinos, 1822
  - hemoglobins, 949–950
  - urea excretion, 1447, 1448*f*
- Embryogenesis/embryonic development
  - blood *see* Hematopoiesis
  - blood vessels *see* Vasculogenesis
  - brains, 2, 3*f*
  - definition, 613
  - digestive system *see* Gut development
  - heat shock response and, 1736
  - HPI axis *see* Hypothalamic–pituitary–interrenal (HPI) axis development
  - lateral line system in bony fishes, 339, 342*f*, 343*f*
  - maternal stress and, 1521
  - nutrient provision *see under* Parental nutrient provision
  - outflow tract, heart, 1015, 1017*f*
  - pancreas *see under* Pancreas
  - respiratory gas exchange *see* Gas exchange during development
  - taste buds, 189
  - zebrafish *see under* Zebrafish
  - *see also* Organ differentiation; Stem cells; *specific organs/systems*
- Emergence behaviors, hypoxia responses, 767, 768*f*
  - evolution, 1855*f*, 1856
  - *see also* Air-breathing fish
- Emotional learning, medial pallium role, 709
- Emulsification, definition, 1301
- End buds *see under* Lampreys
- End-diastolic volume, 1032–1034, 1173
  - definition, 1030, 1215
- End-systolic volume, 1032–1034, 1036
  - definition, 1030, 1215
- Endemic, definition, 2032
- Endocardial cells
  - bulbus arteriosus, 1019–1020, 1021*f*
  - conus arteriosus, 1024–1026, 1027*f*
- Endocardium, 1000–1001
  - definition, 998, 1060
- Endocrine, definition, 1229, 1276
- Endocrine control *see* Hormonal control
- Endocrine disruption, 1522
- Endocrine disruptors, 787, 1522
  - definition, 783, 2069
  - PAHs, 2075
  - PBDEs, thyroid interference, 2076
  - PCBs, steroid-related effects, 2076
  - pesticides, 2074
  - synthetic estrogens, 2076
- Endocrine factors
  - definition, 1429, 1665
  - rectal gland secretion control, 1434
  - *see also specific factors*
- Endocrine pancreas, 1276–1277
  - *see also* Brockmann body; Pancreatic hormones
- Endocrine signaling, definition, 1515
- Endocrine system, 1190
  - definition, 1190
  - gut *see* Gut hormones
  - nervous system *vs.*, 1190
  - regulation of reproduction *see* Reproduction, endocrine regulation
  - social status and, 652, 653*f*
  - *see also* Hormone(s)
- Endocrinology
  - food intake *see* Food consumption/intake
  - growth *see* Growth-regulating hormones
  - *see also* Hormonal control; Hormone(s)
- Endocytosis
  - definition, 1311
  - intestinal transepithelial transport, 1313*f*, 1322, 1324*f*
    - intact proteins, 1313*f*, 1315
    - role in intestinal barrier function, 1325, 1327, 1327*f*
    - *see also* Intestinal absorption
- Endoderm
  - definition, 1284
  - specification, 1284–1285
- Endogenous opioid peptides, 89
  - prodynorphins, 91, 92*t*
  - proenkephalins, 89, 89, 90*t*
  - pronociceptins, 91
  - proopiomelanocortins, 91, 93*t*
- β-Endorphin, stress response role, 1518
- Endoskeleton, definition, 419, 428, 434
- Endostyle
  - definition, 1780
  - larval lampreys, 1780*f*, 1784
- Endosymbiont, definition, 98, 166
- Endothelin, vascular resistance control, 1106
- Endothelin-1, capacitance curve and cardiac output, effect, 1115
- Endothelium
  - definition, 1190, 1395
  - glomerular *see* Glomerular endothelium
  - lymphatic vessels, 1161, 1162*f*, 1164*f*
    - gills, 1165–1166, 1166*f*
    - lungfish, 1165
  - mammalian, hormone inactivation, 1192
- Endothermy/endotherms, **1914**
  - advantages, 1919, 1919*f*
  - cardiac physiology and, 1916
  - cranial *see* Cranial endothermy
  - definition, 1006, 1054, 1119, 1665, 1688, 1695, 1732, 1888, 1903, 1914, 1914–1915
  - elasmobranchs, 1816
  - evolution, 1915, 1919
  - regional *see* Regional endothermy
  - systemic, 1914*f*, 1915
    - locomotory muscle, 1914*f*, 1915, 1919
    - visceral, 1915–1916, 1919*f*, 1920
  - temperature control during diving, 1911*f*, 1912
- Energetic cost
  - aerobic swimming, 1638, 1638*f*
    - measurement, 1638*f*, 1639
  - anaerobic swimming, 1640
  - buoyancy, 523–525
  - growth and reproduction, 1909
  - *see also* Bioenergetics
- Energetic stress, effect on cellular respiration, 956
- Energetics
  - desert pupfish, 1840*f*, 1841
  - growth *see* Growth, bioenergetics
  - growth hormone transgenic fish *see under* Growth hormone transgenesis
  - migration, 1924
  - prey capture *see under* Prey capture
  - prey handling *see under* Prey handling
  - swimming *see* Swimming, energy expenditure
  - *see also* Bioenergetics
- Energy acquisition
  - cost of digestion and assimilation *see* specific dynamic action (SDA)
  - digestion *see* Digestive efficiency
  - foraging *see* Foraging decisions (energetics)
  - prey capture energetics *see* Prey capture
  - prey handling *see* Prey handling
  - *see also* Bioenergetics
- Energy budget, 1564, 1625, 1675*f*, 1676
  - definition, 1665
  - energy allocation and its fitness consequences, 1624*f*, 1625
  - equations, 1743–1744
  - growth trials and biological evaluation of compound feeds, 1670
- Energy conservation, metabolic rate suppression, 1767
- Energy expenditure
  - cultured fish, 1672, 1672*f*
  - exercise *see* Swimming, energy expenditure
  - growth *see* Growth
  - metabolism *see* Metabolic rate (MR); Metabolism
  - specific dynamic action *see* specific dynamic action (SDA)
  - *see also* Bioenergetics
- Energy metabolism, **1566**
  - *see also* Metabolic rate (MR); Standard metabolic rate (SMR)
- Energy partitioning *see under* Bioenergetics
- Enforced unsaturation, definition, 1877
- *Engraulis enerasicolus* (anchovy), muscle fiber anatomy and microvasculature, 1149, 1149*f*, 1150
- Enteric nerve, definition, 1332
- Enteric nervous system, 83*f*
  - anatomy, 1332, 1333*f*
  - definition, 80, 80, 1229, 1292, 1332
  - gastrointestinal blood flow regulation, 1212
  - gut motility control, 1295*f*, 1296
  - innervation, 1332–1333, 1333*f*
  - neurotransmitters/receptors, 1334
    - acetylcholine, 1335
    - amines, 1335, 1335*f*

    amino acids, 1339
    coexistence and chemical coding, 1339
    distribution, 1335*f*
    neuropeptides, 1335*f*, 1336
      see also specific neuropeptides
    nitric oxide, 1335*f*, 1339
    purines, 1339
  see also Autonomic nervous system (ANS)
Enteric neurons, development, 1287, 1289*f*
Enteritis, soyabean-induced, 1330
Enterochromaffin-like (ECL) cells, 1342
Enterocytes, 1312*f*, 1312–1313
  definition, 1229, 1311, 1322
  lipid accumulation, 1329–1330
  microvilli, 1323, 1325*f*, 1326*f*, 1327*f*, 1328*f*, 1328*f*, 1603*f*, 1605
    definition, 1322
  role in intestinal barrier function, 1323, 1324*f*, 1325, 1325*f*, 1326*f*
  tight junction, 1322, 1324, 1324*f*, 1326*f*
    definition, 1322
    see also Gut
Enteroendocrine cell, 1341
  definition, 1341
  see also Gut hormones
Enterokinase, 1304, 1305*f*
Enteropancreatic (EP) system, 1277–1278
  definition, 1276
Enthalpy, definition, 1703
Entrain, definition, 854
Entrainment
  circadian *see under* Circadian rhythms
  respiratory rhythm, 858, 859–860, 860*f*
Entropy, definition, 1564, 1566, 1703
Envelope, viral, 2035
Environment, hypoxic *see* Hypoxic environments
Environmental acclimation/adaptation
  gill ionocytes, freshwater fish, 1364
  insights from global gene expression data, 2058
  salinity *see* Salinity acclimation
  thermal *see* Thermal acclimation/adaptation
  see also specific adaptations
Environmental degradation, effect on temperature preferences, 763
Environmental influences
  appetite/food intake, 1486, 1513
  cell membrane fluidity *see under* Cell membrane fluidity
  cellular respiration, 956
  CO_2 transport and excretion, 914
  energy expenditure during exercise *see under* Swimming, energy expenditure
  energy partitioning/production, 1666*f*, 1668
  epidermal goblet cell numbers, 482
  fast start performance, 595
  fish growth *see* Growth, environmental effects
  food consumption, assessment using bioenergetics modeling, 1678
  gill ionocytes, freshwater fish, 1364
  learning/memory, 710
  metabolism *see* Metabolism, environmental influences
  migration, 1925
  morphology *see* Ecomorphological paradigm
  reproduction, sturgeon, 1802
  respiration, 792, 792*f*
  sex differentiation, 1497
  temperature *see* Temperature effects
  urea excretion, 1447
  see also Climate change impacts; *specific environmental factors*
Environmental interactions, fish behavior–fish physiology interrelations, 648
Environmental monitoring, transgenic fish, applications, 2006
Environmental observations, temperature preferences *see under* Temperature preferences
Environmental requirements, sturgeons, 1800*f*, 1801
Environmental risk assessment, growth hormone transgenesis, 2022*f*, 2023
Environmental sex determination (ESD), 2052
Environmental stress
  biochemical adaptations *see* Biochemical adaptations, general principles
  noncovalent chemical bonding as basis, 1682
  tolerance, triploid fish, 2013
  see also Stress
Environmental variables
  as source of variation in gene expression data, 2057–2058
  see also specific variables
Enzymes
  catalytic rate, 1703, 1705
  characteristics, 1704
    conformational flexibility, 1703, 1704
    specificity, 1704
  digestive *see* Digestive enzymes
  effects of temperature on function, 1696–1697, 1704, 1704*f*
    catalytic rate (turnover number, k_{cat}), 1705
    substrate affinity, 1703*f*, 1704
  homologs, 1703, 1705, 1707
    definition, 1703
  isoforms, 1705
  stabilization/destabilization factors, 1705
    amino acid composition, 1706
    disulfide bonds, 1707
    hydrogen bonding, 1707
    hydrophobic interactions, 1706
    salt bridges (ionic interactions), 1707
    van der Waals interactions, 1707
  substrate affinity, 1703, 1705
  temperature adaptation, protein structure, 1705
    conservation of active site, 1707
    substitution locations, 1707
  see also specific enzymes
Eosinophils, 989*f*, 990
Epaxial muscle, definition, 409
Ependyma, definition, 159
Ephapsis
  definition, 66
  Mauthner cell and, 68
Epibranchial, definition, 449
Epibranchial bone, 1096, 1096*f*
Epibranchial muscle
  holocephalans, 459, 459*f*, 460*f*
  sharks, 450, 450*f*, 453*f*, 454–455
Epicardium, definition, 1060
Epidermal hyperplasia, 472
Epidermis, 476–477, 478
  cell types, 477*f*, 478
    club cells *see* Club cells
    epithelial cells, 477*f*, 478*f*, 479, 479*f*, 481*f*, 482*f*, 483*f*
    goblet cells, 477*f*, 481, 481*f*, 482*f*, 483*f*
    granular cells, 478*f*, 483–484
    paraneurons, 484
    sacciform cells *see* Sacciform cells
    thread cells *see under* Slime glands (hagfish)
  definition, 476
Epinephrine *see* Adrenaline (epinephrine)
Epipelagic, 1888*f*, 1890–1891
Episodic breathing, 860, 861*f*
  definition, 854
Epithalamus
  agnathan, 21
  cartilaginous fish, 32
  definition, 159
  ray-finned fish, 41, 42
Epithelial-like cell line
  definition, 1965
  piscine, 1965*f*, 1967
Epithelium (epithelial cells)
  definition, 1229, 1268, 1322, 1354, 1457
  epidermis *see under* Epidermis
  gill, 1097*f*, 1098, 1099*f*, 1100*f*
  gut *see under* Gut
Epitope, definition, 208
Eptatretus stoutii (Pacific hagfish), slime production, 504–505, 505*f*, 508
Equilibrium, definition, 791, 909, 916, 921, 935, 1119
Equilibrium potential, definition, 871, 1038
Errlo, 163–164
Erythrocytes *see* Red blood cells (RBCs)
Erythrophores, 490*t*, 491, 495, 1971*f*, 1973
  development, 1971*f*
Erythrophoroma, goldfish, 1977
Erythropoiesis, 986, **992–997**
  common features in vertebrates, 992
  definition, 992
  definitive (larval/adult), 993, 994
  genes required (GATA family), 995
    equilibrium with myeloid (PU.1) factor, 996
    expression during embryonic development, 995, 995*f*
    transcription factors, 995–996
  hormonal regulation (erythropoietin, Epo), 986, 992, 993
  MCHC and, 988
  zebrafish model, process steps, 993, 994*f*
Erythropoietin, 986, 992, 993
Escape latency, 592–593
  definition, 587
  environmental influences, 595
Escape responses
  definition, 73, 587
  Mauthner cell role, 59, 73, 74*f*, 78–79, 587–588, 588*f*
    cellular specializations, 77, 77*f*
  optic tectum role, 141
  see also Fast start
Escape sounds, 690
Escape swimming
  motor control systems, 59
  see also Escape responses
Esophagus
  air-breathing role, 1870
  anatomy, 1269, 1269*f*, 1285*f*
  development *see under* Gut development
  ion regulation role, 1421
  musculature, 1271
Essential amino acids *see* Indispensable amino acids (IDAAs)
Essential metals, 2062–2063
  definition, 2062
  dose–response, 2061*f*, 2062–2063
Estivation
  definition, 765, 1444
  Protopterus, 770, 770*f*
    ureotelism and, 1447
17β-Estradiol
  definition, 662
  formation/metabolism, 1504*f*
    steroidogenic shift to progestogens, 1503, 1504*f*
  oogenesis role, 1500, 1501*f*
  sex change role, 665–666, 666*f*, 2053
  sturgeon, 1802
Estrogen(s)
  definition, 656, 1553
  female sexual behavior and, 660–661
  sex differentiation role, 1494
  spermatogenesis role, 631, 631*f*, 633*f*
  see also 17β-Estradiol
Estrogen receptors (ERs), sex differentiation role, 1496, 1496*f*
Estrogenic chemicals, reproductive disruption, 787–788
Estuaries, 1962
  definition, 1960
Ethanol production, anoxic, 1762
  crucian carp, 1825*f*, 1827, 1829
  goldfish, 1827
Ether extract, definition, 1665
Ethmoid tissue, definition, 720

- Ethology, 647
  - definition, 647
  - *see also* Behavior, fish
- Euhyostyly
  - batoids, 425–426
  - definition, 419
- European eel (*Anguilla anguilla*)
  - gill remodeling, 801, 801*f*
  - larva *see* Leptocephali
  - lifecycle, 1938*f*, 1938–1939
    - *see also* Eel migration
  - migration *see* Eel migration
  - Sargasso Sea spawning ground *see under* Sargasso Sea
  - specific dynamic action, 1609*t*, 1611*f*, 1612
  - swimbladder
    - gas composition and pressure, 531, 532
    - physostome morphology, 527, 527*f*
    - *see also* Physostomous gas bladder
  - swimbladder gas gland cells
    - histology, 528, 528*f*
    - metabolism, 528, 529, 530*f*
  - visual pigments, 120
    - chromophore switching, 120–121, 121*f*
- European Wels (*Siluris glanis*), ears, 256*f*
- Eurydendroid cells, definition, 56
- Euryhaline, definition, 1429, 1466, 1960
- Euryhaline fish, 1349, 1467
  - definition, 1111, 1483
  - plasma ion compositions, 1349*t*
  - salinity monitoring *see* Osmosensing
  - venous capacitance, 1115–1116, 1116*f*
  - *see also* Intertidal species; Salinity acclimation
- Euryhalinity, 1373
  - definition, 1373
  - limiting factors, 1353
- Eurythermal, definition, 1197, 1960
- Eurytherms/eurythermy, 1690
  - adaptations, 1203
  - crucian carp, 1826–1827
  - definition, 1688, 1690, 1732, 1824
  - heat shock response, thermal history and, 1734
    - *see also* Heat shock response, fish
- *Eutrigla gurnardus* (grey gurnards), aggressive sounds, 314, 314*f*
- Eutrophication, 1748
  - definition, 1746, 1748
- Eversion, ray-finned fish, 43, 44*f*
  - definition, 37
- Evolution
  - air-breathing fish, 1852–1853, 1855*f*, 1856
    - air-breathing organ (ABO), 1851*f*, 1853, 1853*f*, 1861*f*, 1862
  - alarm substance, 233
  - animal, 7, 7*f*
  - auditory/lateral line mechanosensory systems *see under* Auditory/lateral line CNS, anatomy
  - biogenomic, mitochondria, 967, 967*f*
  - blind cavefish troglomorphism, 1848
  - Bohr effect, 926, 927*f*
    - fish hemoglobins *see under* Bohr effect, fish hemoglobins
  - conserved genes and signaling pathways, 992, 992–995
  - coronary circulation, 1078–1079
  - corticosteroid signaling system, 1480, 1481*f*
  - cranial/spinal nerves *see* Central nervous system (CNS); Cranial nerve(s); Spinal nerves, evolution
  - ears/hearing, 237
  - electrocommunication, 705
  - electrogenesis, 387, 388*f*
  - electroreception, 359, 360*f*, 705
  - endothermy, 1915, 1919
  - fish genomes *see under* Fish genomes
  - gustatory system, 185–186
  - hagfish slime deployment, 510–512
  - hormonal pheromones, 1555
  - jaws and suction feeding, 598, 598*f*
  - kidney, 1396*f*, 1397
  - loss of hemoglobin in icefish, 996
  - multicellular organisms, clades, 7, 7*f*
  - osmoregulatory strategies, 1350, 1352
  - pancreas, 1277, 1279*f*
  - parental care, 673*f*, 675
  - polyploidy role, 2009–2010
  - retia, 941, 942*f*, 1130
  - Root effect/rete systems, 941, 942*f*
  - single-celled organisms, 7, 8
  - studies using robotic fish, 605, 606*f*, 607*f*
  - thermal acclimation/adaptation *see under* Thermal acclimation/adaptation
  - visual communication, 697
  - visual pigments, 111, 113*f*
  - vocal fishes, 686
  - Y chromosome, 2029
  - *see also* Phylogeny
- Evolutionary constraints, fish eyes, 103, 104*f*
- Evolutionary mutant model, definition, 992
- Evolutionary stable strategy (ESS)
  - definition, 1584
  - foraging behavior, 1586
- Evolutionary thermal adaptation, mitochondrial role, 1713
- Exaptation, definition, 597
- Excess post-exercise oxygen consumption (EPOC), 1215, 1640–1641
  - definition, 1215, 1636
- Exchange vessels, definition, 982–983
- Exchanger, definition, 1359
- Excitability, definition, 1045
- Excitable membranes, **1717**
  - definition, 1717, 1719
  - evolutionary thermal adaptation, 1723
    - Antarctic and temperate fish, 1723
    - fish compared with mammals, 1723
  - ion channels, 1718*f*, 1720
  - temperature effects, 1719*f*, 1720
    - action potential conduction velocity, 1721
    - action potential duration, 1722
    - resting membrane potential, 1719*f*, 1720
    - significance of lipids, 1722
  - *see also* Action potential (AP)
- Excitation–contraction coupling
  - cardiac *see* Cardiac excitation–contraction coupling
  - definition, 973, 1045, 1888
- Excitation–thermogenic coupling, heater organ tissue, 1917*f*, 1917–1918
- Excitatory input, definition, 73
- Excretion, 1395
  - ammonia *see* Ammonia excretion
  - carbon dioxide *see* Carbon dioxide (CO_2) transport/excretion
  - definition, 1665, 1675
  - elasmobranchs, 1815
  - energy budget, 1675*f*, 1676
  - metals, 2064
  - organic xenobiotics, 2072*f*, 2073
  - skin role, 474, 835
    - nitrogen excretion in early life stages, 1447
  - urea *see* Urea excretion
  - *see also* Biotransformation
- Excretion systems, extracellular products, 2036
  - type I (T1SSs), 2036
  - type II (T2SSs), 2036
  - type III (T3SSs), 2036
  - type IV (T4SSs), 2036
- Exercise
  - ammonia toxicity and, 1442, 1442*t*
  - capabilities, growth hormone transgenic fish, 2021
  - circulatory system responses, **1215–1220**
    - aerobic swimming *see under* Aerobic swimming
    - anaerobic swimming, 1218
    - coronary blood flow, 1081–1082, 1082*f*
    - future research, 1219
    - gastrointestinal blood flow, 1138, 1139*f*, 1212–1213, 1213*f*
    - sustained swimming, 813*f*, 816
    - vascular resistance, 1107
    - venous capacitance/cardiac output, 1115
  - effects
    - circulatory system *see circulatory system response (above)*
    - cultured fishes, 2089
    - respiration *see* Exercise, respiratory effects
  - energy utilization *see* Swimming, energy expenditure
  - exhaustive
    - anaerobic metabolism and, 1760*f*, 1761, 1762
    - catecholamine release, 1527
  - limiting factors, 817
    - blood oxygen transport, 812, 817, 818
    - gill surface area, 818
    - tissue diffusion, 818
  - metabolic rate and cost of activity, 812
  - osmorespiratory compromise in exercising fish, 1389, 1390*t*, 1391*t*
  - triploid fish, speed and endurance, 2013
  - *see also* Locomotion; Swimming
- Exercise, respiratory effects, **812–819**
  - gas transport, 814
    - gills, ventilation and diffusion, 814
  - gas transport, cardiovascular, 816
    - blood-flow distribution, 817
    - blood oxygen/carbon dioxide content, 816
    - cardiac output, 815*f*, 816
  - osmorespiratory compromise in exercising fish, 1389, 1390*t*, 1391*t*
- Exhaustive exercise
  - anaerobic metabolism and, 1760*f*, 1761, 1762
  - catecholamine release, 1527
- Exocrine, definition, 1229, 1276
- Exocrine pancreas, 1276–1277
  - *see also* Pancreatic enzymes
- Exocytosis, 504, 504
  - definition, 504, 1524
- Exon, definition, 1998
- Exophthalmia, definition, 2032
- Exoskeleton, definition, 428, 434
- Experimental hydrodynamics, **535–546**
  - basic principles *see under* Fluid dynamics
  - flow field interpretation, 542
    - force *see* Force estimation, fluids
    - power, 545, 545*f*
  - fluid velocity measurements, 538
    - particle image velocimetry *see* Particle image velocimetry (PIV)
    - volumetric techniques, 542
  - future developments, 545
  - suction feeding
    - computational modeling, 600
    - fluid speed measurements, 599, 599*f*
    - *in vivo* pressure recording studies, 600
    - ingested volume of water (IVW), 599, 600*f*, 601
- Expressed sequence tag (EST), 2057
  - definition, 2055
- Extant, definition, 419, 428
- Extension of energetic limits, anaerobic pathways, 1649*f*, 1650
- Extensions, integumentary, 487
- External development
  - parental nutrient provision, 679
  - *see also* Oviparity
- External glomerulus, 1397, 1397*f*, 1398*f*, 1399
- Extinct, definition, 428
- Extra-branchial oxygen chemoreceptors, 1755
- Extracellular fluid, definition, 1154, 1155
- Extracellular matrix (ECM), 429
  - definition, 428
- Extracellular products (ECPs), disease role, 2034
  - excretion systems, 2036
  - hemolysins/cytolysins, 2035

    - aerolysin, 2035
  - lipases, 2035
  - proteases, 2034
    - metalloproteases, 2035
    - serine proteases, 2035
- Extraction coefficient, 821, 823
- Extraocular motor nuclei, octavolateral vestibular nuclei projections, 247*f*, 248
- Extraretinal photoreception, **159–165**
  - deep-brain photosensitivity, 160, 160*f*
  - pineal *see* Pineal photoreception
- Extraretinal photoreceptors, 159, 160*f*
  - deep brain, 160, 160*f*
  - pineal, 160*f*
    - lamprey, 161
    - teleosts, 162
- Extrinsic barrier, gut, 1323, 1325*f*
- Extrinsic drumming muscles *see under* Drumming muscles
- Extrinsic innervation
  - autonomic neurons *see under* Autonomic neurons
  - definition, 1229, 1332
  - gut *see under* Gut innervation
- Eye(s)
  - adaptations *see* Visual adaptations
  - anatomy
    - deep-sea fishes *see* Deep-sea fishes, ocular anatomy
    - teleosts, 168, 169*f*
    - *see also specific structures*
  - development, blind cavefish *see* Eye degeneration, blind cavefish
  - evolutionary constraints, 103, 104*f*
  - hagfish, 17, 1772*f*, 1777
  - heat exchangers serving, 1125, 1126*t*
  - lampreys *see under* Lampreys
  - motor control, steering and, 59, 60*f*
  - movement *see* Eye movement
  - optical elements, 104, 105*f*
    - aphakic aperture *see* Aphakic aperture
    - cornea, 105, 105*f*
    - iris *see* Iris
    - lens *see* Lens
    - retina *see* Retina
  - optical principles, 103, 103*f*
  - shape, deep-sea fishes *see under* Deep-sea fishes, ocular anatomy
  - tubular *see under* Deep-sea fishes, ocular anatomy
  - vascular supply, pseudobranch, 932, 932*f*
  - warming, 1916
    - advantages, 1919
    - *see also* Cranial endothermy
  - *see also* Vision
- Eye degeneration, blind cavefish, 1844, 1845*f*, 1846
  - commitment, 1845*f*, 1846
  - differentiation, 1845*f*, 1846
  - growth and maintenance, 1847
  - phenocopy, 1847
  - rescue, 1847
    - genetic, 1847
    - by lens replacement, 1847
    - pharmacological, 1847
  - specification, 1845*f*, 1846
- Eye movement
  - compensatory, 248, 248*f*
  - horizontal, 249*f*, 249–250
  - tectal stimulation, 141, 141*f*
- 'Eye shine', deep-sea fish, 176, 178*f*, 179*f*

F

- F_1F_0 ATPase (complex V), 959–960, 963*f*, 963–965, 1710
  - definition, 959, 966
  - effect of cold acclimation on activity, 1714*f*, 1715
- Facilitated transporters, definition, 1444
- Facultative air breathing, 1854, 1855*f*, 1857
  - definition, 1850
  - *see also* Air-breathing fish
- Fahraeus effect, 980, 1113
  - definition, 977, 1111
- Fahraeus–Lindqvist effect, 980, 1113
  - definition, 977, 1111
- False hermaphroditism, 1490
  - definition, 1490
- Fangs, probeagle shark, 679, 679*f*
- Fanning, 1575
  - physiological mechanisms, 675
- Far field, 271
  - definition, 270
- Farmed fish *see* Cultured fish
- Fascia adherens
  - cardiomyocyte cell–cell connection, 1012, 1012*f*
  - definition, 1006
- Fast start, **587–596**, 519, 578
  - definition, 516, 587
  - future research directions, 595
  - hydrodynamics, 590, 591*f*
  - muscle activity, 589, 590*f*
  - neural control, 588
    - *see also* Mauthner cells
  - performance *see* Fast start performance
  - stage 1, 587–588
    - definition, 587
  - stage 2, 587
    - definition, 587
  - stage 3, 587
- Fast start performance, 591
  - components, 592*f*
  - factors affecting
    - body design, 592*t*, 594
    - environmental factors, 595
  - indicative levels, 592*t*
  - variables, 592*t*
  - variables, kinematic, 592*t*, 593
    - distance–time performance, 592*t*, 593–594, 594*f*
    - maneuverability, 592*t*, 593, 594*f*
- Fast-swimming oceanic species, gill morphometrics, 807*f*, 808, 808*f*
- Fasting, effects, 1513
- Fat deposits, cardiomyocytes, 1010
- Fatigue
  - definition, 1645
  - delay, 1650
  - early signs, 1650
  - *see also* Swim speed–fatigue time (SSFT) relationship
- Fatty acid(s)
  - absorption, 1315, 1316*f*
    - intraenterocytic resynthesis, 1316–1317
    - passive diffusion, 1316
    - transporting proteins *see* Fatty acid-transporting proteins
  - dietary requirements of cultured fish, 1621
  - functions, 1618, 1621
  - saturation, homeoviscous adaptation, 1729*t*, 1730
  - types, 1621
- Fatty acid-transporting proteins, 1316
  - cytosolic, 955
  - plasma membrane, 954
- Favcolae, definition, 1861
- Fear, pain and, 714–715, 717*f*, 718
- Fecal material, nondietary *see* Nondietary fecal material (NDFM)
- Feces
  - definition, 1665
  - *see also* Egestion
- Feed efficiency, definition, 1665
- Feedback
  - definition, 366
  - inner retina *see under* Inner retina
  - negative *see* Negative feedback
- Feedback transducer, definition, 1169
- Feedforward
  - cardiovascular system control *see under* Cardiovascular system control
  - definition, 1169
- Feedforward systems, definition, 1169
- Feed:gain ratio, definition, 1665
- Feeding, **597–602**
  - agnathans, 598
    - hagfish *see under* Hagfish
    - lampreys, 1781, 1783*f*, 1784
      - lamphredin role *see* Lamphredin
  - ammonia toxicity and, 1442
  - cranial muscle role
    - bony fish, 464*f*, 465*t*, 467, 467*f*
    - respiratory rhythm generation and, 861, 861*f*
    - sharks, 452*t*, 454, 455*f*
  - deep-sea fishes *see under* Deep-sea fishes
  - ecomorphology, 597, 600
  - gastrointestinal blood flow effects, 1137*t*, 1207, 1208*t*
    - *see also* Gastrointestinal blood flow (GBF), regulation during digestion
  - heat increment *see* specific dynamic action (SDA)
  - hydrodynamics *see under* Suction feeding
  - hypoxia effects, 1768
  - integration with swimming, 601
    - pectoral fin braking, 601
    - ram speed, 600*f*, 601
  - jaw mechanisms
    - evolution, 598, 598*f*
    - protrusion, 464*f*, 469, 598*f*, 600
  - jawless fish, 598
  - lateral line function, 333, 333*f*, 334*f*
  - motor control systems, 62
  - mucus *see* Mucus feeding
  - pain effects, 716, 717*f*
  - ram, 597
    - speed, 601, 1974*f*
  - rate, tuna, 1907, 1908
  - rectal gland effects, 1435, 1436*f*
  - sensory/neural food handling control, 191
  - sound signal function, 685*t*, 688
  - status, effect on appetite, 1513
  - suction *see* Suction feeding
  - *see also entries beginning food*; *entries beginning meal*; Feeding behavior; Food consumption/intake; Prey capture
- Feeding apparatus amplification, blind cavefish, 1845–1847
- Feeding behavior, 227
  - chemosensory, 227
    - avoidance and spitting of noxious/unpalatable substances, 230, 230*f*
    - historical aspects, 227
    - stimulating substances, 227
      - amino acids, 227–228, 229*f*, 230*f*
    - *see also* Gustation; Olfaction
  - growth hormone transgenic fish, 2021*f*, 2022–2023
  - lens focal length and, 107
  - *see also* Foraging behavior
- Feeding muscles
  - bony fish, 464*f*, 465*t*, 467, 467*f*
  - recruitment in respiratory rhythm generation, 861, 861*f*
  - sharks, 452*t*, 454, 455*f*
- Feeds/feedstuffs
  - bioenergetics and *see under* Bioenergetics
  - consumption *see* Food consumption/intake
- Female calling, 689
- Female fish
  - hormonal pheromones
    - postovulatory prostaglandins, 1557*f*, 1558, 1559*f*, 1559–1560
    - preovulatory steroids, 1554*f*, 1556, 1556*f*, 1559*f*
    - *see also* Hormonal pheromones
  - parental care *see* Maternal care
  - sexual behavior
    - expression of readiness, 659
    - hormonal control, 660
    - nest digging, 657–658
- Female readiness, 659
- Feminizing chemicals, 787–788

- Fenestra ovalis, 262, 264, 264*f*
- Fenestrated, definition, 1395
- Fertilization, external, 613, 614
- Fetal bovine serum (FBS), 1968
  - heat inactivation, 1968
    - definition, 1965
- Fiber-optic oxygen sensors, 847–848, 850*f*
- Fibroblast growth factors (Fgfs), 9
  - definition, 6
- Fibroblast-like cell line
  - definition, 1965
  - piscine, 1965*f*, 1967
- Fibrocartilage
  - cartilaginous fish, 430*f*, 432
  - definition, 428
- Fick equation, 953–954
- Fight responses, periaqueductal gray and, 63
- Filament *see* Gill filament
- Fin rays, 487
  - structure, 565, 567*f*
- Final thermal preferendum, 760
  - definition, 758
- Finite cell line, 1966
  - definition, 1965
- Fins
  - aspect ratio *see* Aspect ratios
  - dorsal *see* Dorsal fins
  - as hydrofoils, 524
  - paired *see* Paired fin(s)
- Fish behavior *see* Behavior, fish
- Fish blood *see* Blood
- Fish brains *see* Brain(s)
- Fish calls *see* Calls
- Fish cell lines, **1965**
  - applications, 1969
  - cryopreservation, 1969
  - culture equipment, 1967
  - culture vessels, 1966*f*, 1967
  - epithelial-like, 1965*f*, 1967
  - fibroblast-like, 1965*f*, 1967
  - growth media, 1967*f*, 1968
  - growth temperature, 1968
  - macrophage-like, 1965*f*, 1967
  - morphologies, 1965*f*, 1967
  - passaging, 1969
  - sources, 1966
- Fish densities, 520
  - lifestyle and, 521
  - measurement, 520–521, 521*f*
  - *see also* Buoyancy
- Fish eyes *see* Eye(s)
- Fish farming *see* Aquaculture/aquaculture settings
- Fish genomes
  - evolution, **1980**
    - ancestral teleost-specific genome duplication and biodiversity, 1981, 1981*f*
    - diversity of transposable elements, 1983
    - future studies, 1985
    - sex chromosomes, 1984
  - sequences, 1980
  - size, 1981
  - zebrafish, 1822
- Fish growth *see* Growth
- Fish gut *see* Gut
- Fish lung *see* Lung, fish
- Fish model(s)
  - appetite regulation, 1510
  - human diseases, **1986**
    - addiction, 1995
    - behavioral conditions, 1995
    - blood diseases *see* Blood diseases
    - cancer *see under* Cancer
    - cardiac conditions, 1991
    - chemical screening, 1989
    - forward genetic screens, 1822, 1987
    - future prospects, 1996
    - hematopoietic, 1990
    - hematopoietic stem cells, 1990
    - icefish and hemoglobin-related diseases, 992, 996
    - *in vivo* imaging, 1989, 1989*f*
    - kidney diseases, 1991*f*, 1992
    - mood disorders, 1996
    - morpholino gene knockdown, 1988
    - muscle diseases, 1993
    - neurodegenerative diseases, 1993
    - neuroscience studies, 1993
    - reverse genetics, 1822, 1987
    - skin diseases, 1994
    - sleep disorders, 1996
    - transgenesis, 1991–1992
    - transgenic *see* Transgenic models
    - transplantation, 1989
  - neuroethology, electrocommunication, 706
  - organisms, 1986*f*, 1987
  - vertebrate neurons *see under* Mauthner cells
  - zebrafish *see under* Zebrafish (*Danio rerio*)
- Fish parasites, host manipulation *see* Host manipulation
- Fish passages *see* Fishways
- Fish physiology
  - culture environments *see under* Aquaculture/aquaculture settings
  - fish behavior and *see* Behavior–physiology interrelations
  - insights from global gene expression data *see under* Global gene expression profiling
  - *see also specific organs/systems*; *specific species*
- Fish reproduction *see* Reproduction
- Fish robots *see* Robotic fish
- Fish skin *see* Integument
- Fisheries conservation
  - implications of swimming performance, 1655–1656
    - swim speed–fatigue time relationship, 1656*f*, 1657*f*, 1660
    - *see also* Swimming performance, applied aspects
  - threats, 1653
    - *see also* Anthropogenic influences/disruptions; *specific threats*
- Fisheries management
  - bioenergetics model application, 1677
  - sound production as tool, 319
- Fishways
  - development/design
    - for Atlantic salmon *vs.* American shad, 1658*f*
    - historical aspects, 1652*f*, 1654*f*, 1656–1657
    - problems, 1658*f*, 1662
  - Pacific salmon, 1950*f*, 1951
- Fitness
  - definition, 777, 1695
  - parasite, 778
    - *see also* Host manipulation
- Fitness (Darwinian)
  - definition, 1588, 1624
  - energy allocation consequences, 1624*f*, 1625
- Fixed action pattern, definition, 387
- Flagellin, 2037
- Flagellum, choanoflagellates, 7
- Flaps, skin, 488
- Flash kinetics, 498
- Flashlight fish, bioluminescence, 499
- Flatfish (Pleuronectiformes)
  - skin patterns, 495*f*, 741
  - specific dynamic action, 1609*t*, 1611*f*, 1614
- Flatworms, 7
- Flight responses, periaqueductal gray and, 63
- Flow fields, interpretation *see under* Experimental hydrodynamics
- Flow-through respirometers, 848–849, 850, 850*f*
- Flow tunnel
  - definition, 535
  - particle image velocimetry configuration, 539*f*
- Flow velocity, foraging activity and, 1591
- Fluid(s)
  - exchange, physiology, **1111–1118**
    - *see also* Venous capacitance; Venous system
  - properties at rest, 978
    - *see also* Hydraulic pressure; Hydrostatic pressure; Oncotic pressure; Osmotic pressure
  - solids *vs.*, 536, 536*f*
  - velocity measurements *see under* Experimental hydrodynamics
- Fluid compartments
  - basic, 1155
  - fish, 1155
  - interstitial–intravascular relationship *see* Interstitial–intravascular fluid compartments
  - mammals, 1155
- Fluid dynamics
  - basic principles, 536
    - added mass *see* Added mass (acceleration reaction)
    - viscosity *see* Viscosity
    - vorticity *see* Vorticity
  - symbols, 537*t*
  - *see also* Hemodynamics; Hydrodynamics
- Fluvial, definition, 1921
- Fluvial habitats, orientation/navigation mechanisms, 1924
- Follicle cells, mutual regulation of oocyte, 1501
- Follicle-stimulating hormone (FSH), 1504
  - definition, 1500
  - regulation of testicular function, 628, 628*f*
  - sturgeon, 1802
  - *see also* Gonadotropin(s)
- Follicular growth, definition, 635
- Food acquisition
  - need for, and associated demands, 744
  - survival guide *see* Survival strategies (food acquisition-associated)
  - *see also entries beginning foraging*;Feeding; Prey capture
- Food-anticipatory activity, circadian rhythmicity, 738–739
- Food availability, predation risk and, foraging decisions, 1591
- Food consumption/intake
  - *ad libitum see Ad libitum* food consumption
  - assessment, 1511, 1671
  - body mass and, 1601*f*, 1603–1604
  - brain and environmental signal interaction, 1484
  - cultured fish *see under* Cultured fish
  - definition, 1597
  - dietary efficiency and, 1598*f*, 1603
  - endocrinology, **1483–1489**
    - *see also* Appetite regulation; Appetite suppression
  - environmental influences, 1486, 1513
    - assessment, bioenergetics model application, 1678
    - water temperature, 1601*f*, 1603–1604
  - growth hormone transgenic fish, 2020
  - growth relationship, 1484, 1484*f*, 1632*f*, 1633
  - need for, 744
  - regulation *see* Appetite regulation
  - simple energy budget, 1675*f*, 1676
  - thermal effects *see* specific dynamic action (SDA)
  - tuna, 1907, 1908
  - *see also* Digestion; *entries beginning meal*
- Food quality, growth rate relationship, 1633
- Foraging behavior, 1585
  - decision-making *see* Foraging decisions (energetics)
  - definition, 1588
  - evolutionary stable strategy, 1586
  - stages, 1585*f*, 1586
- Foraging competition, strategies for dealing with, 747*f*, 748
- Foraging cycle, 1588*f*, 1589
- Foraging decisions (energetics), **1588**
  - how to forage? (searching for food), 1592
    - foraging mode-switching, 1593
    - search path, 1592
    - search speed, 1591*f*, 1592, 1593*f*
  - what to forage? (prey selection), 1593
    - ontogenic diet shifts, 1594

- prey type/size, 1593
  - when to forage? (motivation to feed), 1589
    - external factors, 1590
      - hypoxia, 1590
      - predation threat, 1590
      - temperature, 1590
    - internal factors, 1589
      - hunger, 1589
      - metabolic rate and aerobic scope, 1589
  - where to forage? (habitat selection), 1591
    - flow velocity, 1591
    - food availability and predation risk, 1591
    - territoriality and aggression, 1591
  - *see also* Survival strategies (food acquisition-associated)
- Foraging mode-switching, 1593
- Foraging models, 745
  - diet model, 745
  - IFD (ideal free distribution) model, 746, 747*f*
  - patch model, 745, 746*f*
- Foraging theory, definition, 744
- Force estimation, fluids, 542
  - choosing vortex or momentum analyses, 543, 544*f*
  - momentum analysis, 543, 544*f*
  - vortex analysis, 538*f*, 543
- Forced choice, two-alternative *see* Two-alternative forced choice
- Forced convection, 836–837
  - definition, 829
- Force–frequency relationship, definition, 1045
- Forebrain
  - acoustic/lateral line *see under* Auditory/lateral line CNS, anatomy
  - agnathan *see under* Agnathan brain, functional morphology
  - cartilaginous fish *see* Cartilaginous fish
  - definition, 46
  - *Latimeria*, 47, 53*f*, 54*f*, 54–55
  - lungfishes *see under* Lungfishes
  - ray-finned fish *see under* Ray-finned fish, brains
  - *see also* Hypothalamus; Preoptic area; Telencephalon
- Foregut
  - anatomy, 1269, 1269*f*
  - *see also* Esophagus; Stomach
- Forkhead genes (*Foxg1*), 13–14
  - definition, 6
- Form drag, definition, 1887
- Forward genetic screens, 1822, 1987
- Forward genetics
  - definition, 1819
  - zebrafish, 1822
- Fovea
  - definition, 375
  - *Gnathonemus petersii* (elephantnose fish), 378, 380*f*
- Foveation, active electrolocation, 378, 380*f*
- foxl2/*foxl2* gene, sex differentiation role, 1497, 2029*t*, 2031
- Fractional float volume, 521, 521*t*
- Frank–Starling relationship, 1061, 1061*f*
  - importance in fish hearts, 1061, 1061*f*
- Free field, 271
  - definition, 270
- Free sarcoplasmic reticulum (fSR)
  - cardiomyocytes, 1011, 1011*f*
  - definition, 1006
- Freerun, definition, 736
- Freerunning period, circadian rhythms *see under* Circadian rhythms
- Freeze-fracture, definition, 1395
- Freeze-fracture electron microscopy, definition, 1381
- Freezing, resistance, cultured fish, transgenesis, 2006
- Freezing responses, periaqueductal gray and, 63
- Frequency, definition, 270, 311
- Frequency modulation
  - definition, 366
  - electric organ discharge, 366–367
    - *see also* Time coding
- Freshwater
  - density, 521
  - eel migration, 1938*f*, 1941
  - ionic composition, 1349, 1360*t*, 1361, 1420*t*
  - partial pressure of oxygen ($P\text{O}_2$), 792, 792*f*
- Freshwater elasmobranchs, 1810
- Freshwater fish
  - acclimation/adaptation
    - cortisol role, 1469
    - effect on venous capacitance, 1115, 1116*f*
  - gill ion regulation *see* Freshwater fish, gill ion regulation
  - gill respiratory morphometrics, 808*f*, 809
  - gut ion regulation *see under* Gut ion/acid–base regulation
  - marine species *vs.*
    - mitochondrion-rich cells, 1354–1355, 1355*f*
    - parental care, 672–673
  - osmoregulation *see under* Osmoregulation
  - osmotic challenges, 1359, 1360*t*, 1361, 1367, 1425–1467
  - plasma, ionic composition/concentrations, 1349*t*
  - *see also individual species*
- Freshwater fish, gill ion regulation, **1359–1365**, 1467*f*, 1467–1468, 1950–1951
  - functional regulation to cope with environmental fluctuations, 1364
  - lampreys, 1795*f*, 1796
  - mechanisms, 1360, 1360*f*, 1361*f*
    - Ca^{2+} uptake, 1361*f*, 1363
    - Cl$^-$ uptake/base secretion, 1361*f*, 1362
    - Na$^+$ uptake/acid secretion, 1361, 1361*f*, 1362*f*
    - *see also* Mitochondrion-rich cells (MRCs), freshwater fish
  - *see also* Osmoregulation, hormonal control
- Friagem
  - definition, 765
  - *Hoplosternum* and, 768–769
- Frictional drag, definition, 1887
- Fright reaction, 231–232
  - *see also* Alarm substance
- Fry, Pacific salmon, feeding ground migration, 1947
- Fry paradigm, 1641*f*, 1642
  - limiting factors, 1641*f*, 1642
  - loading factors, 1641*f*, 1642
  - temperature, 1641*f*, 1642
- Fuel delivery, tissues, 953, 957
- Fuel selection, mitochondrial respiration, 964
- Functional anatomy, fish, **416–418**
  - current knowledge, 417
    - muscle, 417
    - skeleton, 417
    - skin, 417
  - historical perspective, 416
  - *see also specific anatomical structures*
- Functional differentiation, fish hemoglobins *see under* Hemoglobin(s), fish
- *Fundulus heteroclitus* (common killifish) *see under* Killifish
- Fusiform, definition, 1819

G

- G-protein, definition, 98, 166
- G-protein coupled receptors (GPCRs)
  - definition, 89
  - olfactory *see* Olfactory receptor(s) (ORs)
- GABA (γ-aminobutyric acid)
  - definition, 398, 1824
  - role in metabolic depression in anoxic crucian carp, 1828*f*, 1829
- GABAergic, definition, 73
- GABAergic system, 63
  - agnathan, 22*f*, 24*f*
  - cartilaginous fish, 32
  - ray-finned fishes, 42, 43
  - sleep in fish and, 775
- Gadiformes, specific dynamic action, 1609*t*, 1611*f*, 1612
- *Gadus morhua see* Cod, Atlantic
- Gain, definition, 1169
- Gain-of-function studies (reverse genetics), 1823
- Gait, definition, 575
- Gait transitions
  - definition, 575
  - pectoral fins, 566
- Galanin, 1336–1337
  - amino acid sequence, 1336*f*
  - appetite stimulation, 1484, 1485*f*, 1512, 1512*t*
- Gallbladder
  - bile storage, 1303, 1304*f*
  - contraction, cholecystokinin role, 1344, 1344*f*
- Ganglion, 80
  - definition, 80, 1332
- Ganglion cells, retinal *see* Retinal ganglion cells
- Ganoid scales
  - definition, 476
  - morphology, 484–486, 485*f*, 486*f*
- Gap junction
  - cardiomyocyte cell–cell connections, 1012, 1012*f*
  - definition, 123, 1006
  - horizontal cells, 124
- Gars
  - circulatory patterns, 1876*f*, 1881*f*, 1882
  - longnose *(Lepisosteus osseus)*, brain, 38*f*, 40*f*, 43–44
    - *see also* Ray-finned fish, brains
  - *see also* Ray-finned fish
- Gas bladder *see* Swimbladder
- Gas-equilibrium curve, blood
  - nonlinear nature, effect on gas exchange efficiency, 826, 827*f*
  - *see also* Oxygen equilibrium curves (OECs)
- Gas exchange *see entries beginning gas exchange*; Gas transport and exchange; Respiration; Ventilation
- Gas exchange during development (respiratory transitions), 814, **838–845**
  - diffusive boundary layer and, 832*f*, 832–833, 839, 840*f*
  - egg capsule and perivitelline fluid, 832, 840, 841*f*, 1575
  - gills, 843, 843*f*, 844*f*
    - body size and, 1578, 1578*f*, 1580
    - diffusing capacity, 814–816, 818
    - ram ventilation, 814, 1909
  - skin, 841, 842*f*, 844*f*
- Gas exchange during development, models and mechanisms, **829–837**
  - convection, 836, 836*f*
  - diffusion, 833
    - ammonia, 835
    - in aqueous media, 833, 833*t*
    - carbon dioxide, 834
    - oxygen, 834
    - through membranes, 834
    - time considerations, 836
  - resistance, 837, 837*f*, 839
  - respiratory cascade, 831, 831*f*
  - respiratory gases, 830
    - properties in aqueous solutions, 830
- Gas exchange organs, 793, 820
  - efficiency *see* Gas exchange organs, efficiency
  - function, 820
  - gas transport system, 820, 821*f*
  - vertebrate models, 820, 821*f*
  - *see also* Air-breathing organs (ABOs); Gill(s)
- Gas exchange organs, efficiency, 823
  - ideal countercurrent exchanger, 823
    - calculation, 824, 824*t*, 825*f*
    - effect of nonlinear nature of blood-gas-equilibrium curve, 826, 827*f*
    - effect of parallel inhomogeneities, 827
    - effects of pulsatile flow, 827
    - efficiency *vs.* total exchange, 825, 826*f*
    - graphical representation, 824, 824*f*
- Gas exchanger, 1120
  - definition, 829
  - *see also* Gas exchange organs

Gas gland cells (swimbladder)
  glucose metabolism, 528, 529f
  nervous system control, 532f, 533
  proton translocation, 529, 530f
  release of carbon dioxide, 528, 529
  structure, 528, 528f
Gas partial pressure in liquid, definition, 526, 820
Gas secretion, swimbladder *see* Swimbladder, gas secretion
Gas solubility, 830
Gas transport and exchange, 793, 820, 821f
  blood acidification effects, 528, 529
  carbon dioxide *see* Carbon dioxide (CO_2) transport/excretion
  carbonic anhydrase *see* Carbonic anhydrase (CA)
  during development *see* Gas exchange during development
  diffusion and resorption from swimbladder, 531, 532
  efficiency and capillary tortuosity, 1145, 1146f
  hemoglobin *see* Hemoglobin(s), fish
  oxygen *see* Oxygen transport/exchange
  oxygen–carbon dioxide interaction *see* Oxygen–carbon dioxide interaction
  red blood cell function *see under* Red blood cells (RBCs), function
  salting-out effect, 529–531, 531f
  triploid fish, 2011
  see also Bohr effect; Diffusion; Gas exchange organs; Retia mirabilia
Gases in solution, Root effect, 930, 930f
Gasotransmitters
  catecholamine release role, 1531
  vascular resistance control, 1107
  see also Hydrogen sulfide (H_2S); Nitric oxide
Gasterosteus aculeatus *see* Three-spined stickleback
Gastric, definition, 1597
Gastric acid secretion
  elasmobranchs, 1813
  role of gut hormones, 1343, 1343f
Gastric emptying rate, 1299
Gastrin, 1342
  gastric acid secretion role, 1343, 1343f
  gastrointestinal blood flow regulation, 1212
Gastrin-releasing peptide (GRP), 1337
  amino acid sequence, 1337f
  appetite suppression, 1512t, 1513
Gastroenteropancreatic (GEP) cells, definition, 1229, 1276
Gastroenteropancreatic (GEP) system, 1277–1278, 1279f
Gastrointestinal artery, 1090–1091, 1133–1134, 1134f, 1135f, 1207
  see also Gastrointestinal circulation
Gastrointestinal blood flow (GBF)
  factors affecting
    exercise, 817, 1138, 1139f, 1212–1213, 1213f
    feeding, 1137t, 1207, 1208t
      see also Gastrointestinal blood flow (GBF), regulation during digestion
    hypercapnia, 1139, 1140f, 1804
    hypoxia, 1139, 1139f
    stress, 1138, 1138f
    temperature, 1140, 1140f, 1212–1213, 1213f
    in unfed animals, 1136
  regulation, under routine conditions, 1136
  regulation during digestion, 1207
    different ways to increase GBF
      blood redistribution, 1209
      cardiac output, 1207, 1209f
    different ways to sense/coordinate blood flow (with food), 1209, 1209f
      chemical stimuli, 1210
      mechanical stimuli, 1209, 1210f
    exercise effects, 1212–1213, 1213f
    integration of mechanical/chemical stimuli and signal relay to vessels, 1211
      extrinsic signals and chemical stimuli, 1211
      extrinsic signals and mechanical stimuli, 1211
      intrinsic signals and chemical stimuli, 1212
    temperature effects, 1212–1213, 1213f
  sturgeons, 1804
  see also Gastrointestinal circulation
Gastrointestinal circulation, **1132–1141**, 975
  liver, 1134
  pancreas, 1136
  see also Gastrointestinal blood flow (GBF)
Gastrointestinal tract *see* Gut
Gastrulation, 1285, 1819, 1821
  definition, 838, 1284, 1819
GATA family (genes), in erythropoiesis *see* Erythropoiesis
Gaze stabilization
  definition, 244
  vestibular connections, 250
Gelatinous tissues, deep-sea fishes, 1957
Gender-specific differences
  appetite regulation, 1513
  see also Female fish; Male fish
Gene(s)
  circadian clock, 741
  conservation of regulatory ability (biochemical adaptations), 1686
  definition, 1998
  introduction, fish transgenesis, 2001
  isolation, fish transgenesis, 2001
  structure, 2000f, 2001
  see also specific genes
Gene construct
  definition, 2016
  generation, growth hormone transgenes, 2016f, 2017
Gene dosage, definition, 2009
Gene duplication, 967, 968f
  opsin genes in shallow-living species, 118, 119f, 120f
  teleost-specific genome duplication, 1981, 1981f
Gene expression
  definition, 1998
  effect of growth hormone transgenesis, 2018f, 2019
  global, profiling *see* Global gene expression profiling
  levels, definition, 1509
  transgenes, 2002f, 2003
Gene incorporation/integration
  definition, 1998
  fish transgenesis, 2002
Gene knockdown
  definition, 1971
  morpholino *see* Morpholinos
Gene manipulation
  definition, 1998
  see also Transgenesis, fish
Gene regulation, conservation of optimal values (biochemical adaptations), 1686
Gene regulation studies, transgenic fish, 2003f, 2004f, 2005
  see also Transgenic models
Gene transmission, transgenes, 2003f, 2004
General viability, growth hormone transgenic fish, 2018f, 2020
Generator potential
  definition, 2078
  olfactory sensory neurons, 2078–2080
    recordings *see* Electro-olfactogram (EOG)
Genetic engineering
  definition, 1998
  fish
    history, 2016
    see also Transgenesis, fish
Genetic rescue, blind cavefish eyes, 1847
Genetic screens
  forward, 1822, 1987
  reverse *see* Reverse genetics
Genetically modified (GM) organisms, 2001
  fish *see* Transgenic fish
Genetics
  comparative genomics, erythroid genes, 996
  definition, 1986
  embryonic to adult globin switch, 994–995
  GATA gene family (red blood cell maturation), 995
  influence on appetite regulation, 1513
  sex determination *see* Sex determination
  triploid fish, 2009
  zebrafish model, 1821
    genome sequencing project, 1822
    see also Reverse genetics
  see also entries beginning gene
Genome
  definition, 1998, 2032
  fish *see* Fish genomes
Genome duplication, 967, 968f
  see also Gene duplication
Genomic analysis
  stress response, 1550
  temperature effects on mitochondria, 1715, 1715f
Genotype, definition, 1998
Genotypic sex, definition, 1490
Geographical population variation, heat shock response, 1736
Geomagnetic orientation, 1923
Germ cell(s), 2047
  autonomous regulation of spermatogenesis, 632, 634
  definition, 2047
  migration, **2046**
    see also Primordial germ cells (PGCs), migration
  primordial *see* Primordial germ cells (PGCs)
Germ layers, definition, 1284
Germ line transmission, definition, 1998
Germline, definition, 1986
Gestation
  definition, 678
  internal *see* Internal gestation
Ghrelin, 1484
  amino acid sequences, 1345, 1345f
  effects, 1345, 1346f
    appetite regulation, 1345, 1346f, 1484, 1485f, 1512t, 1513
    growth hormone secretion, 1345, 1346f
Gibb's free-energy change, 1646, 1648–1649, 1650
  definition, 1645
Gill(s), 793, **1381–1388**
  aerial respiration role, 1872
    related modifications, 1871f, 1872
  anatomy/morphology, **1095–1103**, 804, 805f, 806f
    filaments *see* Gill filament
    interlamellar space (ILS), 1381, 1384f
    lamellae *see* Lamellae, gill
    quantification *see* Gill respiratory morphometrics
    see also specific structures, cell types
  blood flow pattern, 816
  carbon dioxide excretion, 914
  carbonic anhydrase, 900f, 904, 905, 906f
  chemoreceptors
    carbon dioxide, 866, 867f
    oxygen *see* Oxygen chemoreceptors, branchial
  countercurrent arrangement *see* Countercurrent flow
  development, 843, 843f, 1095–1096
    functional, 843, 844f
    see also Gas exchange during development (respiratory transitions)
  diffusion capacity, 806
  hagfish *see under* Hagfish
  hagfish predators, effects of slime, 513–515, 515f
    water flow and gill resistance, 514f
  hormone inactivation, 1192, 1193f
  ion transport/regulation, 1467, 1467f
    freshwater fish *see* Freshwater fish, gill ion regulation
    seawater fish *see* Mitochondrion-rich cells (MRCs), seawater fish

  ionocytes *see* Gill ionocytes
  lamellae *see* Lamellae, gill
  morphological plasticity *see* Gill remodeling
  nerves, 1097*f*, 1098, 1102
  neuroepithelial cells (NECs) *see* Neuroepithelial cells (NECs), gill
  nonvascular aspects, **1381–1388**
    see also Mitochondrion-rich cells (MRCs); Pavement cells (PVCs)
  osmorespiratory compromise *see* Osmorespiratory compromise
  pillar cells *see* Pillar cells
  remodeling *see* Gill remodeling
  resistance, 807
    definition, 803
  salt secretion mechanisms *see* Mitochondrion-rich cells (MRCs), seawater fish
  surface area *see* Gill surface area
  triploid fish, deformities and compensation, 2013
  urea excretion, 1446
  ventilation *see* Ventilation
  vessels *see* Gill vessels
  water transport, 1369
  xenobiotic uptake and excretion, 2071–2074
  see also entries beginning branchial;Gas exchange organs; Respiration
Gill arch
  anatomy, 1095, 1096*f*, 1097*f*
    dorsal portion, 440–442, 441*f*
    ventral portion, 440, 441*f*
  definition, 803
  filaments *see* Gill filament
  innervation, 1102
  morphology, 804, 805*f*
Gill epithelium, 1097*f*, 1098, 1099*f*, 1100*f*
  mitochondria-rich cells *see* Mitochondrion-rich cells (MRCs)
Gill filament
  anatomy, 804, 805*f*, 1097*f*, 1098, 1384*f*
    mechanical support, 1098
    vascular system, 1097*f*, 1099*f*, 1100*f*, 1101*f*, 1102
  definition, 803, 1095
  see also Lamellae, gill
Gill ionocytes, **1381–1388**
  definition, 796, 1381
  historical work, 1381–1382
  identification methods, 1387
  mitochondrion-rich (chloride) cells *see* Mitochondrion-rich cells (MRCs)
  pavement cells *see* Pavement cells (PVCs)
  regulatory function discovery, 1381
Gill pouches, 1095–1096
  definition, 1788
  hagfish, 1774*f*, 1775
  lamprey, 1790*f*, 1791–1792
Gill rakers, 1096, 1096*f*, 1100–1101
  definition, 1095
  nutrient supply, 1101–1102
Gill remodeling, **796–802**, 1393
  crucian carp and goldfish, 796–797, 797*f*, 1393–1394
    mechanisms, 797, 798*f*
    reasons for, 798
    reversibility, 797, 797*f*
  species other than crucian carp and goldfish, 799
    Arapaima gigas, 802, 1393*f*, 1393–1394
    brook trout, 801
    common carp, 799–800, 800*f*
    cyprinids, 799–800, 800*f*
    European eel, 801, 801*f*
    largemouth bass, 801
    mangrove killifish, 800–801, 801*f*
    rainbow trout, 801
Gill respiratory morphometrics, **803–811**
  definition, 803
  diffusion distances, 806
  fish habitat/metabolic requirements and, 807, 808*f*
    air breathers, 808*f*, 809
    fast-swimming oceanic species, 807*f*, 808, 808*f*
    freshwater fishes, 808*f*, 809
    hypoxia dwellers, 810, 810*f*
    marine fishes of intermediate activity, 808*f*, 809
    sluggish marine species, 808*f*, 809
  in relation to fish growth (scaling), 808*f*, 810
  theories on gill dimensions, 807, 807*f*
Gill surface area
  adjustments
    osmorespiratory compromise role
      exercising fish, 1389
      seawater, 1391
    see also Gill remodeling
  large, disadvantages, 799
  metabolic rate and, 816, 1579*f*, 1580–1581, 1909
  total, 804, 806*f*
    body mass relationship, 808*f*
    definition, 803, 804
    determination, 804, 806*f*
      see also Gill respiratory morphometrics
Gill vessels, 1101
  gill arch, 1101, 1101*f*
  gill filaments, 1097*f*, 1099*f*, 1100*f*, 1101*f*, 1102
  secondary *see under* Secondary vessels
Ginglymodi, brain structure, 38*f*
Gland mucus cell
  definition, 504
  see also under Slime glands (hagfish)
Gland thread cells (GTCs) *see under* Slime glands (hagfish)
Glass eels, 1940
  migration, 1938*f*, 1940–1941
  visual pigments, 120–121, 121*f*
Glial cells, definition, 1332
Global climate models (GCM), 1744
  bioclimatic survival envelope, 1744
  complex multi-component, 1745
  simple recruitment–temperature models, 1744
Global gene expression profiling, **2055**
  applications, 2055
  methods, 2056*f*, 2057
  physiological insights into environmental acclimation/adaptation, 2058
    oxygen availability, 2060
    temperature, 2058*f*, 2059
  sources of data variation, 2057
    individual differences, 2058
Global warming *see* Climate change
Globin(s)
  chains, hemoglobin, 887
  phylogenetic tree, 945, 945*f*
  see also specific globins
Globular calcified cartilage
  cartilaginous fish, 431
  definition, 428
Glomerular capsule
  definition, 1771
  hagfish, 1773
  structure, 1396*f*, 1398*f*, 1400, 1402*f*
Glomerular endothelium, 1400, 1400*f*, 1401–1402, 1402*f*, 1403*f*
Glomerular kidneys
  structure, 1412, 1413*f*, 1414*f*
    see also individual species
  urine formation *see under* Kidney(s)
Glomerular layer, olfactory bulb, 204
Glomerular ultrafiltration, 1412, 1413*f*
  definition, 1411
Glomerulus
  definition, 1348, 1444
  structure/histology
    opisthonephros *see under* Opisthonephros
    pronephros (external glomerulus), 1397, 1397*f*, 1398*f*, 1399
Glomus, 1397
  definition, 1788
  opisthonephros, 1404–1406, 1409*f*
  pronephros, 1399*f*, 1400*f*, 1409*f*
Glowfish, 2005*f*, 2006
Glucagon, physiological actions, 1280, 1280*t*
Glucagon-like peptide-1 (GLP-1), 1280, 1280*t*
  effects, 1345
Glucagon-like peptide-2 (GLP-2), 1345
Glucocorticoid(s), 1474, 1537
  corticosterone *see* Corticosterone
  cortisol *see* Cortisol
  cortisone *see* Cortisone
  definition, 1541
  see also Corticosteroid(s)
Glucocorticoid receptors (GRs), 1451*f*, 1452, 1476, 1518
  activation, 1478, 1478*f*
  definition, 1450
  embryogenesis, 1455, 1455*f*
  evolution, 1480, 1481*f*
  molecular biology, 1476, 1477*f*
  tissue expression, 1479
Glucocorticoid response element (GRE), 1478, 1478*f*
  definition, 1474
Gluconeogenesis
  definition, 1534
  stress effects
    catecholamine-mediated, 1536, 1536*f*
    cortisol-mediated, 1538
Glucose
  renal reabsorption, 1415, 1415
  transporters, 954, 1317, 1317*f*
    see also specific transporters
  tubular transport maximum, trout kidney, 1415
Glucosuria, 1415
  definition, 1411
GLUT1, 954
GLUT2, 954, 1317, 1317*f*
GLUT4, 954
GLUT5, 1317, 1317*f*
Glutamate dehydrogenase (GDH), 1811
Glutamatergic, definition, 56
Glutamine synthase (GS)
  elasmobranchs, 1811
  urea cycle, 1445, 1445*f*
Glutamine synthesis, ammonia toxicity-associated, 1440, 1440*f*
Glycinergic, definition, 66, 73
Glycogen
  clusters, cardiomyocytes, 1010
  stores, crucian carp, 1762, 1825*f*, 1828
Glycogenolysis
  definition, 1534
  stress effects
    catecholamine-mediated, 1536
    cortisol-mediated, 1538
Glycolipids
  cell membrane, 1727
  definition, 1725
Glycolysis, 1759*f*, 1760
  role in extension of energetic limits, 1649*f*, 1650
  see also Anaerobic metabolism; Pyruvate
Glycoprotein, definition, 2032
Glycosaminoglycans, definition, 1015
Gnathonemus petersii (elephantnose fish)
  active electrolocation, 375
    behavioral performance during object detection/analysis, 377–378, 378*f*, 379*f*
    EOD modulation/electric imaging, 375, 376*f*, 377*f*
    foveation, 378, 380*f*
    prereceptor mechanisms, 376
    waveform encoding, 380, 382*f*
  Schnauzenorgan, 377, 378, 380*f*, 383*f*
Gnathostomes
  brains, **1–5**
    historical perspective, 3
    organization, 2, 3*f*, 4*f*
    structural variations across taxa, 4
  definition, 26
  see also Bony fish; Cartilaginous fish; *individual species*

Goal-directed locomotion, motor control systems *see under* Motor control systems
Goatfish (*Parupeneus* spp.), sensory barbels, 187, 192
Goblet (mucous) cells
  definition, 476, 1229, 1322
  gill, 1097f, 1099, 1100f
  integumentary, 477f, 481, 481f, 482f, 483f
  intestinal, 1270–1271, 1302f, 1323, 1325f, 1326f, 1327f, 1328f
  see also Mucus
Goldfish (*Carassius auratus*)
  anoxia responses/tolerance, 1825
    ethanol production, 1827
    metabolic depression, 1828, 1829
  brain structure, 708f
  erythrophoroma, 1977
  feeding behavior, 230f
    amino acids and, 228–230, 229f
    gravel pecking, 228f, 228–230
    spitting of noxious/unpalatable substances, 230, 230f
  gill remodeling *see under* Gill remodeling
  hearing
    auditory scene analysis ability, 281–282
    critical masking ratio, 280f, 280–281
    frequency discrimination, 281
    hearing threshold audiogram, 277, 277f
  noxious stimulus avoidance, 709, 709f, 714–715, 715f
  olfactory receptor(s), 217
    neurons, 214–215
  olfactory responses
    cadaverine, 214
    olfactory bulb, 215
    prostaglandins, 212, 213, 213f
    putrescine, 214
    steroid hormones, 212, 212f, 213
  pain response, 715
  pheromones, reproductive, 231, 231f
    hormonal *see under* Hormonal pheromones
  taste
    palatal organ and taste buds, 187, 191–192
    vagal lobe structure, 190f, 191–192, 193f
Gonadal peptides, gonadotropin regulation, 1507
Gonadal sex differentiation *see* Sex differentiation
Gonadal steroid(s), 1502
  definition, 627, 656
  effect on electrocommunication systems, 705
  formation, 1502, 1504f
    steroidogenic shift (estradiol to progestogens), 1503, 1504f
  gonadotropin regulation, 1507
  olfactory responses, 212, 212f
  receptors, sex differentiation role, 1496
  sex change role, 2053
  sex differentiation role, 1494
  sexual behavior role *see under* Sexual behavior
  spermatogenesis regulation *see under* Spermatogenesis, regulation
  stress effects, 1544f, 1546, 1547
    see also Reproduction, stress effects
  stress hormone interaction, 1547
  sturgeon, 1802
  synthesis *see under* Steroidogenesis
  see also specific gonadal steroids
Gonadotropin(s), 1504
  definition, 627, 1541, 1553
  receptors, 1507
  recombinant, 1504
  regulation of synthesis/secretion, 1505
    dopaminergic inhibition, 1505, 1505f
    gonadal peptides, 1507
    gonadal steroids, 1507
    gonadotropin-releasing hormone *see* Gonadotropin-releasing hormone (GnRH)
    kisspeptin, 1505f, 1506
  regulation of testis functions, 627, 628f
  unorthodox sites, 1507
  see also specific gonadotropins
Gonadotropin-releasing hormone (GnRH), 1501f, 1505, 1505f
  appetite regulation role, 1513
  definition, 662, 1500
  expression sites, 1505
    unorthodox, 1507
  sexual behavior role, 660, 667–668
  stress effects on release, 1544f, 1546–1547
  sturgeon, 1802
Gonads
  definition, 1541
  see also entries beginning ovarian; *entries beginning testes/testicular*
Gonochorism, 613–614, 1491, 1491t
  definition, 613, 662
Gonochoristic species, gonadal development, 1492, 1492f
  histology, 1493f
  see also Sex differentiation
Gouramis
  croaking, sound production, 322, 322f, 325
  hearing, auxiliary anatomy, 274
Gradient-index lenses, 104, 104f
  see also Lens
Granular cells, lamprey epidermis, 478f, 483–484
Granule cell layer, olfactory bulb, 204
Granule cells, electrosensory lateral line lobe, latency code to rate code transformation, 381
Granulocytes, 989, 990, 991
  definition, 984, 1541
  inter-species variability, 990f, 991
Granulosa cells, 1501–1502
  gonadotropin receptors, 1507
  steroidogenic shift, 1503
Gravel pecking, goldfish, 228f, 228–230
Gravitoinertial function, 250
  definition, 244
Great Lakes (North America), sea lamprey invasion, 1785
Green fluorescent protein (GFP)
  in cardiac function studies, 1991–1992
  definition, 1998, 2046
  germ cell visualization, 2047f, 2048
  use in fish transgenesis, 2003, 2006
Grey gurnards (*Eutrigla gurnardus*), aggressive sounds, 314, 314f
Ground effect, 523–524
  definition, 520
Group surfacing, 768
Growl, plainfin midshipman vocalization, 322, 323f
Growth
  bioenergetic models, 1626, 1678
    Davis and Warren model, 1623f, 1626
    dynamic energy budget model, 1627, 1627f
    future perspectives, 1628
    von Bertalanffy model, 1624f, 1626
    Winberg model, 1626
    Wisconsin model, 1627
  bioenergetics, **1623**
    compound feed evaluation (growth trials), 1668t, 1669t, 1670, 1673
    energy allocation and its fitness consequences, 1624f, 1625
    energy budget, 1625, 1675f, 1676
    growth efficiencies, 1625
    models *see* Growth, bioenergetic models
  compact myocardium percentage and, 1078, 1079f
  compensatory *see* Compensatory growth
  components, 1623f, 1625
  definition, 1624
  development and *see* Ontogeny
  endocrinology *see* Growth-regulating hormones
  environmental influences *see* Growth, environmental effects
  food intake and, 1484, 1484f
  gill morphometrics and (scaling), 808f, 810
  growth hormone transgene effects *see under* Growth hormone transgenesis
  hair cell number and, 260
  inhibition, cortisol, 1519, 1539
  lampreys, 1785
  patterns, 1486
  rate *see* Growth rate
  reproduction component, 1623f, 1625
  storage growth, 1623f, 1625
  stress effects, **1534**
    catecholamine-mediated, 1537
    cortisol, 1519
    cortisol-mediated, 1539
  structural, 1623f, 1625
  sturgeons, 1803
  triploid fish, 2014
  see also Scaling, body mass
Growth, environmental effects, 1488, **1629**
  abiotic factors, 1630
    ammonia/ammonium levels, 1632
    current speed, 1632
    interactions of abiotic factors, 1633
    low pH levels, 1632
    oxygen, 1630f, 1631
    photoperiod, 1489, 1632
    salinity, 1489, 1630f, 1631
    temperature, 1489, 1629f, 1630, 1744
  assessment using bioenergetics modeling, 1678
  biotic factors, 1633
    food, 1633
      quality, 1633
      quantity, 1484, 1484f, 1632f, 1633
    social interactions *see under* Social interactions
  compensatory growth, 1634
Growth factors
  sex differentiation role, 1497
  see also specific growth factors
Growth hormone (GH), 2017
  action, 1486–1487, 1487f, 2017
    appetite effects, 1512
  control of production/release, 1487, 1487f, 2017
    environmental influences, 1489
    ghrelin role, 1345, 1346f
    peripheral, 1488
  definition, 1466
  distribution, 1486–1487, 1487f
  ion regulation role, 1467f, 1470
  overexpression in transgenic fish *see* Growth hormone transgenesis
Growth hormone transgenesis, **2016**
  aquaculture application, 2004f, 2006, 2022f, 2023
    environmental risk assessment, 2022f, 2023
  behavioral effects, 2021f, 2022
  endocrine effects, 2018
  energetic effects, 2020
    exercise, 2020
    nutritional requirements, 2020
    oxygen uptake, 2020
  gene construct generation, 2016f, 2017
  gene expression effects, 2018f, 2019
  growth effects, 2017, 2017f
    rearing conditions and, 2022f, 2024
  immune effects, 2020
  phenotypic effects, 2018f, 2019
    abnormalities, 2018f, 2019
  transgene expression, 2018
  viability and, 2018f, 2020
Growth hormone–insulin-like growth factor system *see under* Growth-regulating hormones
Growth media, fish cell lines, 1967f, 1968
Growth rate, 1630
  environmental effects *see* Growth, environmental effects
  social status and, 651
  triploid fish, 2014
  tuna family, 1909

- Growth-regulating hormones, **1483–1489**
  - growth hormone—insulin-like growth factor system, 1486, 1487*f*
    - environmental influences, 1488
    - peripheral control, 1488
    - *see also* Growth hormone (GH); Insulin-like growth factor (IGF)
  - insulin, 1487, 1488
  - steroid hormones, 1487
  - thyroid hormones, 1487
- Growth temperature, fish cell lines, 1968
- Grunt trains, plainfin midshipman, 322, 323*f*
- Guanosine triphosphate (GTP)
  - definition, 887
  - erythrocytic concentration, control, 883, 885*f*
- Guarding, nest *see* Nest guarding
- Gulf menhaden (*Brevoortia patronus*), hearing, auxiliary anatomy, 274
- Gulf of Mexico, hypoxic area, 1749
  - climate change effects, 1749
- Guppy, male, sigmoid display, 658
- Gustation, **183–186**
  - anthropogenic disruption, 786
  - definition, 183–184, 218
  - historical aspects, 184
  - neurophysiology *see* Gustation, neurophysiology
  - olfaction *vs.*, 218, 219*f*
  - role in feeding behavior, 227, 228
    - *see also* Feeding behavior, chemosensory
  - terrestrial *vs.* aquatic environments, 183–184
  - *see also* Taste buds
- Gustation, neurophysiology, **218–226**
  - responses to chemical stimuli, 218
    - amino acids, 218, 219*f*, 220*f*, 221*f*, 222*f*
    - bile acids, 219*f*, 221, 223*f*
    - CO_2/pH, 222, 224*f*, 225*t*
    - general, 218
    - quinine, 220, 221*f*, 225*t*
    - strychnine, 220, 221*f*
    - tetrodotoxin, 220, 221*f*
  - responses to mechanical/tactile stimuli, 224, 225*t*
  - signal transduction, 223
    - *see also* Gustatory receptors
  - *see also* Gustatory system morphology
- Gustatory receptors, 185–186, 223, 225
  - carbon dioxide, 868
  - stimulation, 218
    - *see also* Gustation, neurophysiology
  - tuned, 219, 223
- Gustatory system morphology, **187–193**
  - central (brain) pathways, 190, 191*f*
    - descending projections to facial/vagal lobes, 191
    - primary gustatory nucleus (PGN), 190, 190*f*
    - secondary and tertiary nuclei (SGN/TGN), 190
    - telencephalon, gustatory areas, 190
  - evolution, 185–186
  - peripheral sensory system, 187
    - environmental chemical/tactile map, 193
    - taste buds, 187, 188*f*, 189*f*
  - specialist systems in bottom-feeding fish
    - palatal organs and barbels, 191
    - tactile sensory system association, 192
  - *see also* Gustation; Taste buds
- Gut (gastrointestinal tract), **1229–1231**
  - absorption *see* Intestinal absorption
  - accessory digestive organs, 1268–1269, 1269*f*
    - secretions *see under* Gut secretion(s)
    - *see also* Liver; Pancreas
  - acid–base balance role, 1427
    - *see also* Gut ion/acid–base regulation
  - air-breathing role *see under* Air-breathing organs (ABOs)
  - anatomy *see* Gut anatomy
  - barrier function *see* Gut, barrier function
  - blood flow *see* Gastrointestinal blood flow (GBF)
  - definition, 1132
  - development *see* Gut development
  - disease effects *see under* Disease
  - endocrine systems *see* Gut hormones
  - epithelium, 1270
    - definition, 1268
    - intestinal *see* Intestinal epithelium
  - fish *vs.* mammals, 1270, 1271, 1287, 1288*f*
  - function, 1229, 1311
  - hagfish, 1776
  - hyperemia, 1211
  - ion regulation *see* Gut ion/acid–base regulation
  - length, 1602*f*, 1605
    - retention of food and, 1605
  - microbiota
    - bacteria, 1323
    - definition, 1301
    - role in digestion, 1306, 1308
  - motility *see* Gut motility
  - nervous system *see* Enteric nervous system; Gut innervation
  - osmoregulation *see* Osmoregulation
  - secretions *see* Gut secretion(s)
  - species differences
    - anatomy *see under* Gut anatomy
    - barrier function, 1330
  - studies, 1230
  - vascularization, 1133, 1133*f*, 1134*f*, 1135*f*, 1206
    - *see also* Gastrointestinal circulation
  - water absorption, 1318, 1318*f*, 1320, 1370, 1371*f*
  - *see also* Digestion
- Gut, barrier function, **1322–1331**, 1230
  - epithelium, 1322, 1323*f*, 1324*f*
  - functional layers, 1322, 1331
    - extrinsic barrier, 1323, 1325*f*
    - immunological barrier, 1324, 1326*f*
    - intrinsic barrier, 1323, 1325*f*, 1326*f*
  - pathogen translocation, 1328
    - bacteria, 1328, 1328*f*, 1329*f*
    - viruses, 1328
  - regional differences, 1326, 1327*f*, 1328*f*
  - species differences, 1330
  - in stress and disease, 1329, 1330*f*
- Gut anatomy, **1268–1275**, 1419
  - dietary effects, 1273
  - disease effects, 1274
    - *see also under* Gut, barrier function
  - functional, 1602*f*, 1604
  - macroscopic, 1268, 1269*f*, 1302*f*, 1602*f*
    - foregut, 1269, 1269*f*
    - headgut, 1269
    - hindgut, 1269*f*, 1270
    - midgut, 1269*f*, 1270
  - microscopic, 1270
    - mucosa, 1270, 1270*f*
    - myenteric plexus, 1270*f*, 1271
    - smooth muscle, 1270*f*, 1271
    - submucosa, 1270*f*, 1271
  - seasonal variations, 1274, 1274
  - species differences, 1271, 1272*f*
    - intestine, 1272
    - pyloric caeca, 1273, 1273*f*, 1274*f*
    - spiral valve, 1273
    - stomach, 1271
  - temperature effects, 1274
  - *see also individual structures*
- Gut development, **1284–1291**, 1230
  - accessory digestive organs, 1284–1285
    - liver *see under* Liver
    - pancreas *see under* Pancreas
  - differentiation, 1287
    - enteric neuron, 1287, 1289*f*
    - intestinal epithelium, 1287
    - intestinal smooth muscle, 1287, 1289*f*
  - endoderm specification, 1284–1285
  - epithelium, 1285–1286, 1286*f*
    - cell proliferation, 1286
    - differentiation, 1287
  - esophagus, 1285
    - organization, 1286, 1286*f*
  - intestine, 1285
    - organization, 1285, 1286*f*, 1286*f*
  - pharynx, 1285
    - organization, 1286, 1286*f*
  - stomach, 1285
- Gut hormones, **1341–1347**, 1230, 1271
  - CCK *see* Cholecystokinin (CCK)
  - gastric acid secretion role, 1343, 1343*f*
  - gastrin *see* Gastrin
  - ghrelin *see* Ghrelin
  - glucagon-like peptide-1 *see* Glucagon-like peptide-1 (GLP-1)
  - histamine, 1342
  - immunohistochemistry, 1342*f*
  - leptin, 1346
  - serotonin, 1344, 1344*f*
  - somatostatin, 1342
  - *see also* Pancreatic hormones
- Gut innervation, 1230, 1332
  - autonomic system and, 1332
  - extrinsic, 1332, 1333
    - cranial nerves, 1333, 1334*f*
    - elasmobranchs, 1333, 1334*f*
    - spinal nerves, 1333, 1334*f*
    - teleosts, 1333–1334, 1334*f*
  - intrinsic *see* Enteric nervous system
- Gut ion/acid–base regulation, **1419–1428**
  - acid–base balance, gut role, 1427
  - freshwater teleosts, 1425, 1426
  - ion and osmotic regulation
    - feeding freshwater teleosts, 1426
    - feeding marine teleosts, 1426
    - nonfeeding marine teleosts, 1420
      - esophagus and stomach, 1421
      - intestinal NaCl co-transport and water absorption, 1421, 1421*f*
  - ion regulation, freshwater teleosts, 1425
  - marine teleosts
    - feeding, 1426
    - intestinal Cl^- and water absorption directly linked to HCO_3- secretion, 1422, 1423*f*
    - intestinal water absorption indirectly linked to HCO_3- secretion, 1424, 1425*f*
    - nonfeeding *see above*
- Gut motility, **1292–1300**
  - cellular mechanism, 1294, 1295*f*
  - control, 1295, 1295*f*
    - enteric nervous system, 1295*f*, 1295–1296
    - excitatory/inhibitory signaling substances, 1297, 1298*f*, 1299*f*
    - hormonal, 1295*f*, 1296
    - pharmacological studies, 1296
  - definition, 1292
  - development, 1299
  - integrated effects on, 1299
    - gastric emptying rate, 1299
  - patterns, 1292, 1293*f*
    - propagating contractions *see* Propagating contractions
    - standing contractions *see* Standing contractions
  - peristaltic reflex, 1296, 1297*f*
- Gut secretion(s), 1301
  - intestine/accessory organs, 1303
    - bile, 1303, 1303*f*
    - pancreatic *see* Pancreatic secretion
  - mucus, 1301, 1302*f*
  - stomach, 1270
  - water, 1301
  - *see also* Digestion
- Gymnarchomast, 357*f*, 358
  - definition, 350
- *Gymnarchus niloticus* (mormyrid wave-type fish)
  - amplitude coding, 367, 367*f*
  - circulatory adaptations, air breathing, 1881*f*, 1883
  - electric organ development, 413
  - electric organ discharge, 391, 393*f*
    - central control, 401*f*, 401–402, 402*f*, 405*t*
      - time patterns, 407, 407*f*
  - electrocytes, 391

*Gymnarchus niloticus (*mormyrid wave-type fish) (*continued*)
  electroreceptors
    ampullary organs, 355, 356*f*
    development, 410
    tuberous organs, 357*f*, 358
  wave-type gymnotiforms *vs.*, ELL neuron electrophysiology, 371
  see also Electric organ discharge (EOD); Wave-type electric fish
Gymnocypris przewalskii (Qinghai carp), gill remodeling, 799–800, 800*f*
Gymnomast, 357*f*, 358
  definition, 350
Gymnotiform electric fish, 699
  electric organs, development, 414, 414*f*
  electroreceptors
    ampullary, 356, 356*f*
    development, 412
    tuberous, 357*f*, 358
      see also Tuberous electrosensory system, physiology
  pulse-type, EOD time patterns, central control, 403, 403*f*, 404*f*, 405*f*
  wave-type *see* Gymnotiform wave-type fish
  see also Electric organ discharge; Electrocommunication; Tuberous electrosensory system, physiology
Gymnotiform wave-type fish, 366, 367*f*
  EOD time patterns, central control, 403, 406*f*
  wave-type mormyriforms *vs.*, ELL neuron electrophysiology, 371
  see also Electric organ discharge (EOD); Gymnotiform electric fish; Wave-type electric fish
Gymnotus, electrocytes, 391, 392*f*, 393, 1,393*f*
Gymnovarian, definition, 1800
Gynogenesis, 2027*f*, 2028
  chromosome manipulation, 2008
  definition, 613, 2025

H

H+ concentration
  gustatory responses, 222, 224*f*
  ventilatory response *see* Ventilatory response to CO_2/H^+
  see also pH
Habenula
  agnathan, 20, 21, 22*f*, 24*f*
  cartilaginous fish, 32
  ray-finned fish, 40*f*, 41, 42
Habitat
  gill morphology and *see under* Gill respiratory morphometrics
  selection, foraging *see under* Foraging decisions (energetics)
Habituate, definition, 713
Hadal, 166, 1953*f*, 1954
  definition, 98, 166
  see also Deep sea
Haddock (*Melanogrammus aeglefinus*), spawning behavior, 316–317, 318*f*
Haemulon sciurus (blue-striped grunt), hearing audiogram, 277*f*, 277–278
Hagen–Poiseuille equation, 807
Hagfish, **1771**
  accessory hearts, 1074, 1774, 1775
    cardinal heart, 1074, 1774–1775
    caudal heart, 1075
    portal heart, 1074, 1775
  acid–base regulation, 1776
  anaerobic abilities, 1772–1773
  blood, 1773
  brains, 17, 17*f*, 1777
    forebrain, 21
      diencephalon, 21–23, 22*f*
      secondary prosencephalon, 23, 24*f*
      thalamus, 21–23, 22*f*
    hindbrain, 17, 18*f*
    midbrain, 20, 20*f*
    research, 17
  carbon dioxide excretion, 911–912
  central circulation, 1775
  defense mechanisms, 1776
    see also Hagfish slime
  digestive system, 1776
  fascination with, 1772
  feeding, 1773, 1776
    competition avoidance, 513–515
  gills, 1385*f*, 1774*f*, 1775
    acid–base regulation, MRC ionocytes, 1387
    gap junctions between pavement cells, 1382
  hearts, 1775
    accessory *see accessory hearts (above)*
  kidney, 1407–1409, 1409*f*, 1412, 1773
  lampreys *vs.*, 16, 17*f*
  lateral line system, 336, 344–345, 345*f*
  lifecycle, 16
  low metabolic rate, 1772–1773
  misconceptions, 1772
  nervous system, 1777
    brains *see brains (above)*
  osmotic and ionic regulation, 1349*t*, 1350, 1772*t*, 1773
  phylogeny, 1771*f*, 1772
  physical features, 16, 17*f*, 1771*f*, 1772*f*
    eyes, 17, 1772*f*, 1777
    single nostril, 1772*f*, 1772–1773
    teeth, 1773
    tongue muscles, 1773
  pituitary gland structure, 1459, 1461*f*
  plasma ionic concentrations, 1349, 1349*t*
  slime *see* Hagfish slime
  slime glands *see* Slime glands (hagfish)
  subcutaneous sinus, 1116, 1774
  swimming and musculature, 1776
  taxonomy, 16
  unique physiology and anatomy, 1772–1773, 1777*t*
    see also specific structures
  venous system, 1116
  vestibular system, 245–246, 246*f*, 1777
  see also Agnathan(s)
Hagfish slime, **504–515**, 418, 1776
  compared with other marine animals, 504
  definition, 504
  deployment and maturation, 510, 510*f*, 511*f*
  gland *see* Slime glands (hagfish)
  properties, 505
  properties, mechanical, 509, 509*f*
    coherence in still seawater, 510, 511*f*
    interaction with slime threads, 512*f*, 513, 513*f*, 514*f*
    strand formation, 510–512, 512*f*
    viscosity and seawater dilution, 509–510
  structure and function, 513, 514*f*
  volume and concentration, 505*f*, 508
Hagfish slime exudate, definition, 504
Haikouichthys ercaicunensis, 102–103
Hair cells *see* Sensory hair cells
Haldane effect, 793–794, 890, 910–911
  Bohr effect and *see* Bohr–Haldane effect
  definition, 791, 887, 909, 916, 917
  species differences, 911–912
Halecomorphi, brain structure, 38*f*
Half-maximal effective concentration (EC_{50}), definition, 1474
Half-time
  definition, 1190
  hormones, 1191, 1195*t*, 1195–1196
    see also Hormone inactivation, fish
Haplochromine child, definition, 1675
Haploid, definition, 1998
Hatchetfish, bioluminescence, 499, 499*f*
Hatching, hypothalamic–pituitary–interrenal (HPI) axis development stage, 1455
Head
  vascular system
    arterial, 1086*f*, 1089
    venous, 1088*f*
      drainage system, 1092
  see also entries beginning cranial
Head kidney *see* Pronephros (head kidney)
Head region, air-breathing organs *see under* Air-breathing organs (ABOs)
Headgroup modification, homeoviscous adaptation, 1729*t*, 1730
Headgut
  anatomy, 1269
  see also Pharynx
Health, stress effects, 1548
  genomic analysis, 1550
  head kidney role, 1544*f*, 1549
  immune function, 1546*f*, 1549
    acute stress, 1550, 1551
    chronic stress, 1550, 1551
    cortisol role, 1549*f*, 1550
    integrated immune response to stressors, 1551
  neuro–immuno–endocrine network, 1547*f*, 1548
    time course activation, 1548*f*, 1549
  pathologies, 1550*f*, 1551
  see also Stress response, hormonal
Hearing, **276–282**, 241
  abilities, in fish, 242, 276
    frequency (pitch) discrimination, 281, 281*f*
    purposes, 282
    sensitivity determinants, 280
    species variation, 278
  acoustic behavior *see* Sound production
  directional *see* Directional hearing
  evolution, 237
  goldfish *see under* Goldfish (*Carassius auratus*)
  how fish hear *see* Hearing mechanisms
  human-generated sounds *see* Human-generated sounds
  measurement, 276
    auditory evoked potentials, 278
    avoidance conditioning audiogram, 277
    classical conditioning audiogram, 277
  origin of knowledge/historical aspects, 238, 238*f*, 239*f*, 241*f*
    leading scholars, 238, 240*f*
  sharks and rays, **262–269**, 1808
    acoustic attraction thresholds, 268, 268*f*
    auditory sensitivity, 265
    extent of observations, 269
    frequency detection thresholds, 267
    parietal fossa response, 264, 266–267, 267*f*
    sensory end organs and sound pathways, 264
  sound source localization *see* Sound source localization
  what fish hear, **276–282**
    frequency discrimination, 281
    sensitivity, 280
    sounds heard, 282
    species variation, 278
  see also entries beginning auditory
Hearing mechanisms, 258, **292–297**, 259*f*, 273
  afferent coding of sounds *see under* Auditory afferents
  auditory plasticity *see* Auditory plasticity
  auditory transduction *see* Auditory transduction
  auxiliary/unusual anatomy enhancing, 274
  auxiliary/unusual anatomy that enhance hearing, Weberian ossicles *see* Weberian ossicles
  central representation of sounds, 295
    frequency tuning, 295
    role of octavolateral efferents, 296
    temporal response patterns, 296
    transformation from peripheral code to central code, 296
    see also Auditory/lateral line CNS, anatomy
  mormyrid electric fish, 274, 279–280

  swimbladder role, 274
    see also Inner ear–swimbladder relationship
  see also Ears, fish; *entries beginning auditory*
Heart, 974–975, 1030
  accessory *see* Accessory hearts
  action potential *see* Cardiac action potential
  activity in anoxic crucian carp, 1826*f*, 1828, 1829
  anatomy *see* Cardiac anatomy
  autonomic nervous system control, 84, 85*f*, 1068
    see also Intracardiac neurons/neurotransmitters
  conducting system, 1071
    see also Cardiac action potential
  definition, 973, 1030
  endothermic fish, 1916
  formation (human), fish models, 1991
  function (human), fish models, 1991
  hagfish *see under* Hagfish
  hypoxia tolerance, 1224, 1224*f*
  location, 999
  malformations, fish models in, 1991
  myocytes *see* Cardiomyocyte(s)
  neurons in *see* Intracardiac neurons/neurotransmitters
  outflow tract *see* Outflow tract, heart
  output *see* Cardiac output (V_b)
  pacemaker, 1071
  as pump, 978, 981–982, 1030
    see also Cardiac pumping, physiology
  pumping, physiology *see* Cardiac pumping, physiology
  seasonal variation in capillary density, 1145–1146
  shapes and sizes, variation, 1003, 1003*f*
  temperature effects on function *see under* Temperature effects, cardiovascular system
  types, 1080
  see also Cardiovascular system; *entries beginning cardiac*
Heart diseases (human), fish models, 1991
Heart rate (f_H), 1035, 1035*t*
  acute hypoxia response, 1222, 1223*f*
  aerobic swimming-associated increase, mechanism, 1216
  air-breathing cycle and, 1880
  frequency modulation, tunas, 1909, 1912
  pelagic fishes, effect of acute temperature changes, 1898–1900
  related to body mass, 1579*f*, 1582
  related to cardiac output, 816
  temperature effects
    maximum f_H, 1200, 1201*f*, 1202*f*
    resting f_H, 1198, 1199*f*, 1202*f*
    see also Temperature effects, cardiovascular system
Heart rate conditioning
  behavioral studies of polarized light detection, 148*f*, 148–149
  definition, 143
Heat, 1691
  definition, 1688, 1691, 1695
Heat exchange, factors affecting amount, 1120
Heat exchangers, 1121
  of viscera, 1122
  see also Retia mirabilia
Heat increment of feeding (HIF) *see* specific dynamic action (SDA)
Heat of combustion, definition, 1665
Heat production *see* Thermogenesis
Heat shock elements (HSEs), 1735*f*, 1736
Heat shock proteins (HSPs), 1733
  definition, 1515, 1732
  fish, 1732*t*, 1734
    expression in intertidal fish, 1964
    see also Heat shock response, fish
  stressors, 1734, 1734
Heat shock response
  definition, 1732
  discovery (fruit flies), 1733
Heat shock response, fish, 1732*t*, 1734
  cold stress, 1734
  future research directions, 1737
  plasticity, 1734
    development/aging, 1736
    geographical population variation, 1736
    temporal variation, 1735
    thermal history, 1734
    thermal stress intensity, 1734
  regulation
    molecular, 1735*f*, 1735–1736
    physiological, 1737
  thermal/cross tolerance, 1736
  triggers, 1736
Heat shock transcription factors (HSFs), 1735*f*, 1736
Heat stress, heat shock protein induction *see* Heat shock response
Heater organ tissue *see under* Cranial endothermy
Heave, 575–576, 576*f*
  definition, 575
Heavy metals, 2063
  definition, 2062
  see also Metal(s)
Helical capsids, 2035
Hemagglutinating properties, vitellogenins, 645
Hemagglutinin esterase (HE), infectious salmon anemia virus, 2038
Hematocrit, 985, 986, 986*f*
  Antarctic species, 1203
  athleticism and, 986, 987*f*
  blood viscosity and, 980, 980*f*, 987
    temperature effects and, 987
    see also Fahraeus–Lindqvist effect
  definition, 977, 980, 984, 985, 1111, 1154
  mean cell hemoglobin concentration (MCHC) and, 986, 986*f*, 988
  microcirculation, 1113
    see also Fahraeus effect
  physiological factors affecting, 986, 987*f*
  temporary changes, 987, 987*f*
Hematopoiesis, 992, 993, 994*f*
  definition, 992, 1986
  disorders (human), fish models, 1990
    see also Blood diseases
  sites, 992, 993
  see also Erythropoiesis
Hematopoietic stem cells (HSCs), 993–995, 1990
  definition, 992
  increasing, methods, 1990–1991
  transplantation, fish model, 1989–1991
Hematopoietic tissues, 986
Heme, 888, 945
  definition, 1104
  see also Hemoglobin(s), fish
Heme iron, 888, 891–892
  oxidation, 892
Heme oxygenase, definition, 879
Hemibranch, 1096, 1096*f*, 1097*f*
  definition, 929, 1095
Hemichordates
  acorn worms *see* Acorn worms
  CNS induction/neurogenesis suppression in ectoderm, 9
Hemodynamics, 979
  imperfect non-Newtonian fluids, 980, 980*f*
  perfect Newtonian fluids, 979, 979*f*
  vascular resistance significance, 1105
  see also Fluid dynamics
Hemoglobin(s), 944
  Bohr effect *see* Bohr effect
  definition, 1006
  evolution, 944, 945, 945*f*
  fish *see* Hemoglobin(s), fish
Hemoglobin(s), fish, **944–950**, 985–986
  affinity and cooperativity, 945, 946*f*
    see also Oxygen binding, fish hemoglobins
  allostery, 529–530, 889*f*, 889–890, 923–924, 924*f*, 946, 946*f*
    Monod, Wyman, and Changeaux (MWC) model, 946, 947*f*
    Root effect and, 938, 939*f*
    see also R-state hemoglobin; T-state hemoglobin
  anodal, 949
  Bohr effect *see* Bohr effect, fish hemoglobins
  buffer properties, evolution, 927*f*, 928
  cathodal, 949
  concentration *see* Mean cell hemoglobin concentration (MCHC)
  crucian carp, 799, 1829
  embryonic, 949–950
  functional differentiation, 948
    hemoglobin multiplicity, 949
    hypoxia, 948
  gas transport/exchange, **887–892**
    carbon dioxide interactions, 910, 910
      see also Carbon dioxide (CO_2) transport/excretion
    oxygen binding *see* Oxygen binding, fish hemoglobins
    see also under Red blood cells (RBCs), function
  hagfish, 1773
  histidine residues, 888
  isoforms, Root and non-Root-effect, 938
  multiplicity, 888, 949
  oxidation, 891
  oxygen binding *see* Oxygen binding, fish hemoglobins
  oxygen saturation, aerobic swimming, 1216–1217
  R-state *see* R-state hemoglobin
  Root effect *see* Root effect
  structure, 887, 945, 946*f*
  T-state *see* T-state hemoglobin
Hemoglobin A, definition, 1888
Hemolysins, pathogenesis role *see under* Extracellular products (ECPs), disease role
Hemopoiesis
  definition, 1395, 1986
  see also entries beginning hematopoietic
Hemostatics, 978
  see also Hydraulic pressure; Hydrostatic pressure; Oncotic pressure; Osmotic pressure
Henry's law, 830
Hepatic portal system, 1092, 1134–1135
Herbicides, olfactory effects, 2080–2081, 2081, 2081–2082
Herbivore, definition, 1665
Hermaphroditism, 614, 1491, 1491*t*, 2052–2053
  definition, 613, 2046
  false *see* False hermaphroditism
  sequential, 1491, 1491*t*, 2051*t*, 2052–2053
    protandrous fish *see* Protandry/protandrous fish
    protogynous fish *see* Protogyny/protogynous fish
    reciprocal sex-change fish, 2051*t*, 2052–2053
  simultaneous, 1491, 1491*t*, 2051*t*, 2053
  see also Sex change
Herring bodies, 34
Heterochromatin, definition, 616
Heterodontus francisci (horn shark), 266–267, 267*f*
Heterogamety, 1984
  definition, 1980
Heterologous expression, definition, 208
Heteromorphic chromosomes
  definition, 2025
  sex chromosomes, 2027–2028
Heteropneustes fossilis (Indian catfish), suprabranchial chambers, 1868*f*, 1870
Heterospecifics
  definition, 1629
  feeding/growth effects, 1634
  see also Species differences
Heterotherm
  definition, 1689
  regional, definition, 1689
  temporal, definition, 1689
Heterotropic interaction, definition, 887
Heterozygous, definition, 2009

Hiding places, for sleeping, 774–775
High-salt diet, effect on venous capacitance, 1115
Hill coefficient, 888–889
Hill plot, 888–889, 889f
  definition, 887
Hindbrain
  agnathan, 17, 18f, 20f
  cartilaginous fish *see under* Cartilaginous fish, brains
  definition, 46
  information processing, ampullary electrosensory system, 363
  Latimeria, 47, 53f, 54, 54f
  lungfishes *see under* Lungfishes
  ray-finned fish, 3, 4f, 37, 40f
  see also Cerebellum; Isthmus; Rhombencephalon
Hindgut, anatomy, 1269f, 1270
Hippocampus
  definition, 707
  learning role, 709–710
Histamine
  definition, 772
  enteroendocrine cells, 1342
  gastric acid secretion role, 1343, 1343f
  sleep role, 775
Histidine residues, fish hemoglobins, 888
Histophagy, 679–680
Hodological, definition, 46
Holobranch, 1096, 1097f
  definition, 1095
Holocephalans, cranial muscles, 459, 461t
  actions, 461t
  branchiomeric, 459, 459f, 460f
  epibranchial, 459, 459f, 460f
  hypobranchial, 460f, 462
Holocrine secretion, 504
  definition, 504
Holostyly, definition, 419
Home stream olfactory bouquet (HSOB), toxicant effects, 2081, 2082
Homeophasic adaptation, 1685
  definition, 1681
Homeostasis
  cardiovascular system *see* Cardiovascular system control
  definition, 1169, 1515
  disturbed *see* Stress
  osmotic *see* Osmoregulation
  water *see* Water homeostasis
Homeotherm, definition, 1665, 1689
Homeoviscous adaptation (HVA), 1685, 1685f, 1722, 1725–1726, 1729
  adapative remodeling of membranes, 1728f, 1729t, 1730
    cholesterol content, 1723, 1729t, 1731
    deacylation–reacylation, 1729t, 1730
    desaturation and elongation, 1729t, 1730
    fatty acid saturation, 1729t, 1730
    headgroup modification, 1729t, 1730
    plasmalogen content, 1729t, 1731
    sialization, 1729t, 1730
  definition, 1681, 1717, 1725
  see also Cell membrane fluidity
Homing, salmon *see* Salmon homing
Homology, definition, 6, 416
Homomorphic chromosomes, definition, 2025
Homotropic interaction, definition, 887
Hoplosternum littorale, air-breathing cycle, 768–769, 769f
  see also Air-breathing fish
Horizontal cells, 124, 124f
Horizontal eye movements, 249f, 249–250
Hormonal control
  appetite *see under* Appetite regulation
  color changes, 493, 494
  gastrointestinal blood flow, 1212
  growth *see* Growth-regulating hormones
  gut motility, 1295f, 1296
  ion regulation *see* Osmoregulation, hormonal control
  sex differentiation *see* Sex differentiation
  sexual behavior *see under* Sexual behavior
  spermatogenesis *see under* Spermatogenesis, regulation
  vascular resistance *see under* Systemic vascular resistance, remote control
  vitellogenesis, 638, 640f
  see also specific hormones
Hormonal effects
  electrocommunication systems, 705
  venous capacitance/cardiac output, 1114
  see also Hormonal control; *specific hormones*
Hormonal pheromones, **1553**
  definition, 1553
  evolution, 1555
  goldfish and related carps, 1554f, 1555
    as chemical network, 1560f, 1561
    female postovulatory prostaglandin pheromone, 1557f, 1558, 1559f, 1559–1560
    female preovulatory steroid pheromone, 1554f, 1556, 1556f, 1559f
    intrasexual effects, 1561
    male-derived, 1560
    mechanisms of action, 1559, 1559f
    sexually dimorphic responses, 1558f, 1559
    species specificity, 1561
  spawning role *see under* Spawning
  see also Pheromone(s)
Hormone(s), 1190
  circulating, 1191
  definition, 656, 752, 1045, 1190, 1191, 1373, 1466, 1515, 1541, 1665
  effects *see* Hormonal effects
  elasmobranchs, 1814t, 1816
  gut *see* Gut hormones
  inactivation, **1190–1196**
    in fish *see* Hormone inactivation, fish
    in mammals *see* Hormone inactivation, mammals
  noncirculating, 1191
  pituitary *see* Pituitary hormones
  stress response *see* Stress response, hormonal
  types, 1191
    hydrophilic *see* Hydrophilic hormones
    lipophilic *see* Lipophilic hormones
  see also entries beginning endocrine; *specific hormones*
Hormone inactivation, fish
  cardiac output and, 1194f, 1196
  measurement, 1194
    impact of single-tissue inactivation on whole-body hormone clearance, 1192, 1194f
    physiological inactivation of circulating hormones, 1195, 1195f, 1195t
    single-tissue extraction, 1193f, 1194, 1195, 1196t
    whole-body uptake, 1194, 1195
  mechanisms, 1192
    gills, 1192, 1193f
    systemic (non-gill), 1193f, 1194, 1194f, 1195t
Hormone inactivation, mammals, 1191
  cellular uptake from plasma/intracellular degradation, 1191
  dilution, 1191
  mechanisms, 1191
  metabolic inactivation in plasma, 1191
  sites, 1192
Horn shark (*Heterodontus francisci*), acoustic responses, 266–267, 267f
Host manipulation, **777–782**
  definition, 777
  by fish parasites, 778
    Diplostomun spathcaeum, 778, 779f
    future research directions, 781
    Schistocephalus solidus, 780, 780f, 781f
  trophic transmission and, 778
Hot water life *see Cyprinodon* (desert pupfish)
Hourglass mechanism, 738–739
  definition, 736
Hovering, 571
  hydrodynamics, 573
  strategies, 523
Hox genes, definition, 6
HPI axis *see* Hypothalamic–pituitary–interrenal (HPI) axis
5-HT *see* Serotonin
Hue, 490, 492
  definition, 489
Hum, plainfin midshipman vocalization, 322, 323f
Human(s)
  cardiac troponin C *see under* Cardiac troponin C (cTnC)
  diseases
    cancer *see under* Cancer
    fish models *see* Fish model(s)
  influences on fish behavior *see* Anthropogenic influences/disruptions
  sleep, 772
    disorders, fish models, 1996
    duration, 774
    stages, 773
Human-generated sounds, **304–310**
  impacts on fish, 306, 785
    behavioral studies, 307
    conservation perspective, 307–308, 308f
    hierarchy/zones, 307, 307f
    regulation and mitigation, 308
  sources, 304, 305t
Humoral immunity, definition, 1541
Hunger, as motivation to feed, 1589
Huntingtin, 1993
Huntington's disease, fish models use for, 1993
Hyaline cartilage
  definition, 428
  see also Tessellated cartilage
Hybridization
  definition, 2055
  global gene expression profiling methodology, 2056f, 2057
Hybrids, desert pupfish, 1841
Hydration reaction
  carbonic anhydrase, 899, 905–907, 907f
  definition, 899
Hydration shells, water transport considerations, 1368–1369
Hydraulic pressure, 979, 979f
  definition, 977, 1111, 1154, 1161
  transcapillary fluid exchange and, 1155–1156, 1156f
Hydrochloric acid, stomach secretion, 1270, 1302
Hydrodynamic lift, 523
  definition, 516, 520
Hydrodynamics
  experimental *see* Experimental hydrodynamics
  fast start, 590, 591f
  hagfish slime deployment, 510–512, 513f
    effect on gill flow resistance, 513–515, 514f
  paired fin-associated *see* Paired fin hydrodynamics
  suction feeding *see under* Suction feeding
  see also Fluid dynamics
Hydrofoils, 524, 524f
Hydrogen bonding, protein stabilization, 1707
Hydrogen ion (H^+) concentration
  gustatory responses, 222, 224f
  ventilatory response *see* Ventilatory response to CO_2/H^+
  see also pH
Hydrogen sulfide (H_2S)
  avoidance by mangrove killifish, 1962
  catecholamine release role, 1531, 1532
  vascular resistance control, 1107
Hydrolyze, definition, 1276
Hydromineral regulation, stress effects, 1519, 1520f
Hydrophilic hormones, 1191
  definition, 1190
Hydrophilicity, definition, 2069
Hydrophobic, definition, 1725
Hydrophobic interactions, protein stability role, 1706

Hydrophobicity, definition, 2069
Hydrophones, 265–268
  ultrasonic tracking, 1930
Hydrostatic pressure, 978, 979*f*, 1112
  definition, 977, 1111, 1954
  effects on deep-sea fishes *see* Deep-sea fishes, pressure effects
11β-Hydroxysteroid dehydrogenase(s), evolution, 1480–1482, 1481*f*
11β-Hydroxysteroid dehydrogenase type 2
  evolution, 1480–1482, 1481*f*
  mineralocorticoid receptor co-localization, 1477, 1478*f*
5-Hydroxytryptamine (5-HT) *see* Serotonin
Hyoid arch, anatomy, bony fish, 440, 441*f*
Hyostyly, definition, 419
Hypercapnia, definition, 865, 1411, 1800
Hypercapnia responses
  cultured fishes, 2087
  sturgeon, 1801
    blood–oxygen affinity, 1804
    growth, 1803
    gut blood flow, 1139, 1140*f*, 1804
  ventilatory, 865, 866, 869
    see also Ventilatory response to CO_2/H^+
Hypercarbia, definition, 865, 916
Hypercarbic ventilatory response, 865–866, 866*f*
  air-breathing fish, 866, 867*f*, 868
  hypoxic ventilatory response *vs.*, 866
  see also Carbon dioxide (CO_2) chemoreceptors
Hyperemia
  definition, 1132, 1206
  gastrointestinal tract, 1211
  see also Gastrointestinal blood flow (GBF)
Hyperglycemia, 1313
  definition, 1311
Hyperosmotic, definition, 1348, 1373
Hyperoxia
  definition, 865, 1960
  ventilatory response, 866
Hyperphagia, 752–753
  definition, 752
  see also Compensatory growth
Hyperplasia
  cardiomyocytes, 1007
  definition, 796, 1006, 1221, 1483
  epidermal, 472
Hypertrophy
  cardiomyocytes, 1007–1008, 1010
  definition, 1006, 1483
Hyperventilation, definition, 1752–1753
Hypnotic substances, zebra fish response, 775
Hypobranchial, definition, 449
Hypobranchial bone, 1096, 1096*f*
Hypobranchial muscle
  batoids, 457*t*, 458, 458*f*
  holocephalans, 460*f*, 462
  sharks, 452*t*, 453*f*, 454, 454*f*
Hypochromic, definition, 166
Hypocretins *see* Orexins
Hypometabolic rate, definition, 1800
Hypometabolic response, sturgeon, 1803–1804
Hypoosmotic, definition, 1348, 1373
Hypoosmotic fluids, deep-sea fishes, 1957
Hypoosmotic regulation
  definition, 1483
  role of growth hormones, 1489
Hypophysis *see* Pituitary gland
Hypothalamic–pituitary–interrenal (HPI) axis
  activation
    effects of social status, 652–653, 653*f*
    stress, 652
  components, 1451*f*, 1452
    duplicated HPI axis genes, 1453
    hypothalamus, 1451*f*, 1452
    interrenal, 1451*f*, 1452
    nuclear receptors and cortisol signaling, 1451*f*, 1452
    pituitary, 1451*f*, 1452
  cortisol feedback effect, 653
  definition, 1534
  ontogeny, 1453, 1453*f*, 1456, 1456*f*
    see also Hypothalamic–pituitary–interrenal (HPI) axis development
  stress response, 1475, 1476*f*, 1516*f*, 1517, 1517*f*
    see also Stress response, hormonal
Hypothalamic–pituitary–interrenal (HPI) axis development, 1453
  chinook salmon, 1455
  common carp, 1455
  rainbow trout, 1455
  yellow perch, 1455
  zebrafish *see* Hypothalamic–pituitary–interrenal (HPI) axis development, zebrafish
Hypothalamic–pituitary–interrenal (HPI) axis development, zebrafish, 1453
  embryogenesis, 1453
    adrenocorticotropin hormone (ACTH), 1454
    caudal neurosecretory system (CNSS), 1454
    corticotrophin-releasing factor (CRF) signaling systems, 1454, 1454*f*
    cortisol biosynthesis, 1454
    glucocorticoid and mineralocorticoid receptors, 1455, 1455*f*
    pro-opiomelanocortin (POMC), 1454
  hatching, 1455
  mature oocyte, 1453
Hypothalamo–hypophyseal tract
  cartilaginous fish, 34
  neurosecretory cells, 1458, 1459*f*
    see also Neurohypophysis
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Hypothalamus, 1451*f*, 1452
  agnathan, 22*f*, 23–25, 24*f*
  auditory/lateral line connections, 284*f*, 285*f*, 291
  cartilaginous fish, 31*f*, 32
  definition, 159, 649, 1457, 1483, 1509
  food intake regulation, 1484, 1509, 1510*f*
    see also Appetite regulation
  inferior lobe (sensory integration), 190, 191*f*
  Latimeria, 47, 54*f*, 54–55
  lungfishes, 47, 48*f*, 49*f*, 51
  ray-finned fish, 40*f*, 41–43
  see also Brain(s) Hypothalamic–pituitary–interrenal (HPI) axiss
Hypotonic, definition, 2009
Hypovolemia, effect on vascular resistance, 1108
Hypoxantine-3-N-oxide (H3NO), 232
  olfactory detection, 232
  see also Alarm substance
Hypoxemia, definition, 1524
Hypoxia, 1221, 1752
  definition, 765, 791, 944, 1389, 1524, 1746, 1752, 1764, 1772, 1800, 1824, 1833, 1888, 1960, 2055
  diel-cycling, 1746–1748
  effects, 1740*f*, 1741, 1742
    aquaculture environment, 2086
    cellular respiration, 956
    energetic costs of swimming, 1641*f*, 1642
    fast start performance, 595
    fish growth, 1631
    foraging activity, 1590
    metabolism *see* Metabolism, environmental influences
    see also responses (below)
  gene expression profiling and, 2060
  importance of, 1746
  as refuge, 766
  responses, 872, 1752–1753, 1764
    angiogenic, 1147
    behavioral *see* Hypoxia, behavioral responses
    catecholamine release, 1521, 1522*f*, 1526, 1527*f*, 1535*f*, 1535–1536, 1755
    chemoreceptor *see under* Oxygen chemoreceptors, branchial
    circulatory system *see* Hypoxia, circulatory system responses
    heat shock response, 1734
    insights from global gene expression data, 2060
    osmorespiratory compromise, 1389–1390, 1392, 1393
    pelagic fish, 1901
    respiratory *see* Hypoxia, respiratory responses
    shift to anaerobic metabolism, 1761, 1762, 1765*f*, 1766
    sturgeons, 1803–1804
    see also effects (above);*see also* Metabolic rate suppression; *specific responses*
  tolerance/survival *see* Hypoxia tolerance
  see also Anoxia; Hypoxic environments
Hypoxia, behavioral responses, **765–771**, 1768
  altered activity patterns, 765, 1768
  contribution to metabolic rate suppression, 1767*f*, 1768
  courtship behavior/reproductive activity, 1768
  future studies, 770
  surfacing behaviors, 766
    aquatic surface respiration *see* Aquatic surface respiration (ASR)
    emergence, 767, 768*f*
      evolution, 1855*f*, 1856
      see also Air-breathing fish
    parental care and, 769
    predation trade-off, 768
      group surfacing, 768
      postponement of surfacing, 768
      use of cover, 768
  terrestrial behaviors, 770, 770*f*
Hypoxia, circulatory system responses, **1221–1228**
  acute hypoxia, 1222
    cardiac output and stroke volume, 1222, 1223*f*
    heart rate, 1222, 1223*f*
    tolerance of heart, 1224, 1224*f*
    vascular resistance and arterial blood pressure, 1108, 1223
  angiogenic, 1147
  cardiac action potential, 1042
  cardiac output, 1115, 1222, 1223*f*
  chronic hypoxia, 1225, 1225*f*, 1226*f*
    crucian carp, 1226, 1227*f*, 1826*f*, 1828, 1829
    histological changes (cardiac), 1225–1226, 1226*f*
  coronary blood flow, 1082*f*, 1082–1083
  gastrointestinal blood flow, 1139, 1139*f*
  hematocrit increase, 987–988
  hemoglobin, 948
  tolerance of heart, 1224
  vascular resistance, 1108, 1223
  venous capacitance, 1115
Hypoxia, respiratory responses, **1751**
  benefits, 1753*f*, 1754
  gill morphology *see* Gill remodeling
  gill morphometrics, 810, 810*f*
  hyperventilation *see* Hypoxic hyperventilatory response
  osmorespiratory compromise, 1389–1390, 1392, 1393
Hypoxia dwellers, gill respiratory morphometrics, 810, 810*f*
Hypoxia-inducible factor (HIF-1α)
  definition, 796, 1850
  gill remodeling role, 798
  induction (hypoxia response), 1855*f*, 1856
Hypoxia tolerance, 766, 948–949, 1765*f*, 1766–1767
  behavioral (air breathing/emergence), 1963
  crucian carp *see under* Crucian carp
  definition, 1764
  hagfish, 1772–1773
  lampreys, 1795–1796
  physiological mechanisms
    glycogen stores, 1762
    metabolic rate suppression, 1767, 1769

- Hypoxia tolerance (*continued*)
    - tuna, 1909, 1913
    - *see also* Metabolic rate suppression
    - *see also* Hypoxia, responses
- Hypoxic dead zones, 1748
- Hypoxic environments, **1746**
    - causes, 1738, 1741, 1746–1747
        - anthropogenic, 1748
        - global climate change, 1748f, 1749
        - zebrafish natural habitat, 1821
    - dead zones, 1748
        - climate effects, 1749
    - occurrence, 1747, 1747f
    - oxygen minimum zones, 1747f, 1748
- Hypoxic hyperventilatory response, 1752–1753
    - air-breathing fish, 1752f, 1753
    - benefits, 1753f, 1754
    - definition, 1753
    - hypercapnic ventilatory response *vs.*, 866
    - ontogeny, 1755
    - pathways, 1755
        - branchial oxygen chemoreceptors, 1754f, 1755
        - extra-branchial oxygen chemoreceptors, 1755
    - plasticity, 1755
    - water-breathing fishes, 1751f, 1753

I

- I-band
    - cardiomyocyte myofibrils, 1008–1009, 1009f
    - definition, 1006
- Icefish (Channichthyidae), 996f
    - cardiovascular adaptations to temperature, 1203, 1204f
        - bulbus arteriosus, 1018–1019, 1021f, 1203, 1204f
        - vascular supply, 1143, 1147f, 1151
            - capillary size and structure, 1144, 1145f, 1151, 1152f
    - evolutionary loss of hemoglobin, 996
- Icosahedral capsids, 2035
- *Ictalurus see* Catfish (Siluriformes)
- Ideal free distribution (IFD)
    - definition, 1588
    - model, 746, 747f
- Immortal cell line, 1966
    - definition, 1965
- Immune response
    - growth hormone transgenic fish, 2020
    - humoral, definition, 1541
    - innate, definition, 1542
    - stressors, 1551
        - *see also* Health, stress effects
    - subordinate individuals, 653–654
- Immune suppression
    - definition, 1541
    - stress-mediated, 1546f, 1549, 1550
        - cortisol role, 1549f, 1550
- Immune system
    - elasmobranchs, 1816
    - intestinal, 1324–1325, 1326f
    - skin, 471–472, 472f, 473
    - stress effects on function *see under* Health, stress effects
    - triploid fish, 2013
- Immunocompromised, definition, 2032
- Immunoglobulins
    - definition, 678, 1541
    - egg yolk, 679
    - intestinal barrier function role, 1325
- Immunohistochemistry, definition, 1161, 1354
- Immunological barrier
    - gut, 1324, 1326f
    - skin, 471–472, 472f, 473
- Imprinting (learning), sensitive phases, 710
- Imprinting, olfactory *see* Olfactory imprinting
- Impulse, definition, 535
- In-parallel circuits, resistance, 980, 980f
- In-series circulation
    - definition, 1772
    - resistance, 980, 980f, 982
- *in situ*, definition, 1197, 1221
- *in vitro* saline-perfused posterior cardinal vein preparation
    - catecholamine release studies, 1529, 1529f
    - definition, 1524
- *in vivo*, definition, 1132, 1206, 1986
- *in vivo* imaging, fish models, 1989, 1989f
- Inactivation, definition, 1717
- Incidental sound production, 313
- Incipient lethal temperatures (ILTs), 1697
    - acclimation dependence, 1697f, 1698
    - calculation of species eurythermicity, 1696f, 1698
    - empirical determination, tolerance polygon, 1696f, 1697
- Increasing water depth
    - photoreceptor/visual pigment adaptations, 117, 118f
    - *see also* Deep sea
- Indeterminate growth
    - determinate growth *vs.*, 1486
    - *see also* Growth
- Indian catfish (*Heteropneustes fossilis*), suprabranchial chambers, 1868f, 1870
- Indicator dilution method, 1166–1167
    - definition, 1161
- Indirect calorimetry
    - definition, 1566
    - metabolic rate measurement, 846–847, 1566
        - *see also* Respirometers
- Indispensable amino acids (IDAAs)
    - definition, 1617
    - dietary requirements in cultured fish, 1618–1619
        - balance and impact of other nutrients, 1621
        - estimation methods, 1619, 1619f, 1619f
    - *see also* Amino acids
- Individual differences, biological variation, 2058
- Industrial activities, noise generation, 305t, 305–306
- Infectious hematopoietic necrosis virus (IHNV), 2038–2039
    - vaccine development, 2039–2040
- Infectious pancreatic necrosis virus (IPNV), 1328, 2034f, 2035f, 2037
    - vaccine development, 2039
- Infectious salmon anemia, 2038
- Infectious salmon anemia virus (ISAV), 2037
- Influx, definition, 1359
- Information theory, definition, 366
- Infraorbital bones, bony fish, 436f, 438
- Infrasound, definition, 304
- Infundibulum, definition, 1457
- Ingesta
    - definition, 1597
    - particle size, effect on transit time, 1604
    - *see also* Food consumption/intake
- Ingested volume of water (IVW), 599, 600f, 601
- Inheritance, troglomorphic traits in blind cavefish, 1848
- Inhibitory input
    - definition, 73
    - Mauthner cells, 77f, 78
- Initial phase, 664f, 665f
    - definition, 662
- Innate immunity, definition, 1542
- Inner ear
    - anatomy, 252, 253f
        - inter-species variation, 256f, 273
    - biomechanics/sound transmission mechanisms, 258, **270–275**, 259f
        - mechanics of hearing process, 273
        - *see also* Hearing mechanisms; Sound propagation
    - central auditory pathways *see* Auditory/lateral line CNS, anatomy
    - definition, 236, 252
    - macula neglecta *see* Macula neglecta
    - otolithic end organs, 253f, 254, 256f, 273
        - otoliths *see* Otoliths
        - sensory epithelium *see* Sensory epithelia, fish ears
        - *see also* Lagena; Saccule; Utricle
    - semicircular canals *see* Semicircular canal(s)
    - swimbladder relationship *see* Inner ear–swimbladder relationship
- Inner ear–swimbladder relationship, 258, 259f, 274, 274f
    - *see also* Otophysic connections; Weberian ossicles
- Inner retina, **123–130**
    - cells, 124, 124f
        - amacrine, 124f, 125
        - bipolar, 124f, 125
        - ganglion *see* Retinal ganglion cells
        - horizontal, 124, 124f
    - feedback pathways, 124, 126, 130
        - centrifugal fibers and, 126
        - interplexiform cells and, 126
- Innervation
    - definition, 329
    - extrinsic, autonomic neurons *see under* Autonomic neurons
    - gill arch, 1102
    - lateral line *see under* Lateral line
    - vagal, definition, 1341
    - *see also specific nerves*
- Inotropy, definition, 1030
- Insecticide contamination, organophosphates, olfactory effects, 2081
- Insertional mutagenesis, definition, 1819
- Instantiate, definition, 603
- Insulin, 1280, 1280t
    - appetite suppression, 1485f, 1485–1486, 1512t
    - growth regulation, 1487, 1488
- Insulin-like growth factor (IGF)
    - action, 1486–1487, 1487f
    - distribution, 1486–1487, 1487f
    - production/release
        - environmental influences, 1489
        - peripheral control, 1488
- Insulin-like growth factor-1 (IGF-1)
    - definition, 1466
    - ion regulation role, 1467f, 1470
        - cortisol interaction, 1471
    - levels in growth hormone transgenic fish, 2019, 2023
- Intact fish, vascular resistance measurement, 1109, 1109f
- Intact proteins, absorption, 1313f, 1315
- Integument (skin)
    - bioluminescence and *see* Bioluminescence
    - capillaries, 1146
    - coloration *see* Coloration
    - definition, 471
    - extensions, 487
    - functional anatomy, current knowledge, 417
    - functional morphology *see* Integumentary system, functional morphology
    - functions, **471–475**
        - communication and sensory perception, 472f, 473
        - excretion *see under* Excretion
        - ion regulation, 472f, 474, 1362, 1363f
        - locomotion, 472f, 473
        - protection, 471, 472f
        - respiration *see* Cutaneous respiration
        - temperature adaptation, 474
        - transcapillary fluid movement role, 1159
    - mucus secretion, 504
    - myoseptal connections, 553
    - patterns *see* Patterns, integumentary
    - veins, 1093
    - wound repair mechanism, 472
    - *see also entries beginning cutaneous; entries beginning skin*
- Integumentary system, functional morphology, **476–488**, 417–418
    - current knowledge, 417

  general morphology, 476, 477*f*
  integumentary extensions, 487
  microscopic features, 478
    dermis *see* Dermis
    epidermis *see* Epidermis
Intentional jamming, 704, 704*f*
  definition, 699
Intentional sound production *see* Sound production
Inter-spike interval histogram
  auditory afferent, 294, 295*f*
  definition, 292
Interaural level differences (ILD), 301
Interaural time differences (ITD), 301
Interception
  definition, 684
  prey calls, 689–690
Interference, constructive *see* Constructive interference
Interlamellar cell mass (ILCM)
  definition, 791
  regression during hypoxia, 797, 798*f*
    see also Gill remodeling
Interlamellar space (ILS), 1381, 1384*f*
  definition, 1381
Interlamellar vessels, 1099*f*, 1101*f*, 1102
Interleukins, osmosensing role, 1378
Intermediate filaments, definition, 504
Intermediate host
  definition, 777
  Diplostomun spathcaeum, 778–779, 779*f*
  Schistocephalus solidus, 780*f*, 780–781
Internal carotid artery, retia, 1125
Internal gestation, 671–672
  maternal care association, 672, 673*f*
  nutrient provision *see under* Parental nutrient provision
  see also Viviparity/viviparous fish
Interneurons, definition, 56
Interplexiform cells, 124*f*, 126
Interpulse interval, definition, 311
Interrenal cells/tissue, 652, 652–653, 1452
  corticosteroid release, 1475–1476, 1476*f*
  corticosteroid synthesis, 1451*f*, 1452, 1475
  definition, 649, 1474, 1542
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Intersex, definition, 1490
Interspecific, definition, 1573
Interstitial compliance, mammals *vs.* fish, 1159
Interstitial fluid
  definition, 1154, 1155, 1161
  reabsorption/loss *see* Transcapillary fluid movement
Interstitial growth, definition, 428
Interstitial space, definition, 1161
Interstitial–intravascular fluid compartments, **1154–1160**
  fluid movement between *see* Transcapillary fluid movement
  importance of relationship, 1155, 1156*f*
Intertesseral fibers, 429–431
  definition, 428
Intertidal habitats, **1959**
  environmental challenges, 1961
  habitat types, 1961
    estuarine, 1960*f*, 1962
    rocky, 1960*f*, 1961–1962
  oxygenation, adaptations to changes, 1962*f*, 1963
  pH and carbon dioxide, 1962*f*, 1964
  salinity, adaptations to changes, 1962
  temperature, adaptation to changes, 1962*f*, 1964
  tidal cycles, 1959*f*, 1961
    global distribution of rhythm types, 1960*f*, 1961
    tidal range (height), 1961
Intertidal species
  aquatic surface respiration, 766, 767*f*
  osmoregulation, 1962–1963
    elasmobranchs, 1963
    teleosts, 1963
  terrestrial behaviors, 770
  see also Euryhaline fish
Intestinal absorption, **1311–1321**
  definition, 1311
  dietary xenobiotics, 2071–2072
  ion/water, 1318, 1318*f*, 1320, 1370, 1371*f*
    see also Gut ion/acid–base regulation
  macronutrients, 1313
    carbohydrates, 1317, 1317*f*
    lipid *see* Lipid absorption
    protein *see* Protein absorption
  micronutrients, 1319
  osmoregulation trade-off, 1319*f*, 1320
  regionalization, 1319
  transepithelial transport pathways, 1311, 1312*f*, 1313*f*, 1322, 1324*f*
    active transport, 1311–1312
    diffusion, 1311, 1312*f*
    see also under Endocytosis
Intestinal air breathing, 1871
  circulation patterns, 1876*f*, 1884
Intestinal artery, 1086*f*, 1090–1091, 1133*f*, 1133–1134, 1134*f*, 1207
  see also Gastrointestinal circulation
Intestinal bicarbonate
  levels, marine teleosts, 1420*t*, 1422
  secretion, 1420–1421
    acid–base balance role, 1427
    direct role in water absorption, 1422, 1423*f*
    indirect role in water absorption via alkaline $CaCO_3$ precipitation, 1424, 1425*f*
Intestinal brake, 1300
Intestinal bulb, 1602*f*, 1604–1605
  definition, 1597
Intestinal epithelium, 1322, 1323*f*, 1324*f*
  barrier function *see* Gut, barrier function
  development, 1285–1286, 1286
    cell proliferation, 1286
    differentiation, 1287
    see also Gut development
  sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase), 1311–1312
    see also Intestinal absorption
  structure, 1312*f*, 1312–1313, 1313*f*, 1603*f*, 1605
  transport mechanisms *see* Intestinal absorption
Intestinal fluid, uptake, 1318, 1318*f*, 1320, 1370, 1371*f*
  see also Gut ion/acid–base regulation
Intestinal smooth muscle
  differentiation, 1287, 1289*f*
  morphology, 1270*f*, 1271
Intestinal veins, 1086*f*, 1092, 1092–1093
Intestine
  absorption *see* Intestinal absorption
  anatomy, 1269*f*, 1270, 1285*f*, 1603*f*
    see also Gut anatomy
  development *see under* Gut development
  epithelium *see* Intestinal epithelium
  ion regulation role *see* Gut ion/acid–base regulation
  proximal, definition, 1341
  secretions *see under* Gut secretion(s)
  species differences, 1272
  see also Gut
Intra-enterocytic resynthesis, lipids, 1316, 1316*f*
Intrabranchial septum, 1097*f*, 1098
  definition, 1095
Intracardiac neurons/neurotransmitters, 1067, 1069
  action potential effects, 1042
  lungfish, 1069
  ray-finned fish, 1069, 1070*f*
  teleosts, 1069, 1070*f*, 1071*f*
  see also Autonomic nervous system (ANS)
Intracellular degradation, mammalian hormones, 1191
Intracellular fluid
  % of lean-body mass, 1155
  definition, 1155
Intracerebroventricular, definition, 1509
Intramandibular joint (IMJ), 470
Intraperitoneal, definition, 1509
Intrasexual effects, males, hormonal pheromones, 1560*f*, 1561
Intraspecific, definition, 1573
Intravascular compliance, mammals *vs.* fish, 1159
Intravascular fluid, definition, 1154, 1155
Intrinsic, definition, 1060
Intrinsic barrier, gut, 1323, 1325*f*, 1326*f*
Intrinsic drumming muscles *see under* Drumming muscles
Intrinsic intersegmental connectivity, definition, 56
Intron, definition, 1998
Invagination, definition, 1006
Invertebrates, definition, 1986
Inward current, definition, 1038, 1717
Inward rectifier
  definition, 1038
  Kir2 channels, 1040, 1720–1721
Ion(s), intestinal uptake *see under* Intestinal absorption
Ion channels
  definition, 871, 1038, 1717, 1720
  gating, definition, 1718
  inhibition, by membrane cholesterol, 1722
  L-type calcium channel *see* L-type calcium channel (LTCC)
  ligand-gated, definition, 1718
  for membrane excitability, 1720
    see also Excitable membranes
  opening/closing, 1718*f*
  potassium
    calcium-activated, definition, 56
    inward rectifying (Kir2 channel), 1040, 1720–1721
  thermal compensation, 1723
  voltage-gated
    action potential generation, 1717*f*, 1718*f*, 1720
    definition, 1038, 1718
    opening/closing, 1718*f*
    voltage sensor protein domain, 1718, 1720
    see also Action potential (AP)
Ion regulation, 1467
  alkaline lake fish, 1386
  anadromous fish *see under* Anadromy/anadromous fish
  dominant *vs.* subordinate individuals, 653
  gill, 1467, 1467*f*
    freshwater fish *see* Freshwater fish, gill ion regulation
    osmorespiratory compromise *see* Osmorespiratory compromise
    seawater fish *see* Mitochondrion-rich cells (MRCs), seawater fish
    see also Gill ionocytes
  gut *see* Gut ion/acid–base regulation
  hagfish, 1349*t*, 1350, 1772*t*, 1773
  hormonal control *see* Osmoregulation, hormonal control
  skin role, 472*f*, 474, 1362, 1363*f*
  see also Osmoregulation
Ion transport
  active
    definition, 1429
    rectal gland, 1432–1433, 1433*f*, 1811*f*, 1812–1813
    transepithelial, intestine, 1311–1312
  carbonic anhydrase role, 905–907, 907*f*
  mechanisms in freshwater fishes *see* Mitochondrion-rich cells (MRCs), freshwater fish
  mechanisms in seawater fishes *see* Mitochondrion-rich cells (MRCs), seawater fish
  see also Ion regulation
Ion–damage–cytokine model, osmosensing, 1376, 1377*f*
  cytokine coordination of signal transduction, 1377*f*, 1378
    prolactin, 1378, 1378*f*
    TNF-α and interleukins, 1378

- Ion–damage–cytokine model, osmosensing (*continued*)
  - macromolecular damage contribution, 1377, 1377*f*
- Ionic composition
  - freshwater, 1349, 1360*t*, 1361, 1420*t*
  - plasma *see* Plasma ionic compositions
  - seawater, 1349, 1420*t*
- Ionic gradient, definition, 1354
- Ionic strength, effect on rectal gland activity, 1435
- Ionocytes, 1381–1382
  - definition, 1381
  - gills *see under* Gill ionocytes
  - pavement cells *see* Pavement cells (PVCs)
- Ionone ring
  - definition, 110
  - visual pigments, 111, 112*f*
- Iridescent coloration, 490, 490*t*, 694
  - definition, 416, 489
- Iridophores, 490*t*, 491, 492, 492*f*, 1971*f*, 1973
  - development, 1971*f*
    - genetic control, 1975
- Iris, 105, 105*f*
  - autonomic nervous system control, 86, 86*f*, 87*f*
- Iron, heme *see* Heme iron
- Iron acquisition, regulation of bacterial virulence factors, 2037
- Iron availability, regulation of bacterial virulence factors, 2037
- Irradiance, definition, 98, 150
- Islets, pancreatic, 1277, 1278*f*
  - *see also* Brockmann body
- Isodendritic core
  - definition, 772
  - sleep regulation neurons, 776
- Isoforms
  - carbonic anhydrase *see under* Carbonic anhydrase (CA)
  - definition, 899, 1359
- Isolume, definition, 497
- Isometric, definition, 1030, 1573
- Isosmotic, definition, 1348, 1772
- Isotocin, 675–676
  - definition, 670
- Isotonic, definition, 1030
- Isthmus
  - agnathan, 18*f*, 19
  - cartilaginous fish, 28, 34
  - lungfishes, 48*f*, 50
  - ray-finned fish, 41
- Iteroparity/iteroparous, definition, 613, 1800, 1921
- Iteropary, 614–615

J

- Jacob–Stewart cycle, 912
  - definition, 909
- Jamming avoidance response, 372
  - tuberous mechanisms, 372, 372*f*
- Japanese eel (*Anguilla japonica*), spawning area, 1940
- Jaw(s)
  - bony fish
    - lower, 439, 439*f*
    - upper, 435, 438*f*, 438–439
    - *see also* Bony fish cranial muscles
  - deformities, triploid fish, 2011*f*, 2012
  - durophagy adaptations, 435, 438–440
  - evolution, 598, 598*f*
  - feeding mechanics, 597, 600
  - motor control system, 62
  - protrusion, 598*f*, 600
    - definition, 463
    - premaxillary *see* Premaxillary protrusion
- Jawed craniates, peripheral nervous system, evolution/ developmental transformation, 13
- Jawless fish *see* Agnathan(s)
- Jobling, Malcolm, 1610
- Joule (J), 1669
  - definition, 1624
- Junctional sarcoplasmic reticulum (jSR)
  - cardiomyocytes, 1011, 1011*f*
  - definition, 1006
- Juvenile growth rates
  - triploid fish, 2014
  - tuna family, 1909
  - *see also* Growth rate
- Juvenile salmonids, dominance hierarchies, 650, 650*f*, 787
- Juxtaglomerular cells, 1396*f*, 1401, 1404*f*
  - definition, 1395

K

- Ka, definition, 1119
- Kármán gait, 583
  - definition, 581
- Kármán vortex street, 583
  - definition, 581
- *Katsuwonus pelamis* (skipjack tuna), muscle rete, 1124, 1124*f*, 1125*f*, 1126*f*
- Keeping afloat, buoyancy adaptations, 523, 524*f*
- Kelvin scale, 1690–1691
- Keratinization, definition, 471
- Ketone bodies, definition, 1807
- Ketone body metabolism, elasmobranchs, 1815
- 11-Ketotestosterone
  - definition, 662
  - formation, 630*f*, 1504*f*
  - sex change role, 665–666, 666*f*, 2053
  - sexual behavior role, 660
  - social dominance role, 653
  - spermatogenesis role, 629, 630, 631*f*, 660, 1500, 1503*f*
  - stress effects on release, 1547
  - sturgeon, 1802
  - *see also* Androgen(s)
- Key innovation, definition, 463
- Kidney(s), **1411–1418**
  - acid–base balance role, 1415, 1416*f*, 1417*f*
    - bicarbonate reabsorption, 1416, 1416*f*
  - diseases (human), fish models, 1991*f*, 1992
  - elasmobranchs, 1412, 1815–1816
  - evolution, 1396*f*, 1397
  - hagfish, 1407–1409, 1409*f*, 1412, 1773
  - histology, **1395–1410**
    - opisthonephros *see under* Opisthonephros
    - pronephros *see* Pronephros
  - lampreys, 1404–1406, 1409*f*, 1412, 1793*f*, 1796
  - nitrogen excretion, 1416
  - structure, 1411
    - aglomerular kidneys, 1411
    - glomerular kidneys, 1412, 1413*f*, 1414*f*
  - teleosts, 1412, 1413*f*, 1414*f*
  - urine formation, 1412, 1413*f*
    - reabsorption, 1413*f*, 1414
    - secretion, 1413*f*, 1415
    - ultrafiltration *see* Glomerular ultrafiltration
  - water flows, 1371
- Killifish
  - common (*Fundulus heteroclitus*)
    - aerial egg incubation, 1963
    - temperature acclimation, 1964
  - mangrove (*Kryptolebias marmoratus*)
    - gill remodeling, 800–801, 801*f*
    - H_2S avoidance, 1962
  - osmoregulatory abilities, 1963
- Kilopascal (kPa), definition, 520, 765
- Kinematic analysis, definition, 547
- Kinematic viscosity, 536
  - definition, 535
- Kinetic energy, definition, 1073, 1689
- King's Pool, pupfish *see Cyprinodon nevadensis mionectes*
- Kinocilium, 252–253, 254*f*, 255*f*, 255–256, 257*f*
  - definition, 336
  - neuromast, 336, 337*f*, 338*f*
- Kir2 (inward rectifier) channels, 1040, 1720–1721
- Kisspeptin
  - expression sites, 1506
    - unorthodox, 1507
  - gonadotropin regulation, 1505*f*, 1506
  - phylogenetic analysis, 1506, 1506*f*
  - receptors, 1506
- Knollenorgan, 357, 357*f*, 368*f*, 368–369, 369*t*, 379, 410, 701
  - definition, 350, 699
- KOR (κ opioid receptors), 96
- KRAS oncogene, 1991
- Krebs cycle, 1757*f*, 1758
  - definition, 1757–1758
- Krogh coefficient, 804
  - definition, 803
- Krogh radius (*R*), 1142, 1145
- *Kryptolebias marmoratus see under* Killifish
- Kynurenine
  - definition, 208
  - as olfactory stimulant, 210

L

- L-type calcium channel (LTCC), 1048, 1048*f*
  - temperature effects, 1050, 1050*f*
- Laboratory environment, magnetic fields, 727
- Labriform swimming, 567*f*, 570, 571, 572*f*, 572–573
  - definition, 564
- Labyrinth, definition, 244
- Labyrinth fish *see* Anabantoids
- lac Z, definition, 1998
- Lactate
  - conversion to ethanol *see* Ethanol production, anoxic
  - definition, 1888
  - formation/accumulation, 1760*f*, 1761–1762
- Lactate dehydrogenase (LDH), 1761
  - deep-sea fishes, 1957
  - osmobranchs, 1813
- Lactic acid, 1761
- Lacunae
  - definition, 428
  - tesserae, 431–432
- Lagena, 245–246, 246*f*, 252, 253*f*, 254, 255*f*, 256*f*
  - definition, 244
  - hair cell orientations, 257*f*, 257*f*
  - otolith, 256*f*, 257*f*
  - sensory epithelium, 253*f*, 256*f*
  - *see also* Vestibular system
- Lake char (*Salvelinus namaycush*)
  - bile acid release, 211
  - feeding behavior, 228*f*, 228–230
  - olfactory responses
    - bile acids, 211*f*, 211–212
    - electroencephalography, 214*f*
- Lake Michigan, USA, fisheries management, bioenergetics model application, 1677
- Lake whitefish (*Coregonus clupeaformis*)
  - feeding behavior, 228*f*, 228–230
  - olfactory responses
    - electroencephalography, 213, 214*f*
    - prostaglandins, 213, 213*f*, 214*f*, 215
- Lamellae, gill, 793, 804
  - aerobic swimming-associated improvement in oxygen loading, 1217
  - definition, 791, 796, 803, 1095, 1215, 1381
  - morphology, 805*f*, 1096*f*, 1097*f*, 1098, 1099*f*, 1100*f*, 1381, 1382*f*
  - *see also* Gill(s); Pillar cells
- Lamellae, olfactory *see* Olfactory lamellae
- Lamellar frequency, 804
  - definition, 803
  - determination, 804–806, 806*f*
    - *see also* Gill respiratory morphometrics
- Lamina propria
  - definition, 1322
  - role in intestinal barrier function, 1324*f*, 1324–1325, 1326*f*, 1328*f*
- Laminar flow, 979, 979*f*
  - definition, 535
- Laminar flow hood, definition, 1965

- *Lamna ditropis* (salmon shark), tracking, 1931f, 1932
- Lamnid sharks
  - endothermy, 1914
    - cranial, 1915, 1916
    - evolution, 1919
    - systemic, 1915
  - retia
    - muscle, 1124–1125, 1126t
    - visceral, 1122–1123, 1123f, 1124f
  - *see also* Sharks
- *Lampetra fluviatilis*, 17f
  - brain, 17f, 18f, 20f, 22f, 24f
- Lamphredin, 1784
  - definition, 1780
- Lampreys, **1779**, **1788**
  - acid–base regulation, 1792f, 1797
  - air breathing and, 1852–1853
  - Bohr effect, 923
    - evolution, 927
    - molecular mechanisms, 925f, 926
  - brains, 17, 17f
    - forebrain, 21
      - diencephalon, 21, 22f
      - secondary prosencephalon, 23, 24f
    - hindbrain, 17, 18f
    - midbrain, 20, 20f
    - research, 17
  - carbon dioxide excretion, 911–913, 1792f, 1794–1795
  - circulation, 1792f, 1793
  - development, 1785
  - electroreceptive end buds, 350
    - definition, 350
    - development, 410
  - environmental challenges, 1791
  - environmental physiology, **1788**
  - evolutionary aspects, 1781, 1791
  - eyes, 17
    - visual pigment evolution, 111
  - feeding, 1781, 1783f, 1784
    - lamphredin role *see* Lamphredin
  - gill(s)
    - gap junctions between pavement cells, 1382
    - intercalated cell (IC) types, freshwater, 1384f, 1387
    - pouches, 1790f, 1791–1792
    - seawater chloride cells (swMRCs), 1384f, 1387
  - Great Lakes invasion, 1785
  - growth and metabolism, 1785
  - hagfish *vs.*, 16, 17f
  - hypoxia tolerance, 1795–1796
  - integumentary system, 477–478, 478f, 481
    - dermal rays, 487
    - dermis, 478f, 484
    - epidermal granular cells, 478f, 483–484
  - kidneys, 1404–1406, 1409f, 1412, 1793f, 1796
  - larval *see* Ammocoete(s)
  - lateral line system, 336, 344, 345f
  - life cycle, 16, 1779f, 1781, 1788f, 1791
  - locomotion, 1782
  - metamorphosis, 1779f, 1781, 1781f, 1782f, 1783
  - nitrogenous waste production/excretion, 1797
    - ammonia, 1797
    - urea, 1797
  - oral disk *see* Oral disk, lamprey
  - osmoregulation, 1796
    - freshwater, 1349t, 1352, 1793f, 1794f, 1795f, 1796
    - hormonal regulation, 1797
    - seawater, 1349t, 1351, 1795f, 1796
  - parasitic, 1783f, 1784
  - physical features, 16, 17f
  - physiology, **1779**
  - pineal photoreception, 161
  - pituitary gland structure, 1459, 1461f
  - reproduction, 1784
  - respiration, 1789f, 1790f, 1791
  - sea *see* Sea lampreys
  - skeletal muscle system, 1782
  - species, 1781
  - taxonomy, 16
  - upstream migration, 1784
  - velum *see* Velum
  - *see also* Agnathan(s)
- Land use, environmental hypoxia contribution, 1748f, 1749
- Landmarks, orientation and, 721, 722, 724
- Langrangian coherent structures (LCSs), 543
  - definition, 535
- Lanternfish *see* Myctophidae
- Large field motion, detection/analysis, behavioral studies, 145f, 147
- Large myelinated club endings (LMCEs), 70f, 71
  - *see also* Mauthner cells
- Largemouth bass (*Micropterus salmoides*), gill remodeling, 801
- Larva
  - definition, 336
  - developmental migration, Pacific salmon, 1947
  - eel *see* Leptocephali
  - lamprey *see* Ammocoete(s)
  - zebrafish *see* Zebrafish (*Danio rerio*), larvae
- Larval electric organ, 413f, 413–414
  - definition, 409
  - *see also* Electric organ development
- Lateral, definition, 419
- Lateral lemniscus, 287f, 289, 290f
  - definition, 283
- Lateral line
  - central pathways, 284f, 284–285, 285f
    - anatomy *see under* Auditory/lateral line CNS, anatomy
  - definition, 236, 336, 1921
  - development, bony fishes, 339, 342f, 343f
  - evolution
    - brain circuits, 283
    - sensory organs, 283
  - function *see* Lateral line function
  - innervation, 336–337, 337f, 337–338, 340f
    - active movement and, 332
    - studies, 329
  - neuroethology, **329–335**
    - *see also* Lateral line function
  - orientation role, 723, 724
    - blind Mexican cavefish, 722–724, 724f
  - sensory receptor organs *see* Neuromast(s)
  - structure, **336–346**
    - chondrichthyan fishes, 341, 344f
    - jawless fishes, 344, 345f
    - variation among bony fishes, 338, 341f, 342f
- Lateral line function, **329–335**, 242, 336
  - feeding context, 333, 333f, 334f
  - movement and response characteristics, 330
    - canal neuromasts, 331, 332f
    - rheotaxis, 330, 331f
    - superficial neuromasts, 330f, 331f, 332f, 337–338
  - pelagic fish, 1892
  - studies, 329, 334–335
    - challenges, 329, 330f
    - historical, 329, 334–335
    - neuroethology approach, 329–330
- Lateral line nuclei, 286f, 288
  - cross-talk with auditory systems, 288
  - higher-order, 284f, 289
- Lateral mesencephalic complex (LMC) *see under* Auditory/lateral line CNS, anatomy
- Lateral pallium
  - definition, 707
  - learning/memory role, 710
- *Latimeria*, 46
  - brains, 52
    - forebrain, 47, 53f, 54f, 54–55
    - hindbrain, 47, 53f, 54, 54f
    - histological features, 52–53, 54f
    - lungfish brain *vs.*, 47, 47f
    - macroscopical anatomy, 47, 47f, 53f
    - midbrain, 47, 53f, 54, 54f
  - outflow tract, heart, 1016–1018, 1019f
- Lattice compression, sarcomere length–tension relationship and, 1065
- LD_{50}, definition, 2032
- Leaching, consideration in digestibility studies, 1600
- Lead (Pb), biological effects and toxicodynamics, 2066
- Leaky capillaries, 1159
- Lean-body mass
  - body water and, 1155
  - definition, 1154
- Learning and memory, **707–712**
  - applied contexts, 711
    - self-feeding, 711, 712f
  - brain areas involved, 708, 708f
    - amygdala, 709–710
    - cerebellum, 708–709, 709f
    - hippocampus, 709–710
    - telencephalon, 709
  - different forms, 707
  - factors affecting, 710, 711f
  - fish behavior–fish physiology interrelations, 647
  - magnetic field discrimination, laboratory conditioning, 727–728, 728f
  - noxious stimulus avoidance, 709, 709f, 714, 715f
  - optic tectum capabilities, 142
  - sensitive phases for learning/imprinting, 710
  - spatial *see* Spatial learning
  - *see also* Time–place learning (TPL)
- Lecithotrophy, 679
  - definition, 678
- Lemnothalamic, definition, 37
- Lemnothalamus, ray-finned fish, 37, 42
- Lemon shark (*Negaprion brevirostris*)
  - hearing sensitivity measurement, 266–267, 278–279
  - sound frequency discrimination, 266, 281
- Lens
  - accommodation, 106
  - deep-sea fish, 170f, 173, 173f
  - focal length in relation to body size/feeding habitats, 107
  - monofocal, deep-diving species, 108
  - multifocal, 104f, 106
  - optical properties, 104f, 105
  - structure, 105, 105f
  - suspension, 106
- Lens pad, deep-sea fish, 170f, 171f, 172, 178f
- Lens replacement, blind cavefish eye rescue, 1847
- Lentiform body, 1089
  - definition, 1085
- *Lepidosiren*, 46
  - *see also* Lungfishes
- Lepidotrichia *see* Fin rays
- *Lepisosteus osseus* (longnose gar), brain, 38f, 40f, 43–44
  - *see also* Ray-finned fish, brains
- *Lepomis macrochirus* (bluegill sunfish), wake, 541, 541f, 542f
- Leptin
  - appetite suppression, 1346, 1485–1486, 1512t, 1513
  - fish gut role, 1346
- Leptocephali, 1938f
  - definition, 520, 1937
  - geographical distribution, 1937f
  - metamorphosis to glass eels, 1940
  - migration, 1938f, 1939, 1940
  - neutral buoyancy, 521
- Lethal dose, definition, 1695
- Leukemia
  - fish models for studying, 1990f, 1991
  - oncogenes associated, 1990f, 1991
- Leukocrit, 985, 988, 990f
  - definition, 984
- Leukocytes, 988, 989f
  - cortisol effects, 1549f, 1550–1551
  - counting/measures of, 989
  - definition, 984, 984
  - function, 988
  - granulocytes *see* Granulocytes
  - lymphocytes *see* Lymphocytes

- Leukocytes (*continued*)
  - monocytes *see* Monocytes
  - percentage in blood *see* Leukocrit
  - red blood cells *vs.*, 988
  - skin, 473
  - thrombocytes *see* Thrombocytes
  - types, 989f
- Leukophores, 490t, 491, 1971f, 1973
  - development, 1971f
    - genetic control, 1975
- Leydig cells, 617, 617f, 617–618, 1502, 1503f
  - gonadotropin receptors, 628, 1507
  - steroidogenic pathway, 629, 629f, 630f
- Liem's paradox, 1583f, 1585
- Life history stages
  - definition, 1738
  - *see also* Ontogeny
- Lift, hydrodynamic *see* Hydrodynamic lift
- Ligand, definition, 1474, 1703
- Ligand-activated nuclear hormone receptor
  - corticosteroid *see* Corticosteroid receptors
  - definition, 1474
- Ligand-gated channel, definition, 1718
- Light
  - attenuation in water, wavelength envelope, 152, 153f, 155
  - irradiance (surface incident radiation), definition, 98, 150
  - radiance, definition, 99, 150
  - spectral reflectance, definition, 99, 150
  - *see also* Vision
- Light environment, deep sea, 167, 167f
  - luminescence and, 167, 168f, 498
- Light-evoked responses, pineal, 163
  - *see also* Pineal photoreception
- Light organs, 497
  - *see also* Bioluminescence
- Light–dark cycle, phase shifts, 739, 739f
- Limnetic, definition, 744
- Line *see* Cell lines
- Linear acceleration, 246
- Linuron contamination, olfactory effects, 2081
- Lipases, 1281, 1281t, 1304, 1305, 1305f, 1305t, 1306f, 1308–1309
  - pathogenesis role, 2035
- Lipid(s)
  - absorption *see* Lipid absorption
  - accumulation, enterocytes, 1329–1330
  - ATP production role, 952f, 952–953, 955–956
  - definition, 951, 1665, 1807
  - dietary, 1308, 1308f
    - digestion *see* Lipid digestion
  - digestive efficiency, 1600t, 1601
  - inclusions, deep-sea fishes, 1957
  - membrane, 1726f, 1727
    - homeoviscous adaptation *see* Homeoviscous adaptation
    - lipid rafts *see* Lipid rafts
    - *see also* Cell membrane(s); *specific lipids*
  - metabolism *see* Lipid metabolism
- Lipid absorption, 1315, 1316f
  - intestinal regionalization, 1320
  - lipid breakdown products, 1315–1316
  - micelle formation, 1315
  - uptake across brush-border membrane, 1316
    - fatty-acid-transporting proteins, 1316
    - intraenterocytic resynthesis, 1316
    - passive diffusion, 1316
- Lipid-dependent proteins, cell membrane, 1726
- Lipid digestion, 1306f, 1308, 1309f
  - elasmobranchs, 1813
  - *see also* Lipases
- Lipid metabolism
  - cold-induced shifts, 1647
  - elasmobranchs, 1815
- Lipid rafts, 1722, 1728, 1729t
  - definition, 1718
- Lipolytic, definition, 1311
- Lipophilic, definition, 1437
- Lipophilic hormones, 1191
  - definition, 1190
- Lipophilicity, definition, 2069
- Lipopolysaccharides, 2036
- Lipoproteins, elasmobranchs, 1815
- Lipovitellin, 637, 637f
- Livebearing fishes
  - vitellogenesis, 644
  - *see also* Ovoviviparous fish; Viviparity/viviparous fish
- Liver
  - anatomy, 1285f
  - bile production, 1303
  - blood supply, 1134
  - development
    - determination of anterior-to-posterior position, 1287
    - differentiation, 1290, 1290f
  - elasmobranch, buoyancy role, 1809f, 1810
  - fish *vs.* mammal, 1290, 1290f
- Lobe-finned fish *see* Sarcopterygians
- Local contractions, gut, 1293f, 1294
- Local effect, definition, 2078
- Locomotion, **516–519**
  - circadian rhythmicity, 737, 737f
  - current knowledge, 518
  - historical perspective, 517, 517f, 518f
  - lampreys, 1782
  - motor control systems *see under* Motor control systems
  - motor neuron development *see* Motor neurons
  - skin role, 472f, 473
  - *see also* Exercise; Skeletal muscle; Swimming
- Locomotor control *see under* Motor control systems
- Long QT syndrome, 1991–1992
- Long-range migration, 720
  - orientation mechanisms, 720, 723
  - *see also* Migration
- Long terminal repeat (LTR) retrotransposons, 1983
- Long-wave sensitivity, deep-sea fish visual pigments, 179, 181f
- Longitudinal chromatic aberration (LCA), 103–104, 104f
- Longitudinal spherical aberration (LSA), 103–104, 104f
- Longnose gar (*Lepisosteus osseus*), brain, 38f, 40f, 43–44
  - *see also* Ray-finned fish, brains
- Loss-of-balance rocking, as pain response, 715, 718t
- Loss of function, definition, 1359
- Loss-of-function studies (reverse genetics), 1822
  - antisense technology approaches, 1822, 1988
  - targeting-induced local lesions in genomes (TILLING), 1823, 1987–1988
  - zinc finger nucleases, 1823, 1987–1988
- Loss of righting response (LRR), 1698–1699, 1701
  - definition, 1695
- Loud vocalizers, 685f, 685–686, 686f
- Low-density lipoprotein (LDL), elasmobranchs, 1815
- Lower jaw, bony fish, 439, 439f
- Luciferase, definition, 1998
- Lumen, definition, 1229, 1284
- Luminescence, definition, 497
- Luminescent tissues, 497
  - *see also* Bioluminescent fish
- Lunar phasing, migratory fish, 1926
- Lunar rhythms, 742
- Lung, definition, 1850, 1861
- Lung, fish, 1863
  - circulation *see under* Air-breathing fish, circulatory adaptations
  - epithelial and structural complexity, 1861f, 1863f, 1864f, 1865, 1865f, 1866f
  - evolutionary history, 1851f, 1853–1854, 1861f, 1862
    - gas bladder relationship, 1861f, 1863f, 1865
  - morphology, 1863
    - inter-species variation, 1861f, 1863f, 1864f, 1865f, 1866, 1866f
  - ventilation, 1872f, 1873
- Lungfishes, 46
  - ampullary organs, 353, 353f
  - autonomic nervous system, 83
    - cardiac control, 85, 85f
    - chromaffin tissue and, 83–85, 85f
    - intracardiac neurons/neurotransmitters, 1069
  - brains *see* Lungfishes, brains
  - cardiac anatomy, 1068
    - outflow tract, 1016–1018, 1019f
  - cardiorespiratory interactions, 1187
  - circulatory patterns, 1876f, 1878t, 1879f, 1881
  - lymphatic system, 1165
  - pulmonary vasomotor segment *see* Pulmonary vasomotor segment (PAVS)
  - specific dynamic action, 1609t, 1611f, 1612
- Lungfishes, brains, **46–55**
  - forebrain, 49f, 51
    - hypothalamus, 47, 48f, 49f, 51
    - preoptic area, 49f, 51–52
    - telencephalon, 47, 48f, 49f, 52
  - hindbrain, 48, 48f, 49f
    - cerebellum, 47, 50
    - isthmus, 48f, 50
  - histological features, 48
  - *Latimeria* brain *vs.*, 47, 47f
  - macroscopical anatomy, 47, 47f
  - midbrain, 48f, 51
- Luteinizing hormone (LH), 1504
  - definition, 1500, 1553
  - preovulatory surge, 1554f, 1555–1556
  - regulation of testicular function, 628, 628f
  - sexual behavior role, 660, 661
  - sturgeon, 1802
  - *see also* Gonadotropin(s)
- Lymph, 1162f
  - definition, 1161
- Lymph vessels, 1161, 1162f
- Lymphatic circulation
  - definition, 1161
  - *see also* Secondary circulation
- Lymphatic micropumps, lungfish, 1165
- Lymphatic system, 1161
  - anatomy, **1161–1168**
  - early studies, 1162, 1163f
  - functions, 1161
    - protein removal from interstitium, 1156, 1156f
  - *see also* Secondary circulation
- Lymphocytes, 989, 989f
  - cortisol effects, 989, 991
  - definition, 984, 1542
  - elasmobranchs, 1816
  - inter-species variability, 990f, 991
- Lysophospholipid, definition, 1725
- Lysozyme, definition, 1542

M

- M-band
  - cardiomyocyte myofibril, 1008–1009, 1009f
  - definition, 1006
- Macromolecular damage, osmosensing role, 1377, 1377f
- Macronutrients
  - absorption *see under* Intestinal absorption
  - definition, 1311, 1665
- Macrophage, definition, 1542
- Macrophage-like cell line
  - definition, 1966
  - piscine, 1965f, 1967
- Macula neglecta, 252, 253f
  - sharks and rays, 262, 264, 264f, 265f
- Magnesium carbonate ($MgCO_3$), intestinal precipitation, 1420–1421, 1424, 1425f
- Magnetic cues, navigation, 721, 723
- Magnetic field(s), 726
  - detection *see* Magnetic sense
  - Earth, 726–727, 727f
    - navigation role, 732, 734f

  laboratory environment, 727
Magnetic field discrimination, laboratory conditioning, 727–728, 728*f*
Magnetic moment
  definition, 726
  magnetite, 730
Magnetic sense, **726–735**
  behavioral responses to magnetic fields, 727
    cardiac conditioning, 728, 729*f*
    laboratory conditioning, 727, 728*f*
    orientation to magnetic direction, 727
  future research directions, 732
  magnetite-based magnetoreceptor cells and, 726, 730, 731*f*, 732, 733*f*
  neural responses to magnetic fields, 728
    induced electrical signals in ampullary electroreceptors, 729
    trigeminal nerve of teleosts, 728, 730*f*
  neuroanatomy, 732, 733*f*
Magnetite, crystals, 730
Magnetite-based magnetoreceptor cells, 726, 730, 731*f*, 732, 733*f*
Magnetoreception
  elasmobranch, 726, 1808
  see also Magnetic sense
Maintained discharge
  definition, 123
  ganglion cells *see under* Retinal ganglion cells
Maintenance requirement, definition, 1665
Makaira indica (black marlin), swimming speed, 1581
Makaira nigricans see Blue marlin (*Makaira nigricans*)
Male calling, 689, 690
Male fish
  hormonal pheromones, 1560
    intrasexual effects, 1560*f*, 1561
  parental care *see* Paternal care
  sexual behavior
    expression of maturity/readiness, 658
    hormonal control, 660
    nest construction, 657, 657*f*
Male gametes
  final maturation, 632
  see also Spermatogenesis
Male readiness, 658
Male–male effects, hormonal pheromones, 1560*f*, 1561
Malignant tumor
  definition, 1971
  see also Tumor(s)
Mammals, 1155
  Bohr effect, 923
    molecular mechanisms, 924, 924*f*, 925*f*
  fish *vs.*
    cardiomyocyte dimensions, 1007, 1007*t*
    cardiomyocyte excitability, 1723
    circulation, 1877*f*, 1879
    gut *see under* Gut
    liver, 1290, 1290*f*
    red blood cells (RBCs), 993, 995
    tissue contributions to metabolic rate, 951–952, 952*t*
    transcapillary fluid movement, 1159
    venous system anatomy, 1112, 1113*f*
  fluid compartments, 1155
  hormone inactivation *see* Hormone inactivation, mammals
  monotreme, electroreceptive organs, 358
  sleep, 772
    duration, 774
    stages, 773
  see also Animal(s)
Maneuverability, **575–580**
  definitions, 575, 576*f*
  effectors, 576
    body form, 579
    fish size, 578
    types of maneuvers, 577
  fast start performance and, 592*t*, 593, 594*f*
  measurement methods, 576
  see also Paired fin(s)
Maneuvers
  definition, 575
  types, 577
    see also specific types
Mangrove killifish *see under* Killifish
Mangrove swamps, 1962
  definition, 1960
Map, spatial, definition, 720
Marine fishes
  freshwater species *vs. see under* Freshwater fish
  gill morphometrics
    fast-swimming species, 807*f*, 808, 808*f*
    fishes of intermediate activity, 808*f*, 809
    sluggish species, 808*f*, 809
  gut ion regulation *see under* Gut ion/acid–base regulation
  ion transport mechanisms, mitochondrion-rich cells *see* Mitochondrion-rich cells (MRCs), seawater fish
  osmoregulation *see under* Osmoregulation
  osmorespiratory compromise, 1391
  osmotic challenges, 1354, 1367, 1420, 1467
  plasma ion composition, 1349*t*
  see also Seawater; *individual species*
Marlin
  blue *see* Blue marlin (*Makaira nigricans*)
  satellite tracking, 1932, 1933
  swimming speed, black marlin (*Makaira indica*), 1581
Marr, David, robot design considerations/questions, 605, 606, 607–608
Masking, sound *see under* Sound
Mass exponent, definition, 1566
Mass mortality, hypoxia-associated, 1749
Mastication, 1605
Mate choice, role of visual communication, 693, 697–698
Maternal care, 672, 673*f*
  association with internal gestation, 672, 673*f*
  see also Parental care
Maternal stress, offspring development effects, 1521
Maturation-inducing steroid (MIS)
  definition, 1553
  as pheromones, 1556–1557, 1562
  synthesis, 1555–1556
Maturational proteolysis, yolk proteins (YPs), 641, 643*f*, 644*f*
Mature oocyte, HPI-axis transcripts, 1453
Mauthner, Ludwig, 66, 67*f*
Mauthner cells, **66–72**
  action potential, effect of cold, 1721, 1722
  agnathans, 20
  Club endings, 70*f*, 71, 74*f*, 75–76, 78
  definition, 56, 516, 587
  discovery, 66, 67*f*
  features, 67, 67*f*
  functional structure of network, 74*f*, 75
  functions, 587–588, **73–79**, 588*f*
    associated cellular specializations, 77, 77*f*
    escape responses *see under* Escape responses
    prey capture, 74*f*, 75
    voluntary behavior, 74*f*, 75
  inhibitory input, 77*f*, 78
  as model vertebrate neuron, 68
    inhibitory mechanisms, 68, 68–71, 69*f*
    mixed electrical/chemical synapses at primary auditory afferents, 70*f*, 71
    transmitter release mechanisms, 71
  sensory inputs, 67–68, 68*f*, 74*f*, 75–76
    cellular specializations, 77*f*, 78
  time constant, 71–72, 77
Maximal metabolic rate (MMR), 1765
  definition, 812, 1588, 1764
  hypoxia effects, 1767*f*, 1767–1768
    see also Metabolic rate suppression
  measurement, 812
  standard metabolic rate *vs.*, 1765
Maximum cardiac function, temperature effects, 1200, 1201*f*, 1202*f*
Maxwell–Boltzmann distribution
  definition, 1689
  temperature effects, 1691*f*, 1692
Meal composition, SDA impact, 1613*f*, 1614
Meal energy, SDA impact, 1613*f*, 1614
Meal size, SDA impact, 1613*f*, 1614
Meal type, SDA impact, 1614
Mean cell hemoglobin concentration (MCHC), 988, 2009
  definition, 984
  factors affecting, 988
    erythropoiesis, 988
    RBC swelling, 988
  hematocrit and, 986, 986*f*, 988
  RBC swelling, 988
  tuna, 1911
  values, 988
Mean circulatory filling pressure (MCFP)
  blood volume and, 1113, 1114, 1114*f*, 1116, 1116*f*, 1117
  definition, 981, 1111
  physiological factors affecting
    exercise, 1115
    freshwater/saltwater adaptations, 1115–1116
    hormones/drugs, 1114–1115
    temperature, 1115
Mechanical stimuli, gustatory responses, 224, 225*t*
Mechanoreception
  anthropogenic disruption, 785, 785*f*
  elasmobranch, 1809
  see also Lateral line
Mechanoreceptive sensory organs, placodes, 245, 245*f*
Mechanoreceptors
  definition, 854
  respiratory rhythm generation *see under* Respiratory rhythm generation
Mechanosense, definition, 329
Mechanosensory, definition, 336, 1373
Mechanosensory lateral line *see* Lateral line
Meckel's cartilage
  cranial muscle insertions
    batoids, 455, 457*t*
    holocephalans, 461*t*
    sharks, 450, 452*t*
  definition, 449
Medaka
  definition, 2046
  germ cell migration, 2049*f*, 2050–2052
  as model organism, 1986*f*, 1987
    see also Fish model(s)
  sex determination, 2028, 2052
  Y chromosome evolution, 2029
Medial, definition, 419
Medial longitudinal fasciculus
  agnathan, 18*f*, 20, 20*f*
  ray-finned fish, 40*f*, 41
Medial pallium
  definition, 707
  emotional learning role, 709
Median eminence, definition, 1457
Median fins
  maneuverability role, 576*f*, 576–577
  skeletal supports, 443–445, 445*f*
Median–paired fins (MPFs)
  maneuverability role, 576*f*, 576–577
  see also Paired fin(s)
Median–paired fins (MPF) swimmers, 603
  robotic fish, 603
Medulla oblongata
  definition, 854, 973, 1215
  respiratory neuronal activity, 855*f*, 856–857
    see also Respiratory rhythm generation
Meganuclease, definition, 1998
MEGY (M-ENK-Gly-Tyr), 89–91, 90*t*

Meiotic division
  malfunction in triploid fish, 2010, 2011
  oogonia, 1500, 1501*f*
  spermatogenesis *see under* Spermatogenesis
Melanin(s), 490*t*, 491
  synthesis, 491, 1972*t*, 1974*f*, 1975
Melanin-concentrating hormone (MCH), 494
  definition, 489
Melanization-inhibiting factor, 494
Melanization-stimulating factor, 494
Melanocortin receptor 2 (MC2R), 1452
  definition, 1450
Melanocortins, definition, 699
Melanocytes, absent, fish models, 1995
Melanogenesis, 491, 1972*t*, 1974*f*, 1975
Melanogrammus aeglefinus (haddock), spawning behavior, 316–317, 318*f*
Melanoma
  fish models, 1994*f*, 1995
  mutations associated, 1995
  transgenic models, 1977, 1977*f*, 1978, 2004*f*, 2005
  Xiphophorus, 1976*f*, 1977, 2004*f*, 2005
Melanophore-stimulating hormone (MSH), 494
  appetite suppression, 1485, 1485*f*
  definition, 489, 1542
  stress response role, 1518, 1539
Melanophores, 490*t*, 491, 491*f*, 492–493, 1971*f*, 1973
  definition, 1457
  development, 1971*f*
    genetic control, 1974
  melanin synthesis, 491, 1972*t*, 1974*f*, 1975
  peritoneal, 490
  regulation, 493
Melatonin, 162, 165
  chromatophore response and, 741
  color change regulation and, 494
  definition, 98, 159, 772
  diurnal changes, 494
  food intake effects, 1486
  production, 741
  sleep promotion, 738, 775
    zebra fish, 740
Membrane, 1725
  cardiomyocyte, 1008, 1008*f*
  cell *see* Cell membrane(s)
  excitability *see* Excitable membranes
Membrane bone
  definition, 434
  see also Bony fish skeleton
Membrane order, definition, 1725
Membrane proteins, 1725, 1726
  osmosensors, 1376
  transport role
    intake and excretion of xenobiotics, 2072, 2072*f*, 2074
    ions *see* Ion channels
    water transport, 1367–1368
      see also Aquaporins
    see also Transmembrane proteins; *specific membrane proteins*
Memory
  duration, factors affecting, 710
  spatial, 722, 722*f*, 724
  see also Learning and memory
Mercury (Hg), biological effects and toxicodynamics, 2066
Mesangial cell, definition, 1411
Mesangium, definition, 1395
Mesencephalic locomotor region (MLR), 57–58, 58*f*, 59*f*
Mesencephalon *see* Midbrain
Mesenchyme, definition, 1457
Mesoderm
  definition, 1284
  specification, 1285
Mesopelagic, definition, 98, 166, 497
Mesopontine, definition, 56
Messenger RNA (mRNA), definition, 2055
Messenger RNA (mRNA) transcripts, definition, 1354
Metabolic cold adaptation (MCA), 1571
  see also Cold acclimation/adaptation
Metabolic intensity, definition, 829
Metabolic rate (MR), 1566
  basal *see* Basal metabolic rate (BMR)
  deep-sea fishes, 1957
  definition, 829, 1573, 1665, 1764
  desert pupfish, 1838*f*, 1838*f*, 1839
  as dominance predictor, 651
  embryos and alevins, effect of egg size, 1573*f*, 1575, 1576
  enzyme activity, variation with muscle type, 551
  mathematical relationship to body size, 1574–1575, 1575*f*, 1577
  maximal *see* Maximal metabolic rate (MMR)
  measurement and estimation, 847, 1566, 1765
    active (maximum) metabolic rate (AMR), 812, 812
    large fish, 1579*f*, 1580
    multiple activity allocation, 1906*f*, 1907
    see also Respirometers
  nutritional influences, cultured fish, 1672, 1672*f*
  ontogenetic changes after hatching, 1575*f*, 1577
    hypotheses for observed changes, 1578
  oxyconformers and oxyregulators, 1961, 1963
  pelagic fish, 1893
  routine *see* Routine metabolic rate (RMR)
  standard *see* Standard metabolic rate (SMR)
  suppression *see* Metabolic rate suppression
  thermal window (temperature thresholds), 1741–1742, 1742*f*, 1744
  tissue contributions, fish *vs.* mammals, 951–952, 952*t*
  see also Aerobic scope; Cellular respiration; Respiratory metabolism
Metabolic rate suppression, **1764**
  anoxic crucian carp *see under* Crucian carp
  contributors
    behavioral changes, 1767*f*, 1768
    biochemical changes, 1767*f*, 1768
    physiological changes, 1767*f*, 1768
  definition, 1764
  energy conservation, 1767
  hypoxia tolerance and, 1767, 1769
Metabolic requirements, gill morphology and *see under* Gill respiratory morphometrics
Metabolic scaling, 1570
  definition, 1566
Metabolic scope for activity *see* Aerobic scope
Metabolic stress, **1534**
  catecholamine-mediated, 1536, 1536*f*
  cortisol-mediated, 1538
  hypoxia-induced, 1765*f*, 1766
Metabolism
  cellular *see* Cellular metabolism
  definition, 1665
  effect of social status, 653
  environmental influences *see* Metabolism, environmental influences
  intermediary, elasmobranchs *see under* Elasmobranchs
  lampreys, 1785
  stress effects *see* Metabolic stress
  triploid fish, 2012
  see also Metabolic rate (MR)
Metabolism, environmental influences, **1645**, 1580–1581
  aerobic metabolic pathways, 1646
    cold-induced shifts to lipid metabolism, 1647
    mitochondrial functioning, 1646, 1646*f*
  anaerobic metabolic pathways, 1648, 1648*f*
    cold acclimation, 1649
    extension of energetic limits, 1649*f*, 1650
Metabolites, vascular resistance control, 1106
Metabolon, definition, 1419
Metabotropic, definition, 56
Metacercariae, *Diplostomun spathcaeum*, 778–779, 779*f*, 780
Metal(s)
  biological effects and toxicodynamics, 2064
    aluminium, 2065
    cadmium *see* Cadmium (Cd)
    chromium, 2065
    copper *see* Copper toxicity
    lead, 2066
    mercury, 2066
    nickel, 2066
    selenium, 2066
    silver *see* Silver (Ag) toxicity
    thallium, 2067
    tin, 2067
    zinc *see* Zinc toxicity
  biological fate and toxicokinetics, 2063
    absorption, 2062*f*, 2063
    bioaccumulation, bioconcentration, and biomagnification, 2064
    biotransformation, 2064
    distribution, storage, and sequestration, 2062*f*, 2064
    excretion, 2064
  classification, 2061*f*, 2062
    essential *see* Essential metals
    heavy *see* Heavy metals
    light, 2063
    nonessential *see* Nonessential metals
  dose–response, 2061*f*, 2062–2063
  environmental fate and bioavailability, 2063
Metallochaperones (MCs), 2062*f*, 2063, 2064
  definition, 2062
Metalloproteases, pathogenesis role, 2033
Metallothioneins (MTs), 2062*f*, 2063, 2064
  definition, 1515, 2062
Metamere/metameric, definition, 6
Metamorphosis, ammocoetes (larval lampreys), 1779*f*, 1781, 1781*f*, 1782*f*, 1783
Metarterioles, definition, 1104
Metastasis, definition, 1971
Methemoglobin, 892
  definition, 887
N-Methyl-D-aspartate receptor *see* NMDA receptor
Mexican tetra *see Astyanax mexicanus*
Micelle formation, 1315
Michaelis–Menten constant, apparent (K_m), 1703*f*, 1704
Micro-RNA (miR)
  definition, 6
  importance in neuron/sensory cell development, 9
Microarray(s), 2056*f*, 2057
  see also Global gene expression profiling
Microarray analysis, 2056*f*, 2057
  growth hormone transgenesis in fish, 2019
Microbiota, gut *see under* Gut
Micronutrients
  absorption, 1319
  definition, 1311, 1665
Micropterus salmoides (largemouth bass), gill remodeling, 801
Micropumps, lymphatic, lungfish, 1165
Microscopy *see* Cell imaging techniques (microscopy)
Microspectrophotometry
  definition, 98, 110, 116
  visual pigments, 111, 119*f*, 119–120
Microvascular units (MVU)
  blood flow velocity, 1143
  definition, 1142–1143
  skeletal muscle access, 1149–1150
Microvillar olfactory neurons, 195, 200*f*, 201*f*, 214–215
Microvilli
  definition, 1229, 1322, 1354
  enterocyte *see under* Enterocytes
Midbrain
  agnathan, 20, 20*f*
  cartilaginous fish, 27*f*, 31, 31*f*
  definition, 1, 46

  electrosensory processing
    ampullary system, 363–364, 364f
    tuberous system *see under* Tuberous electrosensory system, physiology
  influence on respiratory rhythm generation, 858, 859f
  Latimeria, 47, 53f, 54, 54f
  lungfishes, 48f, 51
  ray-finned fish, 3, 4f, 40f, 41
  see also Optic tectum (OT); Torus semicircularis (TS)
Midgut, anatomy, 1269f, 1270
Midshipman
  bioluminescence, 499, 500f
  plainfin *see* Plainfin midshipman
Migrating motor complexes (MMCs), 1293–1294
Migration, 720, **1921**
  animal research, technological progress, 1929
  anthropogenic impacts, 1926
    barriers, 1653f, 1654, 1925f, 1926
      see also Dams
    climate change, 1927
    salmon, 1950f, 1951
    toxicants, 1926
  classification, 1921f, 1922
  definition, 720, 1921, 1922
  eels *see* Eel migration
  energetics, 1924
  environmental influences, 1925
  lampreys, 1784
  motor control systems, 62
  orientation/navigation mechanisms, 720, 723, 1923
    fluvial habitats, 1924
    geomagnetic cues, 1923
    lunar cues, 1926
    olfactory cues, 1924
    open water, 1922f, 1923
    solar cues, 1922f, 1923
    vertical layers and, 1923–1924
  pelagic fish *see* Pelagic fish, migration
  salmon *see* Salmon migration
  scale and prevalence, 1922
  tracking methods *see under* Pelagic fish, migration
Mimicry, fangblennies and cleaner fish, 156, 156f
Mineralocorticoid(s), 1474
  aldosterone *see* Aldosterone
  DOC *see* 11-Deoxycorticosterone (DOC)
  see also Corticosteroid(s)
Mineralocorticoid receptors (MRs), 1451f, 1452, 1476, 1518
  activation, 1478, 1478f
  definition, 1450
  embryogenesis, 1455, 1455f
  evolution, 1480, 1481f
  11β-hydroxysteroid dehydrogenase type 2 co-localization, 1477, 1478f
  molecular biology, 1476, 1477f
  tissue expression, 1479
Minerals
  digestion, 1306f, 1309
  requirements, cultured fish, 1621
Miniature inverted transposable elements (MITEs), 1983
Minimum turning radius, definition, 575
MitF gene, 1995
Mitigation
  definition, 304
  effects of human-generated sound on fish, 308
Mitochondria, 794, **966–972**, 1710
  advantages/disadvantages, 1709f, 1710
  biogenesis *see* Mitochondrial biogenesis
  cardiomyocyte, 1010, 1010f, 1013, 1909–1911
  definition, 791, 959, 966, 1006, 1354, 1709, 1903
  densities in cells
    cardiomyocytes, 1909–1911
    chloride cells (gill ionocytes), 1383
    muscle fiber (locomotive), 1911
    pavement cells (gills), 1382
  detection techniques, 1387
  endosymbiotic origin, importance of, 960
  environmental influences on functioning, 970, 1646, 1646f
    see also temperature effects (below)
  genetic and genomic variation, 967
    biogenomic evolution, 967, 967f
    gene/genome duplication, 967, 971f
  membranes *see* Mitochondrial membranes
  muscle content *see* Muscle mitochondria
  structure and function, 961, 961f, 968
    fuel selection in muscles, 969
    inner membrane surface area, 969, 969f
      see also Oxidative phosphorylation
    see also Mitochondrial respiration
  temperature effects, 970, 1647, 1709f, 1710–1711, 1712
    acute thermal change, 1711f, 1712
    evolutionary thermal adaptation, 1713
    genomic analyses, 1715, 1715f
    thermal acclimation *see under* Thermal acclimation/adaptation
Mitochondrial biogenesis, 961, 962f
  regulation, 970, 971f
    see also Mitochondria, temperature effects
Mitochondrial DNA (mtDNA), 961
  definition, 959, 966
  evolutionary aspects, 967
Mitochondrial DNA-encoded genes, 961–962, 962f
Mitochondrial enzymes, temperature effects, 1709f, 1710
  acute thermal change, 1712
  thermal acclimation, 1714, 1714f, 1715
Mitochondrial membranes, 1710
  properties, 971
  temperature effects, 1709f, 1710–1711
    acute thermal change, 1712–1713
    evolutionary thermal adaptation, 1713
    thermal acclimation, 1715
Mitochondrial respiration, **959–965**
  ATP production *see* Oxidative phosphorylation
  fuel selection, 964
  regulation of rate, 964, 965
  specializations, **966–972**
    genetic/genomic variation, 967
    structure and function, 968
    see also Mitochondria
Mitochondrial substrates, production, 959–960, 960f
Mitochondrial transport, 952f, 955
Mitochondrion-rich cells (MRCs), 1097f, 1099, 1099f, 1383, 1467, 1467f
  α- and β-cell subtypes, 1385–1386
  accessory cells (AC), 1382f, 1385, 1386
  active chloride elimination, 1384–1385
  ammocoete, 1794f, 1796
  definition, 796, 1354, 1359, 1366, 1381
  differentiation, osmosensing and, 1379, 1380f
  freshwater fish *see* Mitochondrion-rich cells (MRCs), freshwater fish
  gill remodeling role, 797–798, 798f
  identification, 799
  intercalated cells (IC), 1383–1384, 1384f, 1387
  morphological features, 1383f, 1383–1384
    changes, in seawater acclimation, 1386, 1387
    tubular system, 1381, 1383f, 1385
  NKA (Na$^+$/K$^+$–ATPase) *see under* NKA (Na$^+$/K$^+$–ATPase)
  seawater fish *see* Mitochondrion-rich cells (MRCs), seawater fish
  see also Osmorespiratory compromise
Mitochondrion-rich cells (MRCs), freshwater fish, 1359–1360, 1361f, 1467f, 1467–1468
  classification systems, teleosts, 1386
  lampreys, 1794f, 1796
  NKA (Na$^+$/K$^+$-ATPase), 1361f, 1362, 1364, 1467f
  NKCC (Na$^+$, K$^+$, 2Cl$^-$ cotransporter), 1364
  seawater fish *vs.*, 1354–1355, 1355f
  subtypes, 1364
  see also Freshwater fish, gill ion regulation
Mitochondrion-rich cells (MRCs), seawater fish, 1354, 1467, 1467f
  cystic fibrosis transmembrane conductance regulator (CFTR), 1355–1356, 1357f
  freshwater fish *vs.*, 1354–1355, 1355f
  morphology, 1354–1355, 1355f, 1356f, 1386, 1387
  NaCl transport, 1355
    mechanisms, 1355, 1357f
    routes, molecular evidence, 1356
  NKA (Na$^+$/K$^+$-ATPase), 1355–1356, 1357f, 1467f
  NKCC (Na$^+$, K$^+$, 2Cl$^-$ cotransporter), 1355–1356, 1357f
Mitral cell layer, olfactory bulb, 204
Mixed synaptic transmission, auditory afferents, Mauthner cell as model, 70f, 71
Mlh1 and Mlh3, 633
Model organisms, 1987
  definition, 1986
  fish, 1987
    see also Fish model(s)
Modeling
  bioenergetic *see* Bioenergetics models
  body mass scaling, alternative regression models, 1577f, 1577–1578
  cold acclimation, oxygen transport cascade, 1151
  reef fish vision and color, Maxwell triangle, 153f
  suction feeding hydrodynamics, 599
  see also specific models
Moderately dense bodies (MDBs), 1019–1020
Modified chambers, head region, as air-breathing organs, 1867f, 1868
Molecular biology, definition, 2032
Molecular chaperones
  crystallin, 106
  definition, 102, 1732
  heat shock response, 1736
Molecular pathology, **2032**
  bacteria *see* Bacterial pathogenicity
  viruses *see* Viral infection process
Momentum analysis, experimental hydrodynamics, 543, 544f
  vortex analysis *vs.*, 543, 544f
Monoamine neurotransmitters
  definition, 662, 783
  effect of toxicants, 786
  sex change role, 668
  see also specific monoamine neurotransmitters
Monocarboxylate transporters (MCTs), 954
Monochromatic, definition, 692
Monocytes, 989, 989f, 990
  definition, 984, 1542
Monod, Wyman, and Changeaux (MWC) model, fish hemoglobins, 946, 947f
Monofocal lenses, deep-diving species, 108
Mononeuronic regulation
  color changes, 494
  definition, 489
Monopterus cuchia (swamp eel), suprabranchial chambers, 1867f, 1869–1870
Monorhinic, definition, 16
Monotremes, electroreceptive organs, 358
Monovalent, definition, 1311
Mood disorders, fish models, 1996
MOR (μ opioid receptor), 95
Mormyrid electric fish, 699
  brain size, 1581
  electric organ development, 413f, 413–414
  electroreceptors
    ampullary, 355, 356f
    development, 411, 411f, 412f
    tuberous, 357, 357f
      see also Tuberous electrosensory system, physiology
  hearing mechanisms, 274, 279–280
  pulse-type *see* Mormyrid pulse-type fish
  wave-type *see Gymnarchus niloticus (*mormyrid wave-type fish)
  see also Active electrolocation; Electric organ discharge; Electrocommunication; Tuberous electrosensory system, physiology
Mormyrid pulse-type fish, 366, 367f
  amplitude coding, 367, 367f

- Mormyrid pulse-type fish (*continued*)
  - corollary discharge *see* Electric organ corollary discharge (EOCD)
  - electrocytes, 391
  - EOD time patterns, central control, 406, 407f
  - time coding, 368, 368f
  - torus semicircularis, 372, 373
  - *see also* Electric organ discharge (EOD); Pulse-type electric fish
- Mormyrid wave-type fish *see Gymnarchus niloticus (*mormyrid wave-type fish)
- Mormyromasts, 357f, 357–358, 367, 372f, 379, 410
  - definition, 350
  - temporal coding, 381–383
  - *see also* Active electrolocation
- *Morone saxatilis see* Striped bass
- Morph, definition, 744
- Morpholinos, 1822, 1988
  - definition, 1359, 1843, 1998
- Morphological color changes *see under* Color changes
- Morphology, ecomorphological paradigm *see* Ecomorphological paradigm
- Mortality, mass, hypoxia-associated, 1749
- Mosaic expression, definition, 1998
- Mosaics, retinal, 114, 114f, 121
- Mossy fibers, definition, 56
- Motility, gut *see* Gut motility
- Motion detection *see under* Movement
- Motivation to feed *see under* Foraging decisions (energetics)
- Motoneurons *see* Motor neurons
- Motor, definition, 854
- Motor control, tectal outputs, 140, 141f
- Motor control systems, **56–65**
  - feeding, 62
  - jaw control, 62
  - locomotion, goal-directed, 56, 58f
    - body orientation control *see under* Body orientation
    - locomotor pattern generation *see under* Central pattern-generating (CPG) network
    - network, 57–58, 59f
    - phase lag, 56–57, 57f
    - steering and related orienting/eye motor control, 59, 60f, 60f
  - locomotor control/posture stabilization, vestibulospinal neurons, 250
  - migration and spawning, 62
  - networks, 56–57
    - CPG *see* Central pattern-generating (CPG) network
    - locations, 57f
  - neural structures of particular relevance, 63
    - basal ganglia, 63, 63f
    - cerebellum, 64
  - periaqueductal gray (PAG), 57f
    - fight, flight, and freezing responses, 63
  - respiration, 62
  - sucker mouth, 62
- Motor learning, definition, 56
- Motor neurons
  - definition, 131
  - evolution/developmental transformation, 7f, 10
    - acorn worms, 9, 10
    - amphioxus, 10
    - cholinergic receptors, 10
    - craniates, 11
      - jawed, 13
    - tunicates, 11
- Motor nuclei, definition, 854
- Mouth *see* Buccal cavity
- Mouth breathers
  - circulation patterns, 1876f, 1883
  - *see also* Air-breathing fish
- Mouth-brooding, 671f, 671–672, 675–676
- Mouth types, ecomorphological paradigm and, 1584–1585
- Movement, **516–519**
  - current knowledge, 518
  - detection/analysis
    - behavioral studies *see under* Visual capabilities, behavioral studies
    - elasmobranch, 1809
    - large field motion, 147
    - lateral line role *see under* Lateral line function
    - small field motion and speed, 147
    - *see also* Moving objects
  - *see also* Buoyancy; Locomotion
- Moving objects
  - perception
    - electrosensory, tuberous mechanisms, 373
    - speed, behavioral studies, 147, 148f
  - *see also* Movement, detection/analysis
- MPTP-induced neurotoxicity, 1992f, 1993
- mRNA, definition, 2055
- mRNA transcripts, definition, 1354
- mtDNA *see* Mitochondrial DNA (mtDNA)
- Mucin(s)
  - chemical structure, 505
  - epidermal secretion, 504
  - hagfish *see* Hagfish slime
- Mucin vesicles, definition, 504
- Mucosa
  - definition, 1229
  - gut
    - definition, 1268
    - morphology, 1270, 1270f
- Mucosal fold (intestinal), 1270, 1323f, 1325f
  - definition, 1322
- Mucous cells *see* Goblet (mucous) cells
- Mucus
  - definition, 471
  - gastrointestinal tract, 1301, 1302f
    - role in intestinal barrier function, 1270–1271, 1323, 1325f
  - skin, roles, 472f
    - feeding *see* Mucus feeding
    - locomotion, 472f, 473
    - protection, 471–472, 472f
    - reproduction, 472f, 474
- Mucus feeding, 472f, 474, 681, 681f, 682
- Mudskippers (Gobiidae)
  - amphibious adaptations, 770, 1858, 1963
    - air breathing, 770, 1858
      - role in nest oxygenation, 1857f, 1859
  - burrows and egg development, 1963
  - detoxification of hydrogen sulfide, 1962
  - osmoregulatory abilities, 1963
- Müller, Johannes, 1879, 1885
- Múller cells, lampreys, 20
- Müller–Lyer illusion, 144–145
- Müller's paradox, 1880
- Multifactorial sex determination, 2028
  - modifiers, 2028
  - polygenic, 2028
- Multifocal fish lens, 104f, 106
- Multimodal processing, optic tectum, 139
- Multipulsed calls, 687f, 688
- Multistate model, Root effect, 939f, 940
- Multiunit receptive field (MURF), 134
- Muscle(s)
  - diseases (human), fish models, 1993
  - feeding *see* Feeding muscles
  - functional anatomy, current knowledge, 417
  - hagfish slime gland, 504–505
  - respiratory *see* Respiratory muscles
  - skeletal *see* Skeletal muscle
  - slow-oxidative fiber, definition, 1904
  - sound-producing *see* Drumming muscles
  - tumors (human), fish models, 1994
  - *see also specific muscles*
- Muscle action potential (MAP)
  - definition, 547
  - depolarization, 1720
  - detection, by electromyograms (EMG), 551, 552f
  - excitable fibers, 1719–1720
- Muscle activity, fast start, 589, 590f
- Muscle contraction
  - cardiomyocytes *see* Cardiomyocyte contraction
  - definition, 1054, 1888
  - oxygen requirement, anaerobic swimming, 1219
- Muscle mitochondria, 969, 1911
  - temperature effects
    - acute thermal change, 1711f, 1712
    - evolutionary thermal adaptation, 1713
    - thermal acclimation, 1713f, 1714, 1714f
  - *see also* Mitochondria
- Muscle retia, 1123
  - central, 1124, 1124f, 1125f, 1126f, 1126t
  - lateral, 1124–1125, 1126t
- Muscular dystrophy, fish models, 1993
- Mutagenesis
  - chemical, 1822
  - definition, 1986
  - insertional, 1819
    - by retrovirus, 1822
  - screen, definition, 1971
- Myctophidae
  - bioluminescence, 501, 501f
  - definition, 98–99, 166
  - visual adaptations *see* Deep-sea fishes, visual adaptations
- Myelinated axons
  - definition, 123
  - retinal ganglion cells, 126, 131
- Myenteric plexus, 1270f, 1271, 1332–1333, 1333f
  - definition, 1229, 1268
  - *see also* Enteric nervous system
- Myoblast, definition, 1483
- Myocardial power output, 1035t, 1036–1037
  - definition, 1030
  - estimates, 1037
- Myocardium
  - anatomy, 1078, 1079f
  - compact *see* Compact myocardium
  - definition, 1077, 1888
  - oxygen consumption, 1037
  - oxygen supply
    - coronary vessels, 1080, 1081f
      - *see also* Coronary artery; Coronary circulation
    - limitations, 1083
  - spongy *see* Spongy myocardium
  - working, definition, 1007, 1045
  - *see also* Cardiomyocyte(s)
- Myocytes
  - cardiac *see* Cardiomyocyte(s)
  - definition, 1888, 1903
- Myofibrils
  - cardiomyocyte *see under* Cardiomyocyte ultrastructure
  - definition, 1006
- Myofilament, 1047, 1047f
  - definition, 1045, 1060
- Myofilament overlap, sarcomere length–tension relationship and, 1062f, 1063
- Myogenic, definition, 1073
- Myogenic electric organ, 414f, 414–415
  - definition, 409
- Myogenic electrocytes, 389–391, 390f
- Myoglobin
  - definition, 1006, 1221, 1888, 1903
  - evolution, 945, 945f
  - fish, 945–946, 947f
    - concentration, tunas, 1911
    - oxygen binding *see* Oxygen binding, fish myoglobins
- Myography, measurement of vasoactivity in isolated blood vessels, 1108f, 1108–1109
  - *see also* Electromyography (EMG)

Myosepta
  definition, 547, 549–550
  role in force transmission, 553
  stiffness, 553
Myosin filament *see* Thick filaments
Myotomal muscle
  definition, 1888
  undulatory swimming role *see under* Undulatory swimming
  see also Skeletal muscle
Myotomes
  arrangement in fish body, 549, 550*f*
  definition, 547, 1085, 1119
  hagfish, 1776
  see also Muscle(s)
Myripristinae, hearing, auxiliary anatomy, 274
Myxine glutinosa, 17*f*
  brain, 17*f*, 18*f*, 20*f*, 22*f*, 24*f*
Myxinidae *see* Hagfish

N

Na^+, K^+, $2Cl^-$ cotransporter *see* NKCC (Na^+, K^+, $2Cl^-$ cotransporter)
Na^+, uptake *see* Sodium (Na^+) uptake
Na^+/H^+ exchanger
  definition, 909
  RBC *see* Sodium/proton exchange, RBC
  see also under Red blood cells (RBCs)
Na^+/K^+-ATPase *see* NKA (Na^+/K^+–ATPase)
Na^+–Ca^{2+} exchanger (NCX)
  Ca^{2+} efflux, 1051
  Ca^{2+} influx, 1048
Nacre, fish mutant, 1994*f*, 1995
nanos, 2047, 2048
Narcolepsy, 776
  definition, 772
  fish models, 1996
Nasal sacs, 194, 196*t*
Natal homing
  definition, 1946
  salmon *see* Salmon homing
Native state, definition, 1703
Natriuretic peptides (NPs)
  catecholamine release role, 1530
  vascular resistance control, 1106
Natural convection, 836*f*, 836–837
  definition, 829
Navigation
  acoustic behavior, 317, 319*f*
  definition, 1921
  Earth's magnetic field role, 732
  mechanisms in migratory fish *see under* Migration
  see also Spatial orientation
NE-2, definition, 1437
Near field, 271
  definition, 270
Near-shore operations, robotic fish use, 607, 608*f*, 609*f*, 610*f*, 611*f*
Necrosis, definition, 2032
Negaprion brevirostris see Lemon shark
Negative buoyancy, 523, 524, 524*f*
  consequences, 1907
  definition, 159, 516, 520, 1887
  see also Buoyancy
Negative feedback
  cardiovascular system control *see* Cardiovascular system control, negative feedback mechanism
  definition, 1169, 1457
Negative reinforcement, definition, 713
Neoceratodus, 46
  see also Lungfishes
Neofunctionalization, definition, 1980
Neoselachii
  definition, 419, 428
  see also Batoids; Rays; Sharks
Nephric capsule *see* Glomerular capsule
Nephron, definition, 1444
Nephrostome, 1396*f*, 1397, 1397*f*, 1398*f*, 1399*f*, 1401*f*
Nerve plexus, definition, 1332
Nerves
  branchial, 1097*f*, 1098, 1102
  cranial *see* Cranial nerve(s)
  olfactory *see* Olfactory nerves
  spinal *see* Spinal autonomic nerves
  see also specific nerves
Nervous reflexes, gut, 1296, 1297*f*
Nervous system
  autonomic *see* Autonomic nervous system (ANS)
  central *see* Central nervous system (CNS)
  endocrine system *vs.*, 1190
  functional morphology, **1–5**
    see also Brain
  gut *see* Enteric nervous system; Gut innervation
  hagfish, 1777
    see also Hagfish, brains
  see also Brain(s); *entries beginning neural*; Neurons
Nest building, 657, 657*f*
  physiological mechanisms, 675
Nest digging, female fish, 657–658
  compensatory growth effects, 756*f*, 756–757
Nest guarding, 657, 657*f*, 671, 671*f*
  compensatory growth effects, 756–757
Nest oxygenation, mudskippers, 1857*f*, 1859
Nest spawners
  behaviors, 657
  see also Spawning
Neural arch cartilage, cartilaginous fish, 430*f*, 432
Neural code, definition, 366
Neural control
  body orientation *see under* Body orientation
  fast start, 588
    see also Mauthner cells
  vascular resistance, 1105, 1174
  see also Autonomic nervous system (ANS); Central nervous system (CNS); Motor control systems; *specific systems*
Neural crest
  definition, 2, 6
  evolution/developmental transformation, 7*f*, 8
    craniates, 11–13, 12*f*
    tunicates, 11
Neural depression, anoxic crucian carp, 1828
Neural electrocytes, 389, 389*f*
Neural mechanisms, sex change *see under* Sex change
Neural network, definition, 387
Neural plasticity *see* Plasticity
Neural regulation, color changes, 493
Neural responses, magnetic fields *see under* Magnetic sense
Neural tube, 2–3, 3*f*
  definition, 2
Neuraxis, definition, 2
Neuroanatomy, magnetic sense, 732, 733*f*
Neurodegenerative, definition, 1986
Neurodegenerative diseases, fish models in, 1993
Neuroendocrine axis
  definition, 1542
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Neuroepithelial bodies (NEBs), 872, 875, 877
  definition, 871
Neuroepithelial cells (NECs), gill, 872, 1097*f*, 1100, 1754*f*, 1755
  definition, 871
  innervation, 873, 874*f*
  location/distribution, 872, 873*f*
  oxygen sensing role *see* Oxygen chemoreceptors, branchial
Neuroethology
  definition, 329
  electrocommunication as model, 706
  lateral line function studies, 329–330
    see also Lateral line function
  psychoacoustics and, **276–282**
Neurogenesis, suppression in ectoderm, 9
Neurogenic electric organs, 414*f*, 414–415
  definition, 409
Neurogenic placodes, 8, 14
  definition, 6, 283
  see also Placodes
Neurohormones
  appetite regulating, 1510, 1512, 1512*t*
  definition, 1509, 1515
  see also specific neurohormones
Neurohypophysis, 1458
  definition, 1457
  histology, 1458, 1458*f*, 1459*f*, 1460*f*
    adenohypophysis relations, 1458, 1460*f*
    elasmobranchs, 1458–1459, 1460*f*
    lamprey, 1459, 1461*f*
  hormones, 1462
  see also Pituitary gland
Neuro–immuno–endocrine network, stress response, 1547*f*, 1548
  time course activation, 1548*f*, 1549
Neurokinin A (NKA)
  amino acid sequence, 1337, 1338*f*
  gut expression, 1338
  see also Tachykinins
Neuromast(s)
  canal *see* Canal neuromasts
  cupula *see* Cupula
  definition, 236, 329, 336, 1937
  eels, 1942
  papillate, 334–335
    definition, 329
  sensory hair cells, 330*f*, 336, 337*f*, 338*f*
    innervation, 337–338
    orientation, 330–331, 337, 337*f*, 340*f*, 342*f*
  structure and function, 336, 337*f*, 338*f*
  superficial *see* Superficial neuromasts
  see also Lateral line
Neuromeres
  cartilaginous fish, 27–29, 32
  definition, 16, 26
  forebrain *see* Prosomeres
  hindbrain *see* Rhombomere(s)
Neuromeric model, definition, 16
Neuronal nitric oxide synthase (nNOS), 1069
  intracardiac, ray-finned fish, 1069, 1070*f*
Neurons
  action potential generation, 1717*f*, 1720
  evolution, 7, 8
  intracardiac *see* Intracardiac neurons/neurotransmitters
  sensory nerve endings, 189, 192
  see also Motor neurons; *specific neurons*
Neuropeptide(s), 1069
  definition, 649, 656, 1067, 1542
  enteric nervous system, 1332, 1336
  see also specific neuropeptides
Neuropeptide Y (NPY), 1279–1280, 1281, 1337
  amino acid sequence, 1337*f*
  appetite stimulation, 1484, 1512, 1512*t*
  gut expression, 1337
  role in rectal gland secretion control, 1435
Neurophysiology
  gustation *see* Gustation, neurophysiology
  olfaction *see* Olfaction, neurophysiology
Neuroscience, fish models in, 1993
  neurodegenerative diseases, 1993
Neurosecretion, definition, 1457
Neurosecretory cells
  definition, 1483
  hypothalamo-hypophysial system, 1458, 1459*f*, 1459*f*
    see also Neurohypophysis
Neurotoxins
  anthropogenic, 786
  olfactory effects, 2081
Neurotransmitters, 786, 1069, 1190–1191
  anthropogenic disruptions, 786

- Neurotransmitters (*continued*)
  - definition, 649, 783, 1190
  - effect of social status on levels, 652
  - enteric nervous system *see under* Enteric nervous system
  - intracardiac *see* Intracardiac neurons/neurotransmitters
  - monoamine *see* Monoamine neurotransmitters
  - *see also specific neurotransmitters*
- Neutral buoyancy
  - attainment strategies, 1956
  - benthopelagic fish, 521
  - changing depth and, 522, 523
  - definition, 1887
  - keeping afloat and, 523
  - maintenance, 533
  - *see also* Buoyancy
- Neutral zone, definition, 1169
- Neutralizing epitope, definition, 2032
- Neutrophils, 989*f*, 990
  - intestinal epithelium, 1323, 1324–1325, 1326*f*
- New World pupfish *see Cyprinodon* (desert pupfish)
- Newtonian fluids
  - definition, 977
  - hemodynamics, 979, 979*f*
- Niche
  - definition, 1584, 1584
  - species interactions, 1583*f*, 1584
- Nickel (Ni), biological effects and toxicodynamics, 2066
- Nile tilapia, 2058
- Nissl stain, definition, 2
- Nitric oxide (NO)
  - catecholamine release role, 1531
  - fish integument, 474
  - as gut neurotransmitter, 1335*f*, 1339
  - regulation of capillary function, 880, 885
  - vascular resistance control, 1107
- Nitric oxide synthase (NOS)
  - autonomic neurons, 1069
    - *see also* Neuronal nitric oxide synthase
  - definition, 1067
- Nitric oxide (NO) system, definition, 1067
- Nitrogen (N_2), capacitance coefficient, air *vs.* water, 821*t*
- Nitrogen cycling, role of fish, bioenergetics model application, 1679
- Nitrogen excretion, 1437–1438, 1444
  - energy losses, consideration in digestive efficiency studies, 1596*f*, 1600
  - fish embryos, 1448
  - lampreys *see under* Lampreys
  - rates, cultured fish, 1673
  - renal role, 1416
  - *see also* Ammonia excretion; Urea excretion
- Nitrogen-free extractive (NFE), definition, 1665
- Nitrogen metabolism, elasmobranchs, 1812*f*, 1813
- NKA (Na^+/K^+–ATPase)
  - definition, 796, 1437, 1466, 1807
  - intestinal epithelium, 1311–1312
    - *see also* Intestinal absorption
  - mitochondrion-rich cell, 1467
    - freshwater fish, 1361*f*, 1362, 1364, 1467*f*
    - microscopic visualization, 1381, 1382*f*, 1383*f*, 1383–1384
    - seawater fish, 1355–1356, 1357*f*, 1467*f*
  - rectal gland, 1432–1433, 1433*f*, 1435, 1811*f*, 1812–1813
- NKCC (Na^+, K^+, $2Cl^-$ cotransporter)
  - definition, 1354, 1466
  - mitochondrion-rich cell, 1467
    - freshwater fish, 1364
    - seawater fish, 1355–1356, 1357*f*
  - rectal gland, 1432–1433, 1433*f*, 1435, 1811*f*, 1812–1813
- NMDA receptor
  - ammonia toxicity-associated activation, 1440, 1441
  - definition, 1437, 1824
  - expression in anoxic crucian carp, 1829
- Nociceptin (NOC), 91
- Nociceptin opioid receptor (NOP), 96
- Nociception, **713–719**
  - behavioral responses, 715, 715*f*, 716*f*, 717*f*
    - species-specific, 716, 716*f*, 717*f*
  - definition, 713, 713
  - future research perspectives, 719
  - *see also* Pain
- Nociceptors, 713
  - definition, 713
- Nocturnality, 740, 773
- Nodes of Ranvier
  - definition, 387
  - electric organs, 389
- Noise
  - definition, 304
  - hearing sensitivity determinant, 280
  - human-generated *see* Human-generated sounds
- Noise-exposure criteria
  - definition, 304
  - regulatory uses, 308
- Non-Newtonian fluids
  - definition, 977
  - hemodynamics, 980, 980*f*
  - *see also* Blood
- Non-rapid eye movement (non-REM) sleep, 773
- Non-steady-state conditions, implications for oxygen–carbon dioxide interactions, 919, 920*f*
- Non-teleost fishes
  - ampullary organs *see* Ampullae of Lorenzini
  - secondary vessels
    - gill, 1166
    - systemic circulation, 1165
  - *see also specific non-teleosts*
- Nonadrenergic noncholinergic (NANC) neurotransmission, cardiac, 1069
- Noncholinergic, definition, 1524
- Noncholinergic control, catecholamine release *see under* Catecholamine release, control mechanisms
- Noncirculating hormones *see under* Hormone(s)
- Noncovalent chemical bonds
  - as basis for environmental stress, 1682
    - biochemical adaptations *see* Biochemical adaptations, general principles
  - definition, 1681
- Nondietary fecal material (NDFM)
  - definition, 1597
  - determination, 1596*f*, 1598
- Nonessential metals, 2062–2063
  - definition, 2062
  - toxicity, dose–response, 2061*f*, 2062–2063
- Nonesterified fatty acids (NEFAs)
  - ATP synthesis role, 952–953
  - definition, 951
  - stress effects
    - catecholamine-mediated, 1536, 1536*f*
    - cortisol-mediated, 1539
  - tissue delivery/transport systems, 953, 954, 954, 955
    - fish, 956
- Nonoscillating pectoral fins, 565
- Nonspherical topology
  - definition, 387
  - electrocytes, 391
- Noradrenaline (norepinephrine)
  - biosynthesis, 1524, 1525*f*
  - definition, 1389
  - gut expression, 1336
  - release, 1525–1526
    - *see also* Catecholamine release
  - stress response activity, 1516–1517, 1518
    - exercise-associated osmorespiratory compromise, 1389–1390
  - *see also* Catecholamine(s)
- Normocapnia, definition, 1800
- Normoxia, definition, 765, 865, 1389, 1800, 1960
- North America, Great Lakes, sea lamprey invasion, 1785
- Nostrils, single, hagfish, 1772*f*, 1772–1773
- Notochord, persistent, cartilaginous fish, 422, 423*f*
- Notopterids, ampullary electroreceptors, 355, 355*f*
  - development, 410
- Noxious substances/stimuli
  - avoidance/spitting, 230, 230*f*
  - definition, 713
  - learning to avoid, 709, 709*f*, 714, 715*f*
  - *see also* Pain
- NTPs *see* Nucleoside triphosphates (NTPs)
- Nuage, 2046*f*, 2047–2048
  - definition, 2046
- Nuclear layer, retina *see under* Retina
- Nucleic acid, viral, 2034
- Nucleoside triphosphates (NTPs)
  - definition, 887
  - effect on oxygen-binding properties of hemoglobin, 891, 891*f*
  - erythrocytic concentration, control, 883, 885*f*
- Nucleus
  - cardiomyocytes, 1009*f*, 1010, 1013
  - definition, 2, 283
- Nucleus isthmi (NI), 132–133, 133*f*
  - tectum–NI circuitry, 137, 138*f*
- Nucleus of the solitary tract, definition, 854
- Nucleus rostrolateralis, 42
- Nursery lakes, salmon migration, 1947
- Nutrient(s)
  - bioavailability, assessment, 1671
  - blood, appetite/food intake effects, 1486, 1513
  - categories, 1618
  - classes, effect on digestive efficiency, 1601
  - definition, 1666
  - digestion *see* Digestion
  - functions, 1617
  - parental provision *see* Parental nutrient provision
  - requirements *see* Nutritional requirements
  - transporters, 1605
    - *see also* Intestinal absorption
  - *see also* specific nutrients
- Nutrient classes, effect on digestive efficiency, 1600*t*
- Nutrient cycling, role of fish, bioenergetics models applications, 1679
- Nutrient vessels, gill, 1099*f*, 1101*f*, 1101–1102
- Nutritional requirements, 1617
  - cultured fish *see* Cultured fish, dietary requirements
  - definition, 1666
  - growth hormone transgenic fish, 2020
  - triploid fish, 2014
- Nystagmus, optokinetic *see* Optokinetic nystagmus

O

- Object recognition, behavioral studies, 143, 144*f*
- Obligatory air breathing, 1855, 1855*f*, 1857, 1858
  - definition, 1850
  - *see also* Air-breathing fish
- Ocean(s)
  - acidification, 1738
  - biological hot spots, 1929, 1930, 1935
  - coverage and depths, 1953*f*
  - migration corridors, 1935
  - oxygen minimum zones, 1747*f*, 1748
    - *see also* Hypoxic environments
  - zonation, 1953*f*
  - *see also* Seawater
- Ocean currents, leptocephalus transportation and, 1939*f*, 1940
- Oceanic feeding grounds, salmon migration, 1949
- Oceanic species *see* Marine fishes
- Oceanodramy, 1921*f*, 1922
  - definition, 1921
- Octanol–water partition coefficient, 2069
- Octaval nuclei, 286, 286*f*
  - ascending, 284*f*, 286–287
  - descending, 284*f*, 286–287, 287*f*, 288*f*

    secondary, 284f, 289, 289f
  see also Octavolateral nuclei
Octavolateral efferents
  auditory afferents *vs.*, 296–297
  definition, 292
  role, 296
Octavolateral nuclei, 245
  definition, 244
  vestibular subgroups *see* Octavolateral vestibular nuclei
  see also Octaval nuclei
Octavolateral vestibular nuclei
  auxiliary connections for gaze and posture control, 250
  location, 246, 247f
  ocular subgroups
    horizontal, 249, 249f
    projections to extraocular motor nuclei, 247f, 248
  origins, 247
  spinal projections for locomotor control and posture stabilization, 250
  terminations of semicircular canal and utricular afferents, 247f, 248
Ocular anatomy
  deep-sea fishes *see* Deep-sea fishes, visual adaptations
  teleosts, 168, 169f
  see also Eye(s); *specific structures*
Ocular oxygen secretion, pseudobranch role, 933
Odontodes *see* Placoid scales
Odor, definition, 1553
Odorants
  contaminants as, 2079f, 2080
    see also Olfactory toxicity
  definition, 183, 208, 1553
  potency/specificity *see under* Olfaction, neurophysiology
  receptor binding, 208–209, 209f
    see also Olfactory receptor(s) (ORs)
OFF-center response, ganglion cell, 126, 127, 127f
Offspring care *see* Parental care
Offspring–parent conflicts, nutrient provision, 682, 683
Oil spills, respiratory effects, 787
Olfaction, **183–186**
  anthropogenic disruption, 783, 784f
    see also Olfactory toxicity
  definition, 183
  elasmobranch, 1808
  gustation *vs.*, 218, 219f
  historical aspects, 184
  neurophysiology *see* Olfaction, neurophysiology
  roles
    alarm substance detection, 232
    feeding behavior, 227, 228–230
    homing, 233
    reproduction, 230–231
  terrestrial *vs.* aquatic environments, 183–184
  toxicant effects *see* Olfactory toxicity
  see also Chemosensory behavior; Hormonal pheromones; Olfactory system
Olfaction, neurophysiology, **208–217**
  olfactory signal transduction, 208, 209f
  potency/specificity of olfactory stimuli (odorants/pheromones), 209
    amino acids, 209, 210f, 211f, 228–230
    bile acids, 211, 211f
    gonadal steroids, 212, 212f
    prostaglandins, 212, 213f, 214f
  signal processing in olfactory bulb and telencephalon, 215, 216f
  see also Olfactory receptor(s)
Olfactores, definition, 6
Olfactory bulb
  accessory, 204
  agnathan, 23
  cartilaginous fish, 28f, 34, 35
  electroencephalogram responses, 213, 214f, 215
  Latimeria, 47, 47f, 53f, 55
  layers, 204
    glomerular, 204
    granule cell, 204
    mitral cell, 204
    olfactory nerve, 204
      see also Olfactory nerves
  lung fishes, 47, 47f, 52
  morphology, 204
  olfactory neuron projections, 200, 201f, 202f, 206
  ray-finned fish, 40f, 43
  ruffed cells, 204
  signal processing, 215, 216f
Olfactory cues
  orientation, 1924
  see also Pheromone(s)
Olfactory epithelium, 194, 196t, 199f
  nonsensory, 195, 199f, 201
  sensory, 195, 199f
    olfactory receptor neurons *see* Olfactory receptor neurons
Olfactory hypothesis, salmonid homing, 233
  see also under Olfactory imprinting
Olfactory imprinting, in salmon, 233, 721
  molecular mechanisms, 234
Olfactory lamellae, 195, 199f
  development, 194–195
Olfactory nerves, 204
  projections, 204
    extrabulbar, 206
    see also Olfactory bulb
Olfactory pits, development, 194
Olfactory placodes, 194, 195, 195f
Olfactory receptor(s) (ORs), 200, 208–209, 209f, 217
  carbon dioxide, 868
  definition, 1553
  expression, 201, 203f
  hormonal pheromones
    female postovulatory prostaglandins, 1559
    female preovulatory steroids, 1556–1557
    specificity, 1562
  phylogeny, 200–201, 203f
  tuning, 209–210, 214–215, 217
Olfactory receptor genes, 185, 200–201, 208
Olfactory receptor neurons, 184, 195, 201f, 206, 208
  ciliated, 195, 200f, 201f, 202f, 214–215
  crypt cells, 195, 201f
  definition, 1553
  degeneration/regeneration, 202
  microvillar, 195, 200f, 201f, 202f, 214–215
  olfactory bulb projections, 200, 201f, 202f, 206
  receptor expression, 201, 203f
    see also Olfactory receptor(s) (ORs)
Olfactory rosettes, 194–195, 199f
Olfactory sensory neurons (OSNs)
  generator potential, 2078–2080
    recordings *see* Electro-olfactogram (EOG)
  pheromone sensitivity, 1558f, 1559
  toxicants, 2079f, 2081
    see also Olfactory toxicity
Olfactory signal transduction, 208, 209f
Olfactory system
  agnathan, 23
    hagfish, 1777
  alarm substance detection, 232
  cartilaginous fish, 28f, 34
  development, 194, 195f
  historical aspects, 184
  lung fishes, 49f, 52
  morphology, **194–207**
    see also Peripheral olfactory organ
  ray-finned fish, 40f, 43, 44–45
  studies, 184
    San Francisco Bay Bridge Analogy, 185, 185f
    see also Electro-olfactogram (EOG)
  see also see also Olfaction; *specific structures*
Olfactory toxicity, **2078**
  behavioral/physiological responses, 2078, 2079f
  contaminants as odorants, 2079f, 2080
  future studies, 2082
  olfaction-based responses, 2079f, 2081
  olfactory–toxicity interactions, 2082
  OSN toxicants, 2079f, 2081
  see also Chemosensory behavior
Olfactory tract
  central projections, 205, 205f
  see also Olfactory bulb
Oligonucleotide, definition, 2055
Oligopeptide transporter 1 (PepT1), 1314f, 1315
Omnivore, definition, 1666
ON-center response, ganglion cell, 126, 127f
ON-OFF receptive field, ganglion cell, 126, 127, 127f
ON-OFF response, ganglion cell, 126, 127f
Oncogene
  definition, 1971, 1986
  leukemia, 1990f, 1991
Oncorhynchus kisutch see Coho salmon
Oncorhynchus mykiss see Rainbow trout
Oncorhynchus nerka see Sockeye salmon
Oncorhynchus tshawytscha see Chinook salmon
Oncotic pressure, 979
  definition, 977, 1154, 1161
  transcapillary fluid exchange and, 1155–1156, 1156f
Onset of spasm (OS) endpoint, 1695, 1698–1699, 1701
Ontogenetic, definition, 744
Ontogenic diet shifts, 1594
Ontogeny
  definition, 1276, 1624, 1629
  hypoxic ventilatory response, 1755
  life history stages, definition, 1738
  metabolic rate changes, 1575f, 1577
  sound production and, 317
  taste buds, 189–190
  temperature sensitivity, 1739f, 1743f, 1744
  zebrafish, 1820f, 1821
  see also Embryogenesis/embryonic development; Growth; Puberty; Scaling, body mass
Oocytes
  definition, 635, 1542
  growth, 1500, 1501f
  maturation, 1501f, 1502
  mature, HPI-axis transcripts, 1453
  mutual regulation of follicle cells, 1501
  primary, definition, 1999
  see also Ovarian follicle
Oogenesis, 1500, 1501f
  definition, 635
  mutual regulation of follicle cells and oocyte, 1501
  oocyte growth, 1500, 1501f
  oocyte maturation, 1501f, 1502
  oogonial proliferation and meiosis, 1500, 1501f
Oogonia
  definition, 1500
  proliferation, 1500, 1501f
    see also Oogenesis
Oophagy, 679, 679f
  definition, 678
OP (organophosphate) contamination, olfactory effects, 2081
Open-channel flumes, volitional swimming studies, 1659f, 1662
Open respirometers, 848–850, 850f, 1568–1569
Open-system temperature changes
  definition, 1888
  pelagic fish and, 1901
Open-type endocrine cell, definition, 1341
Open water, orientation/navigation mechanisms, 1922f, 1923
Opercular beat rate, effect of pain, 716, 717f
Opercular bones, bony fish, 438, 440f
Operculum/opercular, definition, 929, 1354
Opioid(s), analgesic effects, 95
Opioid peptides
  definition, 89
  endogenous *see* Endogenous opioid peptides

Opioid receptors, 95
  definition, 89
  δ (DOR), 96
  expression sites, 95
  κ (KOR), 96
  μ (MOR), 95
  nociceptin (NOP), 96
  physiological roles, 95
Opisthonephros
  definition, 1788
  hagfish, 1407–1409, 1409f, 1773
  lamprey, 1404–1406, 1409f, 1793f, 1796
  structure/histology, 1400
    glomerulus, 1396f, 1400, 1402f, 1403f
      granules, 1401, 1402f, 1404f
      podocytes, 1396f, 1401–1402, 1403f, 1405f
      vasculature, 1399f, 1400, 1403f
    tubule, 1396f, 1402, 1405f, 1406f, 1407f, 1408f
  see also Kidney
Opsanus tau (oyster toadfish), sound production, 312, 313f
Opsins, 110–111, 160
  adaptations to photic environment, 116–117
    chromophore exchange, 120–121, 121f
    gene duplication in shallow-living species, 118–119, 119f, 120f
    increasing water depth, 118, 118f
    loss of UV sensitivity, 121
  definition, 99, 110, 116, 159, 692
  evolution, 111, 113f, 116, 117f
  extraretinal
    deep brain, 160–161
    pineal *see* Pineal opsins
  structure, 111, 112f
Optic tectum (OT), **131–142**
  agnathan, 20, 20f
  anatomy, 131, 132, 132f, 136f
  ascending connections, 139, 140f
  cartilaginous fish, 27, 28f, 31f, 31–32
  cell types, 132, 133f, 134
  damage, effects of, 131–132
  definition, 56
  descending connections and motor control, 140, 141f
  layers, 132, 133f
  learning capabilities, 142
  multimodal processing, 139
  nucleus isthmi–tectum subsystem, 137, 138f
  ray-finned fish, 3, 4f, 40f, 41–42
  retinotectal projection, 133
    retinotectal map *see* Retinotectal map
  torus longitudinalis–tectum subsystem, 135, 136f, 136f
  torus semicircularis, 136f, 139
  visual processing, 134
    color, 135
    patterns, 135
Optical aberrations, 103–104, 104f
  definition, 99, 102
Optical plasticity, 106
Optical system
  principles, 103, 103f
  see also Eye(s); Vision
Optics, physiological, **102–109**
  adaptive *see* Visual adaptations
  eye evolution, constraints, 103
  optical elements of fish eyes *see under* Eye(s); *specific structures (e.g. lens)*
  principles, 103
Optimal foraging theory (OFT), 1586, 1589
  definition, 1588
Optokinetic nystagmus
  behavioral studies, 145f, 147
  definition, 143
Optomotor response
  behavioral studies, 145f, 147
  definition, 143
Ora retinalis, definition, 102
Oral cavity *see* Buccal cavity
Oral disk, lamprey
  definition, 1780
  development, 1783–1784
  function
    feeding, 1781
    substrate attachment, 1782
  structure, 1779f
Oral hood, larval lamprey, 1780f, 1781, 1784
  definition, 1780
Oral tolerance, 1325, 1327
  definition, 1322
Oreochromis see Tilapia
Orexigenic, definition, 1509, 2016
Orexigenic complex, 1484, 1485f
  anorexigenic complex interactions, 1485, 1485f
  definition, 1483
  see also Appetite stimulation
Orexins, 776
  appetite stimulation, 1484, 1485f, 1512, 1512t
  definition, 772
Organ differentiation
  eyes, blind cavefish, 1845f, 1846
  gonads *see* Sex differentiation
  gut *see under* Gut development
  liver, 1290, 1290f
  pancreas, 1289
  see also Embryogenesis/embryonic development
Organelle
  definition, 1006
  see also specific organelles
Organic chemicals, toxic *see* Toxicology, organic chemicals
Organic compounds, definition, 2069
Organic phosphate
  effects, oxygen-binding properties of hemoglobin, 891, 891f
  metabolism, definition, 879
  see also Nucleoside triphosphates (NTPs)
Organochlorine, definition, 2069
Organogenesis, definition, 838
Organometals, 2062f, 2063
Organophosphate (OP) contamination, olfactory effects, 2081, 2081
Orientation
  body *see* Body orientation
  definition, 1921
  spatial *see* Spatial orientation
Orienting movements
  steering-related, 60, 60f
  see also Body orientation
Ornithine transcarbamylase (OTC), urea cycle, 1445, 1445f
Ornithine–urea cycle, 1439, 1441
  definition, 1437
  see also Urea excretion
Orthodenticle-like 1 (Otx1), 13–14
  definition, 6
Orthologous genes/orthologs, definition, 110, 899, 944, 1341, 1359, 1681
Orthomyxoviridae *see under* Viral diseases
Orthoretroviridae, 2039
Oryzias latipes (medaka), as model organism, 1986f, 1987
  see also Fish model(s)
Oscillating pectoral fins, 566, 569f
Oscillogram, definition, 311
Osmoconformers/osmoconformation
  definition, 1444, 1772, 1807
  elasmobranchs, 1812
  hagfish, 1773, 1776
Osmolality
  definition, 1366
  freshwater, 1349t, 1420t
  plasma
    freshwater fish, 1349t
    seawater fish, 1349t
  seawater, 1349t, 1420t
Osmolarity
  definition, 1960
  gut tissue, GI blood flow regulation and, 1212
Osmolytes
  conservation of composition/concentration, 1683
  definition, 1681, 1807
  protein stabilization, 1684
  TMAO *see* Trimethylammonium N-oxide (TMAO)
Osmoprotectants, definition, 1960
Osmoregulation, **1348–1353**, 1467
  cultured fishes, 2088
  definition, 1348, 1366, 1937, 1946
  diploid and triploid fish, 2011f, 2012, 2013
  eels, 1942
  evolutionary aspects, 1350, 1352
  freshwater fish, 1351
    elasmobranchs, 1349t, 1352
    lampreys, 1349t, 1352, 1793f, 1794f, 1795f, 1796
    teleosts, 1349t, 1351, 1352f
  gut functions, nonfeeding teleosts, 1420, 1420t
    esophagus and stomach, 1421
    intestinal, 1421, 1421f
  hagfish mucin vesicles, ion flow control, 505–506
  hormonal control *see* Osmoregulation, hormonal control
  intertidal fish, 1962–1963
    elasmobranchs, 1963
    teleosts, 1963
  intestinal mechanisms, 1318, 1318f
    nutrient uptake and, 1319f, 1320
    see also Gut ion/acid–base regulation
  seawater fish, 1350, 1385
    elasmobranchs, 1349t, 1351
    hagfish, 1349t, 1350, 1772t, 1773
    lampreys, 1349t, 1351, 1795f, 1796
    salt secretion mechanisms, **1354–1358**
    teleosts, 1349t, 1350, 1350f
    see also Mitochondrion-rich cells (MRCs), seawater fish
  sturgeons, 1804
  see also Gill ionocytes; Ion channels; Ion regulation; Ion transport; Water homeostasis
Osmoregulation, hormonal control, **1466–1473**
  anadromous fish, 1471, 1472f, 1948–1949
  cortisol, 1467f, 1468
    freshwater acclimation role, 1469
    growth hormone/IGF-I axis interaction, 1471
    seawater acclimation role, 1468
  growth hormone and IGF-I, 1467f, 1470
    cortisol interaction, 1471
  lampreys, 1797
  prolactin, 1467f, 1469
  thyroid hormones, 1471
Osmorespiratory compromise, **1389–1394**
  definition, 1389
  effect of branchial remodeling, 1393
    see also Gill remodeling
  effect of changes in branchial diffusion distance, 1392
    acclimation to changing salinity, 1393
    acclimation to soft water, 1392, 1392f
  effect of changes in branchial surface area, 1389
    exercise, 1389, 1390t, 1391t
    seawater fish, 1391
Osmosensing, **1373–1380**
  basic principles of osmosensory signal transduction, 1374, 1375f
  cellular/chloride cell differentiation, 1379, 1380f
    see also Mitochondrion-rich cells (MRCs)
  fish sperm motility role, 1380
  ion–damage–cytokine model
    see Ion–damage–cytokine model, osmosensing
  molecular osmosensors, 1374
    calcium as central stimulus, 1374
    cytoskeletal strain as signal, 1375

    membrane proteins, 1376
  need for environmental salinity sensing, 1373
Osmosensory signal transduction, basic principles, 1374, 1375*f*
Osmosis
  definition, 1348, 1366
  general aspects, 1367
    paracellular water transport, 1368, 1368*f*
    transcellular water transport, 1367–1368, 1368*f*
  see also Aquaporins
Osmotic coefficients, 1368–1369
Osmotic gradient, definition, 1354
Osmotic homeostasis *see* Osmoregulation
Osmotic pressure, 979
  colloid *see* Oncotic pressure
  definition, 977, 1111, 1154, 1161
Osteichthyes *see* Bony fish
Osteoglossiformes
  circulatory patterns, 1876*f*, 1881*f*, 1882
  electric organs, development, 413
  electroreceptors
    ampullary *see under* Ampullary electroreceptors
    development, 410, 411*f*, 412*f*
    tuberous *see under* Tuberous electroreceptors
  see also individual species
Ostial valves, 1000, 1112, 1113*f*
  definition, 998, 1111
  sinoatrial canal, 999*f*, 1000
Otolith (end) organs, 253*f*, 254, 256*f*
  definition, 244, 252, 283
  otoliths *see* Otoliths (otoconia)
  sensory epithelium *see* Sensory epithelia, fish ears
  see also Auditory/lateral line CNS, anatomy; Lagena; Saccule; Utricle
Otolithic afferent, 295
  definition, 292
  see also Auditory afferents
Otoliths (otoconia), 254, 262, 273
  as accelerometers, 299, 299*f*
  definition, 236, 244, 252, 262, 270, 1937
  eel, 1939*f*, 1941
  functional mechanism, 264, 276
  lagena, 256*f*, 257*f*
  morphology, 254, 257*f*
  origin of minerals, elasmobranchs, 263–264
  saccular, 254–255, 256*f*, 257*f*, 258
  sharks and rays, 262–264
  utricular, 246, 256*f*, 257*f*
Otophysans, 276
  auditory/lateral line pathways
    forebrain areas, 290
    octaval nuclei, 287*f*, 287–289
  definition, 283, 298
  Weberian ossicles *see* Weberian ossicles
Otophysic connections, 276, 287*f*, 287–290
  definition, 276, 283
  otophysan species, sound pressure sensitivity, 278*f*, 278–279
  pressure-sensitive nonotopyhsans, 278–279, 279*f*, 279–280
  see also Inner ear–swimbladder relationship
Ototoxins, 785–786
  definition, 783
Otter trawl, path, 1656*f*, 1660
Outflow tract, heart, **1015–1029**, 999
  anatomy, 999*f*, 999–1000, 1001*f*, 1003, 1015, 1016*f*, 1016*f*
  bulbus arteriosus *see* Bulbus arteriosus
  conus arteriosus *see* Conus arteriosus
  definition, 998, 1015
  development, 1015, 1017*f*
  phylogeny, 1015, 1016*f*
  terminology, 1015
Outward current, definition, 1038, 1718
Oval (swimbladder), 527, 527*f*, 531–532
Ovarian differentiation, 1492, 1492*f*, 1493, 2026, 2029, 2030
  endocrine control, 1496*f*
    CYP19A role, 1494, 1496*f*, 2029*t*, 2030
    foxl2 role, 1497, 2029*t*, 2031
    see also Sex differentiation, endocrine control
  genes involved, 2029*t*, 2030
  histology, 1493*f*
  see also Sex differentiation
Ovarian follicle
  definition, 635
  growth, 635–636, 636*f*
    previtellogenic, 635–636, 636*f*
    vitellogenic, 635–636, 636*f*
      see also Vitellogenesis
  see also Oocytes
Ovarian maturation, 635–636, 636*f*
  definition, 635
Ovaries, development in triploid fish, 2009*f*, 2009*f*, 2010–2011
Overfishing, susceptibility, swimming ability and, 1655–1656
  see also Swimming performance, applied aspects
Oviparity, 679
  definition, 613, 678
Oviparous fish, 614
  definition, 838
  parental nutrient provision, 679, 681
Oviposition
  definition, 656, 670
  prostaglandin effects, 659
Ovotestis, definition, 1490
Ovoviviparity, 614
  definition, 613, 678
Ovoviviparous fish
  definition, 838
  parental nutrient provision, 679
  vitellogenesis, 644–645
Owens Valley, 1831*f*, 1833
  pupfish *see Cyprinodon radiosus*
Oxidation, hemoglobin, 891
Oxidative phosphorylation (OXPHOS), 871–872, 952, 952*f*, 954, 962, 1710, 1757*f*, 1758
  coupling/uncoupling, 964
  definition, 871, 951, 959, 966, 1709, 1758
  mitochondrial strategies for increasing capacity, 969, 969*f*
  oxidation by electron transport system, 963, 963*f*, 1758–1759
    see also Electron transport system (ETS)
  phosphorylation, 963
  proton motive force *see* Proton motive force (PMF)
  regulation, 964, 965
  see also Mitochondria
Oxyconformer, 1963
  definition, 1800, 1961
  sturgeon, 1803–1804
Oxygen
  air *vs.* water, 1747
    capacitance coefficient, 821*t*, 822
  availability, global gene expression profiling, 2060
  capacitance coefficient
    blood, 823, 823*f*, 895*t*, 897
    water, 895*t*, 897
      air *vs.*, 821*t*, 822
  cultured fishes and *see under* Aquaculture, physiological responses
  growth effects, 1630*f*, 1631
  measurement methods, 846–847
  partial pressures *see* Partial pressure of oxygen (PO_2)
  tissue extraction *see* Tissue oxygen extraction
  transport and exchange *see* Oxygen transport/exchange
  see also Dissolved oxygen (DO)
Oxygen- and capacity-limited thermal tolerance (OCLT), 1740*f*, 1741
  definition, 1738
Oxygen binding, fish hemoglobins, 793, 888, 945–946, 946*f*
  affinity, 945, 946*f*
    modulating factors, 816–817
    sturgeons, 1804
  affinity, optimal, 894, 895*f*
    quantitative model, 895, 895*t*, 896*f*
      equation solving, 897*f*, 898
      equations used, 897
  Bohr effect *see* Bohr effect, fish hemoglobins
  conformational changes, 889*f*, 889–890
  factors affecting, 890
    anions (particularly organic phosphate), 891, 891*f*
    protons, 890
      see also Bohr effect, fish hemoglobins
    temperature, 891, 1897*f*, 1901, 1912
  hemoglobin multiplicity and, 949
  Hill plot *see* Hill plot
  oxygen equilibrium curves *see* Oxygen equilibrium curves (OECs)
  pelagic fish, thermal effects, 1897*f*, 1901
  Root effect *see* Root effect
  triploid fish, 2012, 2013
  see also Hemoglobin(s), fish; Red blood cells (RBCs), function
Oxygen binding, fish myoglobins, 954–955
  environmental influences, 956
Oxygen chemoreceptors
  branchial *see* Oxygen chemoreceptors, branchial
  extra-branchial, 1755
Oxygen chemoreceptors, branchial, 766, 1100, **871–878**, 1754*f*, 1755
  future research directions, 877–878
  hypoxia response, 873, 877–878
    sensing of hypoxia, 873, 874*f*
    transduction of hypoxic stimulus, 874*f*, 875, 876*f*
  microscopic observations, 872, 873*f*, 874*f*
  neurochemistry, 877
  see also Neuroepithelial cells (NECs), gill
Oxygen consumption (M_{O2})
  definition, 1215, 1389, 1566
  during development, 830, 830*f*
  limiting factors, 817
  maximum, aerobic swimming and, 1216
  measurement methods, 846–847, 1566–1567
    see also Respirometers
  metabolite signals, gastrointestinal blood flow and, 1212
  myocardial, 1037
  SMR measurement, 1567, 1568, 1568*f*, 1569*f*
  during swimming, 1215
    cardiovascular responses *see under* Exercise
  tissue, rate determination, 953–954
  triploid fish, 2013
Oxygen delivery, tissues *see* Tissue oxygen delivery
Oxygen demand
  cardiac, definition, 1077
  gastrointestinal blood flow regulation, 1212
  increased, sources in cultured fishes, 2087
  local regulation of blood flow in response to, 1176, 1176*f*
  oxygen supply and *see* Oxygen supply–demand relationship
Oxygen-dependent ion transport, definition, 879
Oxygen diffusion
  diffusion distance (*D*), 1145
    definition, 1142
  embryonic fish, 1575
    see also Gas exchange during development (respiratory transitions)
  through membranes, 834
  see also entries beginning diffusion; Oxygen transport/exchange
Oxygen equilibrium curves (OECs), 888*f*, 888–889, 893, 894*f*
  definition, 887
  hagfish, 1773

- Oxygen equilibrium curves (OECs) (*continued*)
  - Hill plot *see* Hill plot
  - lampreys, 1792f, 1794
  - nonlinear Bohr–Haldane effect, 916–917, 918
  - nonlinear nature, effect on gas exchange efficiency, 826, 827f
- Oxygen loading, gills, aerobic swimming-associated improvement, 1217
- Oxygen minimum zones (OMZs), 1747f, 1748, 1888f, 1889–1890
  - definition, 1888
- Oxygen secretion
  - ocular, pseudobranch role, 933
  - swimbladder *see under* Swimbladder, gas secretion
- Oxygen sensing *see* Oxygen chemoreceptors, branchial
- Oxygen supply
  - temperature effects
    - hypoxemia, 1740f, 1742
    - summer heat stress, 1743
  - *see also* Oxygen consumption (M_{O2}); Tissue oxygen delivery
- Oxygen supply–demand relationship, 814, 1148, 1149f
  - plasticity and feedback control, 1149, 1150f
  - *see also* Oxygen demand
- Oxygen transport/exchange, 793, 879–880, **893–898**
  - carbon dioxide interactions *see* Oxygen–carbon dioxide interaction
  - during development, 834
    - barriers to oxygen uptake, 1575
  - effect of exercise *see under* Exercise, respiratory effects
  - transport cascade, 894–895, 895f
    - quantitative model to predict optimal blood–oxygen affinity, 895, 895t, 896f
      - equation solving, 897f, 898
      - equations used, 897
    - *see also* Cellular respiration; Oxygen binding, fish hemoglobins; Oxygen diffusion
- Oxygen unloading, tissues, aerobic swimming-associated improvement, 1218
- Oxygen uptake
  - growth hormone transgenic fish, 2020
  - metabolic rate measurement, 1765
  - *see also* Oxygen transport/exchange
- Oxygenation
  - adaptations, intertidal habitats, 1963
  - nest, mudskippers, 1859
- Oxygen–carbon dioxide interaction, **916–920**
  - basis, 917
  - *see also* Bohr–Haldane effect
  - implications of non-steady-state conditions, 919, 920f
- Oxyntic cells, 1270
  - definition, 1229, 1268
- Oxynticopeptic cells, 1270, 1302, 1303f
- Oxyregulator, 1963
  - definition, 1800, 1961
  - sturgeon, 1803–1804
- Oxytocin, 1462
- Oyster toadfish, sound production, 312, 313f

P

- P_{50}
  - definition, 791, 916, 921, 935, 1221, 1524, 1824
  - optimal, 894, 895
  - *see also* Oxygen transport/exchange; Partial pressure of oxygen (PO_2)
- p53 mutant, 1995
- Pacemaker, cardiac, 1071
- Pacemaker cells, 1035
  - action potential, 1040, 1041f
- Pacemaker nucleus, 399–400, 400f, 401–402, 402f, 405t, 700, 702
  - definition, 699
  - morphology, 401f
  - *see also* Electric organ discharge (EOD), central control
- Pacemaker potential *see* Diastolic depolarization
- Pacific hagfish (*Eptatretus stoutii*), slime production, 504–505, 505f, 508
- Pacific Ocean Shelf Tracking (POST) program, 1935
- Pacific salmon
  - chinook salmon *see* Chinook salmon (*Oncorhynchus tshawytscha*)
  - coho salmon *see* Coho salmon (*Oncorhynchus kisutch*)
  - definition, 1946
  - lifecycle, 1945f, 1946
  - migration *see* Pacific salmon migration
  - semelparity, 1945f, 1946
- Pacific salmon migration, 720–721, **1945**
  - adult upriver migration to natal stream, 1949f, 1949f, 1951
  - anthropogenic threats, 1950f, 1951
    - *see also* Dams
  - directed, to coast and natal river, 1949
    - *see also* Salmon homing
  - larval developmental migration, 1947
  - to larval feeding grounds, 1947
  - to ocean feeding grounds, 1949
  - orientation mechanisms, 721
  - smolting and migration to sea, 1948
  - testosterone role, 1950, 1951
  - *see also* Salmon homing
- Pacu, cardiorespiratory interactions, central control, 1183–1184, 1184f, 1185f, 1186f, 1187f
- Paddlefish, 1801
  - ampullary organs, 352
  - reproduction, 1801–1802
  - water flea electrosensing, 364
- *Pagothenia borchgrevinki*, thermal acclimation of cardiac performance, 1203–1204, 1204f
- PAHs (polycyclic aromatic hydrocarbons), 2074
  - benzo[a]pyrene, biotransformation, 2071f, 2072
  - definition, 2069
  - phototoxicity, 2075
- Pain, **713–719**
  - avoidance of noxious stimuli, 714
  - behavioral responses, 715, 715f, 716f, 717f, 718t
    - species-specific, 716, 716f, 717f, 718t
  - controversies, 714
    - perception/significance, 718, 718f
  - definition, 713
  - fear and, 714–715, 717f, 718
  - future research perspectives, 719
  - importance to fish, 718
  - *see also* Noxious substances/stimuli
- Paired fin(s), **564–574**
  - anatomy, 564
  - associated muscles, 568t
  - control, 571, 572f
  - diversity, 564, 565f, 566f
  - hydrodynamics *see* Paired fin hydrodynamics
  - maneuvering role, 571, 576f, 576–577
    - braking, 571
    - hovering, 571
    - yawing, 571
    - *see also* Paired fin hydrodynamics
  - motion/swimming, 565
    - *see also* Pectoral fin(s), motion; Pelvic fin(s)
  - pectoral *see* Pectoral fin(s)
  - pelvic *see* Pelvic fin(s)
- Paired fin hydrodynamics, 571, 572f
  - maneuvering, 566f, 573
    - braking, 573
    - hovering, 573
    - yawing, 573
- Palatal organ and vagal lobe, cyprinids, 191–192, 193f
- Palatoquadrate, definition, 449
- Pallium
  - agnathan, 23, 23, 24f
  - cartilaginous fish, 34f, 34–35
  - definition, 2, 37
  - lateral *see* Lateral pallium
  - *Latimeria*, 47, 53f, 54f, 55
  - lungfishes, 47, 48f, 49f, 52
  - medial *see* Medial pallium
  - ray-finned fish, 3, 4f, 38f, 39, 40f, 43
    - development, 43, 44f
  - *see also* Telencephalon
- Pancreas, **1276–1283**
  - anatomy, 1276, 1278f, 1285f
  - blood supply, 1136
  - development, 1277
    - determination of anterior-to-posterior position, 1287
    - differentiation, 1289
  - endocrine, 1276–1277
  - *see also* Brockmann body; Pancreatic hormones
  - evolution, 1277, 1279f
  - exocrine, 1276–1277
  - *see also* Pancreatic enzymes
  - functions, 1276
  - *see also* Pancreatic secretion
- Pancreatic enzymes, 1281, 1304, 1304f, 1305f, 1305t
  - carbohydrases, 1281t, 1282
  - isoforms, 1304–1305
  - lipases *see* Lipases
  - peptidases, 1281, 1281t
  - physiological actions, 1281, 1281t
  - *see also specific enzymes*
- Pancreatic hormones, 1279
  - evolutionary aspects, 1278, 1279f
  - glucagon, 1280, 1280t
  - insulin *see* Insulin
  - peptide YY, 1280t, 1281
  - physiological actions, 1279, 1280t
  - somatostatin *see* Somatostatins
  - *see also* Gut hormones
- Pancreatic secretion, 1276
  - enzymes *see* Pancreatic enzymes
  - hormones *see* Pancreatic hormones
  - regulation, 1282
- Papillate neuromasts, 334–335
  - definition, 329
- Paracellular transport
  - definition, 1366, 1419
  - water, 1368, 1368f
    - *see also* Water homeostasis
- Paracrine effect, definition, 1229, 1341
- Paracrine factors
  - definition, 1429, 1807
  - rectal gland secretion control, 1434
  - *see also specific paracrine factors*
- Paracrine signaling, 1191
  - definition, 627, 1190, 1515
  - gastrointestinal blood flow regulation, 1212
  - vascular resistance control *see under* Systemic vascular resistance, local control
- Parallel inhomogeneity
  - definition, 820
  - effect on gas exchange efficiency, 827
- Parallel paths, ganglion cells, 128
- Paralogons, 1981f, 1981–1982
  - definition, 1980
- Parameter, definition, 1624
- Paraneurons, epidermal, 484
- Parapineal organ, agnathan, 21, 22f, 161
  - photoreception, 161
- Parasite(s)
  - definition, 777
  - fish phenotype manipulation *see* Host manipulation
  - fitness, 778
  - hosts
    - definitive *see* Definitive host
    - intermediate *see* Intermediate host
    - manipulation *see* Host manipulation
    - target *see* Target host
  - life cycle
    - definition, 777
    - *Diplostomun spathcaeum*, 778–779, 779f
    - *Schistocephalus solidus*, 780f, 780–781
  - load, related to thermal tolerance, 1701
  - transmission, 777–778

    - trophic *see* Trophic transmission
  - way of life, 777
    - *see also life cycle (above)*
- Parasitic lampreys, 1781, 1783*f*, 1784
  - *see also* Lampreys; Oral disk, lamprey
- Parasympathetic nervous system
  - definition, 80
  - neurotransmitters, cardiac action potential effects, 1042
  - *see also* Autonomic nervous system (ANS); Cranial autonomic nerves
- Parenchyma, definition, 1862
- Parent-touching behaviors, 681
- Parental care, **670–677**, 614, 769
  - compensatory growth effects, 756
  - costs and benefits, 673*f*, 674, 674*f*
  - definition, 670
  - evolutionary trajectories and phylogenetic comparisons, 673*f*, 675
  - female *see* Maternal care
  - freshwater *vs.* marine species, 672–673
  - future research directions, 676
  - male *see* Paternal care
  - nutrient provision *see* Parental nutrient provision
  - patterns and diversity, 671
    - explanation, 672
    - form of care, 671, 671*f*
    - sex of caregiver, 672, 673*f*
  - physiological mechanisms, 670, 675
    - defense, 675
    - fanning and brooding, 675, 1575
    - nest building, 675
  - surfacing behaviors and, 769
  - *see also specific forms of care*
- Parental nutrient provision, **678–683**, 614
  - future research directions, 683
  - parent–offspring conflicts, 682, 683
  - posthatch, 681
    - additional benefits, 682
    - mucus feeding, 681, 681*f*, 682
  - prehatch/birth, 678
    - external development, 679
    - internal development, 679
      - adelphophagy *see* Adelphophagy
      - oophagy *see* Oophagy
      - placental relationships, 680, 680*f*
  - reasons for, 678
- Parent–offspring conflicts, nutrient provision, 682
- Parietal fossa, sharks and rays, 262, 264, 264*f*, 265
- Parietal peritoneum, definition, 1395
- Parietal valves, 1091–1092, 1112, 1113*f*
  - definition, 1111
- Parkinson's disease, fish models in, 1993
- Parrotfish (*Chlororus sordidus*), color properties, 155, 155*f*
- Pars distalis
  - definition, 1457
  - histology, 1458, 1458*f*
    - elasmobranchs, 1458–1459, 1460*f*
    - lamprey, 1459, 1461*f*
  - proximal *see* Proximal pars distalis
  - rostral *see* Rostral pars distalis
  - *see also* Adenohypophysis
- Pars intermedia
  - definition, 1457
  - histology, 1458, 1458*f*, 1464, 1464*f*
    - elasmobranchs, 1458–1459, 1460*f*
    - lamprey, 1459, 1461*f*
  - propiomelanocortin expression, 91
  - *see also* Adenohypophysis
- Pars nervosa *see* Neurohypophysis
- Parthenogenesis, 614
  - definition, 613, 2009
- Partial pressure
  - changing depth and, 522
  - definition, 520, 791, 829, 846, 893, 909, 916, 921, , 929, , 935, , 1119, , 1888, , 1903
- Partial pressure of carbon dioxide ($P\text{CO}_2$), effect on blood–oxygen binding, 921, 922*f*
- Partial pressure of oxygen ($P\text{O}_2$)
  - bradycardia and, 1222
  - critical, 1574*f*, 1576
    - definition, 1389, 1573, 1752, 1764
    - related to body mass, 1579*f*, 1582
  - definition, 871, 1221
  - elevation, 935, 941
    - retina, 931, 940–941
    - swimbladder, 932, 940–941
    - *see also* Root effect
  - high values in eyes, association with choroid retia, 1129*t*, 1130
  - seawater *vs.* freshwater, 792, 792*f*
  - *see also* P_{50}
- Particle image velocimetry (PIV), 518, 524, 539, 583
  - continuous laser, 539, 540*f*
  - cross-correlation technique, 539–540, 540*f*
  - definition, 516, 520, 535
  - defocusing, 542
  - holographic, 542
  - horizontal planes, 541, 541*f*
  - pulsed laser, 539, 540*f*
  - stereo, 540
  - 3D reconstruction, 541, 541*f*, 542*f*
  - typical configuration, 539, 539*f*
  - *see also* Digital particle image velocimetry (DPIV)
- Particle motion, definition, 304
- Partition coefficient, octanol–water (K_{ow}), 2070–2071
  - definition, 2069
- *Parupeneus* (goatfish), sensory barbels, 187, 192, 192
- Passaging
  - definition, 1966
  - fish cell lines, 1969
- Passive diffusion
  - transepithelial transport, intestine, 1311, 1312*f*
    - lipid uptake, 1316
  - *see also* Diffusion
- Passive hyperpolarizing potential (PHP) cells, 68, 74*f*, 75–76
  - *see also* Mauthner cells
- Passive stiffness, definition, 1060
- Passive tension
  - definition, 1060
  - titin role, 1063*f*, 1066
- Patch model, foraging, 745, 746*f*
- Patent, definition, 1284
- Paternal care, 672, 673*f*
  - preponderance, explanations, 672–673
  - *see also* Parental care
- Pathogen(s)
  - definition, 2032
  - reproductive effects, 1544
  - translocation across intestinal barriers *see under* Gut
- Pathogenesis
  - bacterial *see* Bacterial pathogenicity
  - definition, 2033
  - viral *see* Viral infection process
- Pathogenic bacteria, 2033*t*
  - *see also* Bacterial pathogenicity
- Pathologies *see* Disease
- Pattern processing, optic tectum, 135
- Patterns, integumentary, 418, 494, 495*f*
  - color pattern formation, genetic control, 1972*t*, 1975
  - zebrafish *see under* Zebrafish (*Danio rerio*)
  - *see also* Coloration
- Pavement cells (PVCs), 1098–1099, 1100*f*, 1354, 1382
  - cytological features, 1382
  - functions, 1382–1383
  - *see also* Gill ionocytes
- PBDEs (polybrominated diphenyl ethers), 2076
  - definition, 2069
- pCa$_{50}$, 1064
  - definition, 1060
- PCBs *see* Polychlorinated biphenyls (PCBs)
- *P*crit *see* Critical oxygen tension (P_{crit})
- PEA (β-phenylethyl alcohol), olfactory imprinting studies, 233–234
- Pearl organs, 488
- Pectoral fin(s), 564
  - anatomy, 565, 567*f*
  - associated muscles, 565, 568*t*
  - control, 571
  - hydrodynamics *see* Paired fin hydrodynamics
  - locomotion/stabilization role, 59
  - maneuvering role, 571
    - braking, 571
    - hovering, 571
    - yawing, 571
    - *see also specific maneuvers*
  - motion, 565
    - gait transitions, 566
    - nonoscillating passive control surfaces, 565
    - oscillating, 566, 569*f*
    - undulating, 566
- Pectoral fin tendons
  - definition, 321
  - sound generation, 324–325, 326*f*
- Pectoral girdle
  - bony fish, 445–446, 446*f*
  - cartilaginous fish, 424
    - batoids, 421*f*, 426
    - chimaeroids, 426
    - sharks, 425
  - definition, 321, 1085
  - sound generation, 324–325, 326*f*
- Pectoral spine
  - definition, 321
  - sound generation, 324–325, 326*f*
- Pedicels, 1412, 1414
  - definition, 1411
  - morphology, 1414*f*
- Pejus temperatures, 1741–1742, 1744
  - definition, 1732, 1734–1735
- Pelagic environment
  - biodiversity, 1929
  - classification, 1888*f*, 1889–1890
- Pelagic fish, **1887**
  - adaptations to pelagic life, 1891
    - body coloration, 1890*f*, 1892
    - body shape, 1890*f*, 1891
    - buoyancy, 1892
    - cardiorespiratory system, 1893
    - metabolic rates and swimming, 1893
    - sensory mechanisms, 1892
    - vertical movements *see under Vertical movements (below)*
  - definition, 1954
  - depth preference, 1894
  - diurnal rhythm, 526, 1934*f*
  - horizontal movements, 1890–1891, 1891*f*
  - migration *see* Pelagic fish, migration
  - species diversity, 1890*f*, 1890–1891
  - trophic guilds, 1956
  - vertical movements, 1891
    - limitations, 1895*f*, 1896*f*, 1897, 1899*f*
    - rapid/repeated, adaptations, 1898
      - cardiac function during acute temperature changes, 1898
      - hypoxia and thermal effects on blood–oxygen binding, 1897*f*, 1901
    - regional endothermy, 1900
  - *see also* Deep-sea fishes; *individual species*
- Pelagic fish, migration, 1890–1891, **1928**, 1891*f*
  - tracking methods
    - acoustic tagging, 1929–1930
    - geopositioning data, archival tagging, 1933, 1933*f*
    - long term tracking, shark and marlin, 1931*f*, 1932
    - multiple data from pop-up tags (PSAT), 1929*f*, 1932
    - satellite transmitters, 1930, 1931
  - tracking programs, multi-species, 1935
- Pelvic fin(s), 564
  - anatomy, 565, 569*f*

Pelvic fin(s) (*continued*)
  associated muscles, 565, 568*t*
  control, 571, 572*f*
  hydrodynamics *see* Paired fin hydrodynamics
  maneuvering role, 571
    braking, 571
    hovering, 571
    yawing, 571
    see also specific maneuvers
  motion, 570
Pelvic girdle
  bony fish, 447, 447*f*
  cartilaginous fish, 424
    batoids, 421*f*
    sharks, 420*f*
  definition, 1085
Pen-2, in Alzheimer's disease, 1993
Pepsin, 1270, 1302, 1305*t*, 1307
Pepsinogen, stomach secretion, 1270, 1302
Peptidases, 1281, 1281*t*
  see also specific enzymes
Peptide hormones, 1191
  see also specific peptide hormones
Peptide tyrosine tyrosine (PYY), 1280*t*, 1281
  amino acid sequence, 1337*f*
  gut expression, 1337
Peptidergic neurons, cartilaginous fish, 31*f*, 33–34
Peptides
  definition, 1190
  see also specific peptides
Perca flavescens (yellow perch), hypothalamic–pituitary–interrenal (HPI) axis development, 1455
Perception *see* Sensory perception
Perch
  silver *see* Silver perch
  yellow, hypothalamic–pituitary–interrenal (HPI) axis development, 1455
Perciformes
  specific dynamic action, 1609*t*, 1611*f*, 1612, 1906*f*, 1908
  see also individual species
Perfluorooctane sulfonate (PFOS), 2077
Performance consequences, prey capture, 1586
Perfusate
  definition, 1190
  hormone inactivation measurements, 1195–1196
Perfuse, definition, 1085, 1104
Perfused organs, vascular resistance measurement, 1109, 1109*f*
Perfusion, definition, 829
Perfusion conductance, gas exchange efficiency and, 825, 826*f*
Perfusion limited, definition, 1437
Peri-stimulus time histogram
  auditory afferent, 294, 295*f*
  definition, 292
Periaqueductal, definition, 56
Periaqueductal gray (PAG), 57*f*
  fight, flight, and freezing responses, 63
Pericardial cavity, definition, 1085
Pericardium
  anatomy, 999, 999*f*
  cyclostomes, 1004
  definition, 998
  elasmobranchs, 1004
  venous capacitance and, 1116
Perichondral bone
  definition, 428
  early cartilaginous fish, 429
Period, definition, 311
Period (Per) genes, 741
Period histogram
  auditory afferent, 294, 295*f*
  definition, 292
Peripheral hormones
  appetite regulation, 1512, 1512*t*
  see also specific hormones
Peripheral nervous system (PNS)
  definition, 6
  evolution/ developmental transformation, 8
    acorn worms, 10
    amphioxus, 10–11
    craniates, 11, 12*f*
      jawed, 13
    tunicates, 11
  see also Autonomic nervous system (ANS)
Peripheral olfactory organ, 194, 196*t*
  development, 194, 195*f*
  nasal sacs, 194, 196*t*
  olfactory epithelium *see* Olfactory epithelium
  olfactory receptor neurons *see* Olfactory receptor neurons
  see also Olfactory system
Peristalsis
  definition, 1229, 1292
  see also Propagating contractions
Peristaltic reflex, 1296, 1297*f*
  see also Gut motility
Peritoneal cavity, definition, 1085
Peritoneal melanophores, 490
Perivitelline fluid (PVF)
  definition, 829, 838
  gas exchange and, 832, 840, 841*f*
Persistent organic pollutants (POPs) *see under* Pollution/pollutants
Personal-care products, definition, 2069
Pesticide(s), 2073*f*, 2074
  current-use (CUPs), 2074
  definition, 2078
  sea lamprey control, 1786
Pesticide contamination, 2080
  olfactory effects, 2079*f*, 2081, 2082
    see also Olfactory toxicity
Pet trade, colorful fish, genetically-modified, 2005*f*, 2006
Petromyzontidae *see* Lampreys
Peyer's patches, 1330–1331
PFOS (perfluorooctane sulfonate), 2077
PGCs *see* Primordial germ cells (PGCs)
PGFs
  definition, 208
  olfactory responses, 212, 213*f*
    effect of pollutants, 788
  see also Prostaglandin(s)
pH
  blood/physiological, 910
    ammonia/ammonium reaction and, 1438
    regulation *see* Red blood cells (RBCs), pH regulation
  effect on blood–oxygen-binding affinity *see* Bohr effect
  environmental/water
    ammonia excretion and, 1438, 1438*f*
    effect on cardiac contractility, 1058, 1059*f*
    gustatory responses, 222, 224*f*
    ventilatory response *see* Ventilatory response to CO_2/H^+
  regulation
    red blood cells *see* Red blood cells (RBCs), pH regulation
    role in conservation of protein structure/function, 1684*f*, 1685
    see also Acid–base balance
Pharmaceuticals
  definition, 2069, 2078
  production, GM fish as biofactories, 2006
Pharmacological rescue, blind cavefish eyes, 1847
Pharmacological studies, gut motility, 1296
Pharyngeal jaw apparatus (PJA), prey processing, 468*f*, 470
Pharynx
  anatomy, 1285*f*
  definition, 1807
  development *see under* Gut development
Phase, definition, 555, 736
Phase lag, 56–57, 57*f*
Phase locking
  auditory afferents, 294, 295*f*
    transformation of peripheral code to central code, 296
  definition, 292, 366
  electric organ discharge and, 367*f*, 368, 368*f*
Phase model, sound source localization, 301
Phase shift, circadian rhythms *see under* Circadian rhythms
Phasically active, definition, 854
Phenocopy
  definition, 1843
  eye degeneration in blind cavefish, 1847
Phenology
  effect of global warming, 1740, 1743*f*
  photoperiod effects on thermal tolerance, 1701
Phenotype
  definition, 777, 1986, 1998
  manipulation by fish parasites *see under* Host manipulation
Phenotypic, definition, 1197
Phenotypic plasticity
  definition, 1833
  see also Cyprinodon (desert pupfish)
Phenotypic sex, definition, 1490
β-Phenylethyl alcohol (PEA), olfactory imprinting studies, 233–234
Pheromone(s)
  definition, 183, 227, 471, 1553, 1807
  hormonal *see* Hormonal pheromones
  nonhormonal, 1555
  potency/specificity *see under* Olfaction, neurophysiology
  receptors, 185
    see also Olfactory receptor(s) (ORs)
  reproductive, 230–231, 231*f*
    see also Hormonal pheromones
  skin-associated, 473
  see also specific pheromones
Pheromone active space
  androstenedione, 1560–1561
  definition, 1553
Phonotaxis
  definition, 298
  plainfin midshipman, 300–301
Phosphatidylcholine, 1308*f*
Phosphocreatine *see* Creatine phosphate
Phosphoglycerides
  cell membranes, 1726*f*, 1727
  definition, 1725
Phospholamban, 1052
Phospholipids
  cell membrane
    arrangement, 1726, 1726*f*
    role, 1728
      see also Cell membrane fluidity
    see also Cell membrane(s)
  definition, 1709, 1725
  mitochondrial membrane, 1710
    thermal effects, 1709*f*, 1710–1711
      acute thermal change, 1712–1713
      evolutionary thermal adaptation, 1713
      thermal acclimation, 1714*f*, 1715
Phosphorous budget, 1679
Phosphorous cycling, role of fish, bioenergetics model application, 1679
Phosphorylation, mitochondrial, 963
  see also Oxidative phosphorylation
Phosphotide, definition, 1725
Phosphovitin (Pv), 637, 637*f*
Phosvitinless vitellogenins, 644
Photic, definition, 1888
Photic environment, 110
  definition, 99, 110, 116
  photoreceptor/visual pigment adaptations, 116
    chromophore exchange, 120, 121*f*

    gene duplication in shallow-living species, 118, 119*f*, 120*f*
    increasing water depth, 117, 118*f*
  variations, 116
Photon capture, retinal modifications in deep-sea fishes, 173, 175*f*, 176*f*
Photon-limited environment, visual adaptations, 167
  see also Deep-sea fishes, visual adaptations
Photoperiod
  appetite/food intake effects, 1486, 1513–1514
  definition, 1629
  growth effects, 1489, 1632
  migratory effects, 1925–1926
  thermal tolerance and, 1701
Photophore
  communication role, 473
  definition, 497
Photopigment
  definition, 99, 159
  see also Visual pigments
Photoreception
  extraretinal *see* Extraretinal photoreception
  retinal *see* Retina
Photoreceptors, 107, **110–115**, 159
  adaptations to photic environment *see under* Photic environment
  cones *see* Cones
  evolution, 116, 117*f*
  extraretinal *see* Extraretinal photoreceptors
  mosaics, 114, 114*f*, 121
  renewal in deep-sea fishes, 174
  rods *see* Rods
  see also Retina
Photosensitivity, deep-brain, 160, 160*f*
Phylogenetic, definition, 1197
Phylogenetic analysis, definition, 89
Phylogeny
  air-breathing fish *see under* Air-breathing fish
  definition, 670, 1276
  hagfish, 1771*f*, 1772
  olfactory receptors, 200–201, 203*f*
  outflow tract, heart, 1015, 1016*f*
  parental care, 673*f*, 675
  see also Evolution
Physiological color changes *see under* Color changes
Physiological optics *see* Optics, physiological
Physiological thermoregulation, definition, 758
Physiology, definition, 1986
Physoclistous gas bladder, 1853–1854
  definition, 1085, 1851
  morphology, 526, 527*f*
  see also Swimbladder
Physoclists
  definition, 1085
  gas bladder *see* Physoclistous gas bladder
  visceral arteries, 1091
Physostomes
  definition, 1085
  gas bladder *see* Physostomous gas bladder
Physostomous gas bladder, 1091, 1853–1854
  definition, 1851
  morphology, 526, 527*f*
  see also European eel (*Anguilla anguilla*), swimbladder; Respiratory gas bladder
Phytate, definition, 1666
Piaractus mesopotamicus see Pacu
Pigment-based colors
  reflected-light signaling, 694
  see also Chromatophore(s)
Pigment cell *see* Chromatophore(s)
Pigment cell tumors, 1977
  goldfish erythrophoroma, 1977
  transgenic skin tumor models, 1977*f*, 1978
  Xiphophorus melanoma, 1976*f*, 1977
Pigment genes, 1972*t*, 1973, 1975
  melanin synthesis, 1972*t*, 1974*f*, 1975
  polymorphisms, 1976
    blind cavefish, 1976
    Danio species, 1976
    stickleback, 1977
  pteridine synthesis, 1972*t*, 1974*f*, 1975
  see also Chromatophore development, genetic control
Pigmentary coloration *see under* Coloration
Pigmentation, **1971**
  human diseases, fish models, 1993*f*, 1994
  see also entries beginning pigment, color
Pike perch (*Stizostedion lucioperca*), ears, 256*f*
Pile drivers, noise generation, 305*t*, 305–306
Pillar cells, 804, 805*f*, 1097*f*, 1098, 1099*f*, 1100*f*
  carbonic anhydrase IV, 905, 906*f*
  definition, 899, 1190
  hormone metabolism, 1192, 1193*f*
  see also Lamellae, gill
Pilotage, 722
  definition, 720
Pineal complex, photoreception *see* Pineal photoreception
Pineal opsins
  lamprey, 161–162
  teleosts, 163
Pineal organ, 162
  agnathan, 21, 22*f*
    lampreys, 161
  cartilaginous fish, 28*f*, 32
  circadian rhythmicity, 165, 741
  definition, 99, 159
  physiology, light-evoked responses, 163
    see also Pineal photoreception
  ray-finned fish, 42
  structure/ultrastructure, 162, 162*f*, 164*f*
    adaptive radiation, diversity, 163, 164*f*
  see also Parapineal organ
Pineal photoreception, 160*f*, 161
  lamprey parapineal/pineal organs, 161
  teleost pineal, 162
    light-evoked responses, 163
    visual pigments, 163
Pineal regulation, color changes, 493, 494
Pinealectomy, effect on circadian rhythms, 741, 741*f*, 742*f*
Piranha, drumming muscles, 324, 326*f*
Piscivores
  definition, 1589
  prey selection
    ontogenic diet shifts and, 1594
    prey size, 1593
Pit organs *see* Superficial neuromasts
Pitch, 575–576, 576*f*, 577
  definition, 575
  see also Maneuverability
Pituicytes, 1458
  definition, 1457
Pituitary adenylate cyclase activating polypeptide (PACAP)
  amino acid sequences, 1338*f*
  catecholamine release role, 1530
  gut expression, 1338
    receptors, 1338
  gut motility role, 1297–1298, 1299*f*
Pituitary gland, **1457–1465**, 1452
  anatomy, 1458, 1458*f*
    macroscopic, 1451*f*
    microscopic *see histology (below)*
  brain relations, 1460, 1461*f*
  definition, 649, 1509, 1542
  elasmobranchs, 1458–1459, 1460*f*
  hagfish, 1459, 1461*f*
  histology, **1457–1465**
    anterior pituitary *see* Adenohypophysis
  hormones *see* Pituitary hormones
  lampreys, 1459, 1461*f*
  neurohypophysis *see* Neurohypophysis
  regulation, color changes, 493
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Pituitary hormones
  adenohypophysis, 1460
    immunocytochemical localization, 1460, 1461*f*, 1461*f*, 1462*f*
  appetite regulation, 1512, 1512*t*
  gonadotropins *see* Gonadotropin(s)
  neurohypophysis, 1462
  see also specific hormones
Pituitary regulation, color changes, 494
pKa, definition, 1119
Placenta, nutrient provision, 679–680
Placoderm
  definition, 1851
  lung-like structures, 1852–1853
Placodes
  definition, 6, 194, 244, 336
  lateral line, 284
  mechanoreceptive sensory organs, 245, 245*f*
  olfactory, 194, 195, 195*f*
  otic, 284
  see also Neurogenic placodes
Placoid scales
  definition, 476
  morphology, 479*f*, 484, 485*f*, 486*f*
    modified, 487
Plainfin midshipman (*Porichthys notatus*)
  phonotaxis, 300–301
  sound production, 322, 323*f*
    sound-producing muscles, 323–324, 324*f*
      sexual dimorphism, 324*f*, 325–326
    vocal neuron control, 326–328, 327*f*
Planktivores
  definition, 1589
  foraging
    prey selection
      ontogenic diet shifts and, 1594
      size, 1593
    search speed, 1592, 1593*f*
Plasma, 984–985, 985*f*
  definition, 984
  skimming, 987*f*, 988
Plasma ionic compositions, 1349, 1350
  freshwater fish, 1349*t*
  hagfish, 1349, 1349*t*
  seawater fish, 1349*t*
Plasma membrane Ca^{2+}-ATPase (PMCA), 1051
  definition, 1045
Plasmalogen, homeoviscous adaptation, 1729*t*, 1731
Plasticity
  auditory *see* Auditory plasticity
  developmental, hypoxic hyperventilatory response, 1755
  electrosensory fishes, 384, 385*f*
  gill morphology *see* Gill remodeling
  heat shock response *see under* Heat shock response
  neural connections, definition, 131
  optical, 106
  phenotypic, definition, 1833
  retinotectal map, 134, 134–135
Platelets, iridophore, 490*t*, 491, 492, 492*f*
Pleiotropic, definition, 2016
Pleiotropy
  definition, 1843
  troglomorphic traits in blind cavefish, 1845*f*, 1848
Plerocercoid, *Schistocephalus solidus*, 780*f*, 780–781
Plesimorphy, definition, 1851
Pleuronectiformes (flatfish)
  skin patterns, 495*f*, 741
  specific dynamic action, 1609*t*, 1611*f*, 1614
Plexiform layer *see under* Retina
Pluripotent epiblast cell, definition, 2046
PMCA *see* Plasma membrane Ca^{2+}-ATPase (PMCA)
Pneumatic duct, 1853–1854
  air-breathing role, 1870
  definition, 1852
  see also Physostomous gas bladder
Podocytes, 1412, 1414*f*, 1414–1415
  definition, 1411

Podocytes (*continued*)
  opisthonephros, 1396f, 1401–1402, 1403f, 1405f
  pronephros, 1399, 1400f, 1400f
Poikilotherms
  adaptive strategies for thermal stresses, 1696–1697, 1703, 1705
  definition, 1509, 1667, 1689, 1695, 1703
Polar body, definition, 1998
Polar headgroup, definition, 1725
Polarized light, detection, behavioral studies, 148, 148f
Pollution/pollutants
  definition, 2062, 2069
  effects on thermal tolerance, 1700t, 1701
  endocrine disruptors *see* Endocrine disruptors
  environmental partitioning, 2070
  fast start performance effects, 595
  intertidal habitats, 1961
  multixenobiotic resistance (MXR), 2074
  persistent organic pollutants (POPs), 2070, 2073f
    emerging risks, 2076
    known contaminants, 2074
  see also Toxicants
Polybrominated diphenyl ethers *see* PBDEs (polybrominated diphenyl ethers)
Polychlorinated biphenyls (PCBs), 2076
  accumulation in fish, bioenergetics model application, 1679
  definition, 1675, 2069
Polycyclic aromatic hydrocarbons *see* PAHs (polycyclic aromatic hydrocarbons)
Polygenic sex determination, 2028
Polymerase chain reaction (PCR), definition, 1474
Polyphyletic character, definition, 387
Polyploid, definition, 2009
Polyploidization, 1981
  definition, 1980
Polypteridae
  ampullary organs, 352
  brain structure, 38f, 42, 43–44, 44f
  circulatory patterns, 1876f, 1877f, 1880f, 1881f, 1882
  see also Bichirs; Reedfish
Pomacentrus ambionensis, UV vision and colors, 156, 157f
Ponyfish, bioluminescence, 501
Pop-up satellite tag, 1932
  definition, 1928
Population dynamics
  effects of environmental temperature changes, 1740
    abundance and productivity, 1741
    community composition, 1741
    geographical distribution, 1740
    species interactions, competitiveness, 1743f, 1744
  monitoring by telemetry, 1930, 1932
Population variation, heat shock response, 1736
Porcupine fish (*Diodon liturosus*), antipredator morphology, 750, 750f
Porichthys notatus see Plainfin midshipman
Porphyropsins, 111, 120, 121f, 121–122
  definition, 99, 110, 116
Portal circulation, 980–981, 1134–1135
  definition, 977
Portal heart, hagfish, 1074, 1775
Portal system
  definition, 1092
  hepatic, 1092, 1134–1135
  renal, 1092
Portal vessel, definition, 1457
Positional cloning, definition, 1986
Positive buoyancy
  definition, 516, 520, 1887
  see also Buoyancy
Positive selection, definition, 966
Post-absorptive processes, SDA contributions, 1615
Post-cardinal vein, definition, 1119
Post-effector mechanisms
  definition, 387
  electrogenesis, 395, 396f
Post-gill (systemic) arteries, 1087f, 1088, 1088f
  anterio-ventral, 1091
  posterio-dorsal, 1089
    head, 1086f, 1089
    segmental, 1087f, 1090
    trunk, 1087f, 1089
    visceral, 1087f, 1090
POST (Pacific Ocean Shelf Tracking) program, 1935
Postcranial axial skeleton, bony fish *see under* Bony fish skeleton
Posterio-dorsal post-gill arteries *see under* Post-gill (systemic) arteries
Posterior, definition, 419
Posterior mesenteric artery (PMA), rectal gland, 1431–1432
Postganglionic neurons, cardiac *see* Intracardiac neurons/neurotransmitters
Postovulatory prostaglandin pheromones, 1557f, 1558, 1559f, 1559–1560
Postprandial, definition, 1206, 1229, 1292, 1341, 1608
Postprandial metabolic response, 1608f, 1610
  see also specific dynamic action (SDA)
Postprandial thermogenesis, 1915–1916, 1919f, 1920
Postsynaptic, definition, 871
Postsynaptic potential
  definition, 398
  electric signal generation, 400–401
Posture control *see* Stability control
Potamodromy, 1921f, 1922
  definition, 1921
Potassium channels
  calcium-activated, definition, 56
  Kir2 channel, 1040, 1720–1721
Potassium current
  cardiac action potential, 1040
  see also Inward rectifier
Potential energy, definition, 1073
Potentiate, definition, 1206
Power, definition, 555, 556
Power analyses, experimental hydrodynamics, 545, 545f
Power output, myocardial, 1035t, 1036–1037
Power spectral analysis, definition, 1178
Power spectral density, definition, 311
PPCPs (pharmaceuticals and personal care products), 2076
Pre-absorptive processes, SDA contributions, 1615
Pre-gill arteries, 1086, 1086f
Precapillary sphincters, 1104–1105
  definition, 1104
Precocious parr, 652
Predation
  studies using biomimetic robotic fish, 605–606, 607f
  trade-off, surfacing *see under* Hypoxia, behavioral responses
  see also entries beginning prey;Predator(s)
Predation risk
  foraging-associated, 1590
    food availability and, 1591
  reduction, migratory eels, 1940–1941, 1942
  survival strategies *see* Survival strategies (food acquisition-associated)
Predator(s)
  biomimetic robots, 606, 607f
  detection, electrosensing, 365
  repulsion, sound production, 685t, 689
  see also Eavesdroppers; Predation
Predator avoidance, 749
  compensatory growth effects, 755
  learning/memory and, 710
  porcupine fish, 750, 750f
  sound production, 685t, 689
Predator strike, 587–588, 588f, 589, 590f
  definition, 587
  neural control, 588–589
  see also Fast start
Predator–prey interactions
  chemosensory behavior, 233
  trade-offs, 1591, 1593
    see also Foraging decisions (energetics)
  utilization by fish parasites, 778
    see also Host manipulation
  vocal, 689–690
Predator–prey trophic link, strength estimation, bioenergetics model application, 1678
Preferred temperatures *see* Temperature preferences
Preglomerular nuclear complex
  auditory/lateral line connections, 284f, 290
  definition, 37
  ray-finned fish, 40f, 41, 42
Prehatch nutrient provision *see under* Parental nutrient provision
Premaxillary protrusion, 469
  definition, 463
  mechanism, 464f, 469
  see also Jaw(s), protrusion
Premotor, definition, 56
Preoptic area
  agnathan, 22f, 23, 24f
  cartilaginous fish, 34
  Latimeria, 54f, 54–55
  lungfishes, 49f, 51–52
  ray-finned fish, 40f, 41–42
Preoptic–thalamic–pretectal (PTP) areas, 140f
  tectal connections, 139, 140f
Preovulatory steroid pheromones, 1554f, 1556, 1556f, 1559f
Prepacemaker nucleus, 702–703
  definition, 699
Preprohormone, definition, 649
Pretectum
  agnathan, 21, 22f
  cartilaginous fish, 32
  ray-finned fish, 40f, 41–42
Previtellogenesis, 635–636, 636f
Prey capture
  behavior, 1585, 1585f
  definition, 463
  electrosensing, 364
  energetics, **1583**, 1594
    competition theory relevance, 1584
    ecomorphological paradigm, 1583f, 1584
    performance consequences of behavioral/morphological changes, 1586
    see also Foraging decisions (energetics)
  Mauthner cell role, 74f, 75
  strategies, 597, 747–748
    manipulation (biting) *see* Biting
    prey type/size, 748
    pursuit, 747f, 747–748
    ram feeding, 597
    sit-and-wait, 747f, 747–748
    suction *see* Suction feeding
  swimming integration *see under* Swimming
  see also Foraging behavior; Predator strike
Prey detection, lateral line function, 333
Prey handling
  definition, 1589
  energetics, 1594
    costs of prey pursuit/capture, 1594
    prey orientation and ingestion, 1594
    see also Foraging decisions (energetics); Prey capture, strategies
Prey handling time, 1593
Prey ingestion, energetics, 1594
Prey orientation, energetics, 1594
Prey processing
  definition, 463
  pharyngeal jaw apparatus, 465t, 468f, 470
Prey pursuit, energetics, 1594
Prey robots, biomimetic, 606, 607f
Prey selection *see under* Foraging decisions (energetics)
Primary circulation, secondary circulation *vs.*, 1162, 1163–1165, 1164f

Primary cultures, 1966
  definition, 1966
Primary lamellae *see* Gill filament
Primary oocyte, definition, 1999
Primary production, definition, 1833
Primary stress response *see under* Stress response, hormonal
Primitive, definition, 283
Primitive condition, definition, 1772
Primitive gut tube, 1285
  definition, 1284
  see also Gut development
Primitive vertebrate feature, definition, 283
Primordial germ cells (PGCs), 2047
  definition, 2046
  migration, 2047f, 2048f, 2050
    medaka, 2049f, 2050–2052
    reasons for, 2052
    visualization with GFP technology, 2047f, 2048
    zebrafish, 2049f, 2050
    see also Sex differentiation
  pseudopodia, 2048f, 2050
  specific markers, 2047
    nuage *see* Nuage
  specification, 2047
    period of, 2048
Principal islet
  definition, 1276
  see also Brockmann body
Prion, definition, 1999
Prismatic calcified cartilage
  cartilaginous fish, 431
  definition, 428
Pro-opiomelanocortin (POMC), 91, 93t, 1451f, 1452
  embryonic expression, 1454
  see also Hypothalamic–pituitary–interrenal (HPI) axis
Pro-toxins, definition, 2034
Proactive coping style, stress, 1520, 1521f
Probiotics, definition, 2033
Procercoids, *Schistocephalus solidus*, 780f, 780–781
Prodynorphins, 91, 92t
Proenkephalin, 89, 90t
Proerythroblasts, 993, 994f
  see also Erythropoiesis
Progeny
  parental care *see* Parental care
  stressor effects, 1543f, 1545
Progestogens
  progesterones, spermatogenesis role, 632, 633f
  steroidogenic shift from estradiol, 1503, 1504f
Programmed rheostasis, 1177
  definition, 1169–1170
Prolactin
  definition, 670, 1466
  ion regulation role, 1467f, 1469
  parental care role, 670
    fanning behavior, 675–676
Prolactin receptor (PRLR), osmosensing role, 1378f
Proliferation, definition, 1966
Prolonged mode (non-sustainable swimming), 1656f, 1659–1660
Promoter, definition, 1971, 1999, 2016
Pronephros (head kidney)
  definition, 1541, 1788
  histology, 1397f, 1399, 1400f, 1401f
  larval lamprey, 1796
  stress response role, 1544f, 1549
    see also Interrenal cells/tissue
Pronociceptins, endogenous opioid peptides, 91
Propagating contractions, 1293, 1293f, 1294f
  definition, 1292
Proprioception, definition, 1807
Prosencephalon *see* Forebrain
Prosomeres
  agnathan, 21, 22f
  cartilaginous fish, 32
Prostaglandin(s)
  definition, 208, 656, 1553
  hematopoietic stem cell increase, 1990–1991
  olfactory responses, 212, 213f, 214f
  PGFs *see* PGFs
  sexual behavior role, 659, 661
Prostaglandin pheromones
  female postovulatory, 1557f, 1558, 1559f, 1559–1560
  sexually dimorphic responses, 1558f, 1559
Protandry/protandrous fish, 663, 664f, 1491, 1491t, 2051t, 2052–2053
  definition, 662
  histological aspects, 663, 664f
  see also Hermaphroditism; Sex change
Protease(s)
  definition, 2033
  pathogenesis role *see under* Extracellular products (ECPs), disease role
Protease inhibitor, definition, 1667
Protection, integument role, 471, 472f
Protein(s)
  absorption *see* Protein absorption
  ATP production role, 952f, 953, 955–956
  conservation of structure/function *see* Protein structure/function, conservation (biochemical adaptations)
  deep-sea fishes, 1957
  definition, 1667
  denaturation *see* Protein denaturation
  destabilization, 1705
  dietary requirements of cultured fish *see under* Cultured fish, dietary requirements
  digestion, 1305t, 1306f, 1307
  digestive efficiency, 1600t, 1601
  effect of temperature *see under* Temperature effects
  hagfish slime threads, 504, 505f, 508
    assembly from intermediate filaments, 508
    conformation, stretched, 509–510, 510f
    elasticity and breaking strength, 509f, 509f, 509–510
    unraveling mechanism, 510–512, 512f, 513f
  heat shock *see* Heat shock proteins (HSPs)
  isoforms, carbonic anhydrase *see under* Carbonic anhydrase (CA)
  membrane *see* Membrane proteins
  muscle *see under* Skeletal muscle
  native state, definition, 1703
  SDA and, gastrointestinal blood flow, 1212
  stabilization, 1705
    osmolytes, 1684
  stabilizing forces
    disulfide bonds, 1707
    noncovalent interactions, 1706, 1707
  synthesis, hypoxia effects, 1769
  see also Amino acids; Enzymes; *specific proteins*
Protein absorption, 1314
  amino acids, 1314, 1314f
  di- and tripeptides, 1314f, 1315
  intact proteins, 1313f, 1315
Protein coat, viral *see under* Viral structure
Protein conformation, definition, 1054, 1703
Protein denaturation, 1705–1706
  definition, 1703, 1732
  as heat shock response trigger, 1735f, 1736
Protein permeability, capillaries, 1159
Protein sequence, definition, 1054
Protein structure/function, conservation (biochemical adaptations), 1682f, 1683
  conservation of appropriate internal milieu, 1683
    organic osmolytes, 1683, 1683f
    pH, 1684f, 1685
Proteolysis, maturational, yolk proteins (YPs), 641, 643f, 644f
Proteolytic enzymes, pancreatic *see* Pancreatic enzymes
Proteomics, definition, 2033
Protogyny/protogynous fish, 663–665, 664f, 2051f, 2051t, 2052–2053
  definition, 662
  diandric, 663–665, 664f
  initial phase, 664f, 665f
    definition, 662
  monandric, 663–665, 664f
  terminal phase, 664f, 665f, 665–666
  see also Hermaphroditism; Sex change
Proton effects, oxygen-binding properties of hemoglobin, 890
  see also Bohr effect, fish hemoglobins
Proton motive force (PMF), 963, 1758–1759
  definition, 959, 966
Proton trapping, definition, 829
Protopterus
  brains
    forebrain, 48f, 49f, 51
      telencephalon, 48f, 49f, 52
    gross morphology, 47, 47f
    hindbrain, 48f, 48–49, 49f
      cerebellum, 50
    histological features, 48f, 48–49, 49f
  estivation *see under* Estivation
  see also Lungfishes
Protopterus dolloi (African lungfish), heart, outflow tract, 1016–1018, 1019f
Proximal, definition, 123, 419
Proximal intestine
  definition, 1341
  see also Intestine
Proximal pars distalis (PPD), histology, 1458, 1458f, 1458f, 1463f, 1464f
  hormone immunocytochemistry, 1460, 1461f
Pseudobranch, 932, 932f, 1095–1096, 1101
  definition, 929, 1095, 1752
  function, 929
    ocular blood supply, 932, 932f
    ocular oxygen secretion, 933
  location, 932–933, 933f
  morphology, 933, 933f
  oxygen sensing and, 1755
Pseudoplacenta, 680, 680f
  definition, 678
Pseudopodia, germ cell, 2048f, 2050
Pseudupleuronectes americanus (white flounder)
  color, 495f
  skin pattern, 495f
Psychoacoustics, **276–282**
  see also Hearing
Psychophysics, definition, 276, 277
Pteridine
  pigmentation, 490t, 491
  synthesis, 1972t, 1974f, 1975
Pterygiophores, 439f, 443–445
Puberty, 617–618
  endocrine profiles, triploid fish, 2010–2011
  sturgeons, 1802
Public anxieties, genetically modified fish, 2007
Pulmonary vasomotor segment (PAVS), 1879f, 1882
  definition, 1877
Pulsatile flow, effect on gas exchange efficiency, 827
Pulse pressure, 979
Pulse train, definition, 311
Pulse-type electric fish, 366
  amplitude coding, 367, 367f, 369t
  gymnotiform, EOD time patterns, central control, 403, 403f, 404f, 405f
  mormyrid *see* Mormyrid pulse-type fish
  time coding, 368, 368f, 369t
  see also Tuberous electrosensory system, physiology
Pulse-type electric organ discharge, 366, 367f, 701, 701f, 702
  see also Electric organ discharge (EOD)
Pupfish *see* *Cyprinodon* (desert pupfish)
Pupils, 105
Purine pigments, 490t, 491
Purines, as gut neurotransmitters, 1339

Purkinje cells, 64
  definition, 56
Purkinje fibers, 1071
Pursuit strategy, prey capture, 747*f*, 747–748
Putrescine, olfactory responses, 214
Pygoplytes diacanthus see Angelfish
Pyloric ceca, 1269*f*, 1270, 1273, 1602*f*
  definition, 1119, 1597
  species differences, 1273, 1273*f*, 1274*f*
Pyrethroids, olfactory effects, 2081
Pyruvate
  fate of, 1760
  generation, 1759*f*, 1760
Pyruvate dehydrogenase (PDH) complex, 1824
  crucian carp, 1825*f*, 1827–1828
PYY *see* Peptide tyrosine tyrosine (PYY)

Q

Q_{10} (temperature coefficient), 1693, 1696
  definition, 951, 1197, 1689
Q_{10dB}, 295–296
  definition, 292
Qinghai carp (*Gymnocypris przewalskii*), gill remodeling, 799–800, 800*f*
Quantile
  definition, 1566
  standard metabolic rate representation, 1569*f*, 1569*t*, 1570
Quantitative trait locus (QTL)
  analysis, inheritance of troglomorphic traits in blind cavefish, 1848
  definition, 1843
Quiet vocalizers, 685*f*, 686, 686*f*
Quinine, gustatory response, 220, 221*f*, 225*t*
Quorum sensing, 2038
  definition, 2034

R

R-state hemoglobin, 889*f*, 889–890, 946, 946*f*
  definition, 526, 887
  see also Hemoglobin(s), fish
Radiance, definition, 99, 150
Radiation, adaptive, air-breathing fish, 1858
Rainbow trout (*Oncorhynchus mykiss*)
  bulbus arteriosus, 1018, 1022, 1024*f*
  cold acclimation, myocyte excitability, 1719*f*, 1721
    sodium and calcium flux, 1723
  feeding behavior
    amino acids and, 228–230, 229*f*, 230*f*
    bottom searching, 228*f*, 228–230
  gill remodeling, 801
  gustatory responses, 219*f*
    amino acids, 219, 219*f*, 220*f*, 222*f*
    bile acids, 221, 223*f*
    carbon dioxide/pH, 222–223, 224*f*
    quinine/strychnine/tetrodotoxin, 220, 222*f*
    tactile stimulation, 224–225, 225*t*
  gustatory system
    brain, neural pathways, 190, 190–191
    taste buds, 187, 189*f*, 189–190
  hypothalamic–pituitary–interrenal (HPI) axis development, 1455
  metabolic rate, abrupt developmental changes, 1577–1578
  noxious stimulus avoidance, 714–715
  olfactory receptor neurons, 214–215
  olfactory responses
    amino acids, 209–210, 210*f*, 211*f*, 216, 228
    electroencephalography, 214*f*
    prostaglandins, 213
  osmotic acclimation, ionocyte proliferation, 1386
  pain response, 715, 716*f*, 716–717, 717*f*, 717*f*, 718*t*
    significance, 718, 718*f*
  respiratory gases in blood, related to swimming speed, 815*f*, 817
  vascular system
    capillary size and structure, 1145*f*, 1152*f*
    cardiac angiogenesis, 1145–1146
    seasonal changes, in red muscle, 1151*f*
Raja clavata (thornback ray), 263*f*, 264
Raja undulata, brain, 27*f*, 31*f*, 34*f*
Ram, definition, 597
Ram feeding, 597
  speed, 601, 1974*f*
Ram ventilation, 852, 1753
  definition, 854, 1629, 1752, 1888, 1903
  growth and, 1632
  pelagic fish, 1891–1892, 1893–1894
Raphe, definition, 449
Rapid eye movement (REM) sleep, 773
RAS oncogene pathway, 1994
Rate code, definition, 366
Rate constant, definition, 1190
Rate-level function
  auditory afferents, 293*f*, 293–294
  definition, 292
Rate processes, effects of temperature *see under* Temperature
Ratfishes *see* Chimaeras
Ray-finned fish
  Bohr effect, 923
    evolution, 926*f*, 927–928
      hemoglobin buffer properties and, 927*f*, 928
    see also Bohr effect, fish hemoglobins
  brains *see* Ray-finned fish, brains
  endogenous opioid peptides
    prodynorphins, 91, 92*t*
    proenkephalins, 90*t*
    pronociceptin, 91
    proopiomelanocortins, 93*t*
  intracardiac neurons/neurotransmitters, 1069, 1070*f*
  SDA *see* Ray-finned fish, specific dynamic action
  skeleton *see* Bony fish skeleton
  taxonomy, 37, 38*f*
  see also Teleosts; *individual species*
Ray-finned fish, brains (functional morphology), **37–45**
  forebrain, 3, 4*f*, 41
    diencephalon, 3, 4*f*, 40*f*, 41
    telencephalon *see under* Telencephalon
  hindbrain, 3, 4*f*, 37, 40*f*
  midbrain, 3, 4*f*, 40*f*, 41
  structural diversity, 4–5, 37, 38*f*
Ray-finned fish, specific dynamic action, 1609*t*, 1612
  Anguilliformes, 1609*t*, 1611*f*, 1612
  Cypriniformes, 1609*t*, 1611*f*, 1612
  Gadiformes, 1609*t*, 1611*f*, 1612
  Perciformes, 1609*t*, 1611*f*, 1612, 1906*f*, 1908
  Pleuronectiformes, 1609*t*, 1611*f*, 1614
  Salmoniformes, 1609*t*, 1611*f*, 1612
  Scorpaeniformes, 1609*t*, 1611*f*, 1612
  Siluriformes, 1609*t*, 1611*f*, 1612
RayBot, 607–608, 608*f*, 609*f*, 610*f*
  swimming performance, 610, 611*f*
Rays, 26
  auditory system structure *see under* Auditory system structure
  hearing *see under* Hearing
  lateral line system, 336, 341, 344*f*
  see also Batoids; Elasmobranchs
Reabsorption, renal, 1413*f*, 1414
Reactive coping style, stress, 1520, 1521*f*
Reactive oxygen species (ROS)
  production, 1710
  role in erythrocytic ion transport, 880, 882
Reactive rheostasis, 1176
  definition, 1170
Reafference, definition, 375
Receiver, 692
  definition, 692
Receptive field
  definition, 99, 123, 131
  ganglion cells, 126, 127*f*
  multiunit, 134
Receptor(s)
  definition, 183, 1466
  gut neurotransmitters *see under* Enteric nervous system
  see also specific receptors
Receptor-mediated endocytosis
  definition, 1190
  hormones, 1191–1192
Receptor tyrosine kinase, definition, 1971
Reciprocal sex-change fish, 2051*t*, 2052–2053
Recombinant gonadotropins, 1504
Rectal gland, dogfish, **1429–1436**
  activation/secretion control, 1434
    endocrine, paracrine, and autocrine regulation, 1434
    extracellular pH and ionic strength, 1435
    molecular mechanisms, 1435
    morphological contributions, 1435, 1436*f*
    nervous control, 1435
  anatomy, 1430
    feeding response, 1435, 1436*f*
    gross morphology, 1430, 1431*f*
    secretory cell morphology, 1431*f*, 1432
    vasculature, 1431, 1431*f*
  fluid, 1429–1430, 1430*t*
  function, 1430, 1434
    studies, 1432
  salt transport
    energetic requirements, 1433
    mechanism, 1432
      ion movements, 1432, 1433*f*, 1811*f*, 1812–1813
Rectum
  definition, 1597
  morphology, 1602*f*
Red blood cells (RBCs), 985
  abnormalities, triploid fish, 2011*f*, 2012
  adrenergic response *see under* Sodium/proton exchange, RBC
  carbonic anhydrase, 899, 900*f*, 904, 905, 906*f*, 910–911, 911*f*, 911–912, 913
    see also Carbon dioxide (CO_2) transport/excretion
  concentration *see* Hematocrit
  definition, 984, 984, 1395
  development *see* Erythropoiesis
  fish *vs.* mammal, 993, 995
  function *see* Red blood cells (RBCs), function
  hemoglobin concentration *see* Mean cell hemoglobin concentration (MCHC)
  NTP concentration, control, 883, 885*f*
  size, 1143–1144
    diploid *vs.* triploid fish, 2010*f*, 2011–2012
  sodium/proton exchange *see* Sodium/proton exchange, RBC
  splenic release, 986, 987*f*, 987–988
  swelling, effect on hematocrit, 987*f*, 988
  volume regulation, 880, 882*f*
  white blood cells *vs.*, 988
  see also Hemoglobin(s), fish
Red blood cells (RBCs), function, 985–986, 992, 993–994
  gas transport/exchange, **879–886**
    carbon dioxide *see* Carbon dioxide (CO_2) transport/excretion
    oxygen transport, 879–880
      see also Oxygen binding, fish hemoglobins
    see also Carbon dioxide (CO_2) transport/excretion; Hemoglobin(s); Oxygen–carbon dioxide interaction
  regulation of capillary function, 884
Red blood cells (RBCs), pH regulation, 880, 881*f*, 912, 913*f*, 913*f*
  effect of temperature and variations in environmental CO_2 levels, 914
  see also Sodium/proton exchange, RBC
Red body *see* Retia mirabilia
Red fluorescent protein (RFP)
  definition, 1999

  - use in fish transgenesis, 2003, 2006
- Red muscle, 550, 550*f*, 556, 1637*f*, 1638
  - definition, 1119, 1636, 1888
  - endothermy, 1915
  - energetics (aerobic swimming), 1638, 1638*f*
    - measurement, 1638*f*, 1639
  - mitochondrial content, 969–970
    - *see also* Muscle mitochondria
  - white muscle *vs.*, work-loop analysis, 560, 561*f*
  - *see also* Skeletal muscle
- Rediploidization, 1981–1982
  - definition, 1980
- Reducing equivalents
  - definition, 959, 966, 1709, 1758
  - oxidation by electron transport chain, 963
  - production, 959–960
- Reedfish
  - ampullary organs, 352
  - brain structure, 38*f*
- Reef fish, color vision/communication, **150–158**
  - anti-communication (camouflage), 151
  - camouflage and communication, 151*f*, 152, 154*f*
    - pointillism, 155, 155*f*
    - yellow and blue coloration, 152, 154*f*
  - color codes and evolution, 150
  - colored aposematically, 151
  - communication and deception, 155, 156*f*
    - cleaner blue (yellow) color guilds, 155
    - mimicry (color), 156
  - communication colors, 152
  - flamboyant signals, evolution, 698
  - hiding and advertisement behavior, 150–151, 153*f*
    - related to lifestyle, 155
    - territorial signals, 151, 155, 156–157
  - partial color blindness, 151
  - UV vision, 156, 157*f*
  - vision diversity, 156
  - visual acuity, 153–154
  - *see also* Coral reefs
- Reflectance, definition, 694
- Reflected-light signaling *see under* Visual communication
- Reflection coefficient, 1156
  - definition, 1154
- Reflexes, gut, 1296, 1297*f*
- Refraction, sound waves, 272
- Refractive index, definition, 99, 102
- Refractory period, definition, 1718
- Refuge, hypoxia as, 766
- Regional endothermy
  - definition, 1888
  - pelagic fish, 1900
    - tuna *see under* Tuna
- Regional heterotherm, definition, 1689
- Regressive traits
  - blind cavefish troglomorphism, 1844
    - *see also* Eye degeneration, blind cavefish
  - definition, 1843
- Regulation, human-generated sounds, 308
- Relay nucleus, electric fish, 399–400, 401*f*, 402, 402*f*, 405*t*
  - *see also* Electric organ discharge (EOD)
- Releasing factor, definition, 1457
- Remodeling, gill *see* Gill remodeling
- Remote monitoring *see* Telemetry, wildlife
- Remote-operated vehicles (ROVs), luminescence observation, 498, 502–503
- Renal *see* Kidney(s)
- Renal corpuscle, structure, 1396*f*, 1397–1398, 1398*f*, 1402*f*
  - adult lamprey, 1404–1406
- Renal portal system, 1092
- Renal tubules
  - structure/histology
    - opisthonephros *see under* Opisthonephros
    - pronephros, 1397*f*, 1399, 1399*f*, 1400*f*
  - tubular secretion, 1413*f*, 1415
  - tubular transport maximum
    - definition, 1411
    - glucose, trout kidney, 1415
- Renin, 1401
  - definition, 1395
- Renin–angiotensin system, vascular resistance control, 1105
- Replication, viral, 2036
- Repolarization
  - cardiac action potential phase, 1040
  - definition, 1038, 1045, 1718
- Reporter gene, definition, 1999
- Reproduction, 1701
  - anthropogenic disruptions, 787
  - appetite regulation and, 1513
  - associated behaviors *see* Sexual behavior
  - diversity of strategies, **613–615**
    - *see also specific strategies*
  - elasmobranchs, 1812
  - endocrine regulation *see* Reproduction, endocrine regulation
  - as growth component, 1623*f*, 1625
  - growth hormone transgenic fish, 2023
  - hypoxia effects, 1768
  - lampreys, 1784
  - organs *see* Testes
  - processes *see* Spermatogenesis; Vitellogenesis
  - stress impacts *see* Reproduction, stress effects
  - sturgeons *see under* Sturgeons, physiology
  - thermal limits in desert pupfish, 1839
  - thermal tolerance and, 1701
  - triploid fish, 2010
  - zebrafish, 1821
- Reproduction, endocrine regulation, **1500–1508**
  - brain–hypophyseal–gonadal (BPG) axis, 1500, 1501*f*
  - gonadal steroids *see* Gonadal steroid(s)
  - gonadotropins *see* Gonadotropin(s)
  - oogenesis *see* Oogenesis
  - spermatogenesis, 1502, 1503*f*
  - sturgeon, 1802
- Reproduction, stress effects, 1519, 1541*f*, 1543
  - chemical stressors, 1544
  - energetic balance and, 1542*f*, 1543
  - inhibitory mechanisms, 1546*f*, 1548
  - insufficient nutrients/food, 1544
  - pathogens, 1544
  - progeny, 1543*f*, 1545
  - reproductive behavior, 1544
  - reproductive hormone release, 1546
  - reproductive hormone–stress hormone interaction, 1545*f*, 1547
  - short term *vs.* long term effects, 1546*f*, 1548
- Reproductive behavior *see* Sexual behavior
- Reproductive hormones
  - stress effects on release, 1544*f*, 1546
  - stress hormone interactions, 1545*f*, 1547
  - *see also* Gonadal steroid(s); Gonadotropin(s); Gonadotropin-releasing hormone (GnRH)
- Reproductive sound signaling, 685*t*, 689
- Reproductive success, compensatory growth effects, 755–756, 756*f*, 756–757
- Reproductive system
  - characteristics of gametes, 616
  - secondary sexual characteristics, development in triploids, 2009*f*, 2010*f*, 2010–2011
  - spermatogenesis *see* Spermatogenesis
  - vitellogenesis *see* Vitellogenesis
  - *see also* Egg(s); *entries beginning ovarian*; *entries beginning testes/testicular*
- Rescue, blind cavefish eyes *see under* Eye degeneration, blind cavefish
- Reserve growth, 1623*f*, 1625
  - *see also* Growth
- Resistance
  - circuits, 980, 980*f*, 982
  - definition, 829, 973, 977, 1111
  - gas exchange during development, 837, 837*f*, 839
  - Newtonian fluids, 979, 979*f*
- Resistance vessels, **1104–1110**
  - anatomy, 1104
  - arteriogenesis, 1142, 1146–1147
  - definition, 982–983
  - systemic, physiology *see* Systemic vascular resistance
- Respiration, **791–795**
  - anthropogenic disruptions, 787
  - associated muscles *see* Respiratory muscles
  - bimodal *see* Bimodal respiration
  - cardiac interactions *see* Cardiorespiratory interactions (CRIs)
  - control
    - CO2/H^+ *see* Ventilatory response to CO_2/H^+
    - oxygen sensing *see* Oxygen chemoreceptors, branchial
    - rhythm *see* Respiratory rhythm generation
  - cutaneous *see* Cutaneous respiration
  - definition, 1675
  - elasmobranchs, 1811
  - environment and, 792, 792*f*
  - exercise effects *see* Exercise, respiratory effects
  - gas transport and exchange *see* Gas transport and exchange
  - lampreys, 1789*f*, 1790*f*, 1791
  - motor control systems, 62
  - rhythm generation *see* Respiratory rhythm generation
  - simple energy budget, 1675*f*, 1676
  - techniques in respiratory physiology *see* Respiratory physiology techniques
  - tissue, 794
    - cellular *see* Cellular respiration
    - mitochondrial *see* Mitochondrial respiration
  - triploid fish, 2012
  - whole-animal *see* Whole-animal respiration
  - *see also* Branchial respiration; Gas exchange organs; Gill(s); Ventilation
- Respiratory adaptations, air-breathing *see under* Air-breathing fish
- Respiratory alkalosis, 866
  - definition, 865
- Respiratory cascade
  - definition, 791, 829
  - during development, 831, 831*f*
- Respiratory epithelium
  - definition, 1862
  - lung/respiratory gas bladder, 1863*f*, 1865, 1865*f*, 1866*f*
  - suprabranchial chambers, 1867*f*, 1867*f*, 1869–1870
- Respiratory exchange ratios (RER), 953, 953*t*
- Respiratory gas bladder, 1863
  - blood flow *see under* Air-breathing fish, circulatory adaptations
  - epithelial and structural complexity, 1861*f*, 1863*f*, 1864*f*, 1865, 1865*f*, 1866*f*
  - evolutionary history, 1851*f*, 1853–1854, 1861*f*, 1862
    - fish lung relationship, 1861*f*, 1863*f*, 1865
  - morphology, 1864–1865
    - inter-species variation, 1861*f*, 1863*f*, 1864*f*, 1865*f*, 1866, 1866*f*
  - ventilation, 1873
  - *see also* Air-breathing fish; Physostomous gas bladder
- Respiratory lamella *see* Lamellae, gill
- Respiratory media, air *vs.* water *see under* Water
- Respiratory metabolism
  - levels, 847
    - measurement methods *see under* Respiratory physiology techniques
  - sturgeons, 1803
  - *see also* Respiration
- Respiratory muscles
  - cranial nerve innervation, 855, 855*f*, 856*f*, 857*f*, 858*f*
  - rhythm generation *see* Respiratory rhythm generation
- Respiratory physiology techniques, 794, **846–853**
  - apparatus, 847

Respiratory physiology techniques (*continued*)
    see also specific apparatus
  body mass considerations, 848
  gill ventilation measurements, 852, 852f
    background, 847
  respiratory (aerobic) metabolism measurements, 846
    apparatus, 848
      see also Respirometers
    consideration of metabolic rate categories/levels, 847
    oxygen measurements, 846–847, 847
Respiratory responses
  CO_2/H^+ *see* Ventilatory response to CO_2/H^+
  hypoxia *see* Hypoxia, respiratory responses
  sustained swimming, 813f
    gill ventilation during exercise, 814
    oxygen consumption and gas transport, 813f, 814, 815f
    see also Exercise, respiratory effects
Respiratory rhythm generation, **854–864**
  air-breathing fish, 862, 863f
  central generator *see* Respiratory rhythm generator (RRG)
  episodic breathing, 860, 861f
  feeding muscle recruitment, 861, 861f
  mechanoreceptors, 855f, 856f, 857f, 859, 860f
  midbrain influences, 858, 859f
Respiratory rhythm generator (RRG), 855f, 856, 856f, 857f, 858f
Respiratory sinus arrhythmia, 1187
  air-breathing fish, 1880
  definition, 1178, 1877
Respiratory system
  cardiovascular system interactions
      see Cardiorespiratory interactions (CRIs)
  pelagic fish, 1893
  see also Gill(s); Respiration; Ventilation
Respiratory toxicants, 787
Respiratory transitions, developmental *see* Gas exchange during development (respiratory transitions)
Respirometers, 848
  closed *see* Closed respirometers
  definition, 846
  open, 848–849, 850, 850f, 1568–1569
  two-chamber (van Dam-type), 852, 852f
Respirometry, 1637
  definition, 1636
Response magnitude, definition, 218
Resting cardiac function, acute temperature effects, 1198, 1199f, 1201f, 1202f
Resting membrane potential (RMP)
  cardiomyocytes, 1039, 1040, 1041
  definition, 398, 871, 1038, 1045, 1718
  effect of temperature, 1719f, 1720
  function, 1719–1720
Resting–routine metabolism
  definition, 846, 847
  measurement, 847
    see also Respirometers
  see also Routine metabolic rate (RMR)
Restriction enzyme, definition, 1999
Rete (retia), **1119–1131**
  definition, 1119
  description and mechanism, 1119
  vascular *see* Retia mirabilia
Rete mirabile *see* Retia mirabilia
Retention efficiency, feeds, 1673
Retia *see* Rete (retia)
Retia mirabilia, **1119–1131**, 931–932, 941
  definition, 526, 791, 909, 929, 935, 973, 1119, 1395, 1888, 1903, 1914
  evolution, 941, 942f, 1130
  factors affecting amount of exchange, 1120
    flow rate in vessels, 1121
    proximity of blood vessels, 1120f, 1120–1121
    surface area of contact between vessels, 1121, 1121f
  gas exchangers, 1120, 1120t
    retina *see* Choroid rete mirabile
    swimbladder *see* Swimbladder rete
  heat exchangers, 1120, 1121, 1122f, 1126t, 1911–1912
    muscle *see* Muscle retia
    serving brain and eyes, 1125, 1126t
    visceral *see* Visceral retia
  see also Root effect
Reticular formation, definition, 131
Reticular system, lampreys/agnathans, 20
Reticulospinal neurons
  definition, 56, 66
  see also Mauthner cells
Retina, 105f, 107, 123
  circadian phenomena, 741
  countercurrent exchangers *see* Choroid rete mirabile
  countercurrent multipliers serving, 1125
  inner *see* Inner retina
  nuclear layer, 124f
    definition, 123
  oxygen partial pressure, elevation, 931, 940–941
    see also Root effect
  photoreceptors *see* Photoreceptors
  physiological significance of Root effect, 931, 940–941
    see also Choroid rete mirabile
  plexiform layer, 124f
    definition, 123
  structure, deep-sea fishes *see under* Deep-sea fishes, ocular anatomy
  warming, advantages, 1919
Retinal (chromophore), 111, 112f
Retinal cone mosaics, 114, 114f, 121
Retinal ganglion cells, 124f, 126
  axons, 126, 131
  color coding, 127, 128f
  definition, 99, 131
  maintained discharges, 128
    cross-correlations, 129
    source, 128
    variability, 129
  parallel paths, 128
  receptive fields, 126, 127f
  tectum–nucleus isthmi system and, 138f
  tectum–torus longitudinalis system and, 136f, 137
Retinal pigment epithelium (RPE), deep-sea fish, 174–175, 175f, 176f
Retinomotor movements, 106
  definition, 99, 102
Retinotectal map, 133, 133f
  development, 134
  plasticity, 134–135
Retinotopic map
  definition, 99, 131
  steering and, 60, 60f
  see also Retinotectal map
Retrotransposons, 1983
  definition, 1980
  long terminal repeat, 1983
Retrovirus, 1819, 1822
Retzius, Magnus Gustav, 238, 240f
Reverse genetics
  definition, 1819, 1821–1822
  fish models, 1821–1822, 1987
Reverse transcriptase, definition, 1980, 2033
Reynolds number, 518
  definition, 516, 535, 536, 536f, 575, 581
Rhabdomyosarcoma, fish models, 1994
Rhabdoviridae, 2038, 2039f
Rhamphychthys, electrocytes, 391, 392f, 393, 393f
Rheostasis
  cardiovascular system control *see* Cardiovascular system control, rheostasis mechanism
  definition, 1170
  programmed *see* Programmed rheostasis
  reactive *see* Reactive rheostasis
Rheotaxis, 1923
  definition, 329, 720, 783, 785, 1921
  effect of sex steroids, 661
  effect of toxicants, 785
  lateral line function, 330, 331f
  negative, 1923, 1924
  positive, 1923, 1924
Rhesus (Rh) glycoprotein, 835, 1439, 1442
  definition, 829, 1437
Rhincodon see Whale shark (*Rhincodon*)
Rhizoprionodon see Sharpnose sharks (*Rhizoprionodon*)
Rhodopsins, 111, 116, 121–122
  definition, 99, 116
  depth adaptations and, 117–118, 120–121
  phylogenetic tree, 117f
Rhombencephalon
  definition, 2
  Latimeria, 47, 47f, 53f, 53–54, 54f
  lungfishes, 47, 47f
    histology, 48f, 48–49, 49f
  see also Hindbrain
Rhombomere(s)
  cartilaginous fish, 28–29
  definition, 6, 66
Rhombomere 4, Mauthner cells, 67–68
Rhythms
  circadian *see* Circadian rhythms
  circannual, 743
  circatidal, 742
  lunar, 742
  respiratory *see* Respiratory rhythm generation
  semilunar, 742
Risk assessment, environmental, growth hormone transgenesis, 2023
Risk-taking behavior
  during compensatory growth, 753
  growth hormone transgenic fish, 2021f, 2023
Riverine ecosystems, effects of dams, 1654
RNA polymerase, definition, 2033
RNA sequencing, 2056f, 2057
Robot, definition, 603
Robotic fish, 603
  biomimetic approach, 604
  classification, 604
  design, 605
  design case studies
    engineering fish-like vehicles for near-shore and benthic operations, 607
      see also RayBot
    testing biological hypotheses using evolving robots, 605, 606f, 607f
  early
    RoboTuna, 603
    TwiddleFish, 603
  future perspectives, 610
RoboTuna, 603
Rocking, as pain response, 715, 718t
Rodlet cells, 1100–1101
Rods
  definition, 99, 150
  visual pigments, 110–111
    adaptations to photic environment *see under* Photic environment
    evolution, 111, 113f, 116, 117f
  see also Photoreceptors
Rolls, 575–576, 576f, 577
  definition, 575
  see also Maneuverability
Root effect, 522, 529–530, **935–943**, 890, 947, 948f
  Bohr effect *vs.*, 921, 923f, 929, 930f, 935–936, 936f
  definition, 516, 520, 526, 791, 887, 909, 916, 921–922, , 929, , 935, , 936, , 944, , 1119
  discovery, 935
  evolution, 941, 942f
  functional role, 930
    gases in solution, 930, 930f

    single-pass root effect, 931
  mechanisms, 938, 947
    multistate model, 939*f*, 940
    subunit differences in oxygen binding at low pH, 940
    two-state allosteric model of hemoglobin and, 938, 939*f*
  pH dependence, 936, 936*f*, 937*f*, 947, 948*f*
  physiological significance, 931, 940, 948
    countercurrent multiplication, 931, 932*f*
      see also Retia
    retina, 931, 940–941
      see also Choroid rete mirabile
    swimbladder, 932, 940–941
  rete systems and, 931–932, 941, 942*f*
  species differences, 936–938, 937*f*, 948*f*
  teleosts, hemoglobin types, 929–930
  variations in magnitude, 936, 937*f*, 937*f*
    hemoglobin isoforms and, 938
  see also Hemoglobin(s), fish
Rostral, definition, 2, 854
Rostral pars distalis
  histology, 1458, 1458*f*, 1462*f*, 1463, 1463*f*
    hormone immunocytochemistry, 1460, 1461*f*, 1461*f*
  propiomelanocortin expression, 91
Rotation, 577
  see also Maneuverability
Route integration, 721–722
  definition, 720
Routine metabolic rate (RMR), 1764–1765
  definition, 812, 1764
  reduction, 1765, 1767, 1767*f*
    see also Metabolic rate suppression
  see also Metabolic rate (MR)
Routine metabolism, definition, 847, 847
Routine swimming *see* Volitional swimming
Rubbing, as pain response, 715, 717–718, 718*t*
Ruffed cells, olfactory bulb, 204
Ryanodine receptors, 1011, 1011*f*, 1909–1911
  definition, 1006, 1903

S

S-layers, 2037
S-start, 587–588, 588*f*
  caudal, 589, 590*f*
  definition, 587
  muscle activity, 589, 590*f*
  neural control, 588
  whole-body, 589, 590*f*
  see also Fast start
Sacciform cells
  definition, 476
  morphology, 481*f*, 482
Saccule, 245–246, 246*f*, 253*f*, 254, 255*f*, 259*f*
  definition, 244
  hair cell orientation, 255–256, 257*f*
  otolith, 254–255, 256*f*, 257*f*, 258
  sensory epithelium, 253*f*, 255*f*, 256*f*, 260
  see also Vestibular system
Saccus vasculosus
  cartilaginous fish, 33*f*, 33–34
  definition, 1457
Salinity
  definition, 1833
  fast start performance effects, 595
  growth effects, 1489, 1630*f*, 1631
  intertidal habitats, 1962
  sensing *see* Osmosensing
Salinity acclimation
  effect on venous capacitance, 1115, 1116*f*
  hormonal control, 1471–1472
    cortisol, 1468, 1948–1949
    growth hormone/IGF-I, 1470
    thyroid hormones, 1471
      see also Osmoregulation, hormonal control
  osmorespiratory compromise, 1393
    salmon, 1948
    sturgeon, 1804–1805
  see also Euryhaline fish; Smoltification
Salinity tolerance, cultured fishes, 2088
Salmo salar see Atlantic salmon (*Salmo salar*)
Salmo trutta see Brown trout (*Salmo trutta*)
Salmon
  Atlantic *see* Atlantic salmon (*Salmo salar*)
  migration *see* Salmon migration
  olfactory imprinting *see under* Olfactory imprinting
  Pacific *see* Pacific salmon
Salmon alphaviruses (SAVs), 2038
Salmon homing, 1949
  chemosensory mechanisms, 233, 721
    evidence base, 233
    olfactory imprinting *see* Olfactory imprinting
Salmon migration, 720–721
  orientation mechanisms, 721
  Pacific salmon *see* Pacific salmon migration
  see also Salmon homing
Salmon shark (*Lamna ditropis*), habitat use and movements, telemetry, 1931*f*, 1932
Salmonids
  juvenile, dominance hierarchies, 650, 650*f*, 787
  specific dynamic action, 1609*t*, 1611*f*, 1612
  see also specific salmonids
Salt bridges, 1707
Salt Creek, 1832*f*, 1832*f*, 1834
  pupfish *see Cyprinodon salinus*
Salt secretion, mechanisms (teleosts), **1354–1358**
  see also Mitochondrion-rich cells (MRCs), seawater fish
Salt transport
  mitochondrion-rich cells *see* Mitochondrion-rich cells (MRCs), seawater fish
  rectal gland *see under* Rectal gland, dogfish
  see also Ion regulation
Salting-out effect, 526, 529, 530–531, 531*f*
  definition, 526
Saltmarshes, 1960*f*, 1962
  definition, 1961
Salvelinus alpinus see Arctic char
Salvelinus fontinalis see Brook trout
Salvelinus namaycush see Lake char Lake char; (*Salvelinus namaycush*)
San Francisco Bay Bridge Analogy, 185, 185*f*
Saratoga Springs, 1831*f*, 1833*f*
  pupfish *see Cyprinodon nevadensis nevadensis*
Sarcolemma (SL), 1046–1047, 1047*f*
  cardiomyocytes, 1008, 1008*f*
    cell–cell connections, 1012, 1012*f*
  definition, 1006
Sarcomere(s), 1055*f*, 1062, 1063*f*
  cardiomyocyte, 1008–1009, 1009*f*
  contractile element
    cardiomyocytes *see* Cardiomyocyte contraction
    definition, 1054
    thick filament *see* Thick filaments
    thin filament *see* Thin filaments
    see also specific contractile elements
  definition, 1006, 1054, 1903
Sarcomere length (SL)–tension relationship, 1062, 1062*f*
  fish heart, 1064, 1065*f*
  lattice compression, 1065
  myofilament calcium sensitivity and, 1060–1061, 1064, 1064*f*
  myofilament overlap, 1062*f*, 1063
  sarcomere importance, 1062, 1063*f*
  thin-filament activation, 1066
  titin and passive tension, 1066
Sarcoplasmic reticulum (SR), 1047, 1049
  calcium-induced calcium release, 1049, 1049*f*
  calcium storage, 1051
  cardiomyocytes, 1011, 1011*f*, 1013
  definition, 1006, 1904
  free *see* Free sarcoplasmic reticulum (fSR)
  intracellular calcium influx, 1049
  junctional *see* Junctional sarcoplasmic reticulum (jSR)
  see also Ryanodine receptors
Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), 1047, 1051
  cardiomyocytes, 1011
  definition, 1006, 1045, 1904
  expression variation within tuna family, 1909–1911, 1913
Sarcopterygians, 46
  brains, functional morphology, **46–55**
    gross morphology, 47, 47*f*
    Latimeria see under Latimeria
    lungfishes *see* Lungfishes, brains
  definition, 46
  endogenous opioid peptides
    prodynorphins, 91, 92*t*
    proenkephalins, 90*t*
    proopiomelanocortins, 93*t*
  evolution, 46
  skeleton *see* Bony fish skeleton
Sargasso Sea, European eel spawning ground, 1937*f*, 1939–1940
  return migration, 1941–1942
  see also Eel migration
Satellite tags, 1931
  definition, 1928
  fishes and sharks, 1932
  PAT pop-up, archival transmitting, 1932
Satellite transmitters, 1930, 1931
Satiety
  definition, 1509
  see also Appetite regulation
Saturation, definition, 1709
Scale jacket, 442
Scaleless fish skin, 477–478, 478*f*
Scales
  elasmoid *see* Elasmoid scales
  ganoid *see* Ganoid scales
  modified, 485*f*, 487
  morphology, 477, 477*f*, 484, 485*f*, 487
  placoid *see* Placoid scales
  protective role, 472*f*, 473
Scaling, definition, 1573
Scaling, body mass, **1573**
  allometric and isometric scaling, 1573, 1574
    scaling exponent (*b*), 1573, 1574
  alternative regression models, 1577*f*, 1577–1578
  embryonic fish, 1575
    egg size limits, 1575
    embryo and alevin oxygen demand, 1574*f*, 1576
    metabolic rate and egg size, 1573*f*, 1575
  gill morphometrics and, 808*f*, 810
  post-hatching development, 1577
    abrupt breakpoints, 1577–1578
    general trends, 1575*f*, 1577
    gill development and gas exchange, 1578, 1578*f*, 1580
    respiratory demand of developing organs, 1578–1580
    swimming efficiency and aerobic scope, 1576*f*, 1577*f*, 1578
    swimming mode and lifestyle, 1580
    water content influence, 1580
  variation between species, 1579*f*, 1580
    brain mass, 1581
    critical partial oxygen pressure, 1582
    gill surface area, 1580–1581
    heart rate, 1582
    skeletal mass, 1581
    standard metabolic rate, 1580–1581
    swimming speed, 1581
Scaling exponent (*b*), 1574
  definition, 1573
Scanning ion-selective electrode technique (SIET)
  definition, 1359
  studies of gill ionocyte Na$^+$ uptake in freshwater fish, 1362, 1362*f*

Scapulocoracoid, definition, 449
Scattering, sound waves, 272
Schiff base
  definition, 110
  visual pigments, 111, 112f
Schistocephalus solidus
  fish phenotype manipulation, 780, 780f, 781f
  life cycle, 780f, 780–781
Schnauzenorgan, *Gnathonemus petersii* (elephantnose fish), 377, 378, 380f, 383f
Schooling chorus, 685t, 688
Schooling fish
  sleep adaptations, 775
  use of eddies, 586
Sciaenidae, hearing, auxiliary anatomy, 274
Sclera
  color, effect of social status, 653
  definition, 649
Scombrids
  endothermy *see* Endothermy/endotherms
  systematics (cladogram), 1904f, 1905–1906
  tunas *vs.* other scombrids
    cardiac calcium cycling, 1909–1911
    red (slow-oxidative locomotor) muscle position, 1910f, 1911
    relative heart mass, 1908f, 1909
    standard metabolic rates, 1906f, 1907
  see also Tuna
Scope for activity (SA) *see* Aerobic scope
Scorpaeniformes, specific dynamic action, 1609t, 1611f, 1612
Scyliorhinus canicula
  brain, 26–27, 27f, 30f, 31f, 34f
  conus arteriosus, 1023–1024, 1025f, 1026f, 1026–1027, 1027f
Scyliorhinus sellaris, conus arteriosus, 1024–1026, 1026f, 1027f
Sea(s) *see* Ocean(s)
Sea bream, robotic, 604
Sea lampreys
  Great Lakes invasion, 1785
  growth phases
    arrested, 1785
    parasitic, 1785
  locomotion, 1782
  metamorphosis, 1782f
  oral disk, 1779f
  parasitic, 1783f, 1784–1785
  population control measures, 1786
  upstream migration, 1784
Seahorses, sound production, 325
Search path, foraging, 1592
Search speed, foraging, 1591f, 1592, 1593f
Seasonal variations
  capillary density, 1145–1146
  gut anatomy, 1271, 1274
  thermal tolerance and *see under* Thermal tolerance
  see also Environmental influences
Seaward migration
  eels, 1938f, 1941
  salmon, 1948
Seawater
  density, 521
  hagfish slime and
    collapse on stirring, 509–510
    contact rupture of mucin vesicles, 505–506, 506f, 507f
    expansion speed at deployment, 510
    hydration process, 510–512, 511f
    seawater volume in deployed slime, 508–509
    water retention related to structure, 513, 514f
  ionic composition, 1349, 1420t
  osmorespiratory compromise, 1391
  partial pressure of oxygen ($P\text{O}_2$), 792, 792f
Seawater acclimation *see* Salinity acclimation
Seawater fishes *see* Marine fishes
Secondary circulation, **1161–1168**, 975, 982, 982f
  anatomy, 1162
    see also Secondary vessels
  definition, 973
  history, 1162, 1163f
  physiological function, 1167
  pressure, 1167
  primary circulation *vs.*, 1162, 1163–1165, 1164f, 1166–1167
  volume, 1166
Secondary lamella *see* Lamellae, gill
Secondary metabolites/compounds, definition, 1667
Secondary octaval (SO) nuclei, 284f, 289, 289f
Secondary prosencephalon
  agnathan, 23, 24f
  see also Forebrain; Hypothalamus; Preoptic area; Telencephalon
Secondary stress response, 1516, 1518
  metabolic effects *see* Metabolic stress
Secondary vessels
  arterioarterial anastomosis, 1162, 1164f
  gill, 1164f, 1165, 1166f
    non-teleostean fish, 1166
  location, 1162
  systemic circulation, 1163, 1164f
    lungfish, 1165
    non-teleostean fish, 1165
    zebrafish, 1165
Secretagog, 1431
  definition, 1429
  see also specific substances
Secretion
  definition, 1229, 1301
  gut *see* Gut secretion(s)
  oxygen
    ocular, pseudobranch role, 933
    swimbladder *see under* Swimbladder
  tubular, 1413f, 1415
Secretion systems, extracellular products, 2036
Secretogog, definition, 1276
Secretory cells, rectal gland, morphology, 1431f, 1432
Sedentary, definition, 1132
Segmental organization, brains *see* Neuromeres
Segmental vessels
  arteries, 1087f, 1090
  veins, 1087f
Seismic surveying, noise generation, 305t, 306
Selenium (Se), biological effects and toxicodynamics, 2066
Self-feeding, 711, 712f
Self-renewal
  definition, 616
  stem cells *see under* Stem cells
Semelparous fish, 614–615, 1938–1939, 1945f, 1946
  definition, 613, 1921, 1938, 1946
  Pacific salmon, 1945f, 1946
Semicircular canal(s), 245–246, 246f, 252, 253f, 254, 256f
  afferents, central terminations, 247f, 248
  definition, 244, 252
  elasmobranchs, 262–263, 263f, 264, 266f
  hagfish, 246f, 1777
  see also Vestibular system
Semilunar rhythms, 742
Seminal fluid
  composition control, 617
  production sites, 617–618
Senescence, definition, 1966
Sensors, ocean, animals as, 1935
Sensory, definition, 855
Sensory epithelia, fish ears, 254, 255
  definition, 236
  lagena, 253f, 256f
  saccular, 253f, 255f, 256f, 260
  utricular, 256f
  see also Sensory hair cells
Sensory ganglion, definition, 336
Sensory hair cells, 252, 254f, 255f
  definition, 237, 252, 336
  neuromasts *see under* Neuromast(s)
  number, fish growth and, 260
  orientation, 255f, 255–256, 257f
    lagena, 257f
    saccular, 255–256, 257f
    utricular, 257f
Sensory information, anthropogenic disruption
  see under Anthropogenic influences/disruptions
Sensory mechanisms, pelagic fish, 1892
Sensory perception
  fish behavior–fish physiology interrelations, 647
  pain *see* Nociception
  skin role, 473
  spatial orientation, 723, 724, 724f
  see also specific sensory systems
Sensory projections, definition, 855
Sensory systems
  electrocommunication *see* Electrocommunication
  integration during navigation, 723–724
  magnetic *see* Magnetic sense
  pain and nociception *see* Nociception; Pain
  spatial orientation *see* Spatial orientation
  visual signals *see* Visual communication
Sensory–motor processing, definition, 73
Septum (septa), definition, 1457
Sequential hermaphroditism *see under* Hermaphroditism
Sequestration, metals, 2062f, 2064
SERCA *see* Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA)
Serine proteases, pathogenesis role, 2035
Serosal, definition, 1419
Serotonin
  gut expression, 1335f, 1336, 1344, 1344f
  gut motility role, 1298, 1299f
Serotonin transport gene, fish mutant, 1996
Serotoninergic neurons
  cartilaginous fish, 29, 31f, 33–34
  ray-finned fishes, 37–39, 42
Serotoninergic system, effects of social status, 653
Serta (serotonin transport gene), 1996
Sertoli cells, 617, 617f
  phagocytotic activity, 620
  proliferation and cyst support, fish, 618–619
  receptors, 629
    gonadotropin, 628, 628f, 629, 1507
  regulation *see under* Spermatogenesis, regulation
  role in birds/mammals, 618
Serum, definition, 1966
Setpoint, definition, 1170
Sewage treatment plants, downstream hypoxia, 1749
Sex change, 663
  definition, 1490
  neural mechanisms, 667
    behavioral changes, 668, 669f
  patterns, 663, 664f, 665f
  size-advantage model, 2052f, 2053
  social control *see* Socially controlled sex change
  steroid hormone patterns/role, 665, 666f, 2053
    stress hormones, 667
  timing of, 2053
  see also Hermaphroditism; Trans-sex phenomenon
Sex chromosomes
  definition, 2025
  rapid evolutionary turnover, 1984
Sex determination, **2025**
  definition, 1490, 2025
  environmental, 2052
  genetic/genotypic, 2026, 2052
    chromosomal, 2025t, 2026f, 2027, 2027f
    medaka fish, 2028, 2052
    multifactorial *see* Multifactorial sex determination
  high plasticity, 2052
    see also Hermaphroditism
  see also Primordial germ cells (PGCs)
Sex differentiation, 2026, 2029
  control *see* Sex differentiation, endocrine control

  - definition, 1490, 1491, 2025
  - environmental influences, 1497
  - genes involved, 2029*t*, 2030
  - histology, 1493*f*
  - morphological aspects, 1491
  - patterns, 1492, 1492*f*
  - stages, 2029
  - *see also* Ovarian differentiation; Testicular differentiation
- Sex differentiation, endocrine control, 1493
  - future research directions, 1498
  - growth factors, 1497
  - sex steroid receptors, 1496, 1496*f*
  - sex steroids, 1494, 1495*f*
  - steroidogenic enzymes, 1494
  - transcription factors, 1497, 2029*t*, 2030
- Sex ratio, definition, 1490
- Sex steroids *see* Gonadal steroid(s)
- Sexual attractiveness, compensatory growth effects, 756
- Sexual behavior, **656–661**
  - chemosensory, 230, 231*f*
    - *see also* Hormonal pheromones
  - compensatory growth effects, 755
  - construction, 657, 657*f*
    - *see also* Nest building
  - courtship *see* Courtship
  - dominance *see* Dominance
  - fish behavior–fish physiology interrelations, 648
  - hormonal control, 659
    - female fish, 660
    - male fish, 660
  - overview, 656
  - stress effects, 1544
  - zebrafish, aquarium and wild, 1821
  - *see also specific behaviors*
- Sexual dimorphism, 658
  - sound production, 317–318
  - zebrafish (*Danio rerio*), 1820
- Sexual identity, electric signaling role, 701
- Sexual maturation, definition, 1483
- Sexual maturity, male display, 658
- Sexual receptivity, female display, 659
- Sexual selection, visual exuberance, 697–698
- Sexuality types, 1491, 1491*t*
  - definition, 1490
  - gonochorism *see* Gonochorism
  - hermaphroditism *see* Hermaphroditism
  - unisexuality, 1491*t*
- Shallow-living species, opsin gene duplication, 118, 119*f*, 120*f*
- Shallow water, sound propagation, 272
- Shape, body *see* Body shape
- Sharks, 26
  - auditory system structure *see under* Auditory system structure
  - caudal heart, 1075
  - cranial muscles *see* Sharks, cranial muscles
  - endothermy *see under* Lamnid sharks
  - hearing *see under* Hearing
  - lamprey parasitism of, 1783*f*, 1784
  - lateral line system, 336, 341, 344*f*
  - rectal gland *see* Rectal gland, dogfish
  - skeletal anatomy, 420*f*, 424
    - appendicular skeleton, 425
    - axial skeleton, 424
    - visceral arches, 425
  - *see also* Elasmobranchs
- Sharks, cranial muscles, 450, 452*t*
  - actions, 452*t*
    - feeding activity, 452*t*, 454, 455*f*
    - respiration activity, 452*t*, 455, 456*f*
  - branchiomeric, 450, 450*f*, 453*f*, 454*f*
  - epibranchial, 450, 450*f*, 453*f*, 454–455
  - extrinsic eye muscles, 452*t*, 454, 454*f*
  - hypobranchial, 452*t*, 453*f*, 454, 454*f*
- Sharpnose sharks (*Rhizoprionodon*)
  - Atlantic *(Rhizoprionodon terraenovae)*, conus arteriosus, 1026–1027, 1028*f*
  - hearing threshold, 268, 268*f*
- Shear
  - definition, 535
  - resistance, fluids *vs.* solids, 536, 536*f*
- Shear flow, 537, 538*f*
- Shelter, use in surfacing strategies, 768
- Ship noise, 305, 305, 305*t*
- Short-range orientation, mechanisms, 710, 711*f*, 721
  - egocentric cues, 721, 721, 724
  - factors influencing choice of, 724
  - learning allocentric spatial cues, 722
  - spatial memory, 721, 722, 722*f*
- Shotgun sequencing, 2056*f*, 2057
- Sialization, homeoviscous adaptation, 1729*t*, 1730
- Sideroblastic anemia, 1990
- Siderophores, pathogenesis role, 2039
- Sight *see* Vision
- Sigmoid display, male guppy, 658
- Sign-conserving, definition, 123
- Sign-inverting, definition, 123
- Signal
  - definition, 684
  - sound *see* Sound signals/signaling
- Signal transduction
  - definition, 1373, 1534
  - gustatory, 223
    - *see also* Gustatory receptors
  - olfactory, 208, 209*f*
    - *see also* Olfactory receptor(s) (ORs)
  - osmosensory, 1374, 1375*f*
    - *see also* Osmosensing
  - *see also specific systems*
- Signaler, definition, 692
- Silurids
  - electric organ, development, 414
  - electroreceptors
    - ampullary, 356, 356*f*, 357*f*
    - development, 414
    - tuberous, 357*f*, 358
  - *see also* Catfish (Siluriformes); *individual species*
- Siluriformes *see* Catfish (Siluriformes)
- *Siluris glanis* (European Wels), ears, 256*f*
- Siluromast, 357*f*, 358
  - definition, 350
- Silver eels, 120, 1942
  - spawning migration, 1937*f*, 1938*f*, 1943
  - visual pigments, 120–121, 121*f*
- Silver perch (*Bairdiella chrysoura*), sound production, 312, 312*f*, 315
  - measurement, 314*f*
- Silver (Ag) toxicity, 2067
  - effects of social status, 654
- Simple squamous epithelium, definition, 1395
- Simultaneous hermaphroditism, 665, 2051*t*, 2053, 2053
  - definition, 662
- Single-bend response, 588, 589*f*, 590
  - definition, 587
  - *see also* C-start
- Single nephron glomerular filtration rate (SNGFR), 1413–1414
- Single nucleotide polymorphisms (SNPs), pigmentation, fish models, 1994–1995
- Single-pass root effect, 931
- Single-tissue extraction, hormone inactivation measurement, 1193*f*, 1194, 1195, 1196*t*
- Sinoatrial (SA) canal, 1000, 1001, 1002
- Sinoatrial (SA) node, 1071
  - definition, 998
- Sinoatrial (SA) valve, 1032
- Sinus venosus, 1030–1031
  - anatomy, 999*f*, 999–1000, 1001
  - cyclostomes, 1004
  - definition, 998
  - *see also* Ductus Cuvier
- Sit-and-wait strategy, prey capture, 747*f*, 747–748
- Size
  - coral reef fish, 151
  - fish eggs, 1575
  - homeostatic control, triploid fish, 2014
  - largest and smallest fish, 1574
    - tuna family, 1909
  - mouth size and feeding, 599, 600
  - related to swimming speeds, 1579*f*, 1581
  - *see also* Growth; Scaling, body mass
- Size-advantage model, 2052*f*, 2053
- Size constancy
  - behavioral studies, 145*f*, 146
  - definition, 143
- Size principle, definition, 387
- Skates, 26
  - lateral line system, 336, 341, 344*f*
  - *see also* Elasmobranchs
- Skein cells, 478*f*
- Skeletal element, definition, 419, 428
- Skeletal muscle
  - capillary supply, 1149
    - variation between species, 1150, 1911
  - elasmobranchs, 1809
  - endothermy, 1914*f*, 1915, 1919
  - fiber types, 550, 1637*f*, 1638
    - biochemistry of action, 550, 551, 1908
    - body location, 550, 550*f*, 1910*f*, 1911
    - capillary supply, 1144*f*, 1145, 1149
    - energetics *see under* Swimming, energy expenditure
    - mechanical functions, 551
    - *see also* Red muscle; White muscle
  - hagfish, 1776
  - lampreys, 1782
  - mechanical work, 556
    - analysis *see* Work-loop analysis
  - mitochondrial content, 969
    - *see also* Muscle mitochondria
  - proteins
    - myosin light chain (MLC), 550–551, 551
    - parvalbumin, 551
    - troponin C, 550, 551
    - troponin T, 551
  - *see also entries beginning muscle*; Myotomes

- Skeleton
  - bony fish *see* Bony fish skeleton
  - cartilaginous fish
    - anatomy *see* Cartilaginous fish, skeletal anatomy
    - tissues *see* Cartilaginous fish, skeletal tissues
  - functional anatomy, current knowledge, 417
  - mass, body size scaling, 1579*f*, 1581
  - muscle force transmission, 553
    - caudal keel and tendons, tuna, 1905*f*, 1907
- Skin *see* Integument (skin)
- Skin diseases (human), fish models, 1994
  - pigmentation, 1993*f*, 1994
  - tumors, 1995
- Skin extensions, 487
- Skin flaps, 488
- Skin respiration, 473–474, 1872*f*, 1873
- Skin tumors
  - human, transgenic models (fish), 1977, 1977*f*, 1978, 1989, 2004*f*, 2005
  - *see also* Pigment cell tumors
- Skipjack tuna (*Katsuwonus pelamis*), muscle rete, 1124, 1124*f*, 1125*f*, 1126*f*
- Skull
  - bony fish, 435, 436*f*, 437*f*
  - cartilaginous fish *see* Chondrocranium
- Skull roof, bony fish, 435–437, 437*f*
- Sleep, **772–776**
  - definition, 772
  - fish, 774
    - behavioral adaptations, 774
      - hiding, 774–775
      - postures, 774

Sleep (*continued*)
        schooling fish, 775
    effect of deprivation, 776
    studies, 775
  humans/mammals, 772
    disorders, fish models, 1996
    duration, 774
    stages, 773
Sleep deprivation, effect on zebrafish, 776
Sleep disorders, 776
Sleeping Beauty transposon, 2002–2003
  definition, 1999
Slime glands (hagfish)
  exudate, 504, 505*f*
    fluid component, osmolytes, 506*t*, 506–507
    forced ejection, 504–505, 510, 510*f*
    mass, stored in glands, 508
    solid components, 506*f*, 508–509
  gland mucus cells (GMCs), 504, 505
    definition, 504
    function, 505
    origin and maturation, 504–505
    pre-release stabilization of mucin vesicles, 506–507
  gland thread cells (GTCs), 483–484, 504, 508, 508*f*
    definition, 504
    function, 508
    loss of plasma membrane at ejection, 510–512
    origin and maturation, 504–505, 508
    thread skeins, 504, 508, 508*f*
  mucin vesicles, 504, 505
    isolated, stabilization tests, 506–507, 507*f*
    osmolarity, swelling and rupture, 505–506, 506*f*
  number, position and structure, 504, 505*f*
  see also Hagfish slime
'Slime hags' *see* Hagfish
Slime layers, bacteria, 2037, 2037
Slime threads, definition, 504
Slip, 575–576, 576*f*
  definition, 575
Slow-oxidative muscle fiber, definition, 1904
Slow waves, definition, 1292
Small field motion, detection/analysis, behavioral studies, 147, 148*f*
Small veins, physiology, 1113
Smell, sense of *see* Olfaction
Smell, system of *see* Olfactory system
Smoltification
  cultured fishes, 2088
  definition, 649, 752, 1483, 1946, 2084
  effect of social status, 652
  growth hormone transgenic fish, 2021–2022
  Pacific salmon, 1945*f*, 1946, 1947, 1948
    see also Salmon migration
Smooth muscle, gut, 1270*f*, 1271
  intestinal *see* Intestinal smooth muscle
Sneaker male, 672–673
  definition, 670
'Snotties' *see* Hagfish
Social behaviors
  fish behavior–fish physiology interrelations, 648
  see also specific behaviors
Social interactions, growth effects, 1633
  presence of conspecifics, 1634
  presence of heterospecifics, 1634
Social learning, 710
Social status, 649–650
  definition, 649
  physiological consequences, 651
    brain neurotransmitters, 652
    endocrine system, 652, 653*f*
    growth, 651
    metabolism, 653
    pain response, 717–718, 718*f*, 718–719
    susceptibility to aquatic toxicants, 654
    susceptibility to disease, 653–654
  see also Dominance hierarchies
Social stress
  effect on gastrointestinal barrier function, 1329
  responses, 651, 652–653, 653*f*
Socially controlled sex change, **662–669**
  associated species, 663
  neural mechanisms, 668, 669*f*
  protandry, 663
  protogyny, 663–665
  see also Sex change
Sockeye salmon (*Oncorhynchus nerka*)
  migration, 1946
    lake-nursery environment, 1947
    to oceanic feeding grounds, 1949
    upriver, 1949*f*, 1951
    see also Salmon migration
  semelparity, 1945*f*
Sodium (Na^{+}) uptake, freshwater fish ionocytes
  gills, 1361, 1361*f*, 1362*f*
  zebrafish embryonic skin, 1362, 1363*f*
Sodium current, cardiac action potential, 1040
Sodium/potassium adenosine triphosphatase *see* NKA (Na^{+}/K^{+}–ATPase)
Sodium/proton exchange, RBC
  β-adrenergically stimulated, 880, 881–882, 884*f*, 912, 913*f*
    definition, 879, 909
    short-circuiting, 919, 920*f*
  definition, 909
  see also Red blood cells (RBCs), pH regulation
Sodium/proton exchanger, definition, 909
Sodium pump *see* NKA (Na^{+}/K^{+}–ATPase)
Sodium–calcium exchanger (NCX)
  Ca^{2+} efflux, 1051
  Ca^{2+} influx, 1048
Sodium–potassium–chloride cotransporter *see* NKCC (Na^{+}, K$^{+,}$ 2Cl^{-} cotransporter)
Soft water, acclimation, 1392, 1392*f*
Solar cues, orientation, 1922*f*, 1923
Soldier-fishes, hearing, auxiliary anatomy, 274
Sole, common (*Solea solea*), thermal tolerance, life stage variation, 1739*f*, 1744
Solids, fluids *vs.*, 536, 536*f*
Sollid, J., 796–797
Somatic, definition, 1624
Somatostatins, 1280, 1280*t*, 1342
  amino acid sequences, 1342, 1343*f*
  enteroendocrine cells, 1342–1343
  gastric acid secretion role, 1343, 1343*f*
  growth hormone inhibition, 1487–1488
  gut motility role, 1299*f*
  rectal gland secretion control, 1435
Somatotopy, definition, 375
Somites, 1287
  definition, 1284
Sonars, noise generation, 305*t*, 306
Sonic hedgehog (Shh)
  definition, 1843
  role in blind cavefish troglomorphism
    eye degeneration, 1845*f*
    feeding apparatus development, 1848
  role in blind cavefish troglomorphism, eye degeneration, 1846, 1847
Sonic motor nuclei *see* Vocal motor nuclei (VMNs)
Sonograms
  bottlenose dolphin, 315, 315*f*
  definition, 311
  grey gurnards, 314*f*
  oyster toadfish, 313*f*
  silver perch, 312*f*
  white seabass, 318*f*
Sonomicrometry
  definition, 547
  limitations, 552
Sound, 270
  ambient noise, 280
  anthropogenic disruptions *see* Human-generated sounds
  attenuation distance, 268
  capture/transmission to inner ear, 258, 259*f*
  central representation *see under* Hearing
  components, 267
    acoustic particle motion *see* Acoustic particle motion
    sound pressure *see* Sound (acoustic) pressure
  duration, 280
  environmental soundscape, 276
    source segregation, 281
  fish interactions, 273
  hearing *see* Hearing; Hearing mechanisms
  hearing sensitivity determinant, 280
  human-generated *see* Human-generated sounds
  masking, 267, 276, 280, 307*f*, 309
    critical ratio (CR), 280, 280*f*
    definition, 304
    spatial detection filtering, 281
  production *see* Sound production
  propagation *see* Sound propagation
  speakers, underwater, 265–266, 267–268
  transmission loss, 272
  uses, behavioral *see* Sound production, behavioral significance
  see also Audiograms; Calls
Sound-exposure level (SEL)
  definition, 304
  regulatory standards, 308–309
Sound frequency
  coding, 294, 294*f*
  tuning, 295
Sound intensity, coding, 293, 293*f*
Sound localization, definition, 237
Sound (acoustic) pressure, 270, 276, 277
  acoustic particle motion *vs.*, 298–299
  definition, 270, 298
  detection, 299
    see also Sound source localization
Sound-pressure level (SPL)
  definition, 304, 311
  regulatory standards, 308–309
Sound-producing muscles *see* Drumming muscles
Sound-producing species, 312
  see also Sound production; Vocal fishes
Sound production, **311–320**, 242, 267–268
  behavioral significance, 311, **684–691**
    agonistic, 685*t*, 688*f*, 689
    chorusing, 685*t*, 688
    disturbance/distress, 685*t*, 689–690
    feeding, 685*t*, 688
    historical aspects, 686
    limitations, 690
    other communication modalities and, 690
    reproductive, 685*t*, 689
    swimming, 685*t*
  as fishery management tool, 319
  incidental, 313
  intentional, 314
    aggressive sounds, 314, 314*f*
    alarm calls, 314, 315*f*, 689–690
    navigation/echolocation, 317, 319*f*
    spawning/advertisement calls *see* Spawning/advertisement calls
  mechanisms/physiology, **321–328**
    vocal neuron control, 326, 327*f*
    vocal organs *see* Vocal organs
  muscles *see* Drumming muscles
  ontogeny and, 317
  origin of knowledge/historical aspects, 238, 238*f*
  reasons for, 313
  sex differences, 317
  sound-producing species, 312
    see also Vocal fishes
  thrashing movement, 265–266
  see also Calls; Sound signals/signaling; Vocalizations
Sound propagation
  definition, 270
  underwater, 270, 271*f*, 298
    shallow water, 272
    sound sources and, 271
    transmission loss, 272

- Sound signals/signaling, **684–691**
  - agonistic, 689
  - avoiding/repelling predators, 689
  - chorusing behavior, 688
  - definition, 684
  - other communication modalities and, 690
  - quiet *vs.* loud, 685, 685f
    - fish producing, 686f
  - reproductive, 689
  - simple *vs.* complex, 687, 687f, 688f
  - *see also* Calls; Sound production; Vocal fishes
- Sound source(s)
  - human-generated sounds, 304, 305t
  - segregation, 298
    - definition, 298
    - *see also* Sound source localization
  - underwater sound propagation and, 271
- Sound source localization, **298–303**
  - behavioral evidence, 299, 300f
  - brain mechanism, 302, 302f
  - theories, 301
    - vector detection and phase model, 301
  - unresolved issues, 302
- Soundscape, 242
  - definition, 237, 304
- Source segregation, 298
  - definition, 298
  - *see also* Sound source localization
- *SOX9* gene, 2029t, 2030
- Soyabean-induced enteritis, 1330
- Spasm, definition, 1695
- Spatial coding, electrolocation *see under* Active electrolocation, central processes
- Spatial learning, 721, 722, 722f, 724
  - hippocampus role, 709–710
  - *see also* Spatial orientation
- Spatial map, definition, 720
- Spatial orientation, **720–725**
  - flexibility in learning/remembering spatial information, 724
  - to magnetic field direction
    - experiments, 727
    - role, 732
  - mechanisms, 720
    - long-range migration *see under* Migration
    - short-range *see* Short-range orientation
  - sensory systems, 723, 724, 724f
  - *see also* Learning and memory
- Spatial patterns, reflected-light signaling, 695, 697f
- Spatial vision, behavioral studies, 145f, 146
- Spawning
  - chorusing behavior, 688
    - *see also* Spawning/advertisement calls
  - compensatory growth effects, 756f, 756–757
  - frequency, tunas, 1909
  - lampreys, 1784
  - motor control systems, 62
  - role of hormonal pheromones, 231, 231f, 1555–1556
    - female postovulatory prostaglandins, 1557f, 1558
    - female preovulatory steroids, 1556f, 1557
    - male-derived pheromones, 1560–1561
    - mass spawning, 1560f, 1561
    - mechanisms, 1559f, 1559–1560
  - simple energy budget and, 1675f, 1676
  - *see also* Egg(s); Reproduction
- Spawning/advertisement calls, 315, 316f, 688
  - haddock, 316–317, 318f
  - mate choice and, 316
  - white seabass, 315–316, 317f, 318f
- Spawning migration
  - eels, 1938f, 1943
  - lampreys, 1784
  - salmon *see* Salmon migration
- Speciation
  - definition, 1980
  - teleost-specific genome duplication (TSGD) and, 1982
- Species differences
  - cardiac action potential *see under* Cardiac action potential
  - gut
    - anatomy *see under* Gut anatomy
    - barrier function, 1330
    - gastric emptying rate, 1300
  - Root effect, 936–938, 937f, 948f
  - standard metabolic rate (SMR), 1571, 1906f, 1907
  - thermal tolerance, 1150, 1695, 1739f, 1740
  - white blood cell composition, 990f, 991
  - *see also individual species*
- Species identity, electric signaling role, 701
- Species preferences, temperatures, 759, 759f, 759t
- Species specificity, hormonal pheromones, 1561
- Specific dynamic action (SDA), **1608**, 1564, 2085
  - cultured fishes, 2087
  - definition, 1564, 1608, 1888, 1904, 2084
  - determinants, 1614
    - body size, 1615
    - body temperature, 1613f, 1615
    - meal composition, 1613f, 1614
    - meal energy, 1613f, 1614
    - meal size, 1613f, 1614
    - meal type, 1614
  - early studies, 1608–1610
  - effect on routine metabolic rate, 812
  - future research directions, 1616
  - gastrointestinal blood flow and, 1212
  - measurement, 1608f, 1610
  - processes contributing to response, 1615
    - post-absorptive, 1615
    - pre-absorptive, 1615
  - taxonomic account, 1609t, 1611
    - Actinopterygii *see* Ray-finned fish, specific dynamic action
    - elasmobranchs, 1609t, 1611, 1611f
    - lungfishes, 1609t, 1611f, 1612
- Specification
  - definition, 1284
  - endoderm, 1284–1285
  - eye, blind cavefish, 1845f, 1846
  - primordial germ cells *see under* Primordial germ cells (PGCs)
  - *see also* Organ differentiation
- Spectral reflectance, definition, 99, 150
- Spectral sensitivity
  - behavioral studies, 145, 145f
  - color vision, 151, 153f, 153–154
  - definition, 99, 150
  - ultraviolet vision, 156, 157f
  - *see also* Vision
- Spectrogram
  - definition, 311
  - *see also* Sonograms
- Spectroscopy
  - definition, 1667
  - feed/animal tissue analyses, 1669
- Speed of objects, detection/analysis, behavioral studies, 147, 148f
- Speed–maneuver limit, 576
  - definition, 575
- Speigel mutant, 1996
- Sperm competition, 672–673
  - definition, 670
- Sperm maturation, 632, 633f, 1502, 1503f
  - *see also* Spermatogenesis
- Sperm motility, osmosensing role, 1380
- Spermatogenesis, 1502
  - consequences of triploidy, 2011
  - cystic, 618, 1977f
    - noncystic *vs.*, 618–619, 621f
      - apoptotic losses, 620
  - definition, 616, 627
  - meiotic division, 622, 623–624, 1500, 1502, 1503f
    - first division, 623–624
    - second (reduction) division, 624
    - spermatocyte ultrastructure, 623–624
  - phases, 620, 625f
    - duration, 622
    - meiotic *see meiotic division (above)*
    - mitotic, 621, 622f
      - *see also proliferative (mitotic) generations (below)*
    - spermiogenic, 622, 625
  - proliferative (mitotic) generations, 622, 624f
    - differentiating (type A_{diff}), 623, 623f
    - last generation (type B), 623
    - number, interspecific variation, 621, 622f
    - primordial spermatogonia (undifferentiated, A_{und}), 622–623, 623f
  - regulation *see* Spermatogenesis, regulation
  - semicystic, 625–626
  - spermatozoa, differentiation (spermiogenesis), 622, 625
  - spermiation (release to ducts) *see* Spermiation
  - steroid hormone blood levels and, 630
- Spermatogenesis, regulation, **627–634**
  - endocrine, 633f, 633–634, 1502, 1503f
    - androgens, 631, 631f, 633f
    - estrogens, 631, 631f, 633f
    - final maturation of male gametes, 632, 633f
    - gonadotropins, 627, 628f
    - phases, 633f
    - progesterones, 632, 633f
    - Sertoli cells, 628f, 629, 633f, 1502, 1503f
    - steroid hormone blood levels, 630
  - future research perspectives, 633
  - germ cell autonomous control, 632, 634
- Spermatogonia
  - definition, 1500
  - renewal/proliferation, 1502, 1502, 1503f
  - *see also* Spermatogenesis
- Spermatogonial stem cells (SSCs)
  - cloned germ cells and cytoplasmic bridges, 618, 623
  - contact with Sertoli cells, 618–619
  - definition, 616
  - functional assay, 622–623
  - self-renewal/differentiation, 628f, 631, 631f, 634
  - *see also* Spermatogenesis
- Spermiation, 619–620, 625–626, 632, 633f
  - definition, 627
- Spermine, olfactory responses, goldfish, 214
- Spermiogenenesis, 622, 625
  - *see also* Spermatogenesis
- Spherical aberration, 103–104, 104f
- Spheroids
  - definition, 1966
  - fish cells in culture, 1966f, 1967–1968
- Sphingolipids
  - cell membranes, 1726f, 1727
  - definition, 1725
- Sphyrind, definition, 1807
- Spike
  - definition, 123
  - *see also* Action potential (AP)
- Spinal autonomic nerves
  - cyclostomes, 82
  - definition, 80, 1332
  - elasmobranchs, 82
  - evolution *see* Spinal nerves, evolution
  - gut innervation, 1333, 1334f, 1334f
  - teleosts, 82, 84f
  - *see also* Autonomic nervous system (ANS)
- Spinal CPG network *see* Central pattern-generating (CPG) network
- Spinal nerves, evolution, **6–15**
  - craniates, 11
    - jawed, 13
  - *see also* Central nervous system (CNS), evolution; Motor neurons
- Spiracle, definition, 419
- Spiral valve, 1270, 1273
  - species differences, 1273
- Spitting, goldfish, 230, 230f
- Splanchnocranium, bony fish, 438–439
- Spleen, RBC release, 986, 987f, 987–988

- Spongy myocardium, 1002, 1003–1004
  - coronary vessels, 1080
  - histology, 1000f
  - structure, 1078, 1079f
  - *see also* Myocardium
- Spontaneous immortalization, 1966
  - definition, 1966
- Spontaneous swimming activity, energetics, 1641
- Spring viremia of carp virus (SVCV), 2038–2039
- Sprint mode (non-sustainable swimming), 1656f, 1659–1660
- Squalene
  - buoyancy and, 521, 521t, 525
  - definition, 520
- Stability
  - anterior myotomal muscle action, 553
  - definition, 575
- Stability control
  - auxiliary vestibular connections, 250
  - maneuvers and, 575, 576f
  - turbulent flow *see under* Turbulence
  - vestibulospinal neurons, 250
  - *see also* Vestibular system
- Stakes
  - skeletal anatomy, 421f
    - appendicular skeleton, 422f, 426
  - *see also* Batoids
- Standard length (SL), definition, 336
- Standard metabolic rate (SMR), 1565, 1567, 1568f, 1764–1765
  - body size and, 1570
  - definition, 812, 846, 847, 1566, 1608, 1645, 1764–1765, 1889, 1904
  - foraging activity relationship, 1589, 1590
  - maximal metabolic rate *vs.*, 1765
  - measurement, 847, 1567, 1568f, 1569f
    - data, 1569t, 1570
    - oxygen consumption (Mo_2), 1567, 1568, 1568f, 1569f
    - SDA determination, 1608f, 1610
    - use of quantiles, 1569f, 1569t, 1570
    - *see also* Respirometers
  - reduction, 1765, 1767f, 1767–1768
    - *see also* Metabolic rate suppression
  - sources of variation
    - experimental, 1570–1571
    - interspecific variation, 1571, 1906f, 1907
    - intraspecific variation, 1571
    - temporal/geographic, 1571
  - *see also* Basal metabolic rate (BMR); Metabolic rate (MR)
- Standard metabolism
  - definition, 846, 847, 1589
  - *see also* Standard metabolic rate (SMR)
- Standing contractions, 1293f, 1294
  - definition, 1292
- Standing waves, sound propagation in shallow water and, 272
- Starling's forces
  - definition, 1154
  - transcapillary fluid movement and, 1155–1156, 1156f
- Startle response, 266
  - definition, 587
  - escape *see* Escape responses
  - withdrawal, 588
- Starvation, effects, 1513
- α-Stat regulation
  - cardiac contractility and, 1056f, 1058
  - definition, 1054
- State, definition, 575
- Static respirometers, 848–849, 849f, 1568
- Steady state, definition, 1045
- Steering, neural mechanism, 59, 60f
- Stem cells
  - definition, 616
  - hematopoietic *see* Hematopoietic stem cells (HSCs)
  - self-renewal, 618, 624f
  - definition, 616
  - spermatogonial *see* Spermatogonial stem cells (SSCs)
- Stenohaline, definition, 1429, 1466, 1961
- Stenothermal, definition, 1197
- Stenotherms, 1690
  - definition, 1689, 1690, 1733
  - heat shock proteins, 1734, 1734
- Stereovilli (stereocilia), 252–253, 254f, 255f
  - definition, 336
  - neuromast, 336, 337f, 338f
- Sterile, definition, 2009
- Sterile triploids, 2007, 2007
- Sterility, cultured fish, transgenesis/chromosome manipulation, 2006, 2007
- Sterilizing techniques, sea lamprey population control, 1786
- Steroid hormones
  - growth regulation, 1487
  - olfactory responses, 212, 212f
  - structures, 1545f
  - *see also* Gonadal steroid(s)
- Steroid pheromones
  - female preovulatory, 1554f, 1556, 1556f, 1559f
  - male-derived, 1560–1561
  - mechanism of action, 1559
    - synchronous ovulation/mass spawning, 1560f, 1561
  - species specificity, 1561
  - *see also* Hormonal pheromones
- Steroid receptors, definition, 662
- Steroidogenesis
  - definition, 1534
  - gonadal steroids, 1494, 1495f, 1502, 1504f
    - shift from estradiol to progestogens, 1503, 1504f
- Steroidogenic enzymes, gonadal steroids, 1494, 1495f
  - sex differentiation role, 1494
- Steroids, definition, 1542, 1553
- Stickleback, three-spined *see* Three-spined stickleback
- *Stizostedion lucioperca* (pike perch), ears, 256f
- Stockholm Convention (2001), 2070, 2076
- Stomach
  - air-breathing organ, 1869f, 1870
  - anatomy, 1269f, 1269–1270, 1602f
    - functional, 1604–1605
    - smooth muscle, 1270f, 1271
    - *see also* Gut anatomy
  - definition, 1597
  - development, 1285
  - gizzard-like, 1605
  - ion regulation role, 1421
  - oxynticopeptic cells, 1270, 1302, 1303f
  - secretions, 1270, 1302, 1305t
    - acid, elasmobranchs, 1813
  - species differences, 1271
  - species without, 1272
- Stomach air breathers, circulation patterns, 1876f, 1884
- Stomachless species, 1272
- Storage, metals, 2062f, 2064
- Storage growth, 1623f, 1625
  - *see also* Growth
- Strain, cytoskeletal, as osmosensory signal, 1375
- Stratum album centrale (SAC), 132, 133f
- Stratum compactum, 553, 553
- Stratum fibrosum et griseum superficiale (SFGS), 132, 133f
- Stratum griseum centrale (SGC), 132, 133f
- Stratum marginale (SM), 132, 133f
- Stratum opticum (SO), 132, 133f
- Stratum periventriculare (SPV), 132, 133f
- Stream capture, definition, 1843
- Streams, flow velocity, foraging activity and, 1591
- Stress, 1450, 1515
  - coping styles, 1520, 1521f, 1522f
  - definition, 1450, 1515, 1542–1543
  - responses, 652
    - effect of social status, 652–653, 653f
    - hormonal *see* Stress response, hormonal
  - social *see* Social stress
- Stress axis *see* Hypothalamic–pituitary–interrenal (HPI) axis
- Stress effects
  - gastrointestinal barrier function, 1329, 1330f
  - gastrointestinal blood flow, 1138, 1138f
  - growth *see under* Growth
  - health *see* Health, stress effects
  - hormonal *see* Stress response, hormonal
  - metabolism *see under* Metabolism
  - reproduction *see under* Reproduction
- Stress-induced analgesia, 716
  - definition, 713
- Stress proteins *see* Heat shock proteins (HSPs)
- Stress response, hormonal, **1515–1523**
  - catecholamines *see under* Catecholamine(s)
  - cortisol *see* Cortisol
  - modifying factors, 1520
    - endocrine disruption, 1522
    - innate individual variation/different coping styles, 1520
    - maternal stress and offspring development, 1521
  - primary response, 1516, 1516f
    - brain–sympathetic–chromaffin cell axis, 1516, 1516f, 1517f
    - beta-endorphin release, 1518
    - hypothalamic–pituitary–interrenal axis, 1475, 1476f, 1516f, 1517, 1517f
    - alpha-melanophore-stimulating hormone release, 1518, 1539
    - thyroid-stimulating hormone release, 1518, 1539
  - reproductive hormone interaction, 1545f, 1547
  - secondary response, 1516, 1518
    - metabolic effects *see* Metabolic stress
  - sex change role, 667
  - sturgeons, 1805
  - tertiary, 1516, 1518
    - growth effects *see* Growth, stress effects
  - *see also specific stress hormones*
- Stressed blood volume, 1114, 1114f, 1117
  - definition, 1111
- Stressed volume (SV), 981
  - *see also* Stressed blood volume
- Stressors, 1515
  - definition, 1515, 1543
- Striped bass (*Morone saxatilis*)
  - capillary size and structure, 1145f, 1152f
  - fish farming, dietary care, 532–533
  - seasonal temperature response, muscle capillarity, 1150, 1150f, 1151
  - temperature preferences, 762, 763f
- Striped marlin (*Tetrapturus audax*), satellite tracking, 1933
- Stroke volume
  - acute hypoxia response, 1222, 1223f
  - definition, 973, 1060, 1889, 1904
  - *see also* Cardiac output (V_b)
- Stroma
  - definition, 194
  - olfactory epithelium, 195
- Structural coloration *see under* Coloration
- Structural colors, reflected-light signaling, 694, 695f, 696f
- Structural growth, 1623f, 1625
  - *see also* Growth
- Strychnine, gustatory response, 220, 221f
- Sturgeons, physiology, **1800**
  - acid–base balance, 1804
  - ampullary organs, 352, 353f
  - blood–oxygen affinity, 1804
  - conus arteriosus
    - function, 1028
    - structure, 1024–1026, 1025f, 1027
  - environmental requirements, 1800f, 1801
  - growth, 1803
  - gut blood flow, 1804

  - osmoregulation, 1804
  - reproduction, 1801
    - endogenous and environmental regulation, 1802
  - respiratory metabolism, 1803
  - stress responses, 1805
  - swimming performance, 1805
- Subculturing *see* Passaging
- Subcutaneous sinus, hagfish, 1116, 1774
- Subfunctionalization, 1982
  - definition, 1980
- Subjective day/night, 739–740, 741
  - definition, 736
- Submucosa
  - definition, 1229
  - gut, 1270f, 1271
    - definition, 1268
- Subordinate individuals
  - dominant individuals *vs.*, 650, 650f, 651
    - coloration, 653
    - cortisol levels, 652–653, 653f
    - growth rate, 651–652
    - immune response, 653–654
    - ionoregulatory ability, 653
    - susceptibility to aquatic toxicants, 654
  - *see also* Dominance hierarchies
- Subpallium
  - agnathan, 23, 23, 24f
  - cartilaginous fish, 34f, 34–35
  - definition, 2, 37
  - *Latimeria*, 47, 55
  - lungfishes, 47, 49f, 52
  - ray-finned fish, 3, 4f, 40f, 43
  - *see also* Telencephalon
- Substance P
  - amino acid sequence, 1338f
  - definition, 699
  - gut expression, 1335f, 1338
  - *see also* Tachykinins
- Substrate affinity, 1705
  - definition, 1703
- Substrate-level phosphorylation, definition, 1758
- Sucker mouth, motor control system, 62
- Suction feeding, 467, 597, 598f
  - associated muscles, 464f, 465t, 467f, 468–469
  - evolution, 598, 598f
  - hydrodynamics, 599
    - fluid movement visualization, 599, 599f
    - ingested volume of water (IVW), 599, 600f, 601
    - modeling, 599
  - performance, 599
- Suction pump
  - cardiac, 981–982
  - definition, 977
- Suctorial feeding
  - definition, 1780
  - parasitic lampreys, 1781, 1783f, 1784
    - *see also* Oral disk, lamprey
- Sump pump
  - cardiac, 981–982
  - definition, 977
- Sun–compass orientation, 739
- Superficial neuromasts, 337, 339f
  - distribution, 330f, 331–332, 337, 340f
  - function, 330f, 331f, 332f, 337–338
  - hair cell orientation, 337, 340f
  - innervation, 337–338, 340f
  - morphological variation, 337, 340f
- Supernumerary tesseral layers, 430f, 432–433
  - definition, 428
- Superparamagnetic (SPM) particles, 730
- Supra-terminal mouth
  - aquatic surface respiration role, 766
  - definition, 765
- Suprabranchial chambers (SBCs), air-breathing fish, 1867f, 1869
  - anabantoids, 1868f, 1870
  - channids, 1869f, 1870
  - circulation patterns, 1876f, 1883f, 1884
  - clariid catfishes, 1867f, 1870
  - Indian catfish, 1868f, 1870
  - swamp eel, 1867f, 1869–1870
  - ventilation, 1873
- Suprachiasmatic nucleus (SCN), 741, 773–774
  - definition, 772
- Supraventricular tachycardia, transgenesis and fish models, 1991–1992
- Surface ocean currents, 1939f, 1940
- Surfacing behaviors *see under* Hypoxia, behavioral responses
- Surfactant, 1865–1866
  - definition, 1862
- Surge, 575–576, 576f, 578
  - definition, 575
- Survival strategies (food acquisition-associated), **744–751**
  - dealing with competition, 747f, 748
  - food capture/consumption, 747, 747f
  - predation risk, 748
    - consequences, 750
    - predator avoidance tactics, 749
  - what should fish eat?, 745
  - where should fish obtain food?, 745
  - *see also* Foraging models
- Suspensorium
  - bony fish, 439–440, 440f
  - cartilaginous fish
    - batoids, 425–426
    - chimaeroids, 426
    - sharks, 425
  - definition, 420
- Swamp eel, suprabranchial chambers, 1867f, 1869–1870
- Swamps, mangrove, 1962
  - definition, 1960
- Swelling, RBC, effect on hemoglobin concentration, 988
- Swimbladder, **526–534**
  - acoustic functions
    - adaptations for high-frequency sensitivity, 279–280
    - sound generation, 322–323
      - *see also* Drumming muscles
    - sound pressure sensitivity, 276, 278–279
  - buoyancy role, 521, 522, 525, 526
    - changing depths, 522
    - keeping afloat, 523
  - countercurrent multipliers serving, 1125
  - deep-sea fishes, 1956
  - definition, 237, 252, 270, 516, 520, 909, 1954
  - gas gland cells *see* Gas gland cells (swimbladder)
  - inflation
    - control, 532f, 533
    - failure, 532
  - inner ear relationship, 258, 259f, 259f, 274, 274f
    - *see also* Otophysic connections
  - metabolic activity, 528
    - countercurrent concentration, 530, 531f
    - gas resorption, 531
    - gas secretion *see* Swimbladder, gas secretion
    - single concentrating effect, 528
  - morphology, 526, 527f
    - gas gland cells, 528, 528f
    - physostome and physoclist fishes, 526, 527f
    - secretory and resorbing sections, 527, 527f
    - wall layers, 527
  - physiological significance of Root effect, 932, 940–941
  - physoclistous *see* Physoclistous gas bladder
  - physostomous *see* Physostomous gas bladder
  - respiratory *see* Respiratory gas bladder
  - rete *see* Swimbladder rete
  - role, 274
- Swimbladder, gas secretion, 528
  - carbon dioxide role, 913–914
  - oxygen, 932, 940–941
    - evolution, 943
- Swimbladder rete, 527, 527f, 931–932, 941, 1128
  - adrenergic control, 532f, 533
  - choroid rete *vs.*, 1125–1127, 1127f
  - countercurrent gas exchange, 530, 530, 531f
  - evolution, 941, 942f, 943
  - morphology, 1128, 1128f
- Swim speed–fatigue time (SSFT) relationship, 1656f, 1659–1660
  - ecological relevance, 1660
  - fisheries conservation implications, 1652f, 1660
  - *see also* Swimming performance, applied aspects
- Swim-tunnel respirometer, 1638f, 1639
  - definition, 1636
  - *see also* Swimming respirometers
- Swimming
  - ability
    - growth hormone transgenic fish, 2021
    - overfishing susceptibility and, 1655–1656
  - acoustic (low-frequency) oscillations produced, 265–266, 267–268
  - activity patterns, hypoxia response, 765, 1768
  - adaptations, pelagic fish, 1893
  - anguilliform, 548, 549f
  - backwards, 577
  - burst *see* Burst swimming
  - carangiform, 548, 549f, 552
  - circulatory system responses *see under* Exercise
  - continuous *see* Continuous swimming
  - decreased levels, as pain response, 716, 716f, 718t
  - elasmobranchs, 1809
  - energy requirement, 1637, 1637
    - *see also* Swimming, energy expenditure
  - energy transfer and momentum, 547
  - escape, 59
    - *see also* Escape responses
  - extension of energetic limits, anaerobic pathways, 1649f, 1650
  - hagfish, 1776
  - integrated responses, **1215–1220**
    - *see also* Exercise, circulatory system responses
  - integration with prey capture, 601
    - braking control, 601
    - ram speed, 601
  - intensity, locomotion categories, 812
  - kinematic analysis, 548
    - definition, 547
    - speed and tail-beat frequency, 548–549, 549f
  - metabolic efficiency, 1578, 1581
    - *see also* Swimming efficiency, bioenergetics
  - modes (movement), 547, 549f
    - metabolic scaling to body mass, 1580
    - *see also specific modes*
  - motor control systems *see under* Motor control systems
  - muscle function during, work-loop analysis, 562
  - paired fins *see* Paired fin(s); Pectoral fin(s); Pelvic fin(s)
  - ‘pay now’ or ‘pay later’, cardiovascular system, 1215
  - pectoral and pelvic fin *see* Pelvic fin(s)
  - performance *see* Swimming performance
  - phase lag, 56–57, 57f
  - sound signal production, 685t, 690
  - speeds *see* Swimming speeds
  - undulatory *see* Undulatory swimming
  - volitional *see* Volitional swimming
  - *see also* Aerobic swimming; Exercise; Locomotion; Maneuverability; Skeletal muscle
- Swimming, energy expenditure, **1636**
  - basis of energy requirement, 1637
  - environmental influences and Fry paradigm, 1641f, 1642
    - dissolved oxygen availability, 1641f, 1642
    - loading factors, 1641f, 1642
    - temperature, 1641f, 1642
  - estimation in wild fish, 1642
  - muscle types and swimming activity, 1637f, 1638
    - aerobic red muscle and sustained aerobic activity, 1638, 1638f

- Swimming, energy expenditure (*continued*)
    - anaerobic white muscle and high-intensity bursts of activity, 1640
    - spontaneous swimming activity, 1641
- Swimming efficiency, bioenergetics
  - burst swimming, anaerobic metabolism, 1908
  - net cost of transport, 812–814, 1903, 1907f, 1908
  - ontogenetic changes, 1578
- Swimming metabolism
  - definition, 846
  - measurement, 847, 847
    - *see also* Swimming respirometers
- Swimming performance
  - applied aspects *see* Swimming performance, applied aspects
  - catecholamine role, 1536
  - sturgeons, 1805
- Swimming performance, applied aspects, **1652**
  - aerobic scope effects, 1658–1659
  - critical swimming speed protocol, 1656f, 1658
  - data origins/historical aspects, 1654f, 1656
  - fish size considerations, 1660–1662
  - limitations of available data, 1662
  - swim speed–fatigue time relationship, 1656f, 1659–1660
    - implications for fisheries conservation, 1656f, 1657f, 1660
  - volitional swimming studies *see* Volitional swimming performance studies
  - *see also* Swimming respirometers
- Swimming respirometers, 848–849, 851, 1658
  - Blaska-type, 851, 851f, 1655f, 1658
  - Brett-type, 851, 851f, 1655f, 1658
  - *see also* Swim-tunnel respirometer
- Swimming speeds
  - cost of transport *vs.*, 1638f, 1639–1640
  - critical *see* Critical swimming speed (U_{crit})
  - effect on oxygen consumption, 812–814, 813f
  - energetic costs, scombrids, 1907f, 1908
  - optimum (U_{opt}), 814
  - related to body size, 1579f, 1581
- Symmorphosis, 1148, 1149f
  - definition, 1143
- Sympathetic chains, definition, 80
- Sympathetic nervous system
  - definition, 80, 1457
  - neurotransmitters, cardiac action potential effects, 1042, 1042
  - *see also* Autonomic nervous system (ANS)
- Sympatric, definition, 656
- Synapomorph, definition, 6
- Synapomorphy, 8
  - definition, 1852
- Synaptic transmission, Mauthner cell as model *see* Mauthner cells
- Synarcual
  - batoid, 421f, 425
  - chimaeroid, 426
  - definition, 420
- Synchronism, definition, 387
- Synchronous air breathing, 769, 1858
- Syncytia (syncytium), definition, 123, 387
- Synencephalon, cartilaginous fish, 32
- Syngnathidae (seahorses), sound production, 325
- Systemic, definition, 1085
- Systemic arteries *see* Post-gill (systemic) arteries
- Systemic effects, definition, 2078
- Systemic endothermy *see under* Endothermy/endotherms
- Systemic vascular resistance, **1104–1110**
  - branchial vascular resistance and, 1132, 1133f
  - control, 1104, 1143, 1173
    - local *see* Systemic vascular resistance, local control
    - remote *see* Systemic vascular resistance, remote control
  - definition, 1221
  - factors affecting, 1107
    - exercise, 1107
    - hypovolemia, 1108
    - hypoxia, 1108, 1223
    - temperature, 1107
  - hemodynamic importance, 1105
  - measurement methods, 1108
    - intact fish, 1109, 1109f, 1110f
    - isolated blood vessels, 1108, 1108f
    - perfused organs, 1109, 1109f
- Systemic vascular resistance, local control, 1104, 1106, 1173
  - metabolites, 1106
  - paracrine-mediated responses, 1106
    - arachidonic acid metabolites, 1106
    - endothelin, 1106
    - gasotransmitters, 1107
- Systemic vascular resistance, remote control, 1104, 1105, 1174
  - hormonal, 1105
    - adrenomedullin, 1106
    - arginine vasotocin, 1106
    - bradykinin, 1106
    - catecholamines, 1105
    - natriuretic peptides, 1106
    - renin–angiotensin system, 1105
    - urotensins, 1106
  - neural, 1105, 1174
- Systemic veins, 1088f, 1092
- Systemic viremia, definition, 2033
- Systole, 982–983, 1031
  - definition, 973, 977, 1030, 1221
  - *see also* Cardiac cycle
- Systolic pressure, definition, 977, 979, 1030

T

- T-state hemoglobin, 889f, 889–890, 946, 946f
  - definition, 526, 887
  - *see also* Hemoglobin(s), fish
- T-tubular network
  - cardiomyocyte membrane, 1008, 1008f, 1011, 1011f
  - definition, 1007
- Tachycardia, definition, 1877
- Tachykinins, 1337
  - amino acid sequences, 1338f
  - gut expression, 1335f, 1338
    - receptors, 1338
  - gut motility role, 1297, 1298f, 1299f
- Tactile stimulation
  - feeding, 227
  - gustation, 224, 225t
- Tagging of Pacific Pelagics (TOPP), 1935
- Tags
  - acoustic, 1928, 1929–1930
    - chat, 1928, 1930–1931
    - listening stations, 1929f, 1930
  - archival, 1928, 1933, 1933f
    - pop-up satellite archival transmitting (PSAT), 1928, 1928f, 1932
  - design, 1928f, 1930
  - multi-species tagging, 1935
  - Single Position Only Tags (SPOT), 1928f, 1931f, 1932–1933
  - survival and attachment, 1932, 1932f
- Tail
  - stability control role, turbulent flows, 585, 586
  - *see also entries beginning caudal*
- Tail-beat frequency, oxygen requirement and, 1219
- Tannins, definition, 1667
- Tapeta, deep-sea fish, 176, 178f, 179f
- Target host, 778
  - definition, 777
  - *Diplostomun spathcaeum*, 779–780
  - *Schistocephalus solidus*, 781
- Tastants, 185
  - definition, 183
- Taste, sense of *see* Gustation
- Taste, system of, morphology *see* Gustatory system morphology
- Taste buds, 225, 1100–1101
  - development, 189
  - distribution, 187, 188f
  - elasmobranch, 1808
  - historical aspects, 184
  - structure, 187, 188f
    - basal cells, 188–189
    - receptor cells, 188
    - synaptic contacts and neurotransmitters, 189
  - *see also* Gustation; Gustatory system morphology
- Taurochenodeoxycholic acid, 1303, 1303f
  - *see also* Bile acids
- Taurocholic acid (TCA), 1303, 1303f
  - gustatory responses, 219f, 221, 222f, 223f
  - *see also* Bile acids
- Tearing (food items), 469
  - associated muscles, 464f, 470
- Tectum *see* Optic tectum (OT)
- Tectum–nucleus isthmi subsystem, 137, 138f
- Tectum–torus longitudinalis subsystem, 135, 136f, 136f
- Teeth, 1269
  - hagfish, 1773
- TEL-AML1 oncogene, 1991
- Telemetry
  - brain temperature, 1918f, 1919
  - definition, 1928–1929
- Telemetry, wildlife
  - definition, 1928–1929
  - satellite data transmission, 1928, 1930, 1931
  - used for environmental biosensing, 1930, 1935, 1935
  - *see also* Tags
- Telencephalon
  - agnathan, 23, 24f
  - cartilaginous fish, 34, 34f
  - definition, 2, 707
  - gustatory areas, 190
  - *Latimeria*, 53f, 54f, 55
  - learning/memory role, 709
  - lungfishes, 47, 48f, 49f, 52
  - olfactory areas, 190, 205–206
    - signal processing, 215, 216f
  - ray-finned fish, 3, 4f, 38f, 40f, 43
    - development, 43, 44f
  - *see also* Pallium; Subpallium
- Teleost-specific genome duplication (TSGD), 1981, 1981f
- Teleosts
  - anoxia tolerance, 1825
    - *see also* Crucian carp
  - autonomic nervous system, 82, 83f, 84f
    - cardiac control, 85, 85f
    - chromaffin tissue and, 82, 85, 85f
    - control of iris, 86, 86f, 87f
    - extrinsic innervation of gut, 1333–1334, 1334f
    - intracardiac neurons/neurotransmitters, 1069, 1070f, 1071f
  - Bohr effect, 923
    - evolution, 926f, 927
      - hemoglobin buffer properties and, 927f, 928
    - molecular mechanisms, 925, 925f
  - brain structure, 38f
  - carbon dioxide excretion, 911, 912, 913
  - cardiac outflow tract *see* Bulbus arteriosus
  - cardiorespiratory interactions, central control *see under* Cardiorespiratory interactions (CRIs)
  - clupeid *see* Clupeids
  - color change regulation, 493
  - definition, 110, 237, 283, 1221, 1474, 1889, 1905
  - electric organ development *see under* Electric organ development
  - electrocommunication *see* Electrocommunication
  - electroreceptors
    - ampullary *see under* Ampullary electroreceptors
    - development, 410
    - tuberous *see* Tuberous electroreceptors

- evolution, 116–117
- GI tract and blood flow regulation *see* Gastrointestinal blood flow (GBF), regulation during digestion
- GI tract circulation, 1133–1134
- gill salt secretion, **1354–1358**
  - *see also* Mitochondrion-rich cells (MRCs), seawater fish
- gills, 1095–1096
- gut ion regulation *see* Gut ion/acid–base regulation
- kidneys, 1412, 1413*f*, 1414*f*
- ocular anatomy, 168, 169*f*
- osmoregulation
  - freshwater species, 1349*t*, 1351, 1352*f*
  - marine species, 1349*t*, 1350, 1350*f*
- otophysan *see* Otophysans
- pineal gland, 162
  - photoreception *see under* Pineal photoreception
  - structure/ultrastructure, 162, 162*f*, 164*f*
- Root effect *see* Root effect
- trigeminal nerve responses to magnetic fields, 728, 730*f*
  - *see also* Magnetic sense
- vascular anatomy *see under* Vascular anatomy
- *see also individual species*

Telescopic vision, 107, 108*f*

Temperature, **1688**
- definition, 1689, 1690, 1695
- effects on fish physiology *see* Temperature effects
- environmental, fitness/success and, 1696
- growth, fish cell lines, 1968
- heat and, 1691
- measurement, 1690
  - scales, 1690–1691
- preferences *see* Temperature preferences
- thermal adaptation/acclimation strategies, 1694
  - *see also* Thermal acclimation/adaptation
- thermal energy and, 1691
- thermal performance curve, 1689*f*, 1690
- thermocline, consequences for diving, 1912
- tolerance *see* Thermal tolerance
- *see also entries beginning thermal*

Temperature adaptations *see* Thermal acclimation/adaptation

Temperature coefficient *see* Q_{10} (temperature coefficient)

Temperature compensation, 1703*f*, 1705
- definition, 1703
- *see also* Thermal acclimation/adaptation

Temperature effects, 1696, 1710, 1741, 1742*f*
- appetite/food intake, 1486, 1513–1514
- Atlantic cod *see under* Cod, Atlantic (*Gadus morhua*)
- blood viscosity, 987
- body pH, 914–915
- cardiovascular function *see* Temperature effects, cardiovascular system
- cell membrane fluidity *see* Homeoviscous adaptation (HVA)
- cellular respiration, 956
- cultured fishes, 2084*f*, 2085*f*, 2086
- energetic costs of swimming, 1641*f*, 1642
- excitable membranes *see under* Excitable membranes
- fast start performance, 595
- food/feeding *see* specific dynamic action (SDA)
- foraging activity, 1590
- gastrointestinal blood flow, 1140, 1140*f*, 1212–1213, 1213*f*
- growth, 1489, 1629*f*, 1630, 1744
- gut anatomy, 1274
- heat shock protein induction *see* Heat shock response
- insights arising from global gene expression data, 2058*f*, 2059
- membrane structure, **1725**
  - homeoviscous adaptation *see* Homeoviscous adaptation (HVA)
  - *see also* Cell membrane(s); Cell membrane fluidity
- metabolism *see* Metabolism, environmental influences
- migration, 1926
- mitochondria *see under* Mitochondria
- muscles, work-loop analysis, 561, 562*f*
- oxygen-binding properties of hemoglobin, 891, 1897*f*, 1901, 1912
- pelagic fish
  - blood–oxygen binding, 1897*f*, 1901
  - cardiac function, 1898
- population dynamics *see under* Population dynamics
- proteins, **1703**
  - adaptation range, fish, 1703
  - enzyme function *see under* Enzymes
  - structural adaptive modifications, 1705
- on rate processes, 1690*f*, 1691
  - Maxwell–Boltzmann distribution, 1691*f*, 1692
  - quantification *see* Q_{10}
  - *see also* Arrhenius plot
- on weak bonds, 1693
- *see also* Critical thermal maximum (CTMax); Critical thermal minimum (CTMin); Thermal acclimation/adaptation

Temperature effects, cardiovascular system, **1197–1205**
- cardiac action potential, 1043, 1043*f*, 1721, 1722
- cardiac excitation–contraction coupling *see under* Cardiac excitation–contraction coupling
- cardiac function
  - maximum, 1200, 1201*f*, 1202*f*
  - pelagic fish, 1898
  - resting, 1198, 1199*f*, 1201*f*, 1202*f*
- cardiac scope, 1200, 1201*f*
- hypoxia tolerance of heart, 1224, 1224*f*
- vascular resistance, 1107
- venous capacitance/cardiac output, 1115
- *see also* Thermal acclimation/adaptation

Temperature preferences, **758–764**
- accuracy and constancy of preferred temperature, 759*f*, 760, 760*f*
- conditions altering preferred temperature
  - major alterations, 759
  - small alterations, 759, 759*t*
- definition, 758
- environmental observations, 761
  - avoidance of dangerous temperatures, 762, 762*f*, 763*f*
  - global climate change/environmental degradation effects, 763
- species preferences, 759, 759*f*, 759*t*
- thermoregulatory system characteristics, 761, 761*f*

Temperature quotient
- definition, 1695
- *see also* Q_{10} (temperature coefficient)

Temperature sensitivity, ontogeny, 1739*f*, 1743*f*, 1744

Temperature tolerance *see* Thermal tolerance

Temperature tolerance polygon, definition, 1695

Temperature–oxygen squeeze, definition, 829

Template matching
- behavioral studies, 144, 144*f*
- definition, 143

Temporal, definition, 1206

Temporal code, definition, 366

Temporal coding
- auditory afferents, 294, 295*f*
- mormyromast receptors, 381–383

Temporal heterotherm, definition, 1689

Temporal response patterns, auditory neurons, 296

Temporal variation, heat shock response, 1735

Terrestrial adaptations, air breathing, 1855*f*, 1858
- *see also* Air-breathing fish

Terrestrial behaviors, hypoxia responses, 770, 770*f*
- *see also* Amphibious fishes

Terrestrial environment, aquatic environment *vs.*, 183
- chemoreception, 183–184

Territoriality
- foraging activity and, 1591
- reef fish, 151, 155, 156–157

Tertiary stress response, 1516, 1518
- growth effects *see* Growth, stress effects

Tessellated cartilage
- cartilaginous fish, 429–431, 430*f*
  - reinforcements, 430*f*, 432–433
- definition, 416, 428

Tesserae, 429–431, 430*f*
- definition, 428
- lacunae, 431–432

Testes
- differentiation *see* Testicular differentiation
- regulation
  - gonadotropins, 627, 628*f*
  - spermatogenesis *see* Spermatogenesis, regulation
- structure *see* Testes, anatomy and histology

Testes, anatomy and histology, **616–626**, 627, 628*f*
- anatomical features, 617
  - development in triploid fish, 2009*f*, 2011
  - variation between species, 617–618, 618*f*, 620*f*
- component parts, 617
  - efferent duct, 617, 619*f*
  - interstitial and germinative compartments, 617, 617*f*
- distribution of spermatogenic cysts, 620
  - evolutionary development, 620
  - seasonal activity, 620
- spermatogenesis process, cystic, 618, 621*f*
- spermatogenesis sequence
  - cytology of differentiating cells, 622
  - phases of spermatogenesis, 620, 625*f*
  - *see also* Spermatogenesis

Testicular differentiation, 1492, 1492*f*, 2026, 2029–2030
- endocrine control, 1496*f*
  - *see also* Sex differentiation, endocrine control
- genes involved, 2029*t*, 2030
- histology, 1493*f*
- *see also* Sex differentiation

Testicular (efferent and spermatic) ducts
- functions, 617
- urinogenital papilla opening, 617–618, 619*f*

Testis–ova, definition, 1490

Testosterone
- definition, 662
- formation/metabolism, 1502–1503, 1504*f*
- olfactory responses, Atlantic salmon, 212
- salmon migration role, 1950, 1951
- stressor effects on release, 1547
- *see also* Androgen(s)

Tetrachromatic color vision, definition, 99, 110, 116

Tetraploidy, 2007
- definition, 1999, 2009

Tetrapodomorpha, 1852
- definition, 1852

Tetrapturus audax (striped marlin), satellite tracking, 1933

Tetrodotoxin (TTX)
- definition, 1206
- gustatory response, 220, 221*f*

Thalamus
- agnathan, 21
  - hagfish, 21–23, 22*f*
  - lamprey, 21, 22*f*
- cartilaginous fish, 28*f*, 32
- ray-finned fish, 40*f*, 41–42

Thallium (Tl), biological effects and toxicodynamics, 2067

Thermal acclimation/adaptation, 760, 1700
- cardiovascular system responses, 1199*f*, 1200
  - adaptations, 1202, 1204*f*
    - *Pagothenia borchgrevinki*, 1203–1204, 1204*f*
  - cardiomyocytes, 1007–1008, 1010, 1011
  - *see also* Temperature effects, cardiovascular system
- definition, 758, 758

Thermal acclimation/adaptation (*continued*)
  evolution, 1701
    enzyme selection, 1705
    ion channel performance, 1723
  homeoviscous adaptation (HVA) *see* Homeoviscous adaptation (HVA)
  intertidal habitats, 1964
  membrane excitability, evolution, 1723
  mitochondrial role, 1713
    cold acclimation *see under* Cold acclimation/adaptation
    warm acclimation, 1647, 1714*f*, 1715
  rate, 1699*f*, 1700
  skin role, 474
  strategies, 1694
  thermal tolerance and, 1700
  see also Cold acclimation/adaptation; *Cyprinodon* (desert pupfish); Thermal tolerance; Warm acclimation
Thermal change, effects *see* Temperature effects
Thermal effects of food (TEF) *see* specific dynamic action (SDA)
Thermal energy
  definition, 1689, 1691
  temperature and, 1691
Thermal history, heat shock response and, 1734
Thermal performance curve, 1689*f*, 1690
Thermal rate coefficient (Q_{10})
  definition, 1889
  effect of acute temperature changes in pelagic fish, 1898–1900
Thermal stress, heat shock protein induction *see* Heat shock response
Thermal tolerance, **1695**
  basis for, 1695
  biological applications, 1702
  desert pupfish, 1836*f*, 1837, 1837*f*
  ecophysiological theory, 1701
  environmental temperatures (T_e) and fitness, 1696
  heat shock proteins and, 1736
  history, 1697
  influencing factors, 1699
    acclimation temperature and fluctuations, 1699*f*, 1700
    environmental stresses, 1700*t*, 1701
    genetic variation, 1701
    multiple factor interactions, 1699
    reproductive status and ontogeny, 1701
    seasonality and photoperiod, 1699*f*, 1701
    triploidy, 2012, 2013
  laboratory measurement, 1697
    chronic lethal method (CLM), 1699
    comparison of approaches, 1698
    critical thermal methodology (CTM), 1697*f*, 1698
    CTMax endpoints, 1698
    CTMin endpoints, 1699
    exposure time, 1699
    incipient lethal temperatures (ILTs), 1696*f*, 1697
    variables, 1699
  oxygen- and capacity-limited *see* Oxygen- and capacity-limited thermal tolerance (OCLT)
  research motivations, 1697
    global climate change, 1702
  seasonal effects, 1699*f*, 1701, 1740–1741
    muscle capillaries, 1150, 1151, 1151*f*
  species variation, 1150, 1695, 1739*f*, 1740
  variation during life cycle, 1701, 1739*f*, 1743*f*, 1744
Thermocline, 1888*f*, 1889–1890
  definition, 1630, 1889
  limitation of vertical movements in pelagic fish, 1895*f*, 1896*f*, 1898, 1899*f*
  temperature, consequences for diving, 1912
Thermodynamics
  enthalpy, definition, 1703
  entropy, definition, 1703
  forces in protein unfolding and stability, 1705–1706
  turnover number, enzyme catalysis (k_{cat}), 1705
  see also Temperature; *specific aspects*
Thermogenesis
  heater organ tissue, 1917*f*, 1917–1918
  metabolic rate measurement, 1765
  post-feeding, 1915–1916, 1919*f*, 1920
  see also Endothermy/endotherms
Thermogenic tissue
  definition, 1914
  heater organ tissue *see under* Cranial endothermy
Thermoregulation
  behavioral *see* Behavioral thermoregulation
  definition, 1695
  physiological, definition, 758
Thermoregulatory system, characteristics, 761, 761*f*
Thick filaments, 1054–1055, 1055*f*
  definition, 1007, 1054
Thin filaments, 1054–1055, 1055*f*
  activation, sarcomere length–tension relationship and, 1066
  definition, 1007, 1054
Thornback ray (*Raja clavata*), inner ear, 263*f*, 264
Thread cell, hagfish *see under* Slime glands (hagfish)
Thread skein, definition, 504
Three-spined stickleback (*Gasterosteus aculeatus*)
  color polymorphism, 1977
  parental care, 671, 671*f*, 675
    costs/benefits, 674
    fanning behavior, 675–676
  sexual behavior
    females, 659
    males
      courtship display, 658–659
      nest construction, 657, 657*f*
  spatial orientation mechanisms, 710, 711*f*, 724
Threshold, definition, 292
Threshold potential, definition, 1038, 1718
Threshold P$_{vO2}$, definition, 1077
Thrichopsis vittata (croaking gouramis), vocalization, 322, 322*f*
Thrombocytes, 989, 989*f*, 990*f*
  definition, 984
  inter-species variability, 990*f*, 991
Thunniform swimming, 548, 549*f*, 1907
  see also Tuna
Thunnus albacores see Yellowfin tuna
Thunnus obesus (bigeye tuna), bulbus arteriosus, anatomy, 1016*f*
Thunnus thynnus see Atlantic bluefin tuna
Thyroid hormones
  definition, 1466
  growth regulation role, 1487
  ion regulation role, 1471
  triiodothyronine (T3) levels in growth hormone transgenic fish, 2019
Thyroid-stimulating hormone (TSH), stress response role, 1518, 1539
Tight capillaries, 1159
Tight junction, enterocytes *see under* Enterocytes
Tilapia
  brain gustatory nuclei (*Oreochromis niloticus*), 190–191
  gill ion-regulating cells, 1385–1386
    Alcolapia grahami, 1386
    freshwater (*Oreochromis mossambicus*), 1386
  growth hormone transgenesis, 2018–2019
    metabolic effects, 2020, 2021
    phenotypic effects, 2019
TILLING (targeting-induced local lesions in genomes) genetics research, 1823, 1987–1988
Tilting, 577
  see also Maneuverability
Time coding, 368
  projections to higher-order neurons, 368
    see also Electrosensory lateral line lobe (ELL)
  pulse-type fish, 368, 368*f*, 369*t*
  wave-type fish, 368, 368*f*, 369*t*
  see also Electric organ discharge (EOD), central control
Time constant
  definition, 66, 73
  Mauthner cells, 71–72, 77
Time–place learning (TPL)
  compensatory growth effects, 755
  definition, 755
  see also Learning and memory
Tin (Sn)
  biological effects and toxicodynamics, 2067
  see also Tributyl tin (TBT)
Tissue(s)
  fuel delivery, 953, 957
  oxygen delivery *see* Tissue oxygen delivery
  oxygen extraction *see* Tissue oxygen extraction
  oxygen unloading, aerobic swimming-associated improvement, 1218
Tissue oxygen delivery, 953, 957
  aerobic swimming-associated increase, mechanism, 1216
  hydrogen sulfide toxicity, intertidal habitats, 1962
  see also Oxygen transport/exchange
Tissue oxygen extraction
  aerobic swimming-associated increase, mechanism, 1216
  definition, 1215
Tissue perfusion, regulation, 1174, 1175*f*
Tissue respiration, 794
  cellular *see* Cellular respiration
  mitochondrial *see* Mitochondrial respiration
Titin, 1063*f*, 1066
Togaviridae *see under* Viral diseases
Tonal sounds, 688
Tone, vascular
  definition, 1111
  venous *see* Venous tone
Tongue, 1269
  hagfish, 1773
Tonically active, definition, 855
Topographic map, definition, 131
TOPP (Tagging of Pacific Pelagics) program, 1931*f*, 1932, 1935
Torpedo marmorata (electric ray), electric lobes, 27, 28*f*
Torus longitudinalis (TL), 132–133, 133*f*
  tectum–TL circuitry, 135, 136*f*
Torus semicircularis (TS), 136*f*, 139
  auditory/lateral line pathways *see under* Auditory/lateral line CNS, anatomy
  definition, 292
  directional acoustic information and, 302
  electrosensory processing
    ampullary system, 363–364, 364*f*
    tuberous system *see under* Tuberous electrosensory system, physiology
  frequency tuning and, 295–296
Total body water, 1155
Total exchange, efficiency *vs*., gas exchange, 825, 826*f*
Total filament length
  definition, 803
  determination, 804–806
  see also Gill respiratory morphometrics
Total gill surface area *see* Gill surface area
Total peripheral resistance, arterial blood pressure and *see under* Arterial blood pressure regulation
Totipotency, definition, 2046
Toxicants
  behavioral effects *see under* Anthropogenic influences/disruptions
  endocrine disruption, 1522
  impact on migration, 1926
  metals *see* Metal(s)
  olfactory effects *see* Olfactory toxicity
  susceptibility, effect of social status, 654
  see also Pollution/pollutants; *specific toxicants*
Toxicodynamics
  definition, 2062, 2069
  metals *see under* Metal(s)
Toxicokinetics
  definition, 2062, 2069

  metals *see under* Metal(s)
Toxicology, metals *see* Metal(s)
Toxicology, organic chemicals, **2069**
  awareness and scope of toxic effects, 2070
    natural toxins, 2070
    Stockholm Convention (2001), 2070, 2076
    toxic action paradigm, 2069f, 2070
  biological fate, 2070
    accumulation in organism and food chain, 2071
    biotransformation, 2070f, 2071f, 2072
    excretion, 2072f, 2073
    uptake, 2071
  synthetic environmental contaminants
    chemical structures, 2073f
    dioxins, 2075
    PCBs *see* Polychlorinated biphenyls (PCBs)
    perfluorooctane sulfonate (PFOS), 2077
    pesticides, 2074
    pharmaceuticals and personal care products (PPCPs), 2076
    polybrominated diphenyl ethers (PBDEs), 2069, 2076
    polycyclic aromatic hydrocarbons (PAHs), 2069, 2074
Trabecula(ae), 1077
  atrium, 1002
  definition, 998, 1067
  ventricle, 1000f, 1002, 1078, 1079f
Trabecular cartilage, tessellated cartilage reinforcements, 430f, 433
Trace-amine-associated receptors (TAARs), 214
Tracking
  oceanic fish, **1928**
    multi-species tagging, 1935
    see also Pelagic fish, migration; Tags
  wildlife *see* Telemetry, wildlife
Trans-sex phenomenon, 2052
  definition, 2046
  see also Hermaphroditism; Sex change
Transactivation, definition, 1466, 1474
Transamination, definition, 1437–1438
Transbranchial deoxygenation, definition, 1877
Transcapillary fluid movement, 1156f, 1157
  fish skin role, 1159
  fluid loss from vasculature into interstitium, 1158, 1158f
  fluid reabsorption from interstitial space into vasculature, 1157, 1157f
  interstitial compliance and, 1159
  mammals *vs.* fish, 1159
  physiological significance, 1158
  Starling's forces and, 1155–1156, 1156f
  vascular permeability and, 1159
Transcellular fluid, definition, 1154, 1155
Transcellular transport
  definition, 1366, 1419
  water, 1367–1368, 1368f
    see also Water homeostasis
Transcription factors
  definition, 1971
  sex differentiation role, 1497, 2029t, 2030
Transcriptome, definition, 2055
Transcriptomics, cold acclimation, 1715, 1715f
Transected, definition, 855
Transepithelial potential, definition, 1389
Transepithelial transport, intestine *see* Intestinal absorption
Transgenesis
  cardiac dysfunction studies, 1991–1992
  definition, 1999, 2001
Transgenesis, fish, 1988f, 1989, 2001
  gene expression, 2002f, 2003
  gene incorporation, 2002
  gene introduction, 2001
  gene isolation, 2001
  gene transmission, 2003f, 2004
  growth hormone *see* Growth hormone transgenesis
  history, 2016
Transgenic, definition, 2016
Transgenic fish
  applications, 2004
    aquaculture, 2004f, 2005
      see also under Growth hormone transgenesis
    as biofactories, 2006
    colorful fish for pet trade, 2006
    environmental monitoring, 2006
    gene regulation studies, 2003f, 2004f, 2005
      see also Transgenic models
  concerns/anxieties, 2007
  growth hormone overexpression *see* Growth hormone transgenesis
Transgenic models, 1989, 2004–2005
  skin tumors, 1977, 1977f, 1978, 1989, 2004f, 2005
Transient cycles, circadian rhythm *see under* Circadian rhythms
Transit time, food, dietary efficiency and, 1598f, 1603
Translational maneuvers, 578
  definition, 575
Translocation of pathogens, across intestinal barriers *see under* Gut
Transmembrane proteins
  carbonic anhydrase isoforms, 901, 903–904, 904f
  definition, 899
  see also Membrane proteins
Transmission loss, 272
Transmitter, definition, 99, 123
Transmitter release mechanisms, Mauthner cell as model, 71
Transmural pressure, 1112
  definition, 977, 1111
Transplantation, fish model, 1989, 1990–1991
Transporter isoforms, definition, 1359
Transporting epithelium, definition, 1457
Transposable elements/transposons
  definition, 1980, 2001
  diversity, 1983
  fish *vs.* mammalian genomes, 1983–1984
  miniature inverted, 1983
  Sleeping Beauty *see* Sleeping Beauty transposon
Trematodes
  definition, 777
  Diplostomun spathcaeum see Diplostomun spathcaeum
Triacylglycerol (TAG), definition, 951
Trial and error learning, 710
Tributyl tin (TBT), 2067
  biotransformation, 2064
  structure, 2062f
  toxicity, 2067
Trichopsis vittata (croaking gouramis), sound production, 322, 322f, 325
3-Trifluoromethyl-4-nitrophenol (TFM), sea lamprey population control, 1786
Trigeminal nerve (TN)
  interaction with gustation, 192
  magnetic field responses in teleosts, 728, 730f
Triglycerides
  buoyancy and, 521, 521t, 525
  definition, 520
Triglycerol, 1308f
Triiodothyronine (T3)
  levels in growth hormone transgenic fish, 2019
  see also Thyroid hormones
Trimethylammonium N-oxide (TMAO)
  depth-related changes, 1683f, 1684
  protein stabilization, 1684
Tripeptides, absorption, 1314f, 1315
Triploblasts, 7, 8, 10
Triploid, definition, 2001, 2009
Triploid fish, physiology, 2007, **2009**
  applications, 2007, 2010, 2014
  artificial and natural occurrence, 2009
  cardiovascular physiology, 2011
  disease resistance, 2013
  hematology and gas transport, 2011
  immunology, 2013
  nutrition and growth, 2014
  reproductive physiology, 2010
  research relevance, 2014
  respiration and metabolism, 2012
  sterile, 2007, 2007
  stress and environmental tolerance, 2013
Trituration, 1605
Troglomorphism
  blind cavefish *see under* Blind cavefish
  definition, 1843
Trophic transmission
  definition, 777
  host manipulation and, 778
Trophotaeniae, 679–680
  definition, 678
Tropical species
  dominance hierarchies, 650
  migration and impacts of dams, 1658–1659
Tropomysin (TM), 1055, 1055f
Trout
  brook *see* Brook trout
  brown *see* Brown trout
  cardiac troponin C *see under* Cardiac troponin C (cTnC)
  rainbow *see* Rainbow trout
True digestive efficiency, definition, 1597, 1598
Truncus arteriosus, 1016–1018, 1019f
  definition, 1015
Trunk
  arterial system, 1087f, 1089
  movements, 57f, 58, 59f
  venous drainage, 1092
    cutaneous veins, 1093
    hepatic portal system, 1092
    renal portal system, 1092
Trypsin, 1281, 1281t, 1304, 1304–1305, 1305f, 1305t, 1306
  definition, 1966
  use in fish cell culture, 1969
Tubercles, breeding, 488
Tuberous electroreceptors, 356, 366–367, 379
  amplitude coding *see* Amplitude coding
  ampullary organs *vs.*, 356–357
  definition, 350, 409
  development *see under* Electroreceptor development
  gymnotid, 357f, 358
  Osteoglossiformes
    Gymnarchus, 357f, 358
    Knollenorgans *see* Knollenorgan
    mormyrids, 357, 357f
    mormyromasts *see* Mormyromasts
  silurids, 357f, 358
  time coding *see* Time coding
  see also Active electrolocation; Electric organ discharge (EOD); Tuberous electrosensory system, physiology
Tuberous electrosensory system, physiology, **366–374**
  amplitude coding *see* Amplitude coding
  electrosensory lateral line lobe neurons *see under* Electrosensory lateral line lobe (ELL)
  midbrain neurons/torus semicircularis, 372
    emergence of selectivity for salient stimuli, 372
    EOD waveform processing, 373
    jamming avoidance response, 372
    moving object processing, 373
  time coding *see* Time coding
  see also Electric organ discharge; Electrocommunication
Tubeshoulders, bioluminescence, 501
Tubular eyes, deep-sea fishes *see under* Deep-sea fishes, ocular anatomy
Tubular secretion, 1413f, 1415
Tubular system, definition, 1381
Tubular transport maximum
  definition, 1411
  glucose, trout kidney, 1415
Tubulovesicular system, definition, 1381

- Tumor(s)
  - malignant, definition, 1971
  - modifier, definition, 1971
  - pigment cell *see* Pigment cell tumors
  - suppressor, definition, 1971
- Tumor necrosis factor-α (TNF-α), osmosensing role, 1378
- Tuna, **1903**
  - bluefin, Atlantic *see* Atlantic bluefin tuna
  - characteristics and taxonomy, 1904*f*, 1905
  - continuous swimming, 1907
    - feeding and behavior, 1907
    - morphology and efficiency, 1905*f*, 1907
  - diving
    - diel behavior, 1934*f*
    - physiological specializations, 1911*f*, 1912
  - endothermy, 1910*f*, 1911
    - advantages, 1919
    - cardiac physiology and, 1916
    - cranial, 1915, 1916–1917
    - effects on tissues, 1912
    - systemic, 1914*f*, 1915
  - metabolic rate
    - cost of burst swimming, 1908
    - cost of continuous swimming, 1907*f*, 1908
    - cost of feeding, 1908
    - costs of growth and reproduction, 1909
    - high rates and aerobic scopes, 1906*f*, 1907
    - measurement, 1580, 1906*f*, 1907
  - physiological specializations for high aerobic capacity, 1909, 1910*f*
  - skipjack *see* Skipjack tuna
  - yellowfin *see* Yellowfin tuna
  - *see also* Pelagic fish; Scombrids
- Tunaxanthin, 694
  - definition, 692
- Tuned/tuning
  - definition, 208, 218
  - gustatory receptors, 219, 223
  - olfactory receptors, 209–210, 214–215, 217
- Tunicates, PNS/ motor neurons, evolution/ developmental transformation, 11
- Tuning curve
  - auditory afferents, 294, 294*f*
  - definition, 292
- Turbidity, fast start performance effects, 595
- Turbulence, 537, 582, 979*f*, 980
  - definition, 535
  - instabilities, 581
    - control *see stability control systems (below)*
  - representations, 582
    - importance of scale, 583, 583*f*
    - physical, 582*f*, 583
    - statistical, 582, 582*f*
  - response latency, 583
  - stability control systems, 584
    - correcting displacements, 585
    - damping instability, 584
    - failure, 585, 585*f*
  - *see also* Eddy–fish interactions
- Turbulence intensity (TI), 585
  - definition, 581
- Turbulent flow *see* Turbulence
- Turbulent kinetic energy (TKE), 582
- TwiddleFish, 603
- Twin cones, 113–114, 114*f*
- Two-alternative forced choice
  - color discrimination studies, 145–146
  - definition, 143
  - object recognition studies, 143–144, 144*f*
- Two-chamber respirometer, 852, 852*f*
- Tympanum, definition, 1807
- Type I-IV excretion systems *see* Excretion systems, extracellular products

U

- Ubiquitin, 1736
- Ultrafiltration *see* Glomerular ultrafiltration
- Ultrasound, definition, 304
- Ultraviolet (UV)
  - coloration, 694, 695*f*
  - patterns and sexual selection, 156–157
  - visual sensitivity
    - loss, salmonids, 121
    - variation between species, 157*f*
  - visual sensitivity, variation between species, 156
- Uncoating, viral, 2036
- Undulating pectoral fins, 566
- Undulatory swimming, **547–554**
  - axial movement pattern, 547–548, 548*f*
  - categories, 547, 549*f*
  - force/power transmission, muscle to skeleton, 553
  - measurement of muscle performance, 552
  - muscle length change and activity, 551, 552*f*
  - myotomal muscle anatomy, 549, 550*f*
    - muscle fiber types, 550
    - regional variation of properties, 550
  - myotomal muscle roles, 553
    - muscle to muscle power transmission, 553
    - stability control, 553
    - stiffness modulation, 553
  - *see also* Swimming
- Unfed animals, factors affecting GBF *see under* Gastrointestinal blood flow (GBF)
- Unisexuality, 1491*t*
- Unpalatable substances, avoidance/spitting, 230, 230*f*
- Unsaturation, definition, 1709
- Unstressed blood volume, 1114, 1114*f*, 1117
  - definition, 981, 1111
- Untranslated region (UTR), definition, 2046
- Upper incipient lethal temperature (UILT), definition, 1197
- Upper jaw, bony fish, 435, 438*f*, 438–439
- Upper uniform-temperature surface layers
  - definition, 1889
  - pelagic fish and, 1889, 1895*f*, 1898
- Upstream migration
  - lampreys, 1784
  - salmon, 1949*f*, 1949*f*, 1951
- Upwelling areas, hypoxia development, 1748
- Urea cycle, 1445, 1445*f*
  - elasmobranchs, 1812*f*, 1813
- Urea excretion, 1446
  - early life stages, 1447, 1448*f*
  - environmental impacts, 1447
  - lampreys, 1797
  - renal role, 1416–1417
  - *see also* Nitrogen excretion
- Urea retention
  - coelacanths, 1446
  - elasmobranchs, 1445, 1446*f*, 1815–1816
- Urea synthesis, 1445, 1445*f*
  - environmental impacts, 1447
- Urea transporters (UTs), 1446
  - rectal gland, 1434
- Ureogenesis, 1441
- Ureotelic, definition, 1437, 1444
- Ureotelic fish, 1441, 1444–1445
  - *see also* Urea excretion
- Ureotelism *see* Urea excretion
- Uricolysis, 1445, 1445*f*
  - definition, 1437, 1444
- Urinary bladder, water homeostasis role, 1372
- Urine formation *see under* Kidney(s)
- Urochordates, tunicates, motor neurons, 11
- Urodele amphibians, ampullary organs, 354
- Urophysis, 1092
  - definition, 1085, 1515
- Urotensins, vascular resistance control, 1106
- USA
  - Great Lakes, sea lamprey invasion, 1785
  - Lake Michigan, fisheries management, bioenergetics model application, 1677
- Utricle, 245–246, 253*f*, 254, 255*f*, 256*f*, 258–259, 259*f*
  - definition, 244
  - hair cell orientation, 257*f*
  - otolith, 246, 256*f*, 257*f*
  - sensory epithelium, 256*f*
  - *see also* Vestibular system
- Utricular afferents, central terminations, 247*f*, 248

V

- V-type proton ATPase, 1438, 1442
  - definition, 1437
- Vaccines, viral diseases, 2039
- Vagal afferents, definition, 1341
- Vagal innervation, definition, 1341
- Vagal preganglionic neurons (VPNs)
  - cardiac *see* Cardiac vagal preganglionic neurons (CVPNs)
  - definition, 1178
- Vagal tone, definition, 1178
- Vagus nerve
  - definition, 1178, 1509
  - *see also* Cardiorespiratory interactions (CRIs)
- Valves, venous, 1091–1092, 1112, 1113*f*
- van Bergeijk, Willem, 237, 301
- van Dam-type respirometer, 852, 852*f*
- Van der Waals interactions, protein stabilization, 1707
- Van't Hoff rule, definition, 1695
- *vasa*, 2047, 2048
  - gene regulation studies with transgenic fish, 2003*f*, 2005
- Vascular, definition, 935
- Vascular anatomy, **1085–1094**
  - arterial *see* Arterial system
  - bony fish, 1086, 1086*f*, 1087*f*, 1088*f*
  - elasmobranchs, 1093
  - hagfish, 1093
  - rectal gland, 1431, 1431*f*
  - venous *see* Venous system
  - *see also* Cardiovascular (circulatory) system
- Vascular cartilage, cartilaginous fish, 430*f*, 432
- Vascular circuits, 980, 980*f*, 1132, 1133*f*
- Vascular permeability, mammals *vs.* fish, 1159
- Vascular plexus, definition, 1457
- Vascular resistance, 1104
  - systemic *see* Systemic vascular resistance
- Vascular tone
  - definition, 1111
  - venous *see* Venous tone
- Vascularized, definition, 935
- Vasculature, fluid reabsorption/loss *see* Transcapillary fluid movement
- Vasculogenesis, 1146
  - definition, 1143
  - scaling, muscle capillary supply, 1148, 1149*f*
- Vasoactive intestinal peptide (VIP)
  - amino acid sequences, 1338*f*
  - catecholamine release role, 1530
  - gut expression, 1338
    - receptors, 1338
  - gut motility role, 1297–1298, 1299*f*
  - rectal gland activation role, 1433*f*, 1434
- Vasoconstriction, definition, 1132, 1206
- Vasodilator, definition, 1132, 1206
- Vasotocin, arginine *see* Arginine vasotocin (AVT)
- Vector, definition, 726
- Vector detection, 301
  - phase model, 301
  - *see also* Sound source localization
- Vegetable diets, effect on intestinal barrier function, 1329–1330
- Veins
  - definition, 973
  - systemic, 1088*f*, 1092
  - *see also* Venous system; *specific veins*
- Velum, 1780*f*, 1781, 1789*f*, 1791
  - definition, 1780, 1790
- Venous
  - definition, 909, 916, 929, 1067, 1119, 1889
  - *see also* Veins
- Venous capacitance, **1111–1118**
  - curves, 1114, 1114*f*, 1116*f*, 1117

  - pericardium and, 1116
  - physiological factors affecting, 1114
    - adaptation to freshwater/saltwater/high-salt diet, 1115
    - drugs, 1114
    - exercise, 1115
    - hormones, 1114
    - hypoxia, 1115
    - temperature, 1115
  - venous compliance and, 1116, 1116f
- Venous compliance, 981, 981f, 1113, 1114
  - measurement, 1116
    - *in vivo*, 1114, 1114f, 1116f, 1117
    - *in vitro*, 1116, 1117f
  - venous capacitance and, 1116, 1116f
  - *see also* Compliance
- Venous drainage
  - head, 1092
  - trunk *see under* Trunk
- Venous return, definition, 1060
- Venous system, 1086, 1112
  - anatomy, 1088f, 1088f, 1091, 1112, 1113f
    - fish *vs.* mammals, 1112, 1113f
  - cardiac output role, 1112–1113
    - *see also* Venous capacitance
  - central, 1088f, 1088f, 1092
  - drainage
    - head, 1092
    - trunk *see under* Trunk
  - functions, 1112
  - hagfish, 1116
  - head *see under* Head
  - physiology
    - capacitance *see* Venous capacitance
    - compliance *see* Venous compliance
    - tone *see* Venous tone
    - venules/small veins, 1113
  - trunk segmental, 1086f
  - *see also* Cardiovascular (circulatory) system; Veins; *specific veins*
- Venous tone, 1113
  - measurement, 1116
- Ventilation, 793, **820–828**, 847
  - air-breathing organs, 1862, 1872f
  - ammonia and, 1439
  - cranial muscle role, sharks, 452t, 455, 456f
  - definition, 791, 829, 846
  - gas-exchange organ efficiency *see* Gas exchange organs, efficiency
  - hagfish, 1775
  - measurement, two-chamber respirometer, 852, 852f
  - metabolic rate suppression and, 1768
    - *see also* Hypoxia, respiratory responses
  - plasticity in gill morphology *see* Gill remodeling
  - ram *see* Ram ventilation
    - *see also* Gas exchange organs; Gill(s); Respiration
- Ventilation rate, 821
  - effect of pain, 716, 716f, 718t
- Ventilation volume, definition, 846, 847, 865
- Ventilation–perfusion ratio, definition, 829
- Ventilatory frequency, definition, 846, 847
- Ventilatory response to CO_2/H^+, **865–870**
  - reflex responses, 865, 866f
    - air-breathing fish, 866, 867f
  - stimulus specificity, 867f, 869
  - *see also* Carbon dioxide (CO_2) chemoreceptors
- Ventilatory stroke volume, definition, 846, 847, 865
- Ventral, definition, 420
- Ventral aorta, 1086f, 1086–1087
  - blood pressure, 1035t
- Ventricle, 1030–1031
  - anatomy, 1002, 1078, 1079f, 1086–1087
    - macroscopic, 999, 999f, 1000, 1001f, 1068
    - microscopic, 1000, 1000f, 1002
    - shape/size variations, 1003, 1003f
  - definition, 2, 998, 1030, 1889, 1905
  - *see also* Cardiac cycle; Myocardium
- Ventricular action potential, 1041, 1041f
- Ventricular pressure, 1032
- Venules, physiology, 1113
- Vertebral centrum, cartilaginous fish, 422, 423f
  - areolar cartilage, 430f, 431, 432
- Vertebral column, bony fish, 442, 443f, 444f
- Vertebrate neurons, Mauthner cell as model *see under* Mauthner cells
- Vertebrates, definition, 16, 26, 37, 1986
- Vertical layers, orientation and, 1923–1924
- Vertical movements, pelagic fish *see under* Pelagic fish
- Vertical zonation
  - definition, 1961
  - intertidal communities, 1960f
- Vertical zonation, intertidal communities, 1961, 1961–1962
- Vestibular, definition, 237
- Vestibular system, 252, **244**
  - anatomy/physiology, **244**
  - definition, 252
  - elasmobranchs, 262–263, 263f, 264, 266f, 1809
  - hagfish, 245–246, 246f, 1777
  - octavolateral nuclei *see* Octavolateral vestibular nuclei
  - organization
    - conservation in other vertebrates, 251
    - sensory periphery, 245, 245f, 246f
      - *see also* Lateral line
  - role in body orientation control, 61, 61f
  - *see also* Semicircular canal(s); Utricle
- Vestibuloocular reflex, 244–245, 248, 249f
  - definition, 244
- Vestibulospinal neurons, locomotor control and posture stabilization, 250
- Viability, growth hormone transgenic fish, 2018f, 2020
- *Vibrio anguillarium*, translocation across intestinal epithelium, 1328
- Viral diseases, 2037
  - Birnaviridae, 2037
    - infectious pancreatic necrosis virus, 2034f, 2035f, 2037
  - control, 2039
    - vaccines, 2039
  - future research directions, 2040
  - Orthomyxoviridae, 2037
    - infectious salmon anemia virus, 2037
  - Orthoretroviridae, 2039
  - Rhabdoviridae, 2038, 2039f
  - Togaviridae, 2038
    - salmon alphaviruses, 2038
- Viral hemorrhagic septicemia virus (VHSV), 2038–2039, 2039f
  - vaccine development, 2040
- Viral infection process, 2035
  - assembly, 2032f, 2036
  - attachment, 2035
  - penetration and uncoating, 2036
  - replication, 2036
  - *see also* Viral diseases
- Viral replication, 2036
- Viral structure, 2034
  - nucleic acid, 2034
  - protein coat, 2034
    - envelope, 2035
    - helical, 2035
    - icosahedral, 2035
- Virion, definition, 2034
- Virulence, definition, 2034
- Virulence factor
  - definition, 2034
  - regulation, 2037
- Virus(es)
  - definition, 2034
  - infection process *see* Viral infection process
  - pathogenic *see* Viral diseases
  - translocation across intestinal epithelium, 1328
- *Vis-a-fronte* cardiac filling, 1033, 1033f
  - definition, 1030, 1073, 1215
- *Vis-a-tergo* cardiac filling, 1031–1032
  - definition, 1030, 1073
- Viscera, endothermy, 1915–1916, 1919f, 1920
- Visceral, definition, 1119
- Visceral arches, cartilaginous fish, 420f, 422
  - batoids, 425
  - chimaeroids, 426
  - sharks, 425
- Visceral arteries, 1087f, 1090
- Visceral endothermy, digestion and, 1919f, 1920
- Visceral peritoneum, definition, 1395
- Visceral retia, 1122
  - Atlantic bluefin tuna, 1122, 1123f
  - historical description, 1122, 1122f
  - lamnid sharks, 1122–1123, 1123f, 1124f
- Viscosity, 979–980
  - blood *see* Blood viscosity
  - definition, 535, 536, 536f, 977
  - kinematic *see* Kinematic viscosity
  - *see also* Reynolds number
- Vision, **98–101**
  - adaptations *see* Visual adaptations
  - anthropogenic influences, 786
  - challenges for amphibious fishes, 1858
  - color *see* Color vision
  - coral reef environment, 152
  - elasmobranch, 1809
  - optics *see* Optics, physiological
  - photoreceptors and pigments *see* Photoreceptors; Visual pigments
  - *see also* Eye(s); Spectral sensitivity; *specific eye structures (e.g. retina)*
- Visual adaptations, 107
  - amphibious vision, 108, 108f
  - deep-sea fishes *see* Deep-sea fishes, visual adaptations
  - lens focal length in relation to body size/feeding habits, 107
  - monofocal lenses in deep-diving species, 108
  - telescopic vision, 107, 108f
- Visual capabilities, behavioral studies, **143–149**
  - archerfish, 145f, 146, 147, 148f
  - color detection, 145
    - color constancy, 146
    - color discrimination, 145
    - spectral sensitivity, 145, 145f
  - future perspectives, 149
  - movement detection/analysis, 147
    - large field motion, 145f, 147
    - small field motion/perception of object speed, 147, 148f
  - object recognition, 143, 144f
  - polarized light detection, 148, 148f
  - spatial vision, 145f, 146
  - zebrafish larvae, 145f, 147, 149
- Visual communication, **692–698**
  - bioluminescent signaling, 693
  - evolution of visual signals, 697
  - overview, 692
  - reef fish, 150–151, 152, 153f
    - *see also* Reef fish, color vision/communication
  - reflected-light signaling, 694
    - pigment-based colors, 694
    - spatial patterns, 695, 697f
    - structural colors, 694, 695f, 696f
  - temporal properties of signals, 696
  - *see also* Coloration
- Visual crypsis, 747f, 747–748
- Visual ecology, reefs, 151
- Visual field enlargement, deep-sea fishes *see under* Deep-sea fishes, ocular anatomy
- Visual looming, 141
  - definition, 131
- Visual pigments, **110–115**
  - adaptations to photic environment *see under* Photic environment
  - chromophore *see* Chromophore
  - cone *see under* Cones

- Visual pigments (*continued*)
  - deep-sea fish *see* Visual pigments, deep-sea fish
  - evolution, 111, 113*f*, 116, 117*f*
  - microspectrophotometry, 111, 119*f*, 119–120
  - pineal, 163
  - rod *see under* Rods
  - structure, 110, 112*f*
  - *see also specific visual pigments*
- Visual pigments, deep-sea fish, 118, 176, 179*f*
  - effect of ambient pressure, 180
  - long-wave sensitivity, 179, 181*f*
  - multipigment species, 178, 180*f*
  - species with single visual pigment, 178, 180*f*
- Visual processing, optic tectum, 134
  - color, 135
  - patterns, 135
- Visual recognition, courtship behavior, 658
- Visual signals *see* Visual communication
- Vitamins
  - definition, 1667
  - digestion, 1306*f*, 1309
  - requirements, cultured fish, 1621
- Vitellogenesis, **635–646**, 1500–1501, 1501*f*
  - applications, 645
  - definition, 635, 656
  - endocrine control, 638, 640*f*
  - in livebearing fishes, 644
  - stages, 635, 640*f*
    - vitellogenin synthesis/secretion, 639, 640*f*
    - vitellogenin uptake and processing into yolk proteins, 640, 640*f*
- Vitellogenins, 614, 635–636, 787–788
  - alternative functions, 645
  - chemistry, 636
  - definition, 613, 635, 678
  - nomenclature/classification, 638, 638*t*, 639*f*
  - phosvitinless, 644
  - receptors, 641, 641*f*, 642*f*
  - structure, 637, 637*f*
  - *see also* Yolk proteins (YPs)
- Viviparity/viviparous fish, 614, 671*f*, 671–672, 679
  - definition, 613, 670, 678, 838
  - parental nutrient provision, 679
  - vitellogenesis, 644–645
- Vocal, definition, 684
- Vocal fishes, 312, 321, 685, 685*t*
  - discovery/evolution of behavior, 686
  - loud, 685*f*, 685–686, 686*f*
  - quiet, 685*f*, 686, 686*f*
  - simple *vs.* complex calls, 687, 688*f*
  - *see also* Calls; Sound production; Sound signals/signaling; Vocalizations
- Vocal motor nuclei (VMNs)
  - control, 326, 327*f*
  - definition, 321
  - location, 326*f*, 326–328
- Vocal organs, 322
  - drumming muscles *see* Drumming muscles
  - pectoral, 324–325, 326*f*
- Vocalizations, 321
  - croaking gouramis, 322, 322*f*
  - plainfin midshipman *see* Plainfin midshipman
  - sonograms/waveforms, 321–322, 322*f*, 323*f*
  - *see also* Calls; Sound production
- Voice behavior, **321–328**
  - *see also* Sound production, mechanisms/physiology
- Volatilization, ammonia, 1440
- Volitional swimming
  - definition, 575
  - maneuver analysis, 576
    - *see also* Maneuverability
  - *see also* Swimming
- Volitional swimming performance studies
  - data, 1660*f*, 1661*f*, 1662
  - open-channel flumes, 1659*f*, 1662
- Voltage-gated channel *see under* Ion channels
- Voltage sensor, definition, 1718
- Volume, secondary circulation, 1166
- Volume regulation
  - definition, 879
  - red blood cells, 880, 882*f*
- Voluntary behavior, Mauthner cell role in prey capture, 74*f*, 75
- von Bertalanffy growth model, 1624*f*, 1626
- von Frisch, Karl, 238–240, 240*f*, 299–300
- Vortex (or vortices), 537
  - analysis, 538*f*, 543
    - momentum analysis *vs.*, 543, 544*f*
  - definition, 516, 535, 581
  - *see also* Eddies; Vorticity
- Vortex filament, 538, 538*f*
  - definition, 535
- Vortex ring, 538, 538*f*, 541, 541*f*, 543
  - analysis, 543, 544*f*, 544–545
  - definition, 535
- Vortex strength, definition, 581
- Vorticity, 537, 538*f*, 583
  - definition, 535, 581
  - estimation, 538–539
    - *see also* Experimental hydrodynamics
  - mathematic representation, 537
  - *see also* Vortex (or vortices)

W

- Wakes, 536–537, 541
  - American eels, 541, 541*f*
  - analysis
    - power, 545, 545*f*
    - vortex, 543, 544, 544*f*
    - *see also* Experimental hydrodynamics
  - bluegill sunfish, 541, 541*f*, 542*f*
  - definition, 535
  - particle image velocimetry (PIV), 541, 541*f*
- Walleye dermal sarcoma virus (WDSV), 2039
- Walleye epidermal hyperplasia virus (WEHV), 2039
- Warm acclimation
  - cardiovascular adaptations/responses, 1199*f*, 1200–1202
    - cardiomyocytes, 1011
    - *see also* Temperature effects, cardiovascular system
  - mitochondrial role, 1647, 1714*f*, 1715
  - *see also* Thermal acclimation/adaptation
- Warm water exposure, gill remodeling *see* Gill remodeling
- Warning coloration, 150, 151, 151*f*
- Water
  - air *vs.* (as respiratory media), 792, 792*f*, 820
    - capacitance coefficients, 820, 821, 821*t*, 822, 822*f*
    - density differences, 820
    - diffusion coefficients, 820, 821
    - oxygen concentration, 792, 1747
      - *see also* Dissolved oxygen (DO)
    - partial pressure of oxygen, 792
    - viscosity differences, 820, 821
  - balance *see* Water homeostasis
  - fresh *see* Freshwater
  - homeostasis *see* Water homeostasis
  - intestinal secretion, 1301
  - intestinal uptake *see under* Intestinal absorption
  - sea *see* Seawater
  - temperature, physiological effects *see* Temperature effects
  - warm, exposure, gill remodeling *see* Gill remodeling
- Water column, definition, 1889
- Water depth, increasing, photoreceptor/visual pigment adaptations, 117, 118*f*
- Water flea, electric field, 359–360, 360*f*
  - paddlefish electrosensing, 364
- Water flow, speed, growth effects, 1632
- Water homeostasis, 1467, **1366–1372**
  - challenges
    - freshwater fish, 1367
    - marine fish, 1367
  - gastrointestinal water transport, 1318, 1318*f*, 1320, 1370, 1371*f*
  - gill water transport, 1369, 1369*f*
  - renal water flows, 1371
  - skin role, 472*f*, 474
  - urinary bladder role, 1372
  - *see also* Gut ion/acid–base regulation; Osmoregulation; Osmosis
- Water transport membrane proteins, 1367–1368
  - aquaporins *see* Aquaporins
- Water withdrawals, impacts, 1655
- Water–blood barrier thickness, definition, 803
- Watt, definition, 1624
- Wave-type electric fish, 366, 367*f*, 368, 368*f*, 369*t*
  - amplitude coding *see under* Amplitude coding
  - gymnotiform *vs.* mormyriform
    - ELL neuron electrophysiology, 371
    - *see also Gymnarchus niloticus (*mormyrid wave-type fish); Gymnotiform wave-type fish
  - time coding, 368, 368*f*, 369*t*
  - *see also* Tuberous electrosensory system, physiology
- Wave-type electric organ discharge, 366, 367*f*, 700*f*, 700–701, 702
- Wavelength, definition, 270
- Wax esters
  - buoyancy and, 521, 521*t*, 523, 525
  - definition, 520
- Weak bonds, effects of temperature, 1693
- Weakly electric fish, 366
  - gymnotiforms *see* Gymnotiform electric fish
  - mormyrids *see* Mormyrid electric fish
- Weber, Ernst-Heinrich, 238, 240*f*
- Weberian ossicles, 258, 259*f*, 274, 274*f*, 276, 277, 279
  - definition, 252, 276
  - historical description, 238, 239*f*
- Whale shark (*Rhincodon*)
  - adult fish size, 1574
  - satellite tag tracking, 1931–1932
  - swimming speed, 1581
- Whistles, bottlenose dolphin, 315, 315*f*
- White blood cells *see* Leukocytes
- White coloration, 694–695
- White flounder *(Pseudopleuronectes americanus*), skin pattern, 495*f*
- White muscle, 550, 550*f*, 556, 1637*f*, 1638
  - definition, 1636, 1889
  - energetics (anaerobic swimming), 1640
  - mitochondrial content, 969–970
  - pelagic fish, 1894
  - red muscle *vs.*, work-loop analysis, 560, 561*f*
  - *see also* Skeletal muscle
- White seabass (*Atractoscion nobilis*), spawning/acoustic behavior, 315–316, 317*f*, 318*f*
- White shark (*Carcharodon carcharias*)
  - behavior, telemetry data, 1929*f*
  - 'White Shark Café' region, 1928*f*
- Whole-animal respiration, 794
  - respiratory exchange ratios (RER), 953, 953*t*
  - techniques *see* Respiratory physiology techniques
  - *see also* Respiration
- Whole-body uptake, hormone inactivation measurement, 1194, 1195
- Whole genome duplication, 1451
  - definition, 1450
  - HPI axis genes, 1453
- Whole transcriptome shotgun sequencing, 2056*f*, 2057
- Wild fish
  - energetic costs of swimming, estimation, 1642
  - *see also individual species*
- Wildlife telemetry *see* Telemetry, wildlife
- Winberg growth model, 1626
- Winkler method, measurement of oxygen concentration, 846–847
- Winter flounder (*Pseudopleuronectes americanus*), skin pattern, 495*f*
- Wisconsin bioenergetics models, 1676
  - growth, 1627

Withdrawal responses, 588
  see also Escape responses
Withdrawals, water, impacts, 1655
Work
  definition, 555, 556
  swimming-related, 555
    skeletal muscle role, 556
      analysis *see* Work-loop analysis
Work loop(s)
  definition, 547, 555
  uses of experimental technique, 552, 553
    see also Work-loop analysis
Work-loop analysis, **555–563**, 552, 553
  cycle frequency, 557, 558
    definition, 555
    effect on work and power, 559, 559*f*
  duty cycle, 557–558
    definition, 555
  history, 556
  lengthening work, 558, 558*f*, 559–560
    definition, 555
  methodology, 556, 558*f*
    isolated muscle preparation, 556–557, 557*f*
  net work, 558*f*, 558–559
    definition, 555
    effects of strain and cycle frequency, 559, 559*f*
  power
    definition, 555, 556
    strain and cycle frequency effects, 559, 559*f*
  results, 559
    muscle functions during swimming, 562
    red *vs.* white muscle, 560, 561*f*
    strain and cycle frequency effects on work and power, 559, 559*f*
    temperature effects, 561, 562*f*
  shortening work, 556, 558, 558*f*, 559–560
    definition, 555
  stimulus/pulse frequency, definition, 555
  strain cycle, 558*f*
    definition, 555
  strain/strain amplitude
    definition, 555
    effects on work and power, 559, 559*f*
Working myocardium
  definition, 1007, 1045
  see also Cardiomyocyte(s); Myocardium
Wound repair mechanism, fish integument, 472

X

Xanthophores, 490*t*, 491–492, 493, 1971*f*, 1973
  development, 1971*f*
    genetic control, 1974
Xenobiotic(s)
  definition, 2062, 2069
  metabolism, definition, 1966
  see also Pollution/pollutants; Toxicants; Toxicology, organic chemicals; *specific xenobiotics*
Xenotransplantation, definition, 1986
Xenoturbella, 7, 8
Xiphophorus, melanoma, 1976*f*, 1977, 2004*f*, 2005

Y

Y chromosome, evolution, 2029
Yawing, 571, 575–576, 576*f*, 577, 577
  definition, 575
  hydrodynamics, 573
  see also Maneuverability
Yellow eels, 1938*f*, 1941
  visual pigments, 120, 121*f*
  see also Eel migration
Yellow perch (*Perca flavescens*), hypothalamic–pituitary–interrenal (HPI) axis development, 1455
Yellowfin tuna (*Thunnus albacares*)
  bulbus arteriosus
    function, 1021, 1021*f*, 1023*f*, 1024*f*
    structure, 1016*f*, 1020*f*, 1020*f*
  morphology, 1890*f*
Yolk, egg
  chemistry, 636
  definition, 635
  immunoglobulins, 679
  proteins *see* Yolk proteins
  see also Lecithotrophy; Yolk proteins
Yolk proteins (YPs)
  definition, 635
  maturational proteolysis, 641, 643*f*, 644*f*
  precursors *see* Vitellogenins
  structure, 637, 637*f*

Z

Z-line, 1062–1063, 1063*f*
  cardiomyocyte myofibrils, 1008–1009, 1009*f*
  definition, 1007, 1060
Zebrafish (*Danio rerio*), **1819**
  appearance, 1819*f*, 1820
    sexual dimorphism, 1820
      see also integumentary pattern (below)
  definition, 2046
  digestive system
    anatomy, 1285*f*
    development, 1284–1285
      see also Gut development
  distribution and ecology, 1821
  embryogenesis, 1284
    germ cell migration, 2049*f*, 2050
    HPI axis *see under* Hypothalamic–pituitary–interrenal (HPI) axis development, zebrafish
    as model, 993, 994*f*
    stages, 1820*f*, 1821
      see also Zebrafish (*Danio rerio*), larvae
  genome, 1822
  golden, pigmentation mutant, 1994–1995
  HPI axis development
    see Hypothalamic–pituitary–interrenal (HPI) axis development, zebrafish
  integumentary pattern, 495
    formation, 1973*f*, 1975
      see also Chromatophore development
    pigment cell types, 1971*f*, 1973
    pigmentation gene mutants, 1972*t*, 1973, 1973*f*
  larvae
    behavioral studies of visual capabilities, 145*f*, 147, 149
    gill development, 843*f*, 843–844
  lifecycle and reproduction, 1820*f*, 1821
  male reproductive organs
    morphology and anatomy, 618*f*, 619*f*
    spermatogenesis, compared with mammal, 621*f*
    spermatogonial divisions, 623, 624*f*
    spermatozoa, development steps, 624, 625*f*
  model organism status, 1986*f*, 1987
    embryonic development, 993
    erythropoiesis, 993, 994*f*
    forward genetics, 1822
    genetic research, 1821
    human hematopoiesis, 992, 993
    muscle tumor studies, 1994
    reverse genetics, 1822
    see also Fish model(s); Fish model(s), human diseases
  nacre mutant, 1994*f*, 1995
  pain response, 715, 716*f*, 716–717, 717*f*, 718*t*
  research uses, 1819
    see also model organism status (above)
  secondary circulation/lymphatic system, 1165
  sleep, 1287
  taxonomy, 1819*f*, 1820
  transgenic, glowfish, 2005*f*, 2006
Zeitgeber, 739, 774
  definition, 736, 772
Zero–one rule, 745
  definition, 744
Zinc finger nucleases, reverse genetics, 1823, 1987–1988
Zinc toxicity, 2067
  effects of social status, 654
Zoonosis, definition, 2034
Zwitter ion
  amino acids, 209
  definition, 208
Zygote, definition, 670